The impact of *Mycobacterium obuense (NCTC13365)* on innate and adaptive immunity

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1 Background – *Defining how IMM-101 affects DC phenotype/function*

-IMM-101 is heat killed whole cell gram positive *Mycobacterium obuense* (NCTC13365)

-IMM-101 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

-Here we present initial studies into the immunological effects of IMM-101, with a focus on dendritic cells (DCs)

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

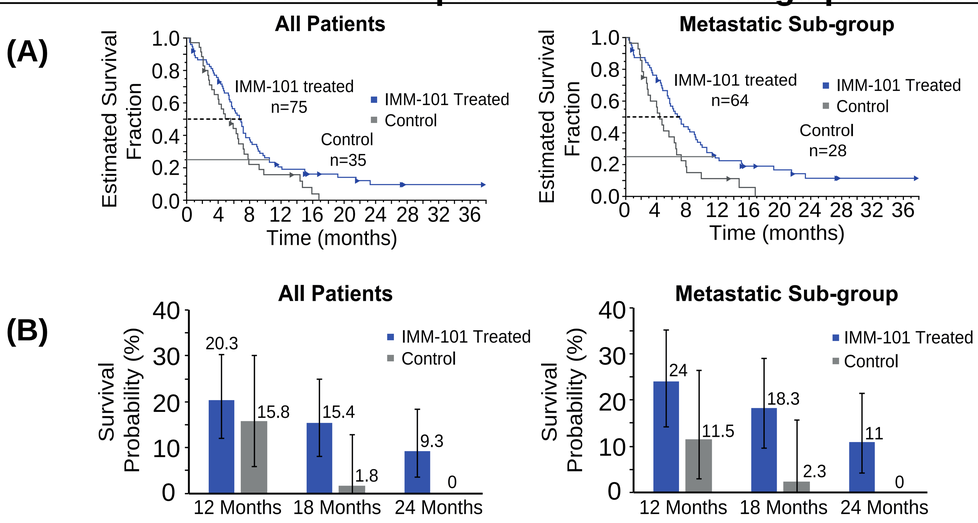
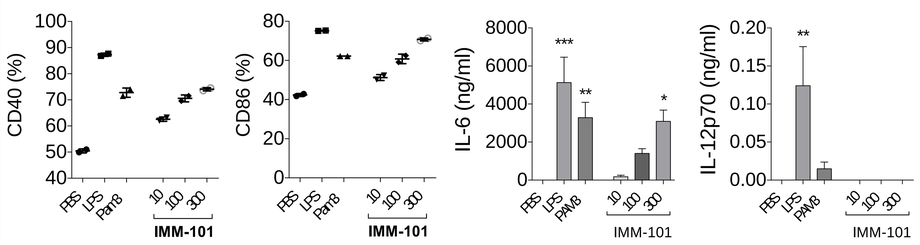


Figure 1. A) Overall survival Kaplan-Meier Curves for the Intention to Treat (ITT) population, shows significant effect of IMM-101 treatment (0.1mL intradermal injection of 10mg/mL) in combination with gemcitabine (1000mg/m^2) in the metastatic group (p= 0.011) compared to control (Gemcitabine alone) and a trend towards protection in all patients (p= 0.075). (B) Survival Probability at 12, 18 and 24 months for ITT population ±SEM.

- ***IMAGE 1 trial showed IMM-101 treatment significantly increased survival in patients with metastatic disease***

3 IMM-101 induces activation/maturation of murine and human DCs

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



(B) ***Human monocyte derived DCs (FACS/CBA)***

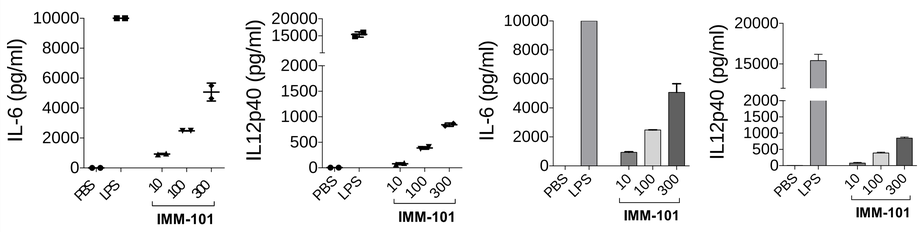


Figure 2. (A) Flow cytometric analysis of the activation/maturation and ELISA of cell culture supernatants from murine GMCSF bone marrow derived DCs (BMDCs) following overnight stimulation with 10, 100 or 300µg/ml IMM-101, PBS, 250ng/ml LPS or 250µg/ml Pam3Cys (Data combined from 3 experiments). (B) Flow cytometric analysis or of the activation/maturation or CBA analysis of culture supernatants from human monocyte derived DCs following overnight culture with 10, 100 or 300 µg/ml IMM-101, PBS, 250ng/ml LPS (one example donor from 2 repeats). (* p<0.05, ** p<0.01, *** p<0.001)

- ***IMM-101 displayed a dose-dependent ability to induce phenotypic activation/maturation and cytokine production by either human or murine DCs***

Better understanding of the interaction of IMM-101 with DCs could help to explain its therapeutic efficacy

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

McDermott *et al.*, 2014, Durable benefit and the potential for long-term survival with immunotherapy in advanced melanoma Cancer Treatment Reviews 40 1056-1064

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

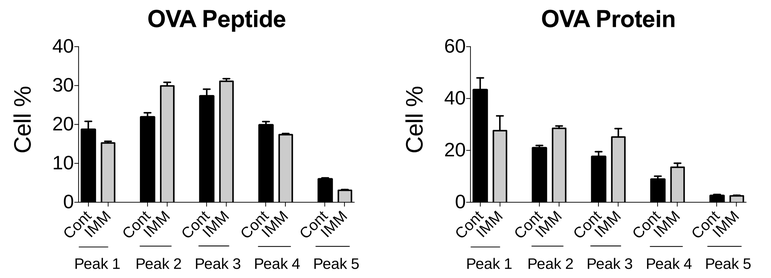


Figure 3. CFSE labelled OVA specific OTII $CD4^+$ T cells were cultured for 72 hours alone ('T cells'), with murine GMCSF bone marrow derived DCs that had been pre-exposed to 300µg/ml IMM-101 ('IMM'), or with control, non-exposed DCs ('Media'), with the addition of OVA peptide (0.01µg/ml) or OVA protein (5 µg/ml). (A) the percentage of T cells in each proliferation peak (±SEM).

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

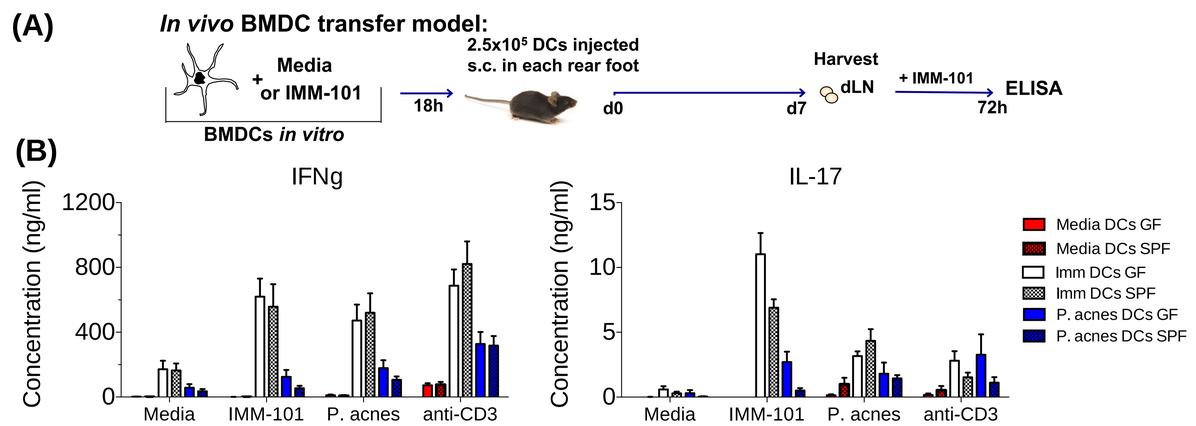


Figure 4. (A) Schematic of DC transfer protocol. Mice (SPF or GF) were injected s.c. with (B) IMM-101 (300µg/ml) activated or control (media) BMDCs (5x10^5 cells/mouse). 7 days later, draining lymph nodes were removed, and LN cells cultured for 72 hours with media, 100µg/ml IMM-101, 10µg/ml *P. acnes* 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 activated DCs adoptively transferred into naïve recipient mice induced elevated IFNγ and IL-17 in vivo in both SPF and GF mice***

-***LN from mice treated with IMM-101 activated DCs showed increased IFNγ and IL-17 responses upon restimulation with P.acnes suggesting a level of antigen cross reactivity***

6 IMM-101 DCs do not need to make IL-12 to induce Interferon-γ *in vivo*

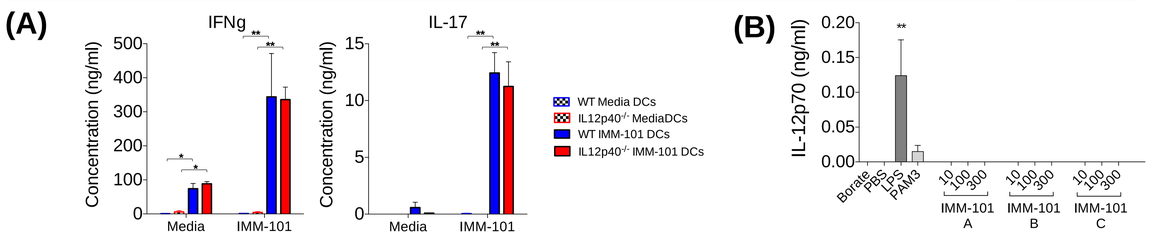


Figure 5. (A) Mice were injected s.c. with IMM-101 activated or control (WT or IL-12p40$^{-/-}$) BMDCs. 7 days later, draining lymph nodes were removed, and LN cells cultured for 72 hours with media, or 100µg/ml IMM-101. (B) IL-12p70 concentration in DC culture supernatants following GMCSF DC stimulation with different preparations of IMM-101, determined by ELISA (±SEM). (* p<0.05, ** p<0.01, *** p<0.001)

- ***IMM-101 stimulated IL-12p40$^{-/-}$ DCs induced an equivalent T cell IFNγ response in vivo to IMM-101 stimulated WT DCs, suggesting DC IL-12 independent Th1 induction***

7 Summary – *How does IMM-101 affect the immune response?*

- IMM-101 is effective at reducing tumour burden in late stage pancreatic cancer
- IMM-101 induces dose dependent activation of in *in vitro* generated DCs
- IMM-101 enhances the antigen processing and presenting ability of DCs
- IMM-101 treated DCs induce IFNγ and IL-17 responses *in vivo* in both SPF and GF mice, suggesting little or no commensal involvement in the effect of IMM-101
- IMM-101 re-stimulated lymph nodes taken from mice injected with *P. acnes* treated DCs secrete IFNγ and IL-17, suggesting antigen cross-reactivity between IMM-101 and *P. acnes*
- IMM-101 treated IL-12 deficient DCs still promote a strong IFNγ response following their *in vivo* transfer, suggesting DC IL-12 independent IFNγ induction