

TCR recognizing G12V mutated RAS and uses thereof**Technical field**

The present invention relates to the field of treating cancer patients who express mutated
5 RAS variant. Herein disclosed is a selection of verified targets for T cell receptors (TCR)
against mutated human rat sarcoma (RAS) peptides, as well as novel T cell receptors
(TCR) which are isolated and/or expressed in an engineered cell, and which specifically
recognize a specific G12V mutated human rat sarcoma (RAS) peptide, wherein the
10 peptide can be a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey
RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, and wherein the
peptide consists of VVVGAVGVGK (SEQ ID NO: 1) or VVGAVGVGK (SEQ ID NO:2) and
is presented in HLA-A*11.

Background

Adoptive cell transfer (ACT) therapies using engineered T cells (TCR-Ts) or chimeric
15 antigen receptor (CAR)-T cells, have gained much traction in recent years. The use of
CAR-T in the treatment of B cell malignancies has demonstrated to be very effective
(Weber et al 2020), however this efficacy is not replicable in the treatment of solid
tumours. TCR-T-based immunotherapy holds much promise for patients with difficult-to-
treat cancers where there are currently no alternative treatment options. A great
20 advantage of TCR-T-based therapy relates to T cells being able to recognise a much
larger number of antigenic targets and from all cellular compartments than chimeric
antigen receptors. The TCR-antigen-HLA interaction is the core component of the
targeting mechanism that allows T cells to kill cancer cells. TCRs also have lower epitope
density requirement for activation compared to chimeric antigen receptors. An important
25 consideration when designing TCR-T-based therapy is the Human Leucocyte Antigen
(HLA) restriction, as any given TCR can only be used to treat patients with the
corresponding HLA genetic background to avoid tissue rejection. This restriction needs to
be taken into consideration when developing a therapeutic TCR and when using such a
TCR in engineered T cells.

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There remains an unmet need in the art for obtaining specific and safe TCRs for difficult-
to-treat cancers.

The T cell Receptor (TCR)

The TCR is the component of the T cell responsible for interacting with and sensing the
35 targets of T cell adaptive immunity. In general terms, the TCR is comprised of a

heterodimeric protein complex presented on the cell surface. Each of the two TCR chains are composed of two extracellular domains, being the variable (V)-region and the constant (C)-region, both of the immunoglobulin superfamily (IgSF) domain, forming antiparallel β -sheets. These are anchored in the cell membrane by a type-I transmembrane domain, which adjoins a short cytoplasmic tail. The quality of the T cells to adapt and detect diverse molecular constituents arises from variations in the TCR chains that are generated during T cell genesis. This variation is generated by somatic recombination in a similar manner to antibody genesis in B cells.

TCR chain diversity

The T cell pool consists of several functionally and phenotypically heterogeneous subpopulations. However, T cells may be broadly classified as $\alpha\beta$ or $\gamma\delta$ according to the somatically rearranged TCR isoform they express at their surface. There exist two TCR chain pair isoforms; TCR alpha (TRA) and TCR beta (TRB) pairs; and TCR gamma (TRG) and TCR delta (TRD) pairs. T cells expressing TRA:TRB pairs are referred to as $\alpha\beta$ T cells, while T cells expressing TRG:TRD pairs are referred to as $\gamma\delta$ T cells.

TCRs of both $\alpha\beta$ and $\gamma\delta$ forms are responsible for the recognition of diverse ligands, or 'antigens', and each T cell generates $\alpha\beta$ or $\gamma\delta$ receptor chains *de novo* during T cell maturation. These *de novo* TCR chain pairs achieve diversity of recognition through generation of receptor sequence diversity in a process called somatic V(D)J recombination after which each T cell expresses copies of a single distinctly rearranged TCR. At the TRA and TRG loci, a number of discrete variable (V) and functional (J) gene segments are available for recombination and juxtaposed to constant (C) gene segments, thus referred to as VJ recombination. Recombination at the TRB and TRD loci additionally includes a diversity (D) gene segment and is referred to as VDJ recombination.

Each recombined TCR possesses potential for unique ligand specificity, determined by the structure of the ligand-binding site formed by the α and β chains in the case of $\alpha\beta$ T cells or γ and δ chains in the case of $\gamma\delta$ T cells. The structural diversity of TCRs is largely confined to three short hairpin loops on each chain, called complementarity-determining regions (CDR), and termed CDR1, CDR2 and CDR3 respectively. Three CDRs are contributed from each chain of the receptor chain pair, and collectively these six CDR loops sit at the membrane-distal end of the TCR extracellular domain to form the antigen-HLA binding sites.

Sequence diversity in each TCR chain is achieved in two modes. First, the random selection of gene segments for recombination provides basal sequence diversity. For example, TRB recombination occurs between 47 unique V, 2 unique D and 13 unique J

germline gene segments. In general, the V gene segment contributes both the CDR1 and CDR2 loops and are thus germline encoded. The second mode to generate sequence diversity occurs within the hypervariable CDR3 loops, which are generated by random deletion of template nucleotides and addition of non-template nucleotides, at the junctions
5 between recombining V, (D) and J gene segments.

Human Leukocyte Antigen (HLA) class I and II

HLAs are complexes on cell surface, encoded by HLA family of genes. They can also be referred to as Major Histocompatibility Complexes (MHC), for simplicity both the gene and MHC will collectively be referred to herein as HLAs. There are two forms of classical HLA
10 complexes: HLA class I (HLAI) and HLA class II (HLAII). There are three classical HLAI genes: *HLA-A*, *HLA-B*, *HLA-C*. These genes encode a membrane-spanning α -chain, which associates with an invariant beta-2-microglobulin (β 2M) chain. The HLAI α -chain is composed of three domains with an immunoglobulin fold: α 1, α 2 and α 3. The α 3 domain is membrane-proximal and largely invariant, while the α 1 and α 2 domains together form the
15 polymorphic membrane-distal antigen-binding cleft. There are six classical HLAII genes: *HLA-DPA1*, *HLA-DPB1*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DRA*, and *HLA-DRB1*. These genes encode paired DP, DQ and DR heterodimeric HLA complexes comprising a α -chain and a β -chain. Each chain has two major structural domains with an immunoglobulin fold, where the α 2 and β 2 domain comprise membrane-proximal and largely invariant modules
20 similar to that of the HLAI α 3 domain. The HLAII α 2 and β 2 domains together form the membrane-distal antigen-binding cleft and are regions of high polymorphism.

The antigen-binding cleft of HLAI and HLAII comprises two anti-parallel α -helices on a platform of eight anti-parallel β -sheets. In this cleft the peptide antigen is bound and presented in an extended conformation. The peptide-contacting residues in HLAI and
25 HLAII are the location of most of the sequence polymorphism, which constitutes the molecular basis of the diverse peptide repertoires presented by different HLA alleles. The peptide makes extensive contacts with the antigen-binding cleft and as a result each HLA allele imposes distinct sequence constraints and preferences on the presented peptides. A given peptide will thus only bind a limited number of HLAs, and reciprocally each allele
30 only accommodates a particular fraction of the peptide collection from a given protein. The set of HLAI and HLAII alleles that is present in each individual is called the HLA genotype, each of the two chromosomes containing a linear haplotype that will, in the absence of recombination, be transmitted intact to progeny cells. The polymorphism of HLAI and HLAII genes and the co-dominant expression of inherited alleles drives very large diversity
35 of HLA haplotypes across the human population, which when coupled to the enormous

sequence diversity of $\alpha\beta$ TCR, presents high obstacles to standardisation of analysis of these HLA-antigen-TCR interactions.

$\alpha\beta$ TCR engagement of HLAI and HLAII

The $\alpha\beta$ TCRs recognize peptides as part of a mixed peptide-HLA (pHLA) binding interface formed by residues of both the HLA and the peptide antigen (altered self). HLAI complexes are presented on the surface of nearly all nucleated cells and are generally considered to present peptides derived from endogenous proteins. T cells can thus interrogate the endogenous cellular proteome of an HLAI-presenting cell by sampling pHLAI complexes of an interacting cell. Engagement of HLAI requires the expression of the TCR co-receptor CD8 by the interacting T cell, thus HLAI sampling is restricted to CD8⁺ $\alpha\beta$ T cells. In contrast, the surface presentation of HLAII complexes is largely restricted to professional APCs and are generally considered to present peptides derived from proteins exogenous to the presenting cell. An interacting T cell can therefore interrogate the proteome of the extracellular microenvironment in which the presenting cell resides. The engagement of HLAII requires the expression of the TCR co-receptor CD4 by the interacting T cell, and thus HLAII sampling is restricted to CD4⁺ $\alpha\beta$ T cells.

TCR:CD3 Complex

Mature $\alpha\beta$ and $\gamma\delta$ TCR chain pairs are presented on the cell surface in a complex with accessory CD3 subunits, denoted ϵ , γ , δ and ζ . These subunits associate with $\alpha\beta$ or $\gamma\delta$ TCRs as three dimers ($\epsilon\gamma$, $\epsilon\delta$, $\zeta\zeta$). This TCR:CD3 complex (TCRsp) forms the unit for initiation of cellular signalling responses upon engagement of an $\alpha\beta$ or $\gamma\delta$ TCR with HLA-restricted antigen. The CD3 accessories associated as a TCR:CD3 complex contribute signalling motifs called immunoreceptor tyrosine-based activation motifs (ITAMs).

CD3 ϵ , CD3 γ and CD3 δ each contribute a single ITAM while the CD3 ζ homodimer contains 3 ITAMs. The three CD3 dimers ($\epsilon\gamma$, $\epsilon\delta$, $\zeta\zeta$) that assemble with the TCR thus contribute 10 ITAMs. Upon TCR ligation with antigen, phosphorylation of the tandem tyrosine residues creates paired docking sites for proteins that contain Src homology 2 (SH2) domains, such as the critical ζ -chain-associated protein of 70 kDa (ZAP- 70). Recruitment of such proteins initiate the formation of TCR:CD3 signalling complexes that are ultimately responsible for T cell activation and differentiation.

$\alpha\beta$ T cells

$\alpha\beta$ T cells are generally more abundant in humans than their $\gamma\delta$ T cell counterparts. A majority of $\alpha\beta$ T cells interact with peptide antigens that are presented by complexes on

the cell surface. Peptide-HLA (pHLA)-recognising T cells were the first to be described and are by far the best characterised. More rare forms of $\alpha\beta$ T cells have also been described. Mucosal-associated invariant T (MAIT) cells appear to have a relatively limited α and β chain diversity and recognise bacterial metabolites rather than protein fragments.

5 The invariant natural killer T-cells (iNK T cells) and germline-encoded mycolyl-reactive T cells (GEM T cells) are restricted to recognition of glycolipids that are cross-presented by non-HLA molecules. iNK T cells are largely considered to interact with CD1d-presented glycolipids, whereas GEM T cells interact with CD1b-presented glycolipids. Additional forms of T cells are thought to interact with glycolipids in the context of CD1a and CD1c,

10 however, such cells are yet to be characterised in significant detail.

Conventional $\alpha\beta$ T cells

The key feature of most $\alpha\beta$ T cells is the recognition of peptide antigens in the context of HLA molecules. These are often referred to as 'conventional' $\alpha\beta$ T cells. Within an individual, self-HLA molecules present peptides from self and foreign proteins to T cells,

15 providing the essential basis for adaptive immunity against malignancies and foreign pathogens, adaptive tolerance towards commensal organisms, foodstuffs, and self. The HLA locus that encodes HLA proteins is the most gene-dense and polymorphic region of the human genome, and there are in excess of 12,000 alleles described in humans. The high degree of polymorphism in the HLA locus ensures a diversity of peptide antigen

20 presentation between individuals, which is important for immunity at the population level.

RAS Oncogenes

Members of the RAS super-family of proteins (KRAS/NRAS/HRAS) are involved in cellular signal transduction pathways, and mutation in RAS protein family members is a canonical

25 driver of oncogenesis. The RAS family members have an identical amino acid sequence in positions 1 through to 86, and more than 99% of all cancer-associated mutations occur at positions 12, 13 or 61 (Prior *et al* 2012). As these positions are in the domains responsible for GTP dephosphorylation into GDP, the mutations lead to constitutive activation of the RAS protein. Since they are a gain of function mutations, one mutated

30 allele expressed at normal level is sufficient to support cancer establishment and growth. Predominant mutations in the RAS occur in KRAS (85%) but also in NRAS (11%) and HRAS (4%). KRAS mutation patterns are dominated by G to A transitions at the second base of codon 12 resulting in G12D mutations, and by G to T transitions at the same base leading to G12V mutations (COSMIC - Catalogue of Somatic Mutations In Cancer

35 <https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=KRAS>). KRAS is frequently mutated

in a variety of hard-to-treat cancers, including pancreatic cancer (~90%), colon cancer (~43%), lung cancer (~30%), and melanoma (~50%).

Pancreatic Ductal Adenocarcinoma

- 5 Pancreatic cancer is the fourth leading cause of cancer death in both the European Union (EU) and the United States (US), with incidences of 7.7 and 7.6 per 100,000 people, respectively (Rawla et al, 2019). Pancreatic ductal adenocarcinoma, an exocrine pancreatic cancer, accounts for 95% of all pancreatic cancers. KRAS mutation is the most frequent mutation (more than 90%) (Hobbs et al, 2013) and the initiating genetic event for
- 10 PDAC. KRAS is found in primary tumours, metastatic tumours and even in pancreatic intraepithelial neoplasia, the earliest preneoplastic stage in PDAC progression. More than three-quarters of patients with pancreatic cancer present with advanced disease, of whom around half have distant metastases at diagnosis (Ducreux et al, 2015). The 5-year life expectancy for pancreatic cancer is about 5% and is essentially
- 15 unchanged over the last 2 decades.

- Surgical resection is the only curative modality for PDAC but, at best, only a fifth of patients are considered operable and even in these cases, the 5-year survival is ~20% (Ducreux et al, 2015). Phase 3 data supports the use of chemotherapy combination
- 20 regimens, such as 5 fluorouracil (5 FU), irinotecan, and oxaliplatin (FOLFIRINOX), or gemcitabine and nab-paclitaxel (Abraxane®), as the preferred first-line treatment options for patients who can tolerate aggressive therapy. However, these combination regimens have been linked to cumulative toxicity, which often necessitates dose reductions or discontinuation of certain or all chemotherapeutic agents prior to disease progression. For
- 25 example, in clinical practice, the FOLFIRINOX regimen is typically limited to 12 cycles (equivalent to 6 months) before therapy de-escalation, or a treatment hiatus is implemented to mitigate toxicity.

- For patients who demonstrate a positive or stable response after 4-6 months of first-line
- 30 therapy, there are various treatment options available, ranging from continued indefinite therapy to complete cessation of treatment.

- In PDAC, the dominant KRAS mutations are G12V and G12D. Clinical experience targeting cell surface expressed mutated KRAS pHLA complexes includes the recent
- 35 report of autologous T cells genetically transduced to express 2 allogeneic TCRs targeting mutated KRAS G12D-HLA-C*08:02 in an adult with relapsed metastatic PDAC (Leidner et al, 2022).

TCR-T-based immunotherapy targeted towards RAS-mutations holds much promise for patients with difficult to treat cancers where there are currently no alternative treatment options such as but not limited to PDAC, colorectal cancer, non-small cell lung cancer and melanoma . Still, there is a need for validating optimal targets and isolating TCRs that

5 recognize these suitable targets with high affinity and specificity without displaying cytotoxicity towards e.g., suboptimal targets or wild-type RAS.

Summary of the invention

In general, the identification of TCRs that have a particularly suitable profile for therapeutic

10 use is not straightforward and typically has a high attrition rate. The current invention thus for the first time discloses several verified suitable RAS targets as well as TCRs that have been stringently de-risked and are therefore suitable therapeutic candidates for use in cancer therapy.

15 The current invention relates to several new T cell receptors (TCR), which are isolated and/or expressed in an engineered cell, and which specifically recognize a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, which consists of VVGAVGVGK (SEQ ID NO: 1)

20 or VVGAVGVGK (SEQ ID NO:2) presented in HLA-A*11, and wherein the individual TCR comprises a paired TCR alpha- and TCR beta-chain, wherein the TCR alpha chain variable domain comprises SC1alpha-CDR1alpha-SC2alpha-CDR2alpha-SC3alpha-CDR3alpha-SC4alpha-SC5alpha, and the TCR beta chain variable domain comprises SC1beta-CDR1beta-SC2 beta-CDR2 beta-SC3beta-CDR3beta-SC4beta-SC5beta,

25 wherein SC is a scaffold region and CDR is a complementarity determining region. In one embodiment, as exemplified in the experimental section, the TCR according to the current invention is a TCR, which, when expressed in an engineered cell, does not have detectable cross-reactivity towards any somatically expressed HLA-presented peptide other than a G12V mutated human rat sarcoma (RAS) peptide, which consists of

30 VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) and which is presented in HLA-A*11 in a cross-reactivity assay.

In aspects, TCRs according to the invention have a higher sensitivity towards SEQ ID NO:1 and/or SEQ ID NO:2 when presented in a HLA-A*11 complex than does a known

35 TCR. Such sensitivity is determined in a cell-cell contact assay wherein peptide-pulsed eAPCs are contacted with TCR-expressing eTPCs, wherein the eTPCs present the TCR to be assessed and wherein the eTPCs have been engineered to contain a Response

Element used to asses the sensitivity in combination with CD3 downregulation. Such an assay is described in Example 3 in more detail.

In aspects, the sensitivity is at least 1.10 fold, such as at least 1.15 fold, such as at least 1.2 fold, such as at least 1.25 fold, such as at least 1.3 fold, such as at least 1.35 fold , such as at least 1.4 fold, such as at least 1.45 fold, such as at least 1.5 fold, such as at least 1.6 fold, such as at least 1.65 fold higher than that of a known TCR.

- 10 A TCR disclosed herein typically comprises:
 - a. a TCR alpha chain CDR3 sequence with at least 75%, such as at least 80% identity to a TCR alpha chain CDR3 sequence selected from the group consisting of SEQ ID NO: 88, SEQ ID NO: 90, and SEQ ID NO: 98, and SEQ ID NO: 141, and SEQ ID NO: 143, and
 - 15 b. a TCR beta chain CDR3 sequence with at least 75%, such as at least 80% identity to a TCR beta chain CDR3 sequence selected from the group consisting of SEQ ID NO: 89, SEQ ID NO: 91, and SEQ ID NO: 99, and SEQ ID NO 142, and SEQ ID NO 144.
- 20 In embodiments, the TCR according to the current invention comprises:
 - a. a TCR alpha chain CDR3 sequence with a TCR alpha chain CDR3 sequence selected from the group consisting of SEQ ID NO: 88, SEQ ID NO: 90, and SEQ ID NO: 98, and SEQ ID NO: 141, and SEQ ID NO: 143, and
 - 25 b. a TCR beta chain CDR3 sequence with a TCR beta chain CDR3 sequence selected from the group consisting of SEQ ID SEQ ID NO: 89, SEQ ID NO: 91, and SEQ ID NO: 99, and SEQ ID NO 142, and SEQ ID NO 144.

In a currently preferred embodiment of the invention the TCR alpha and beta chain variable domain scaffold regions or CDRs of the TCR comprises and/or consists of one of the sequences selected from the group consisting of :

	SEQ ID NO	SEQ ID NO	SEQ ID NO	SEQ ID NO	SEQ ID NO	SEQ ID NO	SEQ ID NO
SC1alpha	18	20	28	126	128	18	20
CDR1alpha	60	62	70	135	137	60	62
SC2alpha	32	34	42	129	131	32	34
CDR2alpha	74	76	84	138	140	74	76

SC3alpha	46	48	56	132	134	46	48
CDR3alpha	88	90	98	141	143	88	90
SC4alpha	7	9	15	123	125	7	9
SC5alpha	104	104	104	104	104	104	104
SC1beta	19	21	29	127	30	19	21
CDR1beta	61	63	71	136	72	61	63
SC2beta	33	35	43	130	44	33	35
CDR2beta	75	77	85	139	86	75	77
SC3beta	47	49	57	133	58	47	49
CDR3beta	89	91	99	142	144	89	91
SC4beta	8	10	16	124	12	8	10
SC5beta	105	105	106	106	106	5162	5162

or sequences having at least 90% such as at least 95%, at least 97% or at least 99%, or 100% identity to said respective sequences.

In currently preferred embodiments, the TCR alpha chain variable, domain scaffold regions, and/or or CDRs comprise and/or consist of one of the sequences selected from the group consisting of SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 117, SEQ ID NO: 145 and SEQ ID NO: 147, wherein the beta chain variable, domain scaffold regions, and/or or CDRs comprise and/or consist of one of the sequences selected from the group consisting of SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 118, SEQ ID NO: 146 and SEQ ID NO: 148.

A TCR according to the current invention can be selected from the group comprising chimeric, humanized and human TCRs.

A TCR according to the invention can comprise constant regions of the alpha and beta chains which have been modified to include cysteine residues to allow covalent connection by di-sulphide bridges or comprise constant regions of the alpha and beta chains which allow non covalent connections between said paired alpha and beta chains. In embodiments, the alpha and/or beta chain comprise(s) a constant region of a TCR-chain of another mammal, such as, but not limited to, a mouse chimeric constant region(s). Further, the alpha and/or beta chain can have been modified to include cysteine residues to allow di-sulphide bridges.

In aspects, the TCR comprises a TCR alpha chain (TRA) and a TCR beta chain (TRB) which are covalently linked, such as through a linker peptide. The TCR alpha chain and/or the TCR beta chain can further be covalently linked to a moiety, and the linked moiety can

comprise an affinity tag or a label. In embodiments, the tag is selected from the group consisting of a CD34 enrichment tag, glutathione-S-transferase (GST), calmodulin binding protein (CBP), protein C tag, Myc tag, Halo tag, HA tag, Flag tag, His tag, biotin tag, and V5 tag. In embodiments, the label is a fluorochrome or a fluorophore such as a fluorescent protein.

In aspects, the covalently linked moiety is selected from the group consisting of an inflammatory agent, cytokine, toxin, cytotoxic molecule, radioactive isotope, an enzyme that catalyses a cell wall sorting reaction, and an antibody or antigen-binding fragment thereof.

The current invention in one aspect relates to a soluble TCR and/or a conjugated soluble TCR according to the current invention. In aspects, the TCR is a bi-specific T cell engager (such as BiTE), or it is an immune mobilizing monoclonal T cell receptor.

The invention further relates to a pharmaceutical composition comprising a soluble protein consisting of all or a functional part of a TCR as disclosed and/or defined herein and at least one pharmaceutically acceptable excipient, carrier and/or stabilizer.

A pharmaceutical composition of the current invention comprises a soluble TCR, a conjugated soluble TCR, a cell expressing a TCR and/or a composition comprising a TCR, wherein the TCR is a TCR according to the current invention. Said soluble TCR can be conjugated to a radionuclide, a chemotherapeutic agent, a toxin or another therapeutically active component, and at least one pharmaceutically acceptable excipient, carrier or stabilizer.

In one aspect, the invention relates to a method of treating a disorder characterized by G12V mutated human RAS expression in a subject comprising administering to said subject a therapeutically effective amount of genetically engineered cells that express a TCR as disclosed and/or defined herein, or a pharmaceutical composition disclosed and/or defined herein, wherein said disorder is a solid or haematological malignancy expressing the peptide of VVVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2), wherein said engineered cell is a T cell, T cell progenitor cell, NK cell, NK progenitor cell or a differentiated stem cell and which is either autologous or allogenic, and wherein about 10^4 to about 10^{10} engineered cells are infused systemically into said subject or injected or pumped directly into a solid tumour, into a blood vessel that feeds a tumour and/or into the volume surrounding a solid tumour.

Said method can be used for treating a disorder in a subject wherein said subject carries an HLA allele selected from HLA-A*01, HLA-A*02, HLA-A*03, HLA-A*11, HLA-A*23, HLA-A*24, HLA-A*25, HLA-A*26, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*32, HLA-A*33, HLA-A*34, HLA-A*66, HLA-A*68, HLA-A*80, HLA-B*07, HLA-B*08, HLA-B*13, HLA-B*14, HLA-B*15, HLA-B*18, HLA-B*27, HLA-B*35, HLA-B*37, HLA-B*38, HLA-B*40, HLA-B*44, HLA-B*49, HLA-B*50, HLA-B*51, HLA-B*52, HLA-B*53, HLA-B*54, HLA-B*55, HLA-B*57, HLA-B*58, HLA-C*01, HLA-C*02, HLA-C*03, HLA-C*04, HLA-C*05, HLA-C*06, HLA-C*07, HLA-C*08, HLA-C*12, HLA-C*14, HLA-C*15, HLA-C*16, HLA-E*01, optionally wherein the HLA allele is selected from the group consisting of HLA-A*01:01, HLA-A*01:02, HLA-A*01:03, HLA-A*02:01, HLA-A*02:02, HLA-A*02:03, HLA-A*02:04, HLA-A*02:05, HLA-A*02:06, HLA-A*02:07, HLA-A*03:01, HLA-A*11:01, HLA-A*11:02, HLA-A*11:03, HLA-A*11:04, HLA-A*11:05, HLA-A*11:09, HLA-A*23:01, HLA-A*24:02, HLA-A*24:03, HLA-A*24:04, HLA-A*24:07, HLA-A*24:10, HLA-A*24:17, HLA-A*25:01, HLA-A*26:01, HLA-A*29:02, HLA-A*30:01, HLA-A*30:02, HLA-A*31:01, HLA-A*32:01, HLA-A*33:01, HLA-A*33:03, HLA-A*34:02, HLA-A*66:01, HLA-A*66:02, HLA-A*68:01, HLA-A*68:02, HLA-A*80:01, HLA-B*07:02, HLA-B*07:05, HLA-B*08:01, HLA-B*13:01, HLA-B*13:02, HLA-B*14:01, HLA-B*14:02, HLA-B*15:01, HLA-B*15:02, HLA-B*15:03, HLA-B*18:01, HLA-B*27:05, HLA-B*35:01, HLA-B*35:02, HLA-B*35:03, HLA-B*35:05, HLA-B*35:08, HLA-B*35:12, HLA-B*37:01, HLA-B*38:01, HLA-B*40:01, HLA-B*40:02, HLA-B*44:02, HLA-B*44:03, HLA-B*49:01, HLA-B*50:01, HLA-B*51:01, HLA-B*51:02, HLA-B*52:01, HLA-B*53:01, HLA-B*54:01, HLA-B*55:01, HLA-B*57:01, HLA-B*58:01, HLA-C*01:02, HLA-C*02:02, HLA-C*03:03, HLA-C*03:04, HLA-C*04:01, HLA-C*05:01, HLA-C*06:02, HLA-C*07:01, HLA-C*07:02, HLA-C*08:01, HLA-C*08:02, HLA-C*12:02, HLA-C*12:03, HLA-C*14:02, HLA-C*15:02, HLA-C*16:01, HLA-E*01:01, HLA-E*01:03. In an embodiment the HLA allele is HLA-A*11, such as HLA-A*11:01.

In addition, said method can be used for treating a said subject, wherein said subject carries an alternate subform of HLA-A*11 other than HLA-A*11:03 or HLA-A*11:04, which alternate subform of HLA-A*11 is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which is specifically recognized by the TCR according to the current invention, such as wherein said subject carries a different HLA-A, HLA-B or HLA-C allele than HLA-A*11:01, that is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which is recognized by the TCR according to the current invention.

Said method is in particular intended for treating a disorder in a subject, wherein said subject carries an HLA-A*11 allele. In addition, said method can be used for treating a said subject, wherein said subject carries an alternate subform of HLA-A*11 other than

HLA-A*11:03 or HLA-A*11:04, which alternate subform of HLA-A*11 is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which is specifically recognized by the TCR according to the current invention, or wherein said subject carries a different HLA-A, HLA-B or HLA-C allele than HLA-A*11, that is capable
5 of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which is recognized by the TCR according to the current invention.

In one aspect, the current invention relates to a TCR disclosed, described and/or defined herein for use as a medicament. In particular, the current invention relates to a TCR
10 disclosed, described and/or defined herein for use in the treatment of a cancer in a patient carrying HLA-A*11, comprising administering to said patient said TCR or a pharmaceutical composition comprising said TCR, wherein said cancer is a solid or haematological malignancy expressing the peptide of SEQ ID NO: 1 or SEQ ID NO: 2, and wherein said TCR or a pharmaceutical composition comprising said TCR is infused systemically into
15 said patient or injected or pumped directly into a solid tumour, into a blood vessel that feeds a tumour and/or into the volume surrounding a solid tumour in said patient.

In aspects, said treatment comprises administering to said patient an engineered cell, wherein said engineered cell is a T cell, T cell progenitor cell, NK cell, NK progenitor cell
20 or differentiated stem cell and which is either autologous or allogenic, and wherein about 10^4 to about 10^{10} engineered cells are infused systemically into said patient or injected or pumped directly into a solid tumour, into a blood vessel that feeds a tumour and/or into the volume surrounding a solid tumour.

25 Typically, the use related to herein is for treating a disorder in a subject, wherein said subject carries a subform of HLA-A*11 capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and thus is recognized by a TCR according to the current invention.

30 In an aspect a TCR according to the invention is capable of specifically recognising both of SEQ ID NO: 1 and SEQ ID NO: 2 when either is presented in a HLA-A*11 complex, thereby allowing for treatment of patients wherein either or both peptides are presented in the HLA-A*11 context.

35 Typically, the use related to herein is for treating a disorder in a subject, wherein said subject carries an alternate subform of HLA-A*11, which alternate subform of HLA-A*11 is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and thus is recognized by a TCR disclosed, described and/or defined herein, such as wherein said

subject carries a different HLA-A, HLA-B or HLA-C allele than HLA-A*11, that is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which is recognized by the TCR according to the current invention.

- 5 Typically, the use related to herein is for treating a disorder in a subject, wherein said subject carries an alternate subform of HLA-A*11 other than HLA-A*11:03 or HLA-A*11:04, which alternate subform of HLA-A*11 is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and thus is recognized by a TCR disclosed, described and/or defined herein,

10

In one aspect, the invention relates to a nucleic acid encoding a TCR comprising a paired TCR alpha- and TCR beta-chain as disclosed, described and/or defined herein.

- 15 In another aspect, the invention relates to a vector comprising a nucleic acid encoding a TCR comprising a paired TCR a - and TCR b chain as disclosed, described and/or defined herein. Said vector can be selected from the group consisting of an expression vector, a cloning vector, viral vector or a non-viral vector. In certain aspects, said vector further comprises a nucleic acid sequence encoding CD8 α and/or CD8 β that can be operably linked to a nucleic acid encoding a tag, such as, but not limited to, wherein the
- 20 nucleic acid encoding a tag is at the 5' upstream of the nucleic acid sequence encoding TCR α and/ or TCR β and/or wherein the tag is a truncated CD34 molecule representing a cell surface enrichment tag. The isolated nucleic acid according to the current invention and the nucleic acid sequence encoding CD8 α and/or CD8 β can optionally be
- 25 interconnected with an internal ribosome entry site or a nucleic acid sequence encoding a self-cleaving peptide, such as selected from the group consisting of P2A, E2A, F2A and T2A.

- In aspects of the invention, the vector disclosed, described and/or defined herein is a nucleic acid vector, viral vector or a nanoparticle vector, wherein the nucleic acid vector
- 30 comprising one or more nucleic acid sequences encoding a TCR comprising a paired TCR alpha- and TCR beta-chains as but are not limited to, linear or circular double stranded DNA, or linear or circular single stranded DNA, that may or may not be chemically modified DNA, linear or circular double stranded RNA, or linear or circular single stranded RNA, that may or may not be chemically modified RNA. The RNA vector
- 35 may contain a 5' CAP, 5' UTR, 3' UTR and 3' poly(A) tail and Internal Ribosome Entry Site (IRES), and may contain substituted nucleotides such as pseudouridine, and may contain replication-directing elements for self-amplification and/or may be circularized. In aspects, the viral vector comprises a packaged nucleic acid payload within one or more surface

envelope receptors that bind to a ligand on a target cell, heterologous viral envelope glycoproteins, fusion glycoproteins, T cell activation or co-stimulation molecules, cytokines or cytokine-based transduction enhancers, and/or transmembrane proteins comprising a mitogenic domain and/or cytokine-based domain exposed on the surface and/or

5 conjugated to the surface of the viral vector. The tropism of the vector can further be retargeted by pseudotyping with a Cocal or a Nipah virus envelope protein, and the Nipah envelope protein can be engineered to bind EpCAM, CD3, CD5, CD4, or CD8 or other marker suitable for targeted cellular delivery of a encapsulated nucleic acid vector. Such viral vectors may be used both ex vivo and in vivo to direct the mRNA payload to a

10 desired immune cell type. In certain aspects, a nucleic acid payload according to the current invention is encapsulated within a nanoparticle vector such as lipid nanoparticle (LNP) comprising ionizable cationic lipid, a sterol, a phospholipid, a non-functionalized PEG-lipid, and a functionalized PEG-lipid wherein the functionalized PEG-lipid may have been conjugated with a binding moiety, and wherein the LNP encapsulates a payload of

15 mRNA. The mRNA payload may contain a linear expression construct with 5' cap, 5' UTR, 3' UTR, poly-A features and IRES, and may contain substituted nucleotides such as pseudouridine, and may contain replication-directing elements for self-amplification. The mRNA payload may also be circular. The LNP can be targeted (tLNP) to deliver payload to cell types based on expression of cell surface markers (Tombácz et al 2021; Breda et al

20 2023) for example LNP may be conjugated to anti-CD5, or anti-CD3, or anti-CD4, or anti-CD8 targeting molecules for targeting CD8⁺ and CD4⁺ T cells (Wada (2018); Dalloul, (2009), Lee (2021)) or CD16 or NK1.1 for targeting NK cells, EpCAM as a targeting epithelial cells targeting molecule or to another marker or a functional ligand thereof that targets cellular delivery to specific target cells. Such tLNP particles may be used both ex

25 vivo and in vivo to direct the mRNA payload to a desired immune cell type.

In aspects, such vectors may be used for ex vivo manufacture of cell therapy products for infusion and/or targeted delivery of vectors in vivo for TCR expression in targeted cell populations. For ex vivo manufacture and cellular manipulation, vectors may be delivered

30 to cells maintained in culture as encapsulated LNPs, as tLNPs, by combination with agents that promote uptake of nucleic acids such as lipofectamine, by electroporation or by inclusion in a viral-genome derived backbone so as to form a viral-like particle (VLP) capable of mediating both integrative or non-integrative manipulation of target cells.

35 Several viral vector systems, modified viruses designed to deliver genetic material into cells, are suitable for such manipulations and include viral vector systems derived from retroviruses such as lentivirus (LV) and gammaretrovirus, systems which are typically used for ex vivo manipulation and manufacture. Viral systems incorporating

vectors may also be used for in vivo delivery of target payloads, typically derived from adeno-associated virus (AAV).

5 A further aspect of the invention relates to an engineered cell expressing the TCR as disclosed, described and/or defined herein and/or the isolated nucleic acid disclosed, described and/or defined herein and/or which comprises a vector disclosed, described and/or defined herein.

10 Optionally, the engineered cell according to the current invention comprises a chromosomal gene knock out of one or more TCR gene(s), one or more HLA gene(s), or both, such as wherein the engineered cell comprises a knockout of an HLA gene selected from the group consisting of an α -1-macroglobulin gene, α -2-macroglobulin gene, α -3-macroglobulin gene, β -1-microglobulin gene, β -2-microglobulin gene, and combinations thereof, or such as, wherein the engineered cell comprises a knockout of a TCR gene
15 selected from a TCR alpha variable region gene, TCR beta variable region gene, TCR constant region gene, and combination thereof. An engineered cell according to the current invention can express CD8a and/or CD8b, and, optionally, the CD8a and/or CD8 b is fused to a truncated CD34 enrichment tag. The engineered cells can be enriched using the truncated CD34 enrichment tag.

20 Typically, the engineered cell disclosed, described and/or defined herein, is a hematopoietic progenitor cell, peripheral blood mononuclear cell (PBMC), cord blood cell, or immune cell. In an embodiment, the immune cell is a T cell, cytotoxic lymphocyte, cytotoxic lymphocyte precursor cell, cytotoxic lymphocyte progenitor cell, cytotoxic
25 lymphocyte stem cell, CD4⁺ T cell, CD8⁺ T cell, CD4/CD8 double negative T cell, gamma delta ($\gamma\delta$) T cell, natural killer (NK) cell, NK-T cell, dendritic cell, or a combination thereof. In one embodiment, the cell is derived from a hematopoietic stem cell (HSC) or an induced pluripotent stem cell (iPSC). The T cell can be a naive T cell, central memory T cell, effector memory T cell, or a combination thereof, or a primary T cell or a cell of a T
30 cell line.

In a currently preferred embodiment, the engineered cell disclosed, described and/or defined herein does not express or has a lower surface expression of an endogenous TCR and is capable of producing a cytokine or a cytotoxic molecule when contacted with
35 a target cell that presents a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the

peptide consists of VVVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO:2) presented in a HLA-A*11 context.

5 In embodiments, the engineered cell according to the current invention is capable of producing TNF-alpha, IL-2, and/or IFN-gamma, and/or a perforin and/or a granzyme, such as granzyme B. An engineered cell according to the current invention can be contacted with a target cell *in vitro* or *ex vivo* and is preferably capable of producing a higher level of cytokine or a cytotoxic molecule when contacted with a target cell with a heterozygous expression of human RAS, such as an at least 1.05-fold higher level of cytokine or a
10 cytotoxic molecule.

In embodiments, the engineered cell according to the current invention is capable of killing a target cell that presents a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey
15 RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the peptide consists of VVVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO:2) presented in a HLA-A*11 context. The killing is determined by a killing assay and the ratio of the engineered cell and the target cell in the killing assay is from 1:64 to 1:1, wherein the target cell is a target cell pulsed with a range of between 0.1 pM and 1 mM of
20 a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the peptide consists of VVVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO:2) presented in a HLA-A*11 context.

25 The engineered cell of the current invention does not induce T cell expansion, cytokine release, or cytotoxic killing when in contact with a target cell that presents a peptide selected from the group consisting of VVGAGGVGK (SEQ ID NO: 3) presented in HLA-A*11 context, or VVGAGGVGK (SEQ ID NO: 4) presented in HLA-A*11 context.

30 In an embodiment, the engineered cell disclosed, described and/or defined herein is capable of killing a higher number of target cells when the target cells have heterozygous expression of a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS)
35 peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the peptide consists of VVVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO:2) presented in a HLA-A*11 complex. Typically, the cell killing is at least 1.05-fold higher in the target cells

that present VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO:2) in HLA-A*11.

In aspects, the target cell is a cell line or a primary cell, such as selected from the group consisting of a cancer cell line, a primary cancer cell line, a transformed cell line, and an immortalized cell line, such as a HEK293-, ARH-77-, COR-L23-, NCI-H441-, SW620-, QGP-1-, or IGR-1- derived cell lines. Such cell lines may be modified to stably express HLA-A*11 and/or luciferase reporters.

The current invention also relates to a population of engineered cells disclosed, described and/or defined herein as well as to a pharmaceutical composition comprising any one or more of the engineered cells or vectors of the current invention, such as wherein the vectors are selected from the group consisting of viral vectors, non-viral vectors, LNPs or any other carrier comprising the nucleic acid sequence described, disclosed and/or defined herein.

Abbreviations and definitions

It is to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise.

Abbreviations

aAM	Analyte antigenic molecule
aAPX	Analyte antigen-presenting complex
APC	Antigen-presenting cell
APX	Antigen-presenting complex
B-cell	B lymphocytes
β2M	Beta 2 Microglobulin
BFP	Blue fluorescent protein
CAR	Chimeric antigen receptor
CAR-T	CAR T cell
CD1b	Cluster of differentiation 1b
CD1d	Cluster of differentiation 1d
CD3	Cluster of differentiation 3

CDR	Complementarity-determining regions
CM	Cargo molecules
CMV	Cytomegalovirus
C-region	Constant region
D-region	Diversity region
DAMPS	Danger associated molecular patterns
DC	Dendritic cells
DNA	Deoxyribonucleic acid
dsDNA	Double stranded DNA
eAPC	Engineered antigen-presenting cell
eAPC-a	Engineered antigen-presenting cell expressing an analyte antigenic molecule
eAPC-p	Engineered antigen-presenting cell that present an analyte antigen-presenting comple: Engineered antigen-presenting cell that presents an analyte antigen-presenting
eAPC-pa	complex and analyte antigenic molecule
eAPCS	Engineered antigen-presenting cell system
eTPC	Engineered TCR-presenting cell
eTPC-t	Engineered TCR-presenting cell that present full-length TCR pairs
eTPCS	Engineered TCR-presenting cell system
FAB	Antibody fragment antigen binding
FACS	Fluorescence-activated cell sorting
FL	Full length
FRT	Flippase recognition target
GEM T-cells	Germ line-encoded mycolyl lipid-reactive T-cells
GFP	Green fluorescent protein
HCMV	Human Cytomegalovirus
HDR	Homology directed recombination
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HLAI	HLA class I
HLAII	HLA class II
IgSF	Immunoglobulin superfamily
iNK T-cells	Invariant natural killer T-cells
IRES	Internal ribosome entry site
ITAM	Immunoreceptor tyrosine-based activation motif
J-donor	Joining donor
J-region	Joining region

LNP	Lipid Nanoparticle
MACS	Magnetic-activated cell sorting
MAGE	Melanoma associated antigen
MAIT	Mucosal-associated invariant T
MG	Minigene
MHC	Major Histocompatibility Complex
MR1	Major histocompatibility complex class I-related gene protein
mRNA	Messenger ribonucleic acid
NCBP	Non-cell based particles
NK T-cells	Natural killer T cells
odeCDR3	Oligonucleotide duplex encoding CDR3
ORF	Open reading frame
PAMPS	Pathogen-associated molecular patterns
PCR	Polymerase chain reaction
PDAC	Pancreatic ductal adenocarcinoma
pHLA	Peptide HLA
pMHC	Peptide MHC
PMSA	Prostate-specific membrane antigen
RFP	Red fluorescent protein
RMCE	Recombinase mediated cassette exchange
RNA	Ribonucleic acid
RT	Reverse Transcription
SH2	Src homology 2
T cells	T lymphocytes
TAA	Tumour-associated-antigens
TALEN	Transcription activator-like effector nucleases
TCR	T-cell Receptor
TCRsp	TCR surface proteins in complex with CD3
tLNP	Targeted Lipid Nanoparticle
TORES	TCR ORF Reconstitution and Engineering System
TRA	TCR alpha
TRB	TCR beta
TRD	TCR delta
TRG	TCR gamma
UFD	Ubiquitin fusion domain
V-C entry	
vector	Variable-Constant entry vector
V (-region)	Variable region

ZAP-70 ζ -chain-associated protein of 70 kDa

Definitions

aAM: Analyte antigenic molecule. Generally, a protein but could also be a metabolite that is expressed by a cell from their genomic DNA and/or a specific introduced genetic
5 sequence. The AM is expressed in the cell and a fragment can then be presented on the cell surface by an APX as cargo or on its own. Either as cargo or not, the AM can then be the target of T-cell receptor bearing cells or related affinity reagents.

Adaptive immunity: A subsystem of the overall immune system that is composed of highly specialized, systemic cells and processes that eliminate pathogens or prevent their
10 growth.

Adjuvants

Adjuvant – substance, which when administered prior, together or after administration of an antigen accelerates, prolong and/or enhances the quality and/or strength of an immune response to the antigen in comparison to the administration of the antigen alone.
15 Adjuvants can increase the magnitude and duration of the immune response induced by vaccination.

A pair of complementary TCR chains: Two TCR chains wherein the translated proteins are capable of forming a TCRsp on the surface of a TCR presenting cell when expressed with the CD3 components.

20 **Affinity:** Kinetic or equilibrium parameter of an interaction between two or more molecules or proteins

Affinity Reagent: Any reagent that is prepared as analyte to probe TCRsp binding and/or stimulation at the cell surface of the eTPC-t in an eTPC:A system

Allele: Variant form of a given gene

25 **Alloreactivity:** Significant activity of a TCR to an HLA other than that for which it was selected; "off-HLA" reactivity

Amplicon: a piece of DNA or RNA that is the source and/or product of artificial amplification using various methods including PCR

Analyte: an entity that is of interest to be identified and/or measured and/or queried in the
30 combined system

Antibody: Affinity molecule that is expressed by specialized cells of the immune system called B-cells and that contains of two chains

Antigen: any molecule that may be engaged by a TCR and results in a signal being transduced within the T cell

- 5 **Analyte antigen:** collectively the eTPC:Antigen system (eTPC:A) representing any entity presenting an antigen for analytical determination

Antibody Drug Conjugates: (ADCs) consist of antibodies, toxic drugs, and linkers. The antibody moiety targets the antigen on the tumour, once bound the ADCs are internalised into the tumour and release the drugs in lysosomes to kill the tumour cells

- 10 **Antigen-binding cleft:** long cleft or groove that is the site at which peptide antigens bind to the MHC-I molecule

APC: Antigen-presenting cell. A cell capable of presenting antigen on its cell surface, generally in the context of an HLA

- 15 **aAPX:** Analyte antigen-presenting complex. A protein that is expressed and presented on the cell surface by nucleated cells from genes/ORF encoding genomic DNA and/or a specific introduced genetic sequence. The APX presents a cargo, being either a peptide or other metabolite molecules.

Autoimmunity: Is the system of immune responses of an organism against its own healthy cells and tissues.

- 20 **Avidity:** the sum total of the strength of binding of two molecules to one another at multiple sites, eg taking into account the valency of each interaction

Bi-Specific T cell engager: artificial bispecific monoclonal antibodies that direct T cell's cytotoxic activity against cancer or a target cell

- 25 **C (-region):** Constant gene segment. One of the gene segments that is used to assemble the T-cell receptor. The c-region is a distinct segment that rather than driving diversity of the TCR, defines its general function in the immune system.

Cargo-loading machinery: Cellular set of proteins that generate and load cargo molecules on APX from proteins or other presented molecules found in the cell.

- 30 **C cloning fragment:** Constant Cloning fragment. Also referred to as a C gene segment cloning fragment. A construct carrying a portion of a C gene segment used to construct a V-C entry vector.

- CD3:** a multi-protein complex of 6 chains, that associate with T cell receptor as three dimers ($\epsilon\gamma$, $\epsilon\delta$, $\zeta\zeta$). This TCR:CD3 complex forms the unit for initiation of cellular signalling responses upon engagement of a $\alpha\beta$ or $\gamma\delta$ TCR with antigen. The CD3 accessories associated as a TCR:CD3 complex contribute signalling motifs called immunoreceptor tyrosine-based activation motifs (ITAMs).
- CDR:** complementarity-determining regions. Short sequences on the antigen-facing end of TCRs and antibodies that perform most of the target binding function. Each antibody and TCR contains six CDRs and they are generally the most variable part of the molecules allowing detection of a large number of diverse target molecules.
- CAR:** Chimeric Antigen Receptor. A fusion protein engineered to contain 2 or more amino acid sequences linked together in a way that does not occur naturally or does not naturally occur in a host cell, which fusion protein can function as a receptor when present on a surface of a cell.
- Cis-acting element:** regions of non-coding DNA that regulate the transcription of nearby ORFs.
- C-part:** Constant part. A small portion of Constant gene segment sequence carried by a J receiving cassette fragment, J receiving cassette and J donor vector to standardise overhang sequences for operation of the TORES to reconstitute TCR ORFs.
- CM:** Cargo molecules. Peptide or metabolite that is presented by an antigen-presenting complex for example a HLA I or HLA II. The CM can be expressed by the cell intrinsically from the genomic DNA, introduced into the culture medium or expressed from a specifically introduced genetic sequence.
- Cognate Antigen:** An antigen, often presented by an HLA, that is recognised in a particular TCR. TCR and antigen are cognate objects.
- Copy-number:** The whole number occurrence of a defined sequence encoded within the genome of a cell.
- Cross-reactivity:** cytotoxic activity of a T cell for a somatically expressed HLA-presented peptide other than the desired target HLA-presented peptide
- Cytogenetic:** The study of inheritance in relation to the structure and function of chromosomes, i.e. determine the karyotype of a cell
- Cytotoxic/Cytotoxicity:** Process in which a T cells releases factors that directly and specifically damage a target cell.

D (-region): Diversity gene segment. One of the gene segments that is used to assemble the T-cell receptor. Each individual has a large number of different variations of these regions making it possible for each individual to arm T cells with a very large variety of different TCR.

- 5 **Dimer:** is an oligomer consisting of two structurally similar monomers joined by bonds that can be either strong or weak, covalent or intermolecular.

DNA: Desoxyribonucleic acid. Chemical name of the molecule that forms genetic material encoding genes and proteins.

- 10 **eAPC/eAPC-p/eAPC-pa:** Engineered antigen-presenting cell, as described in WO 2017/083316. -p indicates that the cell presents an antigen-presenting complex, and -pa indicates that the cell presents an antigen presented in an antigen-presented complex.

EC50: Half maximal effective concentration, concentration effective in producing 50% of the maximal response

Endogenous: Substance that originated from within a cell

- 15 **Eukaryotic conditional regulatory element:** A DNA sequence that can influence the activity of a promoter, which may be induced or repressed under defined conditions

- 20 **Eukaryotic Promoter:** A DNA sequence that encodes a RNA polymerase binding site and response elements. The sequence of the promoter region controls the binding of the RNA polymerase and transcription factors, therefore promoters play a large role in determining where and when your gene of interest will be expressed.

Eukaryotic terminator/Signal terminator: A DNA sequence that are recognized by protein factors that are associated with the RNA polymerase II and which trigger the termination process of transcription. It also encodes the poly-A signal

- 25 **Engineered Cell:** A cell wherein the genome has been engineered through genetic modification modified.

- 30 **Epitope:** An epitope, also known as antigenic determinant, is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells. For example, the epitope is the specific piece of the antigen to which an antibody binds.

Epigenetic insulator sequence: DNA element that disrupts the communication between a regulatory sequence, such as an enhancer or a silencer, and a promoter.

eTPC system: eTPCS, the system by which eTPC-t cells, or libraries thereof, are prepared for combination in the eAPC:eTPC system

FACS/Flow Cytometry: Fluorescence-activated cell sorting. Flow cytometry is a technique by which individual cells can be analyzed en masse for the expression of specific cell surface and/or intracellular markers. A variation of that technique, cell sorting, allows cells that carry a defined set of markers to be retrieved for further analysis.

Family of APX: A set of several similar genes that encode functionally related proteins, which constitute an antigen presentation complex

Flp Recombinase: A recombinase (Flippase, Flp) derived from the 2 μ m plasmid of baker's yeast *Saccharomyces cerevisiae*.

Fluorescent (protein) marker: Molecule that has specific extinction and emission characteristics and which can be detected by Microscopy, FACS and related techniques.

Germline gene segments: (TCR) Gene segments that are naturally occurring in humans.

Gene cis-acting elements: are present on the same molecule of DNA as the gene they regulate whereas trans-regulatory elements can regulate genes distant from the gene from which they were transcribed. Cis-regulatory elements are often binding sites for one or more trans-acting factors.

Genetic barcoding: DNA barcoding is a taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species.

Genomic donor vector: matched to genomic receiver site, designed to deliver donor genetic material

Genomic Receiver Site: A site within the genome for targeted integration of donor genetic material encoded within a Genetic Donor Vector.

Genomic Receiver Site Recycling: The reversion of an occupied genomic receiver site back to the conformation wherein a new analyte (TCR) ORF can be integrated

Haplotype: a physical arrangement of genomic variants that tend to be inherited together.

HLA haplotype: a linear arrangement of HLA alleles along an individual chromosome

Heterospecific recombinase sites: A DNA sequence that is recognized by a recombinase enzyme to promote the crossover of two DNA molecules.

- HLA I:** Human Leukocyte Antigen class I. A gene that is expressed in humans in all nucleated cells and exported to the cell surface where it presents as cargo short fragments, peptides, of internal proteins to T-cell receptors. As such it presents fragments of potential ongoing infections along with intrinsic proteins. The HLA I can additionally
- 5 present as cargo peptides that are added to the culture medium, generated from proteins expressed from introduced genetic elements or generated from proteins that are taken up by the cell. HLA class I genes are polymorphic meaning that different individuals are likely to have variation in the same gene leading to a variation in presentation. Related to HLA class II.
- 10 **HLA II:** Human Leukocyte Antigen Class II. A gene that is expressed in humans in specific cells that are coordinating and helping the adaptive immune response for example dendritic cells. Related to HLA class I. HLA class II proteins are exported to the cell surface where they present as cargo short fragments, peptides, of external proteins to T-cell receptors. As such it presents fragments of potential ongoing infections along with
- 15 intrinsic proteins. The HLA II can additionally present as cargo peptides that are added to the culture medium, generated from proteins expressed from introduced genetic elements or generated from proteins that are taken up by the cell. HLA class II genes are polymorphic meaning that different individuals are likely to have variation in the same gene leading to a variation in presentation.
- 20 **Homologous arms:** A stretch of DNA that has near identical sequence identity to a complement homologous arm and therefore promote the exchange of two DNA molecules by the cellular process, homology directed repair.
- ImmTACs:** Immune mobilising monoclonal T-cell receptors Against Cancer. These bispecifics are designed to activate T cells against cancer and virus-infected cells. An
- 25 ImmTAC is specific to both MHC-peptides (pHLA) located on the surface of the tumor and CD3s located on T cells. The ImmTAC is thus able to directly activate CD3 and its corresponding pathways in activated T cells.
- Immune surveillance:** Process in which the immune system detects and becomes activated by infections, malignancies or other potentially pathogenic alterations.
- 30 **Immunotherapy:** a type of treatment that boosts the body's natural defenses to fight a disease. It uses substances made by the body or in a laboratory to improve or restore immune system function.

Insulator: A DNA sequence that prevents a gene from being influenced by the activation or repression of nearby genes. Insulators also prevent the spread of heterochromatin from a silenced gene to an actively transcribed gene.

Integration: The physical ligation of a DNA sequence into a chromosome of a cell

- 5 **Integration vector:** The product of TORES containing TCR ORFs, and matched to genomic receiver sites, containing genetic elements at the 5' and 3' ends to enable integration.

Integration couple: matched integration vector and genomic receiver site

- 10 **Internal ribosome entry site (IRES):** A DNA sequence that once transcribed encodes a RNA element that allows the initiation of translation in a cap-independent manner

Isoform: any of two or more functionally similar proteins that have a similar but not identical amino acid sequence and are either encoded by different genes or by RNA transcripts from the same gene which have had different exons removed.

- 15 **J (-region):** Joining segment. One of the gene segments that is used to assemble the T-cell receptor. Each individual has a large number of different variations of these regions making it possible for each individual to arm T cells with a very large variety of different TCR.

J donor backbone: Joining donor backbone. The vector backbone into which a J receiving cassette fragment is inserted to create a J receiving cassette vector.

- 20 **J donor vector:** The vector of the two-component vector system that carries the J TCR segment, and donates this segment to the V-C entry vector during reconstitution of a full-length TCR ORF.

J receiving cassette fragment: Joining receiving cassette fragment. A cloning fragment that carries a C-part used to construct a J receiving cassette vector.

- 25 **J receiving cassette vector:** Joining receiving cassette vector. The vector, carrying a C-part, into which a J segment part is inserted to create a J donor vector.

J segment part: Joining segment part. A DNA construct carrying a portion of a J gene segment that is inserted into a J receiving cassette vector to generate a J donor vector.

Karyotype: a complete set of chromosomes

- 30 **Kozak Sequence:** Short sequence required for the efficient initiation of translation

Lipid nanoparticle: Small spherical particles composed of lipid. Used to deliver a payload into a target cell

Major HLA class I: a Family of APX that comprise of the genes HLA-A, HLA-B and HLA-C

- 5 **Matched:** When two components encode genetic elements that direct and restrict the interaction between the complemented components

Meganuclease recognition site: A DNA sequence that is recognized by a endodeoxyribonuclease, commonly referred to as a meganuclease

Metabolite: A molecule created or altered through metabolic pathways of the cell

- 10 **MHC:** Major Histocompatibility Complex. Large locus on vertebrate DNA containing a set of closely linked polymorphic genes that code for cell surface proteins essential for the adaptive immune system.

Mobile genetic element: A DNA sequence that can permit the integration of DNA with the activity of transposases enzymes

- 15 **Monoclone cell line:** A defined group of cells produced from a single ancestral cell by repeated cellular replication

- mRNA splice acceptor site:** At the 5' end the DNA nucleotides are GT [GU in the pre-messenger RNA (pre-mRNA)]; at the 3' end they are AG. These nucleotides are part of the splicing sites. **DONOR-SPLICE:** splicing site at the beginning of an intron, intron 5' left
20 end. **ACCEPTOR-SPLICE:** splicing site at the end of an intron, intron 3' right end.

Multimer: A protein complex consisting of multiple identical monomers. Often used in context of HLA multimer reagent.

Native: an entity that is naturally occurring in the cell

- 25 **Negative Selection Marker:** A selectable marker that confers negative selection of a vector and/or of host organism carrying said marker-bearing vector

- Non-cell-based Particle:** (NCBP) acts in a similar manner to an affinity reagent, inasmuch that the particle presents an analyte antigen or other entity that is to be assessed for TCRsp engagement at the surface of a eTPC-t within and eTPC:A system.
30 However, an NCBP is considered as a larger entity that can further carry genetic or other information that is to act as an identifier, either directly or by proxy, of the presented

analyte antigen or other binding entity. A typical example of an NCBP would be a bacteriophage in a phage-display scenario

Non-coding gene: A non protein coding DNA sequence that is transcribed into functional non-coding RNA molecules

5 **Nucleases:** enzymes that degrade nucleic acids

odeCDR3: oligonucleotide duplex encoding complementarity-determining regions. A synthetic construct carrying CDR3 genetic sequence with terminal overhangs, used in conjunction with the two-component vector system to reconstitute a full-length TCR ORF.

10 **Oncogenic driver mutations:** mutations found in genes that encode for signalling proteins that are critical for maintaining cellular proliferation and survival

Origin of replication: a particular sequence in a vector, plasmid or genome at which replication is initiated.

ORF: Open reading frame. Stretch of genetic material that encodes a translation frame for synthesis of a protein (polypeptide) by the ribosome

15 **Overhang:** A single stranded sequence at the terminus of a double stranded nucleic acid molecule. Often referred to as sticky or cohesive ends.

Para-reactivity: in the present context means reactivity towards an HLA that does not belong to the putative HLA but presents the relevant peptide

20 **PCR:** Polymerase chain reaction in which a specific target DNA molecule is exponentially amplified

Peptide: short string of amino acids between 6 - 30 amino acids in length

Phenotypic analysis: Analysis of the observable characteristics of a cell.

Plasmid: A genetic construct can replicate independently of the chromosomes, typically a small circular DNA strand in the cytoplasm of a bacterium or protozoan.

25 **pMHC:** peptide bound to a Major Histocompatibility Class I Complex (p-MHC)

Polymorphic: Present in different forms in individuals of the same species through the presence of different alleles of the same gene.

Polypeptide: Protein consisting of a stretch of peptides, forming a three-dimensional structure.

Positive Selection Marker: A selectable marker that confers positive selection of a vector and/or host organism carrying said marker-bearing vector

Primer: Short DNA sequence that allows specific recognition of a target DNA sequence for example during a PCR.

- 5 **Professional APC:** any nucleated cell capable of presenting an antigen for sampling by alpha beta and gamma delta T cells presented in HLAII.

Promoter: Regulatory DNA element for the controlled initiation of gene expression.

Recombinase: Enzymes that mediate genetic recombination.

- 10 **Reporter Element:** A genetic element that mediates a reported signal in the organism or vector bearing said element. May be used as a positive or negative selection maker.

Restriction Enzyme Cleavage Sequence: The genetic sequence cleaved by a restriction enzyme, which can be extrinsic or intrinsic to the recognition sequence of said restriction enzyme.

- 15 **Restriction Enzyme Recognition Sequence:** The genetic sequence recognised and engaged by a restriction enzyme.

Selectable marker: A DNA sequence that confers a trait suitable for artificial selection methods

- 20 **Sensitivity:** TCR sensitivity is an assesment of the activation of a TCR, when expressed by an engineered TCR-presenting cell, by a target peptide presented in the relevant HLA complex. The lower the numeric value is, the higher the sensitivity is.

Specific cytotoxicity: killing of cells that express mutated KRAS G12V, but not wild type KRAS, by effector T cells expressing TCR specific for the mutated KRAS G12V peptide presented in an HLA molecule.

- 25 **Splice acceptor site:** A DNA sequence at the 3' end of the intron AM, APX CM or affinity reagent for interaction with cells with TCRsp on the surface, or TCRsp based reagents

Splice donor site: A DNA sequence at the 5' end of the intron

Soluble TCR: not membrane bound

Somatic V(D)J recombination: process after which each T cell expresses copies of a single distinctly rearranged TCR. Refers to recombination at the TRB and TRD loci and additionally include a diversity (D) gene segment.

Suicide gene: A gene that will mediate cell death within the host organism carrying said gene. May be used as a positive or negative selection marker.

Synthetic: an entity that is artificially generated.

Targeted endonucleases: nucleases that can make a double-strand break to the genome and may be used to insert material into the genome

Targeted lipid nanoparticle: A lipid nanoparticle targeted to deliver payload to specific cell types based on expression of surface markers. Used for both ex vivo or in vivo payload delivery

T cell: T lymphocyte. White blood cell that expresses a T-cell receptor on its surface. Selected by the immune system to not react with the own body but have the potential to recognize infections and malignancies as well as reject grafts from most members of the same species.

T cell maturation: process that allows T cells to distinguish cells that belong to the body and are healthy from those that aren't healthy or don't belong to the body at all. Takes place in the thymus

T cell repertoire: T cells distinct set of T cell receptors

TCR: T cell Receptor. Affinity molecule expressed by a subgroup of lymphocytes called T lymphocytes. In humans the TCR recognizes cargo presented by APX CM or APX AM, including fragments from virus or bacterial infections or cancerous cells. Therefore, the TCR recognition is an integral part of the adaptive immune system. The TCR consists of two chains that are paired on the cell surface. The TCR expressed on the surface of each cells is assembled at random from a large pool of varied genes (the v,d,j and c segments) and thus each individual has a pool of T cells expressing a very large and diverse repertoire of different TCRs.

TCRsp: analyte pair of TCR chains that are expressed as TCR surface proteins in complex with CD3

TCR Drug Conjugates (TDC): soluble TCR (instead of an antibody) used to target tumour-specific p-MHCs and deliver a toxic drug into tumour cells

Terminator element: is a section of nucleic acid sequence that marks the end of a gene or operon in genomic DNA during transcription. This sequence mediates transcriptional termination by providing signals in the newly synthesized mRNA that trigger processes which release the mRNA from the transcriptional complex. These processes
5 include the direct interaction of the mRNA secondary structure with the complex and/or the indirect activities of recruited termination factors. Release of the transcriptional complex frees RNA polymerase and related transcriptional machinery to begin transcription of new mRNAs. The termination element is in the template strand of DNA and consists of two inverted repeats separated by half a dozen bases and followed by a
10 run of adenines (A's).

Thymic selection: Immature thymocytes undergo a process of **selection**, based on the specificity of their T-cell receptors. This involves **selection** of T cells that are functional (positive **selection**), and elimination of T cells that are autoreactive (negative **selection**).
15 The medulla of the **thymus** is the site of T Cell maturation.

tCD34: a truncated CD34 enrichment tag, i.e. a CD34 that is truncated so that the biological function of CD34 is reduced or removed. Cells can be enriched if they express such a truncated CD34 enrichment tag,
20

Tumour associated antigens: Tumour antigen is an antigenic substance produced in tumour cells, i.e., it triggers an immune response in the host. Tumor antigens are useful tumour markers in identifying and quantifying tumour cells with diagnostic tests and are potential candidates for use in cancer therapy.

25 **TRA:** TCR alpha encoding locus. One of the four different locus encoding genes that can form a VDJ recombined TCR chain. Translated TCR alpha chain proteins typically pair with translated TCR beta chain proteins to form alpha/beta TCRsp.

TRB: TCR beta encoding locus. One of the four different locus encoding genes that can form a VDJ recombined TCR chain. Translated TCR beta chain proteins typically pair with
30 TCR alpha chain proteins to form alpha/beta TCRsp.

TRD: TCR delta encoding locus. One of the four different locus encoding genes that can form a VDJ recombined TCR chain. Translated TCR delta chain proteins typically pair with translated TCR gamma chain proteins to form gamma/delta TCRsp.

35 **TRG:** TCR gamma encoding locus. One of the four different locus encoding genes that can form a VDJ recombined TCR chain. Translated TCR gamma chain proteins typically pair with translated TCR delta chain proteins to form gamma/delta TCRsp.

Two-component vector system: a single V-C entry vector and a single J donor vector with desired sequences can be combined with a short DNA oligonucleotide duplex encoding CDR3 (odeCDR3) sequence to reconstitute a full length TCR ORF in vitro in a single-tube reaction, in a restriction enzyme and ligase dependent and PCR independent manner.

Type I transmembrane domain: single-pass molecules anchored to the lipid membrane with a stop-transfer anchor sequence and their N-terminal domain targeted to the endoplasmic reticulum lumen during synthesis (and the extracellular space, if mature forms are located on the Plasmalemma).

Type IIS Restriction Enzyme: restriction enzymes that recognize asymmetric DNA sequences and cleave outside of their recognition sequence.

V (-region): Variable region. One of the gene segments that is used to assemble the T-cell receptor. Each individual has a large number of different variations of these regions making it possible for each individual to arm T cells with a very large variety of different TCR.

V-C entry vector: The vector of the two-component vector system that carries the V and C TCR segments, and which receives sequences from the J donor vectors and odeCDR3 during reconstitution of a full-length TCR ORF.

V cloning fragment: Variable Cloning fragment. Also referred to as a V gene segment cloning fragment. A construct carrying a portion of a V gene segment used to construct a V-C entry vector.

Vector: A vector is a genetic construct that carries genetic information. In the present context vector usually describes plasmid DNA vectors. A vector can represent any such construct that can be propagated and selected in a host organism.

Z-score: The z-score is the intensity of a specific peptide as compared to the intensity of all other peptides in the sample, in other words the formula for calculating z-scores takes the raw intensity score of the peptide and subtracts the population mean, and all is divided by the population standard deviation.

Detailed description of invention

The current invention discloses several verified suitable RAS targets as well as TCRs recognising these targets, and which have a particularly suitable profile for therapeutic use
5 in cancer therapy.

The present invention, for the first time, discloses a T cell receptor (TCR), which is isolated and/or expressed in an engineered cell, and which specifically recognizes a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human
10 Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, and wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) or VVGAVGVGK (SEQ ID NO:2) presented in a HLA-A*11 context.

15 In one aspect, the invention relates to a method of treating a disorder characterized by G12V mutated human RAS expression in a subject, such as a human patient, comprising administering to said subject a therapeutically effective amount of genetically engineered cells that express a TCR as disclosed and/or defined herein, or a pharmaceutical composition disclosed and/or defined herein.

20 In an aspect a TCR according to the invention is capable of specifically recognising both of SEQ ID NO: 1 and SEQ ID NO: 2 when either is presented in a HLA-A*11 complex, thereby allowing for treatment of patients wherein either or both peptides are presented in the HLA-A*11 context.

25 G12V RAS

The inventors of the present application have surprisingly found novel functional TCRs that are able to bind to productively engage with functionally presented targets such as RAS targets, a G12V mutated RAS 9-mer (SEQ ID NO: 2) and/or a G12V mutated RAS 10-mer (SEQ ID NO: 1) peptide in the context of HLA-A*11:01 with high affinity and
30 specificity, wherein productive engagement of TCR with said targets triggers a signalling, cytotoxic response in a T cell expressing such TCR.

It is appreciated that the G12V mutation in KRAS corresponds to similar mutations in other RAS proteins such as HRAS and NRAS. Thus, peptides comprising the G12V
35 mutation presented in a HLA context may be derived from a mutated HRAS or mutated NRAS instead, so that the peptide in itself may not reveal whether it stems from a G12V mutated KRAS or a mutated HRAS or a mutated NRAS, regardless of whether the

numbering of the substituted G->V is in the same location as long as the surrounding amino acid residues are the same. Thus, TCRs according to the present invention may equally well bind peptides derived from mutated HRAS or mutated NRAS sharing the corresponding mutation. In such cases, the recognised peptide consists of

5 VVGAVGVGK (SEQ ID NO: 1) or VVGAVGVGK (SEQ ID NO:2).

In one embodiment, as demonstrated in the experimental section, the TCR according to the current invention is a TCR, which, when expressed in an engineered cell, has at the most 4% cross-reactivity towards any somatically expressed HLA-presented peptide other
10 than a G12V mutated human rat sarcoma (RAS) peptide, which consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) and which is presented in a HLA-A*11 complex in a cross-reactivity assay.

A TCR disclosed herein typically comprises:

15 a. a TCR alpha chain CDR3 sequence with at least 75% identity, such as at least 80%identity, to a TCR alpha chain CDR3 sequence selected from the group consisting of SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 98, SEQ ID NO: 141 and SEQ ID NO: 143.

and

20 b. a TCR beta chain CDR3 sequence with at least 75% identity , such as at least 80%identity,to a TCR beta chain CDR3 sequence selected from the group consisting of SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 99, SEQ ID NO: 142 and SEQ ID NO: 144.

25 In embodiments, the TCR according to the current invention comprises:

a. a TCR alpha chain CDR3 sequence with a TCR alpha chain CDR3 sequence selected from the group consisting of SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 98, SEQ ID NO: 141 and SEQ ID NO: 143.

30 and

b. a TCR beta chain CDR3 sequence with a TCR beta chain CDR3 sequence selected from the group consisting of SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 99, SEQ ID NO: 142 and SEQ ID NO: 144.

35 In a currently preferred embodiment of the invention, the TCR alpha chain variable, domain scaffold regions, and/or CDRs comprise and/or consist of one of the sequences selected from the group consisting of SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 117, SEQ ID NO: 145 and SEQ ID NO: 147 and the beta chain variable, domain scaffold

regions, and/or or CDRs comprise and/or consist of one of the sequences selected from the group consisting of SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 118, SEQ ID: 146 and SEQ ID NO: 148.

5 In an even more preferred embodiment of the invention, the TCR comprises an alpha chain paired with a beta chain, wherein such pairs are selected from the group of alpha:beta chain pairs consisting of SEQ ID NO:107 and SEQ ID NO 108; SEQ ID NO: 109 and SEQ ID NO 110; SEQ ID NO: 117 and SEQ ID NO:118; SEQ ID NO: 145 and SEQ ID NO: 146; and SEQ ID NO: 147 and SEQ ID NO: 148.

10

A TCR according to the current invention can be selected from the group comprising chimeric, humanized and/or human TCRs.

A TCR according to the invention may comprise constant regions of the alpha and beta chains which have been modified to include disulphide bonds, either interchain disulfide
15 bridge (Boulter et al 2003) which has been shown to enable high-yield production of stable, soluble protein from refolded inclusion bodies, or including additional TRA Constant domain : TRB Constant domain disulphides or stabilizing mutations on the surface of the constant domains (Robinson et al 2021). In embodiments, the alpha and/or beta TCR chain comprise(s) a constant region from another mammal, such as, but not
20 limited to, a mouse chimeric constant region(s), wherein, the TCRs can comprise a mouse constant region comprising mouse constant region of an alpha chain and mouse constant region of beta chain, or both. Preferably, the TCR comprises both. It is appreciated by those skilled in the art that as well as replacing the constant region(s) with those from mouse, the constant domains of another mammal such as a rat, chimpanzee, gorilla,
25 macaque or rhesus monkey may be used, which are alternative embodiments.

In aspects, the TCR comprises a TCR alpha chain a TCR beta chain which are covalently linked, such as through a linker peptide. The TCR alpha chain and/or the TCR beta chain can further be covalently linked to a moiety, and the linked moiety can comprise an affinity tag or a label. In embodiments, the tag is selected from the group consisting of a CD34
30 enrichment tag, glutathione-S-transferase (GST), calmodulin binding protein (CBP), protein C tag, Myc tag, Halo tag, HA tag, Flag tag, His tag, biotin tag, and V5 tag. In embodiments, the label is a fluorochrome or a fluorophore such as a fluorescent protein. In aspects, the covalently linked moiety is selected from the group consisting of an inflammatory agent, cytokine, toxin, cytotoxic molecule, radioactive isotope, an enzyme
35 that catalyses a cell wall sorting reaction, and an antibody or antigen-binding fragment thereof.

The alpha/beta paired TCRs of the present description may have an introduced disulfide bond between their constant domains. Preferred TCRs of this type include those which have parts of a TRA constant domain sequence and a TRBI or TRB2 constant domain sequence replaced by cysteine residues, the said cysteines forming a disulfide bond

5 between the TRA constant domain sequence and the TRBI or TRB2 constant domain sequence of the TCR. It is appreciated by those skilled in the art that there are few amino acid residues within the TRA and TRB1/TRB2 sequence chains, respectively, that may be modified to cysteine so as to form a disulfide bond.

10 With or without the introduced inter-chain bond mentioned above, the alpha/beta paired TCRs of the present description may have a TRA constant domain sequence and a TRB1 or TRB2 constant domain sequences, and the TRA constant domain sequence and the TRB1 or TRB2 constant domain sequences of the TCR may be linked by the native disulphide bond.

15 The TCR can comprise a human/mouse chimeric TCR. In this regard, the TCR can comprise a mouse constant region of an alpha chain, a mouse constant region of a beta chain, or both mouse constant regions. Preferably, the TCR comprises both alpha and beta mouse constant regions. It is appreciated by those skilled in the art that, as well as

20 replacing the constant region(s) with those from mouse, the constant domains of another mammal such as a rat, chimpanzee, gorilla, macaque or rhesus monkey may be used, which are alternative embodiments.

Alternatively or additionally, the inventive human/ mouse chimeric TCR can comprise any

25 of the alpha chain CDR3s of the invention.

Alternatively or additionally, the inventive human/ mouse chimeric TCR can comprise any of the beta chain CDR3s of the invention.

Specifically recognizing

30 In the context of the present specification, "specifically recognizing" means that a TCR recognises a target peptide presented by a specific HLA allele.

The current invention relates to several new T cell receptors (TCR), which are isolated and/or expressed in an engineered cell, and which specifically recognize a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS

35 (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, which consists of VVGAVGVGK (SEQ ID NO: 1) or VVGAVGVGK (SEQ ID NO:2) presented in a HLA-A*11 complex, and wherein the individual TCR comprises a paired TCR alpha- and TCR beta-chain, wherein the TCR

alpha chain variable domain comprises SC1alpha-CDR1alpha-SC2alpha-CDR2alpha-SC3alpha-CDR3alpha-SC4alpha-SC5alpha, and the TCR beta chain variable domain comprises SC1beta-CDR1beta-SC2 beta-CDR2 beta-SC3beta-CDR3beta-SC4beta-SC5beta, wherein SC is a scaffold region and CDR is a complementarity determining
5 region.

TCRs according to invention, when expressed in an engineered cell, do not cause specific cytotoxic activity towards cells not expressing a G12V mutated RAS peptide.

10 TCRs according to the invention, when expressed in an engineered cell, do not cause specific cytotoxic activity towards cells expressing the wild type human RAS peptide, such as VVGAGGVGK (SEQ ID NO: 3) presented in a HLA-A*11 complex and/or VVGAGGVGK (SEQ ID NO: 4) presented in a HLA-A*11 complex.

15 TCRs according to the invention, when expressed in an engineered cell, do not specifically recognize the G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/ or VVGAVGVGK (SEQ ID NO: 2) presented in an allelic variant of the human leukocyte antigen (HLA) molecule other than an allelic variant of the human leukocyte antigen (HLA) molecule selected from the group
20 consisting of HLA-A*11, HLA-A*30:01, HLA-A*68:02, HLA-B*54:01, HLA-B*35:01, HLA-B*35:02, HLA-B*35:03, HLA-B*35:05, HLA-B*35:12, HLA-B*52:01, HLA-B*53:01, HLA-B*57:01 and HLA-B*58:01.

Furthermore, when expressed in an engineered cell, a TCR according to the invention
25 does not recognise allelic variants of the human leukocyte antigen (HLA) molecule other than one or more allelic variant of the human leukocyte antigen (HLA) molecule selected from the group consisting of HLA-A*68:02, HLA-B*54:01, HLA-B*35:01, HLA-B*35:02, HLA-B*35:03, HLA-B*35:12, HLA-B*57:01 and HLA-B*58:01.

30 In one embodiment, TCRs according to the invention, when expressed in an engineered cell, do not specifically recognizes the G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) presented in an allelic variant of the human leukocyte antigen (HLA) molecule other than the HLA-A*11 and/ or HLA-A*30:01.

35 In one embodiment, TCRs according to the invention, when expressed in an engineered cell, do not specifically recognizes the G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2)

presented in an allelic variant of the human leukocyte antigen (HLA) molecule other than the HLA-A*11 and/ or HLA-A*30:01 and/ or HLA-A*68:02.

5 In one embodiment, TCRs according to the invention, when expressed in an engineered cell, do not specifically recognizes the G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) presented in an allelic variant of the human leukocyte antigen (HLA) molecule other than the HLA-A*11 and/ or HLA-B*54:01.

10 In one embodiment, TCRs according to the invention, when expressed in an engineered cell, do not specifically recognizes the G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) presented in an allelic variant of the human leukocyte antigen (HLA) molecule other than the HLA-A*11 and/ or HLA-B*35:01 and/ or HLA-B*35:02 and/ or HLA-B*35:03, and/ or
15 HLA-B*35:05 and/ or HLA-B*35:12 and/ or HLA-B*52:01 and/ or HLA-B*53:01 and/or HLA-B*57:01 and/ or HLA-B*58:01.

In one embodiment, TCRs according to the invention, when expressed in an engineered cell, do not specifically recognizes the G12V mutated human RAS peptide, wherein the
20 peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) presented in an allelic variant of the human leukocyte antigen (HLA) molecule other than the HLA-A*11.

In one embodiment, TCRs according to the invention do not specifically recognize a G12V
25 mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/ or VVGAVGVGK (SEQ ID NO: 2) presented in a HLA-A*11:03 complex.

In one embodiment, TCRs according to the invention do not specifically recognize a G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID
30 NO: 1) and/ or VVGAVGVGK (SEQ ID NO: 2) presented in a HLA-A*11:03 and HLA-A*11:04 complex.

In one embodiment, as exemplified in the experimental section, the TCR according to the current invention is a TCR, which, when expressed in an engineered cell, has at the most
35 4% cross-reactivity towards any somatically expressed HLA-presented peptide other than a G12V mutated human rat sarcoma (RAS) peptide, which consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) and which is presented in a HLA-A*11 complex in a cross-reactivity assay.

Alloreactivity “off-HLA” recognition by T cell.

Alloreactivity in the context of the present invention means an undesirable reactivity of the TCR towards a peptide-free HLA not belonging to a putative HLA for that TCR, i.e., in the case of a TCR specific for mutated G12V KRAS, an HLA that does not belong to the HLA-A*11 supergroup and at the same time does not present a peptide.

Para-reactivity “on-target-off-HLA” recognition by a T cell

Para-reactivity in the present context means reactivity towards an HLA that does not belong to the putative HLA but presents the relevant peptide, i.e. in the present context an HLA not belonging to the HLA-A*11 supergroup but presenting a G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) or VVGAVGVGK (SEQ ID NO: 2).

Strong cytotoxic activity

Strong cytotoxic activity in the current context means T cell cytotoxic activity towards a target cell population resulting in an increase in specific lysis of the target cell population that is increased more than 4.5-fold when target cells are co-cultured with T cells in a 1:1 ratio compared to a 1:16 or to a 1:64 T cell to target cell ratio.

TCRs of the current invention

- A TCR disclosed herein typically comprises:
- a. a TCR alpha chain CDR3 sequence with at least 80% identity to a TCR alpha chain CDR3 sequence selected from the group consisting of SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 98, SEQ ID NO: 141 and SEQ ID NO: 143.
 - and
 - b. a TCR beta chain CDR3 sequence with at least 80% identity to a TCR beta chain CDR3 sequence selected from the group consisting of SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 99, SEQ ID NO: 142 and SEQ ID NO: 144.

In embodiments, the TCR according to the current invention comprises:

- a. a TCR alpha chain CDR3 sequence with a TCR alpha chain CDR3 sequence selected from the group consisting of SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 98, SEQ ID NO: 141 and SEQ ID NO: 143.
- and

- b. a TCR beta chain CDR3 sequence with a TCR beta chain CDR3 sequence selected from the group consisting of SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 99 and SEQ ID NO: 142 and SEQ ID NO: 144.

5 In a currently preferred embodiment of the invention the TCR alpha and beta chain variable domain scaffold regions or CDRs of the TCR comprises and/or consists of one of the sequences selected from the group consisting of SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 145 SEQ ID NO: 146 and SEQ ID NO: 147 and SEQ ID NO: 148.

10

In a preferred embodiment the TCR comprises a paired alpha and beta chain, wherein the pairs are selected from the group consisting of SEQ ID NO: 107 and SEQ ID NO: 108; SEQ ID NO: 109 and SEQ ID NO: 110; SEQ ID NO: 117 and SEQ ID NO: 118; SEQ ID NO: 145 and SEQ ID NO: 146; and SEQ ID NO: 147 and SEQ ID NO: 148. Such pairs

15 may also encompass sequences having at least 80% sequence identity to said sequences; such as at least 90% sequence identity, such as at least 95% sequence identity, such as at least 98% sequence identity or more.

20 A TCR according to the current invention can be selected from the group comprising chimeric, humanized and/or human TCRs.

A TCR according to the invention can comprise constant regions of the alpha and beta chains which have been modified to include cysteine residues to allow covalent connection by di-sulphide bridges or comprise no covalent connections between said
25 paired alpha and beta chains. In embodiments, the alpha and/or beta chain comprise(s) a constant region of another mammal, such as, but not limited to, a mouse chimeric constant region(s). Further, the alpha and/or beta chain may have been modified to include cysteine residues to allow di-sulphide bridges.

30 In aspects, the TCR comprises a TCR alpha chain a TCR beta chain which are covalently linked, such as through a linker peptide. The TCR alpha chain and/or the TCR beta chain can further be covalently linked to a moiety, and the linked moiety can comprise an affinity tag or a label. In embodiments, the tag is selected from the group consisting of a CD34 enrichment tag, glutathione-S-transferase (GST), calmodulin binding protein (CBP),
35 protein C tag, Myc tag, Halo tag, HA tag, Flag tag, His tag, biotin tag, and V5 tag. In embodiments, the label is a fluorochrome or a fluorophore such as a fluorescent protein. In aspects, the covalently linked moiety is selected from the group consisting of an inflammatory agent, cytokine, toxin, cytotoxic molecule, radioactive isotope, an enzyme

that catalyses a cell wall sorting reaction, and an antibody or antigen-binding fragment thereof.

The current invention in one aspect relates to a soluble TCR and/or a conjugated soluble

5 TCR according to the current invention. In aspects, the TCR is a bi-specific T-cell engager (BiTE), or it is an immune mobilizing monoclonal T-cell receptor.

The TCR in one aspect TCR comprises a paired TCR alpha- and TCR beta-chain comprising a complete assembly of combinations of the following sequences:

	SEQ	SEQ	SEQ	SEQ	SEQ	SEQ	SEQ
	ID NO	ID NO	ID NO	ID NO	ID NO	ID NO	ID NO
SC1alpha	18	20	28	126	128	18	20
CDR1alpha	60	62	70	135	137	60	62
SC2alpha	32	34	42	129	131	32	34
CDR2alpha	74	76	84	138	140	74	76
SC3alpha	46	48	56	132	134	46	48
CDR3alpha	88	90	98	141	143	88	90
SC4alpha	7	9	15	123	125	7	9
SC5alpha	104	104	104	104	104	104	104
SC1beta	19	21	29	127	30	19	21
CDR1beta	61	63	71	136	72	61	63
SC2beta	33	35	43	130	44	33	35
CDR2beta	75	77	85	139	86	75	77
SC3beta	47	49	57	133	58	47	49
CDR3beta	89	91	99	142	144	89	91
SC4beta	8	10	16	124	12	8	10
SC5beta	105	105	106	106	106	5162	5162

10

or sequences having at least 90% such as at least 95%, at least 97% or at least 99%, or 100% identity to said respective sequences.

15

In one aspect the TCR comprises a paired TCR alpha- and TCR beta-chain comprising the following combination of sequences:

TCR#	SC1	CDR1	SC2	CDR2	SC3	CDR3	SC4	SC5
------	-----	------	-----	------	-----	------	-----	-----

TCR-1	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
alpha	NO: 18	NO: 60	NO: 32	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				74	46	88	7	104
TCR-2	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
alpha	NO: 20	NO: 62	NO: 34	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				76	48	90	9	104
TCR-6	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
alpha	NO: 28	NO: 70	NO: 42	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				84	56	98	15	104
TCR-9	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
alpha	NO: 126	NO: 135	NO:	ID	ID	ID	ID	ID
			129	NO:	NO:	NO:	NO:	NO:
				138	132	141	123	104
TCR-10	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
alpha	NO: 128	NO: 137	NO:	ID	ID	ID	ID	ID
			131	NO:	NO:	NO:	NO:	NO:
				140	134	143	125	104
TCR-1	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
beta	NO: 19	NO: 61	NO: 33	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				75	47	89	8	105
TCR-1	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
beta	NO: 19	NO: 61	NO: 33	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				75	47	89	8	5162
TCR-2	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
beta	NO: 21	NO: 63	NO: 35	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				77	49	91	10	105
TCR-2	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
beta	NO: 21	NO: 63	NO: 35	ID	ID	ID	ID	ID

				NO:	NO:	NO:	NO:	NO:
				77	49	91	10	5162
TCR-6	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
beta	NO: 29	NO: 71	NO: 43	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				85	57	99	16	106
TCR-9	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SE
beta	NO: 127	NO: 136	NO:	ID	ID	ID	ID	ID
			130	NO:	NO:	NO:	NO:	NO:
				139	133	142	124	106
TCR-	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
10	NO: 30	NO: 72	NO: 44	ID	ID	ID	ID	ID
beta				NO:	NO:	NO:	NO:	NO:
				86	58	144	12	106

or sequences having at least 90% such as at least 95%, at least 97% or at least 99%, or 100% identity to said respective sequences.

In one aspect the alpha and beta chain CDRs have the following sequences:

CDR1alpha	CDR2	CDR3	CDR1bet	CDR2	CDR3
	alpha	alpha	a	beta	beta
SEQ ID NO:	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID
60	NO: 74	NO: 88	NO: 61	NO: 75	NO: 89
SEQ ID NO:	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID
62	NO: 76	NO: 90	NO: 63	NO: 77	NO: 91
SEQ ID NO:	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID
70	NO: 84	NO: 98	NO: 71	NO: 85	NO: 99
SEQ ID NO:	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID
135	NO: 138	NO: 141	NO: 136	NO: 139	NO: 142
SEQ ID NO:	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID
137	NO: 140	NO: 143	NO: 72	NO: 86	NO: 144

5

In one aspect the alpha and beta chain CDR3s have the following sequences:

CDR3 alpha	CDR3 beta
SEQ ID NO: 88	SEQ ID NO: 89
SEQ ID NO: 90	SEQ ID NO: 91

SEQ ID NO: 98	SEQ ID NO: 99
SEQ ID NO: 141	SEQ ID NO: 142
SEQ ID NO: 143	SEQ ID NO: 144

Nucleic acid sequences

In one aspect, the invention relates to one or more nucleic acid sequences encoding a TCR comprising a paired TCR alpha- and TCR beta-chain as disclosed, described and/or defined herein.

The nucleic acids of the invention are recombinant. As used herein, the term "recombinant" refers to (i) molecules that are constructed outside living cells by joining natural and/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.

A desirable method of modifying the DNA encoding the polypeptide of the description employs the polymerase chain reaction as disclosed by Saiki R K, et al. (Saiki et al., 1988). This method may be used for introducing the DNA into a suitable vector, for example by engineering in suitable restriction sites, or it may be used to modify the DNA in other useful ways as is known in the art.

Nucleic acid sequences encoding a TCR comprising a paired TCR alpha- and TCR beta-chains may be but are not limited to, linear or circular double stranded DNA, or linear or circular single stranded DNA. Such a sequence may contain replication-directing elements for self-amplification and/or may be circularized. The DNA may or may not be chemically modified

Alternatively, nucleic acid sequences encoding a TCR comprising a paired TCR alpha- and TCR beta-chains may be but are not limited to linear or circular single stranded RNA. Such RNA may or may not be chemically modified.

A nucleic acid sequence encoding a TCR may be comprised in or by an RNA vector. Such an RNA vector may contain a 5' CAP, 5' UTR, 3' UTR and 3' poly(A) tail and Internal Ribosome Entry Site (IRES), and may contain substituted nucleotides such as pseudouridine. An RNA vector may contain replication-directing elements for self-amplification and/or may be circularized.

Identity vs similarity

By an amino acid sequence having an amino acid sequence that is at least, for example 95% identical to a reference amino acid sequence, is intended that the amino acid sequence of e.g., the TCR, or CDR3, is identical to the reference sequence, except that
5 the amino acid sequence may include up to 5 point mutations and/or deletions per each 100 amino acids of the reference amino acid sequence. In other words, to obtain a TCR, or CDR3 having an amino acid sequence that is at least 95% identical to a reference amino acid sequence, up to 5% of the amino acids in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids. In an amino
10 acid sequence that is at least 95% identical to a reference amino acid sequence up to 5% of the amount of total amino acids in the reference sequence may be inserted and/or added into the reference sequence. The mutations and/or insertions of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either
15 individually among amino acids in the reference sequence or in one or more contiguous groups within the reference sequence.

In the present invention, a local algorithm program is best suited to determine identity. Local algorithm programs, (such as Smith Waterman) compare a subsequence in one
20 sequence with a subsequence in a second sequence and find the combination of subsequences and the alignment of those subsequences, which yields the highest overall similarity score. Internal gaps, if allowed, are penalized. Local algorithms work well for comparing two or more multi domain proteins, which have a single domain, or just a binding site in common.

25 Methods to determine identity and similarity are codified in publicly available programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to BLASTX, BLASTP, BLASTN, and FASTA (Altschul et al 1990). The BLAST programs are publicly available from NCBI and other
30 sources (BLAST Manual, Altschul et al, 1990). Each sequence analysis program has a default scoring matrix and default gap penalties. In general, a molecular biologist would be expected to use the default settings established by the software program used.

Vectors

In aspects of the invention, the vector disclosed, described and/or defined herein is a
35 nucleic acid vector, viral vector or a nanoparticle vector. In another aspect, the invention relates to a vector system directing the expression of a payload comprising one or more nucleic acid sequences encoding a TCR comprising a paired TCR alpha- and TCR beta-chains as disclosed, described and/or defined herein.

Said nucleic acid vector can be selected from the group consisting of expression vector, a cloning vector, phagemid, cosmid, transposon, episome, artificial chromosome or RNA.

The invention relates to a nucleic acid vector comprising one or more nucleic acid

- 5 sequences encoding a TCR comprising a paired TCR alpha- and TCR beta-chains as but are not limited to, linear or circular double stranded DNA, or linear or circular single stranded DNA, that may or may not be chemically modified DNA, linear or circular double stranded RNA, or linear or circular single stranded RNA, that may or may not be chemically modified RNA. The RNA vector may contain a 5' CAP, 5' UTR, 3' UTR and 3' poly(A) tail and IRES, and may contain substituted nucleotides such as pseudouridine, and may contain replication-directing elements for self-amplification and/or may be circularized.

- 15 In case of a viral vector, can be selected from the group consisting of viruses that package the nucleic acid sequences encoding at least a TCR comprising a paired TCR alpha- and TCR beta-chains that comprise of, but are not limited to, double stranded DNA, single stranded DNA, double stranded RNA or single stranded RNA. Examples of viral vectors are adenoviruses, adeno-associated viruses, retroviruses, lentiviruses, and herpes simplex viruses. The viral vector comprises one or more cell surface receptors that bind to a ligand on a target cell and aid transduction; heterologous viral envelope glycoproteins; fusion glycoproteins; T cell activation or co-stimulation molecules, cytokines or cytokine-based transduction enhancers; and/or transmembrane proteins comprising a mitogenic domain and/or cytokine-based domain exposed on the surface and/or conjugated to the surface of the viral vector. Lentiviral viruses are known to effectively transduce
- 20 mammalian cells. To alter their tropism, lentiviruses may be pseudotyped with various heterologous viral envelopes, the most common one being the envelope glycoprotein from vesicular stomatitis virus (VSV-G) (Joglekar et al 2017). The vector can further be pseudotyped by exchanging the VSV-G for other heterologous viral envelopes glycoproteins, such as those from the Measles virus, Baboon endogenous retrovirus, Cocal virus, Nipah virus or Sendai virus (Gutierrez-Guerrero et al 2020). The Nipah envelope protein can be engineered to bind EpCAM, CD4, or CD8 (Bender et al 2016), CD3, CD5 or other markers suitable for targeted cellular delivery of a TCR-encoding nucleic acid vector.

- 35 Said nanopartical vector can be selected from the group consisting of lipid nanoparticles (LNPs) or nanoparticles (NPs), cationic liposome complexes, cationic polymer complexes, cationic liposome-polymer complexes, exosomes, liposomes, and inorganic nanoparticles. In certain aspects, a nanoparticle vector according to the current invention is a LNP

comprising ionizable cationic lipid, a sterol, a phospholipid, a non-functionalized PEG-lipid, and a functionalized PEG-lipid wherein the functionalized PEG-lipid has been conjugated with a binding moiety. The nanoparticle vector encapsulates a payload in the form of a nucleic acid, such as mRNA, for the purpose of transfecting a target cell. The mRNA payload may contain a linear expression construct with 5' cap, 5' UTR, 3' UTR and poly-A features, IRES, and may contain substituted nucleotides such as pseudouridine, and may contain replication-directing elements for self-amplification. The mRNA payload may also be circular. The LNP can be targeted (tLNP) to deliver payload to cell types based on expression of cell surface markers (Tombácz et al. (2021), Breda et al. (2023)) for example LNP may be conjugated to CD5, CD3, CD4, CD8 amongst others, to be used for targeting CD8⁺ and CD4⁺ T cells (Wada (2018); Dalloul, (2009), Lee (2021)) or CD16 or NK1.1 for targeting NK cells, EpCAM for targeting epithelial cells or to another marker or a functional ligand thereof that targets cellular delivery to specific target cells. Such tLNP particles may be used both ex vivo and in vivo to direct the mRNA payload to a desired immune cell type (Parayath N et al (2020), Siebart J et al (2024), Vavassori V et al (2023)).

In certain aspects, said vector further comprises a nucleic acid sequence encoding CD8 α and/or CD8 β . In certain aspects, said vector further and/or alternatively comprises a nucleic acid sequence encoding a truncated CD34 enrichment tag. The isolated TCR nucleic acid according to the current invention and the nucleic acid sequence encoding CD8 α and/or CD8 β and/or CD34 can operably be encoded within a single expression unit interconnected with an internal ribosome entry site or a nucleic acid sequence encoding a self-cleaving peptide, such as selected from the group consisting of P2A, E2A, F2A and T2A. In certain aspects the isolated TCR nucleic acid according to the current invention and the nucleic acid sequence encoding CD8 α and/or CD8 β and/or CD34 can be encoded by more than one expression unit with their own promoter and polyadenylation sequence.

In aspects, such vectors may be used for ex vivo manufacture of cell therapy products for infusion and/or targeted delivery of vectors in vivo for TCR expression in targeted immune cell populations. For ex vivo manufacture and cellular manipulation, vectors may be delivered to cells maintained in culture as encapsulated LNPs, as tLNPs, by combination with agents that promote uptake of nucleic acids such as lipofectamine, by electroporation or by inclusion in a viral-genome derived backbone so as to form a viral-like particle (VLP) capable of mediating both integrative or non-integrative manipulation of target cells.

Several viral vector systems, modified viruses designed to deliver genetic material into cells, are suitable for such manipulations and include viral vector systems derived from retroviruses such as lentivirus (LV) and gammaretrovirus, systems which are typically used for ex vivo manipulation and manufacture. Viral systems incorporating
5 vectors may also be used for in vivo delivery of target payloads, typically derived from adeno-associated virus (AAV).

In certain aspects, said vector further comprises a nucleic acid sequence encoding element to promote stable integration the nucleic acid into the host cell genome, including
10 but are not limited to; homologous directed arms (HDR), long terminal repeat (LTR) and inverted terminal repeats (ITR).

In the current context, a "nucleic acid vector", is any molecule or composition that has the ability to carry a nucleic acid sequence into a suitable host cell where synthesis of the
15 encoded polypeptide can take place *in vivo*. Recombinant expression vectors designed to maintain the recombinant DNA in bacteria are also included. Delivery vectors include but are not limited to liposomes genetically attenuated live carriers such as viruses and bacteria, biodegradable microcapsules, immune-stimulating complexes (ISCOMs) may also be used to deliver nucleic acid.

20 Typically, and preferably, a vector comprises a nucleic acid sequence that has been engineered, using recombinant DNA techniques that are known in the art, to incorporate a desired nucleic acid sequence (e.g., a nucleic acid sequence of the invention). Desirably, the vector is comprised of any type of nucleotides, including, but not limited to DNA and
25 RNA, which can be single-stranded or double stranded, synthesized or obtained in part from natural sources, and which can contain natural, non-natural or altered nucleotides. The vectors of the invention are not naturally-occurring as a whole. However, parts of the vectors can be naturally-occurring. Preferably, the non-naturally occurring or altered nucleotides or internucleotide linkages do not hinder the transcription or replication of the
30 vector.

The recombinant expression vector of the invention can be any suitable recombinant expression vector and can be used to transform or transfect any suitable host cell. Suitable vectors include those designed for propagation and expansion or for expression
35 or both, such as plasmids and viruses, and are well known to those skilled in the art, as are methods to construct and use them. In a preferred embodiment, the recombinant expression vectors used include a nucleic acid vector, e.g., linear or circular double stranded DNA, or linear or circular single stranded DNA, or linear or circular RNA used to

transduce a host cell during the production of a pharmaceutical preparation, as well as recombinant expression vectors designed to maintain the recombinant DNA in bacteria. Such vectors may direct transient or stable expression. Stable expression can be achieved by integration of nucleic acid construct into the host cell genome, or
5 maintainence of expression vector nucleic acid in episomal or other structures that achieve nucleic acid maintenance and TCR-encoding construct expression.

Desirably, a vector according to the current invention comprises regulatory sequences, such as transcription and translation initiation and termination codons, which are specific
10 to the type of host (e.g., bacterium, fungus, plant, or animal) into which the vector is to be introduced, as appropriate and taking into consideration whether the vector is DNA- or RNA-based. The alpha and beta paired chains of a TCR of the present invention may be encoded by one or more nucleic acid sequences located in separate vectors, or may be encoded by polynucleotides located in the same vector. In preferred embodiments, TCR-
15 alpha and TCR-beta chains of the introduced TCR may be cloned into bicistronic constructs in a single vector.

Optionally, the nucleic acid sequence of the vector may be modified such that it contains codon triplets designed for optimal transcription and translation, and which are optimised
20 for use in host cells, such as, but not limited to insect, bacteria, yeast or human cells.

The vector can include one or more marker genes, which act as markers of integration. Marker of integration include genes encoding the likes of green fluorescent protein (GFP), red fluorescent protein (RFP) and blue fluorescent protein (BFP), EGFP as well as other
25 fluorescent proteins, Streptavidin Binding Protein (SBP), b-galactosidase or chloramphenicol acetyltransferase (CAT). In addition, or alternatively, the recombinant expression vector can include one or more marker genes, which are selected from antibiotic resistance genes, deficiency markers. In some embodiments, selection markers are expressed with a polynucleotide that encodes a gene product of interest (eg a binding
30 protein encompassed by the present invention, such as TCR). In one aspect, the recombinant expression vector includes marker genes, such as the truncated CD34 (CD34t) and/or CD19 that marks the transduced host T cells and acts as a selection marker of recovery. In a preferred embodiment, a truncated CD34t and/or CD19 molecule is expressed alongside the TCR of interest within the human cell.

35 The vectors of the current invention can be designed for transient expression, for stable expression, or for both. Also, the recombinant expression vectors can be designed for constitutive expression or for inducible expression.

A vector as described herein can comprise a native or synthetic promoter operably linked to the nucleotide sequence encoding the TCR, polypeptide, or protein (including functional portions and functional variants thereof), or to the nucleotide sequence which is
5 complementary to or which hybridizes to the nucleotide sequence encoding the TCR, polypeptide, or protein. The selection of promoters, e.g., strong, weak, inducible, tissue-specific and developmental-specific, is within the ordinary skill of the artisan. Similarly, the combining of a nucleotide sequence with a promoter is also within the skill of the artisan. The promoter can be a non-viral promoter or a viral promoter. Preferably strong promoters
10 are used, such as retroviral long terminal repeats (LTRs), cytomegalovirus (CMV), murine stem cell virus (MSCV) U3, phosphoglycerate kinase (PGK), P-actin, ubiquitin, elongation factor (EF)-1a (Tsuji et al., 2005), and a simian virus 40 (SV40)/CD43 composite promoter (Cooper et al., 2004; Jones et al., 2009), and the spleen focus forming virus (SFFV) promoter (Joseph et al., 2008), HCMV IE1 promoter. In a preferred embodiment, the
15 promoter is heterologous to the nucleic acid being expressed.

A vector as described herein can comprise transacting genetic element, such as a microRNA, shRNA and/or lcrRNA sequence is designed to modulate an endogenous transcript with the target cell. A vector as described here in can further comprise further
20 open reading frames that benefit cellular persistence, disease trafficking, metabolic fitness, evasion of immunosuppressive tumour microenvironment or other functional characteristics of a therapeutic cell product.

Host Cells (Engineered cells)

25 Host cells according to the current invention are cells that have been engineered to contain the nucleic acids and expression vectors as described herein. As described herein but not limited to, the host cells may be primary cells derived from a donor, may be engineered, and may be used for immunotherapy (immunotherapy host cell, TCR-T cells), or they may be cultured mammalian cells. Such cultured cells may be engineered, and
30 may e.g. be engineered TCR presenting cells (eTPC). eTPCs may be an adherent cell or a suspended cell, i.e., a cell that grows in suspension. Preferred suitable host cells are of human origin and are known in the art, for instance Jurkat cells. For purposes of amplifying or replicating the recombinant expression vector, the host cell is preferably a prokaryotic cell, e.g., a DH5a cell. Other eukaryotic host cells may include yeast and
35 insect cells.

The present description also relates to a host cell with the integration of a functional TCR that is capable of being expressed from the introduced recombinant vector in the host cell.

In a preferred embodiment, the host cell comprising a TCR of the invention is a human T cell of the CD8⁺ and/ or CD4⁺ subtype. In some embodiments, the host cell is selected from the group consisting of a CD8⁺ T cell, a CD4⁺ T cell, T cell progenitor cell, NK cell, NK progenitor cell or differentiated stem cell. The host cells can be allogeneic, autologous, 5 or engineered. In a preferred embodiment, the host cell used is an autologous human CD8⁺ T cell.

The T cells used according to the current invention can come from any source known in the art. For example, T cells can be differentiated *in vitro* from a hematopoietic stem cell 10 population, or T cells can be obtained from a subject, who may or may not be the patient who is intended to be treated. T cells can be obtained from, e.g., peripheral blood mononuclear cells (PBMCs), bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In addition, the T cells can be derived from one or more T cell lines available in the art. T 15 cells can also be obtained from blood collected from a subject using any number of techniques known to the skilled artisan, such as, but not limited to, density gradient centrifugation, FICOLL™ separation and/or apheresis.

In one embodiment, the primary immune cells comprising a TCR (TCR-T also referred to 20 as effector cells) of the invention are activated and/or expanded *in vitro*. Methods for activating and expanding T cells are known in the art. Generally, such methods include contacting PBMC or isolated T cells with a stimulatory agent and costimulatory agent, such as anti-CD3 and anti-CD28 antibodies, generally attached to a bead or other surface, in a culture medium with appropriate cytokines, such as IL-2. Anti-CD3 and anti- 25 CD28 antibodies attached to the same bead serve as a "surrogate" antigen presenting cell (APC). One example is the Dynabeads® system, a CD3/CD28 activator/stimulator system for physiological activation of human T cells.

A further aspect of the invention relates to an immortalized engineered cell expressing the TCR as disclosed, described and/or defined herein and/or the isolated nucleic acid 30 sequence disclosed, described and/or defined herein, and/or which comprises a vector disclosed, described and/or defined herein.

Optionally, the engineered cell according to the current invention comprises a 35 chromosomal gene knock out of one or more TCR gene(s), one or more HLA gene(s), or both, such as wherein the engineered cell comprises a knockout of an HLA gene selected from the group consisting of an α -1-macroglobulin gene, α -2- macroglobulin gene, α -3- macroglobulin gene, β -1-microglobulin gene, β -2-microglobulin gene, and combinations thereof, or such as wherein the engineered cell comprises a knockout of a TCR gene

selected from a TCR alpha variable region gene, TCR beta variable region gene, TCR constant region gene, and combinations thereof.

An engineered cell according to the current invention can express CD8a and/or CD8b.

- 5 Optionally the CD8 alpha and/or CD8 beta is fused to a truncatedCD34 enrichment tag.
The engineered cells can be enriched using the truncatedCD34 enrichment tag.

- Typically, the engineered cell disclosed, described and/or defined herein, originates from a hematopoietic progenitor cell, peripheral blood mononuclear cell (PBMC), cord blood
10 cell, or immune cell. In an embodiment, the such immune cell is a T cell, cytotoxic lymphocyte, cytotoxic lymphocyte precursor cell, cytotoxic lymphocyte progenitor cell, cytotoxic lymphocyte stem cell, CD4+ T cell, CD8+ T cell, CD4/CD8 double negative T cell, gamma delta (gd) T cell, natural killer (NK) cell, NK-T cell, dendritic cell, or a combination thereof. In one embodiment, the engineered cell is derived from a
15 hematopoietic stem cell (HSC) or an induced pluripotent stem cell (iPSC). The T cell can be a naive T cell, central memory T cell, effector memory T cell, or a combination thereof, or a primary T cell or a cell of a T cell line.

- In a currently preferred embodiment, the engineered cell disclosed, described and/or
20 defined herein does not express, or has a lower surface expression of, an endogenous TCR and is capable of producing a cytokine or a cytotoxic molecule when contacted with a target cell that presents a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the
25 peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO:2) presented in a HLA-A*11 complex.

- In embodiments, the engineered cell according to the current invention is capable of producing TNF-alpha, IL-2, and/or IFN-gamma, and/or a perforin and/or a granzyme, such
30 as granzyme B. An engineered cell according to the current invention can be contacted with a target cell *in vitro* or *ex vivo* and is preferably capable of producing a higher level of cytokine or a cytotoxic molecule when contacted with a target cell with a heterozygous expression of human RAS, such as an at least 1.05-fold higher level of cytokine or a cytotoxic molecule.

- 35 In embodiments, the engineered cell according to the current invention is capable of killing a target cell that presents a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey

RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the peptide consists of VVVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO:2) presented in a HLA-A*11 complex. The killing is determined by a killing assay and the ratio of the engineered cell and the target cell in the killing assay is from 1:64 to 1:1, wherein the target cell is a target cell pulsed with a range of between 1×10^3 nM and 0.1 nM of a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the peptide consists of VVVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO:2) presented in a HLA-A*11 complex. In particular the target cell is a cell monoallelic for HLA-A*11.

The engineered cell of the current invention does not induce T cell expansion, cytokine release, or cytotoxic killing when in contact with a target cell that presents a peptide selected from the group consisting of VVVGAGGVGK (SEQ ID NO: 3) presented in a HLA-A*11 complex, or VVGAGGVGK (SEQ ID NO: 4) presented in a HLA-A*11 complex.

In an embodiment, the engineered cell disclosed, described and/or defined herein is capable of killing a higher number of target cells when contacted with target cells with a heterozygous expression of a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the peptide consists of VVVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO:2) presented in a HLA-A*11 complex. Typically, the cell killing is at least 1.05-fold higher.

In aspects, the target cell is a cell line or a primary cell, such as selected from the group consisting of a cancer cell line, a primary cancer cell line, a transformed cell line, and an immortalized cell line, such as HEK293-, ARH -77-, COR-L23-, NCI-H441-, SW620-, QGP-1-, or IGR-1- derived cell lines. Such cell lines may be modified to stably express HLA-A*11 and/or luciferase reporters.

The current invention also relates to a population of engineered cells disclosed, described and/or defined herein as well as to a pharmaceutical composition comprising any one or more of the engineered cells or vectors of the current invention, such as wherein the vectors are selected from the group consisting of viral vectors, non-viral vectors, LNPs or any other carrier comprising the nucleic acid sequence described, disclosed and/or defined herein.

The engineered cells of the invention can substantially cross-react with cells and kill cells that express a polypeptide that contains the natural amino acid sequence of the peptides, such as G12V mutated RAS 10-mer (SEQ ID NO: 1) and 9-mer (SEQ ID NO: 2), as defined in the aspects of the description. As can be derived from the scientific literature and databases (Rammensee et al 1999), certain positions of HLA binding peptides are typically anchor residues forming a core sequence fitting to the binding motif of the HLA receptor, which is defined by polar, electrophysical, hydrophobic and spatial properties of the polypeptide chains constituting the binding groove. In an aspect, one skilled in the art would have the ability given the teachings of the description to modify the amino acid sequence of a TCR, by modulation of the TCR CDR regions whilst maintaining recognition of the known anchor residues, and would be able to determine whether such TCR variants maintain the ability to bind MHC class I or II molecules/ G12V mRAS 10-mer (SEQ ID NO: 1) and 9-mer (SEQ ID NO: 2) complexes.

In one embodiment, a soluble TCR of the invention can be produced and used. In this embodiment the host cell can be a cultured mammalian cell. The mammalian host cell can be an adherent cell or a suspended cell, i.e., a cell that grows in suspension. Preferred suitable host cells are from human and are known in the art, for instance HEK293 cells and ARH-77 cells. For purposes of amplifying or replicating the recombinant expression vector, the host cell is preferably a prokaryotic cell, e.g., a DH5a cell. Other eukaryotic host cells may include yeast and insect cells.

Transformation of appropriate cell hosts with a DNA construct of the present description is accomplished by well-known methods that typically depend on the type of vector used. With regard to transformation of prokaryotic host cells, see, for example, Cohen et al. (Cohen et al., 1972) and (Green and Sambrook, 2012). Electroporation is also useful for transforming and/or transfecting cells and is well known in the art for transforming yeast-cells, bacterial cells, insect cells and vertebrate cells.

Successfully transformed cells, i.e., cells that contain a DNA construct of the present description, can be identified by well-known techniques such as polymerase chain reaction (PCR). Alternatively, the presence of protein encoded by such a DNA construct in the supernatant can be detected using antibodies.

35 Soluble TCR and/or a conjugated soluble TCR

The current invention in one aspect relates to a soluble TCR and/or a conjugated soluble TCR according to the current invention. In aspects, the TCR is a bi-specific monoclonal

antibody (such as T cell engager (BiTE)), or it is an immune mobilizing monoclonal T-cell receptor against cancer (such as ImmTAC).

The invention further relates to a pharmaceutical composition comprising a soluble protein
5 consisting of all or a functional part of a TCR as disclosed and/or defined herein and at least one pharmaceutically acceptable excipient, carrier or stabilizer.

A pharmaceutical composition of the current invention comprises a soluble TCR, a conjugated soluble TCR, a cell expressing a TCR, or a composition comprising a TCR,
10 wherein the TCR is a TCR according to the current invention. Said soluble TCR can be conjugated to a radionuclide, a chemotherapeutic agent, a toxin, or another therapeutically active component, and at least one pharmaceutically acceptable excipient, carrier or stabilizer.

15 **Pharmaceutical compositions**

A "pharmaceutical composition" is a composition suitable for administration to a human being in a medical setting. Preferably, a pharmaceutical composition has minimal bioburden and is produced according to GMP guidelines.

20 The TCRs, nucleic acids, recombinant expression vectors, and host cells (including populations thereof) according to the present invention, can be formulated into a pharmaceutical composition. In preferred embodiment, the invention provides a pharmaceutical composition comprising any of the TCRs and functional variants thereof, nucleic acids, expression vectors, host cells such as, but not limited to, TCR-Ts (including
25 populations thereof), and a pharmaceutically acceptable carrier. Alternatively, the pharmaceutical composition can comprise a TCR-T material according to the present invention in combination with another pharmaceutically active agents or drugs, such as a chemotherapeutic agent, e.g., asparaginase, busulfan, carboplatin, cisplatin, daunorubicin, doxorubicin, fluorouracil, gemcitabine, hydroxyurea, methotrexate,
30 paclitaxel, rituximab, vinblastine, vincristine, etc.

In a preferred embodiment, the pharmaceutical composition is an autologous, transduced CD8+ and/or CD4+ T cell, infused into a patient following pre-treatment with a conditioning regime, which may include one or more of cyclophosphamide, fludarabine,
35 decitabine or azacytidine.

The pharmaceutical composition of the present description may also include cryoprotective agents (CPAs) such as small-molecular-weight penetrating CPAs (e.g.,

dimethyl sulphoxide [DMSO], glycerol, ethylene glycol, and propylene glycol) and high-molecular-weight non-penetrating agents (e.g., sucrose, polyvinylpyrrolidone, and hydroxyethyl starch). In preferred embodiment the CPA is DMSO or is derived from DMSO.

5

In a preferred embodiment the pharmaceutical composition has minimal bioburden and is produced according to GMP guidelines.

Pharmaceutical compositions of the present description may include at least one host cell expressing a TCR of the present description (TCR-T), in a pharmaceutically acceptable carrier.

Pharmaceutical compositions of the present description may also include pharmaceutically acceptable excipients and/ or stabilizers.

15

In preferred embodiment, the composition is selected for parenteral delivery or for injection/ pumping directly into a solid tumour, into a blood vessel that feeds a tumour or into the volume surrounding a solid tumour. The preparation of such pharmaceutically acceptable compositions is within the ability of one skilled in the art. In certain embodiments, buffers are used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 5 to about 8. In certain embodiments, when parenteral administration is contemplated, the composition is in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising a composition described herein, with or without additional therapeutic agents, in a pharmaceutically acceptable vehicle. In certain embodiments, the vehicle for parenteral injection is sterile distilled water in which composition described herein, with or without at least one additional therapeutic agent, is formulated as a sterile, isotonic solution, properly preserved. In certain embodiments, the preparation involves the formulation of the desired molecule with polymeric compounds (such as polylactic acid or polyglycolic acid), beads or liposomes, that provide for the controlled or sustained release of the product, which are then delivered via a depot injection. In certain embodiments, implantable drug delivery devices are used to introduce the desired molecule.

Injectable formulations are in accordance with the invention. The requirements for effective pharmaceutical carriers for injectable compositions are well-known to those of ordinary skill in the art (see, e.g., *Pharmaceutics and Pharmacy Practice*, J. B. Lippincott Company, Philadelphia, Pa., Banker and Chalmers, eds., pages 238-250 (1982), and

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ASHP Handbook on Injectable Drugs, Trissel, 4th ed., pages 622- 630 (1986)).

Preferably, when administering cells, e.g., T cells, the cells are administered via injection.

Preferably, the carrier is a pharmaceutically acceptable carrier. With respect to
5 pharmaceutical compositions, the carrier can be any of those conventionally used and is limited only by chemico-physical considerations, such as solubility and lack of reactivity with the active compound(s), and by the route of administration. The pharmaceutically acceptable carriers described herein, for example, vehicles, adjuvants, excipients, and diluents, are well-known to those skilled in the art and are readily available to the public. It
10 is preferred that the pharmaceutically acceptable carrier be one which is chemically inert to the active agent(s) and one which has no detrimental side effects or toxicity under the conditions of use.

The choice of carrier will be determined in part by the particular TCR, as well as by the
15 particular method used to administer the TCR. Accordingly, there are a variety of suitable formulations of the pharmaceutical composition of the invention.

Uses of the TCRs, nucleic acids and/or vectors and pharmaceutical compositions comprising the same

20 In one aspect, the current invention relates to a TCR disclosed, described and/or defined herein for use as a medicament. In particular, the current invention relates to a TCR disclosed, described and/or defined herein for use in the treatment of a cancer in a patient carrying HLA-A*11, comprising administering to said patient said TCR or a pharmaceutical composition comprising said TCR, wherein said cancer is a solid or haematological
25 malignancy expressing the peptide of SEQ ID NO: 1 or SEQ ID NO: 2, and wherein said TCR or a pharmaceutical composition comprising said TCR is infused systemically into said patient or injected or pumped directly into a solid tumour, into a blood vessel that feeds a tumour or into the volume surrounding a solid tumour in said patient.

30 In aspects, said treatment comprises administering to said patient an engineered cell expressing the TCR, wherein said engineered cell is a T cell, T cell progenitor cell, NK cell, NK progenitor cell or differentiated stem cell and which is either autologous or allogenic, and wherein about 10^4 to about 10^{10} engineered cells are infused systemically into said patient or injected or pumped directly into a solid tumour, into a blood vessel that
35 feeds a tumour or into the volume surrounding a solid tumour.

Typically, the use related to herein is for treating a disorder in a subject, wherein said subject carries HLA-A*11:01, however it can also be used for treating a disorder in a subject that carries an alternate subform of HLA-A*11, the use related to herein is for

treating a disorder in a subject, wherein said subject carries an alternate subform of HLA-A*11. The use related to herein may typically be for treating a disorder in a subject, wherein said subject carries an alternate subform of HLA-A*11 other than HLA-A*11:03, which alternate subform of HLA-A*11 is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and wherein the HLA when presenting the peptide is recognized by a TCR disclosed, described and/or defined herein, such as wherein said subject carries a different HLA-A, HLA-B or HLA-C allele than HLA-A*11, that is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2, and which is recognized by the TCR according to the current invention. In addition the use related to herein may treating a disorder in a subject, wherein said subject carries an alternate subform of HLA-A*11 other than HLA-A*11:04. Whether a specific TCR is suitable for use in a subject thus depends on the HLA allotypes carried by the subject. Table 25 provides an overview of included and excluded HLAs for TCRs according to the invention.

15 **Medical Treatments**

In one aspect, the invention relates to a method of treating a disorder characterized by G12V mutated human RAS expression in a subject comprising administering to said subject a therapeutically effective amount of genetically engineered cells that express a TCR as disclosed and/or defined herein, or a pharmaceutical composition disclosed and/or defined herein, wherein said disorder is a solid or haematological malignancy expressing the peptide of VVVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2), wherein said engineered cell is a T cell, T-cell progenitor cell, NK cell, NK progenitor cell or a differentiated stem cell and which is either autologous or allogenic, and wherein about 10^4 to about 10^{10} engineered cells are infused systemically into said subject or injected or pumped directly into a solid tumour, into a blood vessel that feeds a tumour and/or into the volume surrounding a solid tumour.

Said method is in particular intended for treating a disorder in a subject, wherein said subject carries an HLA-A*11 allele capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which is recognized by the TCR according to the current invention. In addition, said method can be used for treating a said subject, wherein said subject carries an alternate subform of HLA-A*11 other than HLA-A*11:03 or HLA-A*11:04, which alternate subform of HLA-A*11 is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which is recognized by the TCR according to the current invention, or wherein said subject carries a different HLA-A, HLA-B or HLA-C allele than HLA-A*11, that is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which is recognized by the TCR according to the current invention. Table 25 provides suitable HLAs for TCRs according to the present invention.

In another embodiment the TCRs, the nucleic acid or the expression vector of the description are used in medicine. For example, the medicament can be used in the treatment of a cancer in a patient possessing HLA-A*11:01 comprising administering to
5 said patient said TCR or a pharmaceutical composition comprising said TCR, wherein said cancer is a solid or haematological malignancy expressing the peptide of G12V mutated RAS 10-mer (SEQ ID NO: 1) and 9-mer (SEQ ID NO: 2), and wherein said TCR or a pharmaceutical composition comprising said TCR is infused systemically into said patient or injected or pumped directly into a solid tumour, into a blood vessel that feeds a
10 tumour or into the volume surrounding a solid tumour in said patient.

In aspects a pharmaceutical composition comprises a soluble protein consisting of all or a functional part of one or more TCR(s) or one or more engineered cell(s) or one or more vector(s). In aspects the invention relates to one or more compositions each comprising a
15 soluble protein consisting of all or a functional part of one or more TCR(s) or one or more engineered cell(s) or one or more vector(s). Thus, treatment may comprise mono- or multiplexed TCR-T cell therapy. In particular the invention relates to combination therapies with 9-mer- and 10-mer-reactive TCRs, as well as the TCRs of the invention being included in a larger set of TCRs targeting HLA/peptides for individual patients.

20 The terms "treat," and "prevent" as well as words stemming therefrom, as used herein, do not necessarily imply 100% or complete treatment or prevention. Rather, there are varying degrees of treatment or prevention of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the inventive methods can
25 provide any amount of any level of treatment or prevention of cancer in a mammal. Furthermore, the treatment or prevention provided by the inventive method can include treatment or prevention of one or more conditions or symptoms of the disease, e.g., cancer, being treated or prevented. Also, for purposes herein, "prevention" can encompass delaying the onset of the disease, or a symptom or condition thereof.

30 TCRs, nucleic acids and host cells of the present description, and pharmaceutical compositions thereof, may be administered to a subject in need thereof by routes known in the art, and may vary depending on the type of cancer to be treated.

35 TCRs of the present description may comprise a detectable label selected from the group consisting of a radionuclide, a fluorophore and biotin. TCRs of the present description may be conjugated to a therapeutically active agent, such as a radionuclide, a chemotherapeutic agent, or a toxin.

In preferred embodiments, compositions of the present description are administered to a subject using a single dose.

- 5 Effective doses of host cells expressing TCRs of the present invention include, for example at least about 10^4 , at least about 10^5 , at least about 10^6 , at least about 10^7 , at least about 10^8 , at least about 10^9 , at least about 10^{10} host cells per dose. In one embodiment, host cells of the present description are administered in a dose of between about 10^4 to about 10^{10} cells per dose, preferably in a dose of between about 10^5 to about
10 10^9 cells per dose. In preferred embodiments, a single dose is administered.

- In aspects, TCRs according to the present invention are used in personalised cancer immunotherapy, such as, but not limited to, being administered in combination with a vaccine. In particular the invention relates to a combination of a tumour vaccine with a
15 TCR-T cell therapy.

Medicament

- In one aspect, the invention relates to a method of treating a disorder characterized by G12V mutated human RAS expression in a subject comprising administering to said
20 subject a therapeutically effective amount of genetically engineered cells that express a TCR as disclosed and/or defined herein, or a pharmaceutical composition disclosed and/or defined herein, wherein said disorder is a solid or haematological malignancy expressing the peptide of VVVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2), wherein said engineered cell is a T cell, T cell progenitor cell, NK cell, NK
25 progenitor cell or a differentiated stem cell and which is either autologous or allogenic, and wherein about 10^4 to about 10^{10} engineered cells in combination with a vaccine are infused systemically into said subject or injected or pumped directly into a solid tumour, into a blood vessel that feeds a tumour and/or into the volume surrounding a solid tumour. Alternatively, the vaccine may be administered concomitantly with, before, or after
30 administration of the engineered cells.

In general, the following vaccine compositions may be used as a medicament to prime T cells responses within a subject.

- 35 The invention in another aspect relates to a method of treating a disorder characterized by G12V mutated human RAS expression in a subject comprising administering to said subject a therapeutically effective dose regime of a pharmaceutical composition consisting of a soluble protein that is a hybrid of the variable domains of a TCR as disclosed and/or

defined herein and an activation domain, and where the pharmaceutical composition is administered in combination with a vaccine.

In general, the following vaccine compositions may be used as a medicament to prime
5 and support T cell responses within a subject:

- a) Vaccine contains sequence(s), delivered by RNA, DNA or peptide compositions, including liponanoparticles, in vivo electroporation, incorporation into viral particles, and antibody-mediated trafficking amongst other options, designed to enable either or both of
10 MHC class I and class II presentation of the same target epitope, and hence stimulation of therapeutic CD8 cells as well as helper CD4 cells against the same target, respectively. It is appreciated by those skilled in the art that different formulations and designs of vaccines can be optimised to balance and direct MHC class I vs class II responses, that different routes of administration can be exploited to balance responses, that different
15 timings of prime/boost strategy can be exploited alongside TCR cell therapy, and that different immune-cell tropisms can be designed and exploited to maximise effects.

Presentation of MHC class I epitopes including on professional APCs would be expected to support direct expansion of therapeutic cells, which would be beneficial for directing the
20 initial extent of T cell engraftment and expansion. Such an effect may be important in prophylactic or adjuvant settings, where tumour cell levels may be low as compared to a primary or metastatic setting.

Presentation of MHC class II epitopes on professional APCs would be expected to
25 support the expansion of CD4⁺ T cells directed to the same target epitope, which would be expected to be a key mechanism. CD4⁺ T cells are known to promote antitumor immunity by numerous mechanisms including enhancing antigen presentation, co-stimulation, T cell homing, T cell activation, and effector function (Nelde et al, 2021). These effects are mediated at sites of T cell priming and at the tumor microenvironment (Melssen and
30 Slingluff, 2017). CD4⁺ T cells harbor a crucial role for effective antitumor immunity and could further improve and sustain T cell reactivity (Bijker et al, 2007) and tumor clearance (Zwaveling et al, 2002). CD4⁺ T helper cells induce and intensify more permanent tumor immune control (Spitzer et al, 2017), epitope spreading (Hu et al, 2014), CD8⁺ T cell expansion and survival (Giuntoli et al 2002), as well as tumor immune cell infiltration (Bos and Sherman 2010). In addition, CD4⁺ T cells also exhibit direct antitumor effector
35 function (Quezada et al, 2010).

- b) Vaccine contains sequence(s), delivered by RNA, DNA or peptide compositions (including liponanoparticles, in vivo electroporation, incorporation into viral particles, and antibody-mediated trafficking amongst other options), designed to enable either or both of MHC class I and class II presentation of the additional target epitopes, and hence
- 5 stimulation of therapeutic CD8⁺ cells as well as helper CD4⁺ cells against complementary targets. For example, a vaccine encoding patient-tumour specific neoantigens, or other antigens known to be over-expressed in the specific tumour type of interest, may be used to support a targeted T cell therapy.
- 10 Conceptually, such a vaccine in the form of, for example, a polyvalent mRNA expression construct formulated in an LNP particle, could be administered both prior to, or alternatively subsequent to, a TCR-T, using a prime-boost schedule.
- c) Further designs and administration schedules using vaccines that encode both tumour-
- 15 specific neoantigens as well as KRAS mutations can be administered.

Experimental section

The present invention is further illustrated by the following non-limiting experiments.

Figure legends

- 20 **Figure 1: Summary of a TCR discovery platform focused on delivering clinically safe TCRs**
- The figure summarizes the Key Platform Units (left column) and the platform processes (middle column) that are used for discovery of clinically viable TCRs. A key biological discovery achieved at each step is shown (right column).
- 25 **Step 1.** Antigen Discovery - Unbiased mass spectrometry (MS) is used for identification of peptides presented by mono-allelic HLA antigen presenting cells (eAPCs). This approach allows unbiased HLA-restricted mapping for discovery of biologically relevant, processed and presented peptides.
- Step 2.** TCR discovery - The antigen identified in Step 1 is used for T cell enrichment,
- 30 target-dependent isolation, and paired TCR sequencing, allowing for selective capture of TCR clonotypes and identification of TCR clonotypes' capability of binding target peptides.
- Step 3.** TCR antigen validation – eAPC and (engineered TCR-presenting cell) eTPC cell contact assays are used for functional evaluation of pHLA/ TCR sensitivity. TCR and HLA-restricted peptides are validated and the TCRs are ranked based on functional sensitivity
- 35 in defined cell-cell functional contact assays. TCRs with low sensitivity are removed from further analysis. The discovered TCRs can in turn be used to validate the target antigen presented by the eAPC lines as well as in orthogonal cancer cell lines.

Step 4. TCR-T – Candidate TCRs are integrated into primary T cells to create TCR-Ts and TCR-T potency assays are performed, demonstrating that the TCR-Ts are functional and that they possess selective cytotoxic activity.

Step 5. TCR safety assessment– this step in the TCR discovery platform ensures that only de-risked, clinically viable TCRs progress to the clinic. Cross-reactive epitopes within the human genome are predicted from fingerprint profiles of the TCRs using a computer algorithm. The identified cross-reactive epitopes (hits) are then presented on monoallelic eAPCs and defined cell-cell functional contact assays are performed. TCRs with no identified cross-reactive hits are selected and a defined set of potential allo-reactive HLA alleles is determined by screening against a library of monoallelic HLA-expressing cell lines covering 86 most common HLA haplotypes. All the above steps ensure that the end-products of the TCR discovery platform are clinically viable TCRs.

Figure 2: Detection of G12V mutated KRAS peptides

The mean intensities of peptides released from affinity purified A*11:01 pHLA complexes, as measured by unbiased mass spectrometry on a Bruker TIMS-TOF2 instrument, are shown, with z-scores relative to the mean (-) of all observations in the sample shown for specific observations of nonamer- (9-mer, indicated by a full circle) and decamer- (10-mer, indicated by a full square) peptides derived from G12V mutated KRAS (NQ – the particular peptides were detected but not quantified). G12V_FL: A*11:01 monoallelic eAPCs expressing a full-length version of inactivated KRAS protein with G12V mutations (SEQ ID NO: 6); G12V_MG-UFD: A*11:01 monoallelic eAPCs expressing a minigene with the candidate epitope +/- up to 9 amino acids of flanking sequence (SEQ ID NO: 5). COR-L23 lung cancer cell line: cell line COR-L23 natively expressing mutated KRAS G12V and with lentivirally transduced affinity-tagged HLA-A*11:01. Intensity is plotted on Y-axis, against the sample on X-axis.

Figure 3: Selective expansion of TCRs specific for G12V mutated KRAS peptides.

Frequency distribution of TCR clonotypes enriched from G12V mutated KRAS peptide-stimulated expansions, using tetramers specific for the 10-mer and 9-mer peptides. **A)** 10-mer-specific TCR clonotypes (X axis) (n=54) are plotted against the percentage frequency for each particular clonotype observed (Y-axis). Clonotypes which were counted at least 3 times were considered enriched. TCRs are shown as black bars, * TCR-1 and ** TCR-2, and have been marked retrospectively. **B)** A number of 9-mer-specific TCR clonotypes (n=13) which were enriched at least 3 times (X axis) are plotted against the percentage frequency of the particular clonotype observed (Y-axis). ***TCR-6, *TCR-9 and **TCR-10, are shown as black bars, and have been marked retrospectively.

Figure 4: Functional evaluation of sensitivity of identified G12V mutated KRAS 10-mer- and 9-mer-sensitive TCRs

Sensitivity of the identified G12V mutated KRAS 10-mer and 9-mer specific TCRs was functionally evaluated in eTPC-eAPC cell co-culture assays. Briefly, engineered TCR presenting cell (eTPC) lines as described in WO 2018/083317 were generated to present the G12V mutated KRAS 10-mer and 9-mer sensitive TCRs (eTPC-t) and were co-cultured with engineered antigen presenting cells (eAPC) expressing monoallelic HLA-A*11:01 loaded with a concentration range of the G12V mutated KRAS 10-mer or 9-mer peptides, was measured by detection of the RFP (RFP⁺) signal in combination with CD3 decrease (CD3^{low}) as described in Materials and Methods. Figures 4 A-C show flow panel validation and the gating strategy used for detection of activated RFP⁺CD3^{low} cells. **A)** Shows a representative of an “on target 10-mer-specific TCR” unstimulated with G12V mutated KRAS 10-mer peptide (left panel) or stimulated with G12V mutated KRAS 10-mer peptide (right panel). eTPC cells co-cultured with monoallelic eAPC without the KRAS peptide loaded (i.e. unpulsed) eAPC HLA-A*11:01 cells were used to define the flow gates that capture activated cells i.e., those that showed low CD3 staining with the CD3 antibody and positive RFP signal. Therefore, in panel A, the eTPC cells were not activated and are shown below the set gates, they are RFP negative (RFP⁻) except for some cells with autofluorescence, and CD3^{high} (Left panel). Following co-culture of pulsed eAPC HLA-A*11:01 with the G12V mutated KRAS 10-mer peptide and the eTPCs, the eTPC cells were activated which was observed by increase in RFP⁺, and decrease in CD3 (CD3^{low}) (right panel) and therefore most of the cell population was found within the gates set for defining the RFP⁺CD3^{low} population. **B)** A TCR that reacts with the HLA allele presented on the eAPCs (i.e., HLA-A*11:01) without the target peptide is a representative of a “HLA-reactive TCR”. The RFP/CD3 profile does not change upon stimulation with the G12V mutated KRAS 10-mer (right panel), it is always RFP⁺CD3^{low}. **C)** Shown is a representative of an “unresponsive TCR”, (RFP⁻CD3^{high}) in both unstimulated (left panel) and stimulated cells (right panel). **D and E)** Flow cytometric data was used to construct sigmoidal curves from which the half maximal effective concentration (EC50) values were obtained. The sensitivity of the TCRs was based on the EC50 values. **D)** shows EC50 (nM) distribution obtained from the RFP⁺CD3^{low} values of the G12V mutated KRAS 10-mer-responsive TCRs (n=54) (X-axis) plotted against each TCR. 10-mer G12V mutated KRAS-responsive TCRs, TCR-1 and TCR-2, are marked by full circle, retrospectively. **E)** Shows EC50 (nM) distribution obtained from the RFP⁺CD3^{low} values of the G12V mutated KRAS 9-mer-responsive TCRs (n=13) (X-axis) against each TCR (Y-axis). 9-mer G12V mutated KRAS-responsive TCRs, TCR-6, TCR-9 and TCR are marked by full circle, retrospectively.

Figure 5: Functional evaluation of sensitivity and specificity of the G12V mutated KRAS TCRs pulsed with target peptides

In further examination of the TCR sensitivity, the ability of the 10-mer-specific TCRs to specifically recognize and bind the 9-mer peptides and vice versa was analyzed. The figure shows representative sigmoidal curve profiles of two 10-mer- and three 9-mer-specific TCRs. The sensitivity of the 10-mer-specific TCRs towards the 9-mer peptide and vice versa, as well as to the wild type G12 KRAS peptide, are compared. eTPCs expressing the TCR-1 (**A**) or TCR-2 (**B**) were co-cultured with monoallelic HLA-A*11:01 eAPCs- pulsed with a titration range of G12V mutated KRAS 10-mer, G12V mutated KRAS 9-mer, or corresponding G12 KRAS WT peptides. Percentage response (Y-Axis) of activation, as measured by RFP⁺ CD3^{low}, is plotted against the peptide concentration in nM (X-axis). TCR-1 recognized G12V mutated KRAS 10-mer peptide with high specificity and sensitivity (full triangle), only weakly recognized the 9-mer G12V mutated KRAS peptide (full circle), and did not recognize the WT G12 KRAS peptide (full square). **B**) TCR-2 recognized 10-mer G12V mutated KRAS peptide with high sensitivity and specificity (full triangle) and did not recognize the G12V mutated KRAS 9-mer peptide (full circle), or the WT G12 KRAS peptide (full square). To check the sensitivity of the 9-mer-specific TCRs, eTPCs expressing TCR-6 (**C**), TCR-9 (**D**) or TCR-10 (**E**) were co-cultured with monoallelic HLA-A*11:01 eAPCs pulsed with a titration range of G12V 10-mer, G12V 9-mer and WT 9-mer peptides. Specific activation was again measured by detection of RFP signal in combination with CD3 downregulation. **C-E**) TCR-6, TCR-9 and TCR-10 show specificity and sensitivity towards the G12V mutated KRAS 9-mer peptide (full circle) and to some extent the G12V mutated KRAS 10-mer peptide (full triangle), but not to the WT G12 KRAS peptide (full square). **F**) A sensitivity of a reference TCR (SEQ ID NO: 5164) towards a mutated KRAS 10-mer peptide (full circle), mutated KRAS 9-mer peptide (full triangle) and a wild type KRAS peptide (full square) measured by detection of RFP signal in combination with CD3 downregulation.

Figure 6: Evaluation of G12V mutated KRAS-10-mer- and 9-mer-responsive TCRs to constitutively expressed target peptides

To further validate the interaction between the 10-mer- and 9-mer-sensitive TCRs and their target peptides, we utilized the monoallelic eAPCs that constitutively express the target peptides and measured the response in the eTPCs expressing the 10-mer- or the 9-mer-specific TCRs. **A**) Response of G12V mutated KRAS 10-mer-responsive TCRs (n=54) co-cultured with the monoallelic HLA-A*11:01 eAPCs or the monoallelic HLA-A*11:01 eAPCs constitutively expressing the G12V mutated KRAS peptide as a minigene (MG-UFD) (SEQ ID NO: 5) or a full length (FL) (SEQ ID NO: 6) construct. Percentage response was measured by RFP⁺CD3^{low} signal. KRAS G12V 10-mer specific TCRs

TCRs are shown as black square (TCR-1) and black triangle (TCR-2). **B)** Response of G12V mutated KRAS 9-mer-responsive TCRs (n=13) co- cultured with the monoallelic HLA-A*11:01 eAPCs or the monoallelic HLA-A*11:01 eAPCs constitutively expressing the G12V KRAS peptide as a minigene (MG-UFD) (SEQ ID NO: 5) or a full-length (FL) (SEQ ID NO: 6) construct. Percentage response was measured by RFP⁺CD3^{low} staining. The KRAS G12V 9-mer specific TCRs are shown as an upside-down triangle (TCR-6), a diamond (TCR-9) and a hexagon (TCR-10).

Figure 7: G12V mutated KRAS 10-mer- and 9-mer-specific TCRs mediate potent T cell killing of cells presenting the G12V mutated KRAS peptide presented by HLA-A*11:01

Cytotoxic lysis mediated by the 10-mer-sensitive TCRs, **(A)** TCR-1 - two different KARS G12V MG constructs were used to test the killing potential of the TCR-1. KRAS G12V MG-UFD (SEQ ID NO: 5) in the right panel and KRAS G12V MG-native (SEQ ID NO: 179) in the left panel, **(B)** TCR-2, and 9-mer-sensitive TCRs **(C)** TCR-6, **(D)** TCR-9 and **(E)** TCR-10, quantified as loss of luciferase activity after 24 hour co-culture with target eAPC-pa cells. Target cells are monoallelic eAPC-pa constitutively expressing HLA-A*11:01 in combination with G12V mutated KRAS full length (FL) (SEQ ID NO: 6) peptide or eAPCs expressing HLA-A*11:01_KRAS minigene (MG) (SEQ ID NO: 5) or HLA-A*11:01 only eAPCs (used as off-target control). Y-axis shows % specific lysis, while X-axis shows the effector (TCR-T) to Target ratios. **F)** Cytotoxic activity of TCR-T cells expressing the TCR-1 (TCR-T^{TCR-1}) against a panel of cancer cell lines expressing the mutated KRAS G12V in the HLA-A*11:01 (COR-L23, NCI-H441, SW620, QGP-1), as determined by Luciferase Assay. Cancer cells expressing wt KRAS with or without the HLA-A*11:01 expression (IGR-1_KRASwt-A11 or IGR-1_KRASwt, respectively), were used as controls. The Y-axis shows % specific lysis, while X-axis shows the effector (TCR-T^{TCR-1}) to Target cell ratios. All test conditions were prepared in duplicates. Percentage killing = $100 - ((RLU_{\text{test condition}}) / (RLU_{\text{target only}}) * 100)$; RLU = relative luminescence units. **G)** Longitudinal measurement of cytotoxic activity using Incucyte® live-imaging measurements. Effector TCR-T^{TCR-1} cells were added 24 hours after seeding of the target cancer cells (dashed line). Proliferation and disappearance of the GFP-expressing target cancer cells was measured as the fold-change in GFP+ objects over time after the addition of effector TCR T^{TCR-1} cells and compared to the growth of target cancer cells only. **H)** COR-L23 cells expressing HLA-A*11:01 and G12V are shown with and without the addition of effector TCR-T^{TCR-1} cells over 96 hours. Target cell killing was imaged by Incucyte® live-imaging at 2, 48 and 96 hour time points. Live cancer cells are green, TCR-T^{TCR-1} cells are grey and dying cancer cells are red.

Figure 8: Fingerprinting profile of G12V mutated KRAS 10-mer- and 9-mer-sensitive TCRs

Fingerprinting profile of the G12V mutated KRAS 10-mer- and 9-mer-sensitive TCRs. eTPC-t cell lines expressing the G12V mutated KRAS 10-mer- or 9-mer- sensitive TCRs, were stimulated with a library of monoallelic eAPCs stably expressing HLA-A*11:01 as well as the mutated version of the G12V KRAS peptide (eAPC-pa epitope mutagenesis library). Each cell line expressed a point mutation in a certain position. The Y-axis represents the amino acid substitution and the X-axis the peptide position where the substitution has been introduced. Activation of the eTPC-ts is measured by RFP⁺CD3^{low} signal. **A)** Peptide scan of the KRAS G12V 10-mer- sensitive TCR, TCR-1; **B)** Peptide scan of the KRAS G12V 10-mer- sensitive TCR-2; **C-E)** Peptide scan of the KRAS G12V 9-mer- sensitive TCR-6, TCR-9 and TCR-10; **F)** Peptide scan of the KRAS G12V 10-mer- sensitive TCR, TCR-5, used here as a TCR that did not pass the selection process. The darker the colour, the higher the % response due to that particular amino acid substitution in the peptide sequence.

Figure 9: Cross-reactive profiling of the G12V mutated KRAS 10-mer- and 9-mer-sensitive TCRs

The fingerprint profiles from Figure 8 were analysed by a computer algorithm that generates a fingerprint score which when screened against the whole human proteome allows the prediction of cross-reactive hits (CX). **A)** Predicted cross-reactive hits for the 10-mer-sensitive TCRs, TCR-1 and TCR-2, and two less sensitive TCRs which are shown for comparison, TCR-3 and TCR-4. Based on the Fingerprint score (Y-axis), many cross-reactive hits were predicted for TCR-1, while no cross-reactive hits were predicted for the TCR-2. The less sensitive TCRs, TCR-3 and TCR-4, showed some predicted hits based on their fingerprint score. **B)** The sequences of the predicted cross-reactive hits were subsequently used to generate MG constructs that were inserted into the HLA-A*11:01 presenting cells (eAPC-pa Cross-reactive library). These cells were then contacted with the eTPC cells expressing the TCRs (eTPC-t), TCR-1, TCR-2, as well as TCR-3 and TCR-4, for 18 hours. The response of the eTPC-ts was analysed by flow cytometry by measuring RFP⁺CD3^{low} signals. Although a number of cross-reactive hits were predicted for TCR-1, no actual cross-reactivity was detected in the cell-cell cross-reactive contact assay. TCR-2 had no predicted cross-reactive hits, therefore both TCRs were considered as de-risked TCRs. TCR-3 and TCR-4 showed some predicted hits based on their fingerprint score and some were also confirmed in the functional cell-cell cross-reactive assay. TCRs that showed response in RFP⁺CD3^{low} signal above the 4% threshold in the cell-cell contact assays were considered as cross-reactive and therefore based on these results they were considered as unsafe for use in the clinic. A similar analysis was

performed for the G12V 9-mer-sensitive TCRs. **C)** Predicted cross-reactive hits for the 9-mer-sensitive TCRs, TCR-6, TCR-9 and TCR-10. Two less sensitive TCRs, TCR-7 and TCR-8, are shown for comparison. **D)** Based on the Fingerprint score (Y-axis), no cross-reactive hits were predicted for the TCR-6. TCR-9 and TCR-10 showed some predicted hits based on their fingerprint score but were not confirmed in the functional cell-cell cross-reactive assay. TCR-7 and TCR-8 showed some predicted hits based on their fingerprint score.

Figure 10: Allo- and para-reactivity profiles of the G12V mutated KRAS 10-mer- and 9-mer- sensitive TCRs

To assess the alloreactivity of the TCRs, functional cell-cell contact assays were applied again, wherein the responder cell lines (eTPC-t expressing TCR-1, TCR-2, TCR-6, TCR-9 or TCR-10) were co-cultured with a library of monoallelic HLA-expressing cell lines (eAPC-p alloreactive library) covering the most common haplotypes. 86 different HLA alleles were tested. The read-out of the interaction was TCR-activation, i.e., flow cytometric analysis of RFP⁺CD3^{low} signal. Para-reactivity was assessed by pulsing the library with the G12V mutated KRAS 10-mer peptide in case of TCR-1 and TCR-2, and G12V mutated KRAS 9-mer peptide in case of TCR-6, TCR-9 and TCR-10. **A)** Alloreactivity and para-reactivity profiles of the two G12V 10-mer-sensitive TCRs, TCR-1 (left panel) and TCR-2 (right panel). Y-axis shows the HLA alleles tested, X-axis is the RFP⁺CD3^{low} signal in the absence (NP= no peptide) or presence (G12V) of the G12V mutated 10-mer KRAS peptide. TCR-1 showed no alloreactivity and showed para-reactivity only when the peptide was added to members of the HLA-A*11 group (01, 02, 03, 04, 05 and 09) and weakly para-reactive with HLA-A*30:01. TCR-2 showed some alloreactivity to HLA-A*68:02 and para-reactivity to members of the HLA-A*11 group (01, 02, 05 and 09) and weak para-reactivity to HLA-A*30:01 and HLA-A*68:02. **B)** Alloreactivity and para-reactivity profiles of the G12V mutated KRAS 9-mer-sensitive TCRs, TCR-6 and TCR-9. Y-axis shows the HLA alleles tested, X-axis is the RFP⁺CD3^{low} signal in the absence (NP= no peptide) or presence (G12V) of the G12V mutated KRAS 9-mer peptide. TCR-6 showed very weak alloreactivity (below 4%) to HLA-B*54:01 (NP), and in the presence of G12V mutated KRAS 9-mer peptide signal was detected from members of the HLA-A*11 group (01, 02, 04, 05 and 09) and weakly (below 4%) from HLA-B*54:01. TCR-9 showed alloreactivity to HLA-B*35:01, HLA-B*35:02, HLA-B*35:03, HLA-B*35:12, HLA-B*57:01 and HLA-B*58:01. In the presence of the peptide, signal was detected from HLA-A*11 group (01, 02, 05, 09), HLA-B*35 group (01, 02, 03, 05, 12), HLA-B*52:01, HLA-B*53:01, HLA-B*57:01 and HLA-B*58:01. **C)** TCR-10 showed no alloreactivity and in the presence of peptide, signal from members of the HLA-A*11 group (01, 02, 05 and 09) was detected.

Figure 11. Integration of TCR-1 into primary T cells leads to eradication of KRAS G12V positive tumour cells in a human lung cancer xenograft model.

Human lung cancer cells, COR-L23, naturally expressing the KRAS G12V mutation were retrovirally transduced to express HLA-A*11:01 and firefly luciferase (CORL-L23_A*11) and served as human tumour in vivo model. The CORL-L23_A*11 cells were injected intravenously into the tail vein of NSG mice. Tumour burden was monitored using non-invasive in vivo bioluminescence imaging (BLI) and mice were allocated into three groups with equal distribution of tumor burden in the lungs. Mice were treated with 10 million primary CD8 T cells transduced either with TCR-1 (TCR-T^{TCR-1}, n=10) or with a negative control TCR (TCR-T^{NY-ESO}, n=10) by intravenous tail vein injections. A group of control mice did not receive any TCR-T cells (untreated, n=10). Mice were sacrificed when showing signs of suffering. **A)** Schematic overview of the TCR-1 efficacy study using a CORL-23 human lung tumour in vivo model. **B)** Representative BLI images of CORL-23 bearing mice at baseline 2 days before treatment to 63 days after treatment. Images showing baseline data are representative images of mice prior to allocation of mice into different treatment groups. **C)** Median photon intensity per treatment group from baseline to 63 days after treatment. **D)** Survival analysis of mice after TCR-T treatment.

Figure 12. Generation of Combinatorial mutagenesis library of the CDR3 regions of the alpha or beta TCR chains of TCR-1.

The CDR3 nucleotide sequences from T cell receptor alpha (TRA) and T cell receptor beta (TRB) chains of the parental TCR (TCR-1) were used as a base sequence to generate the diversified CDR3 libraries. **A-C)** Flow plots representing the gating strategy for the response observed in parental TCR-1 **A)** and respective alpha (TRA) **B)** and beta (TRB) **C)** CDR3 libraries after overnight co-culture with eAPC line presenting KRAS WT MG peptide in HLA-A*11:01 (MG KRAS) (middle panels) or eAPC line presenting KRAS G12V mutated MG peptide in HLA-A*11:01 (MG G12V) (right panels). The represented responses correspond to the percentage of cells inside the sorting gate RFP⁺CD3^{low}. The eAPC-HLA-A*11:01 cells without the KRAS wild type or mutated peptide (Null) were used to quantify the background signal (left panels) and set the RFP⁺CD3^{low} gate correctly. **D)** Summary of the response observed in A-C parental TCR-1 and respective alpha (TRA) and beta (TRB) CDR3 libraries after overnight co-culture with eAPC-HLA-A*11:01 line (Null), eAPCs presenting KRAS WT MG peptide in HLA-A*11:01 (MG KRAS) or eAPCs presenting mutated KRAS G12V MG in HLA-A*11:01 (MG G12V). The represented responses correspond to the percentage of cells inside the sorting gate RFP⁺CD3^{low}. The experiment was performed in duplicate (parental TCR) and in triplicate (TRA and TRB CDR3 libraries).

Figure 13. Sequence logo analysis of diversified and selected TRA and TRB CDR3 regions of TCR-1

The CDR3 sequences from Example 7 were subjected to sequence logo analysis. Panels A (alpha) and C (beta) demonstrate the diversification at positions 4-12 (alpha) and 4-13 (beta). Panel B (alpha) demonstrates that arginine (R) was found to be absent at position 6, and lysine (K) to be absent from position 8 in all alpha sequences. Panel D (beta) demonstrates similarly the amino acid residues at positions 6 to 9 and 11 found to be absent in the specified alpha sequences.

Figure 14. Schematic for alignment of scaffold nomenclature used in the specification with TCR chain structure. SC = scaffold, CDR = complementary determining region. SC1, CDR1, SC2, and CDR2 together may be referred together as the variable region, CDR3 is referred to as CDR3, SC4 may be referred to as joining region, and SC5 may be referred to as constant region. The use of the terms "alpha" and "beta" specify the relevant TCR chain.

Materials and Methods

Cell Engineering

Generation of eAPC-p and eAPC-pa by recombinase-mediated cassette exchange (RMCE)

Generation of eAPC-p and eAPC-pa lines was achieved by RMCE as described previously in detail in WO2018-083316, with the exception of the insertion of a different open reading frame (ORF) including the gene of interest (GOI). The cells of this specification were generated as described below. Briefly, ARH-77 HLA-null line was generated using vectors containing the heterospecific recombinase sites flanking a marker (described in Table 1), (SEQ ID NO: 181 and SEQ ID NO: 182). The monoallelic HLA line (eAPC-p) was constructed by RMCE by electroporation with a vector (SEQ ID NO: 183) containing HLA.A*11:01 as a GOI (in the HLA monoallelic library construction described in Example 5 other HLAs were as GOI). After electroporation, cells were incubated in culture medium RPMI 1640 with Glutamax-I + 10% FBS (37°C, 5% CO₂) for two days, before analysis. To generate eAPC-pa lines, eAPC-p were further electroporated with a cassette containing the antigen ORFs as GOI 2 (SEQ ID NO: 5-6, 149 and 179).

eAPC-pa cell lines were generated to be employed in subsequent cell : cell co-culture assays (also interchangeably referred to as cell : cell contact assays or responder assays

throughout this specification). eAPC-pa lines produced were designed to express either the full length KRAS G12V peptide (G12V_FL) or the wild type full length KRAS peptide (KRAS_FL) or a shortened version of the KRAS G12V peptide referred to as the Minigene (G12V_MG). The MG contains candidate epitope +/- up to 9 amino acids of flanking sequence. More specifically, MG expressing cell lines were generated by using different constructs depending on whether or not Ubiquitin fusion degradation substrates (Ub-UFD (MG-UFD) or none (MG-native)) were present.

eAPC-pa cell lines were also used to generate the Epitope Mutagenesis Library wherein each eAPC-pa cell line expresses a mutant variant of a target peptide antigen (Example 5), or for producing the Cross-Reactive Library (Example 5) where each cell line expresses a putative cross-reactive antigen determined by the TCRs unique fingerprinting.

Electroporation conditions

For each reaction, 4x10⁶ cells were electroporated in 500 ml RPMI 1640 with GlutaMAX™ using the Gene Pulser Xcell™ (Bio-Rad) with the following setting: Square Wave 285V, pulse length 12.5 ms and 2 pulses with 1s interval. The cells were electroporated with vectors according to Table 1. For eAPC-p, the DNA concentrations used for the gene of interest (GOI- HLA) encoding vector (SEQ ID NO: 183) was 57mg, 13mg for the Flp vector (CMVpro_FLPo_Sv40pA_V2) and 5mg for the reporter gene vector (pcDNA 3.1 turbo/pMax GFP). For eAPC-pa, eAPC-p lines were additionally electroporated with 57mg of a second GOI containing vector (SEQ ID NO: 1-6 and 141). Following electroporation, the cells were transferred to culture vessels and grown in RPMI 1640 with GlutaMAX™+10% FBS (catalogue number 10500064, Gibco) and 37°C, 5% CO₂.

Table 1. Vectors used for DNA delivery by electroporation.

Vector	Description
F14-GOI-1-F15 (SEQ ID NO:181)	RMCE site 1
FRT-GOI-2-F3 (SEQ ID NO:182)	RMCE site 2
FRT-GOI-2-F3 (SEQ ID NO:183)	Vector carrying the HLA ORF of the genetic sequence to be integrated
FRT-GOI-2-F3 (SEQ ID NO: 5-6, 149 and 179)	Vector carrying the KRAS ORF of the genetic sequence to be integrated
CMVpro_FLPo_Sv40pA_V2	Flp vector encoding an optimized FLIP recombinase
Described in WO2018-083316	

pcDNA 3.1 turbo/pMax GFP Described in WO2018-083316	Vector encoding a reporter gene used to assess DNA delivery efficiency and as a selection marker of integration
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Sorting of polyclonal GFP-expressing cells

Cells electroporated with the GOI or the pcDNA 3.1 turbo/pMax GFP were sorted for transient GFP expression. Cells were harvested (3-4 M cells per electroporation), centrifuged at 200 x g for 5 min, and the supernatant removed. The cell pellet was washed once with DPBS 1X (Gibco) and resuspended in DPBS 1X +2%FBS (Gibco) before sorting in RPMI 1640 with Glutamax-I + 20% FBS +1% Antibiotic Antimycotic solution (cat. number 15240-062, Gibco) in Influx™ instrument (BD Biosciences). Cells positive for the reporter gene (GFP⁺) were sorted as a polyclonal population into 5 mL polypropylene round-bottom tubes FACS tubes containing culture medium. Following sorting, the cell suspension was centrifuged at 200 x g for 5 min, and the supernatant removed. Depending on the number of sorted cells, cells were transferred into a suitable vessel containing appropriate volume of culture medium (RPMI 1640 medium with Glutamax-I +10% FBS (Gibco)), and grown at 37°C, 5% CO₂.

Single cell sorting based on stable GOI integration

To obtain a population of cells constitutively expressing the integrated protein or marker, cells were sorted 9 to 11 days after the first GFP⁺ selection. At that stage, the cells that have successfully integrated the GOI become negative for the pre-existing gene cassettes (BFP⁻ or c-Myc⁻ or RFP⁻), and positive for the integrating cassette (SBP⁺ and GOI (HLA)). Next, about 0.5-2 million ARH-77 cells were centrifuged at 200 x g for 5 min, washed once with cold staining buffer (SB) consisting of DPBS 1X + 2%FBS (Gibco) and the supernatant was removed. The cell pellet was resuspended in staining solution (50 ml for 0.5-2 Million cells) containing the conjugated antibodies (summarised in Tables 6 and 8) in DPBS 1X+2% FBS and the samples were incubated in the dark for 30-45 min at 4°C. Cells were then washed with SB, resuspended in 300-600 ml of SB (depending on the cell number) and transferred to a clean FACS tube. Single cell sorting was achieved through standard cell sorting methodologies using the Influx™ instrument (BD Biosciences). For the sorting of single cells for monoclonal population generation, single cells displaying the phenotype of interest were deposited per well into 96-well plates, containing pre-warmed 200 ml of RPMI 1640 medium with Glutamax-I +20% FBS+1% Antibiotic Antimycotic solution and the cells were grown in the incubator at 37°C, 5% CO₂. Two weeks after sorting, 50 ml of RPMI 1640 medium with Glutamax-I +10% FBS was added to each well. Monoclonal cell populations were used after 3 weeks.

Single cell sorting of eAPC-p

In the eAPC-p (eAPC-HLA) expression of the analyte HLA (GOI) is detected through cell surface HLA staining. Depending on the availability of antibodies, a pan-HLA (i.e., HLA-ABC) or an allele specific antibody (i.e., HLA-A*11:01) can be used (Table 2).

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Table 2. Standard antibodies for eAPC-p staining for monoclonal sorting

HLA Class	Antibody	Cat number	Provider	Antibody amount (mg)	Total staining Volume (ml)
I	Anti-HLA-ABC Alexa-APC-Cy7	311425	BioLegend	0.6	50
Staining buffer (SB) = DPBS1X+2%FBS					

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From eAPC-p, eAPC-pa (eAPC-HLA-analyte antigenic molecule (aAM)) populations can be derived using discrete (a single ORF delivered into an eAPC-p) or pool (mix of several ORF delivered into an eAPC-p population) processes. Cell populations are stained with the antibody panel shown below (Table 3).

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Table 3. Standard eAPC-pa staining for single cell sorting

Antibody	Cat number	Provider	For up to 3M cells	
			Antibody amount	Total staining Volume
Anti-SBP-Alexa-647	sc-101595 AF647	SantaCruz Biotech	0.6 mg	50ml
Anti-c-Myc-Alexa-488	sc-40 AF488	SantaCruz Biotech	0.6 mg	
Staining buffer (SB) = DPBS1X+2%FBS				

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Individual eAPC-pa were selected, and single cell sorted (monoclones) based on loss of signal of the pre-existing gene cassette (c-Myc and red fluorescent proten (RFP) (c-Myc/RFP⁺)) and gain of signal of the selection marker of integration Streptavidin-Binding Peptide SBP (SBP⁺).

Phenotypic screening of monoclonal cell lines

A sample of 50,000 cells of the outgrown monoclonal population were harvested, centrifuged at 200 x g for 5 min. The cell pellet was washed once with cold staining buffer (SB) consisting of DPBS 1X + 2%FBS (Gibco), centrifuged at 200 x g for 5 min, and the supernatant removed. Cell pellets were resuspended in appropriate volume of staining solution (25 ml for 30-100,000 cells) containing the conjugated antibodies in DPBS 1X+2% FBS and incubated in the dark for 30-45 min at 4°C. Cells were washed with cold SB and resuspended in 15 ml of SB and analysed by flow cytometry using the iQue Cell Analyzer (Sartorius).

eAPC-p

The individual eAPC-p monoclonal populations were screened for the expression of the target HLA. Monoclonal populations were stained with a anti-HLA conjugated antibody and analysed by flow cytometry. Depending on the aAPX (HLA) to be analysed and the instrument used, a panel of antibodies conjugated to different fluorochromes targeting different epitopes on the GOI (i.e., HLA Class I) is used (Table 4).

Table 4. Antibodies for phenotypic characterisation of eAPC-p monoclonal population. Staining buffer (SB) = DPBS 1X+2%FBS

HLA Class	Antibody	Cat number	Provider	Antibody amount (mg)	Total staining Volume (ml)
I	Anti-Human HLA-ABC PE-Cy7	561349	BD Biosciences	0.1	25
	Anti-Human HLA-ABC PE-Cy5	555554	BD Biosciences	0.025	25
	anti-Human HLA-ABC V450	561346	BD Biosciences	0.001	25
	anti-Human HLA-C PE	566372	BD Biosciences	0.1	25
	anti-Human HLA-A2 PE-Cy7	561347	BD Biosciences	0.05	25

	anti-Human HLA-A2 BV786	741008	BD Biosciences	0.001	25
	Anti-c-Myc- Alexa-488	sc-40 AF488	SantaCruz Biotech	0.6 ug	25
	anti-c-Myc- Alexa-405	sc-40 AF405	SantaCruz Biotech	0.1	25
	anti-SBP tag- Alexa-488	sc-101595 AF488	SantaCruz Biotech	0.1	25

Monoclonal cell lines with an HLA⁺ fluoresce signal compared to the HLA^{null} eAPC cell line control demonstrate integration of the GOI and thus expression of their analyte aAPX (i.e., HLA-A*11:01), and are therefore validated as eAPC-p cell lines.

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eAPC-pa

The phenotypic characterisation of the eAPC-pa cells is based on the loss of fluorescence of the selection marker(s) in the receiver cassette (c-Myc/RFP⁺), and gain of the selection marker(s) of integration of the donor cassette (SBP⁺) determined by antibody staining.

- 10 Eligible monoclones must retain their HLA allele expression. The screening is performed by flow cytometry as described above. The following antibodies targeting different HLA alleles or analyte antigenic molecules, or antibodies conjugated to different fluorochromes can be used (Table 5).

15 **Table 5. Antibody staining panel for phenotypic characterisation of eAPC-pa**

Antibody	Cat number	Provider	For up to 100 000 cells	
			Antibody amount (mg)	Total staining Volume (ml)
anti-SBP tag-Alexa-488	sc-101595 AF488	SantaCruz Biotech	0.1	25
Anti-c-Myc-Alexa-405	sc-40 AF405	SantaCruz Biotech	0.1	25
Anti-Human HLA-ABC PE-Cy5	561349	BD Biosciences	0.1	25
Staining buffer (SB) = DPBS 1X+2%FBS				

eAPC-pa monoclonal populations that show a strong fluorescent signal from the donor cassette, and a loss of fluorescent signal from the receiving cassette, compared to controls, demonstrate expression of analyte aAM selection marker and therefore inferred

aAM expression. Co-expression of aAPX (i.e., HLA) and the aAM selection marker validates eAPC-pa lines.

Plots of c-Myc vs RFP showing loss of c-Myc/RFP signal in monoclonal population(s) in comparison to the parental eAPC-p, and plots of SBP vs HLA showing gain of the SBP cassette (while retaining the HLA signal), indicating a successful integration event.

To confirm the identity of the aAM-ORF encoded in eAPC-pa monoclonal cell lines, such cell lines were subjected to genetic characterisation by sequencing the Barcode encoded within the 3' UTR of the aAM-ORF.

10 **Genotypic analysis of monoclonal cell lines**

Genomic DNA was extracted using the QIAamp® DNA Mini kit (cat num 51306, Qiagen), Quick-DNA 96 Plus kit (cat number D4070 D4071, Zymo Research) according to the manufacturer's instructions, and PCR was conducted using primers that target adjacent and internal regions of the HLA ORF or aAM-ORF, thereby selectively amplifying successful integration couple events. Comparison was made to an unmodified parental line, wherein the HLA or aAM-ORF is lacking.

PCR reactions were done using either purified genomic DNA (gDNA) or crude cell lysates. For crude cell lysates, 70,000-150,000 ARH-77 derived cells were harvested, pelleted and washed with of 1X DPBS. Cell pellets were resuspended in 20ml of low EDTA TE buffer, the cells were lysed by heating for 10min at 98°C, lysates are centrifuged at >800 x g for 5min and the PCR reaction master mix was prepared according to Table 6.

Table 6. PCR reagents

Reagent	Per reaction (ml)	Cat Number	Provider
5X Phusion HF buffer	6	F-549L	Thermo Fisher Scientific
dNTPs	0.3	10083252	Thermo Fisher Scientific
Phusion Hot Star II DNA polymerase (2U/ml)	0.3	F-549L	Thermo Fisher Scientific
Forward primer (100mM stock)	0.15	-	
Reverse primer (100mM stock)	0.15	-	
H ₂ O	21.1 (22.1)*	129114, 129117	Qiagen
Cell sample	2 (1)*	-	-

*Volumes to add when using purified gDNA.

PCR products were run on a 1% or 2% Agarose gel beside a 50 bp or 1 kb ladder, in 1X TAE buffer, at 120 V for 25-30 min. DNA was stained with 10.000 dilution of SYBR safe DNA gel stain (cat num S33102, Invitrogen Life Technologies) and gel image captured using the Fusion SL UV-camera (Vilber).

HLA amplification

To validate the HLA expression, PCR amplicons were generated with two set of primers that primed on the AAVS1 genomic sequence distal to the region encoded by the homologous arms (Table 7) and using the PCR conditions described in Table 8..

Table 1. PCR and sequencing primers for HLA Class I Barcode PCR

Region	Primer	Sequence	Expected Product size	Sequencing Primer
Barcode	Forward	CATCATCACCATCACCCTGAC	196 bp	CAC CACTGACTATAGTCA
	Reverse	GAATCCTGACTCCTCGACTAG		

Table 8. PCR cycler program for HLA Class I

Step	Temperature	Duration	Number of cycles
1. Pre-heating	98°C	Hold	1
2. Initial Denaturation	98°C	30 sec	1
3. Denaturation	98°C	10 sec	1
4. Annealing	64°C	20 sec	
5. Extension	72°C	20 sec	
6. Go to step 3	-	-	34x (total 35 cycles)
7. Final extension	72°C	5 min	1
8. Cooling	12°C	Hold	1

aAM-ORF amplification

To validate integration of the aAM-ORF in the engineered monoclones, PCR amplification of the aAM-ORF region utilising primers targeting the ORF-Full length Barcode or the ORF-Minigene UGI was applied (Table 9).

The ORF-Minigenes can carry different proteasome degradation signals (none (native) or

Ub-UFD), which will dictate the set of primer to use (Table 9). The PCR amplification conditions for the FL ORF are summarised in Table 10 and for the MGs in Table 11.

Table 9. PCR and sequencing primers for ORF-Full length Barcode, and ORF-Minigene

5 Barcode

Region	ORF	Tag	Primer	Sequence	Product size (bp)	Sequencing Primer
Barcode	Full length	N/A	Fwd	ATCTGGACGGCT TCGACTT	165	GGCTTCGACTTC TGTAGC
		N/A	Rev	ATCCGTATGGTG ACAAGACG		
Barcode	Mini-gene	None	Fwd	CATTTTCAGGTGT CGTGAAGC	450	GGAGCTACAGAA GTCGAAGC
			Rev	ATCCGTATGGTG ACAAGACG		
		UbUFD	Fwd	TCGTCAAGACTC TGACTGGT	400	GTAAGACCATCA CCCTG
			Rev	GGAGCTACAGAA GTCGAAG		

Table 10. PCR cycle conditions for ORF-full length (FL) Barcode amplification

Step	Temperature	Duration	Number of cycles
1. Pre-heating	98°C	Hold	1
2. Initial Denaturation	98°C	30 sec	1
3. Denaturation	98°C	10 sec	1
4. Annealing	64°C	20 sec	
5. Extension	72°C	20 sec	
6. Go to step 3	-	-	34x (total 35 cycles)
7. Final extension	72°C	5 min	1
8. Cooling	12°C	Hold	1

10 Table 11. PCR cycle conditions for ORF-Minigene UGI amplification

Step	Temperature	Duration	Number of cycles
1. Pre-heating	98°C	Hold	1
2. Initial Denaturation	98°C	30 sec	1

3. Denaturation	98°C	10 sec	1
4. Annealing	64°C	20 sec	
5. Extension	72°C	40 sec	
6. Go to step 3	-	-	34x (total 35 cycles)
7. Final extension	72°C	5 min	1
8. Cooling	12°C	Hold	1

Sequencing of the aAM-ORF and HLA amplicons is performed by a third party (Eurofins Genomics). Raw .ab1 files are downloaded and analysed using the Geneious Prime analysis software (2022.1.1, Java Version 11.0.14.1+1 (64 bit)). Sequence reads are annotated and inspected for parameters such as the Sequence %HQ, UGI Mean PHRED, UGI ambiguities and UGI mutations.

Engineering eTPC-t cells presenting a full-length functional TCRαβ using bi-directional DNA delivery vectors.

Electroporation of Jurkat, Clone E6-1

For each reaction, 5x10⁶ cells were electroporated in 500 ml CTS Opti-MEM I Reduced Serum Medium (Thermo Fisher Scientific) using the Gene Pulser Xcell™ (Bio-Rad) with the following setting: Square Wave 265V, pulse length 15 ms and 2 pulses with 1s interval. For integration of the bidirectional donor vectors encoding the TRA and TRB ORFs cells were electroporated with 10ug of the vector encoding the Flp recombinase enzyme (CMVpro-Flp-sv40pA-V2) and 55 mg of the integrating vectors (i.e., TCR encoding vectors). After electroporation, cells were incubated and further expanded in culture medium RPMI 1640 with Glutamax-I (Life Technologies) + 10% FBS (37°C, 5% CO₂) for 7 days prior to the T cell enrichment.

Magnetic Cell Sorting of eTPC-t

Isolation of cells expressing the desired TCR was achieved by following a single round of Miltenyi TCR- Indirect-positive selection and using the MultiMACS Cell24 Separator Plus (Miltenyi Biotec) for simultaneous multisample separation. Engineered T cells were labeled with phycoerythrin (PE)-conjugated anti-TCRαβ antibody (Thermo Fisher Scientific, Cat.Number: 12-9986-42, Working Dilution:1/10) and incubated for 10 min. Cells were washed twice with pre-cooled stain buffer (Dulbecco's phosphate-buffered saline (DPBS) with 2% Foetal bovine serum (FBS)) and resuspended in stain buffer with anti-PE MicroBeads (Miltenyi Biotec, Cat.No:130-048-801, Working Dilution:1/4) according to the manufacturer's instructions and incubated for 15min. All incubations were performed at 4 °C and protected from light. To quantify the integration rate of the bidirectional TCR donor

construct pre-enriched cells, flow through (negative fraction) and positive fraction were analyzed by flow cytometry using the iQue Screener Plus. Engineered T cells (eTPC-t) were cultured and out-grown for 7-10 days and were subsequently subjected to an additional phenotypic analysis prior to cryopreservation.

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Phenotypic analysis by Flow cytometry

For phenotypic analysis cell polyclones were assessed for TCR⁺/CD3⁺ expression and the loss of the pre-existing selection markers (BFP⁺/SBP⁺). The anti-TCRαβ antibody conjugated with PE (Thermo Fisher Scientific, Cat.No: 12-9955-41, 12-9955-42, Working Dilution: 1/25), the anti-CD3 antibody conjugated with BV650 (BD Biosciences, Cat. Number: 564003, 563999, Working Dilution: 1/50), and the anti-SBP antibody conjugated with AF488 (Santa Cruz Biotech, Cat.No: sc-101595 AF488, Working Dilution: 1/50) comprised the antibody panel used for the analysis. Cells were incubated at 4°C, for 20 min, washed twice with pre-cooled stain buffer DPBS containing 2% Foetal bovine serum (FBS) and subsequently resuspended in stain buffer to be analyzed using the iQue Screener Plus instrument. Pure populations of engineered T cells (eTPC-t) were cryopreserved, and cell aliquots were obtained for the mycoplasma test and gDNA sequencing to verify the integration of TRA/TRB at the right genomic locus.

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Genetic characterization by genomic DNA sequencing

Primers used to assess integration of the TRA-ORF, annealed to the TRA-C segment (Reverse primer SEQ ID NO: 163) and the EF1a promoter (Forward primer SEQ ID NO: 162) that is a pre- existing part of the genomic receiving sites. Primers used to assess integration of the TRB-ORF, annealed to the TRB-C segment (Reverse primer SEQ ID NO: 165) and the EF1a promoter (Forward primer SEQ ID NO: 162) that is a pre- existing part of the genomic receiving sites. PCR products were run on a 1% Agarose gel in 1XTAE buffer, using the PowerPac Basic (Bio-Rad), stained with 10,000 dilution of SYBR Safe and analyzed with Fusion SL (Vilber Lourmat).

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Amplicon sequencing primers, (SEQ ID NO: 164) and (SEQ ID NO: 166) were used to amplify TRA-C and TRB-C segments to genotypically confirm the TRA/ TRB integrated ORFs.

Table 12. PCR reaction for amplification of TRA or TRB.

PCR mix	µl per reaction
5xPhusion HF buffer	

dNTPs (20mM)	0.25
Phusion HSII	0.25
Fwd 100uM	0.15
Rev 100mM	0.15
H2O	18.2
Cell sample / DNA	1

Table 13. PCR cycling reactions for amplification of TRA or TRB.

PCR conditions common for TRA/TRB			
Initial Denaturation	98°C	30sec	1 cycle
Denaturation	98°C	10sec	35 cycles
Annealing	69°C	20sec	
Extension	72°C	15sec	
Final Extension	72°C	5min	1 cycle
Hold	12°C	hold	

5 *Engineering of luciferase expressing eAPC-pa lines using RMCE*

eAPC target cell lines expressing HLA-A*11:01 and KRAS G12V we generated using RMCE as described in earlier section. RMCE was further used to introduce Firefly luciferase into the eAPC line expressing HLA-A*11:01.

10 *Engineering of luciferase expressing eAPC-pa lines using directed endonuclease*

A polycistronic construct expressing EF-1 alpha promotor-driven Firefly Luciferase and green fluorescent protein (GFP) was used as homology-directed repair (HDR) template to introduce these markers into eAPC lines expressing HLA-A*11:01 and KRAS by directed endonuclease-mediated knock-in into the CCR5 locus. Firefly Luciferase and green

15 fluorescent protein (GFP) was separated by a P2A cleavage site in the HDR construct.

For nucleofection, 2 million cells were prepared in 20 µl Lonza SF buffer. Pre-incubated RNP and DNA mixture was added to cells and mixed gently. This mixture was then transferred into 16-well Nucleocuvette Strips (Lonza Bioscience) and pulsed with the code EN-138. For every 2 million cells, 40pmol RNP and 2ug plasmid DNA were used. After

20 nucleofection, pre-warmed RPMI with 10% FBS was gently added to the cuvette and the cuvette was placed in a 37°C tissue culture incubator for 15 min for cell recovery.

Monoclones of the engineered lines were generated by fluorescence activated cell sorting (FACS) using GFP as the selection marker.

*Engineering of HLA-A*11:01 and Luciferase expressing cancer cell lines*

5 VSV-G pseudotyped third-generation lentivirus purchased from Vectorbuilder was used to introduce HLA-A*11:01 ORF into commonly used cancer cell lines expressing the G12V mutant KRAS or wild type KRAS. The lentiviral vector additionally contained Firefly Luciferase and GFP ORFs with the three ORFs separated by T2A and P2A self-cleavage sites and expression of all driven by the EF-1 alpha promoter.

10 50,000 cells per transduction were seeded the day before transduction. 1,000,000-5,000,000 p24 transduction units of virus-like particles (VLPs) were added per transduction followed by LentiBoost (Sirion Biotech) at a 100 fold dilution. Cells were incubated for 10 min at RT and spinoculated at 30 min and 800g at 32°C. Following
15 spinoculation cells were placed in a tissue culture incubator. On the next day media was exchanged. Seven days after transduction, purity of cell lines was measured and the cells were sorted to monoclones or polyclones with a purity of >90% using FACS and GFP expression.

20 Table 14. Summary of mammalian cell lines used through out the specification

Unmodified cell line	Source	Specification
COR-L23	ECACC	92031919
SW620	ATCC	CCL-227
IGR-1	DSMZ	ACC 236
NCI-H441	ATCC	HTB-174
QGP-1	JCRB	JCRB0183
HEK 293	DSMZ	ACC305
ARH-77	DSMZ	ACC512
Jurkat clone E6.1	Merck	88042803-1VL

Primary Cells

Collection of healthy donor peripheral blood and leukapheresis products were undertaken with written consent, granted by the Swedish Ethical Review Authority. Peripheral blood
25 mononuclear cells and primary human CD8⁺ T cells were isolated from apheresis product

from healthy donors using CliniMACS CD8⁺ reagent according to the manufacturer's instructions (Cat. Number 170-076-703, Miltenyi Biotec).

CD8⁺ T cell activation and nucleofection

- Cryopreserved CD8⁺ T cells were recovered for 24 hours in RPMI-1640 medium with 10% FBS before activation. For activation, CD8⁺ T cells were plated at an initial concentration of 1 million cells/mL in AIM V (Cat. Number 11534536, Thermo Fisher Scientific) medium in presence of 5% serum replacement (SR) (Cat. Number A2596102, Thermo Fisher Scientific) and 100U/ml IL-2 (Cat. Number 130-097-748, Miltenyi Biotec). T Cell TransAct (Cat. Number 130-111-160, Miltenyi Biotec) was added to the culture at a 1:100 dilution.
- After 72 hour stimulation, activated CD8⁺ T cells were centrifuged and gently resuspended in P3 buffer with supplement, as part of the P3 Primary Cell 4D-Nucleofector™ X Kit (Cat. Number V4XP-3032, Lonza Bioscience) at 2 million cells per 20 µl. 30 pmol ribonucleoprotein (RNP) and 2 mg plasmid deoxynucleic acid (DNA) were pre-incubated and added into the suspension. This mixture was then transferred into 4D-Nucleofector cuvette (Cat. Number V4XP-3032, Lonza Bioscience) and pulsed with code EH115. After nucleofection, pre-warmed AIM V medium with 5% SR and 100U/ml IL-2 was gently added to the cuvette and the cuvette was placed in a 37°C tissue culture incubator for 15 min to allow for cell recovery. After recovery, the cells were transferred to prewarmed AIM V medium with 5%SR, 100U/ml IL-2 and 2 mM HDR enhancer. After overnight incubation, nucleofected cells were collected by centrifugation and replated in fresh AIM V medium with 5%SR, 100U/ml IL-2, without HDR enhancer to allow expansion. 7 days after nucleofection, CD34⁺ cells were enriched using CD34 MicroBead Kit according to the manufacturer's instructions (Cat. Number 130-046-702, Miltenyi Biotec).

25 Identification of G12V KRAS peptides via mass spectrometry

Metal affinity chromatography

- ARH-77 cells were cultured in RPMI supplemented with 10% FBS at 37 °C and 5% CO₂. Harvested cells were washed once with ice-cold PBS and lysed in ice-cold lysis buffer (150 mM Sodium chloride (NaCl), 1x PBS, 1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 5 mM Imidazole, 0,2 mM iodoacetamide, 5% glycerol, and 1x Halt protease inhibitor cocktail (Thermo Scientific)), vortexed and incubated at 4 °C for 20 minutes. Cleared lysate was mixed with HisPur™ nickel–nitriloacetic acid (Ni-NTA) Resin (Thermo Scientific) and rotated for 2 hours at 4 °C. After removal of the lysate unbound fraction, the resin was washed twice with high salt buffer (250 mM NaCl, 1x PBS, 25 mM Imidazole) and twice with low salt buffer (50 mM NaCl, 1x PBS). Washed beads were harvested in low salt wash buffer and transferred to spin columns (Thermo

Scientific). The bound fraction was eluted in 15% Acetonitrile and 0.2% TFA and ultrafiltered over 3kD Ultra Centrifugal Filter (Amicon). The peptide fraction was reduced and alkylated in a one-step reaction with 10 mM Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 50 mM chloro-acetamide and in 100mM Triethylammonium bicarbonate buffer (TEAB) shaking for 30 minutes at 37°C. The sample was then immediately re-acidified in 5% FA and subjected to solid phase extraction on Hypersep Retain-CX columns (Thermo). The sorbent was activated with methanol (MeOH) and equilibrated with 0.2% TFA prior to sample loading. The column was later washed with MeOH and then water prior to elution of the absorbed peptide fraction in elution buffer (MeOH, 5% NH₄OH). Samples were transferred to HPLC-glass vials, vacuum-dried and stored at -20 °C prior to LC-MS/MS analysis.

Mass spectrometry

Peptides were re-suspended in 10 mL Solvent (10% formic acid (FA), Milli-Q) prior LC-MS/MS analysis. Each sample was analyzed on a timsTOF Pro2 (Bruker, United States) connected to a nanoElute system (Bruker, United States), injecting 2 mL from each sample vial. The nanoElute system was equipped with an UHPLC analytical column (Aurora Ultimate nanoflow with CSI fitting; 25 cm x 75 mm ID, 1.7 mm C18; IonOpticks) heated to 50 °C, and connected to a CaptiveSpray (Bruker, United States) ion source. Mobile phase buffers consisted of Solvent A (2% ACN, 0.1% FA, Milli-Q) and Solvent B (ACN, 0.1% FA). The peptides were eluted during a 55 min gradient with a flow rate set to 0.250 mL/min. The gradient started at 2% of solvent B, raised to 4% within 2 min, to 18% within 33 min, to 26% within 7 min, to 36% within 3 min, to 95% within 1 min, and maintained at 95% for 9 minutes. The nano spray voltage was set to 1.5 kV, with dry gas flow set to 3 L/min and 180 °C.

The mass spectrometer acquisition was configured to operate in a data dependent acquisition (DDA) mode, using trapped ion mobility spectrometry (TIMS) with the scan mode set to Parallel Accumulation–Serial Fragmentation (PASEF), high sensitivity detection and positive mode. The precursor scan range was set between m/z 100 and 1700. The TIMS 1/K₀ values ranged from 0.7 and 1.42, with the ramp and accumulation times of 166 ms, and a duty cycle of 100%. The PASEF precursor region polygon left boundary was set at m/z 180 for low 1/K₀ and m/z 1300 at max 1/K₀, and the right boundary at m/z 1700 for both high and low 1/K₀ (0.7 and 1.42). The quadrupole energy was set to 5.0 eV and low mass m/z 200. The collision cell energy was set to 10 eV and collision RF to 1500.0 Vpp.

Data analysis

The MS acquisition data files were processed using PEAKS Online X (Bioinformatics Solutions Inc., Canada) using database search, De Novo sequencing, post-translational modifications (PTM) search, and SPIDER algorithms. The library search was performed against a composite FASTA file including standard Human proteome library sequences and short sequences corresponding to SNP and indel events known to be present in ARH-77 and supplemented with the KRAS mutations included in the experiment; a FASTA file with a list of common LC-MS/MS contaminants was included in the search. The precursor and fragment mass error tolerances were set to 15 ppm and 0.05 Da, respectively, and the peptide lengths ranged between 7 and 25 amino acids. The De Novo search parameters were set to be the same as the library search with the Average Local Confidence (ALC) set to 50%. The PTM search was set to detect all built-in modifications, and with De Novo ALC set to 15%. The SPIDER algorithm was employed to find mutations and sequence variants. The peptide false discovery rate (FDR) was set to 1% and the proteins $-10\log P \geq 20$ and proteins unique peptides ≥ 1 . The results from PEAKS Online X search were further analyzed using Bruker-provided and proprietary software (Python scripts) implementing the clustering methods of Andreatta et al (2017) so as to confirm concordance to expected pHLA repertoire characteristics to assess the quality of the immunopeptides identified.

20 TCR Discovery

Naïve CD8⁺ T cell enrichment

Cryopreserved PBMCs from healthy HLA-A*11:01 donors were thawed and rested overnight at 10 Million cells/ mL in RPMI1640 with 2mM L-glutamine and 10%FBS. The next day, PBMCs were counted using the NucleoCounter NC-200 (ChemoMetec) and enriched using the EasySep™ Mouse Naïve CD8⁺ T Cell Isolation Kit (StemCell) following the manufacturer's instructions. Purity of the CD8 naïve T cells was validated by flow cytometry using surface antibody staining against CD45R0, CD27, CD95, CD8 and CD4 and analysis on the cell analyzer BD LSRFortessa.

30 *Selective expansion of target-specific TCRs*

Enriched naïve CD8 T cells were co-cultured with engineered antigen presenting cells (eAPCs) constitutively expressing the HLA-A*11:01 at a ratio of 20:1 (CD8⁺ T cells: eAPC). In a round bottom 96 wells plate, 5000 naïve CD8⁺ T cells were co-cultured with 250 eAPCs and 1mM KRAS G12V-10-mer peptide in 100mL of OpTmizer CTS supplemented with 2mM GlutaMax and 10% human serum for 7 days. 1 day and 7 day after the seeding, 50mL of interleukin 2 (IL-2) at 100 U/mL and 25 U/mL respectively was added to support expansion of specifically activated T cells by the KRAS G12V-10-mer peptide.

Harvesting and sorting of KRAS G12V specific T cells

- 14 days after expansion, cells were harvested and counted using the NucleoCounter NC-200. Cells were stained with A*11:01 G12V KRAS MHC tetramer conjugated with
- 5 phycoerythrin (Cat Number WD04069 PE 50, Immudex) and A*11:01 WT KRAS MHC conjugated with APC (allophycocyanin) tetramer (Cat Number WD05135 AP 50, Immudex) for 30min at room temperature (RT). A*11:01 G12V MHC tetramer-stained CD8⁺ T cells were magnetically enriched using anti-phycoerythrin (PE) Microbeads (Miltenyi) following the manufacturer's instructions. The enriched fraction was stained for
- 10 extracellular markers against cMyc, SBP and CD8 to distinguish eAPC from CD8⁺ T cells and CD8⁺ A*11:01 G12V KRAS MHC-PE⁺ and A*11:01 WT KRAS MHC- cells were single cell sorted using FACS sorting.

TCR selection

- 15 The identified unique TCRs from each human donor were extracted and counted. The absolute count and frequency of one TCR pair relative to the total number of pairs identified per donor was determined. A total count of more than 3 observations was considered to be an expanded clonotype.

20 *Peptide Pulse assay*

eTPC-t and eAPC cells harvesting

- An aliquot of actively growing cultures of eTPC-t cells and eAPC cells ($0.4-1.0 \times 10^6$ cells/mL) was taken for cell counting using CountBright Absolute Counting Beads (ThermoFisher) read on an iQue Screener Plus (Sartorius). The desired number of cells
- 25 were harvested, washed once with complete media and resuspended at a concentration of 0.7M/mL in complete media (RPMI 1640 with L-glutamin and 10%FBS).

Contacting eTPC-t and eAPC in an eTPC:eAPC system with exogenous antigenic molecules

- 30 Co-cultured assay were performed in round bottoms 96 wells plate at a 1:2 eTPC to eAPC ratio. 100uL of eAPC (70,000 cells) were seeded together with 50uL of eTPC-t (35,000) and 50uL of peptide was added. Peptide concentration used ranged from 0 to 10 mM final. Cells were co-cultured for 18h at 37C before functional reading by flow cytometry.

35 **Plasmid production**

Vector library assembly and cloning

The construction of vectors described in the examples comprises a variety of methods well known to those skilled in the art, and specific reaction compositions are outlined in

detail in WO2019/016175. The following key materials in table 15 were used in the described procedures:

Table 15:

Product	Supplier	Supplier Number
Acc651	New England BioLabs	R0599L
BbsI HF	New England BioLabs	R3539L
DH5alpha competent cells	Thermo Fisher Scientific	18265017
DNA clean and concentrator kit	Zymo Research	D4030
EcoRI	New England BioLabs	R3101S
NotI	New England BioLabs	R3189L
QIAamp DNA Mini kit	Qiagen	51306
QIAquick Gel Extraction kit	Qiagen	28704
Qiagen Plasmid Plus Midi kit	Qiagen	12945
T4 ligase	New England BioLabs	M0202L
T4 ligase buffer 10x	New England BioLabs	B0202S
XbaI	New England BioLabs	R0145S
XhoI	New England BioLabs	R0146S

5

Oligonucleotide duplex encoding CDR3 (odeCDR3) assembly

odeCDR3 were routinely assembled by annealing partially complementary single stranded oligonucleotides. A detailed description of reaction composition and conditions is provided in WO2019/016175. The following key materials in table 16 were used in the described

10 procedures:

Table 16

Product	Supplier	Supplier Number
T4 ligase buffer 10 x	New England BioLabs	B0202S
T4 PNK	New England BioLabs	M0201L

TCR reconstitution

Operation of a TORES2 to reconstitute full-length TCR ORFs is described in detail in WO2019/016175. The following key materials in table 17 were used in the described

15 procedures:

Table 17:

Product	Supplier	Supplier Number
Bsal-HF	New England BioLabs	R3535L
rCutSmart buffer 10 x	New England BioLabs	B6004S
DH5alpha competent cells	Thermo Fisher Scientific	18265017
NotI-HF	New England BioLabs	R3189L
QIAamp DNA Mini kit	Qiagen	51306
T4 Ligase	New England BioLabs	M0202L
T4 Ligase buffer 10 x	New England BioLabs	B0202S

Fluorescence activated cell sorting (FACS)

Single HLA-multimer positive CD8⁺ T cells were sorted by FACS for amplification and sequencing of TCR chains. This was achieved through standard cell sorting methodologies using a *BDInflux* instrument. Briefly, cells were stained with HLA-multimer reagent on ice for 10 mins, then with CD3 and CD8 antibodies as markers of CD8⁺ T cells. Cells with CD3+CD8+Multimer+ signal were sorted to PCR plate pre-loaded with 5 µL of nuclease-free water. Specimens were snap-frozen until subsequent processing. The following key materials in table 18 were used in the described procedures:

Table 18:

Product	Supplier	Supplier Number
A*11:01 G12V KRAS MHC tetramer (PE)	Immudex	WD05135
Anti-CD8 (clone RPA-T8) (BV510)	BD	563256
Anti-CD3 (clone SK7) (APC-H7)	BD	560176

Sequencing of TCR alpha and beta chains from single T cells

Singly FACS-sorted T cells were subjected to a two-step amplification process that entails a V-region specific primer collection for each TRA and TRB, followed by paired nested PCR reactions that create TRA and TRB amplicons for sequence analysis as described in WO2019/016175. This procedure is described previously (Han et. al. 2014). The following materials in table 19 were used in the described procedures:

Table 19

Product	Supplier	Supplier Number
2x Reaction Mix	Thermo Scientific	12574035
5X Phusion HF Buffer	Thermo Fisher Scientific	F-549S
dNTPs	Thermo Fisher Scientific	10297018
Nuclease free water	Qiagen	129114
Phusion Hot Start II DNA Polymerase	Thermo Fisher Scientific	F-549S
SuperScript® III One- Step RT-PCR System with Platinum® Taq High Fidelity DNA Polymerase	Thermo Scientific	12574035

Responder assay (eAPC:eTPC co-culture assays)

- This procedure was used to evaluate and validate the specificity and sensitivity of a TCR for an antigen presented on a defined HLA background, evaluate cross-reactivity, and/or define an alloreactivity profile for specific TCRs.

Cell line preparation

- Selected analyte eAPC-pa and eTPC-t were thawed at least 4 days before their planned responder assay setup to allow cell recovery and growth. Both the eAPC-pa and eTPC-t cells were maintained independently in log-phase with cell concentrations between the 0.2-1 Million/ ml in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco) supplemented with 10%FBS (Gibco).

Assay set up and Cell Staining

- eAPC-pa and eTPC-t cells were separately counted using iQue flow cytometer (Sartorius) and plated in 2:1 ratio (70,000 eAPC-pa and 35,000 eTPC-t cells per well) in 200 µl final volume in a 96 well round-bottom plates and incubated overnight (18 hours) at 37 °C, 5% CO₂, 90 % RH. Cell analysis was conducted through cell surface marker staining and flow cytometry analysis using the iQue instrument (Sartorius) using the following antibody panel (Table 20). The antibodies were added directly into the cell suspension in the 96-well round-bottom assay plate. After overnight incubation, 25 µl of the antibody mix was directly added to the culture plates, and cells were mixed using the microplate shaker at

room temperature for 5 sec and incubated at 2-8°C for 40-60 min. Following the incubation, cells were centrifuged for 4 min at 400 x g and the supernatants were aspirated. The cell pellets were resuspended in 20 µl of 1.5X CellFix (4% Paraformaldehyde) solution and mixed at room temperature using microplate shaker 5 sec. Cells were incubated at 2-8 °C for 15 min before acquisition on the iQue cytometer.

Table 20. The antibody staining panel for the eTPC-t. Antibody mix prepared in Staining Buffer consisting of 2% FBS (Gibco) in DPBS (Thermo Fisher Scientific)

Antibody	Catalogue number	Supplier
BD Horizon™ BV786 Mouse anti-Human CD80	564159	BD Biosciences
BD Pharmigen™ Alexa Fluor 488 Mouse Anti-Human CD8	557696	BD Biosciences
BD OptiBuild™ BV510 Anti-Human CD3	740202	BD Biosciences

10 *Quality Control Measures*

eAPC-pa

The eAPC-pa were subjected to two quality control procedures: a) genotyping confirmation by direct Sanger sequencing and b) intracellular Flag staining. Genotyping was described in the cell engineering section.

Intracellular FLAG staining

To confirm ORF minigene expression, proteasomal degradation was inhibited with addition of MG-132 (Cat number 474787, Merck), according to manufacturer's instructions and followed by intracellular FLAG staining. Following proteosome inhibition, eAPC-pa were stained with 25µl of Death Cell Marker (DCM) Aqua BV-510 dye (Zombie Aqua™ Fixable Viability Kit, Cat num. 423102, BioLegend) diluted 1:100 with DPBS and incubated in dark while shaking for 20 mins at RT. Cells were washed with 250µl Stain buffer (DPBS +2%FBS), centrifuged at 400g for 4 minutes, and supernatant removed. Intracellular Flag staining was performed using the Cytotfix/Cytoperm Fixation/Permeabilization solution kit (Cat num. 554714, BD Biosciences) according to manufacturer's instructions. For cellular

- permeabilization, cell pellets were resuspended in 100µl Cytotfix/Cytoperm solution and incubated for 20 mins at 4°C. Washed twice with 250µl PermWash Buffer (diluted 1:10 with water) at 400g for 3 minutes. Supernatant was removed and cells were stained with anti-DYKDDDDK-PE (FLAG) antibody (Cat num 130-128-816, Miltenyi) for 30-60 minutes
- 5 at 4°C in the dark. Following the incubation, the cells were washed twice with 250µl PermWash Buffer at 400g for 3 minutes. Cell pellets were resuspended in 20µl of Stain Buffer (DPBS +2%FBS) or 1% PFA and incubated for 10-15 min at RT and data was acquired on the iQue instrument.

10 *eTPC-t genotyping*

The eTPC-t were subjected to quality control by genotyping confirmation using Sanger sequencing to confirm integration of the desired TCR chains. Described in detail in the Cell engineering section.

15 Cross-reactivity assay

Mutagenesis epitope libraries for fingerprinting

Mutagenesis epitope libraries for the G12V 10-mer peptide and the G12V 9-mer peptides were created by substituting all possible naturally occurring single amino acids at each position within the G12V mutated KRAS 9-mer (VVGAVGVGK) and 10-mer

- 20 (VVGAVGVGK) peptides, Table 21 and Table 22, respectively.

Table 21. Mutagenesis epitope library for the 10-mer G12V KRAS peptide.

Antigen mutation position	Mutation aa	Antigen mutation	Antigen mutation position	Mutation aa	Antigen mutation
1	A	V1A	6	A	G6A
1	C	V1C	6	C	G6C
1	D	V1D	6	D	G6D
1	E	V1E	6	E	G6E
1	F	V1F	6	F	G6F
1	G	V1G	6	H	G6H
1	H	V1H	6	I	G6I
1	I	V1I	6	K	G6K
1	K	V1K	6	L	G6L
1	L	V1L	6	M	G6M
1	M	V1M	6	N	G6N
1	N	V1N	6	P	G6P
1	P	V1P	6	Q	G6Q

1	Q	V1Q	6	R	G6R
1	R	V1R	6	S	G6S
1	S	V1S	6	T	G6T
1	T	V1T	6	V	G6V
1	W	V1W	6	W	G6W
1	Y	V1Y	6	Y	G6Y
2	A	V2A	7	A	G7A
2	C	V2C	7	C	G7C
2	D	V2D	7	D	G7D
2	E	V2E	7	E	G7E
2	F	V2F	7	F	G7F
2	G	V2G	7	H	G7H
2	H	V2H	7	I	G7I
2	I	V2I	7	K	G7K
2	K	V2K	7	L	G7L
2	L	V2L	7	M	G7M
2	M	V2M	7	N	G7N
2	N	V2N	7	P	G7P
2	P	V2P	7	Q	G7Q
2	Q	V2Q	7	R	G7R
2	R	V2R	7	S	G7S
2	S	V2S	7	T	G7T
2	T	V2T	7	V	G7V
2	W	V2W	7	W	G7W
2	Y	V2Y	7	Y	G7Y
3	A	V3A	8	A	V8A
3	C	V3C	8	C	V8C
3	D	V3D	8	D	V8D
3	E	V3E	8	E	V8E
3	F	V3F	8	F	V8F
3	G	V3G	8	G	V8G
3	H	V3H	8	H	V8H
3	I	V3I	8	I	V8I
3	K	V3K	8	K	V8K
3	L	V3L	8	L	V8L
3	M	V3M	8	M	V8M
3	N	V3N	8	N	V8N

3	P	V3P	8	P	V8P
3	Q	V3Q	8	Q	V8Q
3	R	V3R	8	R	V8R
3	S	V3S	8	S	V8S
3	T	V3T	8	T	V8T
3	W	V3W	8	W	V8W
3	Y	V3Y	8	Y	V8Y
4	A	G4A	9	A	G9A
4	C	G4C	9	C	G9C
4	D	G4D	9	D	G9D
4	E	G4E	9	E	G9E
4	F	G4F	9	F	G9F
4	H	G4H	9	H	G9H
4	I	G4I	9	I	G9I
4	K	G4K	9	K	G9K
4	L	G4L	9	L	G9L
4	M	G4M	9	M	G9M
4	N	G4N	9	N	G9N
4	P	G4P	9	P	G9P
4	Q	G4Q	9	Q	G9Q
4	R	G4R	9	R	G9R
4	S	G4S	9	S	G9S
4	T	G4T	9	T	G9T
4	V	G4V	9	V	G9V
4	W	G4W	9	W	G9W
4	Y	G4Y	9	Y	G9Y
5	C	A5C	10	A	K10A
5	D	A5D	10	C	K10C
5	E	A5E	10	D	K10D
5	F	A5F	10	E	K10E
5	G	A5G	10	F	K10F
5	H	A5H	10	G	K10G
5	I	A5I	10	H	K10H
5	K	A5K	10	I	K10I
5	L	A5L	10	L	K10L
5	M	A5M	10	M	K10M
5	N	A5N	10	N	K10N

5	P	A5P	10	P	K10P
5	Q	A5Q	10	Q	K10Q
5	R	A5R	10	R	K10R
5	S	A5S	10	S	K10S
5	T	A5T	10	T	K10T
5	V	A5V	10	V	K10V
5	W	A5W	10	W	K10W
5	Y	A5Y	10	Y	K10Y

Table 22. Mutagenesis epitope library for the 9-mer G12V KRAS peptide.

Antigen mutation position	Mutation aa	Antigen mutation	Antigen mutation position	Mutation aa	Antigen mutation
1	A	V1A	6	A	G6A
1	C	V1C	6	C	G6C
1	D	V1D	6	D	G6D
1	E	V1E	6	E	G6E
1	F	V1F	6	F	G6F
1	G	V1G	6	H	G6H
1	H	V1H	6	I	G6I
1	I	V1I	6	K	G6K
1	K	V1K	6	L	G6L
1	L	V1L	6	M	G6M
1	M	V1M	6	N	G6N
1	N	V1N	6	P	G6P
1	P	V1P	6	Q	G6Q
1	Q	V1Q	6	R	G6R
1	R	V1R	6	S	G6S
1	S	V1S	6	T	G6T
1	T	V1T	6	V	G6V
1	W	V1W	6	W	G6W
1	Y	V1Y	6	Y	G6Y
2	A	V2A	7	A	V7A
2	C	V2C	7	C	V7C
2	D	V2D	7	D	V7D
2	E	V2E	7	E	V7E
2	F	V2F	7	F	V7F

2	G	V2G	7	G	V7G
2	H	V2H	7	H	V7H
2	I	V2I	7	I	V7I
2	K	V2K	7	K	V7K
2	L	V2L	7	L	V7L
2	M	V2M	7	M	V7M
2	N	V2N	7	N	V7N
2	P	V2P	7	P	V7P
2	Q	V2Q	7	Q	V7Q
2	R	V2R	7	R	V7R
2	S	V2S	7	S	V7S
2	T	V2T	7	T	V7T
2	W	V2W	7	W	V7W
2	Y	V2Y	7	Y	V7Y
3	A	G3A	8	A	G8A
3	C	G3C	8	C	G8C
3	D	G3D	8	D	G8D
3	E	G3E	8	E	G8E
3	F	G3F	8	F	G8F
3	H	G3H	8	H	G8H
3	I	G3I	8	I	G8I
3	K	G3K	8	K	G8K
3	L	G3L	8	L	G8L
3	M	G3M	8	M	G8M
3	N	G3N	8	N	G8N
3	P	G3P	8	P	G8P
3	Q	G3Q	8	Q	G8Q
3	R	G3R	8	R	G8R
3	S	G3S	8	S	G8S
3	T	G3T	8	T	G8T
3	V	G3V	8	V	G8V
3	W	G3W	8	W	G8W
3	Y	G3Y	8	Y	G8Y
4	C	A4C	9	A	K9A
4	D	A4D	9	C	K9C
4	E	A4E	9	D	K9D
4	F	A4F	9	E	K9E

4	G	A4G	9	F	K9F
4	H	A4H	9	G	K9G
4	I	A4I	9	H	K9H
4	K	A4K	9	I	K9I
4	L	A4L	9	L	K9L
4	M	A4M	9	M	K9M
4	N	A4N	9	N	K9N
4	P	A4P	9	P	K9P
4	Q	A4Q	9	Q	K9Q
4	R	A4R	9	R	K9R
4	S	A4S	9	S	K9S
4	T	A4T	9	T	K9T
4	V	A4V	9	V	K9V
4	W	A4W	9	W	K9W
4	Y	A4Y	9	Y	K9Y
5	A	G5A			
5	C	G5C			
5	D	G5D			
5	E	G5E			
5	F	G5F			
5	H	G5H			
5	I	G5I			
5	K	G5K			
5	L	G5L			
5	M	G5M			
5	N	G5N			
5	P	G5P			
5	Q	G5Q			
5	R	G5R			
5	S	G5S			
5	T	G5T			
5	W	G5W			
5	Y	G5Y			

The mutated peptides were then used as aAM-ORFs to prepare eAPC-pa cells as described in the cell engineering section.

Responder assay was then performed with the TCR candidates as described in the responder assay section.

Derivation of Finger print score and identification of cross-reactive candidates

- 5 A matrix describing the response data, as percentage of maximal signal (position x amino acid), is converted into a PSSM (position-specific score matrix), using a fixed value multiplier. The PSSM matrix is then systematically aligned to all possible peptides in the human proteome (UniProt), thus identifying all epitope-length peptides where the linear series of residues are all positively scored by the PSSM, irrespective of the intensity of the
10 scoring values per se, and which routinely comprises the base list of potential cross-reactivity candidates.

- To provide a mechanism for relative ranking, the PSSM is converted to a log-odds score matrix, applying a PSI-BLAST style pseudo-count (Altschul et al 2009), the
15 positive values of which are used to derive a relative scoring metric for each candidate (referred to as flat score). Candidates are also scored for similarity to the HLA-A*11:01 using a pseudocount-corrected log-odds matrix derived from bulk immunopeptidomics data of eluted peptides from that HLA. The epitope sequence is additionally aligned
20 and scored to all possible peptides within the human proteome using BLASTP “short” E and p-value scores with a BLOSUM62 matrix, so as to identify any additional candidates that may be closely structurally related. The above algorithms are implemented as custom python scripts.

Cross-reactivity eAPC-pa Library construction

- 25 The fingerprint score was then screened against the whole human proteome to find matches to these tolerated positions and therefore predict potential cross-reactive hits. According to the fingerprint score, a number of cross-reactive hits were predicted and used to generate the cross-reactivity eAPC-pa library. ORFs of the identified cross-reactive hits were engineered into eAPC-p (HLA-A*11:01 monoallelic cells) by RMCE as
30 described in the cell engineering section. The resulting eAPC-pa cells were then used in the responder assays with the eTPC-t expressing the TCRs.

eAPC-p HLA Monoallelic library construction

- A library of 86 monoallelic HLA-expressing eAPCs covering the most common HLA
35 haplotypes was generated. The HLAs used to construct the library are summarised in Table 23. The eAPC-p cells were generated by RMCE as described in the cell engineering section.

Table 23. A list of HLA haplotypes used in the alloreactivity assay. The HLA sequences were obtained from EBI (<https://www.ebi.ac.uk/ipd/imgt/hla/>) and the EBI accession numbers are listed under HLA name.

HLA-A subtype alleles	HLA-B subtype alleles	HLA-C subtype alleles	HLA-E subtype alleles
HLA-A*01:01 HLA00001	HLA-B*07:02 HLA00132	HLA-C*01:02 HLA00401	HLA-E*01:01 HLA00934
HLA-A*01:02 HLA00002	HLA-B*07:05 HLA00137	HLA-C*02:02 HLA00404	HLA-E*01:03 HLA00936
HLA-A*01:03 HLA00003	HLA-B*08:01 HLA00146	HLA-C*03:03 HLA00411	
HLA-A*02:01 HLA00005	HLA-B*13:01 HLA00152	HLA-C*03:04 HLA00413	
HLA-A*02:02 HLA00007	HLA-B*13:02 HLA00153	HLA-C*04:01 HLA00420	
HLA-A*02:03 HLA00008	HLA-B*14:01 HLA00157	HLA-C*05:01 HLA00427	
HLA-A*02:04 HLA00009	HLA-B*14:02 HLA00158	HLA-C*06:02 HLA00430	
HLA-A*02:05 HLA00010	HLA-B*15:01 HLA00162	HLA-C*07:01 HLA00433	
HLA-A*02:06 HLA00011	HLA-B*15:02 HLA00165	HLA-C*07:02 HLA00434	
HLA-A*02:07 HLA00012	HLA-B*15:03 HLA00166	HLA-C*08:01 HLA00445	
HLA-A*03:01 HLA00037	HLA-B*18:01 HLA00213	HLA-C*08:02 HLA00446	
HLA-A*11:01 HLA00043	HLA-B*27:05 HLA00225	HLA-C*12:02 HLA00453	
HLA-A*11:02 HLA00044	HLA-B*35:01 HLA00237	HLA-C*12:03 HLA00455	
HLA-A*11:03 HLA00045	HLA-B*35:02 HLA00238	HLA-C*14:02 HLA00462	
HLA-A*11:04 HLA00046	HLA-B*35:03 HLA00239	HLA-C*15:02 HLA00467	
HLA-A*11:05 HLA00047	HLA-B*35:05 HLA00241	HLA-C*16:01 HLA00475	

HLA-A*11:09 HLA01310	HLA-B*35:08 HLA00244		
HLA-A*23:01 HLA00048	HLA-B*35:12 HLA00249		
HLA-A*24:02 HLA00050	HLA-B*37:01 HLA00265		
HLA-A*24:03 HLA00053	HLA-B*38:01 HLA00267		
HLA-A*24:04 HLA00054	HLA-B*40:01 HLA00291		
HLA-A*24:07 HLA00057	HLA-B*40:02 HLA00293		
HLA-A*24:10 HLA00060	HLA-B*44:02 HLA00318		
HLA-A*24:17 HLA00067	HLA-B*44:03 HLA00319		
HLA-A*25:01 HLA00071	HLA-B*49:01 HLA00340		
HLA-A*26:01 HLA00073	HLA-B*50:01 HLA00341		
HLA-A*29:02 HLA00086	HLA-B*51:01 HLA00344		
HLA-A*30:01 HLA00089	HLA-B*51:02 HLA00346		
HLA-A*30:02 HLA00090	HLA-B*52:01 HLA00362		
HLA-A*31:01 HLA00097	HLA-B*53:01 HLA00364		
HLA-A*32:01 HLA00101	HLA-B*54:01 HLA00367		
HLA-A*33:01 HLA00104	HLA-B*55:01 HLA00368		
HLA-A*33:03 HLA00106	HLA-B*57:01 HLA00381		
HLA-A*34:02 HLA00109	HLA-B*58:01 HLA00386		
HLA-A*66:01			

HLA00112			
HLA-A*66:02 HLA00113			
HLA-A*68:01 HLA00115			
HLA-A*68:02 HLA00117			
HLA-A*80:01 HLA00130			

Cytotoxic assays

TCR-T cells (effector cells) were generated by directed endonuclease-mediated knock-in of the TCRs (TCR-T^{TCR-1}, TCR-T^{TCR-2}, TCR-T^{TCR-6}, TCR-T^{TCR-9} and TCR-T^{TCR-10}) into the endogenous TRAC locus and subsequently enriched for successfully edited cells, as described in material and methods, using CD34 microbead kit (Miltenyi Biotec, according to manufacturer's instructions). Target cells were used in cytotoxicity assays with varied effector-to-target (E:T) ratio which involve monoallelic eAPCs constitutively expressing cognate HLA-A*11:01 in combination with antigen KRAS G12V FL, as well as relevant control cell lines expressing HLA-A*11:01 KRAS G12V MG and HLA-A*11:01 (off-target control); or common cancer cell lines natively expressing KRAS G12V or KRAS wt, retrovirally transduced to express HLA-A*11:01. All target cells recombinantly express Firefly Luciferase. Target cells were plated at 10,000 cells (eAPC) or 20,000 cells (cancer lines) per well in a 96-well plate. Adherent cancer cells were left to adhere and TCR-T cells were 2-fold serially diluted to obtain E:T ratios from 1:1 to 1:64. For assessing ED₅₀ values of TCR-1, TCR-2, TCR-6, TCR-9 and TCR-10, target cells expressing HLA-A*11:01 were pulsed with varying concentrations of KRAS G12V (Peptides & Elephants) in the range of 0.1pM-1μM. Following washing, peptide-pulsed target cells or no peptide control target cells were co-cultured with TCR-T cells at an E:T ratio of 1:4. All test conditions were prepared in duplicates. After 24h of co-culture, target cell killing was assessed via luciferase activity of remaining viable cells in co-cultures compared to target only control wells and calculated as: % killing = 100 – ((RLU test condition)/(RLU target only)*100); RLU = relative luminescence units.

CORL23 lung cancer mouse model

All animal procedures were performed in accordance with the approved Research Institutes of Sweden (RISE) protocol. Female NSG mice (NOD SCID gamma, NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ) were purchased from Charles River Laboratories, Germany.

The mice were housed in micro-isolator cages under sterile conditions at the RISE facilities at temperature 20-26°C, humidity 40-70% and 12 hour light/12 hour dark cycle.

5 The NSG (NOD SCID gamma, NOD.Cg-Prkdcscidll2rgtm1Wjl/SzJ) mouse strain is genetically engineered to be severely immunodeficient and is an industry standard for xenograft studies as it enables engraftment and treatment using human cells without rejection (Ito et al 2002; Maletzki et al 2020). Experiments were carried out with n=10 mice per group.

10 Mice were monitored weekly using Bioluminescence imaging (BLI) to measure tumour burden and continuously for well-being to measure overall survival.

CORL-L23_A*11 cells were resuspended in Dulbecco's Phosphate-Buffered Saline (DPBS) and 100 µl containing 0.5×10^6 cells was injected for engraftment.

15 Primary CD8⁺ T cells were prepared in Dulbecco's Phosphate-Buffered Saline (DPBS) as described in the Primary CD8⁺ T cell isolation, activation and nucleofection section, above. One day before treatment, cryopreserved TCR-T cells TCR^{TCR-1} and TCR^{NY-ESO} were thawed and cultured overnight. On the day of treatment TCR-T cells were resuspended in Dulbecco's Phosphate buffered saline (DPBS) and 100 µl containing 10×10^6 cells injected into the tail vein for engraftment.

Reagents

25 Human recombinant IL-2 (Miltenyi Biotec) was prepared according to manufacturer's instructions in Phosphate Buffered Saline (PBS) + 0.1 % human serum albumin to a final concentration of 1.5×10^6 International Unit (IU)/ml.

VivoGlo Luciferin (Promega) was prepared according to manufacturer's instructions in DPBS to a final concentration of 15 mg/ml and injected intraperitoneally prior to tumour imaging.

Bioluminescence Imaging Data analysis

35 Bioluminescence images (BLI) were analysed using Kuant v2.4 image software (Vilber). BLI intensities were quantified as the background subtracted photon intensity (photons per second per steradian, ph/s/sr) in a consistently sized region of interest drawn around the lungs of each animal. When more than image per group and time-point was available, intensities were averaged. Kaplan-Meyer survival curves were generated to summarize survival across the groups.

Incucyte longitudinal live imaging based killing assay

The day prior to co-culture, TCR T cells were thawed and cultured as described above. Additionally, 20 000 GFP expressing target cells in 100 µl cell line culture media were added per well of a poly-D Lysine coated 96wp and left to adhere while imaged every 1-2h for 22-24h with a 20x objective. Quantification of the GFP+ target cell proliferation at 24h was used to calculate required TCR-T numbers to be added for E:T of 1:16, 1:4 and 1:1 in 100 µl. AnnexinV red staining reagent (Sartorius) was added to the co-culture at a 1:800 dilution. Imaging was continued every 1-2h for a total of 5 days. Target cells were masked based on phase and intensity in the green channel, while dead and dying cells were masked based on phase and intensity in the red channel. Quantification was performed as object count per well or a fold-change from the first scan. At each scan 4-9 fields are separately imaged and averaged per assay well.

15 *Data analysis*

Quantified Incucyte data was generated using Incucyte software 2022A Rev1 and 2022VB Rev2. Data was graphed using Graphpad Prism version 10.

Examples

20 The TCR discovery platform described in Figure 1, begins with the identification of HLA-restricted peptides derived from a target of interest (Step 1), which is then used in the TCR discovery (Step 2). Next, is the TCR and Antigen validation step (Step 3) where the platform's main focus is on delivering an effective and safe (derisked) TCR (Steps 4 and 5). The following examples describe each platform process and the key biological
25 discovery of each step as shown in Figure 1.

Example 1. Mass Spectrometric identification of G12V mutated KRAS peptides presented by mono-allelic HLA-A*11:01 eAPCs.

Herein is described the unbiased HLA-restricted peptide mapping for biologically relevant, processed and presented G12V mutated KRAS peptides, using mass spectrometry (MS)
30 (Figure 2). Antigen presenting cells (eAPC) engineered to express single HLA class I allele (HLA-A*11:01) were generated as described in WO 2018/083316. The HLA-A*11:01 open reading frame (ORF) also encoded a C-terminal 6xHistidine tag for capture by metal affinity chromatography as previously described (WO 2018/083316). The monoallelic eAPCs were
35 transfected with either a full length (FL) version of inactivated KRAS protein with G12V mutations (G12V_FL) (SEQ ID NO: 6); or a minigene (MG) with the candidate epitope +/- up to 9 amino acids of flanking sequence (G12V_MG-UFD) (SEQ ID NO: 5). Additionally,

an orthogonal cell line (COR-L23), that natively expresses G12V mutated KRAS has been lentivirally transduced with affinity-tagged HLA-A*11:01, and was used in the assay.

Peptides released from affinity purified HLA-A*11:01 pHLA complexes were measured by unbiased mass spectrometry on a Bruker TIMS-TOF2 instrument. Figure 2 shows the z-scores relative to mean intensity of all observations for the specific observations of 2 peptides derived from the G12V mutated KRAS – the nonamer (9-mer) and decamer (10-mer). In general, the z-score is the intensity of a specific peptide as compared to the intensity of all other peptides in the sample. This means that calculating z-scores takes the raw intensity score of the peptide and subtracts the population mean and the resulting figure is divided by the population standard deviation. Z-scores are calculated from $\log_{10}$ transformed data. Across all three cell lines, 2 epitopes of the G12V mutated KRAS were observable (these two KRAS peptide variants have been shown previously by Bear *et al* 2021). The z-scores in the G12V_FL sample suggest that the G12V mutated KRAS 10-mer is better processed than the G12V mutated KRAS 9-mer peptide (which was detected but not quantified). However, both peptides were detected in the G12V_MG sample and the presence of both was also confirmed in the orthogonal cell line COR-L23.

In conclusion, the analysis confirmed 2 processed and presented G12V mutated KRAS peptides restricted to HLA-A*11:01 class I allele.

Example 2. Selective expansion of TCR clonotypes specific for identified G12V mutated KRAS peptides.

After confirming the existence of the target peptides, the next step is to use the identified mutated KRAS G12V 10-mer and 9-mer peptides to discover TCR clonotypes that specifically bind them (Figure 1 Key platform Unit 2. TCR discovery).

To discover G12V mutated KRAS -10-mer-specific TCRs, naïve CD8 T cells derived from healthy donor peripheral blood mononuclear cells (PBMCs) were co-cultured with monoallelic engineered antigen presenting cells (eAPCs) constitutively expressing the HLA-A*11:01 in the presence of the 10-mer peptide for 7 days. Therefore, in this experimental set up, naïve CD8 T cells expressing a TCR capable of recognizing the KRAS G12V 10-mer peptide loaded and presented by the eAPC were selectively activated. Upon activation, CD8 T cells proliferated and expansion was supported by addition of exogenous interleukin 2 (IL-2) for 7 days. Expanded CD8 T cells were stained with Major Histocompatibility Complex (MHC) multimer (a PSMA tetramer that contains the KRAS peptide) and magnetically enriched before single cell sorting by fluorescence activated cell sorting (FACS). Alpha beta TCR pairs were sequenced and the frequency of appearance of each

TCR clonotype was counted (Figure 3 A). If a TCR clonotype was observed at least 3 times it was considered enriched.

To discover G12V mutated KRAS 9-mer- specific TCRs, the same process as described
5 above for the 10-mer was applied. Briefly naïve CD8⁺ T cells derived from healthy donor
peripheral blood mononuclear cells (PBMCs) were co-cultured with monoallelic engineered
antigen presenting cells (eAPCs) constitutively expressing the HLA-A*11:01 in the presence
of the 9-mer peptide for 7 days. Briefly, naïve CD8⁺ T cells expressing a TCR capable of
10 recognizing the KRAS G12V 9-mer peptide loaded and presented by the eAPC were
selectively activated. Upon activation CD8⁺ T cells proliferated and expansion was
supported by addition of exogenous interleukin 2 (IL-2) for 7 days. Expanded CD8⁺ T cells
were stained with MHC multimer (a Prostate Specific Membrane Antigen (PSMA) derived
tetramer that contains the KRAS peptide) and magnetically enriched before single cell
15 sorting by fluorescence activated cell sorting (FACS). Alpha beta TCR pairs were
sequenced and the frequency of appearance of each TCR clonotype was counted (Figure
3 B). If a TCR clonotype was counted at least 3 times it was considered enriched.

In conclusion, we have successfully expanded and enriched TCR clonotypes that have the
capability of recognizing the G12V mutated KRAS 10-mer and 9-mer peptides presented
20 by HLA-A*11:01.

**Example 3. Validation of sensitivity between the identified G12V mutated KRAS-
specific TCRs and HLA-A*11:01-presented G12V mutated KRAS peptides**

Following the discovery of the peptides derived from the mutated KRAS and the TCRs
25 specific for these peptides, the TCR sensitivity was determined (Figure 1, Step 3).

To functionally evaluate the sensitivity of the identified G12V mutated KRAS 10-mer and 9-
mer-specific TCRs, cell-cell contact assays with peptide-pulsed eAPCs contacted with TCR-
expressing eTPCs (eAPC-p – eTPC-t) were applied as described briefly here. Firstly, the
30 TCRs were expressed into engineered TCR presenting cells (eTPCs) containing a synthetic
reporter element that responds to TCRsp engagement as described in WO 2018/083317.
Briefly, the eTPCs have been engineered to contain a Response element that comprises of
a Driver-Activator component and an Amplifier-Report component, wherein both units utilize
synthetic promoters. The Driver is a synthetic promoter that is responsive to the native TCR
35 signalling pathways, encoding three sets of tandem transcription factor binding sites for
NFAT-AP1-NFkB (3xNF-AP-NB). Upon transcriptional activation, the Driver induces
expression of the Activator protein, a synthetic designed transcription factor derived by

fusion of the Herpes VP16 activation domain, the GAL4 DNA binding domain and two nuclear localization signals at the N- and C-terminals (NV16G4N), to which the cognate DNA recognition sequence is present 6 times in tandem in the Amplifier promoter. Both the Driver and Amplifier promoters utilize the core promoter sequence (B recognition element (BRE), TATA Box, Initiator (INR) and transcriptional start site) from HCMV IE1 promoter, immediately 3' of the respective transcription factor binding sites. The Amplifier upon transcriptional activation drives expression of the reporter, red fluorescent protein (RFP). To functionally evaluate the sensitivity of the enriched G12V mKRAS 10-mer or 9-mer specific TCRs, the responder eTPC-ts expressing the G12V mutated KRAS 10-mer or 9-mer -specific TCRs were co-cultured with the monoallelic eAPCs presenting the HLA-A*11:01 that were either unpulsed or pulsed with a concentration gradient of either the G12V mutated KRAS 10-mer peptide, the G12V mutated KRAS 9-mer peptide or the corresponding wild type (WT) peptides. Following the peptide pulsed cell-cell contact assays, the cells were stained with CD3 antibodies and the samples were analysed for CD3 expression and RFP signal using flow cytometry. CD3 downregulation reflects the internalisation of the CD3/TCR complex following its interaction with its target and is usually associated to the strength of the interaction (avidity). The more sensitive a TCR is the more downregulation in the CD3 should be observed. It is initially a safety mechanism that the T cells have to avoid overactivation and the risk of damaging healthy cells. Stimulation of the TCR/CD3 complex results in RFP expression and CD3 downregulation by the eTPC-t, which is proportional to the strength of activation. The decrease in CD3 in combination with RFP is therefore used as a signal of TCR engagement and activation.

As an example, the flow cytometric profile of three representative 10-mer-responsive TCRs is shown in Figure 4 A-C and described below. Figure 4A is a representative of a 10-mer-specific TCR (on Target TCR) that is unpulsed with G12V mutated KRAS 10-mer peptide (left panel) or pulsed with G12V mutated KRAS 10-mer peptide (right panel). The unpulsed eAPC HLA-A*11:01 cells stained high with the CD3 antibody and exhibited low RFP expression. Pulsing of the eAPC HLA-A*11:01 with the G12V mutated KRAS 10-mer peptide, led to increased number of cells with the RFP⁺CD3^{low} signature, thus confirming TCR activation. Figure 4B is a representative of a TCR that reacts with the HLA allele presented on the eAPCs, HLA-A*11:01, regardless of whether the target antigen is present (HLA-reactive). The RFP/CD3 profiles did not change when the cells were pulsed with the G12V mutated KRAS 10-mer, but remained RFP⁺CD3^{low}. Figure 4C is a representative of a TCR that is unresponsive to the mutated G12V 10-mer epitope, the cells have RFP⁻/CD3^{high} signature in both unpulsed (left panel) and pulsed cells (right panel).

The flow cytometric data obtained from the peptide pulse assays for all the 10-mer- and 9-mer-responsive TCRs was used to construct sigmoidal curves from which the half maximal effective concentration (EC50) values were obtained. The data from the peptide pulse assays is represented in two ways – Figure 4 which shows that many of the 10-mer- and 9-mer-responsive TCRs identified in the TCR discovery step are sensitive to the G12V mutated KRAS 10-mer or the 9-mer peptides. This is shown by plotting the TCRs against the EC50 – increased sensitivity is shown with EC50 values moving towards the right. The EC50 for TCR-1 and TCR-2 were respectively, 1.26 nM (95% CI 0.985 to 1.66) and 9.83 nM (95% CI 8.07 to 11.99). The EC50 of TCR-6 was 16.89 nM (95% CI 11.23 to 24.65) and the EC50 values of TCR-9 and TCR-10 were respectively, 2.14 nM (95% CI 1.73 to 2.64) and 5.69 nM (95% CI 4.87 to 6.60). Many of the identified TCRs showed high sensitivity. Secondly, Figure 5 focuses on the TCRs that showed specificity and the obtained data is presented to demonstrate the sensitivity of the identified TCRs to other peptides (for example sensitivity of the 10-mer-specific TCRs to the 9-mer peptide and vice versa as well as to the wild type KRAS G12 peptide (data presented Figure 5). In Figure 5 the 10-mer TCR, TCR-1, showed high sensitivity when incubated with eAPC HLA-A*11:01 cells pulsed with the G12V mutated KRAS 10-mer peptide (EC50 1.26 nM (95% CI 0.985 to 1.66)), while the same TCR also showed poor sensitivity when incubated with eAPC HLA-A*11:01 cells pulsed with the G12V mutated KRAS 9-mer peptide (EC50 421 nM (95% CI 349 to 511)). TCR-1 did not bind the WT G12 KRAS peptide (Figure 5A). The 10-mer TCR, TCR-2, also showed high sensitivity when incubated with eAPC HLA-A*11:01 cells pulsed with the G12V mutated KRAS 10-mer peptide (EC50 9.83 nM (95% CI 8.07 to 11.99)) but did not bind when incubated with eAPC HLA-A*11:01 cells pulsed with the G12V mutated KRAS 9-mer peptide or the WT G12 KRAS peptide (Figure 5B).

The 9-mer TCR, TCR-6, showed high sensitivity when incubated with eAPC HLA-A*11:01 cells pulsed with the G12V mutated KRAS 9-mer peptide, EC50 16.89 (95% CI 11.23 to 24.65) and detectable but lower sensitivity when incubated with eAPC HLA-A*11:01 cells pulsed with the G12V mutated KRAS 10-mer peptide, EC50 113nM (95% CI 99.58 to 128.5) and no interaction with the WT G12 KRAS peptide was observed (Figure 5C). TCR-9 showed high sensitivity when incubated with eAPC HLA-A*11:01 cells pulsed with the G12V mutated KRAS 9-mer peptide, EC50 2.14 nM (95% CI 1.72 to 2.64) and detectable but lower sensitivity when incubated with eAPC HLA-A*11:01 cells pulsed with the G12V mutated KRAS 10-mer peptide, EC50 48.12 nM (95% CI 41.35 to 55.98) and no substantial interaction with the WT G12 KRAS peptide was observed EC50 1506 nM (95% CI 1302 to 1741) (Figure 5D). TCR-10 showed high sensitivity when incubated with eAPC HLA-A*11:01 cells pulsed with the G12V mutated KRAS 9-mer peptide, EC50 5.69 nM (95% CI 4.87 to 6.60) and detectable but lower sensitivity when incubated with eAPC HLA-A*11:01

cells pulsed with the G12V mutated KRAS 10-mer peptide, EC50 171 nM (95% CI 157.7 to 185.5) and no interaction with the WT G12 KRAS peptide was observed (Figure 5E). A reference TCR (SEQ ID NO: 5164) showed sensitivity towards eAPC HLA-A*11:01 cells pulsed with the G12V mutated KRAS 10-mer peptide (EC50 2.1 nM (95% CI 1.840 to 2.397) (Full circle), while the same TCR failed to bind the eAPC HLA-A*11:01 cells pulsed with the G12V mutated KRAS 9-mer peptide (Full triangle) or the WT G12 KRAS peptide (Full square) (Figure 5F).

Next, to further validate the interaction between the G12V mutated KRAS 10-mer- and 9-mer-responsive TCRs and the mutated KRAS peptides, an assay was performed to look for a response when the target peptide is endogenously expressed and presented by the antigen presenting cells, Figures 6. Therefore, monoallelic HLA-A*11:01 eAPCs or the monoallelic HLA-A*11:01 eAPC line constitutively expressing the G12V mutated KRAS peptide either as a minigene (MG) (SEQ ID NO: 5) or as a full-length peptide (FL) (SEQ ID NO: 6) were co-cultured with responder eTPC-ts expressing the G12V mutated KRAS 10-mer-responsive TCRs (Figure 6A) or the G12V mutated KRAS 9-mer-responsive TCRs (Figure 6B). The response of eTPC-t responder cells was measured by RFP signaling in combination with CD3 downregulation (RFP⁺CD3^{low}).

A partial response of the responder cells expressing the G12V mutated KRAS 10-mer-responsive TCRs after incubation with the eAPC HLA-A*11:01 expressing the (SEQ ID6) was observed compared to the eAPC HLA-A*11:01 expressing the SEQ ID 5 where we see a large gradient in the response (Figure 6A). This is expected as the use of the MG creates a more sensitive system because of the shorter sequence. Also higher intensity detection of the peptides in the MS data is observed (Figure 2). Only a subset responded to the eAPC-HLA-A*11:01 loaded with SEQ ID NO: 6 in RFP⁺CD3^{low} measurements, compared to the cells loaded with the SEQ ID NO: 5. Similarly, a partial response of the responder cells expressing the G12V mutated KRAS 9-mer-responsive TCRs after incubation with the eAPC HLA-A*11:01 expressing the (SEQ ID NO: 6) was observed compared to the eAPC HLA-A*11:01 expressing the SEQ ID NO: 5 (Figure 6B). However importantly, the TCRs identified in the peptide pulse sensitivity assays were also detected in this experiment and are marked retrospectively.

In conclusion, several G12V mutated KRAS sensitive 10-mer- and 9-mer-responsive TCRs were identified. Furthermore, the TCR – antigen functional validation step within the platform demonstrated that the selected TCRs mediate functional activation by peptide

pulse of mutated KRAS G12V in the context of HLA-A*11:01 but did not mediate functional activation by the WT KRAS peptide.

Example 4: The G12V mutated KRAS specific TCRs mediate potent T cell killing of cells presenting the G12V mutated KRAS peptide in the HLA-A*11:01 context

As part of the TCR de-risking process, the cytotoxic potency of the G12V mutated KRAS 10-mer- and 9-mer-specific TCRs was analysed by using primary T cells transduced with the TCRs (TCR-T^{TCR-1}, TCR-T^{TCR-2}, TCR-T^{TCR-6}, TCR-T^{TCR-9} and TCR-T^{TCR-10}), co-cultured with luciferase-expressing eAPCs and the ONE-Glo Luciferase Assay System (Promega) according manufacturer's instructions. The cytotoxic potency of the primary T cells transduced with the TCR-1 was also assessed on a panel of cancer cell lines expressing the KRAS G12V mutation within the HLA:A*11:01 context.

TCR-T cells (effector cells) were generated by directed endonuclease-mediated knock-in of the TCRs (TCR-T^{TCR-1}, TCR-T^{TCR-2}, TCR-T^{TCR-6}, TCR-T^{TCR-9} and TCR-T^{TCR-10}) into the endogenous TRAC locus and subsequently enriched for successfully edited cells, as described in material and methods, using CD34 microbead kit (Miltenyi Biotec, according to manufacturer's instructions). Monoallelic eAPCs (Target cells, ARH-77) constitutively expressing HLA-A*11:01 in combination with endogenously expressing G12V mutated KRAS full length (FL) peptide, as well as relevant control cell lines expressing HLA-A*11:01 G12V mutated KRAS minigene (MG) peptide and HLA-A*11:01 only (off-target control), were used in cytotoxicity assays with variation in effector-to-target (E:T) ratio. For variation in E:T ratio, target cells were plated at 10,000 per well in a 96-well plate and TCR-T cells were 2-fold serially diluted to obtain E:T ratios of 1:1 to 1:64. For assessing EC50 values of TCR-1, TCR-2, TCR-6, TCR-9 and TCR-10, target cells expressing HLA-A*11:01 were pulsed with varying concentrations of G12V mutated KRAS 7-16 peptide (Peptides & Elephants) in the range of 0.1pM-1µM. Upon thorough washing, peptide-pulsed target cells or no peptide control target cells were co-cultured with TCR-T cells at an E:T ratio of 1:4. All test conditions were prepared in duplicates. After 24h of co-culture, target cell killing was assessed via luciferase activity of remaining viable cells in co-cultures compared to target only control wells and calculated as: % killing = 100 – ((RLU test condition)/(RLU target only)*100); RLU = relative luminescence units.

Rapid apoptotic death of the target cells was induced by TCR-T cells expressing the TCR-1 against the target cells expressing the KRAS FL (SEQ ID NO: 6) , KRAS MG-UFD (SEQ ID NO: 5) (Figure 7A). Weaker cytotoxic activity of the TCR-T^{TCR-1} towards target cells expressing KRAS MG-native construct (SEQ ID NO: 179) (Figure 7 A, right panel) . Additionally, absence of killing of off-target eAPC expressing HLA-A*11:01 confirms the

absence of overt off-target reactivity. TCR-T cells expressing the TCR-2 (TCR-T^{TCR-2}) also showed similar toxicity towards cells expressing the KRAS MG-native construct (SEQ ID NO: 179) or KRAS FL (SEQ ID NO: 6) (Figure 7B). No significant off-target lysis of cells expressing only the HLA-A*11:01 was observed. TCR-T cells expressing the 9-mer specific TCRs (TCR-T^{TCR-6}, TCR-T^{TCR-9} and TCR-T^{TCR-10}), also showed similar toxicity towards target cells expressing the KRAS MG-UFD (SEQ ID NO: 5) or KRAS FL (SEQ ID NO: 6) (Figure 7D and E). No significant off-target lysis of cells expressing only the HLA-A*11:01 was observed.

10 The cytotoxic killing potential of the effector TCR-T^{TCR-1} cells on a panel of cancer cell lines (Figure 7 F-H). Using immortalized cell lines from public domain collections, a set of model lines that are known to natively express KRAS G12V and have a gradient of HLA-A*11:01 surface density was generated. The cell lines expressing KRAS G12V or KRASwt as negative controls, were transduced to express HLA-A*11:01 as well as Firefly luciferase and nuclear localizing green fluorescent protein (GFP) to support luciferase and real-time imaging based killing assays, respectively. The cell lines have HLA-A*11:01 surface median fluorescent intensities (MFI)s from 39 503 (COR-L23_A11) to 491 (QGP-1_A11) covering an almost 100-fold range as measured by flow cytometry (Table 24). KRAS expression levels were measured using digital droplet polymerase chain reaction (ddPCR) and with the exception of COR-L23_A11 are largely consistent across the cell lines.

Table 24. Characteristics of cancer lines used in cytotoxicity assays

	COR-L23_A11	NCI-H441_A11	SW620_A11	QGP-1_A11	IGR-1_A11
KRAS status	G12V	G12V	G12V	G12V	wild type
KRAS expression ^a	22	5	5	8	3
HLA-A11 density ^b	39 503	11 593	9 540 ^c	491 ^c	18 151

25 ^aKRAS expression relative to the housekeeping gene TATA-box binding protein (TBP)

^bHLA-A*11 surface density is the median fluorescence intensity (MFI) established by antibody staining and flow cytometry.

^cSW620 and QGP-1 are also positive for HLA-A*24:02, which is cross-recognized by the antiHLA-A* antibody and used to establish the HLA-A*11 MFI therefore possibly

30 overestimated.

F) Luciferase activity-based cell-cell killing assays. Firefly luciferase enzymatically converts in an ATP-dependent reaction chromogenic luciferin to oxyluciferin emitting luminescence that can be sensitively and efficiently measured using a plate-based

35 spectrophotometer. Firefly luciferase when ectopically expressed in cells is highly

sensitive and specifically measures cell viability as its protein levels and enzymatic activity rapidly declines in non-viable cells and when exposed to most cell culture media (Coombe et al, 1998). Cytotoxicity assays were performed by luciferase activity readout after a 24h co-culture of TCR-T and target cancer cell lines at varying T:E (16:1, 4:1 and 1:1) ratios compared to cancer cell line expressing KRASwt. The target cell killing was assessed via luciferase activity of remaining viable cells in co-cultures compared to target only control wells and calculated as: % killing = $100 - ((\text{RLU test condition})/(\text{RLU target only}) * 100)$; RLU = relative luminescence units. Very strong killing of all cancer lines across varying KRAS expression levels covering almost 100-fold decrease in HLA-A*11 surface levels was observed. Killing was near-complete at T:E of 1:1 but was also notably strong at T:E of 4:1 with signs of killing observed even at T:E ratios of 16:1, without any indication of killing of the IGR-1_A11 control line expressing KRASwt or IGR-1_A11 only cell line. The results confirmed that the cytotoxic activity of the TCR-T^{TCR-1} cells is potent enough to deliver strong killing of cell lines derived from cancers that natively express KRAS G12V.

G) Incucyte longitudinal live imaging based killing assay was used to gain additional insight into the cytotoxic killing capacity of TCR-T^{TCR-1} cells during prolonged co-culture. The Incucyte platform delivers images from TCR-T^{TCR-1}: target cell co-cultures at set intervals over extended culture periods and is commonly used for real-time quantification of target cell number and death (Lanigan et al, 2020). Expression of nuclear localized GFP by viable target cells was used to measure loss of the target cells through quantification of green objects as well as dead and dying cells following staining with AnnexinV (red objects). The longitudinal assay provides insights into sustained target cell killing and progressive target cell loss at low E:T ratios. The established set of cancer cell lines was measured at E:T ratios of 1:1, 1:4 and 1:16 with TCR-T^{TCR-1}. Target cell loss was quantified as the fold-change of GFP+ positive objects from the first imaging. Effector TCR-T^{TCR-1} cells were added 24 hours after seeding of the target cancer cells (dashed line). Change in the GFP expression was the strongest at E:T 1:1 resulting in complete killing already at 24 hours after effector cell addition across all cancer cell lines. Evident effect was observed at the other E:T ratios (1:4 and 1:16). Cultures without the addition of the effector cells showed continued growth. IGR-1_A11 cells that do not express mutated KRAS G12V showed continued growth even in the presence of the TCR-T^{TCR-1} cells. **H)** To gain additional insight into the mechanism behind the observed target cell killing, apoptotic dead and dying cells were quantified through analysis of the AnnexinV staining and compared to the GFP+ target cell count at 1:16 E:T ratio generated at 2, 48 and 96 hours after addition of the TCR-T^{TCR-1} cells. The analysis clearly shows that the loss of GFP+ target cells is accompanied by a strong increase in AnnexinV positive objects. This demonstrates that target cell loss is accompanied by cell death and a majority of cells die through apoptosis, as expected for cytotoxic T-cell activity (Cassioi et al, 2022).

In conclusion, potent and selective toxicity of primary T cells expressing TCR-1 against cells presenting the G12V mutated KRAS peptides, in HLA-A*11:01 was observed. While target cells expressing the wild type KRAS or the wild type KRAS presented in HLA-A*11:01 were not killed by the TCR-T^{TCR-1} demonstrating no overt off-target reactivities.

Example 5. Cross-reactive and allo-reactive profiling of G12V mutated KRAS TCRs.

The TCRs that passed the functional evaluation sensitivity tests were next triaged into assays that determined their safety profile and ultimately end up with de-risked TCRs (Figure 1, Step 5). The first step in the safety assessment, the G12V mutated KRAS 10-mer and 9-mer TCRs undergo profiling for fingerprinting (Figure 8). The present example shows the fingerprinting of G12V mutated KRAS 10-mer and 9-mer TCRs using positional scanning peptide matrix. Two peptide libraries, containing all possible naturally occurring single amino acids at each position within the G12V mutated KRAS 9-mer (VVGAVGVGK) and 10-mer (VVVGAVGVGK) peptides, were created (the Epitope Mutagenesis Library). Each library peptide was then engineered into eAPC to generate a library of monoallelic HLA-A*11:01 eAPCs stably expressing the mutated version of the KRAS epitope. The eAPC cells were then co-cultured for 18 hours with TCR-Ts expressing TCRs (either TCR-1, TCR-2, TCR-6, TCR-9 or TCR-10). Activation of the responder cells was measured by RFP signal in combination with CD3 downregulation, using flow cytometry. The results were then plotted as a fingerprint profile, wherein the "y-axis" represents the amino acid substitutions and the "x-axis" the position where it each substitution has been introduced. The darker the colour, the higher the response due to that particular amino acid substitution in the epitope sequence. In this example, fingerprint profiles of the 5 are presented (Figure 8 A-E), included is also a fingerprint profile of a failed TCR, TCR-5 (Figure 8D). The fingerprint profiles demonstrate that the TCRs are unique in their ability to recognize the target peptide and reveal where tolerances are in that TCR-target peptide engagement. Based on these tolerances, a computer algorithm was generated and used to obtain a fingerprint score. The fingerprint score is then screened against the whole human proteome to find matches to these tolerated positions and therefore predict potential cross-reactive hits (Figure 9 A and C). According to the fingerprint score, a number of cross-reactive hits were predicted for TCR-1 but no cross-reactive hits were predicted for TCR-2 (Figure 9A). Two less sensitive TCRs, TCR-3 and TCR-4, were included as a comparison in the prediction assay and both showed predicted cross-reactive hits. The sequences from the predicted cross-reactive hits were then used to create minigene (MG) constructs that were introduced into the monoallelic HLA-A*11:01 eAPCs. Subsequently, the cells were co-cultured with the eTPC-t responder cells expressing the TCRs, TCR-1 and TCR-2 or the TCR-3 and TCR-4. To assess the cross-

reactivity potential of the TCRs, the activation of the eTPC-t responder cells was measured by flow cytometry following the co-culture. A threshold of 4% was applied as a cut off for cross-reactivity and anything above this threshold was considered unsafe for clinical use. Even though TCR-1 showed a number of predicted cross-reactive hits, the cross-reactivity assay has confirmed high specificity and no cross-reactive hits for either of the 10-mer responsive TCRs. TCR-3 and TCR-4 have shown some cross-reactivity based on the predicted hits (Figure 9B).

Both, prediction of cross-reactive hits based on the Fingerprint score as well as the cross-reactivity assay were performed also for the 9-mer-responsive TCRs, TCR-6, TCR-9 and TCR-10. A number of cross-reactive hits were predicted for TCR-9 and 1 hit was predicted for TCR-10 (Figure 9C), however following the cross-reactivity cell-cell contact assay all three 9-mer specific TCRs were confirmed to be derisked (safe) and non-cross-reactive (Figure 9D).

As part of the safety profile, allo-reactivity and para-reactivity were determined for the remaining TCRs (Figure 10). Here, the responder eTPC-ts expressing either the 10-mer- or the 9-mer-responsive TCRs were co-cultured for 18 hours with a library of monoallelic HLA-expressing eAPCs covering the most common HLA haplotypes. The response was measured by flow cytometry by detection of RFP expression and reduction in CD3 signal (RFP⁺CD3^{low}). TCR-1, showed no allo-reactivity. After pulsing the HLA eAPC library with the G12V mutated KRAS peptide, peptide-specific activation (para-reactivity) via several members of the HLA-A*11 supergroup was observed and to some extent activation through the HLA-A*30:01. TCR-2 was slightly allo-reactive with HLA-A*68:02. Para-reactivity was observed following peptide pulse assay with the G12V mutated KRAS with fewer members of the HLA-A*11 supergroup, HLA-A*30:01 and to some extent HLA-A*68:01. Regarding the allo-reactivity of the 9-mer-specific TCRs (Figure 10 B-C), TCR-6 was weakly allo-reactive with HLA-B*54:01 and para-reactive with members of the HLA-A11 supergroup and to some extent with HLA-B*54:01. TCR-9 was allo-reactive with HLA-B*35:01, HLA-B*35:02, HLA-B*35:03, HLA-B*35:12, HLA-B*57:01 and HLA-B*58:01. Para-reactivity was observed following peptide pulse assay with the G12V mutated KRAS with fewer members of the HLA-A*11 supergroup, HLA-B*35:01, HLA-B*35:02, HLA-B*35:03, HLA-B*35:05 and HLA-B*35:12 and HLA-B*52:01, HLA-B*53:01, HLA-B*57:01, HLA-B*58:01. TCR-10 was not allo-reactive and para-reactive with members of the HLA-A11 supergroup. The allo-reactivity and para-reactivity results are summarized in Table 25 below.

Table 25.

TCR-ID	Recognition (Alloreactivity) "off-HLA"	Specific recognition (Parareactivity) "on-target-off-HLA"
TCR-1	ND [^]	A*11:01, A*11:02, A*11:03, A*11:04, A*11:05, A*11:09; A*30:01
TCR-2	A*68:02	A*11:01, A*11:02, A*11:05, A*11:09, A*30:01, A*68:02
TCR-6	B*54:01	A*11:01, A*11:02, A*11:04, A*11:05, A*11:09; B*54:01
TCR-9	B*35:01, B*35:02, B*35:03, B*35:12, B*57:01, B*58:01	A*11:01, A*11:02, A*11:05, A*11:09; B*35:01, B*35:02, B*35:03, B*35:05, B*35:12, B*52:01, B*53:01, B*57:01, B*58:01
TCR-10	ND [^]	A*11:01, A*11:02, A*11:05, A*11:09
[^] Not detected		

In conclusion, we have identified and validated G12V mutated KRAS 10-mer- and 9-mer-specific TCRs, with no identified cross-reactive hits and a defined set of potential allo-reactive HLA alleles. With the knowledge gained from the HLA allo-reactivity assays it is possible to proactively mitigate clinical risks and further tailor the use of the validated TCRs during clinical trials depending on the HLA profile of each patient.

Example 6. Integration of TCR-1 into primary T cells leads to eradication of KRAS G12V positive tumour cells in an in vivo efficacy model

An *in vivo* mouse study was conducted to confirm efficacy of TCR-1 transduced T-cells (TCR-T^{TCR-1}) against KRAS G12V positive tumour cells. Human lung cancer cells (COR-L23) positive for human G12V KRAS mutation were retrovirally transduced to express HLA-A*11:01 and firefly luciferase (CORL-L23_A.11). All cells were prepared as described in Materials and Methods. The CORL-L23_A.11 cells were injected intravenously into the tail vein and engraftment was confirmed 3 days later using Bioluminescence imaging (BLI) and mice were allocated into treatment groups to ensure equal distribution of tumour burden in the lungs across the treatment groups (n=10 mice per group). Upon confirmed engraftment, mice were treated with either 10 million TCR-T^{TCR-1} or a control TCR (TCR-T^{NY-ESO}) to control for unspecific activity of the donor CD8⁺ T cells. The control TCR-T cells were generated through the introduction of an irrelevant TCR recognizing an epitope from New York Esophageal squamous cell carcinoma 1 (NY-ESO 1) presented on HLA-A*02:01 into T cells from the same donor. Both TCR-T treated

groups were compared to an untreated group. Use of the luciferase expressing CORL-L23_A.11 cells enables non-invasive measurements of the tumour burden using in vivo bioluminescence imaging (BLI). Expected treatment efficacy would manifest in rapid and sustained reduction of tumour burden as well as an increase in overall survival (Bear et al. 2021, Wang et al 2016). Tumour burden was monitored for 9 weeks using BLI and mice were sacrificed when showing signs of suffering. Mice treated with TCR-T^{TCR-1} displayed complete tumour eradication already at day 7 after treatment confirming the high potency of the TCR-T^{TCR-1} cells. Analysis of the tumour burden for nine weeks after treatment revealed sustained tumour eradication with no signs of tumour recurrence. In contrast, untreated and TCR-T^{NY-ESO} treated groups, showed a markedly increased tumour burden with time and a high mortality rate, while no adverse effects were observed as a result of the TCR-T treatment.

In conclusion, we have demonstrated that TCR-T cells expressing the TCR-1 effectively exert their cytotoxic activity in vivo, resulting in sustained and specific tumour clearance of a cancer model natively expressing the KRAS G12V mutation. Tumour clearance was sustained for nine weeks after treatment, while the control groups experienced progressive increase in tumour burden and mortality.

20

Example 7. Diversification and selection of TRA and TRB CDR3 regions of TCR-1.

The diversification and selection of TCR ORFs is desirable to engineer TCR's chain pairs with altered sensitivities, affinities and/ or specificities. The TCR ORF Reconstitution and Engineering system 2 (TORES²) (described in WO2019016175) was developed for rapid generation of collections of TCR chains that are systematically altered from the original target sequence. To identify variants with comparable RFP⁺CD3^{low} responses to the TCR-1, the CDR3 regions of TCR-1 were diversified as described in this example.

CDR3 nucleotide sequences from T cell receptor alpha (TRA) chain and T cell receptor beta (TRB) chain of a parental TCR (TCR-1) were used as base sequences to generate the diversified combinatorial TRA or TRB libraries. A combination of two degenerate codons containing mixed nucleotide bases (as shown in Table 26) was substituted into the sequences encoding the CDR3 from TRA or TRB of parental TCR-1, so that the sequences were translated into an amino acid sequence that may contain one to two amino acids distinct from the parental TCR-1 CDR3 amino acid sequence. Due to high conservation of cysteine (C) and phenylalanine (F) at the beginning and end of the CDR3 respectively, the degenerate codons containing mixed bases can only be introduced in the positions between the first (C+1 – the first amino acid after the cysteine) and last codon

(F-1– the first amino acid before the phenylalanine) of CDR3. The positions selected for diversification are inside the CDR3 range C+1 and F-1 and this diversification generates a combinatorial library with repeats.

- 5 In the current example, the generation of combinatorial library of TRA or TRB CDR3 regionsutilized the degenerate codons NAS, WKK, NCC and SKG. These codons use standard mixed bases designated using the International Union of Biochemistry (IUB) code and the expected translated amino acids are described in Table 26. Thus, each oligo is produced with one or two codon mix bases in the positions discussed, so that all possible codon triplets are provided for each mix base and all positions between C+1 and F-1 are represented by all possible codons. Table 24 shows all 24 available codons that are represented by the degenerate codons NAS; WKK, NCC, and SKG:

Table 26: Standard mixed bases designated using the International Union of Biochemistry (IUB) code and the expected translated amino acids used for the diversification of TRA and TRB CDR3 sequences

Codon Mix bases	Codon	AA
NAS	GAC	D
NAS	GAG	E
NAS	CAC	H
NAS	AAG	K
NAS	AAC	N
NAS	CAG	Q
NAS	TAG	Stop
NAS	TAC	Y
NCC	GCC	A
NCC	CCC	P
NCC	TCC	S
NCC	ACC	T
SKG	GGG	G
SKG	CTG	L
SKG	CGG	R
SKG	GTG	V
WKK	TGT	C
WKK	TTT	F
WKK	ATT	I

WKK	TTG	L
WKK	ATG	M
WKK	AGG	R
WKK	AGT	S
WKK	TGG	W
Total codons	24	

- 5 In the CDR3 TRA and CDR3 TRB of parental TCR-1 the amino acids between C+3 (the third amino acid after the cysteine) and F-2 (the second amino acid before the phenylalanine) were diversified and resulted in diversification of 9 and 10 amino acid residues with an expected number of 20,568 and 25,086 nucleotide variants, respectively.
- 10 Apart from the introduction of degenerate codons with mixed bases into the sequences encoding CDR3, the general design of the oligonucleotide duplex encoding CDR3 (odeCDR3) for TRA and TRB follows previously described specifications (WO2019016175). The odeCDR3 is designed to be flanked by two single stranded 4-nucleotide DNA overhangs, overhang on the 5' end ($\pm 1-5'$) and an overhang on the 3' end ($\pm 1-3'$). The overhangs are designed to permit directed ligase dependent cloning to the 3'
- 15 end of the TCR variable (V) segment encoded in the entry vector (Overhang $\pm 1-5'$) and the 5' end of the TCR joining (J) (Overhang $\pm 2-3'$) fragment during reconstitution, as described in Materials and Methods). The complementary forward and reverse strand of oligonucleotides encoding CDR3 are designed and ordered as separated oligonucleotide
- 20 pools.

Production of combinatorial TCR plasmid libraries combined with TCR ORF
Reconstitution and Engineering System

- The assembly of oligonucleotide duplex encoding CDR3 (odeCDR3) and consecutive
- 25 TCR reconstitution using TORES² follows previously described specifications (described in WO2019016175). Briefly, the forward and reverse oligonucleotide pools encoding CDR3 of TRA or TRB were combined in a single tube in addition to T4 polynucleotide kinase and respective buffer containing ATP. By incubating this reaction at 37 °C for 30 min the forward and reverse sequences anneal and 5' position of each oligonucleotide is
- 30 phosphorylated to allow future ligation. This product was used for cyclic restriction enzyme digestion and ligation cloning to generate the plasmids containing a library of diversified CDR3 at TRA or TRB of TCR-1.

The reconstitution of the combinatorial TCR libraries starts by producing a pool of plasmids containing a single TCR chain (TRA or TRB) and diversified odeCDR3. For such, the diversified odeCDR3 pool were combined in a single reaction with donor vector
5 containing joining (J) gene segments equal to the parental TCR-1 and an entry vector containing variable and constant (V-C) gene segments equal to the parental TCR-1. In this reaction, a type IIS restriction enzyme (BsaI) was added to cleave the entry and donor vectors by recognizing the sequence 5'-GGTCTC(N1)/(N5)-3' and generate 4 nucleotide overhangs. The compatibility of the overhangs allows the assembly of odeCDR3 and J-
10 fragment in the donor in the order V-odeCDR3-J-C that were ligated by adding T4 DNA ligase. For such the reaction mix was incubated for 4 min at 37 °C and 5 min at 16 °C that repeats for 30 cycles. After the 30 cycles, the T4 DNA ligase was deactivated at 80 °C and a negative selection performed by adding restriction enzyme Not I that cleaves by-products by recognizing the sequence 5'-GCGGCCGC. This product generates library of
15 plasmids containing diversified TRA or TRB chain and is used for transformation of electrocompetent *E. coli*.

The diversified single chains (TRA or TRB) were electroporated into Electrocompetent *E. coli* (NEB® 10-beta, cat: C3019, New England Biolabs) using MicroPulser Electroporator
20 (Bio-rad). The transformed cells were resuspended in stable outgrowth medium (NEB) and incubated for 1h at 37 °C under rotation and absence of antibiotics. Once finalized the recovery phase, the cells were transferred to lysogeny broth (LB) containing 100 µg/mL carbenicillin and incubated for 4h at 37 °C under rotation. To increase the yield of plasmid, the cells were washed in LB and transferred to a new flask containing twice the
25 concentration of carbenicillin (200 µg/mL) and incubated overnight at 25 °C under rotation. The cells were kept in culture for an additional day or until optical density at 600 nm (OD600) above 2.0 was reached when measured using cell density meter and then harvested for plasmid extraction using the Plasmid Maxi Kit (cat: 12963, QIAGEN) following the manufacturer instructions. The extracted plasmids were quantified and
30 validated through genotyping via sanger sequencing and diagnostic digest using set of restriction enzymes.

Using the single chain-encoding plasmids described above, plasmids containing full TCR chain pair sequence were produced wherein one of the chains (TRA or TRB) contained
35 none, one, or two mutations in the CDR3 region, and the complementing chain (TRA or TRB) is identical to the parental TCR-1 sequence. Plasmids that have a diversified CDR3 in the alpha chain (Alpha library) were produced by combining in a single reaction the TRA (TCR entry vector) containing variants at CDR3, the TRB (TCR donor vector) with a

sequence from the parental TCR and a bi-directional terminator vector. Plasmids that have a diversified CDR3 in the beta chain (Beta library) were produced by combining in a single reaction the TRA (TCR entry vector) with sequence from the parental TCR, the TRB (TCR donor vector) containing variants at CDR3 and a bi-directional terminator

5 vector. In these reactions, a type IIS restriction enzyme (Esp3I) was added to cleave the entry and donor vectors by recognizing the sequence 5'-CGTCTC(N1)/(N5)-3' and generate 4 nucleotide overhangs. The compatibility of the overhangs allows the assembly of TRB and bi-directional terminator vector in the donor vector that were ligated by adding T4 DNA ligase, the reaction mix was incubated for 4 min at 37 °C and 5 min at 16 °C that

10 repeats for 30 cycles. After the 30 cycles, the T4 DNA ligase was deactivated at 80 °C and a negative selection performed by adding restriction enzymes Sall, MluI and NotI which cleave plasmids that do not contain the full TCR sequence by recognizing the restriction enzyme site sequences 5'-GTCGAC, 5'-ACGCGT and 5'-GCGGCCGC, respectively. This product generates a library of plasmids each containing a TCR chain

15 pairs in which one of the TRA or TRB chains is diversified, and therefore each individual plasmid represents a single TCR variant. This plasmid product is used for transformation of electrocompetent *E. coli* following the protocol described above, and plasmids extraction using Plasmid Maxi Kit (QIAGEN). To validate the final plasmid products, a small sample of the plasmids are submitted to sanger sequence where the nucleotide

20 sequence of TCR is confirmed. Additionally, a small sample of the plasmids were digested using a pool of restriction enzymes and the digestion profile visualized in agarose gel under UV light.

Production of diversified TCR cell libraries (dTCL)

25 The cell libraries were generated through the delivery and integration of diversified TCR plasmid libraries into Jurkat cell line ETR-21 containing the RFP responder unit. The cell production follows previously described specifications (WO 2018 083317). Briefly, the plasmid pool was combined with 5 million Jurkat cells and a vector containing the recombinase flippase. Cells were then electroporated using the Gene Pulser Xcell System

30 (Bio-rad). The transient expression of flippase enzyme in the transfected cells allows for the integration of sequences encoding TCR chain pairs at FRT-F3 sites. Integration can be detected by absence of BFP or GFP marker (i.e., receiver site selection marker) expression and presence of surface expression of CD3 and TCR. The transfected cells were cultured in RPMI 1640 supplied with 10% fetal bovine serum and expanded for 7

35 days. The integration efficiency was evaluated using flow cytometry using anti-human CD3 and anti-human TCR antibodies. The cell lines with successful integration of TCR were enriched using magnetic cell separation (MACS) wherein cells expressing CD3 (CD3⁺) or TCR (TCR⁺) were enriched using positive selection. The expansion and

enrichment process were repeated until each pool of cells (cell library) reached a purity (TCR⁺BFP⁻ or TCR⁺GFP⁻) above 80%. The final output of the workflow is a diversified polyclonal pool of TCR presenting cells (eTPC-t) with a collection of diversified TCR chains (TRA or TRB), in which, each individual cell expresses a single TCR variant. For
5 each parental TCR, two libraries are generated containing mutations on alpha and beta chain and are used for further characterization through cellular assays.

Screening responder assay and sorting of responder variants

To evaluate whether specific mutations in the CDR3 of the TRA or TRB chain are
10 beneficial for specific and sensitive recognition of mutated G12V KRAS 10 mer peptide, each polyclonal pool of eTPC-t were co-cultured with monoallelic engineered antigen presenting cells (eAPC) eAPC HLA-A*11:01 presenting the peptide(s) in an assay corresponding to the responder assay. The pool of eTPC-ts was incubated with eAPCs at a ratio of 1:2 (final cell confluency 0.5 million cells/mL). To ensure that each TCR variant
15 is represented more than fifty times in the assay, this assay was set-up in large vessel (cell culture flask T-75). This co-culture was incubated overnight to allow the interaction of each eTPC-t cell with eAPCs resulting in an increase of intracellular RFP fluorescence in case of successful TCR activation. On the following day, this co-culture was stained with anti-CD3, anti-CD8 and anti-CD80 antibodies to allow the distinction between eAPCs,
20 unaffected eTPC-t and eTPC-t with downregulated CD3. The responding variants were identified by expression of RFP and downregulation of CD3. Therefore, the responding TCR population was gated (RFP⁺CD3^{low}) and isolated using bulk sorting strategy using a BDInflux instrument (Figure 12). The responding variants were bulk sorted and frozen as cell pellets until subsequent processing.

25

Library Preparation & Next Generation Sequencing (NGS)

Genomic DNA was extracted from the diversified TCR cell library (original libraries before a responder assay) and respective bulk sorted population (responder variants after a responder assay) using quick-DNA plus kit (cat.: D4070/ D4074/ D4068, Zymo research)
30 following the manufacturer instructions. After DNA quantification, the diversified TCR cell library and respective bulk sorted population were subjected to an amplification process targeting solely the sequences encoding the diversified chains. For such, forward and reverse primers binding the C-region and V-region of TRA or TRB were used, respectively. An amplification product size of 150 to 200 bp was expected. The Phusion plus DNA
35 polymerase (Cat. F630, Thermo Fisher Scientific) was used for this amplification following the protocol provided by the manufacturer. The Polymerase Chain Reaction (PCR) products were purified using DNA clean & concentrator kit (Zymo research) according to specification from manufacturer. After purification, the PCR products were quantified using

Qubit and nanodrop, and the presence of expected band (PCR product size) visualized via product loading at agarose gel. The products were submitted for amplicon sequencing using Illumina through an external service provider (Eurofins genomics). In this system, 5M paired reads are measured per sample.

5

Table 27 - List of primers used for library preparation of KRAS G12V libraries – Parental TCR-1 – and combinatorial libraries alpha TRA and beta TRB

TC R ID	Condition	Forward sequence	Reverse sequence
TC R-1 TRA CD R3 libra ry	Whole library	SEQ ID NO: 167 TATCATGCAGAGTCTCTCAGCT GGTACACG	SEQ ID NO: 168 CAACTTCACCATCACAGCC TCACAAG
	RFP+CD3	SEQ ID NO: 169	SEQ ID NO: 170
	Low eAPC	ATCTGCGTACAGTCTCTCAGCT	CAACTTCACCATCACAGCC
	KRAS WT	GGTACACG	TCACAAG
	MG		
	RFP+CD3		SEQ ID NO: 172
	Low eAPC	SEQ ID NO: 171	CAACTTCACCATCACAGCC
	G12V	ATGCTTCCTAAGTCTCTCAGCT	TCACAAG
	KRAS	GGTACACG	
	MG		
TC R-1 TRB CD R3 libra ry	Whole library	SEQ ID NO: 173 GCTGACCTGAATGGCTCAAACA CAGCGACC	SEQ ID NO: 174 TCTGAGCTGAATGTGAACG CCTTG
	RFP+CD3	SEQ ID NO: 175	SEQ ID NO: 176
	Low eAPC	CGACGTAGTCATGGCTCAAACA	TCTGAGCTGAATGTGAACG
	KRAS WT	CAGCGACC	CCTTG
	MG		
	RFP+CD3		SEQ ID NO: 178
	Low eAPC	SEQ ID NO: 177	TCTGAGCTGAATGTGAACG
	G12V	AGTCTCGGCAATGGCTCAAACA	CCTTG
	KRAS	CAGCGACC	
	MG		

10 Variant Identification

The amplicon sequencing data was obtained as merged paired reads in FASTQ format and the CDR3 nucleotide sequences were analyzed. From each FASTQ, unique CDR3

nucleotide sequences were extracted and associated with number of counts that corresponds to the number of times this particular CDR3 sequence was observed in a specific sample. The lists were generated for each diversified TCR cell library and respective bulk sorted cells. For normalization of data, the calculation of frequency per condition was applied (equation 1). The variants that successfully recognized the G12V KRAS presented in HLA-A*11:01 were enriched and therefore were expected to have higher frequency compared to a reference library (diversified TCR cell library before responder assay). To represent this enrichment, the frequency of a CDR3 variant in a sorted gate was correlated with frequency in starting reference library (equation 2).

10 Variants that have enrichment value above 1 successfully recognized the G12V KRAS and were therefore enriched. Identified TCR-1 G12V KRAS recognizing variants are listed in Tables 28 (TRA CDR3) and 29 (TRB CDR3).

15 Table 28. TRA diversified CDR3

SEQ ID NO:		SEQ ID NO:		SEQ ID NO:		SEQ ID NO:	
184	CALALTGG GNKLTF	621	CALIGTGG GNRLTF	1058	CALILTGG GSKHTF	1495	CALINTGG GNALTF
185	CALCLTGG GNKLTF	622	CALIGTGG GNVLTF	1059	CALILTGG GSKMTF	1496	CALINTGG GNCLTF
186	CALDLTGG GNKLTF	623	CALIGTGG GNWLTF	1060	CALILTGG GSKSTF	1497	CALINTGG GNGLTF
187	CALELTGG GNKLTF	624	CALIGTGG GQKLTF	1061	CALILTGG GSKVTF	1498	CALINTGG GNHLTF
188	CALGLTGG GNKLTF	625	CALIGTGM GNKLTF	1062	CALILTGG GSMLTF	1499	CALINTGG GNKFTF
189	CALIATGG GNKLTF	626	CALIGTMG GNKLTF	1063	CALILTGG GSQLTF	1500	CALINTGG GNKITF
190	CALICTGG GNKLTF	627	CALIGTPG GNKLTF	1064	CALILTGG GSRLTF	1501	CALINTGG GNKKTF
191	CALIFTGG GNKLTF	628	CALIGTQG GNKLTF	1065	CALILTGG GVKMTF	1502	CALINTGG GNKQTF
192	CALIHTGG GNKLTF	629	CALIGTVG GNKLTF	1066	CALILTGG GVKPTF	1503	CALINTGG GNMLTF
193	CALIITGGG NKLTF	630	CALIHAGG GNKLTF	1067	CALILTGG GVKTTF	1504	CALINTGG GNPLTF

194	CALILMGG GNKLTF	631	CALIHGDD GNKLTF	1068	CALILTGG GWKWTF	1505	CALINTGG GNRLTF
195	CALILTGG FNKLTF	632	CALIHGGG GNKLTF	1069	CALILTGG GWWLTF	1506	CALINTGG GNSLTF
196	CALILTGG GGKLTF	633	CALIHIGG GNKLTF	1070	CALILTGG HNKMTF	1507	CALINTGG GNVLTF
197	CALILTGG GHKLTF	634	CALIHTAG GNKLTF	1071	CALILTGG HNSLTF	1508	CALINTGG GNWLTF
198	CALILTGG GNALTF	635	CALIHTDG GNKLTF	1072	CALILTGGI MKLTF	1509	CALINTKG GNKLTF
199	CALILTGG GNDLTF	636	CALIHTGG GCKLTF	1073	CALILTGGI NRLTF	1510	CALINTNG GNKLTF
200	CALILTGG GNE LTF	637	CALIHTGG GKKLTF	1074	CALILTGG KNELTF	1511	CALINTQG GNKLTF
201	CALILTGG GNFLTF	638	CALIHTGG GNLTF	1075	CALILTGG KNILTF	1512	CALINTRG GNKLTF
202	CALILTGG GNKITF	639	CALIHTGG GNKITF	1076	CALILTGG KNKMTF	1513	CALINTSG GNKLTF
203	CALILTGG GNKNTF	640	CALIHTGG GNKMTF	1077	CALILTGG KNQLTF	1514	CALINTTG GNKLTF
204	CALILTGG GNKYTF	641	CALIHTGG GNKRTF	1078	CALILTGG KNSLTF	1515	CALINTVG GNKLTF
205	CALILTGG GNLLTF	642	CALIHTGG GNKVTF	1079	CALILTGG KNVLTF	1516	CALINTYG GNKLTF
206	CALILTGG GNMLTF	643	CALIHTGG GNNLTF	1080	CALILTGG LNALTF	1517	CALIPTAG GNKLTF
207	CALILTGG GNQLTF	644	CALIHTGG GNRLTF	1081	CALILTGG LNILTF	1518	CALIPTCG GNKLTF
208	CALILTGG GNRLTF	645	CALIHTGG GNTLTF	1082	CALILTGG LNQLTF	1519	CALIPTDG GNKLTF
209	CALILTGG GNTLTF	646	CALIHTGG QNK LTF	1083	CALILTGG LWK LTF	1520	CALIPTGG GNALTF
210	CALILTGG GNWLTF	647	CALIHTGG RNKLTF	1084	CALILTGG MIKLTF	1521	CALIPTGG GNKGTF
211	CALILTGG GNYLTF	648	CALIHTGM GNKLTF	1085	CALILTGG MNALTF	1522	CALIPTGG GNKMTF

212	CALILTGG GPKLTF	649	CALIHTGN GNKLTF	1086	CALILTGG MNQLTF	1523	CALIPTGG GNQLTF
213	CALILTGG GQKLTF	650	CALIHTIGG NKLTF	1087	CALILTGG MNSLTF	1524	CALIPTGG GNRLTF
214	CALILTGG GSKLTF	651	CALIHTKG GNKLTF	1088	CALILTGG NNALTF	1525	CALIPTGG GNSLTF
215	CALILTGG KNKLTF	652	CALIHTSG GNKLTF	1089	CALILTGG NNRLTF	1526	CALIPTGG GNTLTF
216	CALILTGG MNKLTF	653	CALIHTVG GNKLTF	1090	CALILTGG PNELTF	1527	CALIPTGN GNKLTF
217	CALILTGG QNKLTF	654	CALIHTYG GNKLTF	1091	CALILTGG QNELTF	1528	CALIPTKG GNKLTF
218	CALILTGG RNKLTF	655	CALIIGGG NKLTF	1092	CALILTGG QNKETF	1529	CALIPTMG GNKLTF
219	CALILTGG YNKLTF	656	CALIIMGG GNKLTF	1093	CALILTGG RNMLTF	1530	CALIPTPG GNKLTF
220	CALILTHG GNKLTF	657	CALIITGGG NKATF	1094	CALILTGG RNWLTF	1531	CALIPTQG GNKLTF
221	CALILTKG GNKLTF	658	CALIITGGG NKITF	1095	CALILTGG SNCLTF	1532	CALIPTRG GNKLTF
222	CALILTLG GNKLTF	659	CALIITGGG NKQTF	1096	CALILTGG SNELTF	1533	CALIPTTG GNKLTF
223	CALILTMG GNKLTF	660	CALIITGGG NMLTF	1097	CALILTGG SNGLTF	1534	CALIQMGG GNKLTF
224	CALILTPG GNKLTF	661	CALIITHGG NKLTF	1098	CALILTGG SNHLTF	1535	CALIQTGG GNKLTF
225	CALILTQG GNKLTF	662	CALIITNGG NKLTF	1099	CALILTGG SNKCTF	1536	CALIQTGG GNALTF
226	CALILTSG GNKLTF	663	CALIITPGG NKLTF	1100	CALILTGG SNKHTF	1537	CALIQTGG GNCLTF
227	CALILTYG GNKLTF	664	CALIITQGG NKLTF	1101	CALILTGG SNKSTF	1538	CALIQTGG GNELTF
228	CALIMTGG GNKLTF	665	CALIITRGG NKLTF	1102	CALILTGG SNLLTF	1539	CALIQTGG GNILTF
229	CALIPTGG GNKLTF	666	CALIITTGG NKLTF	1103	CALILTGG SNNLTF	1540	CALIQTGG GNKFTF

230	CALISTGG GNKLTF	667	CALIITVGG NKLTF	1104	CALILTGG SNPLTF	1541	CALIQTGG GNKSTF
231	CALITTGG GNKLTF	668	CALIKIGG GNKLTF	1105	CALILTGG SNRLTF	1542	CALIQTGG GNKYTF
232	CALIWTGG GNKLTF	669	CALIKMGG GNKLTF	1106	CALILTGG SNVLTF	1543	CALIQTGG GNPLTF
233	CALLLTGG GNKLTF	670	CALIKTAG GNKLTF	1107	CALILTGG SQKLTF	1544	CALIQTGG GNRLTF
234	CALQLTGG GNKLTF	671	CALIKTCG GNKLTF	1108	CALILTGG TNCLTF	1545	CALIQTGG GNTLTF
235	CALRLTGG GNKLTF	672	CALIKTEG GNKLTF	1109	CALILTGG TNKATF	1546	CALIQTGG GNWLTF
236	CALTLTGG GNKLTF	673	CALIKTGF GNKLTF	1110	CALILTGG TNKGTf	1547	CALIQTHG GNKLTF
237	CALVLTGG GNKLTF	674	CALIKTGG GHKLTF	1111	CALILTGG TNKMTF	1548	CALIQTG GNKLTF
238	CALDITGG GNKLTF	675	CALIKTGG GNKDTF	1112	CALILTGG TNKSTF	1549	CALIQTRG GNKLTF
239	CALGITGG GNKLTF	676	CALIKTGG GNKITF	1113	CALILTGG TNQLTF	1550	CALIQTSG GNKLTF
240	CALIDLGG GNKLTF	677	CALIKTGG GNLLTF	1114	CALILTGG TNRLTF	1551	CALIQTVG GNKLTF
241	CALIELGG GNKLTF	678	CALIKTGG GNMLTF	1115	CALILTGG TNSLTF	1552	CALIRGGG GNKLTF
242	CALIFLGG GNKLTF	679	CALIKTGG GRKLTF	1116	CALILTGG TNWLTF	1553	CALIRIGG GNKLTF
243	CALIGLGG GNKLTF	680	CALIKTHG GNKLTF	1117	CALILTGG VNGLTF	1554	CALIRTCG GNKLTF
244	CALIHLGG GNKLTF	681	CALIKTKG GNKLTF	1118	CALILTGG VNKPTF	1555	CALIRTEG GNKLTF
245	CALIILGGG NKLTF	682	CALIKTQG GNKLTF	1119	CALILTGG VNRLTF	1556	CALIRTGG GCKLTF
246	CALILATG GNKLTF	683	CALIKTSG GNKLTF	1120	CALILTGG VNSLTF	1557	CALIRTGG GDKLTF
247	CALILGAG GNKLTF	684	CALIKTTG GNKLTF	1121	CALILTGG VVKLTF	1558	CALIRTGG GNKATF

248	CALILGDG GNKLTF	685	CALILACG GNKLTF	1122	CALILTGG WCKLTF	1559	CALIRTGG GNKFTE
249	CALILGEG GNKLTF	686	CALILAEG GNKLTF	1123	CALILTGG WNALTF	1560	CALIRTGG GNKITF
250	CALILGGQ GNKLTF	687	CALILAGG GNKHTE	1124	CALILTGG WNDLTF	1561	CALIRTGG GNKMTF
251	CALILGHG GNKLTF	688	CALILAGG GNKMTF	1125	CALILTGG WNELTE	1562	CALIRTGG GNKRTE
252	CALILGMG GNKLTF	689	CALILAGG GNKNTE	1126	CALILTGG WNMLTF	1563	CALIRTGG GNKSTF
253	CALILGTG GNKLTF	690	CALILAGG GNKPTF	1127	CALILTGG WNQLTF	1564	CALIRTGG GNKVTE
254	CALILITGG NKLTF	691	CALILAGG GNPLTF	1128	CALILTGG YNGLTF	1565	CALIRTGG GNLLTF
255	CALILLTG GNKLTF	692	CALILAGG GNRLTF	1129	CALILTGG YNKGTE	1566	CALIRTGG GNMLTF
256	CALILNTG GNKLTF	693	CALILAGG GYKLTF	1130	CALILTGG YNKVTE	1567	CALIRTNG GNKLTF
257	CALILPTG GNKLTF	694	CALILAGQ GNKLTF	1131	CALILTGG YNMLTF	1568	CALIRTRG GNKLTF
258	CALILQTG GNKLTF	695	CALILAGT GNKLTF	1132	CALILTGH GNKPTF	1569	CALIRTVG GNKLTF
259	CALILTEG GGKLTF	696	CALILAGY GNKLTF	1133	CALILTGIG NKMTF	1570	CALIRTYG GNKLTF
260	CALILTGF GGKLTF	697	CALILAHG GNKLTF	1134	CALILTGIG NKYTE	1571	CALISMGG GNKLTF
261	CALILTGG GCNLTF	698	CALILANG GNKLTF	1135	CALILTGIH NKLTF	1572	CALISTCG GNKLTF
262	CALILTGG GGNLTF	699	CALILASG GNKLTF	1136	CALILTGL GNKATF	1573	CALISTFG GNKLTF
263	CALILTGG GNAKTF	700	CALILAYG GNKLTF	1137	CALILTGL GNKDTF	1574	CALISTGG GNELTF
264	CALILTGG GNLATF	701	CALILCEG GNKLTF	1138	CALILTGL GNKSTF	1575	CALISTGG GNGLTF
265	CALILTGG GNLITF	702	CALILCGG GLKLTF	1139	CALILTGM GNKATF	1576	CALISTGG GNKATF

266	CALILTGG GNLMTF	703	CALILCGG GNKHTF	1140	CALILTGM GNKITF	1577	CALISTGG GNKCTF
267	CALILTGG GNLNTF	704	CALILCGG GNRLTF	1141	CALILTGM GSKLTF	1578	CALISTGG GNKDTF
268	CALILTGG GNLRTF	705	CALILCGG GVKLTF	1142	CALILTGM GVKLTF	1579	CALISTGG GNKHTF
269	CALILTGG GNSKTF	706	CALILCGG HNKLTF	1143	CALILTGN GAKLTF	1580	CALISTGG GNKMTF
270	CALILTGG GNVKTF	707	CALILCGG NNKLTF	1144	CALILTGN GNKATF	1581	CALISTGG GNKPTF
271	CALILTGG GNWKTF	708	CALILCNG GNKLTF	1145	CALILTGQ GNKGTF	1582	CALISTGG GNLLTF
272	CALILTGG HGKLTF	709	CALILCPG GNKLTF	1146	CALILTGQ GNKITF	1583	CALISTGG GNMLTF
273	CALILTGG NAKLTF	710	CALILDAG GNKLTF	1147	CALILTGQ GNKMTF	1584	CALISTGG GNRLTF
274	CALILTGG NGKLTF	711	CALILDCG GNKLTF	1148	CALILTGQ GNKPTF	1585	CALISTGG GNSLTF
275	CALILTGG NSKLTF	712	CALILDGN GNKLTF	1149	CALILTGRL NKLTF	1586	CALISTGG GNTLTF
276	CALILTGG QGKLTF	713	CALILDIGG NKLTF	1150	CALILTGR MNKLTF	1587	CALISTHG GNKLTF
277	CALILTIGG GKLTF	714	CALILDNG GNKLTF	1151	CALILTGS GNKGTF	1588	CALISTKG GNKLTF
278	CALILTNG GGKLTF	715	CALILDRG GNKLTF	1152	CALILTGS GNKMTF	1589	CALISTLG GNKLTF
279	CALILTQG GGKLTF	716	CALILEGG GNKMTF	1153	CALILTGS GNKQTF	1590	CALISTMG GNKLTF
280	CALILTRG GGKLTF	717	CALILEIGG NKLTF	1154	CALILTGS GNPLTF	1591	CALISTRG GNKLTF
281	CALILTTG GGKLTF	718	CALILEVG GNKLTF	1155	CALILTGS GWKLTF	1592	CALISTSG GNKLTF
282	CALILTWG GGKLTF	719	CALILFAG GNKLTF	1156	CALILTGS MNKLTF	1593	CALISTVG GNKLTF
283	CALIMLGG GNKLTF	720	CALILFGG DNKLTF	1157	CALILTGT GNALTF	1594	CALISTYG GNKLTF

284	CALIPLGG GNKLTF	721	CALILFRG GNKLTF	1158	CALILTGT GNKATF	1595	CALISVGG GNKLTF
285	CALIRLGG GNKLTF	722	CALILFSG GNKLTF	1159	CALILTGT GNKMTF	1596	CALITTGG GAKLTF
286	CALITDGG GNKLTF	723	CALILGGG GNKHTF	1160	CALILTGT GNKQTF	1597	CALITTGG GNCLTF
287	CALITLGG GNKLTF	724	CALILGGG GNKNTF	1161	CALILTGTY NKLTF	1598	CALITTGG GNGLTF
288	CALIWLGG GNKLTF	725	CALILGGG GNKPTF	1162	CALILTVG GNKDTF	1599	CALITTGG GNILTF
289	CALIYLGG GNKLTF	726	CALILGGG GNMLTF	1163	CALILTVG GNKATF	1600	CALITTGG GNKATF
290	CALLPTGG GNKLTF	727	CALILGGG GNQLTF	1164	CALILTVG GNKITF	1601	CALITTGG GNKCTF
291	CALQITGG GNKLTF	728	CALILHDG GNKLTF	1165	CALILTVG GNKMTF	1602	CALITTGG GNKGTf
292	CALVITGG GNKLTF	729	CALILIAGG NKLTF	1166	CALILTVG GNPLTF	1603	CALITTGG GNKHTF
293	CALAGTG GGNKLTF	730	CALILIDG NKLTF	1167	CALILTVG GNQLTF	1604	CALITTGG GNKNTF
294	CALAHTG GGNKLTF	731	CALILIEG NKLTF	1168	CALILTVG VNKLTF	1605	CALITTGG GNKQTF
295	CALALAGG GNKLTF	732	CALILIGG AKLTF	1169	CALILTVG GNKATF	1606	CALITTGG GNKVTF
296	CALALEGG GNKLTF	733	CALILIGG NALTF	1170	CALILTVG GTKLTF	1607	CALITTGG GNRLTF
297	CALALIGG GNKLTF	734	CALILIGG NKATF	1171	CALILTVG NKLTF	1608	CALITTGG GNTLTF
298	CALALLGG GNKLTF	735	CALILIGG NKITF	1172	CALILTHG DNKLTF	1609	CALITTGG NKLTF
299	CALALNGG GNKLTF	736	CALILIGG NKNTF	1173	CALILTHG FNKLTF	1610	CALITTLG GNKLTF
300	CALALSGG GNKLTF	737	CALILIGG NKSTF	1174	CALILTHG GDKLTF	1611	CALITTMG GNKLTF
301	CALALTCG GNKLTF	738	CALILIGG NPLTF	1175	CALILTHG GIKLTF	1612	CALITTQG GNKLTF

302	CALALTGG GAKLTF	739	CALILIGGG NSLTF	1176	CALILTHG GNCLTF	1613	CALITTTG GNKLTF
303	CALALTGG GCKLTF	740	CALILIGGG NTLTF	1177	CALILTHG GNDLTF	1614	CALITTWG GNKLTF
304	CALALTGG GNALTF	741	CALILIGGG NVLTF	1178	CALILTHG GNLTF	1615	CALIVTAG GNKLTF
305	CALALTGG GNFLTTF	742	CALILIGGG SKLTF	1179	CALILTHG GNKMTF	1616	CALIVTEG GNKLTF
306	CALALTGG GNILTF	743	CALILIGGS NKLTF	1180	CALILTHG GNKPTF	1617	CALIVTFG GNKLTF
307	CALALTGG GNKCTF	744	CALILIPGG NKLTF	1181	CALILTHG GNNLTF	1618	CALIVTGG GNKATF
308	CALALTGG GNKDTF	745	CALILIQGG NKLTF	1182	CALILTHG GNPLTF	1619	CALIVTGG GNKGTTF
309	CALALTGG GNKETTF	746	CALILIRGG NKLTF	1183	CALILTHG GNRLTF	1620	CALIVTGG GNKITF
310	CALALTGG GNKHDTF	747	CALILISGG NKLTF	1184	CALILTHG GNSLTF	1621	CALIVTGG GNKNLTF
311	CALALTGG GNKMTF	748	CALILKGG GHKLTF	1185	CALILTHG GNTLTF	1622	CALIVTGG GNKPTF
312	CALALTGG GNKVTF	749	CALILKGG GNKMTF	1186	CALILTHG GNVLTF	1623	CALIVTGG GNPLTF
313	CALALTGG GNKYTF	750	CALILLAG GNKLTF	1187	CALILTHG GNYLTF	1624	CALIVTIGG NKLTF
314	CALALTGG GNLLTF	751	CALILLDG GNKLTF	1188	CALILTHG GQKLTF	1625	CALIVTLG GNKLTF
315	CALALTGG GNMLTF	752	CALILLGG FNKLTF	1189	CALILTHG GSKLTF	1626	CALIVTPG GNKLTF
316	CALALTGG GNNLTF	753	CALILLGG GCKLTF	1190	CALILTHGI NKLTF	1627	CALIVTQG GNKLTF
317	CALALTGG GNRLTF	754	CALILLGG GGKLTF	1191	CALILTHG KNKLTF	1628	CALIVTSG GNKLTF
318	CALALTGG GNTLTF	755	CALILLGG GNALTF	1192	CALILTHGL NKLTF	1629	CALIVTTG GNKLTF
319	CALALTGG GNWLTF	756	CALILLGG GNCLTF	1193	CALILTHG MNKLTF	1630	CALIVTVG GNKLTF

320	CALALTGG GQKLTf	757	CALILLGG GNDLTf	1194	CALILTHG QNKLTf	1631	CALIWGG GGNKLTf
321	CALALTGN GNKLTf	758	CALILLGG GNELTf	1195	CALILTHG SNKLTf	1632	CALIWSGG GNKLTf
322	CALALTYG GNKLTf	759	CALILLGG GNGLTf	1196	CALILTHG TNKLTf	1633	CALIWTGG GNALTf
323	CALALTlg GNKLTf	760	CALILLGG GNHLTf	1197	CALILTHLG NKLTf	1634	CALIWTGG GNKATf
324	CALALTNG GNKLTf	761	CALILLGG GNILTf	1198	CALILTHV GNKLTf	1635	CALIWTGG GNLLTf
325	CALALTPG GNKLTf	762	CALILLGG GNKATf	1199	CALILTIGG NALTf	1636	CALIWTGG GNPLTf
326	CALALTRG GNKLTf	763	CALILLGG GNKPTf	1200	CALILTIGG NKHTf	1637	CALIWTGG GNQLTf
327	CALALTVG GNKLTf	764	CALILLGG GNKSTf	1201	CALILTIGG NRLTf	1638	CALIWTGG GNSLTf
328	CALALTYG GNKLTf	765	CALILLGG GNKWTF	1202	CALILTIGG NYLTf	1639	CALIWTHG GNKLTf
329	CALALVGG GNKLTf	766	CALILLGG GNPLTf	1203	CALILTIGG QKLTf	1640	CALIWTKG GNKLTf
330	CALAMTG GGNKLTf	767	CALILLGG GNWLTf	1204	CALILTIGG VKLTf	1641	CALIWTRG GNKLTf
331	CALAPTGG GNKLTf	768	CALILLGG GSKLTf	1205	CALILTIGG YKLTf	1642	CALIWTSG GNKLTf
332	CALARTG GGNKLTf	769	CALILLGG HNKLTf	1206	CALILTIGH NKLTf	1643	CALIWTVG GNKLTf
333	CALATTGG GNKLTf	770	CALILLGG MNKLTf	1207	CALILTIGK NKLTf	1644	CALIYTAG GNKLTf
334	CALCDTG GGNKLTf	771	CALILLGG PNKLTf	1208	CALILTIHG NKLTf	1645	CALIYTGg GAKLTf
335	CALCHTG GGNKLTf	772	CALILLGG QNKLTf	1209	CALILTKG DNKLTf	1646	CALIYTGg GNKCTf
336	CALCKTG GGNKLTf	773	CALILLGG SNKLTf	1210	CALILTKG ENKLTf	1647	CALIYTGg GNKITf
337	CALCLAGG GNKLTf	774	CALILlGY GNKLTf	1211	CALILTKG GAKLTf	1648	CALIYTGg GNKPTf

338	CALCLGG GKNLTF	775	CALILLHG GNLTF	1212	CALILTKG GNDLTF	1649	CALIYTGG GNLLTF
339	CALCLLGG GNLTF	776	CALILLQG GNLTF	1213	CALILTKG GNHLTF	1650	CALIYTGG GNMLTF
340	CALCLPGG GNLTF	777	CALILMAG GNLTF	1214	CALILTKG GNKFTF	1651	CALIYTGG KNLTF
341	CALCLSGG GNLTF	778	CALILMEG GNLTF	1215	CALILTKG GNKHTF	1652	CALIYTHG GNLTF
342	CALCLTAG GNLTF	779	CALILMGG GDKLTF	1216	CALILTKG GNKITF	1653	CALIYTLG GNLTF
343	CALCLTGG ANKLTF	780	CALILMGG GNCLTF	1217	CALILTKG GNKPTF	1654	CALIYTNG GNLTF
344	CALCLTGG CNLTF	781	CALILMGG GNHLTF	1218	CALILTKG GNKQTF	1655	CALIYTS GNLTF
345	CALCLTGG ENLTF	782	CALILMGG GNLTF	1219	CALILTKG GNKVTF	1656	CALIYTWG GNLTF
346	CALCLTGG GGKTF	783	CALILMGG GNKCTF	1220	CALILTKG GNKYTF	1657	CALKLTGG GNKGT
347	CALCLTGG GNLTF	784	CALILMGG GNKVTF	1221	CALILTKG GNRLTF	1658	CALKLTGG GNKIT
348	CALCLTGG GNHLTF	785	CALILMGG GNSLTF	1222	CALILTKG GPKLTF	1659	CALKLTGG GNKMT
349	CALCLTGG GNLTF	786	CALILMGG MNLTF	1223	CALILTKG GQKTF	1660	CALKLTGG GNMLTF
350	CALCLTGG GNKCTF	787	CALILMGL GNLTF	1224	CALILTLG NKLTF	1661	CALKLTGG GRKTF
351	CALCLTGG GNKFTF	788	CALILMQG GNLTF	1225	CALILTLG GNDLTF	1662	CALKLTGG GWKTF
352	CALCLTGG GNKGT	789	CALILMSG GNLTF	1226	CALILTLG GNKIT	1663	CALKLTSG GNLTF
353	CALCLTGG GNKMT	790	CALILNAG GNLTF	1227	CALILTLG GNPLTF	1664	CALLLLGG GNLTF
354	CALCLTGG GNKPTF	791	CALILNGG GNKATF	1228	CALILTLG GNQLTF	1665	CALLLMGG GNLTF
355	CALCLTGG GNKSTF	792	CALILNNG GNLTF	1229	CALILTLG GNVLTF	1666	CALLLTDG GNLTF

356	CALCLTGG GNLLTF	793	CALILNRG GNKLTF	1230	CALILTLG GNYLTF	1667	CALLLTEG GNKLTF
357	CALCLTGG GNPLTF	794	CALILNWG GNKLTF	1231	CALILTLG GSKLTF	1668	CALLLTFG GNKLTF
358	CALCLTGG GNRLTF	795	CALILPDG GNKLTF	1232	CALILTLG GTKLTF	1669	CALLLTGG GCKLTF
359	CALCLTGG GNSLTF	796	CALILPEG GNKLTF	1233	CALILTLGP NKLTF	1670	CALLLTGG GNALTF
360	CALCLTGG GNTLTF	797	CALILPGG GNKMTF	1234	CALILTLGT NKLTF	1671	CALLLTGG GNCLTF
361	CALCLTGG GNYLTF	798	CALILPGG GNKPTF	1235	CALILTMA GNKLTF	1672	CALLLTGG GNELTF
362	CALCLTGG GSKLTF	799	CALILPHG GNKLTF	1236	CALILTMG DNKLTF	1673	CALLLTGG GNHLTF
363	CALCLTGG INKLTF	800	CALILPIGG NKLTF	1237	CALILTMG GNFLTF	1674	CALLLTGG GNKATF
364	CALCLTGG KNKLTF	801	CALILPKG GNKLTF	1238	CALILTMG GNILTF	1675	CALLLTGG GNKGTf
365	CALCLTGG MNKLTF	802	CALILPRG GNKLTF	1239	CALILTMG GNKMTF	1676	CALLLTGG GNKHf
366	CALCLTGG QNKLTF	803	CALILPWG GNKLTF	1240	CALILTMG GNKPTF	1677	CALLLTGG GNKKTF
367	CALCLTGG SNKLTF	804	CALILQCG GNKLTF	1241	CALILTMG GNKVTF	1678	CALLLTGG GNKMTF
368	CALCLTGL GNKLTF	805	CALILQDG GNKLTF	1242	CALILTMG GNQLTF	1679	CALLLTGG GNKPTF
369	CALCLTGQ GNKLTF	806	CALILQEG GNKLTF	1243	CALILTMG GNSLTF	1680	CALLLTGG GNPLTF
370	CALCLTIG GNKLTF	807	CALILQGG GNKATF	1244	CALILTMG GNYLTF	1681	CALLLTGG GNTLTF
371	CALCLTLG GNKLTF	808	CALILQGG RNKLTF	1245	CALILTMG GQKLTF	1682	CALLLTGG QNKLTF
372	CALCLTPG GNKLTF	809	CALILQHG GNKLTF	1246	CALILTMG GYKLTF	1683	CALLLTGG RNKLTF
373	CALCLTRG GNKLTF	810	CALILQWG GNKLTF	1247	CALILTMG PNKLTF	1684	CALLLTHG GNKLTF

374	CALCLTTG GNKLTF	811	CALILSCG GNKLTF	1248	CALILTMG RNKLTF	1685	CALLLTIG GNKLTF
375	CALCMTG GGNKLTF	812	CALILSGG GNKGTF	1249	CALILTNG ANKLTF	1686	CALLLTNG GNKLTF
376	CALCNTG GGNKLTF	813	CALILSGGI NKLTF	1250	CALILTNG CNKLTF	1687	CALLLTVG GNKLTF
377	CALCPTG GGNKLTF	814	CALILSNG GNKLTF	1251	CALILTNG GNALTF	1688	CALLLTWG GNKLTF
378	CALCQTG GGNKLTF	815	CALILSRG GNKLTF	1252	CALILTNG GNHLTF	1689	CALLLVGG GNKLTF
379	CALDDTG GGNKLTF	816	CALILSSG GNKLTF	1253	CALILTNG GNKATF	1690	CALMFTG GGNKLTF
380	CALDGTG GGNKLTF	817	CALILSWG GNKLTF	1254	CALILTNG GNKHTF	1691	CALMG TG GGNKLTF
381	CALDLGG GGNKLTF	818	CALILTAG CNKLTF	1255	CALILTNG GNKMTF	1692	CALMHTG GGNKLTF
382	CALDLTAG GNKLTF	819	CALILTAG GEKLTF	1256	CALILTNG GNKVTF	1693	CALMKTG GGNKLTF
383	CALDLTGG GAKLTF	820	CALILTAG GNALTF	1257	CALILTNG GNKYTF	1694	CALMLIGG GNKLTF
384	CALDLTGG GNALTF	821	CALILTAG GNDLTF	1258	CALILTNG GNMLTF	1695	CALMLLGG GNKLTF
385	CALDLTGG GNFLTF	822	CALILTAG GNKATF	1259	CALILTNG GNNLTF	1696	CALMLTCG GNKLTF
386	CALDLTGG GNKATF	823	CALILTAG GNKVTF	1260	CALILTNG GNPLTF	1697	CALMLTGA GNKLTF
387	CALDLTGG GNLLTF	824	CALILTAG GNKWTF	1261	CALILTNG GNQLTF	1698	CALMLTG GCNKLTF
388	CALDLTGG GNMLTF	825	CALILTAG GNKYTF	1262	CALILTNG GNRLTF	1699	CALMLTG GFNKLTF
389	CALDLTGG GNPLTF	826	CALILTAG GNLLTF	1263	CALILTNG GNSLTF	1700	CALMLTG GGNCLTF
390	CALDLTGG GNQLTF	827	CALILTAG GNQLTF	1264	CALILTNG GNTLTF	1701	CALMLTG GGNELTF
391	CALDLTGG GNRLTF	828	CALILTAG GNVLTF	1265	CALILTNG GNWLTF	1702	CALMLTG GGNFLT

392	CALDLTGG GNYLTF	829	CALILTAG GNYLTF	1266	CALILTNG GSKLTF	1703	CALMLTG GGNILTF
393	CALDLTGG GQKLTF	830	CALILTAG GSKLTF	1267	CALILTNG HNKLTF	1704	CALMLTG GGNKATF
394	CALDLTGG GSKLTF	831	CALILTAG HNKLTF	1268	CALILTNGI NKLTF	1705	CALMLTG GGNKMTF
395	CALDLTGG PNKLTF	832	CALILTAGI NKLTF	1269	CALILTNGL NKLTF	1706	CALMLTG GGNKVTF
396	CALDLTGG SNKLTF	833	CALILTAG NNKLTF	1270	CALILTNG PNKLTF	1707	CALMLTG GGNPLTF
397	CALDLTGM GNKLTF	834	CALILTAGT NKLTF	1271	CALILTNG QNKLTF	1708	CALMLTG GGNSLTF
398	CALDLTGQ GNKLTF	835	CALILTAN GNKLTF	1272	CALILTNG SNKLTF	1709	CALMLTG GGNVLTF
399	CALDLTGT GNKLTF	836	CALILTAV GNKLTF	1273	CALILTNG VNKLTF	1710	CALMLTG GGSKLTF
400	CALDLTGV GNKLTF	837	CALILTCG ENKLTF	1274	CALILTNG YNKLTF	1711	CALMLTG GLNKLTF
401	CALDLTMG GNKLTF	838	CALILTCG GNFLTf	1275	CALILTNG GNKLTF	1712	CALMLTG GQNKLTF
402	CALDLTPG GNKLTF	839	CALILTCG GNKITF	1276	CALILTPA GNKLTF	1713	CALMLTG GRNKLTF
403	CALDLTQG GNKLTF	840	CALILTCG GNMLTF	1277	CALILTPG GDKLTF	1714	CALMLTG GTNKLTF
404	CALDPTG GGNKLTF	841	CALILTCG GNPLTF	1278	CALILTPG GNCLTF	1715	CALMLTG GYNKLTF
405	CALDQTG GGNKLTF	842	CALILTCG GNSLTF	1279	CALILTPG GNKRTF	1716	CALMLTIG GNKLTF
406	CALDTTGG GNKLTF	843	CALILTCGI NKLTF	1280	CALILTPG GNMLTF	1717	CALMLTKG GNKLTF
407	CALDVTG GGNKLTF	844	CALILTCG NNKLTF	1281	CALILTPG GNPLTF	1718	CALMLTLG GNKLTF
408	CALEATGG GNKLTF	845	CALILTCG SNKLTF	1282	CALILTPG GQKLTF	1719	CALMLTNG GNKLTF
409	CALECTG GGNKLTF	846	CALILTCG TNKLTF	1283	CALILTPG GSKLTF	1720	CALMLTPG GNKLTF

410	CALEGTG GGNKLTF	847	CALILTDG ANKLTF	1284	CALILTQG ANKLTF	1721	CALMLTRG GNKLTF
411	CALEKTGG GNKLTF	848	CALILTDG DNKLTF	1285	CALILTQG GAKLTF	1722	CALMLTSG GNKLTF
412	CALELIGG GNKLTF	849	CALILTDG GAKLTF	1286	CALILTQG GDKLTF	1723	CALMLTYG GNKLTF
413	CALELSSG GNKLTF	850	CALILTDG GCKLTF	1287	CALILTQG GLKLTF	1724	CALMNTG GGNKLTF
414	CALELTAG GNKLTF	851	CALILTDG GHKLTF	1288	CALILTQG GMKLTF	1725	CALMVTG GGNKLTF
415	CALELTEG GNKLTF	852	CALILTDG GNCLTF	1289	CALILTQG GNALTF	1726	CALMWTG GGNKLTF
416	CALELTGG GAKLTF	853	CALILTDG GNDLTF	1290	CALILTQG GNDLTF	1727	CALMYTG GGNKLTF
417	CALELTGG GGKLTF	854	CALILTDG GNFLTF	1291	CALILTQG GNHLTF	1728	CALNLGG GGNKLTF
418	CALELTGG GLKLTF	855	CALILTDG GNILTF	1292	CALILTQG GNILTF	1729	CALNLLGG GNKLTF
419	CALELTGG GNE LTF	856	CALILTDG GNKH TF	1293	CALILTQG GNKDTF	1730	CALNLMG GGNKLTF
420	CALELTGG GNGLTF	857	CALILTDG GNKVTF	1294	CALILTQG GNKETF	1731	CALNLTIG GNKLTF
421	CALELTGG GNHLTF	858	CALILTDG GNKWTF	1295	CALILTQG GNKMTF	1732	CALNPTG GGNKLTF
422	CALELTGG GNILTF	859	CALILTDG GNLLTF	1296	CALILTQG GNKQTF	1733	CALPGTG GGNKLTF
423	CALELTGG GNKH TF	860	CALILTDG GNMLTF	1297	CALILTQG GNKSTF	1734	CALPLAGG GNKLTF
424	CALELTGG GNKITF	861	CALILTDG GNRLTF	1298	CALILTQG GNKVTF	1735	CALPLTAG GNKLTF
425	CALELTGG GNKVTF	862	CALILTDG GNVLTF	1299	CALILTQG GNLLTF	1736	CALPLTGG GPKLTF
426	CALELTGG GNMLTF	863	CALILTDG GNYLTF	1300	CALILTQG GNNLTF	1737	CALPLTPG GNKLTF
427	CALELTGG GNQLTF	864	CALILTDGI NKLTF	1301	CALILTQG GNRLTF	1738	CALQDTG GGNKLTF

428	CALELTGG GNSLTF	865	CALILTDGL NKLTF	1302	CALILTQG GNSLTF	1739	CALQFTG GGNKLTF
429	CALELTGG GNTLTF	866	CALILTDG PNKLTF	1303	CALILTQG GNTLTF	1740	CALQGTG GGNKLTF
430	CALELTGG GNYLTF	867	CALILTDG SNKLTF	1304	CALILTQG GNYLTF	1741	CALQHTG GGNKLTF
431	CALELTGG GQKLTF	868	CALILTDG TNKLTF	1305	CALILTQG GSKLTF	1742	CALQLCG GGNKLTF
432	CALELTGG HNKLTF	869	CALILTDN GNKLTF	1306	CALILTQG LNKLTF	1743	CALQLIGG GNKLTF
433	CALELTGG INKLTF	870	CALILTDQ GNKLTF	1307	CALILTQG SNKLTF	1744	CALQLLGG GNKLTF
434	CALELTGG KNKLTF	871	CALILTDG GNKLTF	1308	CALILTQH GNKLTF	1745	CALQLQG GGNKLTF
435	CALELTGG SNKLTF	872	CALILTEA GNKLTF	1309	CALILTRG CNKLTF	1746	CALQLTDG GNKLTF
436	CALELTGG WNKLTF	873	CALILTEG ANKLTF	1310	CALILTRG DNKLTF	1747	CALQLTEG GNKLTF
437	CALELTGS GNKLTF	874	CALILTEG GAKLTF	1311	CALILTRG GAKLTF	1748	CALQLTFG GNKLTF
438	CALELTGY GNKLTF	875	CALILTEG GNLTF	1312	CALILTRG GIKLTF	1749	CALQLTGG ANKLTF
439	CALELTHG GNKLTF	876	CALILTEG GNKFTF	1313	CALILTRG GNCLTF	1750	CALQLTGG FNKLTF
440	CALELTIG GNKLTF	877	CALILTEG GNKMTF	1314	CALILTRG GNETLF	1751	CALQLTGG GCKLTF
441	CALELTW GGNKLTF	878	CALILTEG GNKQTF	1315	CALILTRG GNFLTF	1752	CALQLTGG GNCLTF
442	CALELVGG GNKLTF	879	CALILTEG GNQLTF	1316	CALILTRG GNLTF	1753	CALQLTGG GNETLF
443	CALEPTGG GNKLTF	880	CALILTEG GNRLTF	1317	CALILTRG GNKFTF	1754	CALQLTGG GNHLTF
444	CALEQTG GGNKLTF	881	CALILTEG GNVLTF	1318	CALILTRG GNKGTF	1755	CALQLTGG GNILTF
445	CALERTG GGNKLTF	882	CALILTEG GNWLTF	1319	CALILTRG GNKITF	1756	CALQLTGG GNKNTF

446	CALESTGG GNKLTF	883	CALILTEG GNYLTF	1320	CALILTRG GNKPTF	1757	CALQLTGG GNKVTF
447	CALETTGG GNKLTF	884	CALILTEG GQKLTF	1321	CALILTRG GNKWTF	1758	CALQLTGG GNPLTF
448	CALEWTG GGNKLTF	885	CALILTEG GSKLTF	1322	CALILTRG GNKYTF	1759	CALQLTGG GNQLTF
449	CALEYTGG GNKLTF	886	CALILTEG KNKLTF	1323	CALILTRG GNMLTF	1760	CALQLTGG GNSLTF
450	CALFLTGD GNKLTF	887	CALILTEG NNKLTF	1324	CALILTRG GNNLTF	1761	CALQLTGG GNTLTF
451	CALFLTGG GGKLTF	888	CALILTEGT NKLTF	1325	CALILTRG GNPLTF	1762	CALQLTGG GNVLTF
452	CALFLTGG GKKLTF	889	CALILTEG YNKLTF	1326	CALILTRG GNRLTF	1763	CALQLTGG GNYLTF
453	CALFLTGG GNKDTF	890	CALILTEN GNKLTF	1327	CALILTRG GNVLTF	1764	CALQLTGG PNKLTF
454	CALFLTGG GNKGTF	891	CALILTFD GNKLTF	1328	CALILTRG GNWLTF	1765	CALQLTGN GNKLTF
455	CALFLTIG GNKLTF	892	CALILTFG GAKLTF	1329	CALILTRG GNYLTF	1766	CALQLTGT GNKLTF
456	CALFLTWG GNKLTF	893	CALILTFG GNFLTF	1330	CALILTRG GQKLTF	1767	CALQLTIG GNKLTF
457	CALGATG GGNKLTF	894	CALILTFG GNKATF	1331	CALILTRG GSKLTF	1768	CALQLTNG GNKLTF
458	CALGCTG GGNKLTF	895	CALILTFG GNKQTF	1332	CALILTRG HNKLTF	1769	CALQLTPG GNKLTF
459	CALGDTG GGNKLTF	896	CALILTFG GNSLTF	1333	CALILTRG KNKLTF	1770	CALQLTSG GNKLTF
460	CALGFTG GGNKLTF	897	CALILTFG GNTLTF	1334	CALILTRGL NKLTF	1771	CALQLTW GGNKLTF
461	CALGHTG GGNKLTF	898	CALILTFG GNVLTF	1335	CALILTRG PNKLTF	1772	CALQLVG GGNKLTF
462	CALGKTG GGNKLTF	899	CALILTFG GYKLTF	1336	CALILTRG QNKLTF	1773	CALQMTG GGNKLTF
463	CALGLEG GGNKLTF	900	CALILTFH GNKLTF	1337	CALILTRG RNKLTF	1774	CALQNTG GGNKLTF

464	CALGLGG GGNKLTF	901	CALILTFQ GNKLTF	1338	CALILTRG WNLKTF	1775	CALRLIGG GNKLTF
465	CALGLIGG GNKLTF	902	CALILTGA GNKATF	1339	CALILTRN GNKLTF	1776	CALRLLGG GNKLTF
466	CALGLNG GGNKLTF	903	CALILTGA GNKGTf	1340	CALILTRS GNKLTF	1777	CALRLTGG CNKLTF
467	CALGLPG GGNKLTF	904	CALILTGA GNKMTF	1341	CALILTSA GNKLTF	1778	CALRLTGG GAKLTF
468	CALGLQG GGNKLTF	905	CALILTGA GNKSTF	1342	CALILTSG ANKLTF	1779	CALRLTGG GGKLTF
469	CALGLSG GGNKLTF	906	CALILTGA GNKVTF	1343	CALILTSG GAKLTF	1780	CALRLTGG GNALTF
470	CALGLTEG GNKLTF	907	CALILTGA GNQLTF	1344	CALILTSG GNALTF	1781	CALRLTGG GNETF
471	CALGLTFG GNKLTF	908	CALILTGC GCKLTF	1345	CALILTSG GNETF	1782	CALRLTGG GNGLTF
472	CALGLTGC GNKLTF	909	CALILTGC GNKITF	1346	CALILTSG GNFLTF	1783	CALRLTGG GNKMTF
473	CALGLTGG ANKLTF	910	CALILTGC GNKPTF	1347	CALILTSG GNGLTF	1784	CALRLTGG GNKQTF
474	CALGLTGG GAKLTF	911	CALILTGDI NKLTF	1348	CALILTSG GNHLTF	1785	CALRLTGG GNKSTF
475	CALGLTGG GDKLTF	912	CALILTGE GNKITF	1349	CALILTSG GNILTF	1786	CALRLTGG GNPLTF
476	CALGLTGG GIKLTF	913	CALILTGE GNKNTF	1350	CALILTSG GNKATF	1787	CALRLTGG GNRLTF
477	CALGLTGG GLKLTF	914	CALILTGE GNKPTF	1351	CALILTSG GNKFTF	1788	CALRLTGG SNKLTF
478	CALGLTGG GMKLTF	915	CALILTFG GEKLTF	1352	CALILTSG GNKMTF	1789	CALRLTNG GNKLTF
479	CALGLTGG GNCLTF	916	CALILTFG GNKKTf	1353	CALILTSG GNKQTF	1790	CALRLTPG GNKLTF
480	CALGLTGG GNHLTF	917	CALILTFG GNKPTF	1354	CALILTSG GNKVTF	1791	CALRLTQG GNKLTF
481	CALGLTGG GNKETf	918	CALILTGG ANALTF	1355	CALILTSG GNKYTF	1792	CALRLTRG GNKLTF

482	CALGLTGG GNKFTF	919	CALILTGG ANHETF	1356	CALILTSG GNMLTF	1793	CALRLTSG GNKLTF
483	CALGLTGG GNKITF	920	CALILTGG ANKNTF	1357	CALILTSG GNNLTF	1794	CALSATGG GNKLTF
484	CALGLTGG GNKKTF	921	CALILTGG CNELTF	1358	CALILTSG GNQLTF	1795	CALSDTGG GGNKLTF
485	CALGLTGG GNKMTF	922	CALILTGG DNCLTF	1359	CALILTSG GNYLTF	1796	CALSKTGG GNKLTF
486	CALGLTGG GNKPTF	923	CALILTGG DNKCTF	1360	CALILTSG GQKLTF	1797	CALSLGG GGNKLTF
487	CALGLTGG GNKQTF	924	CALILTGG DNKNTF	1361	CALILTSG HNKLTF	1798	CALSLLGG GNKLTF
488	CALGLTGG GNKYTF	925	CALILTGG DNQLTF	1362	CALILTSG MNKLTF	1799	CALSLMG GGNKLTF
489	CALGLTGG GNLLTF	926	CALILTGG ENNLTF	1363	CALILTSG QNKLTF	1800	CALSLTCG GNKLTF
490	CALGLTGG GNNLTF	927	CALILTGG FNALTF	1364	CALILTSGT NKLTF	1801	CALSLTDG GNKLTF
491	CALGLTGG GNQLTF	928	CALILTGG FNGLTF	1365	CALILTSG VNKLTF	1802	CALSLTEG GNKLTF
492	CALGLTGG GNTLTF	929	CALILTGG FNKATF	1366	CALILTSH GNKLTF	1803	CALSLTGA GNKLTF
493	CALGLTGG GNVLTF	930	CALILTGG FNKFTF	1367	CALILTSIG NKLTF	1804	CALSLTGG ANKLTF
494	CALGLTGG GNWLTF	931	CALILTGG FNKKTF	1368	CALILTSM GNKLTF	1805	CALSLTGG GAKLTF
495	CALGLTGG GNYLTF	932	CALILTGG FNKQTF	1369	CALILTSN GNKLTF	1806	CALSLTGG GDKLTF
496	CALGLTGG GSKLTF	933	CALILTGG GAKATF	1370	CALILTSQ GNKLTF	1807	CALSLTGG GIKLTF
497	CALGLTGG GTKLTF	934	CALILTGG GAKHTF	1371	CALILTSR GNKLTF	1808	CALSLTGG GMKLTF
498	CALGLTGG MNKLTF	935	CALILTGG GAKMTF	1372	CALILTSS GNKLTF	1809	CALSLTGG GNALTF
499	CALGLTGG RNKLTF	936	CALILTGG GAKQTF	1373	CALILTSV GNKLTF	1810	CALSLTGG GNDLTF

500	CALGLTGG TNKLTF	937	CALILTGG GAKYTF	1374	CALILTTGA NKLTF	1811	CALSLTGG GNFLTF
501	CALGLTGG VNKLTF	938	CALILTGG GARLTF	1375	CALILTTG GCKLTF	1812	CALSLTGG GNHLTF
502	CALGLTGG YNKLTF	939	CALILTGG GASLTF	1376	CALILTTG GNCLTF	1813	CALSLTGG GNKATF
503	CALGLTGI GNKLTF	940	CALILTGG GCKGTF	1377	CALILTTG GNElTF	1814	CALSLTGG GNKETF
504	CALGLTGL GNKLTF	941	CALILTGG GCKRTF	1378	CALILTTG GNFLTF	1815	CALSLTGG GNKFTF
505	CALGLTGR GNKLTF	942	CALILTGG GCKVTF	1379	CALILTTG GNHLTF	1816	CALSLTGG GNKGTF
506	CALGLTGS GNKLTF	943	CALILTGG GCKWTF	1380	CALILTTG GNKHlTF	1817	CALSLTGG GNKHlTF
507	CALGLTIG GNKLTF	944	CALILTGG GCSLTF	1381	CALILTTG GNKITF	1818	CALSLTGG GNKMTF
508	CALGLTLG GNKLTF	945	CALILTGG GDKATF	1382	CALILTTG GNKMTF	1819	CALSLTGG GNKNlTF
509	CALGLTPG GNKLTF	946	CALILTGG GDKSTF	1383	CALILTTG GNLLTF	1820	CALSLTGG GNKQTF
510	CALGLTSG GNKLTF	947	CALILTGG GDMLTF	1384	CALILTTG GNMLTF	1821	CALSLTGG GNKVTF
511	CALGLTTG GNKLTF	948	CALILTGG GEGlTF	1385	CALILTTG GNRLTF	1822	CALSLTGG GNMLTF
512	CALGLTVG GNKLTF	949	CALILTGG GEKlTF	1386	CALILTTG GNTLTF	1823	CALSLTGG GNQLTF
513	CALGLTW GGNKLTF	950	CALILTGG GEKYTF	1387	CALILTTG GSKLTF	1824	CALSLTGG GNSLTF
514	CALGLTYG GNKLTF	951	CALILTGG GFKPTF	1388	CALILTTG HNKLTF	1825	CALSLTGG GNWLTF
515	CALGLVG GGNKLTF	952	CALILTGG GFKRTF	1389	CALILTTG NNKLTF	1826	CALSLTGG GNYLTF
516	CALGNTG GGNKLTF	953	CALILTGG GFKSTF	1390	CALILTTGP NKLTF	1827	CALSLTGG GQKLTF
517	CALGPTG GGNKLTF	954	CALILTGG GGALTF	1391	CALILTTSG NKLTF	1828	CALSLTGG GSKLTF

518	CALGQTG GGNKLTF	955	CALILTGG GGDLTF	1392	CALILTVG ANKLTF	1829	CALSLTGG GVKLTF
519	CALGRTG GGNKLTF	956	CALILTGG GGELTF	1393	CALILTVG GEKLTF	1830	CALSLTGM GNKLTF
520	CALGSTG GGNKLTF	957	CALILTGG GGKITF	1394	CALILTVG GHKLTF	1831	CALSLTGY GNKLTF
521	CALGVTG GGNKLTF	958	CALILTGG GGKKTf	1395	CALILTVG GIKLTF	1832	CALSLTHG GNKLTF
522	CALGWTG GGNKLTF	959	CALILTGG GGKPTF	1396	CALILTVG GNFLTF	1833	CALSLTIG GNKLTF
523	CALGYTG GGNKLTF	960	CALILTGG GGKVTF	1397	CALILTVG GNLTF	1834	CALSLTNG GNKLTF
524	CALHLTAG GNKLTF	961	CALILTGG GGKWTF	1398	CALILTVG GNKGTf	1835	CALSLTPG GNKLTF
525	CALHLTDG GNKLTF	962	CALILTGG GGKYTF	1399	CALILTVG GNKITF	1836	CALSLTQG GNKLTF
526	CALHLTGG GNLTF	963	CALILTGG GGSLTF	1400	CALILTVG GNKMTF	1837	CALSLTRG GNKLTF
527	CALHLTGG GNKPTF	964	CALILTGG GGVLTF	1401	CALILTVG GNKSTF	1838	CALSLTTG GNKLTF
528	CALHLTGG QNKLTF	965	CALILTGG GGWLTF	1402	CALILTVG GNKVTF	1839	CALSLTVG GNKLTF
529	CALHLTQG GNKLTF	966	CALILTGG GHKATF	1403	CALILTVG GNPLTF	1840	CALSLTYG GNKLTF
530	CALHLTRG GNKLTF	967	CALILTGG GHKDTF	1404	CALILTVG GNTLTF	1841	CALSQTG GGNKLTF
531	CALHLTTG GNKLTF	968	CALILTGG GHKETf	1405	CALILTVG GNVLTF	1842	CALSRTG GGNKLTF
532	CALHYTG GGNKLTF	969	CALILTGG GHKVTF	1406	CALILTVG GNYLTF	1843	CALTDTGG GNKLTF
533	CALIAIGG GNKLTF	970	CALILTGG GHKWTF	1407	CALILTVG GSKLTF	1844	CALTFTGG GNKLTF
534	CALIATCG GNKLTF	971	CALILTGG GHQLTF	1408	CALILTVG SNKLTF	1845	CALTGTG GGNKLTF
535	CALIALEG GNKLTF	972	CALILTGG GIKMTF	1409	CALILTVGT NKLTF	1846	CALHTTGG GNKLTF

536	CALIATGG GHKLTF	973	CALILTGG GKWTF	1410	CALILTVG YNKLTF	1847	CALTLEGG GNKLTF
537	CALIATGG GNELTF	974	CALILTGG GLKPTF	1411	CALILTVH GNKLTF	1848	CALTLHGG GNKLTF
538	CALIATGG GNKATF	975	CALILTGG GMILTF	1412	CALILTVQ GNKLTF	1849	CALTLLGG GNKLTF
539	CALIATGG GNKFTF	976	CALILTGG GMKATF	1413	CALILTWE GNKLTF	1850	CALTLTEG GNKLTF
540	CALIATGG GNKHTF	977	CALILTGG GMKCTF	1414	CALILTWG DNKLTF	1851	CALTLTFG GNKLTF
541	CALIATGG GNKRTF	978	CALILTGG GNAATF	1415	CALILTWG GCKLTF	1852	CALTLTGG ANKLTF
542	CALIATGG GNKSTF	979	CALILTGG GNAFTF	1416	CALILTWG GNDLTF	1853	CALTLTGG GNCLTF
543	CALIATGG GNLLTF	980	CALILTGG GNAMTF	1417	CALILTWG GNQLTF	1854	CALTLTGG GNHLTF
544	CALIATGG GNRLTF	981	CALILTGG GNAPTF	1418	CALILTWG GNRLTF	1855	CALTLTGG GNKATF
545	CALIATGG GNTLTF	982	CALILTGG GNCATF	1419	CALILTWG GNVLTF	1856	CALTLTGG GNKFTF
546	CALIATIGG NKLTF	983	CALILTGG GNCNTF	1420	CALILTWG GSKLTF	1857	CALTLTGG GNKGTF
547	CALIATYG GNKLTF	984	CALILTGG GNCPTF	1421	CALILTWG VNKLTF	1858	CALTLTGG GNKHTF
548	CALICTGG GNELTF	985	CALILTGG GNCVTF	1422	CALILTWN GNKLTF	1859	CALTLTGG GNKPTF
549	CALICTGG GNKATF	986	CALILTGG GNDITF	1423	CALILTWQ GNKLTF	1860	CALTLTGG GNKQTF
550	CALICTGG GNKQTF	987	CALILTGG GNDMTF	1424	CALILTWR GNKLTF	1861	CALTLTGG GNKSTF
551	CALICTGG GNMLTF	988	CALILTGG GNDVTF	1425	CALILTWW GNKLTF	1862	CALTLTGG GNMLTF
552	CALICTGG GNTLTF	989	CALILTGG GNEFTF	1426	CALILTYE GNKLTF	1863	CALTLTGG GNNLTF
553	CALICTGY GNKLTF	990	CALILTGG GNEHTF	1427	CALILTYG ANKLTF	1864	CALTLTGG GNSLTF

554	CALICTIGG GNKLTf	991	CALILTGG GNEITf	1428	CALILTYG GLKLTf	1865	CALTLTGG GQKLTf
555	CALICTKG GNKLTf	992	CALILTGG GNEMTf	1429	CALILTYG GNCLTf	1866	CALTLTGG GSKLTf
556	CALICTMG GNKLTf	993	CALILTGG GNEVTf	1430	CALILTYG GNFLTf	1867	CALTLTGG GVKLTf
557	CALICTNG GNKLTf	994	CALILTGG GNEYTf	1431	CALILTYG GNKATf	1868	CALTLTGG PNKLTf
558	CALICTRG GNKLTf	995	CALILTGG GNFMTf	1432	CALILTYG GNKGTf	1869	CALTLTGG QNKLTf
559	CALICTTG GNKLTf	996	CALILTGG GNFNTf	1433	CALILTYG GNKMTf	1870	CALTLTGN GNKLTf
560	CALICTVG GNKLTf	997	CALILTGG GNGVTF	1434	CALILTYG GNKNTf	1871	CALTLTIG GNKLTf
561	CALICTYG GNKLTf	998	CALILTGG GNHFTf	1435	CALILTYG GNNLTf	1872	CALTTLTG GNKLTf
562	CALIDGGG GNKLTf	999	CALILTGG GNHITf	1436	CALILTYG GNQLTf	1873	CALTLTMG GNKLTf
563	CALIDIGG GNKLTf	1000	CALILTGG GNIATf	1437	CALILTYG GNSLTf	1874	CALTLTNG GNKLTf
564	CALIDSGG GNKLTf	1001	CALILTGG GNIITf	1438	CALILTYG GQKLTf	1875	CALTLTQG GNKLTf
565	CALIDTEG GNKLTf	1002	CALILTGG GNIPTf	1439	CALILTYG GSKLTf	1876	CALTLTRG GNKLTf
566	CALIDTGG ANKLTf	1003	CALILTGG GNIQTf	1440	CALILTYG SNKLTf	1877	CALTLTSG GNKLTf
567	CALIDTGG GNALTF	1004	CALILTGG GNIRTF	1441	CALILTYG WNLKTF	1878	CALTLT TG GNKLTf
568	CALIDTGG GNKGTf	1005	CALILTGG GNIVTF	1442	CALILTYH GNKLTf	1879	CALTLYTG GNKLTf
569	CALIDTGG GNKHTf	1006	CALILTGG GNMFTf	1443	CALILTYN GNKLTf	1880	CALTM TG GGNKLTf
570	CALIDTGG GNKMTf	1007	CALILTGG GNMMTF	1444	CALILTYS GNKLTf	1881	CALTNTGG GNKLTf
571	CALIDTGG GNQLTf	1008	CALILTGG GNMTTF	1445	CALILTYW GNKLTf	1882	CALTRTGG GNKLTf

572	CALIDTKG GNKLTF	1009	CALILTGG GNMWTF	1446	CALILVEG GNKLTF	1883	CALTSTGG GNKLTF
573	CALIDTLG GNKLTF	1010	CALILTGG GNMYTF	1447	CALILVGG GAKLTF	1884	CALVTGTTG GNKLTF
574	CALIDTMG GNKLTF	1011	CALILTGG GNNATF	1448	CALILVGG GGKLTF	1885	CALVCTG GGGNLTF
575	CALIDTNG GNKLTF	1012	CALILTGG GNNCTF	1449	CALILVGG GNALTF	1886	CALVDTG GGGNLTF
576	CALIDTSG GNKLTF	1013	CALILTGG GNNGTF	1450	CALILVGG GNILTF	1887	CALVGTG GGGNLTF
577	CALIDTWG GNKLTF	1014	CALILTGG GNNITF	1451	CALILVGG GNKATF	1888	CALVLAGG GNKLTF
578	CALIDTYG GNKLTF	1015	CALILTGG GNNMTF	1452	CALILVGG GNKNTF	1889	CALVLDGG GNKLTF
579	CALIEGGG GNKLTF	1016	CALILTGG GNNNTF	1453	CALILVGG GNKPTF	1890	CALVLIGG GNKLTF
580	CALIEMGG GNKLTF	1017	CALILTGG GNNVTF	1454	CALILVGG GNKVTF	1891	CALVLLGG GNKLTF
581	CALIEPGG GNKLTF	1018	CALILTGG GNPATF	1455	CALILVGG GNMLTF	1892	CALVLSGG GNKLTF
582	CALIEVGG GNKLTF	1019	CALILTGG GNPFTF	1456	CALILVGG GNPLTF	1893	CALVLTGG GIKLTF
583	CALIFPGG GNKLTF	1020	CALILTGG GNPITF	1457	CALILVGG GNQLTF	1894	CALVLTGG GNHLTF
584	CALIFTDG GNKLTF	1021	CALILTGG GNPQTF	1458	CALILVGG GNSLTF	1895	CALVLTGG GNILTF
585	CALIFTEG GNKLTF	1022	CALILTGG GNPVTF	1459	CALILVGG GRKLTF	1896	CALVLTGG GNKGTF
586	CALIFTFG GNKLTF	1023	CALILTGG GNPYTF	1460	CALILVGG MNKLTF	1897	CALVLTGG GNKKTF
587	CALIFTGG GNALTF	1024	CALILTGG GNQETF	1461	CALILVGP GNKLTF	1898	CALVLTGG GNKYTF
588	CALIFTGG GNKFTF	1025	CALILTGG GNQFTF	1462	CALILVPG GNKLTF	1899	CALVLTGG GNLLTF
589	CALIFTGG GNKITF	1026	CALILTGG GNQMTF	1463	CALILVQG GNKLTF	1900	CALVLTGG GNQLTF

590	CALIFTGG GNKMTF	1027	CALILTGG GNQNTF	1464	CALILVRG GNKLTF	1901	CALVLTGG GNSLTF
591	CALIFTGG GNKPTF	1028	CALILTGG GNQPTF	1465	CALILVSG GNKLTF	1902	CALVLTGG GNWLTF
592	CALIFTGG GNKVTF	1029	CALILTGG GNQYTF	1466	CALILWGG GNKHTF	1903	CALVLTGG GNYLTF
593	CALIFTGG GNKWTF	1030	CALILTGG GNRMTF	1467	CALILYDG GNKLTF	1904	CALVLTGG GTKLTF
594	CALIFTGG VNKLTF	1031	CALILTGG GNRPTF	1468	CALILYGG GEKLTF	1905	CALVLTGG LNKLTF
595	CALIFTHG GNKLTF	1032	CALILTGG GNRQTF	1469	CALILYGG GNKCTF	1906	CALVLTGG QNKLTF
596	CALIFTGG NKLTF	1033	CALILTGG GNRVTF	1470	CALILYGG GNKQTF	1907	CALVLTGG RNKLTF
597	CALIFTLG GNKLTF	1034	CALILTGG GNRYTF	1471	CALILYKG GNKLTF	1908	CALVLTGG SNKLTF
598	CALIFTMG GNKLTF	1035	CALILTGG GNSMTF	1472	CALILYNG GNKLTF	1909	CALVLTGN GNKLTF
599	CALIFTRG GNKLTF	1036	CALILTGG GNSQTF	1473	CALILYQG GNKLTF	1910	CALVLTGP GNKLTF
600	CALIFTSG GNKLTF	1037	CALILTGG GNSVTF	1474	CALILYYG GNKLTF	1911	CALVLTGQ GNKLTF
601	CALIFTTG GNKLTF	1038	CALILTGG GNTFTF	1475	CALIMTGG GCKLTF	1912	CALVLTGT GNKLTF
602	CALIGEGG GNKLTF	1039	CALILTGG GNTMTF	1476	CALIMTGG GNHLTF	1913	CALVLTGV GNKLTF
603	CALIGGGG GNKLTF	1040	CALILTGG GNTQTF	1477	CALIMTGG GNKPTF	1914	CALVLTHG GNKLTF
604	CALIGTAG GNKLTF	1041	CALILTGG GNVATF	1478	CALIMTGG GNLLTF	1915	CALVLTKG GNKLTF
605	CALIGTDG GNKLTF	1042	CALILTGG GNVCTF	1479	CALIMTGG GNMLTF	1916	CALVLTLG GNKLTF
606	CALIGTEG GNKLTF	1043	CALILTGG GNVDTF	1480	CALIMTGG GNQLTF	1917	CALVLTPG GNKLTF
607	CALIGTFG GNKLTF	1044	CALILTGG GNVITF	1481	CALIMTGG GNRLTF	1918	CALVLTVG GNKLTF

608	CALIGTGG GCKLTF	1045	CALILTGG GNVMTF	1482	CALIMTGG GNVLTF	1919	CALVLTW GGNKLTF
609	CALIGTGG GGKLTF	1046	CALILTGG GNWGTf	1483	CALIMTKG GNKLTF	1920	CALVLVGG GNKLTF
610	CALIGTGG GNHLTF	1047	CALILTGG GQILTF	1484	CALIMTNG GNKLTF	1921	CALVMTG GGNKLTF
611	CALIGTGG GNILTF	1048	CALILTGG GQKATF	1485	CALIMTRG GNKLTF	1922	CALVNTG GGNKLTF
612	CALIGTGG GNKCTF	1049	CALILTGG GQKKTF	1486	CALIMTSG GNKLTF	1923	CALVSTGG GNKLTF
613	CALIGTGG GNKETf	1050	CALILTGG GQKPTF	1487	CALIMTVG GNKLTF	1924	CALWLTG GGNKQTF
614	CALIGTGG GNKITF	1051	CALILTGG GQKYTF	1488	CALIMTWG GNKLTF	1925	CALWLTK GGNKLTF
615	CALIGTGG GNKMTF	1052	CALILTGG GQRLTF	1489	CALIMTYG GNKLTF	1926	CALWLTN GGNKLTF
616	CALIGTGG GNKQTF	1053	CALILTGG GRRLTF	1490	CALINDGG GNKLTF	1927	CALWLTY GGNKLTF
617	CALIGTGG GNKYTF	1054	CALILTGG GSCLTF	1491	CALINTAG GNKLTF	1928	CALYLTGG GNKQTF
618	CALIGTGG GNMLTF	1055	CALILTGG GSELTF	1492	CALINTCG GNKLTF	1929	CALYLTGG GVKLTF
619	CALIGTGG GNPLTF	1056	CALILTGG GSKATF	1493	CALINTGG GAKLTF	1930	CALYLTGG YNKLTF
620	CALIGTGG GNQLTF	1057	CALILTGG GSKFTF	1494	CALINTGG GDKLTF	1931	CALYWTG GGNKLTF

Table 29. TRB diversified CDR3 chains

SEQ ID NO:		SEQ ID NO:		SEQ ID NO:	
1932	CASAPSGDY DQPQHF	2179	CASSHSGDY NQPQHF	2426	CASSPSGDY DQHCHF
1933	CASCPSGDY DQPQHF	2180	CASSIGGDYD QPQHF	2427	CASSPSGDY DQHLHF
1934	CASQPSGDY DQPQHF	2181	CASSISGDAD QPQHF	2428	CASSPSGDY DQISHF

1935	CASSASGDY DQPQHF	2182	CASSISGDHD QPQHF	2429	CASSPSGDY DQITHF
1936	CASSCSGDY DQPQHF	2183	CASSISGDPD QPQHF	2430	CASSPSGDY DQKAHF
1937	CASSDSGDY DQPQHF	2184	CASSISGDYA QPQHF	2431	CASSPSGDY DQKVHF
1938	CASSES GDY DQPQHF	2185	CASSISGDYD FPQHF	2432	CASSPSGDY DQLHHF
1939	CASSFSGDY DQPQHF	2186	CASSISGDYD GPQHF	2433	CASSPSGDY DQLSHF
1940	CASSHSGDY DQPQHF	2187	CASSISGDYD NPQHF	2434	CASSPSGDY DQMHHF
1941	CASSISGDYD QPQHF	2188	CASSISGDYD QE QHF	2435	CASSPSGDY DQM SHF
1942	CASSKSGDY DQPQHF	2189	CASSISGDYD QFQHF	2436	CASSPSGDY DQNSHF
1943	CASSLSGDY DQPQHF	2190	CASSISGDYD QKQHF	2437	CASSPSGDY DQRCHF
1944	CASSMSGDY DQPQHF	2191	CASSISGDYD QLQHF	2438	CASSPSGDY DQRNHF
1945	CASSPAGDY DQPQHF	2192	CASSISGDYD QPAHF	2439	CASSPSGDY DQSKHF
1946	CASSPSGDA DQPQHF	2193	CASSISGDYD QPNHF	2440	CASSPSGDY DQSMHF
1947	CASSPSGDF DQPQHF	2194	CASSISGDYD QPSHF	2441	CASSPSGDY DQSNHF
1948	CASSPSGDG DQPQHF	2195	CASSISGDYD QPTHF	2442	CASSPSGDY DQTMHF
1949	CASSPSGDH DQPQHF	2196	CASSISGDYD QRQHF	2443	CASSPSGDY DQTNHF
1950	CASSPSGDW DQPQHF	2197	CASSISGDYD QWQHF	2444	CASSPSGDY DQTPHF
1951	CASSPSGDY DFPQHF	2198	CASSISGDYD QYQHF	2445	CASSPSGDY DQVAHF
1952	CASSPSGDY DIPQHF	2199	CASSISGDYD RPQHF	2446	CASSPSGDY DQVCHF

1953	CASSPSGDY DMPQHF	2200	CASSISGDYD TPQHF	2447	CASSPSGDY DQVHHF
1954	CASSPSGDY DQAQHF	2201	CASSISGDYD YPQHF	2448	CASSPSGDY DQVSHF
1955	CASSPSGDY DQCQHF	2202	CASSISGDYK QPQHF	2449	CASSPSGDY DQVVHF
1956	CASSPSGDY DQDQHF	2203	CASSISGQYD QPQHF	2450	CASSPSGDY DQWHHF
1957	CASSPSGDY DQEQHF	2204	CASSKSGDY DEPQHF	2451	CASSPSGDY DQWLHF
1958	CASSPSGDY DQFQHF	2205	CASSKSGDY DIPQHF	2452	CASSPSGDY DQYLHF
1959	CASSPSGDY DQGQHF	2206	CASSKSGDY DQAQHF	2453	CASSPSGDY DTQQHF
1960	CASSPSGDY DQHQHF	2207	CASSKSGDY DQCQHF	2454	CASSPSGDY DYPFHF
1961	CASSPSGDY DQIQHF	2208	CASSKSGDY DQDQHF	2455	CASSPSGDY DYPGHF
1962	CASSPSGDY DQKQHF	2209	CASSKSGDY DQFQHF	2456	CASSPSGDY EQGQHF
1963	CASSPSGDY DQLQHF	2210	CASSKSGDY DQIQHF	2457	CASSPSGDY GDPQHF
1964	CASSPSGDY DQMQHF	2211	CASSKSGDY DQLQHF	2458	CASSPSGDY GQHQHF
1965	CASSPSGDY DQPAHF	2212	CASSKSGDY DQMQHF	2459	CASSPSGDY GQPSHF
1966	CASSPSGDY DQPCHF	2213	CASSKSGDY DQPKHF	2460	CASSPSGDY GQPTHF
1967	CASSPSGDY DQPGHF	2214	CASSKSGDY DQPVHF	2461	CASSPSGDYI QPTHF
1968	CASSPSGDY DQPHHF	2215	CASSKSGDY DQQQHF	2462	CASSPSGDY KNPQHF
1969	CASSPSGDY DQPMHF	2216	CASSKSGDY DQSQHF	2463	CASSPSGDY MQPTHF
1970	CASSPSGDY DQPNHF	2217	CASSKSGDY DQTQHF	2464	CASSPSGDY NQDQHF

1971	CASSPSGDY DQPPHF	2218	CASSKSGDY DQWQHF	2465	CASSPSGDY NQIQHF
1972	CASSPSGDY DQPSHF	2219	CASSKSGDY NQPQHF	2466	CASSPSGDY NQKQHF
1973	CASSPSGDY DQPVHF	2220	CASSLSGDA DQPQHF	2467	CASSPSGDY NQRQHF
1974	CASSPSGDY DQRQHF	2221	CASSLSGDF DQPQHF	2468	CASSPSGDY NQYQHF
1975	CASSPSGDY DQSQHF	2222	CASSLSGDY DEPQHF	2469	CASSPSGDY PQLQHF
1976	CASSPSGDY DQTQHF	2223	CASSLSGDY DHPQHF	2470	CASSPSGDY PQRQHF
1977	CASSPSGDY DQVQHF	2224	CASSLSGDY DLPQHF	2471	CASSPSGDY QPPQHF
1978	CASSPSGDY DQYQHF	2225	CASSLSGDY DMPQHF	2472	CASSPSGDY RQPGHF
1979	CASSPSGDY DVPQHF	2226	CASSLSGDY DNPQHF	2473	CASSPSGDY SQPGHF
1980	CASSPSGDY DYPQHF	2227	CASSLSGDY DQAQHF	2474	CASSPSGDY WQPSHF
1981	CASSPSGDY NQPQHF	2228	CASSLSGDY DQDQHF	2475	CASSPSGFV DQPQHF
1982	CASSQSGDY DQPQHF	2229	CASSLSGDY DQEQHF	2476	CASSPSGFYL QPQHF
1983	CASSRSGDY DQPQHF	2230	CASSLSGDY DQGQHF	2477	CASSPSGHY DQPHHF
1984	CASSSSGDY DQPQHF	2231	CASSLSGDY DQIQHF	2478	CASSPSGIGD QPQHF
1985	CASSTSGDY DQPQHF	2232	CASSLSGDY DQKQHF	2479	CASSPSGMY DQPAHF
1986	CASSVSGDY DQPQHF	2233	CASSLSGDY DQLQHF	2480	CASSPSGMY SQPQHF
1987	CASSWSGDY DQPQHF	2234	CASSLSGDY DQPEHF	2481	CASSPSGNY DQPAHF
1988	CASSYSGDY DQPQHF	2235	CASSLSGDY DQPLHF	2482	CASSPSGPE DQPQHF

1989	CASTPSGDY DQPQHF	2236	CASSLSGDY DQPNHF	2483	CASSPSGPY DQGQHF
1990	CASAESGDY DQPQHF	2237	CASSLSGDY DQPPHF	2484	CASSPSGPY DQRQHF
1991	CASAHSGDY DQPQHF	2238	CASSLSGDY DQPSHF	2485	CASSPSGQY TQPQHF
1992	CASAISGDYD QPQHF	2239	CASSLSGDY DQPVHF	2486	CASSPSGRY DQYQHF
1993	CASAKSGDY DQPQHF	2240	CASSLSGDY DQQQHF	2487	CASSPSGTID QPQHF
1994	CASAPSGDY DPPQHF	2241	CASSLSGDY DQRQHF	2488	CASSPSGTW DQPQHF
1995	CASAPSGDY DQCQHF	2242	CASSLSGDY DQVQHF	2489	CASSPSGVF DQPQHF
1996	CASAPSGDY DQDQHF	2243	CASSLSGDY DQYQHF	2490	CASSPSGVY CQPQHF
1997	CASAPSGDY DQFQHF	2244	CASSLSGDY DVPQHF	2491	CASSPSGYYI QPQHF
1998	CASAPSGDY DQKQHF	2245	CASSMSGDF DQPQHF	2492	CASSPSGYYL QPQHF
1999	CASAPSGDY DQLQHF	2246	CASSMSGDS DQPQHF	2493	CASSPSHDY DYPQHF
2000	CASAPSGDY DQPAHF	2247	CASSMSGDW DQPQHF	2494	CASSPSHDY VQPQHF
2001	CASAPSGDY DQPEHF	2248	CASSMSGDY DHPQHF	2495	CASSPSIDQD QPQHF
2002	CASAPSGDY DQPHHF	2249	CASSMSGDY DQGQHF	2496	CASSPSIDYQ QPQHF
2003	CASAPSGDY DQPNHF	2250	CASSMSGDY DQNQHF	2497	CASSPSKDA DQPQHF
2004	CASAPSGDY DQPPHF	2251	CASSMSGDY DQPCHF	2498	CASSPSKDY DQRQHF
2005	CASAPSGDY DQQQHF	2252	CASSMSGDY DQPLHF	2499	CASSPSLLYD QPQHF
2006	CASAPSGDY DQSQHF	2253	CASSMSGDY DQPSHF	2500	CASSPSMDY DVPQHF

2007	CASAPSGDY DQTQHF	2254	CASSMSGDY DQPTHF	2501	CASSPSNFY DQPQHF
2008	CASAQSGDY DQPQHF	2255	CASSMSGDY DQRQHF	2502	CASSPSRDY DMPQHF
2009	CASARSGDY DQPQHF	2256	CASSMSGDY DQSQHF	2503	CASSPSRDY DQPTHF
2010	CASASSGDY DQPQHF	2257	CASSMSGDY DVPQHF	2504	CASSPSRDY DQPYHF
2011	CASAVSGDY DQPQHF	2258	CASSMSGDY DWPQHF	2505	CASSPSRDY DQRQHF
2012	CASCPSGDS DQPQHF	2259	CASSNSGDF DQPQHF	2506	CASSPSSIYD QPQHF
2013	CASCPSGDY DPPQHF	2260	CASSNSGDM DQPQHF	2507	CASSPSTCY DQPQHF
2014	CASCPSGDY DQKQHF	2261	CASSNSGDY DAPQHF	2508	CASSPSWDY DQPHHF
2015	CASCPSGDY DQPPHF	2262	CASSNSGDY DHPQHF	2509	CASSPSYDY DCPQHF
2016	CASCPSGDY DQTQHF	2263	CASSNSGDY DQE QHF	2510	CASSPSYDYL QPQHF
2017	CASCPSGDY QQPQHF	2264	CASSNSGDY DQFQHF	2511	CASSPTGDY DQPCHF
2018	CASCPSGDY VQPQHF	2265	CASSNSGDY DQIQHF	2512	CASSPTGDY DQPGHF
2019	CASCPSGLY DQPQHF	2266	CASSNSGDY DQPAHF	2513	CASSPTNDY DQPQHF
2020	CASCVSGDY DQPQHF	2267	CASSNSGDY DQPSHF	2514	CASSPVGDY DKPQHF
2021	CASCYSGDY DQPQHF	2268	CASSNSGDY DQVQHF	2515	CASSPVGDY DQPRHF
2022	CASDLSGDY DQPQHF	2269	CASSPAGDY DDPQHF	2516	CASSPYGDY DPPQHF
2023	CASDPSGDY DQVQHF	2270	CASSPAGDY DMPQHF	2517	CASSPYGYY DQPQHF
2024	CASDPSGDY TQPQHF	2271	CASSPAGDY DQH QHF	2518	CASSQAGDY DQPQHF

2025	CASDSSGDY DQPQHF	2272	CASSPAGDY DQLQHF	2519	CASSQSGDA DQPQHF
2026	CASEPAGDY DQPQHF	2273	CASSPAGDY DQPEHF	2520	CASSQSGDY DEPQHF
2027	CASEPSEDY DQPQHF	2274	CASSPAGDY DQVQHF	2521	CASSQSGDY DMPQHF
2028	CASEPSGDG DQPQHF	2275	CASSPAGDY DQYQHF	2522	CASSQSGDY DQAQHF
2029	CASEPSGDY DQSQHF	2276	CASSPAGPY DQPQHF	2523	CASSQSGDY DQEQHF
2030	CASEPSGDY PQPQHF	2277	CASSPCGDY DQPMHF	2524	CASSQSGDY DQGQHF
2031	CASFPQGDY DQPQHF	2278	CASSPCGHY DQPQHF	2525	CASSQSGDY DQHQHF
2032	CASFPRGDY DQPQHF	2279	CASSPDGDY DAPQHF	2526	CASSQSGDY DQIQHF
2033	CASFPSGDM DQPQHF	2280	CASSPDGDY DGPQHF	2527	CASSQSGDY DQLQHF
2034	CASGCSGDY DQPQHF	2281	CASSPEGDY DCPQHF	2528	CASSQSGDY DQMQHF
2035	CASGESGDY DQPQHF	2282	CASSPFGDT DQPQHF	2529	CASSQSGDY DQNQHF
2036	CASGPSGDF DQPQHF	2283	CASSPFGDY DMPQHF	2530	CASSQSGDY DQPNHF
2037	CASGPSGDY DQDQHF	2284	CASSPGGDY DQPCHF	2531	CASSQSGDY DQPSHF
2038	CASGPSGDY DQEQHF	2285	CASSPGGDY HQPQHF	2532	CASSQSGDY DQQQHF
2039	CASGPSGDY DQLQHF	2286	CASSPHDDY DQPQHF	2533	CASSQSGDY DQRQHF
2040	CASGPSGDY DQPIHF	2287	CASSPIADYD QPQHF	2534	CASSQSGDY DQSQHF
2041	CASGPSGDY DQPPHF	2288	CASSPIGDYQ QPQHF	2535	CASSQSGDY DQTQHF
2042	CASGPSGDY DQTQHF	2289	CASSPILDYD QPQHF	2536	CASSQSGDY DQVQHF

2043	CASGVSGDY DQPQHF	2290	CASSPKG DY DIPQHF	2537	CASSQSGDY DVPQHF
2044	CASHPMGDY DQPQHF	2291	CASSPKG DY VQPQHF	2538	CASSQSGDY NQPQHF
2045	CASHPSGDY DQGQHF	2292	CASSPNGCY DQPQHF	2539	CASSQTGDY DQPQHF
2046	CASIPSGDED QPQHF	2293	CASSPPGDS DQPQHF	2540	CASSRAGDY DQPQHF
2047	CASIPSGDYD QCQHF	2294	CASSPPGDY DPPQHF	2541	CASSRSGDA DQPQHF
2048	CASKPHGDY DQPQHF	2295	CASSPPGNY DQPQHF	2542	CASSRSGDG DQPQHF
2049	CASKPSGDY DQVQHF	2296	CASSPQGAY DQPQHF	2543	CASSRSGDY DHPQHF
2050	CASKPSGDY YQPQHF	2297	CASSPQGPY DQPQHF	2544	CASSRSGDY DQAQHF
2051	CASLPSGDQ DQPQHF	2298	CASSPQKDY DQPQHF	2545	CASSRSGDY DQFQHF
2052	CASLPSGDY MQPQHF	2299	CASSPRGDY FQPQHF	2546	CASSRSGDY DQIQHF
2053	CASLPSGRY DQPQHF	2300	CASSPRGDY NQPQHF	2547	CASSRSGDY DQLQHF
2054	CASMNSGDY DQPQHF	2301	CASSPRWDY DQPQHF	2548	CASSRSGDY DQPAHF
2055	CASMPSGDY DQE QHF	2302	CASSPSACY DQPQHF	2549	CASSRSGDY DQPCHF
2056	CASMPSGDY DQPDHF	2303	CASSPSADY DQTQHF	2550	CASSRSGDY DQPHHF
2057	CASMPSGDY DQS QHF	2304	CASSPSADYI QPQHF	2551	CASSRSGDY DQPPHF
2058	CASNESGDY DQPQHF	2305	CASSPSDDY DQGQHF	2552	CASSRSGDY DQPSHF
2059	CASNPKG DY DQPQHF	2306	CASSPSDDY DQLQHF	2553	CASSRSGDY DQRQHF
2060	CASNPSGDG DQPQHF	2307	CASSPSEDP DQPQHF	2554	CASSRSGDY DQS QHF

2061	CASNPSGDY DQPCHF	2308	CASSPSFDY DQNQHF	2555	CASSRSGDY DQTQHF
2062	CASNPSGDY DQPTHF	2309	CASSPSFDY DQPFHF	2556	CASSRSGDY DTPQHF
2063	CASNPSGDY DQVQHF	2310	CASSPSFPYD QPQHF	2557	CASSRSGDY DYPQHF
2064	CASPPSGDY DQWQHF	2311	CASSPSGAY CQPQHF	2558	CASSSSGDF DQPQHF
2065	CASQPSGDY DQPLHF	2312	CASSPSGAY DCPQHF	2559	CASSSSGDP DQPQHF
2066	CASQPSGDY DQPPHF	2313	CASSPSGCY DQPLHF	2560	CASSSSGDY DHPQHF
2067	CASQPSGDY DQPSHF	2314	CASSPSGCY TQPQHF	2561	CASSSSGDY DKPQHF
2068	CASQPSGDY DQPTHF	2315	CASSPSGDA DQEQHF	2562	CASSSSGDY DMPQHF
2069	CASQPSGDY DWPQHF	2316	CASSPSGDA DQFQHF	2563	CASSSSGDY DQHQHF
2070	CASQPSGDY YQPQHF	2317	CASSPSGDA DQGQHF	2564	CASSSSGDY DQKQHF
2071	CASRPSGDQ DQPQHF	2318	CASSPSGDA DQHQHF	2565	CASSSSGDY DQLQHF
2072	CASRPSGDS DQPQHF	2319	CASSPSGDA DQKQHF	2566	CASSSSGDY DQMQHF
2073	CASRPSGDY DQKQHF	2320	CASSPSGDA DQPSHF	2567	CASSSSGDY DQNQHF
2074	CASRPSGDY DQPAHF	2321	CASSPSGDA DQQQHF	2568	CASSSSGDY DQPDHF
2075	CASRPSGDY DQPNHF	2322	CASSPSGDA DQRQHF	2569	CASSSSGDY DQPHHF
2076	CASRPSGFY DQPQHF	2323	CASSPSGDA DQSQHF	2570	CASSSSGDY DQPNHF
2077	CASSAAGDY DQPQHF	2324	CASSPSGDA DQVQHF	2571	CASSSSGDY DQPPHF
2078	CASSASGDG DQPQHF	2325	CASSPSGDA DQWQHF	2572	CASSSSGDY DQPSHF

2079	CASSASGDW DQPQHF	2326	CASSPSGDD DQSQHF	2573	CASSSSGDY DQPTHF
2080	CASSASGDY DIPQHF	2327	CASSPSGDF DIPQHF	2574	CASSSSGDY DQQQHF
2081	CASSASGDY DQDQHF	2328	CASSPSGDF DQAQHF	2575	CASSSSGDY DQRQHF
2082	CASSASGDY DQGQHF	2329	CASSPSGDF DQDQHF	2576	CASSSSGDY DQSQHF
2083	CASSASGDY DQPAHF	2330	CASSPSGDF DQGQHF	2577	CASSSYGDY DQPQHF
2084	CASSASGDY DQPCHF	2331	CASSPSGDF DQIQHF	2578	CASSTSGDF DQPQHF
2085	CASSASGDY DQPHHF	2332	CASSPSGDF DQLQHF	2579	CASSTSGDG DQPQHF
2086	CASSASGDY DQPKHF	2333	CASSPSGDF DQMQUHF	2580	CASSTSGDY DAPQHF
2087	CASSASGDY DQPNHF	2334	CASSPSGDF DQNQHF	2581	CASSTSGDY DEPQHF
2088	CASSASGDY DQPPHF	2335	CASSPSGDF DQPEHF	2582	CASSTSGDY DQAQHF
2089	CASSASGDY DQPSHF	2336	CASSPSGDF DQPFHF	2583	CASSTSGDY DQCQHF
2090	CASSASGDY DQQQHF	2337	CASSPSGDF DQPGHF	2584	CASSTSGDY DQKQHF
2091	CASSASGDY DQRQHF	2338	CASSPSGDF DQPMHF	2585	CASSTSGDY DQLQHF
2092	CASSASGDY DQSQHF	2339	CASSPSGDF DQPNHF	2586	CASSTSGDY DQPHHF
2093	CASSASGDY DQVQHF	2340	CASSPSGDF DQPSHF	2587	CASSTSGDY DQPKHF
2094	CASSASGDY DQYQHF	2341	CASSPSGDF DQRQHF	2588	CASSTSGDY DQPLHF
2095	CASSCCGDY DQPQHF	2342	CASSPSGDF DQVQHF	2589	CASSTSGDY DQPNHF
2096	CASSCSGDF DQPQHF	2343	CASSPSGDF DQYQHF	2590	CASSTSGDY DQPSHF

2097	CASSCSGDY DQEQHF	2344	CASSPSGDF DTPQHF	2591	CASSTSGDY DQPVHF
2098	CASSCSGDY DQHGHF	2345	CASSPSGDF NQPQHF	2592	CASSTSGDY DQYQHF
2099	CASSCSGDY DQIQHF	2346	CASSPSGDF VQPQHF	2593	CASSTSGDY DTPQHF
2100	CASSCSGDY DQLQHF	2347	CASSPSGDG DQCQHF	2594	CASSTYGDY DQPQHF
2101	CASSCSGDY DQPPHF	2348	CASSPSGDG DQFQHF	2595	CASSVSGDF DQPQHF
2102	CASSCSGDY DQPSHF	2349	CASSPSGDG DQKQHF	2596	CASSVSGDW DQPQHF
2103	CASSCSGDY DQPTHF	2350	CASSPSGDG DQSQHF	2597	CASSVSGDY DDPQHF
2104	CASSCSGDY DQQQHF	2351	CASSPSGDG DQTQHF	2598	CASSVSGDY DEPQHF
2105	CASSCSGDY NQPQHF	2352	CASSPSGDG DQVQHF	2599	CASSVSGDY DGPQHF
2106	CASSDSGDA DQPQHF	2353	CASSPSGDH DQIQHF	2600	CASSVSGDY DIPQHF
2107	CASSDSGDF DQPQHF	2354	CASSPSGDH DQPSHF	2601	CASSVSGDY DMPQHF
2108	CASSDSGDY DCPQHF	2355	CASSPSGDHI QPQHF	2602	CASSVSGDY DQAQHF
2109	CASSDSGDY DMPQHF	2356	CASSPSGDM DQAQHF	2603	CASSVSGDY DQCQHF
2110	CASSDSGDY DQEQHF	2357	CASSPSGDM DQRQHF	2604	CASSVSGDY DQFQHF
2111	CASSDSGDY DQIQHF	2358	CASSPSGDM NQPQHF	2605	CASSVSGDY DQIQHF
2112	CASSDSGDY DQNHQHF	2359	CASSPSGDN DGPQHF	2606	CASSVSGDY DQKQHF
2113	CASSDSGDY DQPLHF	2360	CASSPSGDP DQEQHF	2607	CASSVSGDY DQLQHF
2114	CASSDSGDY DQPTHF	2361	CASSPSGDP DQRQHF	2608	CASSVSGDY DQPEHF

2115	CASSDSGDY DQPWHF	2362	CASSPSGDP DQWQHF	2609	CASSVSGDY DQPNHF
2116	CASSDSGDY DQYQHF	2363	CASSPSGDP NQPQHF	2610	CASSVSGDY DQPVHF
2117	CASSDSGDY DRPQHF	2364	CASSPSGDS DQWQHF	2611	CASSVSGDY DQQQHF
2118	CASSDSGNY DQPQHF	2365	CASSPSGDT DMPQHF	2612	CASSVSGDY DQRQHF
2119	CASSDSGWY DQPQHF	2366	CASSPSGDV DQEQHF	2613	CASSVSGDY DQTQHF
2120	CASSESGDA DQPQHF	2367	CASSPSGDW DQAQHF	2614	CASSVSGDY DTPQHF
2121	CASSESGDF DQPQHF	2368	CASSPSGDW DQGQHF	2615	CASSVSGDY DYPQHF
2122	CASSESGDY DMPQHF	2369	CASSPSGDW DQIQHF	2616	CASSVSGDY NQPQHF
2123	CASSESGDY DQAQHF	2370	CASSPSGDW DQKQHF	2617	CASSVSLDYG QPQHF
2124	CASSESGDY DQEQHF	2371	CASSPSGDW DQPCHF	2618	CASSWAGDY DQPQHF
2125	CASSESGDY DQFQHF	2372	CASSPSGDW DQPSHF	2619	CASSWSGDC DQPQHF
2126	CASSESGDY DQHQHF	2373	CASSPSGDW DQQQHF	2620	CASSWSGDF DQPQHF
2127	CASSESGDY DQIQHF	2374	CASSPSGDW DQRQHF	2621	CASSWSGDH DQPQHF
2128	CASSESGDY DQKQHF	2375	CASSPSGDW DQSQHF	2622	CASSWSGDM DQPQHF
2129	CASSESGDY DQLQHF	2376	CASSPSGDY AQPLHF	2623	CASSWSGDP DQPQHF
2130	CASSESGDY DQMQHF	2377	CASSPSGDY CQPPHF	2624	CASSWSGDQ DQPQHF
2131	CASSESGDY DQNPQHF	2378	CASSPSGDY CQYQHF	2625	CASSWSGD WDQPQHF
2132	CASSESGDY DQPAHF	2379	CASSPSGDY DANQHF	2626	CASSWSGDY DLPQHF

2133	CASSESGDY DQPTHF	2380	CASSPSGDY DAPGHF	2627	CASSWSGDY DQHQHF
2134	CASSESGDY DQPVHF	2381	CASSPSGDY DEKQHF	2628	CASSWSGDY DQIQHF
2135	CASSESGDY DQRQHF	2382	CASSPSGDY DFEQHF	2629	CASSWSGDY DQPCHF
2136	CASSESGDY DQTQHF	2383	CASSPSGDY DFIQHF	2630	CASSWSGDY DQPSHF
2137	CASSFSGDY DQPQHF	2384	CASSPSGDY DFKQHF	2631	CASSWSGDY DQPTHF
2138	CASSFSGDY DEPQHF	2385	CASSPSGDY DFQQHF	2632	CASSWSGDY DQVQHF
2139	CASSFSGDY DLPQHF	2386	CASSPSGDY DHAQHF	2633	CASSWSGDY DTPQHF
2140	CASSFSGDY DMPQHF	2387	CASSPSGDY DHHQHF	2634	CASSWSGDY QQPQHF
2141	CASSFSGDY DNPQHF	2388	CASSPSGDY DHIQHF	2635	CASSWSGGY DQPQHF
2142	CASSFSGDY DQAQHF	2389	CASSPSGDY DHLQHF	2636	CASSYSGDY DQPQHF
2143	CASSFSGDY DQCQHF	2390	CASSPSGDY DHPCHF	2637	CASSYSGDY DAPQHF
2144	CASSFSGDY DQDQHF	2391	CASSPSGDY DHPSHF	2638	CASSYSGDY DLPQHF
2145	CASSFSGDY DQHQHF	2392	CASSPSGDY DHYQHF	2639	CASSYSGDY DQDQHF
2146	CASSFSGDY DQIQHF	2393	CASSPSGDY DIPEHF	2640	CASSYSGDY DQGQHF
2147	CASSFSGDY DQLQHF	2394	CASSPSGDY DIPIHF	2641	CASSYSGDY DQIQHF
2148	CASSFSGDY DQM QHF	2395	CASSPSGDY DITQHF	2642	CASSYSGDY DQLQHF
2149	CASSFSGDY DQNQHF	2396	CASSPSGDY DLIQHF	2643	CASSYSGDY DQNQHF
2150	CASSFSGDY DQPAHF	2397	CASSPSGDY DLPFHF	2644	CASSYSGDY DQPAHF

2151	CASSFSGDY DQPPHF	2398	CASSPSGDY DLTQHF	2645	CASSYSGDY DQPCHF
2152	CASSFSGDY DQPSHF	2399	CASSPSGDY DMEQHF	2646	CASSYSGDY DQPHHF
2153	CASSFSGDY DQSQHF	2400	CASSPSGDY DMKQHF	2647	CASSYSGDY DQSQHF
2154	CASSFSGDY DVPQHF	2401	CASSPSGDY DMMQHF	2648	CASSYSGDY DQVQHF
2155	CASSFSGDY NQPQHF	2402	CASSPSGDY DMPNHF	2649	CASSYSGDY DTPQHF
2156	CASSGAGDY DQPQHF	2403	CASSPSGDY DMPSHF	2650	CASSYSGDY DVPQHF
2157	CASSGSGDY DEPQHF	2404	CASSPSGDY DMTQHF	2651	CASTGSGDY DQPQHF
2158	CASSGSGDY DMPQHF	2405	CASSPSGDY DMVQHF	2652	CASTISGDYD QPQHF
2159	CASSGSGDY DQHQHF	2406	CASSPSGDY DNEQHF	2653	CASTPSGAY DQPQHF
2160	CASSGSGDY DQVQHF	2407	CASSPSGDY DNIQHF	2654	CASTPSGDC DQPQHF
2161	CASSHAGDY DQPQHF	2408	CASSPSGDY DNKQHF	2655	CASTPSGDY DQFQHF
2162	CASSHSGDA DQPQHF	2409	CASSPSGDY DNVQHF	2656	CASTPSGDY DQKQHF
2163	CASSHSGDF DQPQHF	2410	CASSPSGDY DPEQHF	2657	CASTPSGDY DQMQHF
2164	CASSHSGDP DQPQHF	2411	CASSPSGDY DQAAHF	2658	CASTPSGDY DQPAHF
2165	CASSHSGDY DHPQHF	2412	CASSPSGDY DQACHF	2659	CASTPSGDY DQTQHF
2166	CASSHSGDY DLPQHF	2413	CASSPSGDY DQAEHF	2660	CASTPSGDY DQVQHF
2167	CASSHSGDY DNPQHF	2414	CASSPSGDY DQAIHF	2661	CASTPSGDY DQYQHF
2168	CASSHSGDY DQDQHF	2415	CASSPSGDY DQAMHF	2662	CASTQSGDY DQPQHF

2169	CASSHSGDY DQIQHF	2416	CASSPSGDY DQAPHF	2663	CASTRSGDY DQPQHF
2170	CASSHSGDY DQKQHF	2417	CASSPSGDY DQASHF	2664	CASVKSGDY DQPQHF
2171	CASSHSGDY DQMQUHF	2418	CASSPSGDY DQDAHF	2665	CASVPSDDY DQPQHF
2172	CASSHSGDY DQPEHF	2419	CASSPSGDY DQDCHF	2666	CASVPSWDY DQPQHF
2173	CASSHSGDY DQPHHF	2420	CASSPSGDY DQEAHF	2667	CASVPVGDY DQPQHF
2174	CASSHSGDY DQPPHF	2421	CASSPSGDY DQESHF	2668	CASWLSGDY DQPQHF
2175	CASSHSGDY DQPVHF	2422	CASSPSGDY DQFNHF	2669	CASWPSGDY DQSQHF
2176	CASSHSGDY DQVQHF	2423	CASSPSGDY DQGEHF	2670	CASYPDGDY DQPQHF
2177	CASSHSGDY DQYQHF	2424	CASSPSGDY DQGSHF	2671	CASYPSGDY CQPQHF
2178	CASSHSGDY DWPQHF	2425	CASSPSGDY DQHAHF	2672	CASYPSGDY QQPQHF

Equations

Equation 1

5

$$\text{Frequency}_{CDR3} = \frac{\text{Counts of a CDR3 variant}}{\text{Total counts in a sample}}$$

Equation 2

10

$$\text{Enrichment} = \frac{\text{Frequency}_{CDR3} \text{ Sorting condition}}{\text{Frequency}_{CDR3} \text{ RL}}$$

In conclusion, we have identified 1748 CDR3 alpha chain variants (SEQ ID NO: 184 – SEQ ID NO: 1931, Table 28) and 741 CDR3 beta chain variants (SEQ ID NO: 1932 – SEQ ID NO: 2672, Table 29) of the parental TCR-1. These are all valid candidates to pair with each other or one of the native TCR1 chains to make a TCR of improved properties.

Example 8. Sequence logo analysis of diversified and selected TRA and TRB CDR3 regions of TCR-1.

To illustrate the level of conservation in the candidates generated and selected in Example 7, sequence logos were generated (figure 13) demonstrating that certain residues are represented with a higher frequency in the diversified sequences (frequency plots, panel A (TCR alpha) and B (TCR beta), and that certain residues are absent from the diversified sequences (absence plots, panel A (TCR alpha) and B (TCR beta)).

Logos were generated using custom python scripts in combination with a package called logomaker. Specifically, the logos were computed by generating a counts matrix for each position for each amino acid. The counts matrix was then used as a basis to generate the different logos. For bitscores, counts data was converted into a bitscore. For frequency the counts were converted to frequency of amino acid at the specific position. For absence, each amino acid not present in the counts at the position was assigned a value of 1 and the scale was summed. Once the data is appropriately transformed from the counts the new matrix was submitted to the logomaker functions to generate images.

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Items I

- 5 1. A T-cell receptor (TCR), which is isolated and/or expressed in an engineered cell, specifically recognizing a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or

10 VVGAVGVGK (SEQ ID NO:2) presented in a HLA-A*11 complex, and wherein the TCR comprises a paired TCR alpha- and TCR beta-chain, wherein the TCR alpha chain variable domain comprises SC1alpha-CDR1alpha-SC2alpha-CDR2alpha-SC3alpha-CDR3alpha-SC4alpha-SC5alpha, and the TCR beta chain variable

15 domain comprises SC1beta-CDR1beta-SC2 beta-CDR2 beta-SC3beta-CDR3beta-SC4beta-SC5beta, wherein SC is a scaffold region and CDR is a complementarity determining region.

2 2. The TCR according to item 1, wherein the TCR, when expressed in an engineered cell, does not cause specific cytotoxic activity towards cells not expressing a G12V mutated RAS peptide.

20 3. The TCR according to any of items 1 to 2, wherein the TCR, when expressed in an engineered cell, does not cause specific cytotoxic activity towards cells expressing the wild type human RAS peptide, such as VVGAGGVGK (SEQ ID NO: 3) /or VVGAGGVGK (SEQ ID NO: 4).

25 4. The TCR according to any of the preceding items, wherein the TCR, when expressed in an engineered cell, does not specifically recognize cells expressing the G12V mutated human RAS peptide, wherein the peptide is VVGAVGVGK (SEQ ID NO: 1) and/ or VVGAVGVGK (SEQ ID NO: 2) presented in an allelic variant of the human leukocyte antigen (HLA) molecule other than an allelic variant of the human leukocyte antigen (HLA) molecule selected from the group consisting

30 of HLA-A*11, HLA-A*30:01, HLA-A*68:02, HLA-B*54:01, HLA-B*35:01, HLA-B*35:02, HLA-B*35:03, HLA-B*35:05, HLA-B*35:12, HLA-B*52:01, HLA-B*53:01, HLA-B*57:01 and HLA-B*58:01 serotype.

35 5. The TCR according to any one of the preceding items wherein the TCR when expressed in an engineered cell does not specifically recognise other allelic variants of the human leukocyte antigen (HLA) molecule other than an allelic variant of the human leukocyte antigen (HLA) molecule selected from the group consisting of HLA-A*68:02, HLA-B*54:01, HLA-B*35:01, HLA-B*35:02, HLA-B*35:03, HLA-B*35:12, HLA-B*57:01 and HLA-B*58:01 serotype.

6. The TCR according to any of the preceding items, wherein the TCR, when expressed in an engineered cell, does not specifically recognize the G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) presented in an allelic variant of the human leukocyte antigen (HLA) molecule other than the HLA-A*11 serotype.
7. The TCR according to item 1, wherein the TCR does not specifically recognize a G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/ or VVGAVGVGK (SEQ ID NO: 2) presented in a HLA-A*11:03 complex
8. The TCR according to claim 1, wherein the TCR does not specifically recognize a G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/ or VVGAVGVGK (SEQ ID NO: 2) presented in a HLA-A*11:03 and HLA-A*11:04 complex.
9. The TCR according to any of the preceding items, wherein the TCR, when expressed in an engineered cell, has at the most 4% cross-reactivity towards any somatically expressed HLA-presented peptide other than a G12V mutated human rat sarcoma (RAS) peptide, which consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) and which is presented in a HLA-A*11 complex in a cross-reactivity assay.
10. The TCR according to any of the preceding items comprising:
- a TCR alpha chain CDR3 sequence with at least 75% identity to a TCR alpha chain CDR3 sequence selected from the group consisting of SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 98, SEQ ID NO: 141 and SEQ ID NO: 143
 - and
 - a TCR beta chain CDR3 sequence with at least 80% identity to a TCR beta chain CDR3 sequence selected from the group consisting of SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 99, SEQ ID NO: 142 and SEQ ID NO: 144.
11. The TCR according to any of the preceding items comprising:
- a TCR alpha chain CDR3 sequence with a TCR alpha chain CDR3 sequence selected from the group consisting of SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 98, SEQ ID NO: 141 and SEQ ID NO: 143
 - and
 - a TCR beta chain CDR3 sequence with a TCR beta chain CDR3 sequence selected from the group consisting of SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 99, SEQ ID NO: 142 and SEQ ID NO: 144

12. The TCR according to any one of the preceding items, wherein the TCR comprises a paired TCR alpha- and TCR beta-chain comprising a complete assembly of combinations of the following sequences:

	SEQ ID NO	SEQ ID NO	SEQ ID NO	SEQ ID NO	SEQ ID NO	SEQ ID NO	SEQ ID NO
SC1alpha	18	20	28	126	128	18	20
CDR1alpha	60	62	70	135	137	60	62
SC2alpha	32	34	42	129	131	32	34
CDR2alpha	74	76	84	138	140	74	76
SC3alpha	46	48	56	132	134	46	48
CDR3alpha	88	90	98	141	143	88	90
SC4alpha	7	9	15	123	125	7	9
SC5alpha	104	104	104	104	104	104	104
SC1beta	19	21	29	127	30	19	21
CDR1beta	61	63	71	136	72	61	63
SC2beta	33	35	43	130	44	33	35
CDR2beta	75	77	85	139	86	75	77
SC3beta	47	49	57	133	58	47	49
CDR3beta	89	91	99	142	144	89	91
SC4beta	8	10	16	124	12	8	10
SC5beta	105	105	106	106	106	5162	5162

5

or sequences having at least 90% such as at least 95%, at least 97% or at least 99%, or 100% identity to said respective sequences.

- 10 13. The TCR according to any of the preceding items, wherein the TCR comprises a paired TCR alpha- and TCR beta-chain comprising the following combination of sequences:

TCR#	SC1	CDR1	SC2	CDR2	SC3	CDR3	SC4	SC5
TCR-1	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
alpha	NO: 18	NO: 60	NO: 32	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				74	46	88	7	104
TCR-2	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
alpha	NO: 20	NO: 62	NO: 34	ID	ID	ID	ID	ID

				NO: 76	NO: 48	NO: 90	NO: 9	NO: 104
TCR-6 alpha	SEQ ID NO: 28	SEQ ID NO: 70	SEQ ID NO: 42	SEQ ID NO: 84	SEQ ID NO: 56	SEQ ID NO: 98	SEQ ID NO: 15	SEQ ID NO: 104
TCR-9 alpha	SEQ ID NO: 126	SEQ ID NO: 135	SEQ ID NO: 129	SEQ ID NO: 138	SEQ ID NO: 132	SEQ ID NO: 141	SEQ ID NO: 123	SEQ ID NO: 104
TCR-10 alpha	SEQ ID NO: 128	SEQ ID NO: 137	SEQ ID NO: 131	SEQ ID NO: 140	SEQ ID NO: 134	SEQ ID NO: 143	SEQ ID NO: 125	SEQ ID NO: 104
TCR-1 beta	SEQ ID NO: 19	SEQ ID NO: 61	SEQ ID NO: 33	SEQ ID NO: 75	SEQ ID NO: 47	SEQ ID NO: 89	SEQ ID NO: 8	SEQ ID NO: 105
TCR-1 beta	SEQ ID NO: 19	SEQ ID NO: 61	SEQ ID NO: 33	SEQ ID NO: 75	SEQ ID NO: 47	SEQ ID NO: 89	SEQ ID NO: 8	SEQ ID NO: 5162
TCR-2 beta	SEQ ID NO: 21	SEQ ID NO: 63	SEQ ID NO: 35	SEQ ID NO: 77	SEQ ID NO: 49	SEQ ID NO: 91	SEQ ID NO: 10	SEQ ID NO: 105
TCR-2 beta	SEQ ID NO: 21	SEQ ID NO: 63	SEQ ID NO: 35	SEQ ID NO: 77	SEQ ID NO: 49	SEQ ID NO: 91	SEQ ID NO: 10	SEQ ID NO: 5162
TCR-6 beta	SEQ ID NO: 29	SEQ ID NO: 71	SEQ ID NO: 43	SEQ ID NO: 85	SEQ ID NO: 57	SEQ ID NO: 99	SEQ ID NO: 16	SEQ ID NO: 106

TCR-9	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SE
beta	NO: 127	NO: 136	NO: 130	ID NO: 139	ID NO: 133	ID NO: 142	ID NO: 124	ID NO: 106
TCR-10	SEQ ID NO: 30	SEQ ID NO: 72	SEQ ID NO: 44	SEQ ID NO: 86	SEQ ID NO: 58	SEQ ID NO: 144	SEQ ID NO: 12	SEQ ID NO: 106
beta								

or sequences having at least 90% such as at least 95%, at least 97% or at least 99%, or 100% identity to said respective sequences.

5

14. The TCR according to any of the preceding items, wherein the alpha and beta chain CDRs have the following sequences:

CDR1 alpha	CDR2 alpha	CDR3 alpha	CDR1 beta	CDR2 beta	CDR3 beta
SEQ ID NO: 60	SEQ ID NO: 74	SEQ ID NO: 88	SEQ ID NO: 61	SEQ ID NO: 75	SEQ ID NO: 89
SEQ ID NO: 62	SEQ ID NO: 76	SEQ ID NO: 90	SEQ ID NO: 63	SEQ ID NO: 77	SEQ ID NO: 91
SEQ ID NO: 70	SEQ ID NO: 84	SEQ ID NO: 98	SEQ ID NO: 71	SEQ ID NO: 85	SEQ ID NO: 99
SEQ ID NO: 135	SEQ ID NO: 138	SEQ ID NO: 141	SEQ ID NO: 136	SEQ ID NO: 139	SEQ ID NO: 142
SEQ ID NO: 137	SEQ ID NO: 140	SEQ ID NO: 143	SEQ ID NO: 72	SEQ ID NO: 86	SEQ ID NO: 144

15. The TCR according to any of the preceding items, wherein the alpha and beta chain CDR3s have the following sequences:

CDR3 alpha	CDR3 beta
SEQ ID NO: 88	SEQ ID NO: 89
SEQ ID NO: 90	SEQ ID NO: 91
SEQ ID NO: 98	SEQ ID NO: 99
SEQ ID NO: 141	SEQ ID NO: 142
SEQ ID NO: 143	SEQ ID NO: 144

10

16. The TCR according to any of the preceding items, wherein the TCR alpha chain variable, domain scaffold regions, and/or or CDRs comprise and/or consist of one of the sequences selected from the group consisting of SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 117, SEQ ID NO: 145 and SEQ ID NO: 147 , and wherein the beta chain variable, domain scaffold regions, and/or or CDRs comprise and/or consist of one of the sequences selected from the group consisting of SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 118, SEQ ID NO: 146 and SEQ ID NO: 148 .
17. The TCR according to any of the preceding items, wherein the TCR is selected from the group comprising chimeric, humanized and/or human TCRs.
18. The TCR according to any of the preceding items wherein the constant regions of the alpha and beta chains have been modified to include cysteine residues to allow covalent connection by di-sulphide bridges.
19. The TCR according to any of items 1-16, wherein there are no covalent connections between said paired alpha and beta chains.
20. The TCR according to any of items 1-17, wherein the alpha and/or beta chain comprise(s) a constant region of another mammal.
21. The TCR according to item 18, wherein the alpha and/or beta chain comprise(s) a mouse chimeric constant region(s).
22. The TCR according to any of items 1- 19, wherein the alpha and/or beta chain have been modified to include cysteine residues to allow di-sulphide bridges.
23. The TCR according to any of items 1-20, wherein the TCR alpha chain and the TCR beta chain are covalently linked.
24. The TCR according to any of items 1-21, wherein the TCR alpha chain and the TCR beta chain are covalently linked through a linker peptide.
25. The TCR according to any of the preceding items wherein the TCR alpha chain and/or the TCR beta chain are covalently linked to a moiety.
26. The TCR according to item 23, wherein the covalently linked moiety comprises an affinity tag or a label.
27. The TCR according to item 24, wherein the tag is selected from the group consisting of a truncated CD34 enrichment tag, glutathione-S-transferase (GST), calmodulin binding protein (CBP), protein C tag, Myc tag, Halo tag, HA tag, Flag tag, His tag, biotin tag, and V5 tag.
28. The TCR according to item 24, wherein the label is a fluorochrome or a fluorophore such as a fluorescent protein.
29. The TCR according to item 23, wherein the covalently linked moiety is selected from the group consisting of an inflammatory agent, cytokine, toxin, cytotoxic

- molecule, radioactive isotope, an enzyme that catalyses a cell wall sorting reaction, and an antibody or antigen-binding fragment thereof.
30. A soluble TCR and/or a conjugated soluble TCR, wherein the TCR is a TCR as defined in any of items 1-27.
- 5 31. The TCR according to item 28, wherein the TCR is a bi-specific T-cell engager (BiTE), or is an immune mobilizing monoclonal T-cell receptor.
32. A pharmaceutical composition comprising a soluble protein consisting of all or a functional part of a TCR as defined in any of items 1-29 and at least one pharmaceutically acceptable excipient, carrier or stabilizer.
- 10 33. A pharmaceutical composition comprising a soluble TCR, a conjugated soluble TCR, a cell expressing a TCR or a composition comprising a TCR, wherein the TCR is a TCR as defined in any of items 1-29.
34. A pharmaceutical composition comprising a soluble protein consisting of all or a functional part of a TCR as defined in any of items 1-29, wherein said soluble
- 15 protein has been conjugated to a radionuclide, a chemotherapeutic agent, a toxin or another therapeutically active component, and at least one pharmaceutically acceptable excipient, carrier or stabilizer.
35. A method of treating a disorder characterized by G12V muted human RAS expression in a subject comprising administering to said subject a therapeutically
- 20 effective amount of genetically engineered cells that express a TCR as defined in any of items 1-29, or a pharmaceutical composition as defined in any one of items 30-32, wherein said disorder is a solid or haematological malignancy expressing the peptide of VVVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2), wherein said engineered cell is a T-cell, T-cell progenitor cell, NK cell, NK
- 25 progenitor cell or a differentiated stem cell and which is either autologous or allogenic, and wherein about 10^4 to about 10^{10} engineered cells are infused systemically into said subject or injected or pumped directly into a solid tumour, into a blood vessel that feeds a tumour or into the volume surrounding a solid tumour.
- 30 36. The method of treating a disorder in a subject according to item 33, wherein said subject carries a HLA-A*11 allele.
37. The method of treating a disorder in a subject according to any of items 33-34, wherein said subject carries an alternate subform of HLA-A*11 other than HLA-A*11:03, which alternate subform of HLA-A*11 is capable of specifically presenting
- 35 the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which is recognized by the TCR according to any of items 1-29.
38. The method of treating a disorder in a subject according to any of items 33-34, wherein said subject carries an alternate subform of HLA-A*11 other than HLA-

A*11:03 or HLA-A*11:04, which alternate subform of HLA-A*11 is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which is recognized by the TCR according to any of items 1-29.

- 5 39. The method of treating a disorder in a subject according to any of items 33-35, wherein said subject carries a different HLA-A, HLA-B or HLA-C allele than HLA-A*11, that is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which is recognized by the TCR according to any of items 1-29.
40. The TCR according to any of items 1-29 for use as a medicament.
- 10 41. The TCR according to any of items 1-29 for use in the treatment of a cancer in a patient carrying HLA-A*11, comprising administering to said patient said TCR or a pharmaceutical composition comprising said TCR, wherein said cancer is a solid or haematological malignancy expressing the peptide of SEQ ID NO: 1 and/or SEQ ID NO: 2, and wherein said TCR or a pharmaceutical composition comprising said TCR is infused systemically into said patient or injected or pumped directly
- 15 into a solid tumour, into a blood vessel that feeds a tumour or into the volume surrounding a solid tumour in said patient.
42. The TCR for use according to item 38, for use in the treatment of a cancer in a patient carrying HLA-A*11:01, comprising administering to said patient an engineered cell, wherein said cancer is a solid or haematological malignancy
- 20 expressing the peptide of SEQ ID NO: 1 or SEQ ID NO:2, wherein said engineered cell is a T cell, T cell progenitor cell, NK cell, NK progenitor cell or differentiated stem cell and which is either autologous or allogenic, and wherein about 10^4 to about 10^{10} engineered cells are infused systemically into said patient or injected or pumped directly into a solid tumour, into a blood vessel that feeds a tumour or into
- 25 the volume surrounding a solid tumour.
43. The TCR for use according to item 38 or 39, for use in treating a disorder in a subject, wherein said subject carries an alternate subform of HLA-A*11 other than HLA-A*11:03, which alternate subform of HLA-A*11 is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which is recognized
- 30 by the TCR according to any of items 1-29.
44. The TCR for use according to item 38 or 39, for use in treating a disorder in a subject, wherein said subject carries an alternate subform of HLA-A*11 other than HLA-A*11:03 and HLA-A*11:04, which alternate subform of HLA-A*11 is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which
- 35 is recognized by the TCR according to any of items 1-29.
45. The TCR for use according to item 38 or 39, for use in treating a disorder in a subject, wherein said subject carries a different HLA-A, HLA-B or HLA-C allele than HLA-A*11, that is capable of specifically presenting the peptide of SEQ ID

- NO: 1 or SEQ ID NO: 2 and which is recognized by the TCR according to any of items 1-29.
46. A nucleic acid encoding a TCR comprising a paired TCR alpha- and TCR beta-chain as defined in any one of items 1-29.
- 5 47. A vector comprising a nucleic acid encoding a TCR comprising a paired TCR alpha- and TCR beta-chain as defined in any one of the items 1-29
48. A vector according to item 47 which is a vector selected from the group consisting of an expression vector, a coding vector, non-viral or viral vector.
49. The vector according to item 47 or 48 wherein the vector further comprises a
10 nucleic acid sequence encoding CD8 α and/or CD8 β .
50. The vector according to item 49 wherein the nucleic acid sequence encoding CD8 α or CD8 β is operably linked to a nucleic acid encoding a tag.
51. The vector according to item 50 wherein the nucleic acid encoding a tag is at the 5' upstream of the nucleic acid sequence encoding CD8 α or CD8 β such that the tag
15 is fused to the N-terminus of CD8 α or CD8 β when expressed.
52. The vector according to item 50 or 51 wherein the tag is a truncated CD34 enrichment tag.
53. The vector according to any of items 49-52, wherein the isolated nucleic acid of item 46 and the nucleic acid sequence encoding CD8 α and/or CD8 β according to
20 any of items 49-52 are interconnected with an internal ribosome entry site or a nucleic acid sequence encoding a self-cleaving peptide.
54. The vector according item 54, wherein the self-cleaving peptide is selected from the group consisting of P2A, E2A, F2A and T2A.
55. The vector according to any of items 47-54, wherein the vector is a nucleic acid
25 vector selected from the group comprising an expression vector, a cloning vector, phagemid, cosmid, transposon, episome, artificial chromosome or RNA, a nanoparticle, or a viral vector.
56. The vector according to item 55 wherein the vector is a viral vector and is selected from the group comprising adenoviruses, adeno-associated viruses, retroviruses,
30 lentiviruses, and herpes simplex viruses.
57. The viral vector according to item 55 wherein the viral vector is pseudotyped,
58. The vector according to item 55, wherein the vector is a nanoparticle vector selected from the group comprising lipid nanoparticles (LNPs), targeted LNP (tLNP), nanoparticles (NPs), cationic liposome complexes, cationic polymer
35 complexes, cationic liposome-polymer complexes, exosomes, liposomes, and inorganic nanoparticles,
59. The vector according to any one of items 55 to 58 wherein the nucleic acid vector, the viral vector, or the nanoparticle vector comprises one or more cell surface

- receptors that bind to a ligand on a target cell, heterologous viral envelope glycoproteins, fusion glycoproteins, T-cell activation or co-stimulation molecules, cytokines or cytokine-based transduction enhancers, and/or transmembrane proteins comprising a mitogenic domain and/or cytokine-based domain exposed on the surface and/or conjugated to the surface of the viral vector.
- 5
60. The vector according to any of items 47-57, wherein the vector is pseudotyped with a Cocal or a Nipah virus envelope proteins.
61. The vector according item 60, wherein the Nipah envelope protein is engineered to bind EpCAM, CD4, CD8, CD3 or CD5 or other marker suitable for targeted cellular delivery of a TCR-encoding nucleic acid vector..
- 10
62. The vector according to any of items 47-58, wherein the vector comprises a lipid nanoparticle comprising ionizable cationic lipid, a sterol, a phospholipid, a non-functionalized PEG-lipid, a functionalized PED-lipid wherein the functionalized PEG-lipid has been conjugated with a binding moiety.
- 15
63. The vector according to any of items 47-58, wherein the vector comprises a lipid nanoparticle (LNP) or tLNP comprising a PEG lipid and further comprising one or more of a phospholipid, an ionizable cationic lipid, a sterol, a co-lipid and a further PEG-lipid or combination thereof and wherein LNP or tLNP comprises mRNA. The LNP or tLNP can be engineered to bind EpCAM, CD4, CD8, CD3 or CD5 or other marker suitable for targeted cellular delivery of a TCR-encoding nucleic acid vector
- 20
64. An engineered cell expressing the TCR as defined in any of items 1-29.
65. An engineered cell which comprises and/or expresses the isolated nucleic acid of item 46, or a vector according to any of items 47-63.
66. The engineered cell according to item 64 or 65, wherein the engineered cell
- 25
- comprises a chromosomal gene knock out of one or more TCR gene(s), one or more HLA gene(s), or both.
67. The engineered cell according to any of items 64-66, wherein the engineered cell comprises a knockout of an HLA gene selected from the group consisting of an alpha-1-macroglobulin gene, alpha-2-macroglobulin gene, alpha-3-macroglobulin gene, beta-1-microglobulin gene, beta-2-microglobulin gene, and combinations thereof.
- 30
68. The engineered cell according to any of items 64-67, wherein the engineered cell comprises a knockout of a TCR gene selected from a TCR alpha variable region gene, TCR beta variable region gene, TCR constant region gene, and combination thereof.
- 35
69. The engineered cell according to any of items 64-68, wherein the engineered cell expresses CD8a and/or CD8b, optionally the CD8 alpha and/or CD8 beta is fused to a truncated CD34 enrichment tag.

70. The engineered cell according to any of items 64-69, wherein the engineered cells are enriched using the truncated CD34 enrichment tag.
71. The engineered cell according to any of items 64-70, wherein the engineered cell is a hematopoietic progenitor cell, peripheral blood mononuclear cell (PBMC), cord blood cell, or immune cell.
72. The engineered cell according to item 71, wherein engineered cell is an immune cell and is selected from the group comprising a T cell, cytotoxic lymphocyte, cytotoxic lymphocyte precursor cell, cytotoxic lymphocyte progenitor cell, cytotoxic lymphocyte stem cell, CD4⁺ T cell, CD8⁺ T cell, CD4/CD8 double negative T cell, gamma delta (gd) T cell, natural killer (NK) cell, NK-T cell, dendritic cell, or a combination thereof.
73. The engineered cell according to item 71 or 72 wherein the cell is derived from a hematopoietic stem cell (HSC) or an induced pluripotent stem cell (iPSC).
74. The engineered cell according to item 72 or 73, wherein the cell is a T cell and is a T cell, central memory T cell, effector memory T cell, or a combination thereof.
75. The engineered cell according to any of items 72-74, wherein the T cell is a primary T cell or a cell of a T cell line.
76. The engineered cell according to any of items 72-75 wherein the T cell does not express or has a lower surface expression of an endogenous TCR.
77. The engineered cell according to any of items 66-76, wherein the engineered cell is capable of producing a cytokine or a cytotoxic molecule when contacted with a target cell that presents a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) presented in a HLA-A*11 complex.
78. The engineered cell according to item 77, wherein, wherein the engineered cell is contacted with the target cell *in vitro* or *ex vivo*.
79. The engineered cell according to item 77 or 78, wherein the cytokine is TNF-alpha, IL-2, and/or IFN-gamma.
80. The engineered cell according to any of items 77-79, wherein the cytotoxic molecule is a perforin and/or a granzyme, such as granzyme B.
81. The engineered cell according to any of items 77-80, wherein the engineered cell is capable of producing a higher level of cytokine or a cytotoxic molecule when contacted with a target cell with a heterozygous expression of human RAS.
82. The engineered cell according to item 81, wherein the engineered cell is capable of producing an at least 1.05-fold higher level of cytokine or a cytotoxic molecule.

83. The engineered cell according to any of items 77-82, wherein the engineered cell is capable of killing a target cell that presents a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) presented in a HLA-A*11 complex.
84. The engineered cell according to item 83, wherein the killing is determined by a killing assay.
85. The engineered cell according to item 83 or 84, wherein the ratio of the engineered cell and the target cell in the killing assay is from 1:64 to 1:1.
86. The engineered cell according to any of items 77-85, wherein the target cell is a target cell pulsed with a range of between 1×10^3 nM and 0.1 nM of a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) presented in a HLA-A*11 complex.
87. The engineered cell according to item 86, wherein the target cell is a cell monoallelic for HLA-A*11.
88. The engineered cell according to any of items 77-87, wherein the engineered cell is capable of killing a higher number of target cells when contacted with target cells with a heterozygous expression of a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) presented in a HLA-A*11 complex.
89. The engineered cell according to item 88, wherein the cell killing is at least 1.05-fold higher.
90. The engineered cell according to any of items 77-89, wherein the target cell is a cell line or a primary cell.
91. The engineered cell according to item 86, wherein the target cell is selected from the group consisting of a cancer cell line, a primary cancer cell line, a transformed cell line, and an immortalized cell line.
92. The engineered cell according to item 91, wherein the target cell is a HEK293-, ARH -77-, COR-L23-, NCI-H441-, SW620-, QGP-1-, or IGR-1 derived cell line.
93. The engineered cell according to any of items 77-92, wherein the engineered cell does not induce T cell expansion, cytokine release, or cytotoxic killing when in

- contact with a target cell that presents a peptide selected from the group consisting of VVGAGGVGK (SEQ ID NO: 3) presented in a HLA-A*11 complex, or VVGAGGVGK (SEQ ID NO: 4) presented in a HLA-A*11 complex.
94. A population of engineered cells according to any one of items 77-93.
- 5 95. A pharmaceutical composition comprising any one or more of the engineered cells or vectors according to any of items 47 to 94.
96. The pharmaceutical composition according to item 95, wherein the vectors are selected from the group consisting of viral vectors, LNPs or any other carrier comprising the nucleic acid sequence according to item 46.
- 10 97. A pharmaceutical composition comprising a soluble protein consisting of all or a functional part of one or more TCR(s) as defined in any of items 1-30, or one or more vector(s) according to any one of items 46 to 63, or one or more engineered cell(s) according to any one of items 64 to 94.
- 15 98. A T-cell receptor (TCR), which is isolated and/or expressed in an engineered cell, specifically recognizing a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO:2) presented in a HLA-A*11 complex, and wherein the
- 20 TCR comprises a paired TCR alpha- and TCR beta-chain, wherein the TCR alpha chain variable domain comprises SC1alpha-CDR1alpha-SC2alpha-CDR2alpha-SC3alpha-CDR3alpha-SC4alpha-SC5alpha, and the TCR beta chain variable domain comprises SC1beta-CDR1beta-SC2 beta-CDR2 beta-SC3beta-CDR3beta-SC4beta-SC5beta, wherein SC is a scaffold region and CDR is a complementarity
- 25 determining region.
99. The TCR according to item 98, comprising:
- a. a TCR alpha chain CDR3 sequence with a TCR alpha chain CDR3 consisting of SEQ ID NO: 88,
- 30 b. a TCR beta chain CDR3 sequence with a TCR beta chain CDR3 sequence selected from the group consisting of SEQ ID NOs:1932-2672.
100. The TCR according to any of the preceding items 98 or 99, wherein the TCR is selected from the group comprising chimeric, humanized and/or human TCRs.
- 35 101. The TCR according to any of items 98-100 wherein the constant regions of the alpha and beta chains have been modified to include cysteine residues to allow covalent connection by di-sulphide bridges.

102. The TCR according to item 98, comprising:
- a. a TCR alpha chain CDR3 sequence with a TCR alpha chain CDR3 selected from the group consisting of SEQ ID NOs: 185-1931.
- 5 b. a TCR beta chain CDR3 sequence with a TCR beta chain CDR3 sequence consisting of SEQ ID NO: 89.
103. The TCR according to item 98, wherein the TCR alpha chain is selected from the group consisting of SEQ ID Nos: 2673-4420 and the TCR beta chain is SEQ ID NO: 108
- 10 104. The TCR according to item 98, wherein the TCR alpha chain is SEQ ID NO: 107 and the TCR beta chain is selected from the group consisting of SEQ ID NOs: 4421-5161.
105. The TCR according to any one of items 98-104, wherein the TCR, when expressed in an engineered cell, does not cause specific cytotoxic activity towards
- 15 cells not expressing a G12V mutated RAS peptide.
106. The TCR according to any one of items 98-105, wherein the TCR, when expressed in an engineered cell, does not cause specific cytotoxic activity towards cells expressing the wild type human RAS peptide, such as VVGAGGVGK (SEQ ID NO: 3) /or VVGAGGVGK (SEQ ID NO: 4).
- 20 107. The TCR according to any one of items 98-106, wherein the TCR, when expressed in an engineered cell, does not specifically recognize cells expressing the G12V mutated human RAS peptide, wherein the peptide is VVGAVGVGK (SEQ ID NO: 1) and/ or VVGAVGVGK (SEQ ID NO: 2) presented in an allelic variant of the human leukocyte antigen (HLA) molecule other than an allelic variant
- 25 of the human leukocyte antigen (HLA) molecule selected from the group consisting of HLA-A*11, HLA-A*30:01, HLA-A*68:02, HLA-B*54:01, HLA-B*35:01, HLA-B*35:02, HLA-B*35:03, HLA-B*35:05, HLA-B*35:12, HLA-B*52:01, HLA-B*53:01, HLA-B*57:01 and HLA-B*58:01 serotype.
- 30 108. The TCR according to any one of items 98-107 wherein the TCR when expressed in an engineered cell does not specifically recognise other allelic variants of the human leukocyte antigen (HLA) molecule other than an allelic variant of the human leukocyte antigen (HLA) molecule selected from the group consisting of HLA-A*68:02, HLA-B*54:01, HLA-B*35:01, HLA-B*35:02, HLA-B*35:03, HLA-B*35:12, HLA-B*57:01 and HLA-B*58:01 serotype.
- 35 109. The TCR according to any of items 98-108, wherein the TCR, when expressed in an engineered cell, does not specifically recognize the G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO:

- 1) and/or VVGAVGVGK (SEQ ID NO: 2) presented in an allelic variant of the human leukocyte antigen (HLA) molecule other than the HLA-A*11 serotype.
110. The TCR according any one of items 98-109, wherein the TCR does not specifically recognize a G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/ or VVGAVGVGK (SEQ ID NO: 2) presented in a HLA-A*11:03 complex
111. The TCR according to any one of items 98-110 wherein the TCR does not specifically recognize a G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/ or VVGAVGVGK (SEQ ID NO: 2) presented in a HLA-A*11:03 and HLA-A*11:04 complex.
112. The TCR according to any of items 98-111, wherein the TCR, when expressed in an engineered cell, has at the most 4% cross-reactivity towards any somatically expressed HLA-presented peptide other than a G12V mutated human rat sarcoma (RAS) peptide, which consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) and which is presented in a HLA-A*11 complex in a cross-reactivity assay.
113. The TCR according to any of items 98-112, wherein there are no covalent connections between said paired alpha and beta chains.
114. The TCR according to any of items 98-113, wherein the alpha and/or beta chain comprise(s) a constant region of another mammal.
115. The TCR according to item 114, wherein the alpha and/or beta chain comprise(s) a mouse chimeric constant region(s).
116. The TCR according to any of items 98-115, wherein the alpha and/or beta chain have been modified to include cysteine residues to allow di-sulphide bridges.
117. The TCR according to any of items 98-116, wherein the TCR alpha chain and the TCR beta chain are covalently linked.
118. The TCR according to any of items 98-117, wherein the TCR alpha chain and the TCR beta chain are covalently linked through a linker peptide.
119. The TCR according to any of the preceding items wherein the TCR alpha chain and/or the TCR beta chain are covalently linked to a moiety.
120. The TCR according to item 119, wherein the covalently linked moiety comprises an affinity tag or a label.
121. The TCR according to item 120, wherein the tag is selected from the group consisting of a CD34 enrichment tag, glutathione-S-transferase (GST), calmodulin binding protein (CBP), protein C tag, Myc tag, Halo tag, HA tag, Flag tag, His tag, biotin tag, and V5 tag.
122. The TCR according to item 121, wherein the label is a fluorochrome or a fluorophore such as a fluorescent protein.

123. The TCR according to item 121, wherein the covalently linked moiety is selected from the group consisting of an inflammatory agent, cytokine, toxin, cytotoxic molecule, radioactive isotope, an enzyme that catalyses a cell wall sorting reaction, and an antibody or antigen-binding fragment thereof.
- 5 124. A soluble TCR and/or a conjugated soluble TCR, wherein the TCR is a TCR as defined in any of items 98-123.
125. The TCR according to item 124, wherein the TCR is a bi-specific T-cell engager (BiTE), or is an immune mobilizing monoclonal T-cell receptor.
- 10 126. A pharmaceutical composition comprising a soluble protein consisting of all or a functional part of a TCR as defined in any of items 98-125 and at least one pharmaceutically acceptable excipient, carrier or stabilizer.
127. A pharmaceutical composition comprising a soluble TCR, a conjugated soluble TCR, a cell expressing a TCR or a composition comprising a TCR, wherein the TCR is a TCR as defined in any of items 98-126.
- 15 128. A pharmaceutical composition comprising a soluble protein consisting of all or a functional part of a TCR as defined in any of items 98-127, wherein said soluble protein has been conjugated to a radionuclide, a chemotherapeutic agent, a toxin or another therapeutically active component, and at least one pharmaceutically acceptable excipient, carrier or stabilizer.
- 20 129. A method of treating a disorder characterized by G12V muted human RAS expression in a subject comprising administering to said subject a therapeutically effective amount of genetically engineered cells that express a TCR as defined in any of items 1-125, or a pharmaceutical composition as defined in any one of items 126-128, wherein said disorder is a solid or haematological malignancy
- 25 expressing the peptide of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2), wherein said engineered cell is a T-cell, T-cell progenitor cell, NK cell, NK progenitor cell or a differentiated stem cell and which is either autologous or allogenic, and wherein about 10^4 to about 10^{10} engineered cells are infused systemically into said subject or injected or pumped directly into a solid tumour,
- 30 into a blood vessel that feeds a tumour or into the volume surrounding a solid tumour.
130. The method of treating a disorder in a subject according to item 129, wherein said subject carries a HLA-A*11 allele.
- 35 131. The method of treating a disorder in a subject according to any of items 129-130, wherein said subject carries an alternate subform of HLA-A*11 other than HLA-A*11:03, which alternate subform of HLA-A*11 is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which is recognized by the TCR according to any of items 98-125.

132. The method of treating a disorder in a subject according to any of items 129-130, wherein said subject carries an alternate subform of HLA-A*11 other than HLA-A*11:03 or HLA-A*11:04, which alternate subform of HLA-A*11 is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which is recognized by the TCR according to any of items 98-125.
133. The method of treating a disorder in a subject according to any of items 129-130, wherein said subject carries a different HLA-A, HLA-B or HLA-C allele than HLA-A*11:01, that is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which is recognized by the TCR according to any of items 98-125.
134. The TCR according to any of items 98-125 for use as a medicament.
135. The TCR according to any of items 98-125 for use in the treatment of a cancer in a patient carrying HLA-A*11, comprising administering to said patient said TCR or a pharmaceutical composition comprising said TCR, wherein said cancer is a solid or haematological malignancy expressing the peptide of SEQ ID NO: 1 and/or SEQ ID NO: 2, and wherein said TCR or a pharmaceutical composition comprising said TCR is infused systemically into said patient or injected or pumped directly into a solid tumour, into a blood vessel that feeds a tumour or into the volume surrounding a solid tumour in said patient.
136. The TCR for use according to item 135, for use in the treatment of a cancer in a patient carrying HLA-A*11, comprising administering to said patient an engineered cell, wherein said cancer is a solid or haematological malignancy expressing the peptide of SEQ ID NO: 1 or SEQ ID NO:2, wherein said engineered cell is a T cell, T cell progenitor cell, NK cell, NK progenitor cell or differentiated stem cell and which is either autologous or allogenic, and wherein about 10^4 to about 10^{10} engineered cells are infused systemically into said patient or injected or pumped directly into a solid tumour, into a blood vessel that feeds a tumour or into the volume surrounding a solid tumour.
137. The TCR for use according to item 135 or 136, for use in treating a disorder in a subject, wherein said subject carries an alternate subform of HLA-A*11 other than HLA-A*11:03, which alternate subform of HLA-A*11 is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which is recognized by the TCR according to any of items 98-125.
138. The TCR for use according to item 135 to 138, for use in treating a disorder in a subject, wherein said subject carries an alternate subform of HLA-A*11 other than HLA-A*11:03 and HLA-A*11:04, which alternate subform of HLA-A*11 is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which is recognized by the TCR according to any of items 98-125.

139. The TCR for use according to item 135 or 136, for use in treating a disorder in a subject, wherein said subject carries a different HLA-A, HLA-B or HLA-C allele than HLA-A*11, that is capable of specifically presenting the peptide of SEQ ID NO: 1 or SEQ ID NO: 2 and which is recognized by the TCR according to any of items 98-125.
140. A nucleic acid encoding a TCR comprising a paired TCR alpha- and TCR beta-chain as defined in any one of items 98-125.
141. A vector comprising a nucleic acid encoding a TCR comprising a paired TCR alpha- and TCR beta-chain as defined in any one of the items 98-125
142. A vector according to item 141, which is a vector selected from the group consisting of an expression vector, a coding vector, non-viral or viral vector.
143. The vector according to item 141 or 142, wherein the vector further comprises a nucleic acid sequence encoding CD8 α and/or CD8 β .
144. The vector according to item 143, wherein the nucleic acid sequence encoding CD8 α or CD8 β is operably linked to a nucleic acid encoding a tag.
145. The vector according to item 144, wherein the nucleic acid encoding a tag is at the 5' upstream of the nucleic acid sequence encoding CD8 α or CD8 β such that the tag is fused to the N-terminus of CD8 α or CD8 β when expressed.
146. The vector according to item 144 or 145, wherein the tag is a truncated CD34 enrichment tag.
147. The vector according to any of items 141-146, wherein the isolated nucleic acid of item 41 and the nucleic acid sequence encoding CD8 α and/or CD8 β according to any of items 143-146 are interconnected with an internal ribosome entry site or a nucleic acid sequence encoding a self-cleaving peptide.
148. The vector according item 147, wherein the self-cleaving peptide is selected from the group consisting of P2A, E2A, F2A and T2A.
149. The vector according to any of items 141 to 148, wherein the vector is a nucleic acid vector and comprises one or more cell surface receptors that bind to a ligand on a target engineered cell, heterologous viral envelope glycoproteins, fusion glycoproteins, T-cell activation or co-stimulation molecules, cytokines or cytokine-based transduction enhancers, and/or transmembrane proteins comprising a mitogenic domain and/or cytokine-based domain exposed on the surface and/or conjugated to the surface of the viral vector.
150. The vector according to any of items 141-149, wherein the vector is pseudotyped with a Cocal or a Nipah virus envelope proteins.
151. The vector according item 150, wherein the Nipah envelope protein or the LNP is engineered to bind EpCAM, CD4, CD8, CD3 and CD5.

152. The vector according to any of items 141-151, wherein the vector comprises a lipid nanoparticle comprising ionizable cationic lipid, a sterol, a phospholipid, a non-functionalized PEG-lipid, a functionalized PED-lipid wherein the functionalized PEG-lipid has been conjugated with a binding moiety.
- 5 153. The vector according to any of items 141-152, wherein the vector comprises a lipid nanoparticle (LNP) comprising a PEG lipid and further comprising one or more of a phospholipid, an ionizable cationic lipid, a sterol, a co-lipid and a further PEG-lipid or combination thereof and wherein LNP comprises mRNA.
154. An engineered cell expressing the TCR as defined in any of items 98-125.
- 10 155. An engineered cell which comprises and/or expresses the isolated nucleic acid of item 136, or a vector according to any of items 141-153.
156. The engineered cell according to item 154 or 155, wherein the engineered cell comprises a chromosomal gene knock out of one or more TCR gene(s), one or more HLA gene(s), or both.
- 15 157. The engineered cell according to any of items 154-156, wherein the engineered cell comprises a knockout of an HLA gene selected from the group consisting of an alpha-1-macroglobulin gene, alpha-2-macroglobulin gene, alpha-3-macroglobulin gene, beta-1-microglobulin gene, beta-2-microglobulin gene, and combinations thereof.
- 20 158. The engineered cell according to any of items 154-157, wherein the engineered cell comprises a knockout of a TCR gene selected from a TCR alpha variable region gene, TCR beta variable region gene, TCR constant region gene, and combination thereof.
- 25 159. The engineered cell according to any of items 154-158, wherein the engineered cell expresses CD8a and/or CD8b, optionally the CD8 alpha and/or CD8 beta is fused to a truncated CD34 enrichment tag.
160. The engineered cell according to any of items 154-159, wherein the engineered cells are enriched using the truncated CD34 enrichment tag.
- 30 161. The engineered cell according to any of items 154-160, wherein the engineered cell is a hematopoietic progenitor cell, peripheral blood mononuclear cell (PBMC), cord blood cell, or immune cell.
162. The engineered cell according to item 161, wherein the immune cell is a T cell, cytotoxic lymphocyte, cytotoxic lymphocyte precursor cell, cytotoxic lymphocyte progenitor cell, cytotoxic lymphocyte stem cell, CD4+ T cell, CD8⁺ T cell, CD4/CD8 double negative T cell, gamma delta (gd) T cell, natural killer (NK) cell, NK-T cell, dendritic cell, or a combination thereof.
- 35 163. The engineered cell according to item 161 or 162 wherein the cell is derived from a hematopoietic stem cell (HSC) or an induced pluripotent stem cell (iPSC).

164. The engineered cell according to item 156 or 163, wherein the T cell is a T cell, central memory T cell, effector memory T cell, or a combination thereof.
165. The engineered cell according to any of items 162-164, wherein the T cell is a primary T cell or a cell of a T cell line.
- 5 166. The engineered cell according to any of items 162 to 165, wherein the T cell does not express or has a lower surface expression of an endogenous TCR.
167. The engineered cell according to any of items 154-166, wherein the engineered cell is capable of producing a cytokine or a cytotoxic molecule when contacted with a target cell that presents a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) presented in a HLA-A*11 complex.
- 10 168. The engineered cell according to item 167, wherein, wherein the engineered cell is contacted with the target cell *in vitro* or *ex vivo*.
169. The engineered cell according to item 167 or 168, wherein the cytokine is TNF-alpha, IL-2, and/or IFN-gamma.
170. The engineered cell according to any of items 167 to 169, wherein the cytotoxic molecule is a perforin and/or a granzyme, such as granzyme B.
- 20 171. The engineered cell according to any of items 167-170, wherein the engineered cell is capable of producing a higher level of cytokine or a cytotoxic molecule when contacted with a target cell with a heterozygous expression of human RAS.
- 25 172. The engineered cell according to item 171, wherein the engineered cell is capable of producing an at least 1.05-fold higher level of cytokine or a cytotoxic molecule.
173. The engineered cell according to any of items 167-172, wherein the engineered cell is capable of killing a target cell that presents a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) presented in a HLA-A*11 complex.
- 30 174. The engineered cell according to item 173, wherein the killing is determined by a killing assay.
- 35 175. The engineered cell according to item 173 or 174, wherein the ratio of the engineered cell and the target cell in the killing assay is from 1:64 to 1:1.

176. The engineered cell according to any of items 173-175, wherein the target cell is a target cell pulsed with a range of between 1×10^3 nM and 0.1 nM of a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) presented in a HLA-A*11 complex.
177. The engineered cell according to item 176, wherein the target cell is a cell monoallelic for HLA-A*11.
178. The engineered cell according to any of items 173-177, wherein the engineered cell is capable of killing a higher number of target cells when contacted with target cells with a heterozygous expression of a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) presented in a HLA-A*11 complex.
179. The engineered cell according to item 178, wherein the cell killing is at least 1.05-fold higher.
180. The engineered cell according to any of items 154-178, wherein the target cell is a cell line or a primary cell.
181. The engineered cell according to item 180 wherein the target cell is selected from the group consisting of a cancer cell line, a primary cancer cell line, a transformed cell line, and an immortalized cell line.
182. The engineered cell according to item 181, wherein the target cell is HEK293-, ARH -77-, COR-L23-, NCI-H441-, SW620-, QGP-1-, or IGR-1- derived cell lines.
183. The engineered cell according to any of items 150-182, wherein the engineered cell does not induce T cell expansion, cytokine release, or cytotoxic killing when in contact with a target cell that presents a peptide selected from the group consisting of VVGAGGVGK (SEQ ID NO: 3) presented in a HLA-A*11 complex, or VVGAGGVGK (SEQ ID NO: 4) presented in a HLA-A*11 complex.
184. A population of engineered cells according to any one of items 150-183.
185. A pharmaceutical composition comprising any one or more of the engineered cells or vectors according to any of items 150-184.
186. The pharmaceutical composition according to item 185, wherein the vectors are selected from the group consisting of non-viral vectors, viral vectors, LNPs or any other carrier comprising the nucleic acid sequence according to item 141-149.

187. The nucleic acid according to item 46 or the vector according to any one of items 47-63, wherein the nucleic acid encoding a TCR comprising a paired TCR alpha- and TCR beta-chain is linear or circular double stranded DNA
188. The nucleic acid according to item 46 or the vector according to any one of items 47-63, wherein the nucleic acid encoding a TCR comprising a paired TCR alpha- and TCR beta-chain is linear or circular single stranded DNA.
189. The nucleic acid according to item 187 or 188, wherein the DNA is chemically modified.
190. The nucleic acid according to item 46 or the vector according to any one of items 47-63, wherein the nucleic acid encoding a TCR comprising a paired TCR alpha- and TCR beta-chain is linear or circular double stranded RNA
191. The nucleic acid according to item 46 or the vector according to any one of items 47-63, wherein the nucleic acid encoding a TCR comprising a paired TCR alpha- and TCR beta-chain is linear or circular single stranded RNA.
192. The nucleic acid according to item 190 or 191, wherein the RNA is chemically modified.
193. The vector according to any one of items 47 to 63 wherein the RNA contains a 5' CAP, a 5' UTR, a 3' UTR and 3' poly(A) tail, and an internal ribosome entry site (IRES).
194. The vector according to item 193 wherein the vector is circularized
195. The vector according to item 193 or 194 wherein the vector contains replication-directing elements for self-amplification.

ITEMS II

1. A T-cell receptor (TCR), which is isolated and/or expressed in an engineered cell, specifically recognizing a G12V mutated human rat sarcoma (RAS) peptide, wherein the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/ or VVGAVGVGK (SEQ ID NO:2) presented in a HLA-A*11 complex, and wherein the TCR comprises a paired TCR alpha- and TCR beta-chain, wherein the TCR alpha chain variable domain comprises SC1alpha-CDR1alpha-SC2alpha-CDR2alpha-SC3alpha-CDR3alpha-SC4alpha-SC5alpha, and the TCR beta chain variable domain comprises SC1beta-CDR1beta-SC2 beta-CDR2 beta-SC3beta-CDR3beta-

SC4beta-SC5beta, wherein SC is a scaffold region and CDR is a complementarity determining region.

2. The TCR according to item 1, wherein the TCR, when expressed in an engineered cell, does not cause specific cytotoxic activity towards cells not expressing a G12V mutated RAS peptide.
3. The TCR according to any of items 1 to 2, wherein the TCR, when expressed in an engineered cell, does not cause specific cytotoxic activity towards cells expressing the wild type human RAS peptide, such as VVGAGGVGK (SEQ ID NO: 3) and/or VVGAGGVGK (SEQ ID NO: 4).
4. The TCR according to any of the preceding items, wherein the TCR, when expressed in an engineered cell, does not specifically recognize the G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/ or VVGAVGVGK (SEQ ID NO: 2) presented in an allelic variant of the human leukocyte antigen (HLA) molecule other than an allelic variant of the human leukocyte antigen (HLA) molecule selected from the group consisting of HLA-A*11, HLA-A*30:01, HLA-A*68:02, HLA-B*54:01, HLA-B*35:01, HLA-B*35:02, HLA-B*35:03, HLA-B*35:05, HLA-B*35:12, HLA-B*52:01, HLA-B*53:01, HLA-B*57:01 and HLA-B*58:01 serotype.
5. The TCR according to any of the preceding items wherein the TCR, when expressed in an engineered cell, does not specifically recognise other allelic variants of the human leukocyte antigen (HLA) molecule other than an allelic variant of the human leukocyte antigen (HLA) molecule selected from the group consisting of HLA-A*68:02, HLA-B*54:01, HLA-B*35:01, HLA-B*35:02, HLA-B*35:03, HLA-B*35:12, HLA-B*57:01 and HLA-B*58:01 serotype.
6. The TCR according to any of the preceding items, wherein the TCR, when expressed in an engineered cell, does not specifically recognize the G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) presented in an allelic variant of the human leukocyte antigen (HLA) molecule other than the HLA-A*11 serotype.
7. The TCR according to item 1, which does not specifically recognize a G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK

(SEQ ID NO: 1) and/ or VVGAVGVGK (SEQ ID NO: 2) presented in a HLA-A*11:03 complex.

- 5 8. The TCR according to item 1, which does not specifically recognize a G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/ or VVGAVGVGK (SEQ ID NO: 2) presented in a HLA-A*11:03 and HLA-A*11:04 complex.
- 10 9. The TCR according to any of the preceding items, wherein the TCR, when expressed in an engineered cell, has at the most 4% cross-reactivity towards any somatically expressed HLA-presented peptide other than a G12V mutated human rat sarcoma (RAS) peptide, which consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) and which is presented in a HLA-A*11 complex in a cross-reactivity assay.
- 15 10. The TCR according to any of the preceding items comprising:
- 20 a. a TCR alpha chain CDR3 sequence with at least 75% identity to a TCR alpha chain CDR3 sequence selected from the group consisting of SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 98, SEQ ID NO: 141 and SEQ ID NO: 143
- and
- b. a TCR beta chain CDR3 sequence with at least 75% identity to a TCR beta chain CDR3 sequence selected from the group consisting of SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 99, SEQ ID NO: 142, and SEQ ID NO: 144.
- 25 11. The TCR according to any of the preceding items comprising:
- 30 a. a TCR alpha chain CDR3 sequence with a TCR alpha chain CDR3 sequence selected from the group consisting of SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 98 , SEQ ID NO: 141 and SEQ ID NO: 143
- and
- b. a TCR beta chain CDR3 sequence with a TCR beta chain CDR3 sequence selected from the group consisting of SEQ ID SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 99, SEQ ID NO: 142 and SEQ ID NO: 144.
- 35

12. The TCR according to any one of the preceding items, wherein the TCR comprises a paired TCR alpha- and TCR beta-chain comprising a complete assembly of combinations of the following sequences:

	SEQ ID NO	SEQ ID NO	SEQ ID NO	SEQ ID NO	SEQ ID NO	SEQ ID NO	SEQ ID NO
SC1alpha	18	20	28	126	128	18	20
CDR1alpha	60	62	70	135	137	60	62
SC2alpha	32	34	42	129	131	32	34
CDR2alpha	74	76	84	138	140	74	76
SC3alpha	46	48	56	132	134	46	48
CDR3alpha	88	90	98	141	143	88	90
SC4alpha	7	9	15	123	125	7	9
SC5alpha	104	104	104	104	104	104	104
SC1beta	19	21	29	127	30	19	21
CDR1beta	61	63	71	136	72	61	63
SC2beta	33	35	43	130	44	33	35
CDR2beta	75	77	85	139	86	75	77
SC3beta	47	49	57	133	58	47	49
CDR3beta	89	91	99	142	144	89	91
SC4beta	8	10	16	124	12	8	10
SC5beta	105	105	106	106	106	5162	5162

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or sequences having at least 90% such as at least 95%, at least 97% or at least 99%, or 100% identity to said respective sequences.

- 10 13. The TCR according to any of the preceding items, wherein the TCR comprises a paired TCR alpha- and TCR beta-chain comprising the following combination of sequences:

TCR#	SC1	CDR1	SC2	CDR2	SC3	CDR3	SC4	SC5
TCR-1	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
alpha	NO: 18	NO: 60	NO: 32	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				74	46	88	7	104
TCR-2	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
alpha	NO: 20	NO: 62	NO: 34	ID	ID	ID	ID	ID

				NO: 76	NO: 48	NO: 90	NO: 9	NO: 104
TCR-6 alpha	SEQ ID NO: 28	SEQ ID NO: 70	SEQ ID NO: 42	SEQ ID NO: 84	SEQ ID NO: 56	SEQ ID NO: 98	SEQ ID NO: 15	SEQ ID NO: 104
TCR-9 alpha	SEQ ID NO: 126	SEQ ID NO: 135	SEQ ID NO: 129	SEQ ID NO: 138	SEQ ID NO: 132	SEQ ID NO: 141	SEQ ID NO: 123	SEQ ID NO: 104
TCR-10 alpha	SEQ ID NO: 128	SEQ ID NO: 137	SEQ ID NO: 131	SEQ ID NO: 140	SEQ ID NO: 134	SEQ ID NO: 143	SEQ ID NO: 125	SEQ ID NO: 104
TCR-1 beta	SEQ ID NO: 19	SEQ ID NO: 61	SEQ ID NO: 33	SEQ ID NO: 75	SEQ ID NO: 47	SEQ ID NO: 89	SEQ ID NO: 8	SEQ ID NO: 105
TCR-1 beta	SEQ ID NO: 19	SEQ ID NO: 61	SEQ ID NO: 33	SEQ ID NO: 75	SEQ ID NO: 47	SEQ ID NO: 89	SEQ ID NO: 8	SEQ ID NO: 5162
TCR-2 beta	SEQ ID NO: 21	SEQ ID NO: 63	SEQ ID NO: 35	SEQ ID NO: 77	SEQ ID NO: 49	SEQ ID NO: 91	SEQ ID NO: 10	SEQ ID NO: 105
TCR-2 beta	SEQ ID NO: 21	SEQ ID NO: 63	SEQ ID NO: 35	SEQ ID NO: 77	SEQ ID NO: 49	SEQ ID NO: 91	SEQ ID NO: 10	SEQ ID NO: 5162
TCR-6 beta	SEQ ID NO: 29	SEQ ID NO: 71	SEQ ID NO: 43	SEQ ID NO: 85	SEQ ID NO: 57	SEQ ID NO: 99	SEQ ID NO: 16	SEQ ID NO: 106

TCR-9	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SE
beta	NO: 127	NO: 136	NO: 130	ID NO: 139	ID NO: 133	ID NO: 142	ID NO: 124	ID NO: 106
TCR-10	SEQ ID NO: 30	SEQ ID NO: 72	SEQ ID NO: 44	SEQ ID NO: 86	SEQ ID NO: 58	SEQ ID NO: 144	SEQ ID NO: 12	SEQ ID NO: 106
beta								

5 or sequences having at least 90% such as at least 95%, at least 97% or at least 99%, or 100% identity to said respective sequences.

14. The TCR according to any of the preceding items, wherein the alpha and beta chain CDRs have the following sequences:

CDR1alpha	CDR2 alpha	CDR3 alpha	CDR1 beta	CDR2 beta	CDR3 beta
SEQ ID NO: 60	SEQ ID NO: 74	SEQ ID NO: 88	SEQ ID NO: 61	SEQ ID NO: 75	SEQ ID NO: 89
SEQ ID NO: 62	SEQ ID NO: 76	SEQ ID NO: 90	SEQ ID NO: 63	SEQ ID NO: 77	SEQ ID NO: 91
SEQ ID NO: 70	SEQ ID NO: 84	SEQ ID NO: 98	SEQ ID NO: 71	SEQ ID NO: 85	SEQ ID NO: 99
SEQ ID NO: 135	SEQ ID NO: 138	SEQ ID NO: 141	SEQ ID NO: 136	SEQ ID NO: 139	SEQ ID NO: 142
SEQ ID NO: 137	SEQ ID NO: 140	SEQ ID NO: 143	SEQ ID NO: 72	SEQ ID NO: 86	SEQ ID NO: 144

10 15. The TCR according to any of the preceding items, wherein the alpha and beta chain CDR3s have the following sequences:

CDR3 alpha	CDR3 beta
SEQ ID NO: 88	SEQ ID NO: 89
SEQ ID NO: 90	SEQ ID NO: 91
SEQ ID NO: 98	SEQ ID NO: 99
SEQ ID NO: 141	SEQ ID NO: 142

SEQ ID NO: 143 SEQ ID NO: 144

16. An engineered cell expressing a TCR as defined in any one of items 1-13, wherein the engineered cell is selected from the group consisting of an immune cell derived from, a peripheral blood mononuclear cell (PBMC), a cord blood cell, a hematopoietic progenitor cell, an induced pluripotent stem cell, and a primary immune cell collected from any bodily tissue.
17. A nucleic acid encoding a TCR comprising a paired TCR alpha- and TCR beta-chain as defined in any one of items 1-13.
18. A vector comprising a nucleic acid encoding a TCR comprising a paired TCR alpha- and TCR beta-chain as defined in any one of the items 1-13.
19. A vector according to item 16, wherein the vector is a vector selected from the group consisting of an expression vector, a coding vector, non-viral or a viral vector, wherein said vector the ability to carry said nucleic acid sequence into a suitable host cell where synthesis of the encoded polypeptide takes place.
20. A pharmaceutical composition comprising a soluble protein consisting of all or a functional part of one or more TCR(s) as defined in any of items 1-13, or one or more engineered cell(s) according to item 14 or one or more vector(s) according to items 16 or 17.
21. The TCR according to any of items 1-13, an engineered cell according to item 14, a vector according to items 16 or 17 or a pharmaceutical composition according to item 18 for use in the treatment of a cancer in a patient carrying HLA-A*11, comprising administering to said patient said TCR, engineered cell, vector and/or a pharmaceutical composition comprising said TCR, wherein said cancer is a solid or haematological malignancy expressing the peptide of SEQ ID NO: 1 and/ or SEQ ID NO: 2, and wherein said TCR, engineered cell, vector and/or a pharmaceutical composition comprising said TCR is infused systemically into said patient or injected or pumped directly into a solid tumour, into a blood vessel that feeds a tumour or into the volume surrounding a solid tumour in said patient.

CLAIMS

1. A T-cell receptor (TCR), which is isolated and/or expressed in an engineered cell, specifically recognizing a G12V mutated human rat sarcoma (RAS) peptide, wherein
5 the peptide is a mutated human Kirsten RAS (KRAS) peptide, a mutated human Harvey RAS (HRAS) peptide, or a human neuroblastoma RAS (NRAS) peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/ or VVGAVGVGK (SEQ ID NO:2) presented in a HLA-A*11 complex, and wherein the TCR comprises a paired TCR alpha- and TCR beta-chain, wherein the TCR alpha
10 chain variable domain comprises SC1alpha-CDR1alpha-SC2alpha-CDR2alpha-SC3alpha-CDR3alpha-SC4alpha-SC5alpha, and the TCR beta chain variable domain comprises SC1beta-CDR1beta-SC2 beta-CDR2 beta-SC3beta-CDR3beta-SC4beta-SC5beta, wherein SC is a scaffold region and CDR is a complementarity determining region.
- 15 2. The TCR according to claim 1, wherein the TCR, when expressed in an engineered cell, is capable of specifically recognising SEQ ID NO: 1 and SEQ ID NO: 2 when presented in a HLA-A*11 complex.
3. The TCR according to claim 1 or 2, wherein the TCR, when expressed in an
20 engineered cell, has a higher sensitivity toward SEQ ID NO: 1 and SEQ ID NO: 2 when presented in a HLA-A*11 complex than does a known TCR.
4. The TCR according to claim 3, wherein the sensitivity is at least 1.05 fold higher than that of a known TCR.
- 25 5. The TCR according to claim 1 to 4, wherein the TCR, when expressed in an engineered cell, does not cause specific cytotoxic activity towards cells not expressing a G12V mutated RAS peptide
- 30 6. TCR according to any of claims 1 to 5, wherein the TCR, when expressed in an engineered cell, does not cause specific cytotoxic activity towards cells expressing the wild type human RAS peptide, such as VVGAGGVGK (SEQ ID NO: 3) and/or VVGAGGVGK (SEQ ID NO: 4).
- 35 7. The TCR according to any of the preceding claims, wherein the TCR, when expressed in an engineered cell, does not specifically recognize the G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/ or VVGAVGVGK (SEQ ID NO: 2) presented in an allelic variant of the human leukocyte antigen (HLA) molecule other than an allelic variant of the human leukocyte antigen
40 (HLA) molecule selected from the group consisting of HLA-A*11, HLA-A*30:01, HLA-

A*68:02, HLA-B*54:01, HLA-B*35:01, HLA-B*35:02, HLA-B*35:03, HLA-B*35:05, HLA-B*35:12, HLA-B*52:01, HLA-B*53:01, HLA-B*57:01 and HLA-B*58:01 serotype.

8. The TCR according to any of the preceding claims wherein the TCR, when expressed
5 in an engineered cell, does not specifically recognise other allelic variants of the human leukocyte antigen (HLA) molecule other than an allelic variant of the human leukocyte antigen (HLA) molecule selected from the group consisting of HLA-A*68:02, HLA-B*54:01, HLA-B*35:01, HLA-B*35:02, HLA-B*35:03, HLA-B*35:12, HLA-B*57:01 and HLA-B*58:01 serotype.
- 10
9. The TCR according to any of the preceding claims, wherein the TCR, when expressed in an engineered cell, does not specifically recognize the G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) presented in an allelic variant of the human leukocyte
15 antigen (HLA) molecule other than the HLA-A*11 serotype.
10. The TCR according to any of the preceding claims, which does not specifically recognize a G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/ or VVGAVGVGK (SEQ ID NO: 2) presented in a
20 HLA-A*11:03 complex.
11. The TCR according to any of the preceding claims, which does not specifically recognize a G12V mutated human RAS peptide, wherein the peptide consists of VVGAVGVGK (SEQ ID NO: 1) and/ or VVGAVGVGK (SEQ ID NO: 2) presented in a
25 HLA-A*11:03 and HLA-A*11:04 complex.
12. The TCR according to any of the preceding claims, wherein the TCR, when expressed in an engineered cell, has at the most 4% cross-reactivity towards any somatically expressed HLA-presented peptide other than a G12V mutated human rat sarcoma
30 (RAS) peptide, which consists of VVGAVGVGK (SEQ ID NO: 1) and/or VVGAVGVGK (SEQ ID NO: 2) and which is presented in a HLA-A*11 complex in a cross-reactivity assay.
13. The TCR according to any of the preceding claims comprising:
- 35 a. a TCR alpha chain CDR3 sequence with at least 75% identity to a TCR alpha chain CDR3 sequence selected from the group consisting of SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 98, SEQ ID NO: 141 and SEQ ID NO: 143 and

- b. a TCR beta chain CDR3 sequence with at least 75% identity to a TCR beta chain CDR3 sequence selected from the group consisting of SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 99, SEQ ID NO: 142, and SEQ ID NO: 144.

5 14. The TCR according to any of the preceding claims comprising:

- a) a TCR alpha chain CDR3 sequence with a TCR alpha chain CDR3 sequence selected from the group consisting of SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 98, SEQ ID NO: 141 and SEQ ID NO: 143

10 and

- b) a TCR beta chain CDR3 sequence with a TCR beta chain CDR3 sequence selected from the group consisting of SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 99, SEQ ID NO: 142 and SEQ ID NO: 144.

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15. The TCR according to any one of the preceding claims, wherein the TCR comprises a paired TCR alpha- and TCR beta-chain comprising a complete assembly of combinations of the following sequences:

	SEQ	SEQ	SEQ	SEQ	SEQ	SEQ	SEQ
	ID NO	ID NO	ID NO	ID NO	ID NO	ID NO	ID NO
SC1alpha	18	20	28	126	128	18	20
CDR1alpha	60	62	70	135	137	60	62
SC2alpha	32	34	42	129	131	32	34
CDR2alpha	74	76	84	138	140	74	76
SC3alpha	46	48	56	132	134	46	48
CDR3alpha	88	90	98	141	143	88	90
SC4alpha	7	9	15	123	125	7	9
SC5alpha	104	104	104	104	104	104	104
SC1beta	19	21	29	127	30	19	21
CDR1beta	61	63	71	136	72	61	63
SC2beta	33	35	43	130	44	33	35
CDR2beta	75	77	85	139	86	75	77
SC3beta	47	49	57	133	58	47	49
CDR3beta	89	91	99	142	144	89	91
SC4beta	8	10	16	124	12	8	10
SC5beta	105	105	106	106	106	5162	5162

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or sequences having at least 90% such as at least 95%, at least 97% or at least 99%, or 100% identity to said respective sequences.

- 5 16. The TCR according to any of the preceding claims, wherein the TCR comprises a paired TCR alpha- and TCR beta-chain comprising the following combination of sequences:

TCR#	SC1	CDR1	SC2	CDR2	SC3	CDR3	SC4	SC5
TCR-1	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
alpha	NO: 18	NO: 60	NO: 32	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				74	46	88	7	104
TCR-2	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
alpha	NO: 20	NO: 62	NO: 34	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				76	48	90	9	104
TCR-6	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
alpha	NO: 28	NO: 70	NO: 42	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				84	56	98	15	104
TCR-9	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
alpha	NO: 126	NO: 135	NO:	ID	ID	ID	ID	ID
			129	NO:	NO:	NO:	NO:	NO:
				138	132	141	123	104
TCR-10	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
alpha	NO: 128	NO: 137	NO:	ID	ID	ID	ID	ID
			131	NO:	NO:	NO:	NO:	NO:
				140	134	143	125	104
TCR-1	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
beta	NO: 19	NO: 61	NO: 33	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				75	47	89	8	105
TCR-1	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
beta	NO: 19	NO: 61	NO: 33	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				75	47	89	8	5162

TCR-2	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
beta	NO: 21	NO: 63	NO: 35	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				77	49	91	10	105

TCR-2	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
beta	NO: 21	NO: 63	NO: 35	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				77	49	91	10	5162

TCR-6	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
beta	NO: 29	NO: 71	NO: 43	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				85	57	99	16	106

TCR-9	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SE
beta	NO: 127	NO: 136	NO:	ID	ID	ID	ID	ID
			130	NO:	NO:	NO:	NO:	NO:
				139	133	142	124	106

TCR-10	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
beta	NO: 30	NO: 72	NO: 44	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				86	58	144	12	106

or sequences having at least 90% such as at least 95%, at least 97% or at least 99%, or 100% identity to said respective sequences.

- 5 17. The TCR according to any of the preceding claims, wherein the alpha and beta chain CDRs have the following sequences:

CDR1alpha	CDR2	CDR3	CDR1	CDR2	CDR3
	alpha	alpha	beta	beta	beta
SEQ ID NO:	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID
60	NO: 74	NO: 88	NO: 61	NO: 75	NO: 89
SEQ ID NO:	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID
62	NO: 76	NO: 90	NO: 63	NO: 77	NO: 91
SEQ ID NO:	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID
70	NO: 84	NO: 98	NO: 71	NO: 85	NO: 99

SEQ ID NO:	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID
135	NO: 138	NO: 141	NO: 136	NO: 139	NO: 142
SEQ ID NO:	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID
137	NO: 140	NO: 143	NO: 72	NO: 86	NO: 144

18. The TCR according to any of the preceding claims, wherein the alpha and beta chain CDR3s have the following sequences:

CDR3 alpha	CDR3 beta
SEQ ID NO: 88	SEQ ID NO: 89
SEQ ID NO: 90	SEQ ID NO: 91
SEQ ID NO: 98	SEQ ID NO: 99
SEQ ID NO: 141	SEQ ID NO: 142
SEQ ID NO: 143	SEQ ID NO: 144

19. The TCR according to any of the preceding claims wherein the TCR comprises a paired TCR alpha- and TCR beta-chain wherein the alpha chain comprises as CDR3 SEQ ID NO: 88 and the beta chain comprises as CDR3 SEQ ID NO: 89.

20. The TCR according to claim 19 wherein the alpha chain further comprises as CDR1 SEQ ID NO: 60 and as CDR2 SEQ ID NO: 74, and wherein the beta chain further comprises as CDR1 SEQ ID NO: 61 and as CDR2 SEQ ID NO: 75.

21. The TCR according to claim 20 wherein the TCR alpha- and beta chain comprises the following combination of sequences:

TCR#	SC1	CDR1	SC2	CDR2	SC3	CDR3	SC4	SC5
TCR-1	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
alpha	NO: 18	NO: 60	NO: 32	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				74	46	88	7	104
TCR-1	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
beta	NO: 19	NO: 61	NO: 33	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				75	47	89	8	105

22. The TCR according to claim 20 wherein the TCR alpha- and beta chain comprises the following combination of sequences:

TCR#	SC1	CDR1	SC2	CDR2	SC3	CDR3	SC4	SC5
TCR-1	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
alpha	NO: 18	NO: 60	NO: 32	ID	ID	ID	ID	ID

				NO:	NO:	NO:	NO:	NO:
				74	46	88	7	104
TCR-1	SEQ ID	SEQ ID	SEQ ID	SEQ	SEQ	SEQ	SEQ	SEQ
beta	NO: 19	NO: 61	NO: 33	ID	ID	ID	ID	ID
				NO:	NO:	NO:	NO:	NO:
				75	47	89	8	5162

23. An engineered cell expressing a TCR as defined in any one of claims 1-22, wherein the engineered cell is selected from the group consisting of an immune cell derived from, a peripheral blood mononuclear cell (PBMC), a cord blood cell, a hematopoietic progenitor cell, an induced pluripotent stem cell, and a primary immune cell collected from any bodily tissue.
24. A nucleic acid encoding a TCR comprising a paired TCR alpha- and TCR beta-chain as defined in any one of claims 1-122.
25. A vector comprising a nucleic acid encoding a TCR comprising a paired TCR alpha- and TCR beta-chain as defined in any one of the claims 1-22.
26. A vector according to claim 25, wherein the vector is a vector selected from the group consisting of an expression vector, a coding vector, non-viral or a viral vector, wherein said vector has the ability to carry said nucleic acid sequence into a suitable host cell where synthesis of the encoded polypeptide takes place.
27. A pharmaceutical composition comprising a soluble protein consisting of all or a functional part of one or more TCR(s) as defined in any of claims 1-22, or one or more engineered cell(s) according to claim 23 or one or more vector(s) according to claims 25 or 26.
28. The TCR according to any of claims 1-18, an engineered cell according to claim 19, a vector according to claims 21 or 22 or a pharmaceutical composition according to claim 18 for use in the treatment of a cancer in a patient carrying HLA-A*11, comprising administering to said patient said TCR, engineered cell, vector and/or a pharmaceutical composition comprising said TCR, wherein said cancer is a solid or haematological malignancy expressing the peptide of SEQ ID NO: 1 and/ or SEQ ID NO: 2, and wherein said TCR, engineered cell, vector and/or a pharmaceutical composition comprising said TCR is infused systemically into said patient or injected

or pumped directly into a solid tumour, into a blood vessel that feeds a tumour or into the volume surrounding a solid tumour in said patient.

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