Citrullinated glucose-regulated protein 78 is a candidate target for cancer immunotherapy

V Brentville[1], J Chua[1], S Atabani[2], P Symonds[1], K Cook[1], R Choudhury[1], I Daniels[1], S Shah[1], and L Durrant[1,2].

[1]Scancell Holdings plc, Oxford UK, [2]Nottingham University, Nottingham, UK

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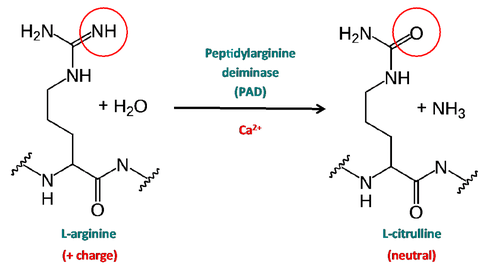
INTRODUCTION

- CD4 T cells are potent effectors but CD4 responses to self antigens are often attenuated.
- Cellular stress induces autophagy which leads to modification of proteins recognised by the immune system (1). One such modification is citrullination (cit).
- In the absence of inflammation, immunity is regulated, whereas in its presence CD4 responses to modified self-antigens are stimulated (2).
- Autophagy is upregulated in rapidly proliferating cancer cells. Cancer cells citrullinate proteins (3).
- Stressful conditions in tumour microenvironment leads to presentation of modified peptides on MHC class II which are targets for CD4 T cells. We have shown that these can be harnessed for tumour therapy (4,5,6).
- The ER chaperone protein glucose-regulated protein 78 (GRP78) is a master regulator of ER stress. ER stress triggered induction of GRP78 leads to enhanced survival of cancer cells and an association of GRP78 expression is linked to tumour progression (7).
- GRP78 has been shown to also be involved in Ca^{2+} homeostasis and is required for stress induced autophagy (8). Citrullinated GRP78 has also been defined as an autoantigen in Type 1 diabetes (9).
- In this study we identify T cell responses to citrullinated GRP78 peptides in HLA transgenic mice and show responses to a single peptide can be restricted through multiple HLA alleles. We demonstrate responses to be citrulline specific, CD4 mediated and show efficient tumour therapy. We show a repertoire of CD4 T cells to citrullinated GRP78 in both healthy donors and ovarian cancer patients but suggest that the repertoire may be attenuated in cancer patients.



Citrullination. A modification that occurs within stressed cells. Peptidylarginine deiminase (PADs) enzymes are activated and convert arginine to citrulline by altering the positively charge aldimine group (=NH) group of arginine to the neutrally charged ketone group (=O) of citrulline.

Identification of T cell responses to citrullinated GRP78 peptides restricted through HLA-DR4, HLA-DR1 and HLA-DP4

Table 1. Predicted binding scores of 5 selected GRP78 peptides to HLA-DR4, DR1 and DP4 using IEDB prediction software. **R** = arginine changed to citrulline.

coordinates	sequence	DR*0401 binding score	DR*0401 predicted cores	DR*0101 binding score	DR*0101 predicted cores	DP*0401 binding score	DP*0401 predicted cores
189-208	TIAGLNVMRIINEPTAAAIA	0.9	VMRIINEPT IAGLNVMRI	7.28	VMRIINEPT IAGLNVMRI	59.16	AGLNVMRII IAGLNVMRI LNVMRIINE
315-334	EDFSETLTRAKFEELNMDLF	11.45	FSETLTRAK	54.2	SETLTRAKF FSETLTRAK LTRAKFEEL	0.13	LTRAKFEEL
327-346	EELNMDLFRSTMKPVQKVLE	6.65	MDLFRSTMK FRSTMKPVQ	28.99	FRSTMKPVQ LFRSTMKPV MDLFRSTMK	19.99	LFRSTMKPV LNMDLFRST
460-478	VTIKVYEGERPL7KDNHLL	10.08	YEGERPLTK	44.67	KVYEGERPL YEGERPLTK	17.85	KVYEGERPL YEGERPLTK
501-520	EIDVNGILRVTAEDKGTGNK	11.98	ILRVTAEDK	35.86	IDVNGILRV ILRVTAEDK	59.87	DVNGILRVT IDVNGILRV

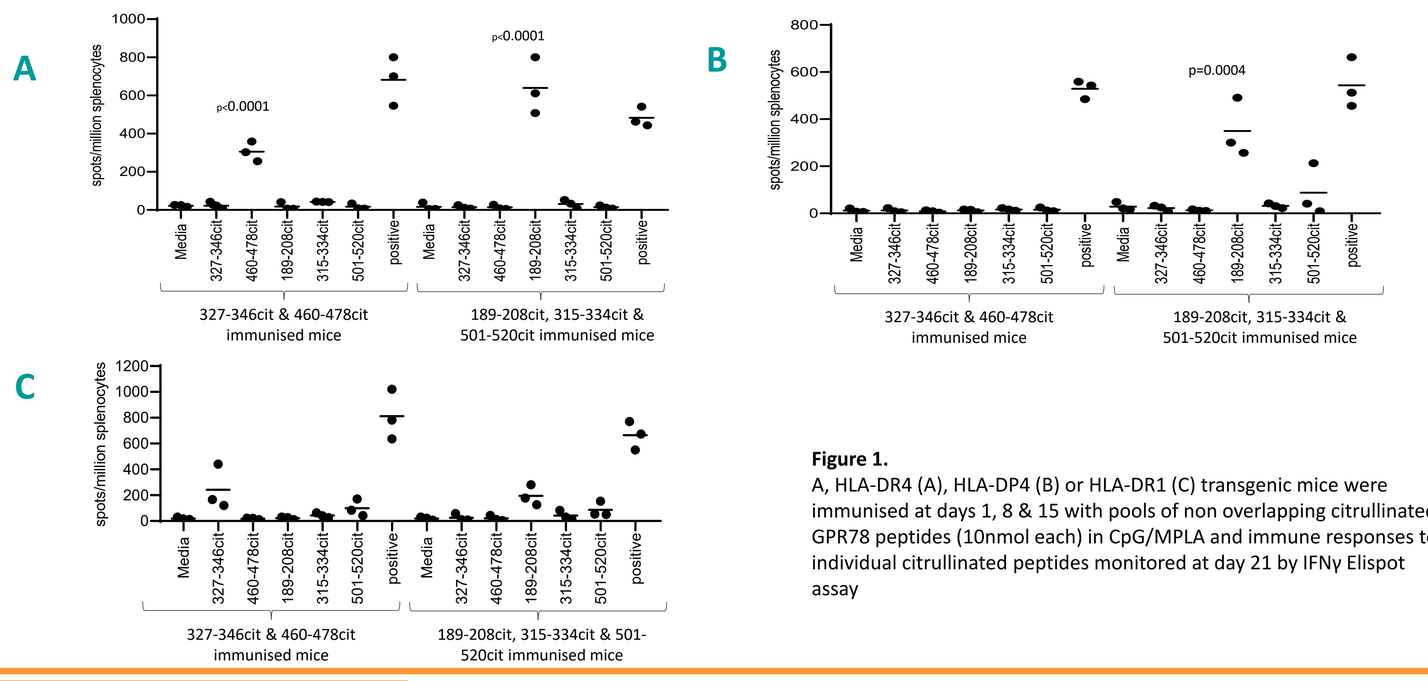


Figure 1.
A, HLA-DR4 (A), HLA-DP4 (B) or HLA-DR1 (C) transgenic mice were immunised at days 1, 8 & 15 with pools of non overlapping citrullinated GPR78 peptides (10nmol each) in CpG/MPLA and immune responses to individual citrullinated peptides monitored at day 21 by IFNγ Elispot assay

HLA-DP4 and DR1 restricted responses to citrullinated GRP78 189-208 are citrulline specific and CD4 mediated.

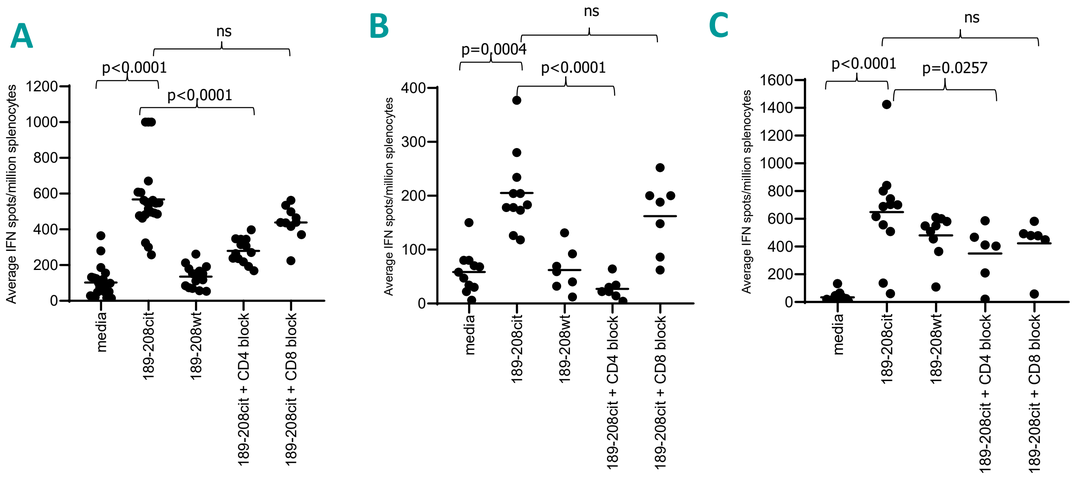


Figure 2.
HLA-DP4 (A), HLA-DR1 (B) or HLA-DR4 (C) transgenic mice were immunised with citrullinated (cit) 189-208 peptide in CpG/MPLA on days 1, 8 & 15. On day 21, immune responses to cit and native (wt) peptides were monitored by IFNγ Elispot assay. Responses to cit peptide were also assessed in the presence of CD4 (clone OKT4 or GK1.5) or CD8 (clone 2.43) blocking antibody (20ug/ml).

Immunisation with citrullinated GRP78 189-208 peptide elicits tumour therapy

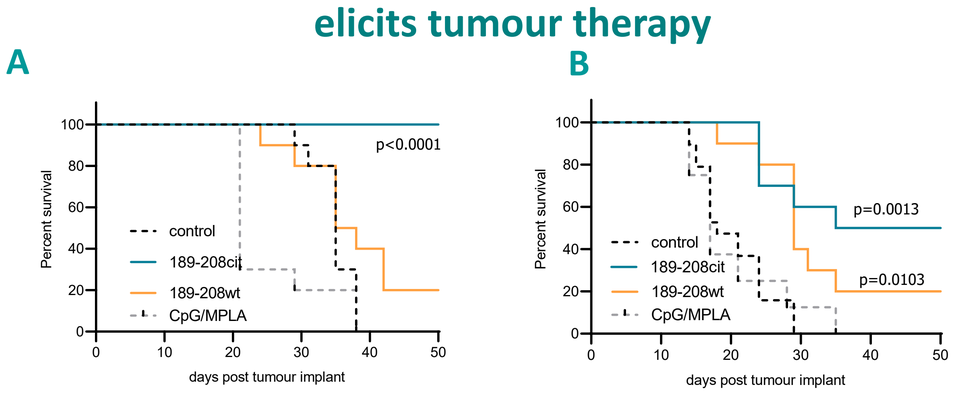


Figure 3.
HLA-DP4 (A) or HLA-DR4 (B) transgenic mice were challenged with B16 tumour cells expressing DP4 or DR4 under an IFNγ inducible promotor. 4, 11 and 18 days later mice were immunised with citrullinated (cit) or native (wt) GRP78 189-208 peptide in CpG/MPLA alongside CpG/MPLA only or unimmunised control. Tumour growth and survival was monitored, N=10/group.

Healthy humans show a repertoire of CD4 T cells able to respond to citrullinated GRP78 189-208 peptide which is attenuated in cancer patients

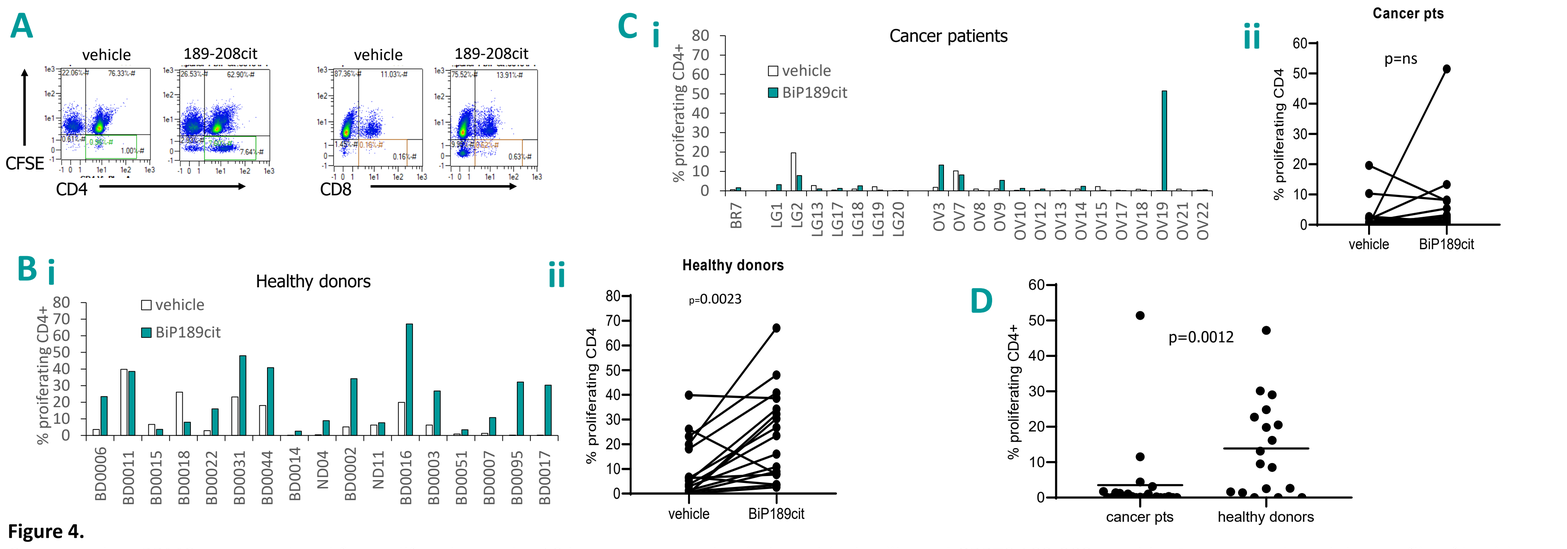


Figure 4.
Cancer patient PBMCs were analysed for proliferation at day 10 in response to stimulation with citrullinated GRP78 189-208 peptide. A, an example staining profile of healthy donor PBMCs responding to citrullinated GRP78 189-208 peptide. A panel of healthy donors (B) and cancer patients (C) (OV – ovarian, LG – lung, BR – breast) PBMCs were analysed for proliferation in response to citrullinated GRP78 189-208 peptide. Results shown as percentage proliferating CD4+ cells shown for each donor (i) and combined (ii). D, Comparison of proliferating CD4 cells in healthy donors and cancer patients in response to citrullinated GRP78 189-208 peptides. Data normalised against background control.

CONCLUSIONS

- **Citrullinated GRP78 peptides stimulate T cell responses restricted through HLA-DP4, HLA-DR1 and HLA-DR4.**
- **T cell responses restricted through HLA-DP4 and HLA-DR1 are CD4 mediated and citrulline specific.**
- **Citrullinated GRP78 189-208 peptide vaccination mediates efficient tumour therapy suggesting citrullination of GRP78 within tumours and the presentation of citrullinated GRP78 189-208 peptide on MHC class II.**
- **Healthy donors and cancer patients show a repertoire of CD4 T cells capable of responding to citrullinated GRP78 189-208 peptide.**
- **Responses in cancer patients appear attenuated compared to healthy donors**
- **Targeting of citrullinated GRP78 by vaccination may help overcome this.**

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