The effect of IMM-101, a heat-killed whole cell preparation of *Mycobacterium obuense* (NCTC 13365) on dendritic cells and the adaptive immune response

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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/pre clinical efficacy

IMM-101 in combination with first line therapies improves treatment outcomes in clinical and preclinical settings

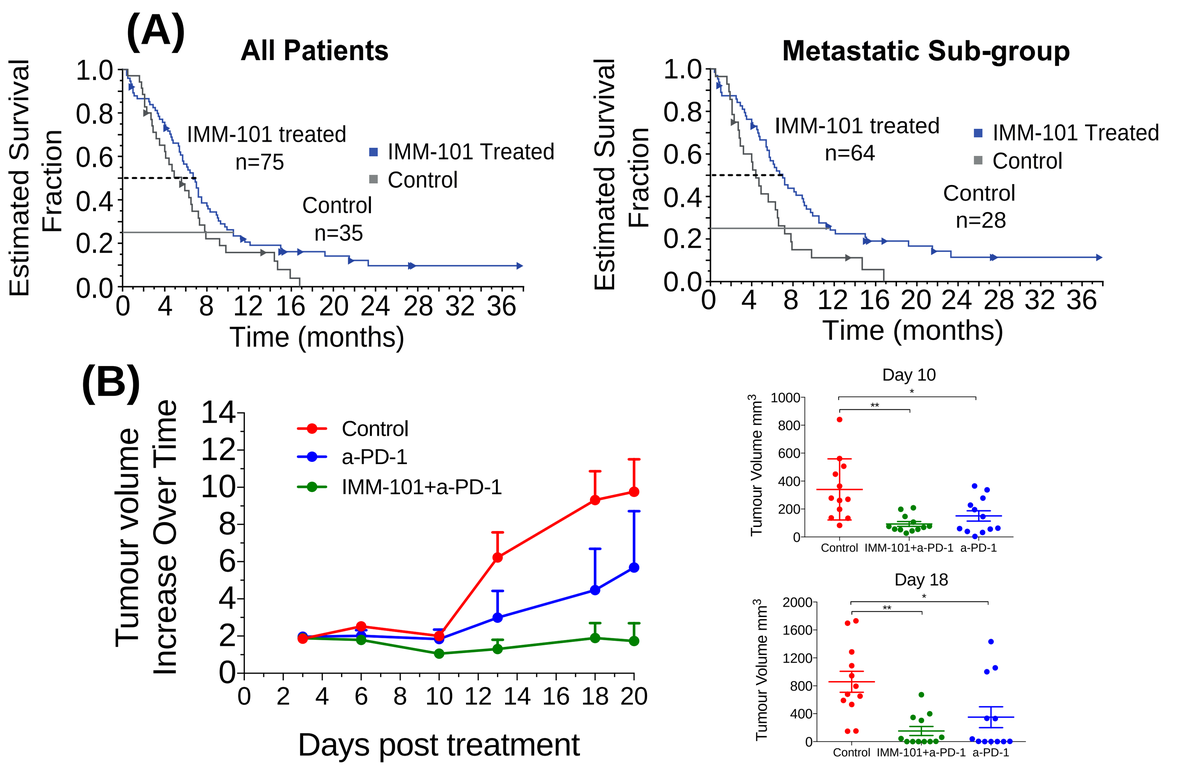
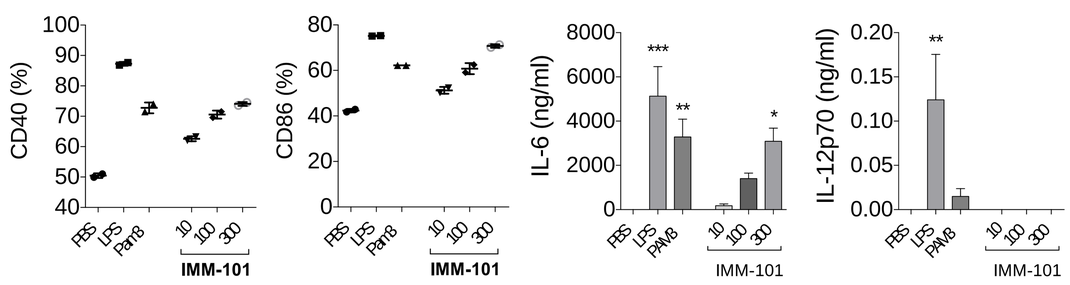


Figure 1. A) Overall survival Kaplan-Meier Curves for the Intention to Treat (ITT) population, shows significant effect of IMM-101 treatment (0.1mL intradermal injection of 10mg/mL) in combination with gemcitabine (1000mg/m^2) in the metastatic group (p= 0.01) compared to control (Gemcitabine alone) and a trend towards protection in all patients (p= 0.075). (B) BALB/C Mice were injected subcutaneous (S.C.) with 1x10^6 EMT-6 mouse mammory tumour cells. On the day tumour mean volume reached 80-120mm^2 (around day 7), treatment commenced with10mg/kg/injection of Anti-PD-1 twice weekly, a combination of 0.1mg/mouse of IMM-101 (daily) and Anti-PD-1 or Vehicle. (* p<0.05, ** p<0.01, *** p<0.001).

3 IMM-101 *In vitro*

IMM-101 induces activation of murine and human DCs

(A) *Mouse GMCSF bone marrow derived DCs (FACS/ELISA)*



(B)

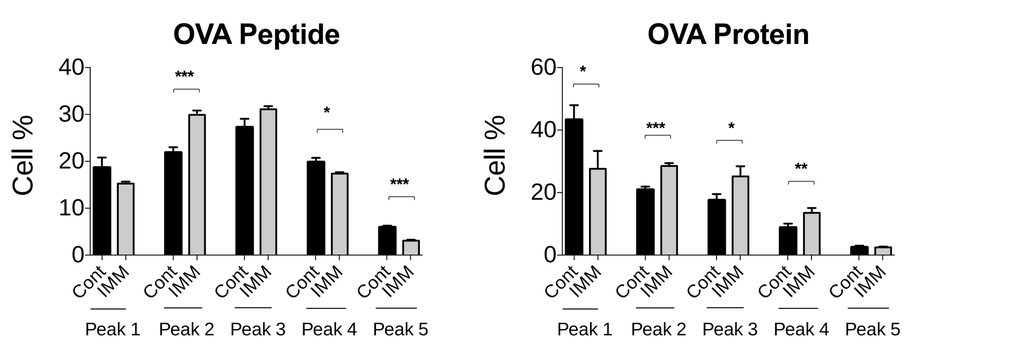
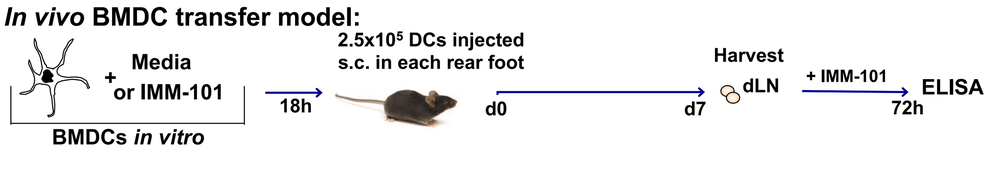


Figure 2. (A) Flow cytometric analysis of the activation and ELISA of cell culture supernatants from murine GMCSF bone marrow derived DCs (BMDCs) following overnight stimulation with 10, 100 or 300µg/ml IMM-101, PBS, 250ng/ml LPS or 250µg/ml Pam3Cys (Data combined from 3 experiments). (B) CFSE labelled OVA specific OTII $CD4^+$ T cells were cultured for 72 hours alone, with murine GM-CSF bone marrow derived DCs that had been pre-exposed to 300µg/ml IMM-101 ('IMM'), or with control, non-exposed DCs, with the addition of OVA peptide (0.01µg/ml) or OVA protein (5 µg/ml). (±SEM) (* p<0.05, ** p<0.01, *** p<0.001).

4 IMM-101 *In vivo*

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

(A)



(B)

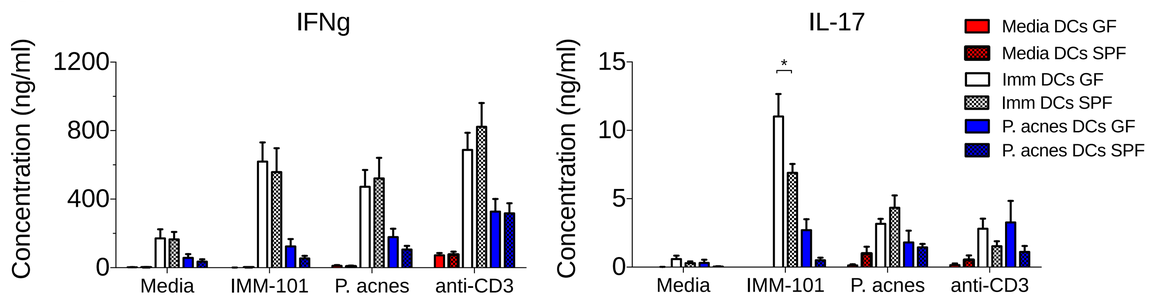


Figure 3. (A) Schematic of DC transfer protocol. Mice (SPF or GF) were injected S.C. with (B) IMM-101 (300µg/ml) activated or control (media) BMDCs (5x10^5 cells/mouse). 7 days later, draining lymph nodes were removed, and LN cells cultured for 72 hours with media, 100µg/ml IMM-101, 10µg/ml *P. acnes* 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 generates an increase in IFN-γ production from a number of cell types

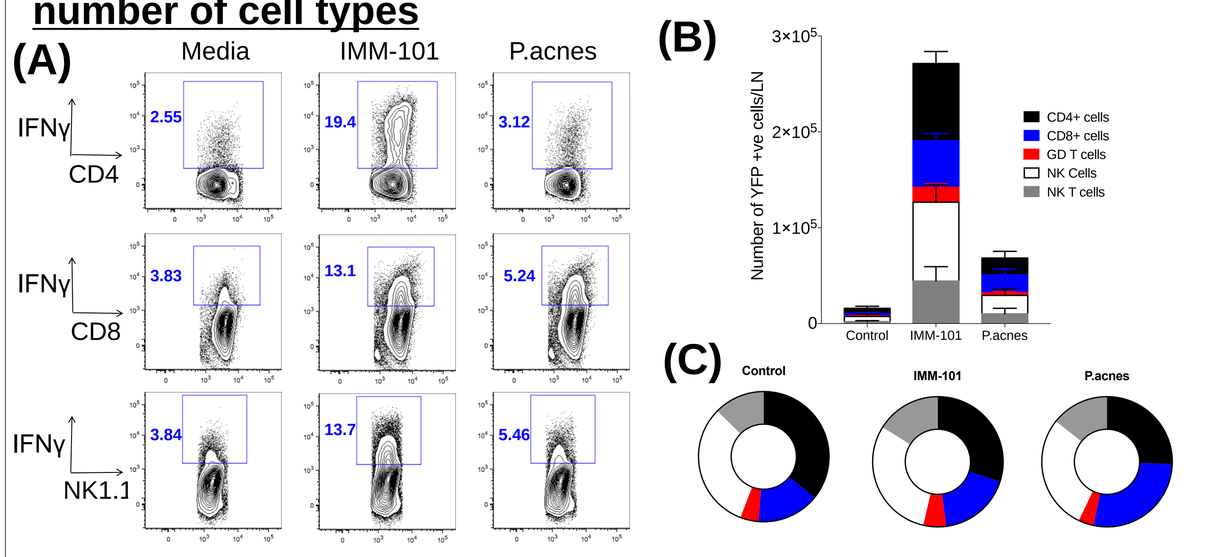


Figure 4. (A) GMDCs stimulated with media, IMM-101 (300µg/ml) or P.acnes (10µg/ml) were injected S.C. into the footpad of GREAT IFN-γ reporter mice. 7 days later the draining LNs were removed and stimulated with PMA/ionomycin before being stained and analysed by FACS. The number (B) or the percentage (C) of YFP expressing IFN-γ producing cells in the draining LNs of mice treated with IMM-101, P.acnes or media DCs. Significant differences were detected. (±SEM).

IMM-101 generates an IFN-γ response independent of IL-12

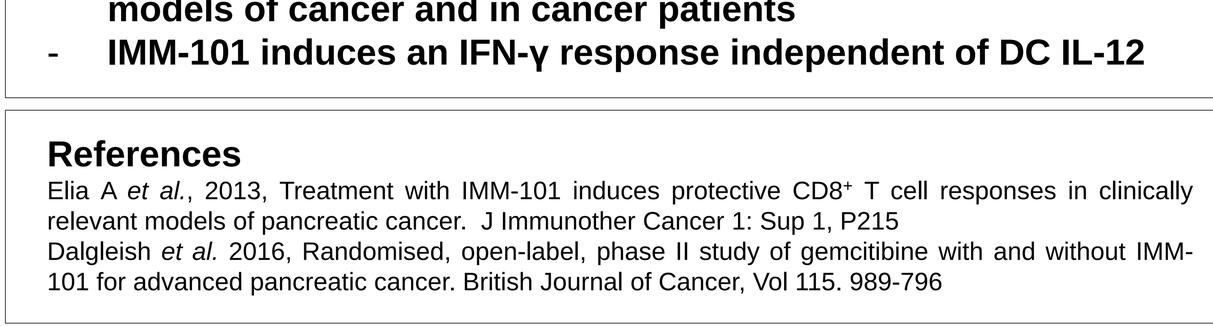


Figure 5. (A) GMDCs were stimulated *in vitro* with IMM-101 at different concentrations for 72 hours, supernatants were then taken and measured for IL-12p70 via ELISA. (B) IL-12p35$^{-/-}$, IL-12p40$^{-/-}$ and WT DCs were stimulated with IMM-101 (300µg/ml) and media before being transferred S.C. into the footpad. Draining LNs were then removed and stimulated with media and IMM-101 for 72 hours, supernatants were taken and tested for IFN-γ via ELISA. (±SEM). (* p<0.05, ** p<0.01, *** p<0.001).

- **IMM-101 in combination with first line therapies improves treatment outcomes in humans and pre-clinical models**
- **IMM-101 can act via dendritic cells to induce an IFN-γ and IL-17 response *in vivo***
- **IMM-101 also enhances the ability of dendritic cells to process and present antigen**
- **IMM-101 has shown significant improvements in murine models of cancer and in cancer patients**
- **IMM-101 induces an IFN-γ response independent of DC IL-12**

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