772P Discovery and exploration of a live bacterial consortium (MB097) as co-therapy to enhance immune checkpoint inhibitor response in melanoma patients

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Abstract

Background: The gut microbiome of cancer patients impacts response to Immune Checkpoint Inhibitor (ICI) therapy. However, neither which bacteria, nor their mechanism of influence on immunotherapy are yet well characterised. Microbiotica's precision microbiome profiling of melanoma patients recruited to the MelResist (Cambridge, UK) study has identified a consortium of 9 diverse bacteria, including 4 novel species, which correlate with immune checkpoint inhibitor response across multiple published melanoma cohorts. This consortium, MB097, is being developed as an anti-PD1 co-therapy for melanoma patients. We are working to understand how the bacteria interact with the immune system to influence ICI response.

Methods: Human monocyte-derived dendritic cells were incubated with live bacteria (individually or as a consortium) firstly anaerobically, then aerobically. The stimulated dendritic cells (DCs) were also used to activate primary allogenic cytotoxic T lymphocytes (CTL) and NK cells. CTL and NK activation was assessed by intracellular FACS and tumour cells (SKOV3).

Results: MB097 bacteria, individually or as a consortium, strongly activated dendritic cells, upregulating maturation markers CD83 and CD86. Importantly, the strains were more potent inducers of IL-12 than IL-10 (up to 30-fold higher) resulting in a higher IL-12:IL10 ratio than other stimuli, LPS, PolyI:C and other bacteria. These MB097 bacteria strains stimulated DCs, which triggered CTLs to upregulate granzyme B, perforin and IFNγ, and kill tumour cells.

Conclusions: MB097 is a consortium of bacteria strongly linked to ICI response across multiple published series. In vitro, the bacteria activated dendritic cells, which in turn activated CTLs and NK cells. Interestingly, the bacteria that most potently induced CTL activation triggered the highest IL-12:IL-10 ratio released from DCs. These bacteria included 3 novel species. This IL-12 axis was less tightly linked to NK cell activation, suggesting other, as yet undefined, mechanisms may influence these cell interactions.

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Signature Predicts Response Across Multiple Melanoma Cohorts

We identified a discrete signature of bacteria differentially represented in patients that responded to ICI therapy as compared to non-responders in the MelResist study. This microbiome signature was extended and refined using additional shotgun metagenomic datasets from published advanced melanoma cohorts[1-4]. As a biomarker, the signature was 91% accurate at predicting response to ICI when all four studies were combined (Fig 1A). Importantly, it was similarly precise when all 4 cohorts were analysed separately (Fig 1B). The signature was validated on independent datasets

Fig 1A: Signature predicts response in 4 studies combined

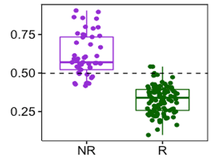
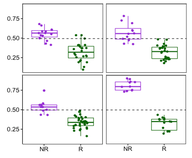


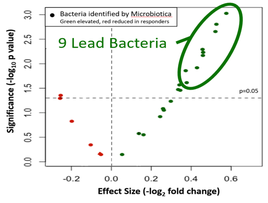
Fig 1B: Signature predicts response in all 4 studies separately



MB097: A Consortium Derived from the Microbiome Signature

The microbiome signature was reduced to 26 core species. This was heavily skewed to bacteria overrepresented in patients that responded to immunotherapy (Fig 2). This indicates the association of the microbiome with ICI therapy outcome is primarily driven by bacteria that enhance the anti-tumour immune response. A consortium of 9 bacteria most significantly associated with response - MB097 - was established for testing.

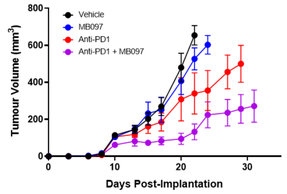
Fig 2: Volcano plot showing association of 26 core bacteria with response



MB097 Shows Anti-Tumour Efficacy In Vivo

To validate MB097, we tested it in a mouse syngeneic tumour model (MCA-205). MB097 was administered by oral gavage and itself had a small but reproducible impact on tumour growth. Most noticeably, MB097 synergised with anti-PD1 treatment to potently inhibit tumour growth (Fig 3).
These results validate the consortium of bacteria identified by a patient-first big data analysis of multiple melanoma studies.

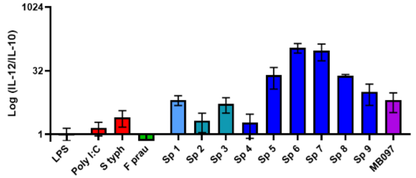
Fig 3: Anti-Tumour Efficacy of MB097 in Mouse Syngeneic Model



MB097 Activates Dendritic Cells

To understand how the bacteria associated with ICI response interact with the host immune response, monocyte-derived dendritic cells (DCs) were stimulated with the MB097 strains. All the strains except one (Sp 1) triggered maturation of the DCs similarly to the control stimuli (LPS, Poly I:C, *S. typhi* and *F. prausnitzii*), as demonstrated by CD86 and CD83 upregulation. The MB097 strains were more potent than the controls stimuli at inducing IL12 as compared to IL10 from the DCs, as shown by the IL12:IL10 ratio.

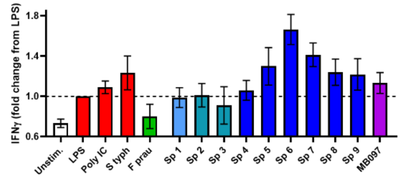
Fig 4: Induction of IL12:IL10 ratio from moDCs



MB097-stimulated DCs Activate Cytotoxic T Lymphocytes

Cytotoxic T Lymphocytes (CTLs) are the key immune cell in the anti-tumour response following ICI treatment and are potently activated by dendritic cells and IL-12. To understand the impact of bacteria associated with ICI response on this cell type, purified allogeneic CTLs were co-cultured with DCs that had previously been stimulated by the MB097 strains. Activation of DCs by MB097 drove strong CTL stimulation, as measured by IFNγ release (Fig 5), granzyme B and perforin upregulation and an increase in tumour cell killing potential. MB097 was as good, or better than, the positive control stimuli.

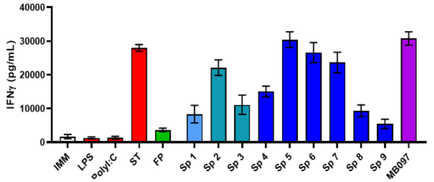
Fig 5: Induction of CTLs by DCs stimulated by MB097



MB097-stimulated DCs Activate NK Cells

NK Cells are also important in the anti-tumour response and are potently activated by DCs and IL-12. As with the CTLs, NK cells were co-cultured with DCs that had previously been stimulated by MB097. MB097 triggered strong NK cell activation, as measured by IFNγ release (Fig 6), granzyme B and perforin upregulation and an increase in tumour cell killing potential. As with CTL induction, MB097 was as good, or better than, the positive control stimuli.

Fig 6: Induction of NK cells by DCs stimulated by MB097



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