Isolation and characterisation of TCRs that recognise Citrullinated and Homocitrullinated post translationally modified peptides

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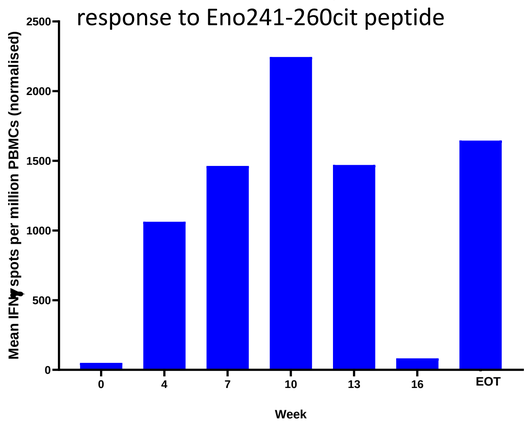
Introduction

- Under conditions of cellular stress, proteins can be post translationally modified causing them to be recognised by the immune system. One such stress induced post translational modification (siPTM) modification is citrullination, the conversion of arginine residues to citrulline by peptidylarginine deiminase (PAD) enzymes.
- We are currently running A Phase 1/2, Multicentre, Open-Label Study of Modi-1 in Patients with Breast, Head and Neck, Ovarian, or Renal Cancer (The ModiFY Study) study. The Moditope® vaccine incorporates two citrullinated vimentin peptides (Vim28cit and Vim415cit), and a citrullinated α-enolase peptide (Eno241cit), each conjugated to the toll-like receptor (TLR)1/2 ligand adjuvant AMPLIVANT®.
- Another post translational modification is the carbamylation of lysine to homocitrulline. This reaction occurs when isocyanic acid reacts with the amine (NH2) groups on lysine to yield homocitrulline. The carbamylation of amine groups leads to a change in molecular charge, which in turn alters antigenic properties and can lead to the generation of unique T cell and antibody epitopes.

T cell responses to post-translationally modified peptides

A Vaccinated Ovarian cancer patient: T cell response to Eno241-260cit peptide



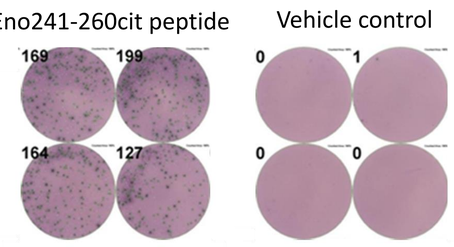
Ovarian cancer patient HLA type:

A1, A3, B7, B8, Cw6, Cw7, DR15, DR17, DQ2, DQ6, DP4

Healthy donor HLA type:

A2, A11, B40, B44, DR13, DR16, DQ5, DQ6, DP4, DP10

B IFNγ ELISpot Response to Enolase 241cit peptide



C IFNγ ELISpot Response Aldo 204-219 hcit peptide

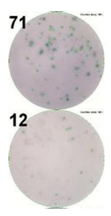
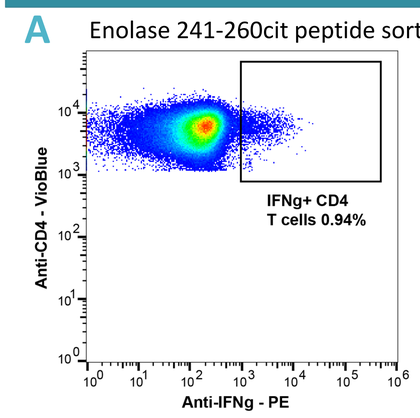


Figure 1. (A) T cell response to Eno241-260cit peptide (VIGMDVAASEFF{cit}SGKYDLD) in PMBCs cultured from an ovarian cancer patient enrolled on the ModiFY study measured by IFNγ ELISpot assay. PMBCs were cultured in the presence of Eno241-260cit peptide for 7-10 days with cytokine support. Patient was vaccinated at week 0, 4, 7 and 13. (B) IFNγ ELISpot assay was performed at the end of the culture period (C) IFNγ ELISpot assay performed on cultured PBMCs in the presence of Aldo204-219Hcit peptide (YVTE-Hcit-VLAAVY-Hcit-ALSD), PBMCs cultured from a healthy donor following 3 rounds of stimulations using peptide pulsed dendritic cells and activated B cells.

Sorting peptide reactive T cells

A Enolase 241-260cit peptide sort



B Aldolase 204-219Hcit peptide sort

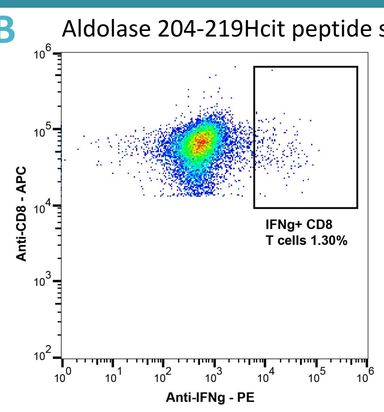
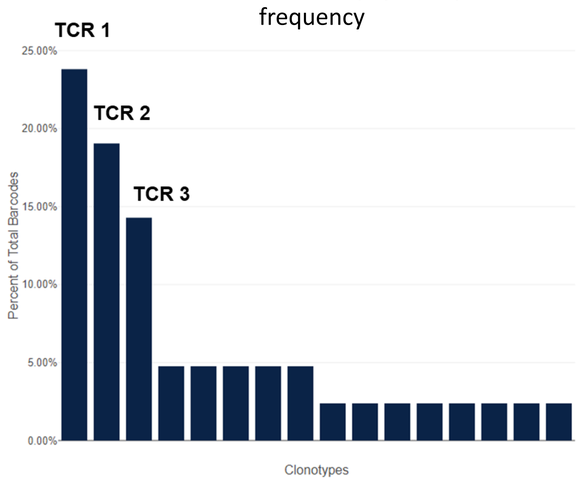


Figure 2. T cells were sorted using IFNγ secretion assay following stimulation with the relevant peptide. (A) Ovarian cancer patient PBMCs sorted at week 25-EOT. Blocking and depletion assays demonstrated this response was meditated by CD4 T cells (data not shown). (B) Healthy donor Aldo204-219Hcit T cells were sorted following overnight stimulation; 6 positive ELISpot wells were pooled for sorting.

TCR sequencing and gene expression profile

Sorted CD4+ Eno 241-260cit peptide specific TCR frequency



Sorted CD4+ Eno 241-260cit peptide specific Gene expression profile

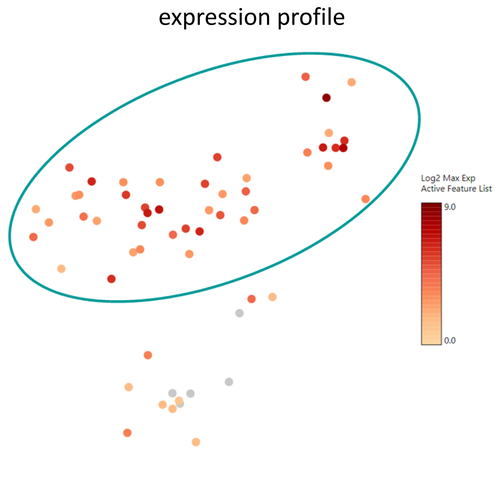
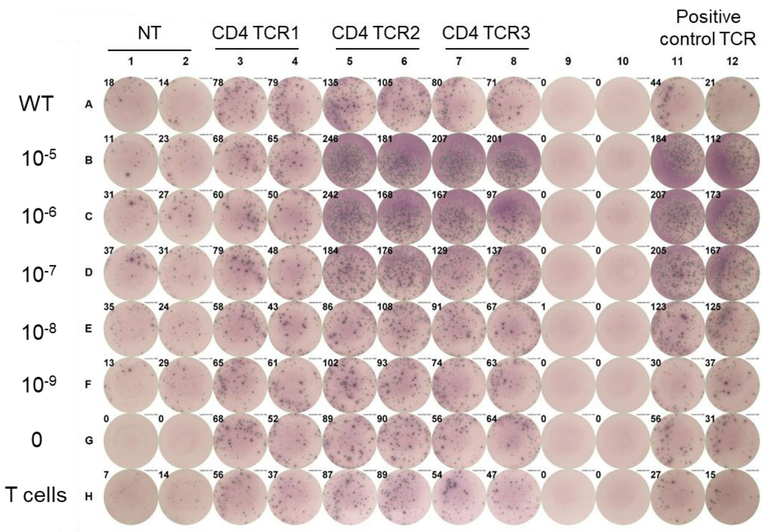


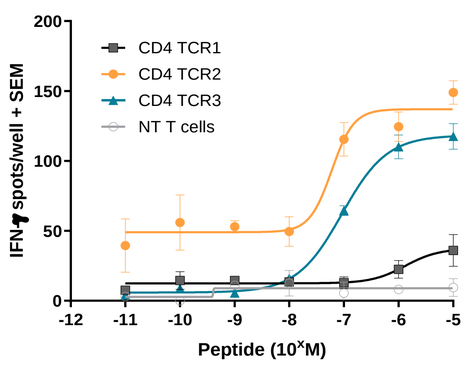
Figure 3. TCR and RNA sequencing performed on sorted T cells. TCR and RNA sequencing was performed using the 10X 5' GEX + TCR kits and sequencing performed by Source BioScience (UK) for the Eno241-260cit specific T cells and by iRepertoire (USA) for the Aldo204-219Hcit specific T cells. Only data from the Eno241-260cit specific sequencing is shown here, 3 TCR with the highest frequency was selected, these T cells are CD4+ IFNγ+ and Granzyme B +, Cytotoxic CD4+ T cells.

Peptide recognition using TCR transduced T cells

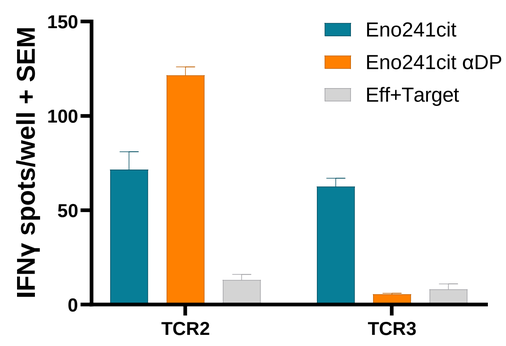
A TCR transduced T cells: Recognition of Eno241-260cit peptide pulsed B cells



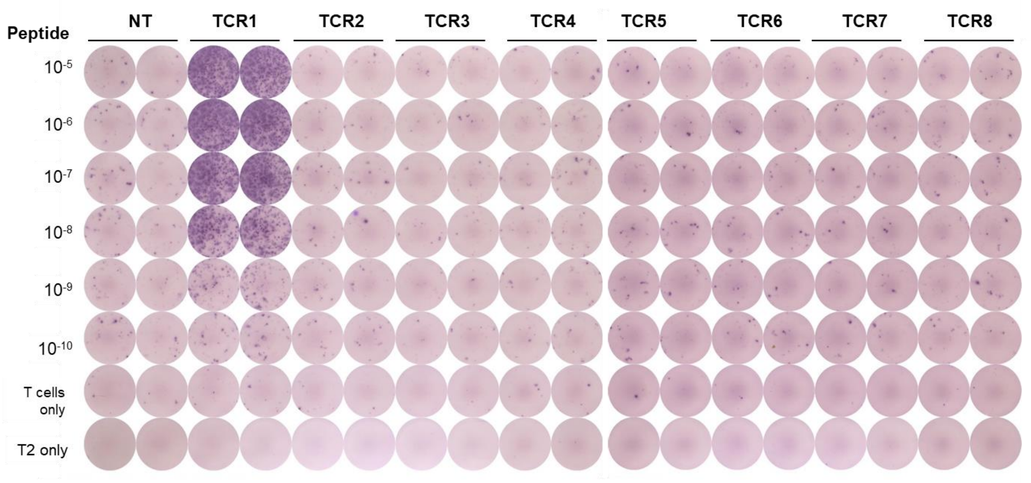
Peptide titration on B cells



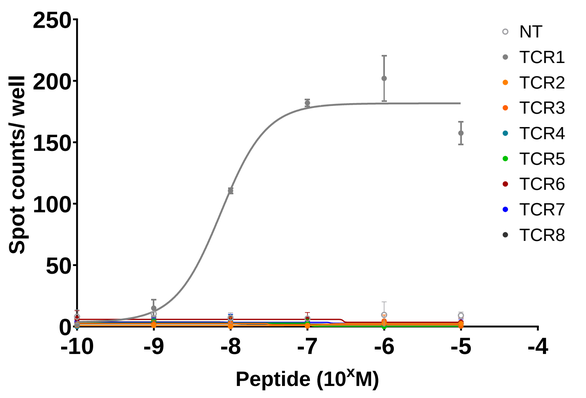
Anti HLA-DP blocking T cell response



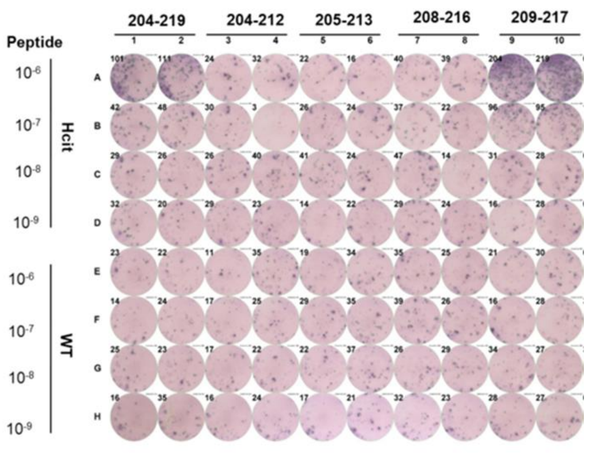
B TCR transduced T cells: Recognition of Aldo 204-219Hcit peptide pulsed T2 cells



Peptide titration on T2 cells



Identification of minimal epitope



NT = non transduced T cells, WT = wildtype peptide not containing Citrulline or Homocitrulline.

Figure 4 Recognition of peptide pulsed targets by TCR transduced T cells. Lentivirus constructs were generated for the highest frequency TCRs. Primary T cells were transduced with lentivirus TCR constructs, an IFNγ ELISpot assay was performed 11-13 days Later. **(A)** Eno241-260cit TCR responses to peptide pulsed activated B cells, 3 TCRs were selected and only TCRs 2 and 3 showed a specific response to Eno241-260cit, this response was blocked by an anti HLA-DP4 antibody for TCR3 but not for TCR 2. (B) Aldo204-219Hcit responses to peptide pulsed T2 cells (HLA-A2+) was determined for the 8 highest frequency TCRs, only TCR 1 showed a response, this response is HLA-A2 restricted, and the minimal epitope was defined as 209-217 VLAAVY-Hcit-AL.

Conclusions

- TCRs specific for the post translationally modified peptides Eno241-260cit and Aldo204-219Hcit were isolated and charactersied.
- One TCR specific for Aldo204-219Hcit TCR was isolated from a healthy donor, this TCR recognises a 9mer peptide 209-217 VLAAVY-Hcit-AL presented in the context of HLA-A2.
- Two Eno241-260cit specific TCRs were isolated, one is restricted by HLA-DP (patient is DP4), the restriction of the second is still to be determined.
- TCRs showed no or minimal recognition of wildtype peptide indicating the TCRs are specific for the post translationally modified peptide only.
- These TCRs can be used in the cellular immunotherapy field.
- Approximately 70% of the population are HLA-DP4 positive, the HLA-DP4 restriction of Eno241-260cit TCR means it is more broadly applicable to a larger population than HLA-A2 restricted therapies.