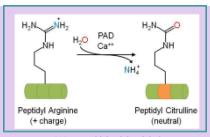
# Healthy individuals have a repertoire of CD4 cells that recognize and respond to citrullinated peptides – a possible new target for cancer immunotherapy?

Ruhul Hasan Choudhury[1], Ian Daniels[1], Vicky Brentville[1], Katherine Cook[1], Peter Symonds[1], Poonam Bilimoria[1]
Suha Atabani, Rachael Metheringham[1], Lindy Durrant[1,2].

1. Scancell Holdings plc, Oxford, UK. 2. Nottingham University, Nottingham, UK.

## Introduction




- Autophagy is triggered in response to cellular stress such as nutrient deprivation, hypoxia and DNA damage.
- Citrullination is a post-translational modification which occurs during autophagy.
- Citrullination is carried out by peptidylarginine deiminase (PAD) enzymes and involves conversion of arginine to citrulline.
- Cancer cells citrullinate proteins. Ubiquitous cytoskeletal protein Vimentin and glycolytic enzyme α-Enolase are citrullinated by PAD enzymes.
- In the absence of inflammation, immunity is regulated, whereas in its presence CD4 responses to modified self-antigens are stimulated [(1)].
- Stressful conditions in tumour microenvironment and inflammation leads to presentation of modified peptides on MHC class II which are a target for CD4 T cells. We have shown that these can be harnessed for tumour therapy [(2,3)].
- Citrulline specific CD4 responses have been previously shown in rheumatoid arthritis patient.
- Here we show that healthy individuals have a T cell repertoire to citrullinated peptides which in some donors responses appears to be pre-existing. Finally, we also show a similar repertoire exists in cancer patients and suggest that potentially this repertoire could be boosted by immunization to assist in tumor eradication.

## Results

### CD25 depletion uncovers response to citrullinated peptides in healthy donors

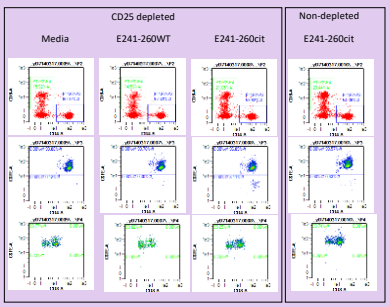
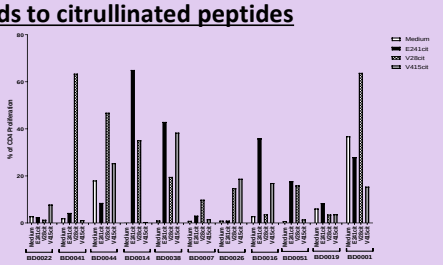



Figure 1: Response to E241cit and wild peptides in healthy donor. CD25 depleted or undepleted PBMCs from healthy donors were labelled with CFSE and stimulated with 10μg/ml citrullinated or wt peptides. Proliferation was determined by FACS at day 10. CD4 proliferation was similar to background in non-depleted PBMCs stimulated with E241cit. CD4 proliferation was observed after CD25 depletion with E241cit stimulated but not with E241wt. v

### Healthy donor have a repertoire of CD4 T cells that responds to citrullinated peptides

Figure 2: Response to E241cit, V28 and V415 in healthy donors after CD25 depletion. Proliferation was determined by FACS at day 9 and 10.. Variable responses to citrullinated peptides were seen among donors. Most donors responded to at least one peptide. BD0038 donor responded to all three peptides .




### CD4 responses are highly clonal with TEMRA and effector memory phenotype

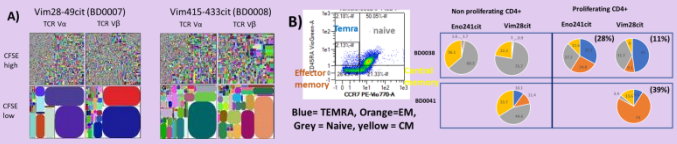



Figure 3: Characterisation of responses to citrullinated peptides. A, Proliferating (CFSE low) CD4+ cells were sorted on MoFlow sorter (Beckman Coulter) at day 10 from two responding donors and sent for TCR repertoire analysis by iRepertoire Inc. Repertoire data for TCR Vα and Vβ is shown as Tree plots where each spot denotes a specific V-J CDR3 and the spot size denotes frequency. B, Proliferating CD4+ cells at day 10 from two donors were analysed for phenotype using CD45RA and CCR7 antibodies.

## Results

### Proliferating CD4 cells express activation marker CD134 and contain IFNγ and GranB

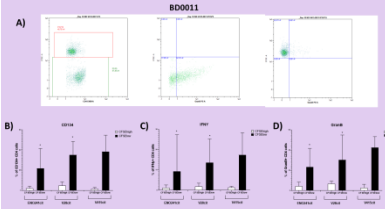



Figure 4: Expression of activation and cytotoxic markers on proliferating CD4 cells. A) GranB expression on proliferating and non-proliferating CD4 cells in response to V415cit. B) Significantly high proportion of CD4 cells express CD134, IFNγ and GranB in proliferating population compared to non-proliferating population in response E241cit and V28cit.

### Healthy Donor showed both Naïve and memory response to citrullinated peptides

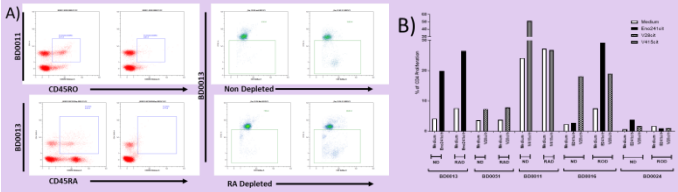



Figure 5: Response to E241cit, V28 and V415 in healthy donors after CD45RA or CD45RO depletion. A) Depletions for CD45RA or CD45RO are very effective. BD0013 donor showed response to E241cit which persists after CD45RA depletion. B) Memory (BD0013, BD0051, BD0024) and naïve (BD0016) responses were observed to E241cit and V28cit. Naïve response was observed to V415cit with BD0011.

### T cells repertoire also exists in cancer patients that can respond to citrullinated peptides

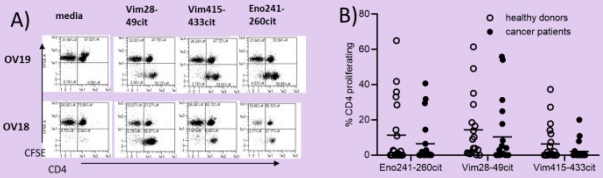



Figure 6: Response to E241cit, V28 and V415 in ovarian cancer patients. Proliferation was determined by FACS at day 9 and 10. double of background proliferation was considered significant. Variable responses to citrullinated peptides were seen among donors. A) OV18 and OV19 showed CD4 responses to all three peptides. B) No significant differences in response were observed between healthy donor and ovarian cancer patients.

## Conclusions

- Citrullinated Vimentin and α-Enolase peptides can stimulate CD4 T cell response. CD25 depletion can unmask or enhance responses.
- Proliferating CD4 T cells express activation marker CD134, IFNγ and Granzyme B.
- CD4 responses are highly clonal and showed TEMRA (highly cytotoxic effector memory cells) or effector memory phenotype.
- Some donors showed a pre-existing (memory) responses to citrullinated peptides.
- Repertoire to citrullinated peptides exists in cancer patients that could potentially be boosted by immunization to assist in tumor eradication.

## References

1. Feitsma AL *et al.* Identification of citrullinated vimentin peptides as T cell epitopes in HLA-DR4-positive patients with rheumatoid arthritis. Arthritis Rheum 2010 Jan;62(1):117-25
2. Brentville VA, Metheringham RL, Gunn B, Symonds P, Daniels I, Gijon M, Cook K, Xue W, Durrant LG. Citrullinated vimentin presented on MHC-II in tumor cells is a target for CD4+ T cell-mediated antitumor immunity. Cancer Research 2016 Feb 1;76(3):548-60.
3. Cook K, Daniels I, Symonds P, Pitt T, Gijon M, Xue W, Metheringham R, Durrant L, Brentville V. Citrullinated α-enolase is an effective target for anti-cancer immunity. Oncoimmunology. 2017 Nov 6;7(2):e1390642