Predicting response to anti-PD-1 therapy from metagenomic sequencing data with machine learning

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BACKGROUND

- The body of evidence **linking gut microbiota with the response to cancer therapy** has been rapidly growing in the past few years.
- In 2018 works by groups of Wargo, Gajewski and Zitvogel [1-3] have shaped the field, describing the correlation between gut microbiota and response to immune checkpoint blockade (ICB).
- Those studies also reported the **increase of ICB response rate by gut microbiota modulation.**

CHALLENGE

- Two analytical techniques for studying microbiome - 16S rRNA sequencing and Shotgun Metagenomic Sequencing (SMS) - are widely applied, but the taxonomy-based approach of 16S shaped the way majority of research is conducted.
- 16S sequencing is a cost-effective way to analyze the taxonomic composition of a sample, however it is limited to genus/species level description, not allowing for functional explanation.
- In crucial research [1-3] the SMS potential was not used to its fullest. **Certain bacterial taxa were identified as associated with response to checkpoint blockade therapy.** On the other hand no functional explanation was provided for these findings.
- Beneficial bacterial strains identified in each paper were inconsistent between studies, and the findings were **not statistically significant validated on external cohort data.**
- The work by Gharaibeh and Jobin [4] presented the machine learning model that utilizes SMS-derived functional data. However, this **model was not validated on external cohort, and stratification potential was not demonstrated.**

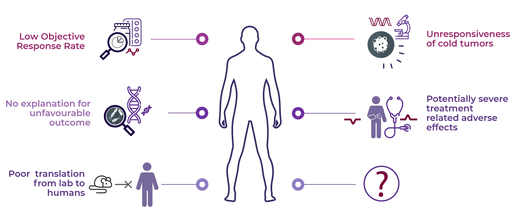

Fig. 1. Challenges for the immune checkpoint blockade therapies that are linked with the role of gut microbiota.

DATASETS

- We used two cohorts of melanoma patients undergoing anti-PD-1 therapy:
  - Gopalakrishnan et al. [1]
  - Matson et al. [2]
- All patients' stool was collected prior to anti-PD-1 therapy.
- Samples were sequenced using Shotgun Metagenomic Sequencing technology.
- Response was measured according to RECIST 1.1 guidelines.
- In Gopalakrishnan cohort patients had Time To Progression reported.
- Original datasets were analyzed with regard to outliers and metadata consistency.

DATASET	MATSON	GOPALAKRISHNAN
Responders	15	14
Non-Responders	24	11
Total	39	25

ANALYSIS OUTLINE

- Data analysis and interpretation was done using **Translational Microbiome Platform** (TMP) developed by Ardigen.
- Shotgun sequencing data of described cohorts were processed with **Microbiome Scout** tool that identified **Metagenomic Features** associated with the analyzed phenotype.
- Selection performance was measured by ROC AUC of used classifier and by statistical significance (p-value) of subgroups separation in Time To Progression (TTP) analysis.

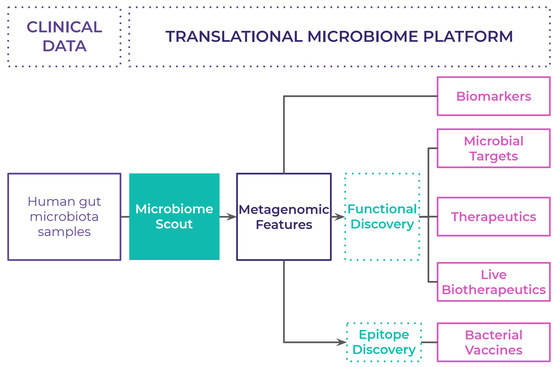

Fig. 2. Scheme of Ardigen's process of microbiome derived Dx/Tx development.

METHODS

- We used all SMS samples for building a study-specific feature space. Ardigen's Microbiome Scout builds metagenomic Feature Space with up to 30% of novel metagenomic sequences (Fig. 3a).
- All the metagenomic features sequences were encoded into functionally-valid space (Fig. 3b).
- For classification problems Random Forest and Logistic Regression algorithms were used as implemented in Ardigen's Microbiome Scout (Fig. 3c).
- Each model was validated in 100x repeated 5-fold cross-validation.

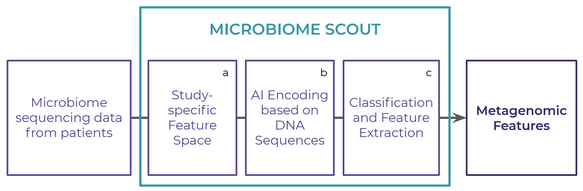

Fig. 3. Scheme of Microbiome Scout procedure with separate stages of building study-specific feature space (a), encoding obtained features into functionally-valid space (b) and machine learning classifiers (c).

VALIDATION SCHEMES

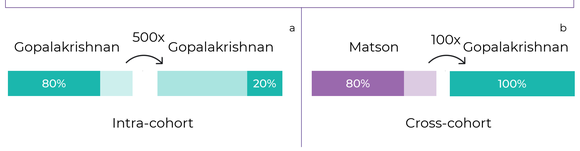

Fig. 4. Visualisation of two validation schemes. In intra-cohort scheme (a) both parts - training and validation - were done using the same dataset randomly split into test and training sets (80:20 ratio). In cross-cohort scheme 80% of training dataset was used for training as well, but validation was done on another cohort. In the presented study validation was always done on Gopalakrishnan cohort, due to the fact that TTP data as well as benchmark ROC AUC were reported only for this cohort.

OUR APPROACH OUTPERFORMS BENCHMARK

- We compared our **intra-cohort predictions** of response to anti-PD-1 therapy in metastatic melanoma to the best model published to date [4].
- Our model **outperforms benchmark** with AUC=0.81 compared to AUC=0.71 reported by Gharaibeh [4].

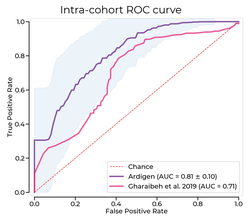

Fig. 5. ROC curve of intra-cohort predictions of response to anti-PD-1 in Gopalakrishnan cohort (purple, AUC=0.81) compared to the benchmark [4] (pink, AUC=0.71).

MICROBIOME-BASED STRATIFICATION

- To evaluate how machine learning predictions reflect the clinical benefit we performed **TTP** analysis.
- All patients predicted not to respond to anti-PD-1 therapy progressed within 90 days (median=60.5).
- Patients predicted to respond stayed **progression-free for significantly longer period of time** (median=144.5).

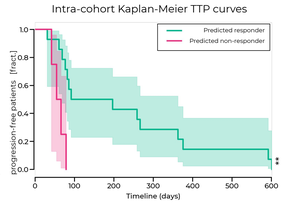

Fig. 6. Kaplan-Meier TTP curves of patients predicted to respond (green) and not to respond (pink) to anti-PD-1 treatment. Patients predicted to respond stayed progression-free significantly longer (p<0.005).

CROSS-COHORT CLASSIFICATION

- Intra-cohort performance of model was analyzed for the benchmark purposes only.
- To prove the significance of model it was **validated on the external cohort.**
- The model trained on samples from the Matson cohort allowed for stratification of Gopalakrishnan cohort with ROC AUC=0.76, which is also outperforming the intra-cohort benchmark [4].

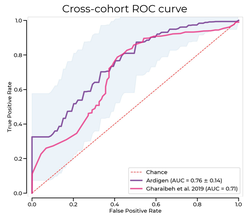

Fig. 7. ROC curve of intra-cohort prediction of response to anti-PD-1 in Gopalakrishnan cohort (purple, AUC=0.76) compared to the intra-cohort benchmark [4] (pink, AUC=0.71).

CROSS-COHORT TIME TO PROGRESSION PREDICTION

- The group predicted to respond to therapy had significantly longer TTPs (median=66) compared to those predicted not to respond (median=92).
- **This is the first reported stratification of checkpoint blockade patients with an external dataset used for validation.**
- Unprecedented cross-cohort performance of our model indicates presence of gut microbiome' functional features influencing therapy outcomes that are **shared by patients across studies**.

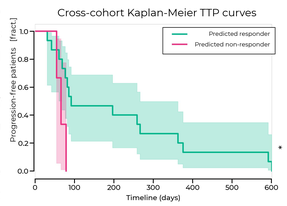

Fig. 8. Kaplan-Meier TTP curves of patients predicted to respond (green) and not to respond (pink) to anti-PD-1 treatment. Patients predicted to respond stayed progression-free significantly longer (p<0.05).

FEATURE SELECTION

- We scored all Metagenomic Features with their contribution to patient stratification.
- We identified a set of roughly a dozen features whose contribution to the stratification greatly exceeds all the remaining features.

FUNCTIONAL DISCOVERY

- We identified that feature ranked as first (upregulated in responders in both cohorts) is associated with **arginine synthesis pathway** and, as previously described, **arginine enhances T-cell antitumor activity** [5, 6].
- Features ranked as 2nd, 6th and 8th were identified as involved in **bacterial evolution and defense.**
- Features ranked as 8th and 9th indicate the role of **phages** in shaping the response to checkpoint blockade.

Fig. 9. Scheme of Functional Discovery procedure that allows for the identification of structure (a), enzyme (b) and metabolite-based (c) features' role in modulating response to therapy.

EPITOPE DISCOVERY

More details: P682

- Epitope Discovery pipeline identified **1262 peptides** with high (>0.8) score of immunogenicity.
- 796 (61%) of those were **strong binders (<50nM).**
- We discovered previously undescribed immunogenic peptides as well as homologous to sequences of known cancer associated proteins.

Fig. 10. Scheme of Epitope Discovery procedure that assesses immunogenicity of peptides derived from Metagenomic Features.

OUTLOOK AND DEVELOPMENT DIRECTIONS

- **Gut microbiota should be regarded as one of the crucial factors driving patients' response to checkpoint blockade therapy.**
- Functional traits in gut microbiota allow for **patients stratification in anti-PD-1 treatment of metastatic melanoma** with a mechanistic explanation.
- The small number of samples in cohorts (n=25 and n=39) is a significant limiting factor of presented work.
- Ardigen is running clinical studies **(NCT04136470 and NCT04145232)** to gather stool along with blood (PBMC) and tumor (FFPE), to be analyzed with Translational Microbiome Platform.

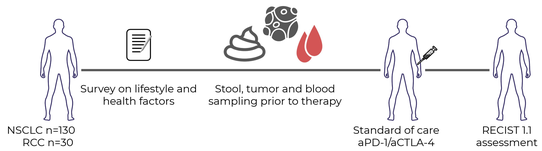

Fig. 11. Scheme of Ardigen's non-interventional clinical studies. We are gathering stool, blood (PBMC) and tumor (FFPE) samples from patients undergoing standard of care anti-PD-1 or anti-CTLA-4 therapy in NSCLC (n=130) and RCC (n=30). Response is measured with RECIST 1.1 criteria.

LITERATURE

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