Targeting citrullinated vimentin and enolase with cytotoxic CD4 T cells, relies upon MHC-II expression by tumors, reduces myeloid suppressor cells and directly kills tumor cells

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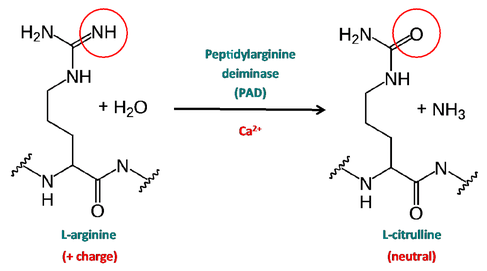
INTRODUCTION

- 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).
- Cancer cells citrullinate proteins (3). Citrullinated proteins in cancer cells include ubiquitous cytoskeletal protein Vimentin and glycolytic enzyme α-Enolase.
- Stressful conditions in tumour microenvironment leads to presentation of modified peptides on MHC class II which is a target for CD4 T cells. We have shown that these can be harnessed for tumour therapy (4,5,6).
- Peptide vaccines require adjuvants for efficient T cell stimulation and TLR ligands have been shown to have such adjuvant properties. Mixtures of peptide with TLR agonists have been shown to be efficient for induction of CD4 and CD8 T cell responses however linkage of the peptide directly to the TLR ligand have been shown to enhance responses(7).
- Amplivant® Technology developed by ISA Pharmaceuticals makes use of a synthetic TLR2 agonist that can be chemically coupled to a synthetic long peptide (SLP). Amplivant ® linked SLPs allow better processing and presentation by dendritic cells for the induction of both CD8 and CD4 T cell responses(8).
- In this study we characterise the immune responses from a peptide vaccine (Modi-1) targeting citrullinated Vimentin and α-Enolase and show efficient tumour therapy is mediated by CD4 T cells. We examine the effects of direct peptide conjugation to Amplivant ® on the stimulation of citrullinated peptide specific CD4 T cells in a mouse model and show a repertoire of CD4 T cells specific to Modi-1 in both healthy donors and ovarian 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.

Citrullinated vimentin and enolase peptides can be combined into a single vaccine (Modi-1) with no observed immunodominance and provides effective tumour therapy

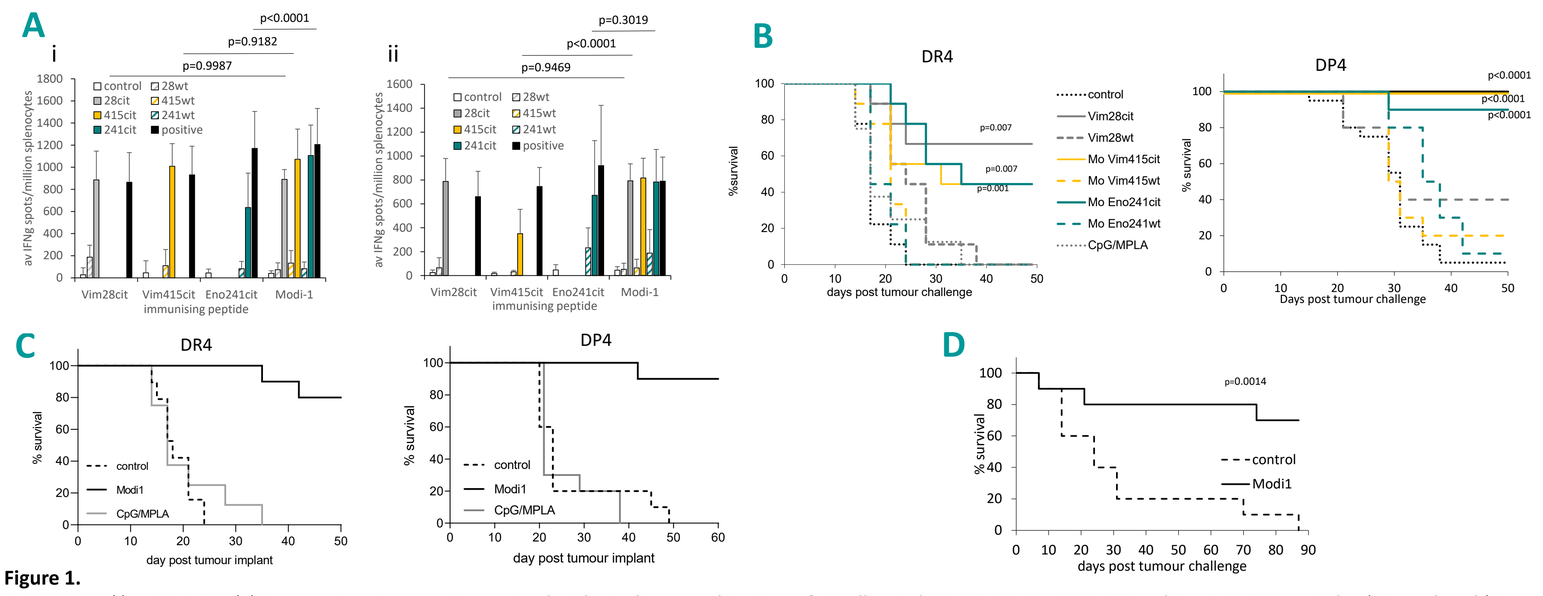


Figure 1.
A, HLA-DR4 (i) or HLA-DP4(ii) transgenic mice were immunised with single or combination of citrullinated Vim28-49, Vim415-433 and Eno241-260 peptides (10nmol each) in CpG/MPLA and immune responses to citrullinated or native (wt) peptides monitored by IFNγ Elispot assay. HLA-DR4 or DP4 transgenic mice were challenged with B16F1 tumour cells expressing DR4 or DP4 under an IFNγ inducible promoter and four days later mice were immunised with individual citrullinated or native peptides (B) or the Modi-1 combination (C) alongside CpG/MPLA adjuvant only and tumour growth and survival monitored. D, HLA-DP4 transgenic mice were challenged with ID8 ovarian cells constitutively expressing HLA-DP4 and four days later mice were immunised with Modi-1 combination and tumour growth and survival monitored. N=10/group.

Modi-1 mediates rapid tumour regression which correlates with increase in CD4 T cell infiltrate and decrease in myeloid suppressor cells

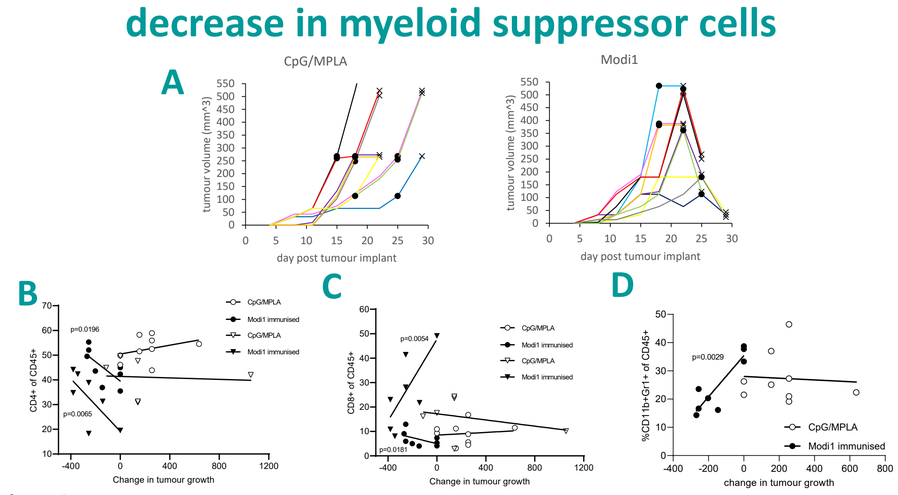


Figure 2.
HLA-DP4 transgenic mice challenged with B16F1 tumour cells expressing DP4 under an IFNγ inducible promoter were immunised with Modi-1 in CpG/MPLA or CpG/MPLA only when tumours reached 5-10mm diameter. Tumour size was monitored (A), circles (•) denote the immunisation point and crosses (X) the analysis point. Tumours were analysed by flow cytometry for percentage of CD4 (B) or CD8 (C) T cells among CD45+ cells and percentage of MDSCs among CD45+ cells (D) and numbers were correlated to tumour size change. Tumour size change was calculated as tumour volume at analysis minus tumour volume at immunisation. Data shown is from two independent studies (circles and triangles) in which n>6

CD4 T cells drive Modi-1 vaccine mediated tumour therapy

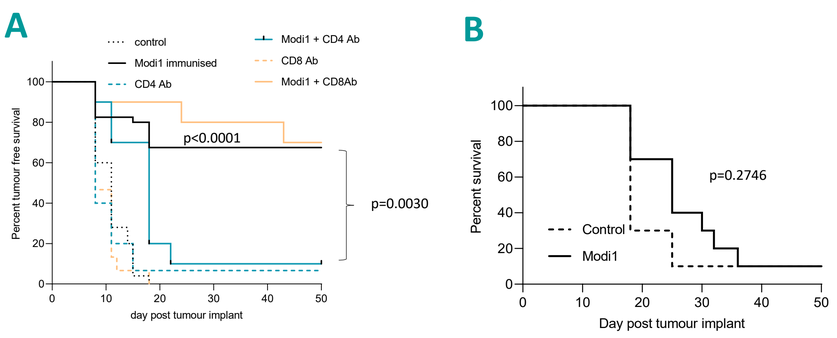


Figure 3.
A, HLA-DP4 transgenic mice were challenged with B16 cells expressing DP4 under an IFNγ inducible promoter and four days later mice were immunised with Modi-1 in the presence of CD4 or CD8 depletion antibodies. B, HLA-DP4 transgenic mice were challenged with B16F1 tumour expressing appropriate MHCI but lacking MHCII expression and four days later mice were immunised with Modi-1. Tumour growth and survival monitored. N>8/group.

Linkage of peptide to TLR2 agonist enhances immune responses and promotes tumour therapy with lower dose vaccine

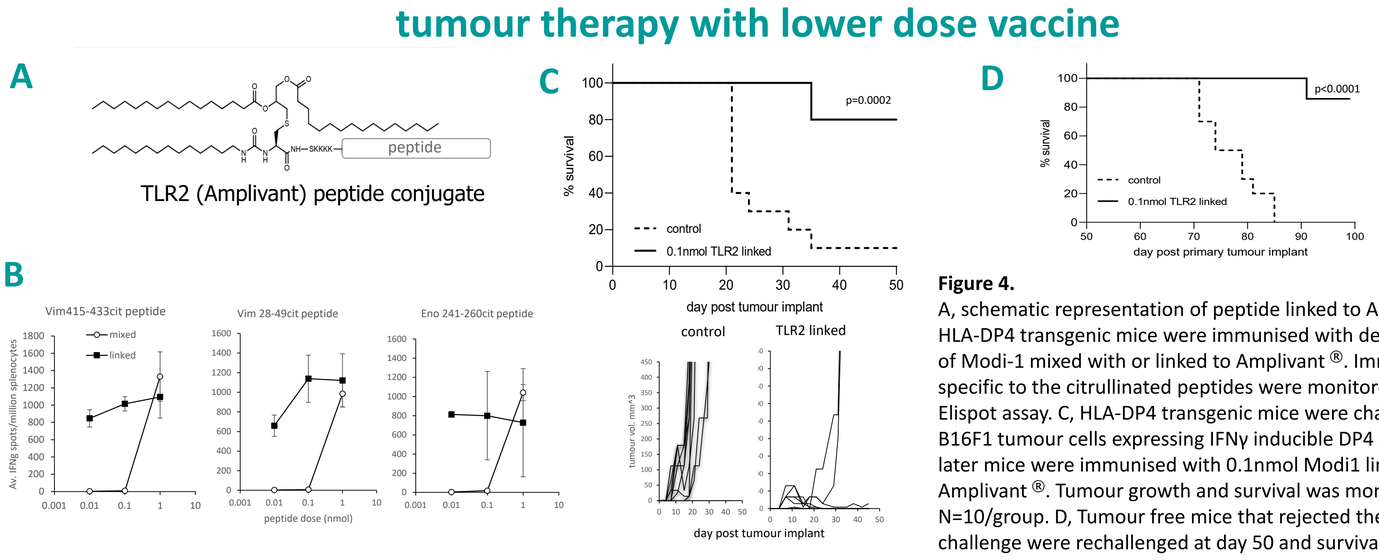


Figure 4.
A, schematic representation of peptide linked to Amplivant ®. B, HLA-DP4 transgenic mice were immunised with decreasing doses of Modi-1 mixed with or linked to Amplivant ®. Immune responses specific to the citrullinated peptides were monitored by IFNγ Elispot assay. C, HLA-DP4 transgenic mice were challenged with B16F1 tumour cells expressing IFNγ inducible DP4 and four days later mice were immunised with 0.1nmol Modi1 linked to Amplivant ®. Tumour growth and survival was monitored, N=10/group. D, Tumour free mice that rejected the first tumour challenge were rechallenged at day 50 and survival monitored.

Ovarian cancer patients have a repertoire of CD4 T cells that respond to Modi-1

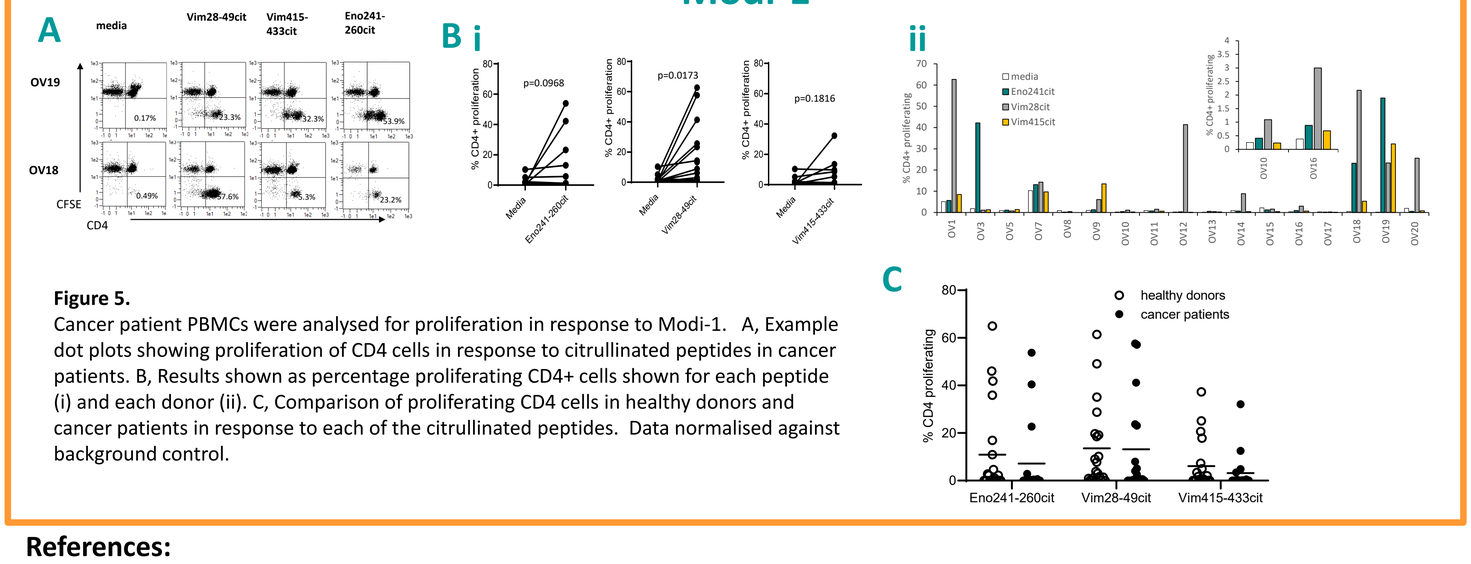


Figure 5.
Cancer patient PBMCs were analysed for proliferation in response to Modi-1. A, Example dot plots showing proliferation of CD4 cells in response to citrullinated peptides in cancer patients. B, Results shown as percentage proliferating CD4+ cells shown for each peptide (i) and each donor (ii). C, Comparison of proliferating CD4 cells in healthy donors and cancer patients in response to each of the citrullinated peptides. Data normalised against background control.

CONCLUSIONS

- **Citrullinated vimentin and α-enolase peptides can be combined into Modi-1 vaccine with no sign of immunodominance**
- **Modi-1 vaccine provides rapid tumour therapy against established tumours**
- **Modi-1 tumour therapy is dependent upon CD4 T cells and direct tumour recognition**
- **Conjugation of peptide to TLR2 agonist (Amplivant ®) enhances responses 10-100 fold**
- **Low dose Amplivant ® linked Modi-1 provides efficient tumour therapy and establishes immunological memory to protect from tumour rechallenge**
- **Ovarian cancer patients show a repertoire of CD4 T cells capable of responding to Modi-1**
- **Modi-1-Amplivant ® conjugates should be considered for translation into the clinic**

References:

1. Ireland JM, Unanue ER. Autophagy in antigen presenting cells results in presentation of citrullinated peptides to CD4 T cells. J Exp Med. 2011 Dec 19;208(13):2625-32.
2. 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
3. Jiang Z et al. Investigating citrullinated proteins in tumour cell lines. World J Surg Oncol. 2013 Oct 7;11:260.
4. Brentville VA, *et al.* 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
5. Cook K *et al*. Citrullinated alpha-enolase is an effective target for anti-cancer immunity. Oncoimmunology, 2018. **7**(2): p. e1390642.
6. Brentville V *et al.* T cell repertoire to citrullinated self-peptides in healthy humans is not confined to the HLA-DR SE alleles; Targeting of citrullinated self-peptides presented by HLA-DP4 for tumour therapy. Oncoimmunology, 2019. **8**(5): p. e1576490
7. Zom GG *et al*. Efficient Induction of Antitumor Immunity by Synthetic Toll-like Receptor Ligand–Peptide Conjugates. Cancer Immunol Res. 2014 Aug; 2(8):756-64
8. Zom GG *et al*. TLR2 ligand-synthetic long peptide conjugates effectively stimulate tumor-draining lymph node T cells of cervical cancer patients. Oncotarget. 2016 Aug;10.18632/oncotarget.11512