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

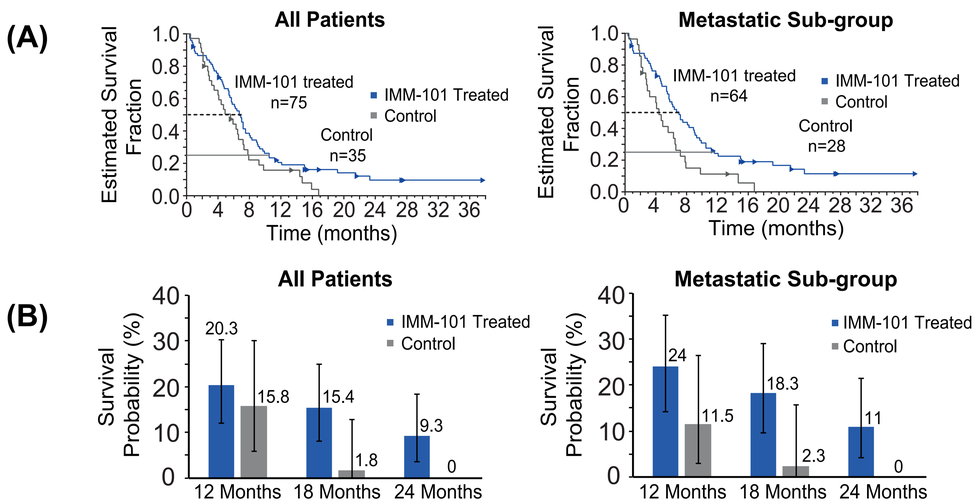

Figure 1. A) Overall survival Kaplan-Meier Curves for the Intention to Treat (ITT) population, shows significant effect of IMM-101 treatment (0.1mL intradermal injection of 10mg/mL) in combination with gemcitabine (1000mg/m²) in the metastatic group (p= 0.011) compared to control (Gemcitabine alone) 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***
- ***Profile of curves as expected from an immunomodulating agent (McDermott et al., 2014)***

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

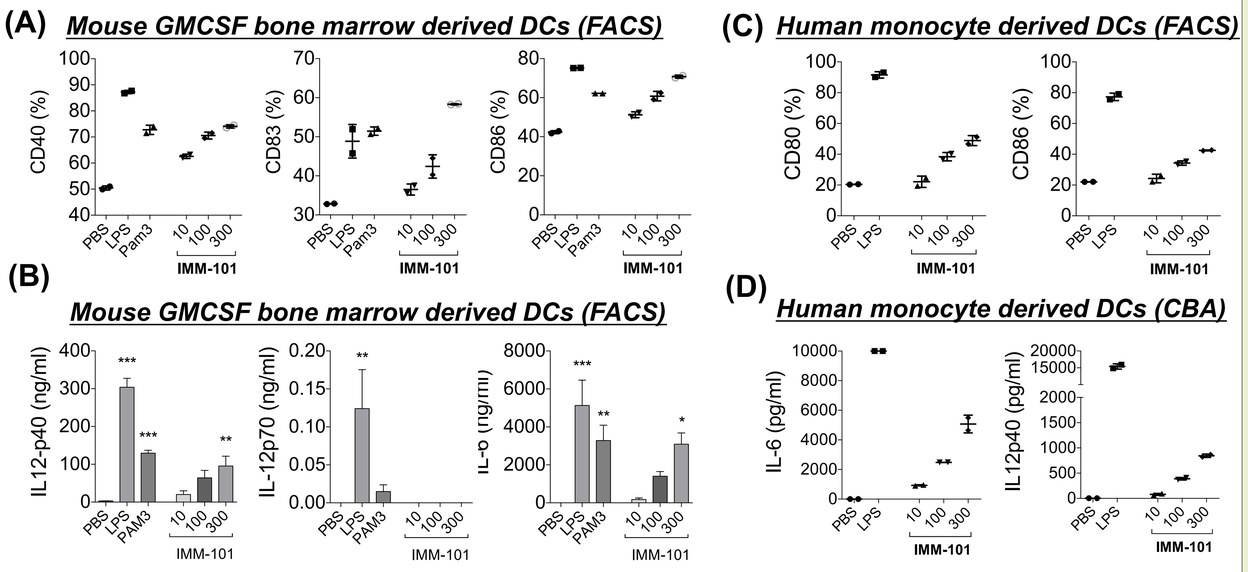

Figure 2. (A) Flow cytometric analysis of the activation/maturation and (B) ELISA of cell culture supernatants from murine GMCSF bone marrow derived DCs (BMDCs) following overnight stimulation with 10, 100 or 300µg/ml IMM-101, PBS, 250ng/ml LPS or 250µg/ml Pam3Cys (Data combined from 3 experiments). (C) Flow cytometric analysis or of the activation/maturation or (D) 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 or 20µg/ml heat killed *Propionibacterium acnes* (P. acnes) (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***
- ***IMM-101 failed to trigger DC IL-12p70 production in either human or murine DCs***

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

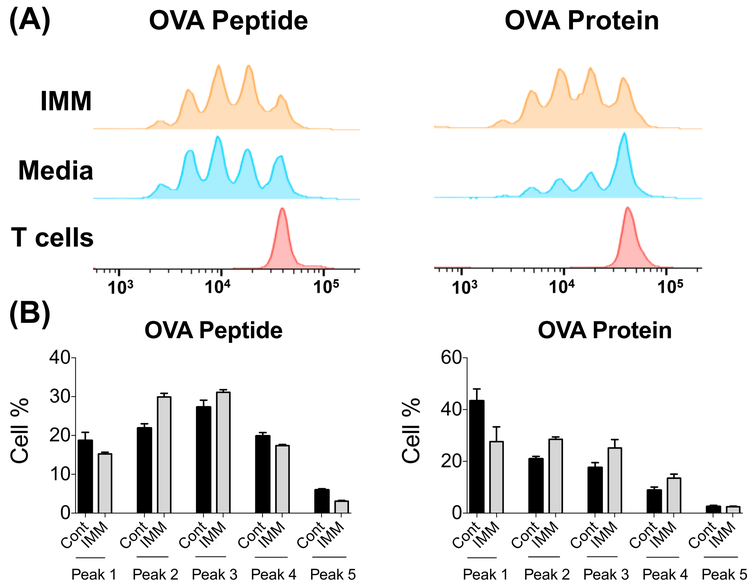

Figure 3. CFSE labelled OVA specific OTII CD4⁺ T cells were cultured for 72 hours alone ('T cells'), with murine GMCSF bone marrow derived DCs that had been pre-exposed to IMM-101 ('IMM'), or with control, non-exposed DCs ('Media'), with the addition of OVA peptide (0.01µg/ml) or protein (5 µg/ml). (A) Flow cytometric histograms showing cell proliferation CFSE dilution in OTII T cells and (B) 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***
- ***IMM-101 caused a slight shift in T cell proliferation towards peak 2 and 3, suggesting a possible role in antigen presentation***

5 IMM-101 activated DCs induce IFNγ and IL-17 *in vivo*

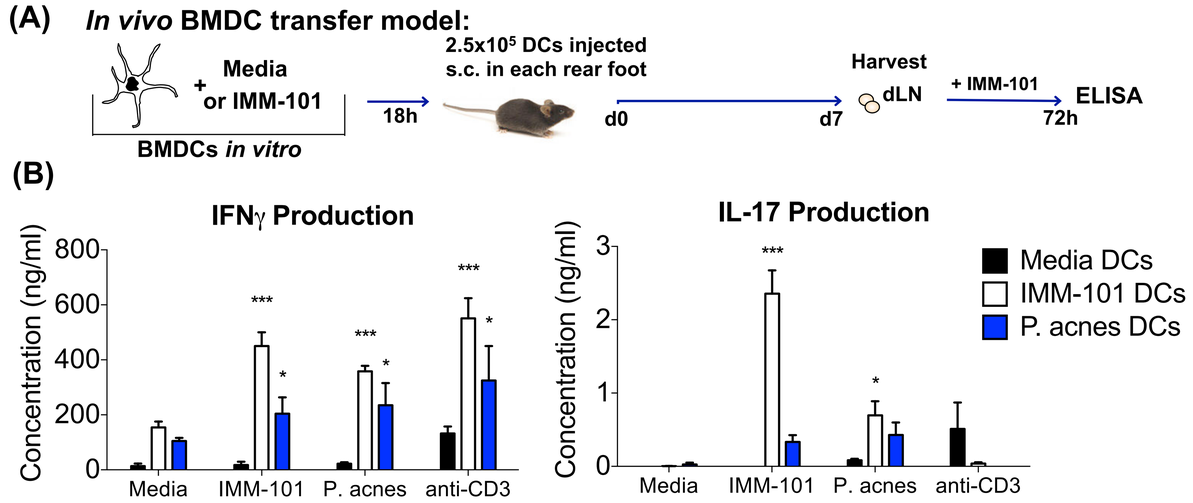

Figure 4. (A) Mice were injected s.c. with IMM-101 activated or control (media) GMCSF bone marrow derived DCs. 7 days later, draining lymph nodes were removed, and LN cells cultured for 72 hours with media, 100µg/ml IMM-101, 10µg/ml *P. acnes* or 16.67µg/ml plate bound anti-CD3. (B) Cytokine levels in culture supernatants were determined by ELISA (±SEM). (* p<0.05, ** p<0.01, *** p<0.001)

- ***IMM-101 activated DCs adoptively transferred into naïve recipient mice induced elevated IFNγ and IL-17 in vivo, with no significant induction of either IL-10 or IL-13 (data not shown)***
- ***Relative IMM-101:anti-CD3 levels of cytokine would indicate induction of T cell IFNγ, and non-T cell IL-17, by IMM-101 activated DCs***

Better understanding of the ability of IMM-101 to influence DC activation and function could help 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