The Immunomodulatory Effects of Cancer Therapy on IFN-gamma Responses in the Periphery

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BACKGROUND

- Interferon-γ (IFN-γ) has long been linked to cytostatic and cytotoxic activity and antitumor mechanisms.
- At the tumour site, IFN-γ is prognostic, predictive and involved in mechanistic signatures that are emerging as important biomarkers for the effectiveness of immuno-oncology therapies (Gajewski *et al.,* 2013; Galon *et al.,* 2013).
- However, repeated tissues biopsies can be challenging and unduly taxing to patients. It is therefore critical to assess whether measuring levels of IFN-γ in the periphery can be as useful to determine a patient's response.
- To date the limiting step to this approach has been level of detection in the serum.
- Quanterix's single molecule array (Simoa™) technology provides ultrasensitive measurements, achieving orders-of-magnitude greater sensitivity than conventional immunoassay platforms.
- Here we describe the use of the Simoa™ technology to measure levels of IFN-γ in serum samples from advanced pancreatic cancer patients recruited to a Phase II clinical trial with a novel immunotherapeutic agent.

MATERIALS and METHODS

- Serum samples were obtained from patients recruited to IMAGE-1 (Immune Modulation and Gemcitabine Evaluation-1), a randomised, open-label, Phase II, first-line, proof of concept study (NCT01303172) designed to explore safety and tolerability of a non-specific immunostimulant, IMM-101 (heat-killed *Mycobacterium obuense*; NCTC 13365) with gemcitabine (GEM) in advanced pancreatic ductal adenocarcinoma (Dalgleish *et al.*, 2016).
- Patients were randomised (2:1) to IMM-101 (0.1 mL intradermal injection of 10 mg/mL) + GEM (1000 mg/m^2 intravenously for 3 consecutive weeks out of 4; n=75), or GEM alone (n=35). Per protocol, this could be continued to a 12-cycle maximum.
- For quantification of peripheral IFN-γ from patients, an ultrasensitive IFN-γ Simoa™ Immunoassay (Serum/Plasma LLOQ = 0.035 pg/ml) validated and manufactured to clinical laboratory standards was used for analyzing serum samples in a CLIA certified laboratory (Myriad RBM, Austin, TX, USA). Serum samples were diluted 1:2 and the sandwich immunoassay was performed on the fully automated Quanterix Simoa HD-1 analyzer.
- 107 samples were analyzed from a total of 54 patients (37 from the GEM + IMM-101 group, 17 from the GEM alone group), selected for the availability of serum samples at two selected time points pre- and post-treatment.
- Six samples (5.6%) were below the LLOQ and reported as LLOQ/2, without impacting the statistical analysis.

RESULTS

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

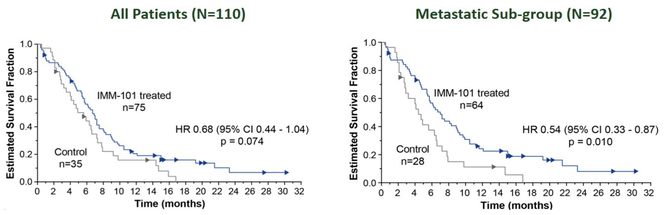

Figure 1. Median overall survival (OS) in the intent-to-treat population was 6.7 months for IMM-101 + GEM v 5.6 months for GEM alone; while not significant, the hazard ratio (HR) numerically favoured IMM-101 + GEM [HR, 0.68 (95% CI, 0.44–1.04, P=0.074)]. In a pre-defined metastatic subgroup (84% of patients recruited), OS was significantly improved from 4.4 to 7.0 months in favour of IMM-101 + GEM [HR, 0.54, 95% CI 0.33–0.87, P=0.01].

The IFN-γ Simoa™ immunoassay is able to detect circulating levels of IFN-γ in the serum of advanced pancreatic cancer patients.

Samples were obtained from patients at the time of screening and/or randomization, prior to the start of any therapy (n=53) and post-treatment at week 13 (prior to the beginning of GEM cycle 4 and ± 6 injections of IMM-101; n=42) and if a sample at week 13 was not available, it was substituted with a sample obtained at week 9 (prior to the beginning of GEM cycle 3 and ± 4 injections of IMM-101; n=12).

We measured the circulating levels of IFN-γ in the serum of advanced pancreatic cancer patients (Figure 2). We found a significant increase in levels detected post-treatment (geometric mean 0.351pg/ml, 95% CI 0.256, 0.482pg/ml) compared to those measured pre-treatment (0.129pg/ml, 95% CI 0.101, 0.166pg/ml).

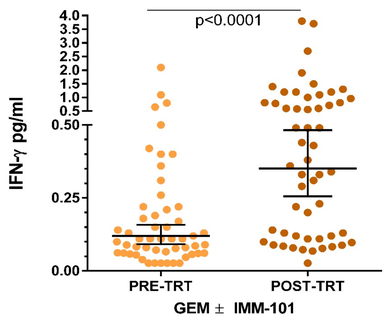

Figure 2. Individual levels of IFN-γ protein measured by IFN-γ Simoa™ immunoassay in serum samples of advanced pancreatic cancer patients recruited to IMAGE-1, prior to the start of treatment (n=53) and post-treatment (n=54). The bars indicate geometric mean ± 95% CI. Statistical analysis was performed by Wilcoxon matched-pairs signed rank test.

Treatment with IMM-101 significantly boosts circulating levels of IFN-γ in the serum of patients receiving IMM-101 + GEM compared to GEM alone.

We investigated whether the increase of IFN-γ following start of treatment was more pronounced in those pancreatic cancer patients that had also received IMM-101, a non specific immunostimulator, which had been shown pre-clinically to induce Type-1 immune responses and in particular boosts IFN-γ secretion (Crooks *et al.,* SITC Annual Meeting 2016).

We measured circulating levels of IFN-γ from samples which had been grouped on the basis of treatment (GEM, n=17 vs GEM ± IMM-101, n=37; Figure 3). We found that the increase in IFN-γ levels post-treatment was significantly elevated only in the group that had received GEM + IMM-101 (pre-treatment geometric mean 0.125pg/ml, 95% CI 0.0892, 0.175pg/ml v post-treatment 0.440pg/ml, 95% CI 0.304, 0.636pg/ml).

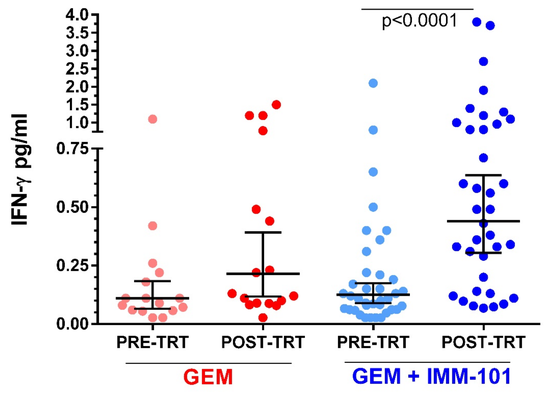

Figure 3. Individual levels of IFN-γ protein measured by IFN-γ Simoa™ immunoassay in serum samples from advanced pancreatic cancer patients pre-treatment and after receiving either GEM alone (n=33) or GEM + IMM-101 (n=74). The bars indicate geometric mean ± 95% CI. Statistical analysis was performed by Anova, Kruskal-Wallis test followed by Dunn's multiple comparisons.

Increased circulating levels of IFN-γ following treatment with GEM + IMM-101 may be indicative of improved response to immunotherapy.

We investigated whether within the group of patients that received GEM + IMM-101, the IFN-γ levels in those patients with an OS above the expected median of 6.7 months (n=26) were increased compared to those in patients with an OS below the expected median (n=11).

We hypothesized that increased circulating levels of IFN-γ may be indicative of enhanced immune function and suggestive of improved response to immunotherapy and disease outcome. Whereas any conclusion remains speculative at this point given the limited number of samples, it nonetheless provides an interesting insight and justifies further investigation.

We report that the increase in circulating IFN-γ levels post-treatment was significantly elevated only in the group that had an OS > 6.7 months (pre-treatment geometric mean 0.128pg/ml, 95% CI 0.0868, 0.188pg/ml v post-treatment 0.537pg/ml, 95% CI 0.359, 0.805pg/ml, Figure 4).

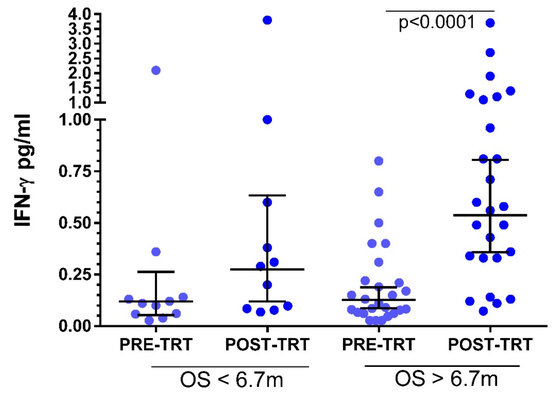

Figure 4. Individual levels of IFN-γ protein measured by IFN-γ Simoa™ immunoassay in serum samples from advanced pancreatic cancer patients pre- and post-treatment with GEM + IMM-101 categorized on the basis of their survival below (n=22) or above (n=52) the expected median. The bars indicate geometric mean ± 95% CI. Statistical analysis was performed by Anova, Kruskal-Wallis test followed by Dunn's multiple comparisons.

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These clinical data are in agreement with pre-clinical results showing IMM-101 induces IFN-γ secretion by a broad range of cellular sources *in vivo*.

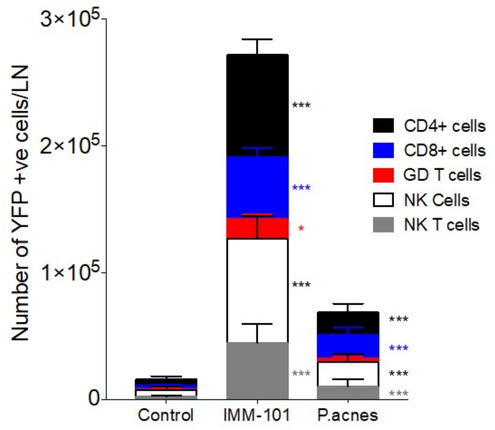

Figure 5. Bone marrow (C57BL/6 WT mice)-derived Dendritic Cells generated using GM-CSF were stimulated with media, IMM-101 (300µg/ml) *or Proponibacterium acnes* (10µg/ml) for 18 hrs then injected (2.5x10^5) s.c. into the footpad of IFN-γ YFP reporter mice. Draining Lymph Nodes (LN) were harvested 7 days later, stained and analyzed by FACS. The number of IFN-γ producing cells in the draining LNs are shown (mean ± SEM). Significant differences were detected (*p<0.05, **p<0.01,*** p<0.001).

The ability of IMM-101 to induces IFN-γ secretion in vivo is lost in Baft3-/- mice suggesting a requirement for CD103+ dendritic cells in this pathway .

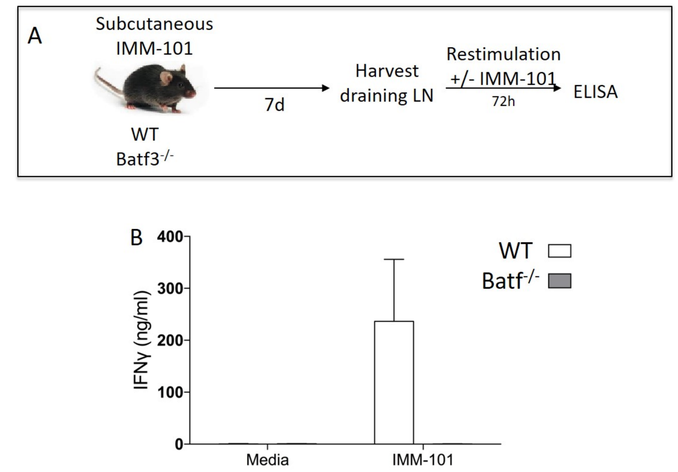

Figure 6. C57BL/6 WT or Batf3-/- mice were injected s.c. in the footpad with IMM-101 (300µg). Draining LNs were harvested 7 days later and restimulated for 72 hours with IMM-101 or media alone. A) Schematic of experimental procedure B) IFN-γ levels were measured by ELISA in the supernatants following restimulation.

CONCLUSIONS

- IFN-γ Simoa™ immunoassay is able to successfully detect changes in circulating levels of IFN-γ protein in the serum of advanced pancreatic cancer patients pre- and post-treatment with GEM ± IMM-101, a non-specific immunostimulant.
- In light of the observation that IMM-101 in combination with GEM increases survival in late stage pancreatic cancer, we were able to show a significant increase in the circulating levels of IFN-γ protein in serum samples from patients treated with IMM-101.
- The ability of IMM-101 treatment to enhance IFN-γ responses *in vivo* has been confirmed pre-clinically.
- Despite the preliminary nature of our work, it is intriguing to speculate whether a rise in circulating IFN-γ levels in serum samples of patients may be suggestive of improved response to immunotherapy and ultimately disease outcome. Further investigations are warranted.