Multi-Technology Meta-Analysis Identifies Bacterial Signatures Delineating Clinical Response to Immune Checkpoint-Inhibitor Therapy in Melanoma Cohorts

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SECOND GENOME

Abstract

A growing body of evidence supports the influence of the gut microbiota on clinical response to cancer therapy, especially in the context of immune checkpoint inhibitors (ICI). While several studies present insight into bacteria differentially abundant in responders and non-responders to ICI, they are limited by biases arising from variations in cohorts, processing workflows, and analysis pipelines. In this context, microbiome-based discovery of an enhancer of response to ICI rests on accurate identification of bacterial strains and functions conferring a host benefit across cohorts.

Herein, five published datasets which investigated links between the gut microbiome and response to ICI were re-analyzed to identify strains differentially abundant between responders and non-responders. No overlap in significantly differentially abundant strains was observed across cohorts or even between datasets from the same cohort sequenced on both shotgun metagenomics and 16S rRNA amplicons. Utilizing a random effects model, we performed a cross-cohort multi-technology meta-analysis (MTMA) to integrate results from these isolated analyses and identified 37 strains that were concordantly enriched in responders across datasets. From these response-associated strains, we identified peptides and evaluated them for activity on primary human T cells. Several peptides induced secretion of effector cytokines (e.g. IFN-γ, IL-2) demonstrating their immunomodulatory functions.

The MTMA approach presented herein resolves a key challenge with microbiome meta-analysis by enabling integration of datasets generated by different technologies, allowing identification of cross-cohort strain signatures that can be used to nominate, test and identify peptides derived from these strains with therapeutic potential.

Comparison of microbiome datasets is challenging

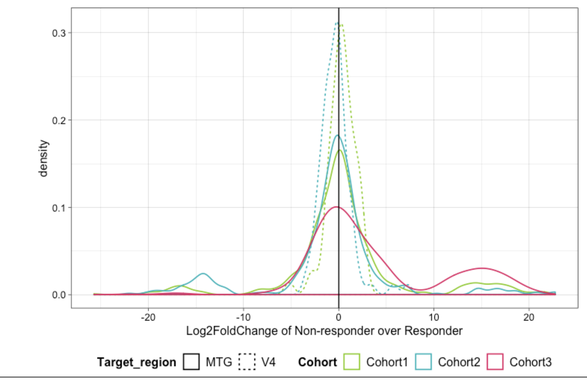
- Gut microbiota has emerged as key in modulating response to immune checkpoint inhibitor (ICI) therapy in melanoma, but overlap in bacterial taxa reported as response-associated is limited.
- **Can a robust bioinformatics pipeline identify bacterial modulators (strains and associated peptides) of ICI-response in melanoma patients supported across multiple cohorts?**

Cohort and DNA-profiling technology induced biases limit comparison of isolated datasets

- Stool from 3 cohorts (5 datasets) of melanoma patients on anti-PD1 ICI therapy was included.
- Microbiome of responders (R) & non-responders (NR) were compared.

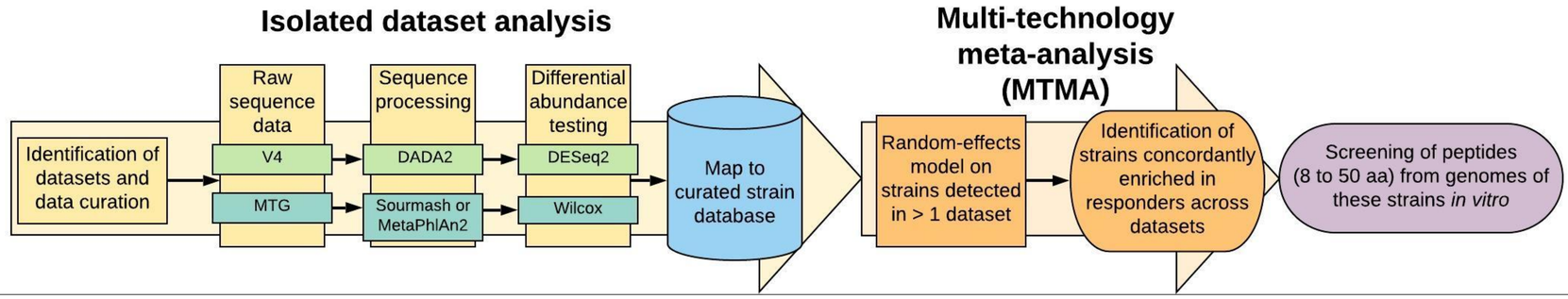
Dataset	Target region	Design
Cohort 1, *Matson et al.*	V4	R:15, NR:26
	MTG	R:14, NR:24
Cohort 2, *Gopalakrishnan et al.*	V4	R:30, NR:13
	MTG	R:14, NR:11
Cohort 3, *Frankel et al.*	MTG	R:3, NR:7

- **Distribution of effect sizes are cohort and DNA-profiling technology dependent.**



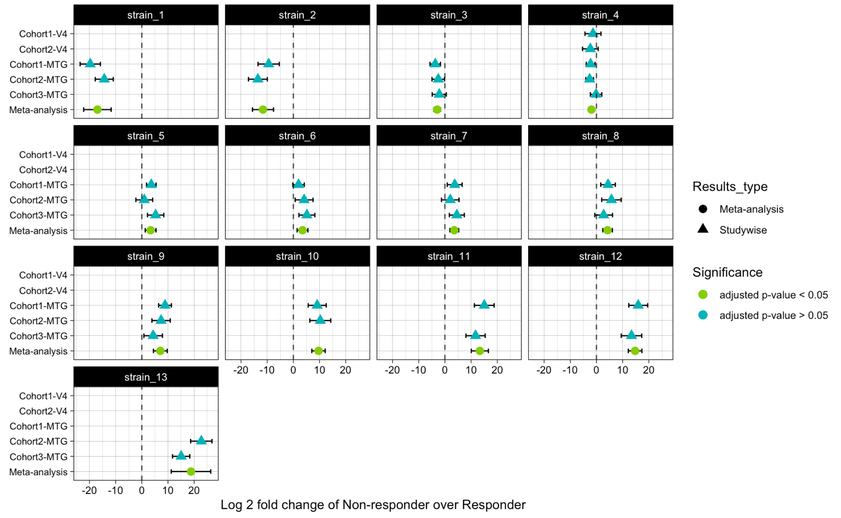
•*Matson et al.* (2018) Science.
•*Gopalakrishnan et al.* (2017) Science.
•*Frankel et al.* (2017) Neoplasia.

Multi-technology meta-analysis enables integration of datasets across DNA-profiling technologies at the strain-level

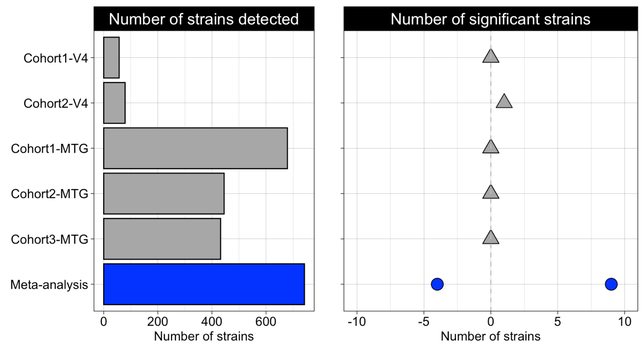


MTMA identifies previously unreported strains associated with response to ICI in melanoma

- **Meta-analysis identified strains significantly enriched in responders or non-responders.**
- Significance: Benjamini-Hochberg-corrected REM p values < 0.05.



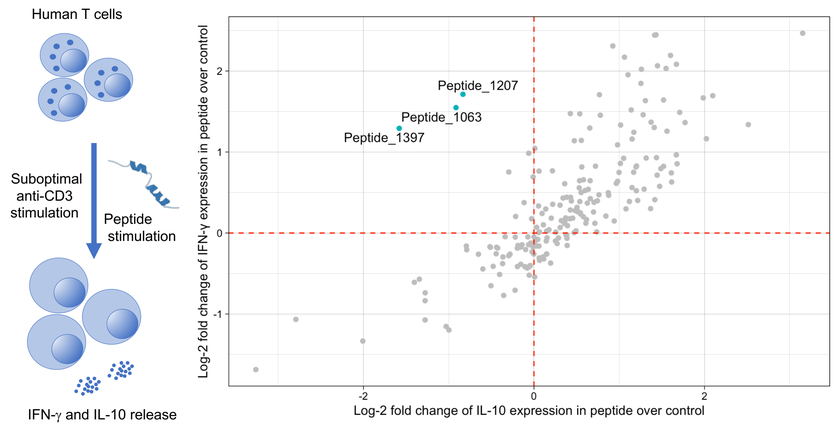
- **Meta-analysis is more sensitive at identifying significant changes compared to individual studies.**



- 37 strains that were associated with response across cohorts were identified from multiple meta-analyses that integrated only V4 or only MTG or both V4 and MTG datasets.

Peptides derived from response-associated strains modulate immune response to T cells

- 205 peptides were selected for screening from the response-associated strains.
- Human T cells isolated from from blood of healthy donors (n = 2) were stimulated with anti-CD3 and each of the peptides or a control peptide (OVA). Cell supernatants were collected after 48 hours and levels of IFN-γ and IL-10 were measured using Meso-scale discovery.



- **Peptides (blue dots) derived from response-associated strains induce the pro-inflammatory cytokine IFN-γ and reduce the anti-inflammatory cytokine IL-10 in activated T cells.**

Conclusions

- **We successfully identified response-associated strains that are corroborated by multiple cohorts, and in some cases multiple DNA-profiling technologies and are not identified in analysis of the individual datasets.**
- **Annotation to the strain-level enabled identification of peptides that modulated host immune response of T cells and promoted secretion of pro-inflammatory and reduction of anti-inflammatory cytokines.**
- The multi-technology meta-analysis framework developed herein allows integration of datasets across DNA-profiling technologies and pinpoints specific strains associated with a disease condition. This analysis workflow lays the foundation for integrating the growing body of microbiome data and thereby enables the identification of robust microbiome modulators of disease.