Abstract 1218P: Antitumor activity and safety of a novel PD-1 vaccine (PD1-Vaxx) alone and in combination with two chimeric HER-2 peptide vaccine (B-Vaxx) in syngeneic Balb/c mice and canines

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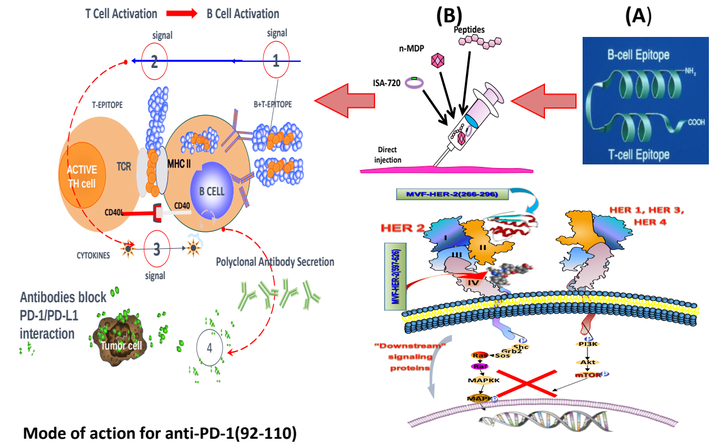
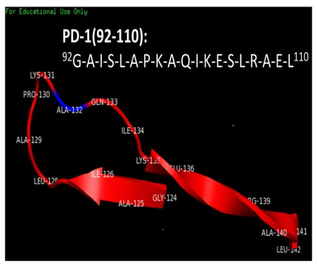
Background

Monoclonal antibodies (MAbs) targeting PD-1 such as Nivolumab or Pembrolizumab have shown activity across a variety of cancers. MAbs such as Herceptin and Perjeta are approved in selected cancers overexpressing HER2. The use of these MAbs is limited by high costs, side effects and development of resistance. In contrast, chimeric B-cell cancer vaccines incorporating a 'promiscuous' T cell epitope could present safer and cheaper alternatives. They elicit a specific immune response that induces memory B & T cell responses, while reducing immune evasion, suppression and resistance. We have translated two HER2 combination peptide vaccines (B-Vaxx) to the clinic in a phase 1/2b trial to safely deliver cancer immunotherapies to advanced cancer patients. We have created and established the development of a novel B-cell peptide vaccine (PD1-Vaxx) with high immunogenicity that binds to human PD-1 and produces tumor inhibition *in vivo* in two animal models of colon cancer. We describe the CT-26 & CT-26/HER-2 tumor models in Balb/c mice used to test for anti-tumor effects of anti-PD-1 immunization therapy alone and in combination with anti-HER2 immunization therapy. The antigenic activity and toxicity profile was investigated in mice and beagle dogs.

B-Vaxx and PD1-Vaxx proposed mechanism of action

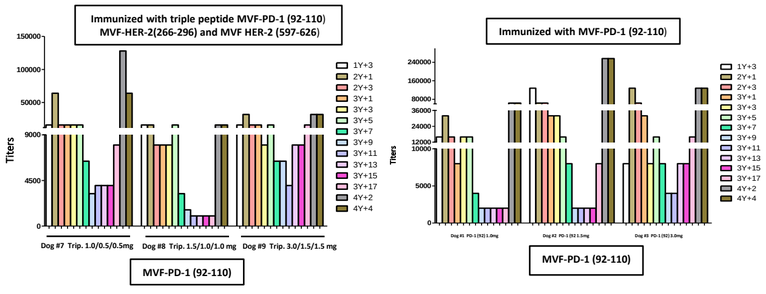


Mode of action for anti-PD-1(92-110) and anti- HER-2 antibodies



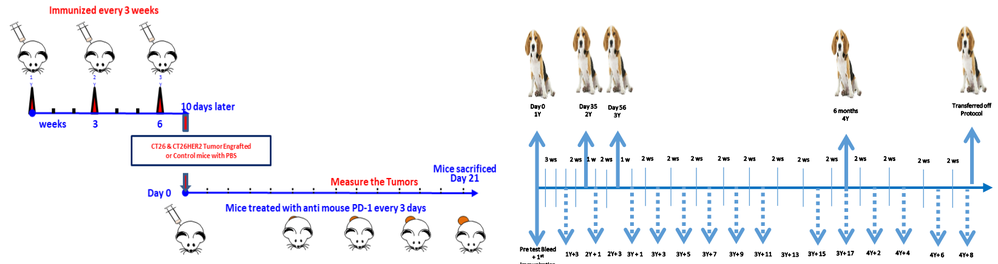
Chimeric PD-1 or HER-2 peptides incorporating a Measles virus fusion protein epitope (MVF) "promiscuous" T cell epitope **(A)** emulsified with nor-MDP adjuvant in ISA720 vehicle **(B)** elicit polyclonal antibodies. The B & T epitope is presented to antigen presenting cells (APCs) or B cells **(1)** without processing, the T cell epitope binds MHC class II and activates the T cell **(2)**. Cytokines are liberated **(3)** to help B cells make anti-peptide polyclonal antibodies. Both B-Vaxx and PD1-Vaxx follow similar pathway. The B-Vaxx antibodies bind and eliminate the tumor cells overexpressing HER-2. The PD1-Vaxx antibodies inhibit interaction of PD-1 with PD-L1 on tumor cell.

Immunogenicity of Combination Peptide Vaccines



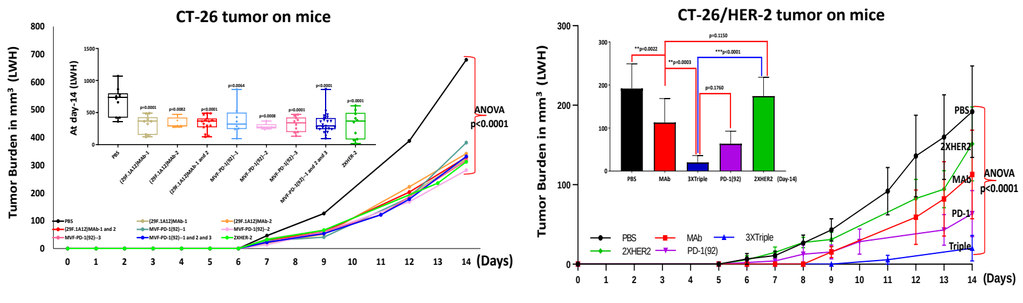
Dogs were immunized with MVF-PD-1(92-110) alone and in combination with HER-2 combo. Booster shots were given 3 times at 3 weeks interval and a final 6 months boost. Figure shows immunogenicity over the vaccination schedule.

Vaccination and Challenge Scheme



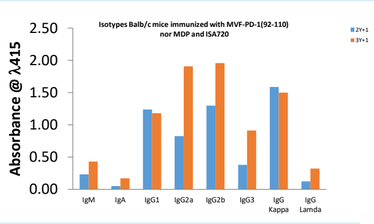
Vaccination and Challenge Scheme. Balb/c mice and beagle dogs were immunized with MVF-PD-1 (92-110) peptide vaccine constructs emulsified with ISA 720. Animals were boosted twice at 3 weeks interval. Antibody titers were determined by ELISA. 2 weeks after the final boost $1x10^5$ tumor cells from CT26 or CT26/HER-2 tumor lines were transplanted s.c. Control mice either were challenged with $1x10^5$ tumor cells and treated with anti-PD-1 antibody (29F.1A12) twice a week for the duration of the experiment.

Antitumor activity of PD-1 and HER-2 combo in CT26 and CT26-HER-2 syngeneic tumor models

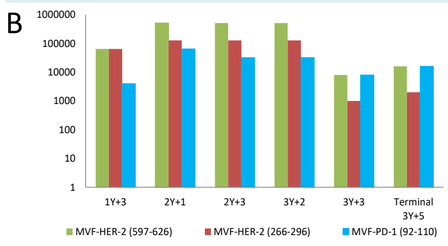


CT-26 & CT-26/HER-2 Tumor Challenge in Balb/c mice. Mice were immunized with PD-1 Peptide, 2XHER2 Peptides, triple (2XHER2+ PD-1 Peptides) at 3 week's interval and challenged 10 days after third vaccination , PBS served as negative control and mice treated with anti-mouse PD-1 monoclonal antibody (Mab, 29F.1A12) twice weekly; Tumor volume mm^3 (LWH) was measured over the course of 14 days post challenge. One-way analysis of variance (one-way ANOVA) and followed by the Tukey's multiple comparisons test were used to compare data in multiple groups or data between groups in multiple groups by GraphPad (Prism 8.1.2.), n=5-10.

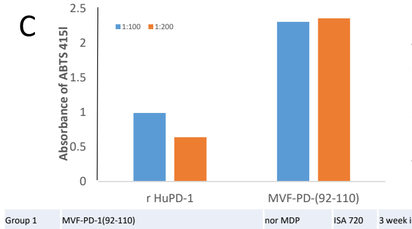
Isotypes of PD-1 vaccination in Balb/c



Immunogenicity of B-Vaxx + PD1-Vaxx



Antigenicity of MVF-PD-1(92-110) against Recombinant PD-1 Protein and Peptide Immunogen

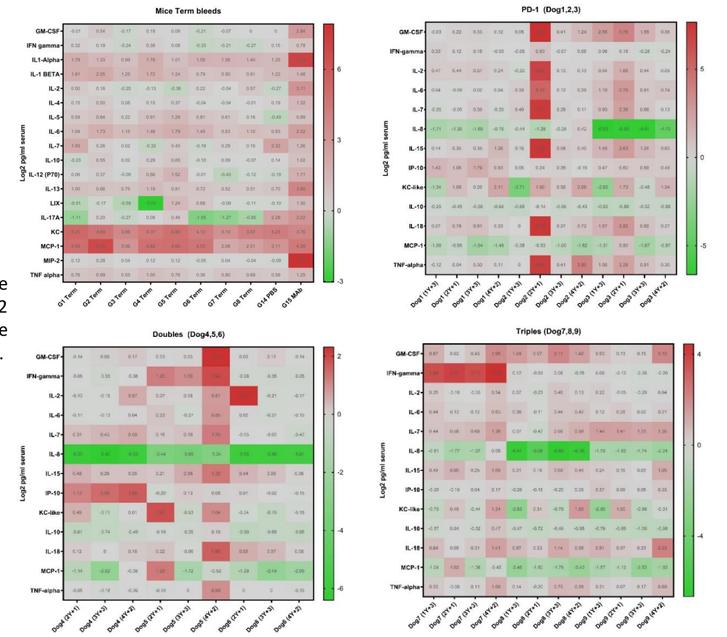


A. Isotype expression of Balb/c mice immunized with MVF-PD-1 (92-110). Results showed significant antigen specific IgG responses. IgG1, IgG2a and 2b were predominantly present in 2Y+1 and 3Y+1 bleeds. **B.** Sera pools from BALB/c mice immunized with 3 peptides **[HER-2(266-296)**, **HER-2(597-626)**, & **PD-1(92-110)]** were tested by ELISA. Sera samples 1Y+3, 2Y+1, 2Y+3, and 3Y+2 were taken before CT26/HER-2 tumor challenge. Samples 3Y+3 and 3Y+5 were taken at 1 week and 3 weeks post challenge respectively. Robust HER-2 and PD-1 antibody responses were elicited in all vaccinated mice with predominantly IgG1 antibodies. **C.** Sera from PD-1 immunized mice showed binding activity to recombinant human PD-1

Group 1	MVF-PD-1(92-110)	nor MDP	ISA 720	3 week interval
Group 2	MVF-PD-1(92-110)	no adjuvant	ISA 720	3 week interval
Group 3	MVF-PD-1(92-110)	nor MDP	ISA 720	2 week interval
Group 4	MVF-PD-1(92-110)	no adjuvant	ISA 720	2 week interval
Group 5	MVF-PD-1(92-110)	nor MDP	ISA 51	3 week interval
Group 6	MVF-PD-1(92-110)	no adjuvant	ISA 51	3 week interval
Group 7	MVF-PD-1(92-110)	nor MDP	ISA 51	2 week interval
Group 8	MVF-PD-1(92-110)	no adjuvant	ISA 51	2 week interval
Group 14	PBS control Group			
Group 15	29F.1A12 MAb positive control group			

Treatment	Dog ID	concentration	dose each peptide
MVF-PD-1 (92-110) nor-MDP, ISA720	#1-CGA	low dose	1.0mg
	#2-CGB	medium dose	1.5mg
	#3-CJV	high dose	3.0mg
MVF-HER-2 (266-296) & MVF-HER-2 (597-626) nor-MDP, ISA720	#4-CKN	low dose	0.5mg/0.5mg
	#5-CLS	medium dose	1.0mg/1.0mg
	#6-CME	high dose	1.5mg/1.5mg
MVF-PD-1 (92-110)& MVF-HER-2 (266-296) & MVF-HER-2 (597-626) nor-MDP, ISA720	#7-CMT	low dose	1.0mg/0.5mg/0.5mg
	#8-CMW	medium dose	1.5mg/1.0mg/1.0mg
	#9-CNE	high dose	3.0mg/1.5mg/1.5mg
MVF, nor-MDP, ISA720	#10-CNF	Negative control	1.5mg

PD-1 Peptide epitope overview effects on cytokine expressions



Heatmap visualization applied to all PD-1 treated Mice and dogs' cytokine data.

- Cytokine profiling was performed using a 13-plex and 18-plex marker panel on Luminex platform for dogs and mice, respectively.
- GM-CSF, IFN-gamma, IL-2, IL-6, IL-7, IL-8, IL-15, IP-10, KC-like, IL-10, IL-18, MCP-1 and TNF-alpha were assessed for concentration in sera by quantitative multiplexing.
- Values are log2-transformed normalized cytokine concentration. This visualization illustrates some differences across different treatments and which cytokines are secreted in response to treatment.
- For the PD-1 treated dogs, there is an obvious increase in the secretion of cytokines in a dose dependent manner. Means as the dose of the drug increases, there is a higher expression of cytokines as well. This effect is higher in GM-CSF, IL-2, IL-6, IL-7, IL-15, IL-18 and TNF-α.
- In PD-1 vaccinated mice however, the overall increase is in KC and MCP-1 cytokines. Both Il-1 alpha and Beta, as well as IL-6 show a predominant expression in terminal bleeds and the changes are comparable to mAb treated group.

Results and Conclusions

- We show robust HER-2 and PD-1 antibody responses in mice and beagle dogs.
- Treatment with PD1-Vaxx and mAb (29F.1A12) had significant reduction in tumor growth in the CT-26 Balb/c model as compared to PBS treated negative controls
- Combined triple vaccination (PD1-vaxx and B-Vaxx) was more effective in the CT-26/HER-2 carcinoma cell line in syngeneic Balb/c which exhibited superior activity compared to the positive gold control anti-mouse PD-1 (CD279) mAb.
- The PD-1 vaccine demonstrated antitumor effect in mouse colon cancer showing no evidence of toxicity or autoimmunity in mice, rabbits and canine.
- Combination immunotherapy of HER2 with PD-1 vaccine may offer a promising new approach to control cancer development/progression and could provide improved outcomes while sparing patients the toxicity of chemotherapy.
- A phase 1 clinical trial with the PD-1 vaccine is under planning.

References

1: Kaumaya PTP. Hum ***Vaccin Immunother***. 2015;11(6):1368-86.
2: Bekaii-Saab,T and Kaumaya PTP et al., Online First on February 25, 2019; DOI: 10.1158/1078-0432. ***Clinical Cancer Research*** (CCR-18-3997)

Acknowledgements

Funding Imugene Ltd, NIH R01 CA CA84356 & NIH R21 CA13508 to PTPK