Defining the immunomodulatory effects of IMM-101: a promising co-therapy for cancer.

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1. Background

- IMM-101 is a non-specific immunomodulator containing heat-killed, whole cell *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).*
- Defining how IMM-101 exerts its immunomodulatory effects is key for advancing its use as a cancer therapy.

2. How does IMM-101 influence DC activation & function *in vitro?*

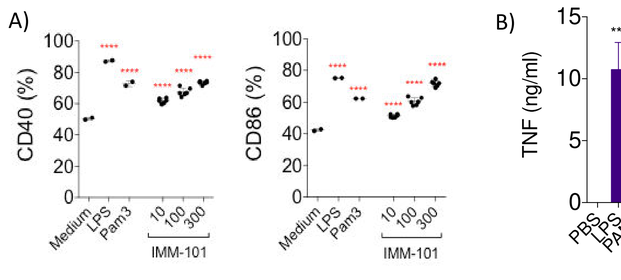
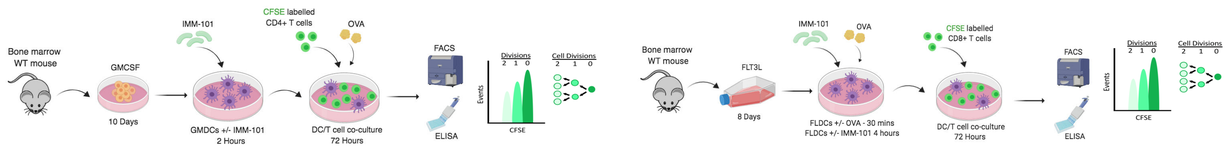


Figure 1 - IMM-101 causes dose-dependent activation of DCs *in vitro* without triggering detectable IL-12p70. GMDCs were stimulated with LPS or Pam3Csk4 at 250ng/ml, or IMM-101 at 10, 100 or 300µg/ml for 18h before cells and supernatants were analysed by A) flow cytometry and B) ELISA, respectively. Similar DC activation was also seen in murine DCs generated with FLT3-L and human moDCs. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Data representative of >3 experiments.



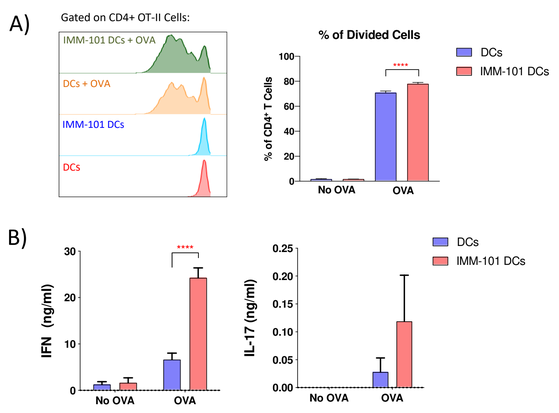


Figure 2 - IMM-101 enhances the ability of DCs to activate OVA-specific $CD4^+$ T cells *in vitro*. GMDCs were incubated for 2h +/- 300µg/ml IMM-101, then co-cultured with CFSE labelled $CD4^+$ T cells +/- 5µg/ml OVA protein for 72h. **A)** The ability of IMM-101 to enhance DC induced T cell proliferation in response to OVA was assessed via CFSE dilution. **B)** DC-induced cytokine secretion was measured by ELISA. *p<0.05, ****p<0.0001. Data representative of 3 experiments.

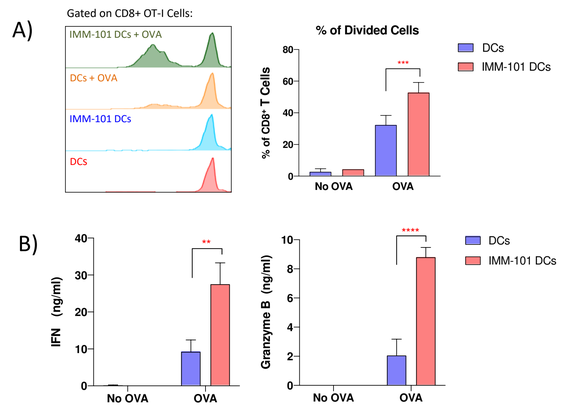


Figure 3 - IMM-101 enhances the ability of DCs to activate OVA-specific $CD8^+$ T cells *in vitro*. FLDCs were incubated for 30 mins +/- 5µg OVA protein, then for 4 hours +/- 300µg/ml IMM-101. DCs were then co-cultured with CFSE labelled $CD8^+$ T cells for 72h. **A)** The ability of IMM-101 to enhance DC induced T cell proliferation in response to OVA was assessed via CFSE dilution. **B)** DC-induced cytokine secretion was measured by ELISA. *p<0.05, **p<0.01, ****p<0.0001. Data representative of 4 experiments.

3. What response do IMM-101 activated DCs induce *in vivo*?

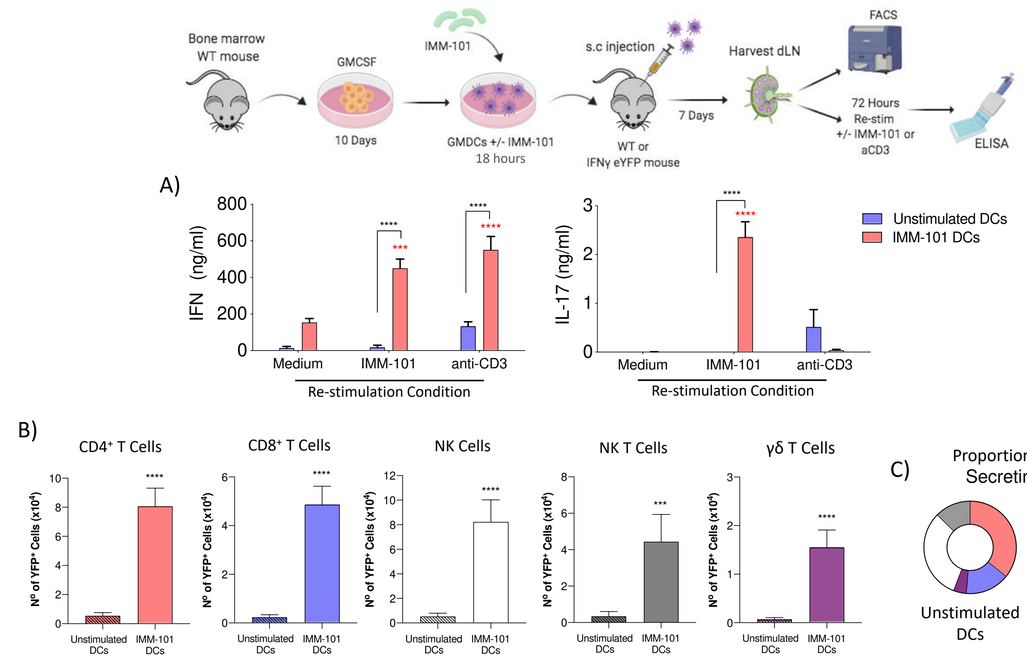


Figure 4 - Adoptive transfer of IMM-101 activated DCs induces IFNγ production by a range of cell types. GMDCs +/- 300µg/ml IMM-101 were injected s.c into WT or IFNγ-eYFP reporter mice. After 7d, popliteal lymph nodes were harvested. **A)** Isolated lymphocytes were cultured for 72h either alone or with 300µg IMM-101 or 0.5µg αCD3, and cytokine secretion measured by ELISA. IL-4, IL-5, & IL-13 were not induced (data not shown). Proportions of YFP^+ cells measured by FACS were used to calculate **B)** numbers or **C)** proportion of IFNγ secreting cells. IL-17 secretion was also increased (data not shown). *p<0.05, ***p<0.001, ****p<0.0001. Red stars denote comparison to medium.

4. Role of IL-12 in IMM-101 activated DC IFNγ induction?

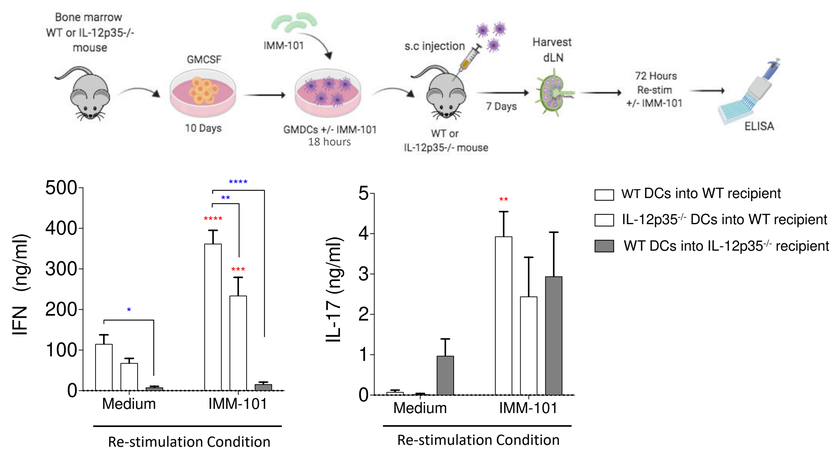


Figure 5 - IFNγ induction by IMM-101 DCs does not require their ability to produce IL-12, but *recipient* IL-12 is essential. WT or IL-12p35$^{-/-}$ GMDCs stimulated with IMM-101 were injected subcutaneously into WT or IL-12p35$^{-/-}$ mice. After 7d, draining LNs were harvested, and isolated lymphocytes were cultured for 72h +/- IMM-101. Cytokine secretion was measured by ELISA. *p<0.05, **p<0.01, ****p<0.0001. Red stars denote comparison to medium. Data representative of 3 experiments.

5. Is there a role for commensal cross-reactivity?

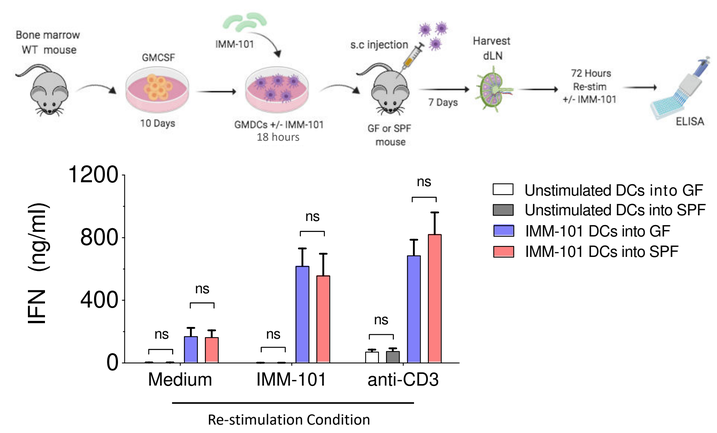


Figure 6 - IFNγ induction by IMM-101 DCs does not require recipient pre-conditioning by commensals. GMDCs +/- 300µg/ml IMM-101 were injected subcutaneously into SPF or gnotobiotic (GF) mice. After 7d, draining LNs were harvested and isolated lymphocytes cultured for 72h +/- 300µg/ml IMM-101 or 0.5µg αCD3. Cytokine secretion was measured by ELISA. In addition to IFNγ, no significant differences were found for any other cytokine in GF animals. Data representative of 3 experiments.

6. What response does IMM-101 directly induce *in vivo*?

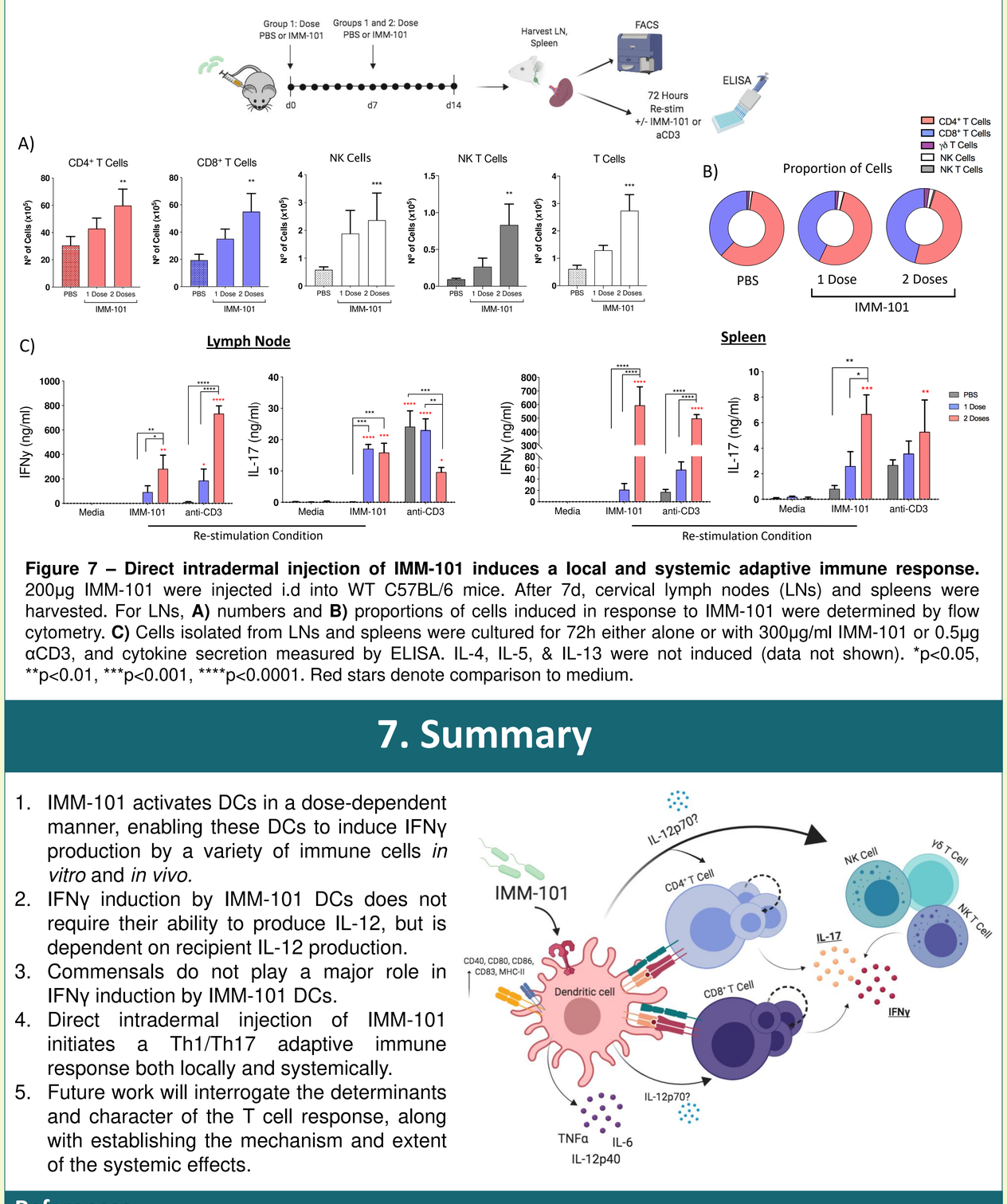


Figure 7 – Direct intradermal injection of IMM-101 induces a local and systemic adaptive immune response. 200µg IMM-101 were injected i.d into WT C57BL/6 mice. After 7d, cervical lymph nodes (LNs) and spleens were harvested. For LNs, **A)** numbers and **B)** proportions of cells induced in response to IMM-101 were determined by flow cytometry. **C)** Cells isolated from LNs and spleens were cultured for 72h either alone or with 300µg/ml IMM-101 or 0.5µg αCD3, and cytokine secretion measured by ELISA. IL-4, IL-5, & IL-13 were not induced (data not shown). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Red stars denote comparison to medium.

7. Summary

1. IMM-101 activates DCs in a dose-dependent manner, enabling these DCs to induce IFNγ production by a variety of immune cells *in vitro* and *in vivo.*
2. IFNγ induction by IMM-101 DCs does not require their ability to produce IL-12, but is dependent on recipient IL-12 production.
3. Commensals do not play a major role in IFNγ induction by IMM-101 DCs.
4. Direct intradermal injection of IMM-101 initiates a Th1/Th17 adaptive immune response both locally and systemically.
5. Future work will interrogate the determinants and character of the T cell response, along with establishing the mechanism and extent of the systemic effects.

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

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