

Peptide sequence	Patient ¹	Predicted to bind at least 1 reported HLA type with a Rank score of $\leq 5.0\%$ ²	Number of patient alleles predicted to bind with a rank score of $\leq 5.0\%$ ³	Number of patient alleles predicted to bind with a rank score of $\leq 2.0\%$ ⁴	Peptide SEQ ID NO.	CLT Antigen NO.
ISKPLIYY	OvCa53	YES	2	0	SEQ ID NO. 3	1
ISKPLIYY	OvCa65	YES	2	2	SEQ ID NO. 3	1
ATLQAAILYEK	OvCa66	YES	1	1	SEQ ID NO. 4	2
ATLQAAILYEK	OvCa59	YES	1	1	SEQ ID NO. 4	2

¹ Schuster et al., 2017, PNAS, 114(46), E9942–E9951; PXD007635 database

² Predicted binding to patient's reported HLA Class I types at a Rank score of $\leq 5.0\%$ score.

³ Number of patient HLA Class I types predicted to bind with a rank score of $\leq 5.0\%$.

⁴ Number of patient Class I types predicted to bind with a rank score of $\leq 2.0\%$.

The results presented here in Examples 1 and 2 are in whole or part based upon data generated by the The Cancer Genome Atlas (TCGA) Research Network (<http://cancergenome.nih.gov/>); and the Genotype-Tissue Expression (GTEx) Project (supported by the Common Fund of the Office of the Director of the National Institutes of Health, and by NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS).

Example 3 – Assays to demonstrate T cell specificity for CLT antigens in ovarian cancer patients

(a) Staining reactive T cells with CLT antigen peptide pentamers

The presence and activity of circulating CD8 T cells specific for CLT antigens in ovarian cancer patients can be measured by using HLA Class I/peptide-pentamer (“pentamer”) staining and/or *in vitro* killing assays. Thus, application of these methodologies to CLT antigens discovered using the methods elucidated in Example 1 & 2 (Table 1 and 2, Figures 1-4) can be used to demonstrate the existence of therapeutically relevant T cell responses to the CLT antigens in cancer patients.

For these studies, CD8 T cells isolated from patient blood are expanded using various cultivation methods, for example anti-CD3 and anti-CD28 coated microscopic beads plus Interleukin-2. Expanded cells can then be stained for specific CLT antigen-reactivity of their T cell receptors using CLT peptide pentamers, which

consist of pentamers of HLA Class I molecules bound to the relevant CLT Antigen peptide in the peptide-binding groove of the HLA molecule. Binding is measured by detection with phycoerythrin or allophycocyanin-conjugated antibody fragments specific for the coiled-coil multimerisation domain of the pentamer structure. In addition to the pentamer stain, further surface markers can be interrogated such as the memory marker CD45RO and the lysosomal release marker CD107a.

Association of pentamer positivity with specific surface markers can be used to infer both the number and state (memory versus naive/stem) of the pentamer-reactive T cell populations

Pentamer stained cells may also be sorted and purified using a fluorescence activated cell sorter (FACS). Sorted cells may then be further tested for their ability to kill target cells in *in vitro* killing assays. These assays comprise a CD8 T cell population, and a fluorescently labelled target cell population. In this case, the CD8 population is either CLT antigen-specific or CD8 T cells pentamer-sorted and specific for a positive-control antigen known to induce a strong killing response such as Mart-1. The target cells for these studies may include peptide-pulsed T2 cells which express HLA-A*02, peptide-pulsed C1R cells transfected with HLA-A*02,03 or B*07 or ovarian cancer cells lines previously shown to express the CLTs/CLT antigens, or patient tumor cells. Peptides used to pulse the T2 or C1R cells include CLT antigen peptides or positive control peptides. Target cells may be doubly labelled with vital dyes, such as the red nuclear dye nuclight rapid red which is taken up into the nucleus of healthy cells. Additional evidence of target cell attack by specific T cells may be demonstrated by green caspase 3/7 activity indicators that demonstrate caspase 3/7-mediated apoptosis. In this way, as target cells are killed, by apoptosis mediated by CD8 T cells, they lose their red fluorescence and gain green fluorescence due to the caspase 3/7 activity intrinsic to apoptosis. Thus, application of such killing assays to pentamer-sorted, CLT antigen-specific CD8 T cells can be used to enumerate the cytotoxic activity of CLT-antigen-specific T cells in *ex vivo* cultures of ovarian cancer patient T cells.

(b) HERVfest analyses of T cell specificity in ovarian cancer patients

Functional expansion of specific T cells (fest) technology has been used to identify specific tumor-derived epitopes present in the “mutation-associated neoantigen” (MANA) repertoire found in tumor cells of patients who have responded to checkpoint-blockade therapies (Anagnostou et al., Cancer Discovery 2017; Le et

al., Science 2017). Application of this technology to CLT antigens discovered using the methods elucidated in Example 1 & 2 (Table 1 and 2, Figures 1-4) can confirm the existence of therapeutically relevant T cell responses to the CLT antigens in cancer patients.

Like other assays (e.g., ELISPOT) to identify epitope-specific T cells in a subject who has undergone immune exposure, “fest” technologies derive their specificity by expanding the cognate T cells in *ex vivo* cultures that include antigen-presenting cells and suitable antigenic peptides. The technique differs from other immunological assays in that it utilizes next-generation sequencing of the T cell receptor (TCR) mRNA present in these amplified cultures (specifically: TCRseq targeting the TCR-V β CDR3 region) to detect the specific TCRs that are expanded in the cells cultured with the target peptides (preselected to match the HLA type of the patient, using standard HLA-binding algorithms). Application of TCRseq to tumor tissues in the same patient, harvested after successful checkpoint-blockade therapy, can then be used to determine which TCRs/T cells detected in the *ex vivo*, peptide-stimulated cultures, are also present at the site of immune-suppression of the cancer. In the case of MANAfest, the method is used to identify specific TCRs that recognize MHC-presented neoantigen peptides that evolve in each patient’s tumor and are also detected in the T cells in the patients’ tumors, permitting the identification of the functionally relevant neoantigen peptides among the thousands of possible mutant peptides found by full-exome sequencing of normal and tumor tissues from each patient (Le et al., Science 2017).

Application of MANAfest (Anagnostou et al., 2017 Cancer Discovery) technology to CLT antigens is done as follows. Step 1: Peptides predicted to contain epitopes that efficiently bind selected HLA supertypes are identified in CLT antigens. Step 2: PBMCs from appropriate patients are selected, and matched by HLA type to the peptide library selected in step 1. Step 4: PBMCs from these patients are separated into T cell and non-T cell fractions. Non-T cells are irradiated (to prevent proliferation), added back to the patient’s T cells, and then divided into 20-50 samples, and cultivated in T cell growth factors and individual CLT-specific synthetic peptides (selected in step 1) for 10 to 14 days. Step 4: TCRseq (sequencing of the epitope-specific TCR-V β CDR3 sequences) is performed on all wells to identify the cognate T cells/TCRs that have been amplified in the presence of the test peptides; specificity of these TCRs is determined by comparison to TCRs detected in