The effects of combination treatment of IMM-101, a heat-killed whole cell preparation of *Mycobacterium obuense* (NCTC 13365) with checkpoint inhibitors in pre-clinical models

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1 Background

- IMM-101 is heat killed whole cell gram positive *Mycobacterium obuense* (NCTC13365)
- IMM-101 is proposed to induce a protective CD8⁺ response in clinically relevant models of pancreatic cancer (*Elia et al. 2013*)
- The IMAGE-1 Phase II clinical trial (NCT01303172) with IMM-101 demonstrated long term survival of patients with metastatic pancreatic cancer *(Dalgleish et al. 2016)*
- Here we present studies into the immunological effects of IMM-101, and both its pre-clinical and clinical efficacy as a combination treatment

2 Clinical Efficacy

IMM-101 increases survival in patients with late stage pancreatic cancer

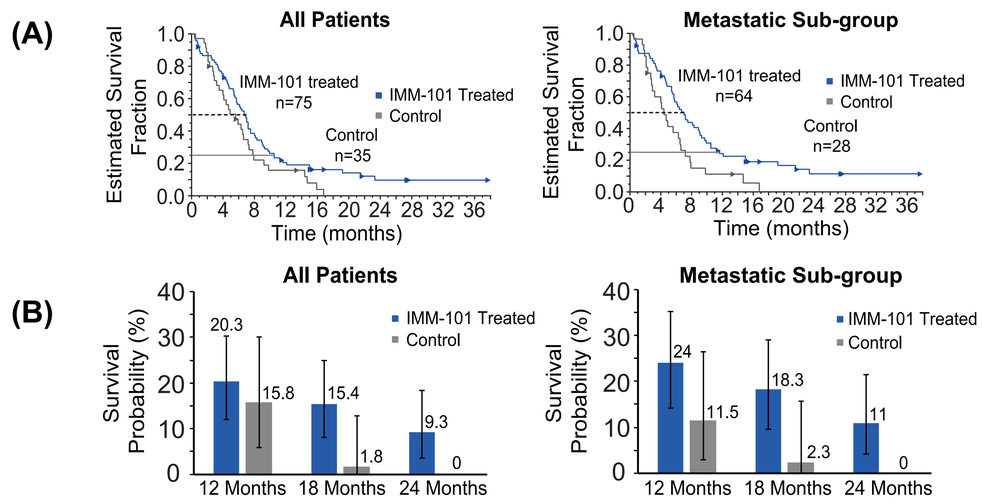

Figure 1. A) Overall survival Kaplan-Meier Curves for the Intention to Treat (ITT) population, shows significant effect of IMM-101 treatment (0.1mL intradermal injection of 10mg/mL) in combination with gemcitabine (1000mg/m²) in the metastatic group (p= 0.01) compared to control (Gemcitabine alone) and a trend towards protection in all patients (p= 0.075). (B) Survival Probability at 12, 18 and 24 months for ITT population ±SEM.

4 Pre Clinical Efficacy and Combination Treatment

IMM-101 reduces tumour burden with anti PD-1 treatment

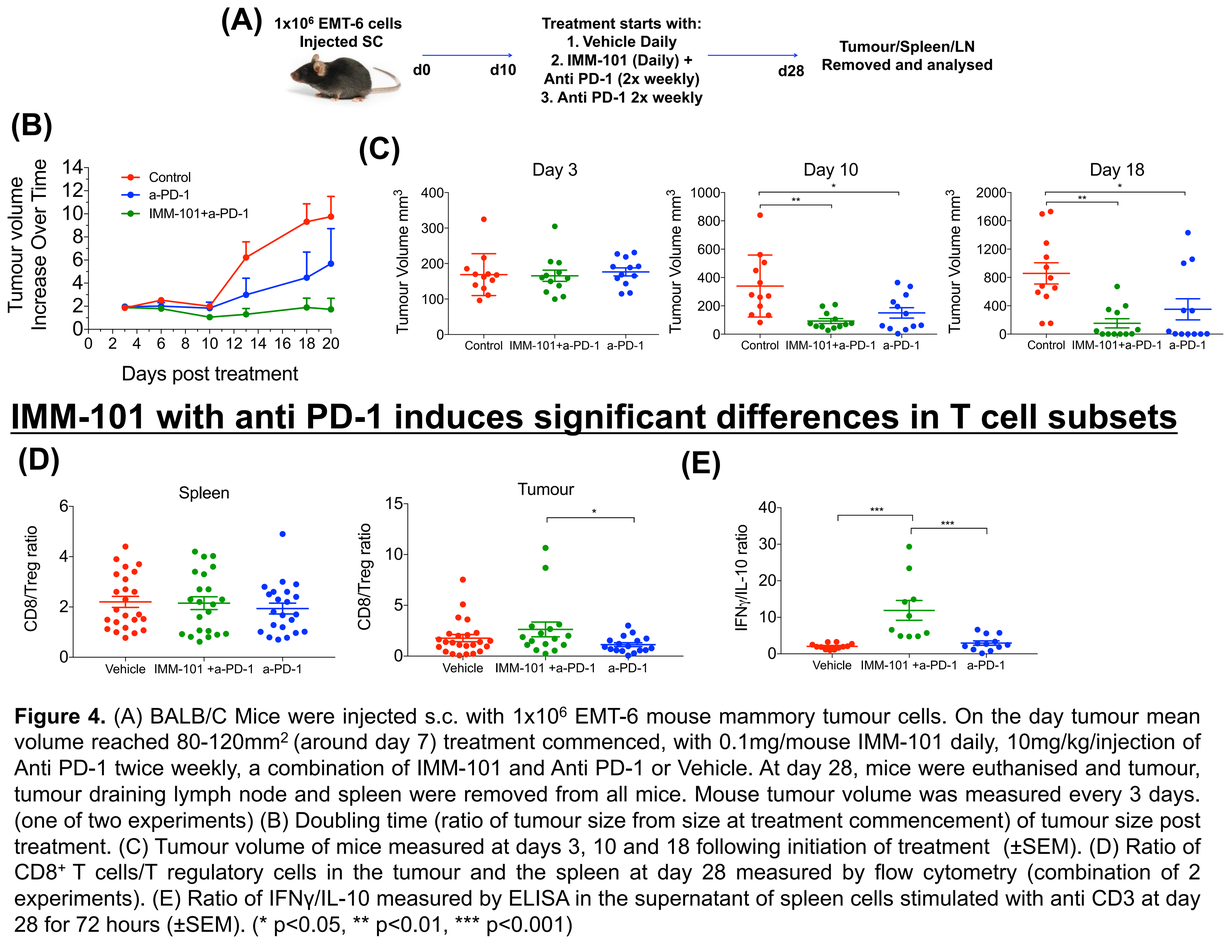

Figure 4. (A) BALB/C Mice were injected s.c. with 1x10⁶ EMT-6 mouse mammory tumour cells. On the day tumour mean volume reached 80-120mm² (around day 7) treatment commenced, with 0.1mg/mouse IMM-101 daily, 10mg/kg/injection of Anti PD-1 twice weekly, a combination of IMM-101 and Anti PD-1 or Vehicle. At day 28, mice were euthanised and tumour, tumour draining lymph node and spleen were removed from all mice. Mouse tumour volume was measured every 3 days. (one of two experiments) (B) Doubling time (ratio of tumour size from size at treatment commencement) of tumour size post treatment. (C) Tumour volume of mice measured at days 3, 10 and 18 following initiation of treatment (±SEM). (D) Ratio of CD8⁺ T cells/T regulatory cells in the tumour and the spleen at day 28 measured by flow cytometry (combination of 2 experiments). (E) Ratio of IFNγ/IL-10 measured by ELISA in the supernatant of spleen cells stimulated with anti CD3 at day 28 for 72 hours (±SEM). (* p<0.05, ** p<0.01, *** p<0.001)

3 Mechanism of Action

IMM-101 treated DCs induce IFNγ and IL-17 *in vivo* in SPF and GF mice

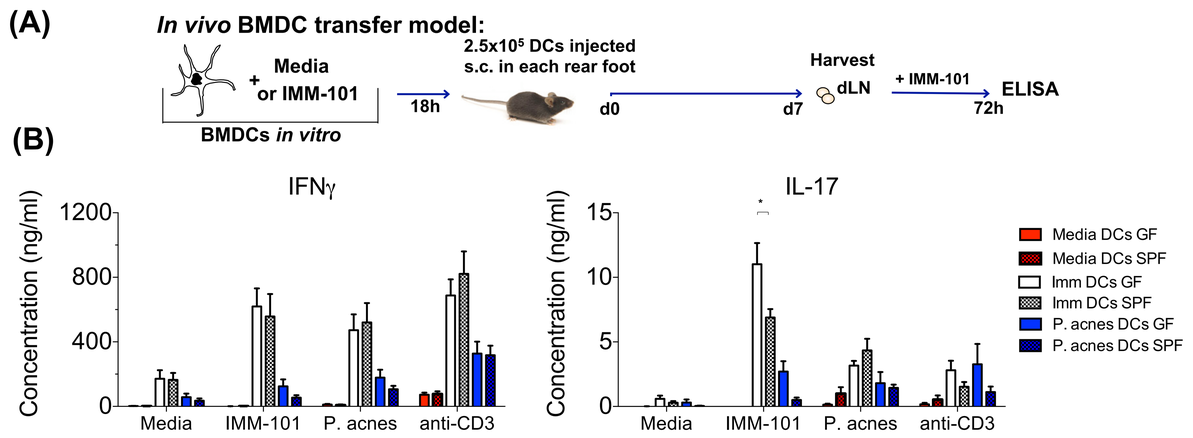

Figure 2. (A) Schematic of dendritic cell (DC) transfer protocol. Specific pathogen free (SPF) or germ free (GF) mice were injected s.c. with (B) IMM-101 (300µg/ml) activated or control (media) bone marrow derived DCs (BMDCs) (5x10⁵ cells/ mouse). 7 days later, draining lymph nodes were removed, and LN cells cultured for 72 hours with media, 100µg/ml IMM-101, 10µg/ml *P. acnes* or 0.5µg/well plate bound anti CD3. Cytokine levels in culture supernatants were determined by ELISA (±SEM). (* p<0.05, ** p<0.01, *** p<0.001) (B is representative of 2 combined studies)

IMM-101 enhances DC antigen processing and/or presentation ability

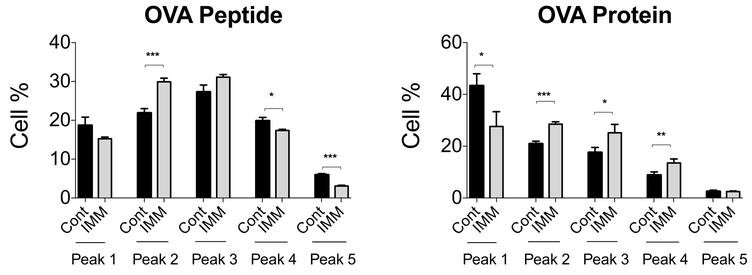

Figure 3. CFSE labelled Ovalbumin (OVA) specific OTII CD4⁺ T cells were cultured for 72 hours alone ('T cells'), with murine GM-CSF BMDCs that had been pre-exposed to 300µg/ml IMM-101 ('IMM'), or with control, non-exposed DCs ('Media'), with the addition of OVA peptide (0.01µg/ml) or OVA protein (5 µg/ml). (A) the percentage of T cells in each proliferation peak (±SEM). (* p<0.05, ** p<0.01, *** p<0.001)

- **IMM-101 increased the ability of DCs to induce OVA specific T cell proliferation compared to control in the presence of OVA protein, suggesting an effect on antigen uptake and/or processing**

5 Summary

- **IMM-101 in combination with Gemcitabine increases survival in late stage pancreatic cancer**
- **IMM-101 can act via dendritic cells to induce an IFNγ and IL-17 response *in vivo***
- **IMM-101 also enhances the ability of dendritic cells to process and present antigen**
- **IMM-101 in combination with anti PD-1 antibodies reduces tumour burden in a murine model of EMT-6 mouse breast tumour cells**
- **IMM-101 significantly increases the intratumoural CD8 T cell/Treg ratio in combination with anti PD-1 compared to anti PD-1 alone**
- **IMM-101 has shown significant survival improvements in both human and murine models of cancer and in cancer patients**

Immunomodulatory co-therapies have the potential to provide significant increases in survival in cancer therapy

References

Elia A *et al.*, 2013, Treatment with IMM-101 induces protective CD8⁺ T cell responses in clinically relevant models of pancreatic cancer. J Immunother Cancer 1: Sup 1, P215

Dalgleish *et al.* 2016, Randomised, open-label, phase II study of gemcitibine with and without IMM-101 for advanced pancreatic cancer. British Journal of Cancer, Vol 115. 989-796