Defining the immunomodulatory effects of IMM-101: a promising, novel 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. Does IMM-101 activate DCs in vitro?

Figure 1 - DC uptake of IMM-101 in vitro. Isolated C57BL/6 bone marrow cells were cultured with GMSCF for 10d, with media changed on d3, 6 and 8. At d10, GMDCs were incubated with 300µg/ml IMM-101, then co-cultured with CFSE labelled CD4+ T cells and visualised using ImageStream.

Figure 2 - IMM-101 causes dose-dependent activation of DCs in vitro. GMDCs were incubated for 2h +/- 300µg/ml IMM-101, then co-cultured with CFSE labelled CD4+ T cells and measured by FACS. *p<0.05, **p<0.01, ***p<0.001. Data representative of 3 experiments.

Figure 3 - 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 and 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 CD4+ T cell proliferation was assessed via CFSE dilution. B) DC induced cytokine secretion was measured by ELISA. *p<0.05, **p<0.01, ***p<0.001. Data representative of 2 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 recipient cell types. GMDCs +/- 300µg/ml IMM-101 were injected subcutaneously into WT or IFNγ−/− YFP reporter mice. After 7d, draining lymph nodes were harvested. A) Isolated lymphocytes were cultured for 72h either alone or with 300µg/ml IMM-101 or 0.5µg/ml CD3. CD40, CD80 and IFNγ secretion measured by ELISA. Type 2 cytokines were not induced (data not shown). B) Proportions of YFP+ cells were assessed using FACS to calculate numbers of IFNγ secreting cells. Although total numbers of IFNγ+ cells increased in IMM-101 DC recipients (bar graph), proportions of IFNγ+ cells remained unchanged (pie chart). As well as IFNγ, IL-17 secretion was also increased (not shown). *p<0.05, **p<0.001.

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 is dependent on CD4+ and CD8+ T cell help. A) WT or IL-12p35−/− GMDCs stimulated with IMM-101 were injected subcutaneously into WT mice, and B) WT GMDCs activated with IMM-101 were injected 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<0.001. 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 incubated 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/ml CD3. 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. Summary

• IMM-101 triggers dose-dependent activation of DCs in vitro.
• IMM-101 activated DCs induce IFNγ production by CD4+, CD8+ and γδ T cells, NK cells and NKT cells in vivo.
• IFNγ induction by IMM-101 DCs does not require their ability to produce IL-12, but is dependent on recipient IL-12 production.
• The microbiod does not play a major role in the IFNγ response induced by IMM-101 DCs.
• Future work is looking to characterise the in vivo response to IMM-101 following intradermal injection, focussing on DCs, CD4+ and CD8+ T cells, and monocytes.

References
Elia A et al., 2013. Treatment with IMM-101 induces protective CD8+ T cell response in clinically relevant models of pancreatic cancer. J Immunother Cancer 1 Suppl 1, P218

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