Abstract 1453: Development of a novel PD-1 vaccine and in combination with two Chimeric HER-2 peptide vaccine provides synergistic inhibition of tumor growth in a syngeneic Balb/c model challenged with CT26/HER-2 carcinoma cell line

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Background: Therapeutic blockade of the signaling axis between PD-1 and PD-L1 with monoclonal antibodies has shown remarkable clinical success in the treatment of cancer and demonstrated impressive activity across a broad set of cancer subtypes. Contrary to treatment with monoclonal antibodies, chimeric B-cell vaccine candidates have the advantage of producing a specific immune response that can potentially induce memory B & T cell responses, while reducing immune evasion and suppression. Key Vaxx is a B-cell peptide cancer vaccine which aims to induce the body to produce polyclonal antibodies that block PD-1 signaling, and thus produce an antitumor effect similar to Keytruda and Opdivo. Key Vaxx has shown great potential in preclinical studies. It outperformed an industry-standard mouse anti-PD-1 antibody in a mouse model of HER2+ colorectal cancer. When used in combination with our HER-2 combination vaccine B-Vaxx (see poster XXX) we obtained synergistic inhibition of tumor growth in a colon carcinoma Balb/c model challenged with CT26/HER-2 cell line. The combined vaccines were safe and had no evidence of toxicology or autoimmunity.

**PD-1 Peptide epitope selection, design, synthesis & characterization**

Several predictive immunogenicity algorithms were used to prioritize four highest scoring B-cell epitope sequences of human PD-1. Amino acids 32-50, 45-64, 73-90 and 91-120 were chosen for evaluation based on the X-ray structure of PD-1:PD-L1. Peptides were synthesized & characterized.

**SPR analyses of PD-1 peptides binding to Nivolumab & rhPD-L1**

The specificity of the selected PD-1 peptides was determined by surface plasmon resonance (SPR) spectroscopy (Biacore T200, at 25°C). The binding affinities to immobilized human PD-L1 (nPD-L1) and Nivolumab on CMS sensor chip. From these binding studies, we concluded that the PD-1 peptides 45-64, 73-90 and 91-120 can recognize the PD-L1 and Nivolumab and can act as inhibitors of PD-1:PD-L1.

**Recognition of mouse PD-1 by anti-PD-1 rabbit antibodies**

**Immunogenicity of individual MVF-PD-1 peptides in Balb/c mice**

Immunogenicity of PD-1 vaccines. The PD-1 peptide vaccines were formulated in Montanide ISA 720 (1:1) and 100 μg nor-MDP. Female Balb/c mice (Charles River Laboratories) at the age of 5 to 6 wk were immunized three times at 3 wk intervals with 100 μg peptide vaccine, and 15 d after the third immunization, the mice were challenged s.c. with CT-26 tumor cells (100,000 per mouse). Sera pools 2Y Winter 2, 3Y Winter 3, and 3Y Winter 2 were determined by ELISA. Each PD-1 peptides elicited high titters of antipeptide antibodies.

**Combination of two HER-2 vaccine: B-Vaxx**

**Composite Data CT-26 Tumor LWH**

Mean plots of tumor growth in syngeneic Balb/c mice (5/6 group) immunized with four PD-1 MVF vaccine constructs: PD-1 (32-50), PD-1 (45-64), PD-1 (73-90) PD-1 (91-120), control (irrelevant peptide), + control anti-mouse PD-1 MAb (29F.1A12). Mice were challenged 15 days after 3rd vaccination with CT26 carcinoma cells (1×10⁵) and tumor growth evaluated. Mice vaccinated with MVF-PD-1 (92-110) showed significant inhibition of tumor growth at Day 14 suggesting that this epitope could be a useful inhibitory vaccine.

**Vaccinations with combo Key-Vaxx and B-Vaxx followed by CT26/HER-2 challenge**

**Mean plots of tumor growth in syngeneic Balb/c mice (5/6 group) immunized with four PD-1 MVF vaccine constructs: PD-1 (32-50), PD-1 (45-64), PD-1 (73-90) PD-1 (91-120), control (irrelevant peptide), + control anti-mouse PD-1 MAb (29F.1A12). Mice were challenged 15 days after 3rd vaccination with CT26 carcinoma cells (1×10⁵) and tumor growth evaluated. Mice vaccinated with MVF-PD-1 (92-110) showed significant inhibition of tumor growth at Day 14 suggesting that this epitope could be a useful inhibitory vaccine.**

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**Results and Conclusions**

**-** Robust HER-2 and PD-1 antibody responses in all vaccinated mice.

**-** Combined vaccination is effective in reducing tumor growth in a Balb/c syngeneic model of colon carcinoma.

**-** Triple vaccination is more effective than PD-1 vaccine alone.

**-** Triple Vaccination efficacy greater than the positive control gold standard anti-mouse PD-1 monoclonal antibody (29F.1A12).

**-** The vaccine combination was found to be safe and did not appear to exhibit toxicity or autoimmunity.

**-** Triple combination vaccine provides synergistic inhibition of tumor growth with no evidence of toxicity or autoimmunity.

**-** A Phase 1b human clinical trial with the vaccine B-Vaxx is under planning (IMUGENE).

**References**

1: Kaumaya TPT. Hum Vaccin Immunother. 2015;11(2):1386-86.
2: Bekaii-Saab T, Kaumaya TP et al., Online First on February 25, 2019; DOI: 10.1158/1078-0432.CCR-18-3997

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