Meta-analysis on the specificity of microbiome-based signatures for predicting immune checkpoint inhibitor therapy response in non-small cell lung cancer patients

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INTRODUCTION

Immune checkpoint inhibitor (ICI) cancer therapies have emerged as a potent option for treatment of non-small cell lung cancer (NSCLC). The varying response rates and immune related adverse events indicate a need for a prognostic biomarker. The aim of the presented study is to perform a meta-analysis of 16S amplicon sequencing datasets across cancer types examining the possibility of an ICI therapy response biomarker based on the human gut microbiome.

METHODS

The presented research covers five 16S amplicon sequencing datasets [1-5] with a total of 191 patients (95 responder, 96 non responder), encompassing both NSCLC (82) and melanoma (109) patients. All raw sequencing data were analyzed using identical bioinformatic processes and evaluated with a compositional data analysis (CoDA) approach. Bayesian-multiplicative replacement was used for elimination of the zeroes in the count data. Centered log-ratio transformation (CLR) was applied for transformation. Subsequently, principal component analysis (PCA) was calculated to visualize variance introduced by factors, such as cancer type or datasets. To identify microorganisms with the highest predictive power for ICI therapy response, differential abundance analysis with Linear discriminant analysis Effect Size (LEfSe) as well as ANOVA-Like Differential Gene Expression Analysis (ALDEx) was performed.

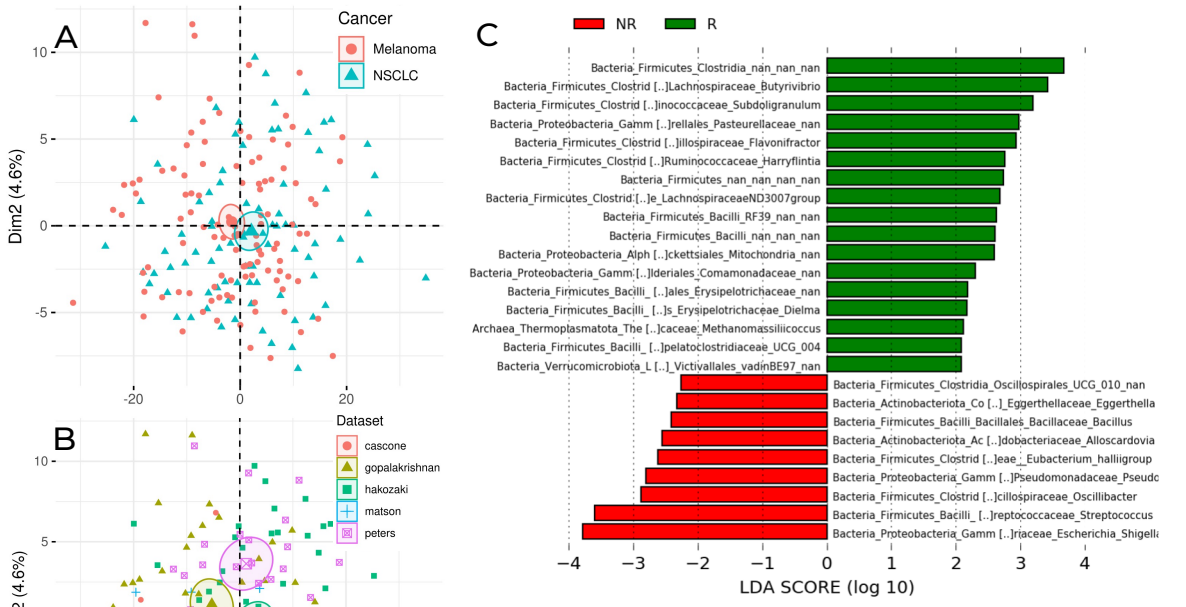

Figure 1. A&B) PCA of the CLR normalized dataset at genus level coloured and with the focus points for the different cancer types (A) and datasets (B). C) LDA Score of the significantly different taxa discovered by the LEfSe analysis (p-value threshold < 0.05, LDA threshold > 2), green coloured bars show organisms enriched in responder (R), red coloured bars show organisms enriched in non-responder (NR).

Taxonomy	p t-test	p t-test corrected	p Wilcoxon	p Wilcoxon corrected	difference R/NR
Bacteria;Firmicutes;Clostridia;nan;nan;nan	0,00299	0,25055	0,00039	0,11583	1,15213
Bacteria;Firmicutes;nan;nan;nan;nan	0,00688	0,28640	0,00254	0,22128	1,50814
Bacteria;Firmicutes;Clostridia;[...];Subdoligranulum	0,00693	0,30122	0,00406	0,26154	1,94534
Bacteria;Proteobacteria;[...];Escherichia-Shigella	0,01740	0,37105	0,03084	0,36644	-1,84489
Bacteria;Firmicutes;Clostridia;[...];Ruminococcaceae;nan	0,00617	0,31110	0,01480	0,39166	0,71229
Bacteria;Firmicutes;Clostridia;[...];[Eubacterium] hallii group	0,04086	0,48329	0,02783	0,41941	-1,50192
Bacteria;Actinobacteriota;Coriobacteriia;[...];Eggerthella	0,05465	0,50876	0,03066	0,44272	-1,29045

Table 1. Difference and p-values (corrected and uncorrected) of the Welch t-tests and Wilcoxon tests of the ALDEx differential abundance analysis

RESULTS

The PCA shows that cancer types have no major impact on the variation in the meta-analysis dataset (Fig. 1A). By comparison, the origin of data introduces higher variation, with the Cascone *et al.* (NSCLC) and Matson *et al.* (melanoma) datasets grouping around a similar focus point (Fig. 1B). The results of the presented LEfSe analysis indicate that the order *Clostridia*, more specifically the genera *Butyrivibrio*, *Subdoligranulum, Flavonifractor, Harryflintia* are enriched in patients responding to ICI therapy (Fig. 1C). Opposing, organisms from the genera *Escherichia_Shigella, Streptococcus, Oscillibacter*, and *Pseudomonas* are more abundant in non-responders. Contrary to the LEfSe analysis, CoDA based ALDEx recovers no significant association from datasets. However, the p-values of the Welch t-tests and Wilcoxon rank sum tests, performed by ALDEx analysis, indicate that the taxa with the lowest p-values are similar to the ones recovered by LEfSe (Tab. 1).

DISCUSSION

These results align with findings of Gopalakrishnan *et al.* and Routy *et al.* who report associations of several *Clostridiales* with ICI therapy response [1, 6]. The presented results contribute to the existing pool of research for a specific ICI response gut microbiome signature and suggest that such a biomarker could be applicable across different cancer types. Even though the more mathematically robust CoDA approach yields no significant results, the recovered associations of LEfSe include common bacterial taxa. While several of these taxa overlap with previous findings, the strength and applicability of these methods remain to be discussed. The presented preliminary data give hope for a tumor-agnostic clinical ICI response prediction biomarker, which shall help to improve cancer therapy outcomes for NSCLC and melanoma patients alike.

References

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