Influence of microbiota on the effectiveness of immunotherapeutic drugs in the treatment of metastatic solid tumors

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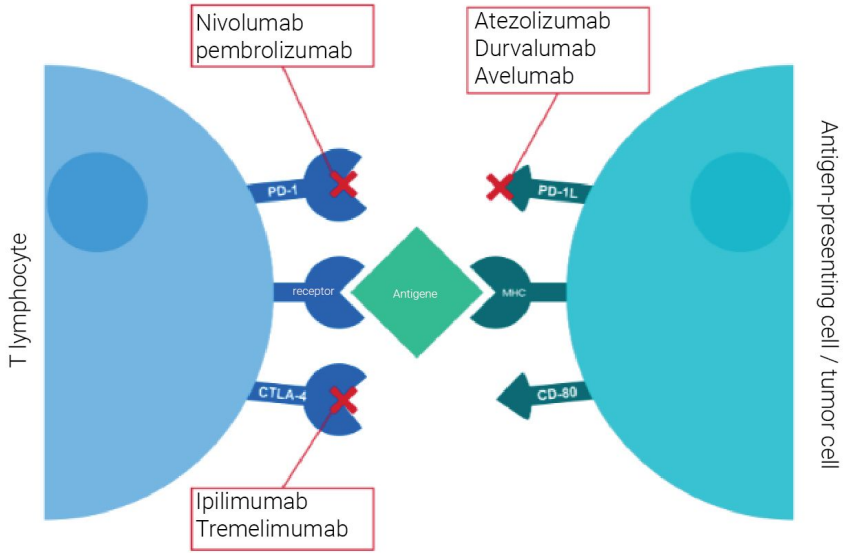
Checkpoint immunotherapy

Immunological checkpoints are a system of mechanisms that regulate the triggering of autoimmune processes.

Tumor cells can use such checkpoints to prevent the activation of tumor-specific lymphocytes, thus gaining resistance to the action of the immune system.

One of the promising methods of tumor immunotherapy is the prevention of stopping the immune response by tumor cells through interaction with checkpoints.



Fecal microbiota in oncology

According to the literature, the quantitative and qualitative composition of the fecal microbiota may correlate with the response to cancer therapy.

Some families are considered more favorable for therapy, others less. Accordingly, the composition of the microbiota can have a predictive effect at the stage of choosing a therapy method, and fecal microbiota transplantation (FMT) can increase the effectiveness of therapy.

However, for immunotherapy, there is still no work to analyze the composition of the microbiota and its effect on therapy.

Aim: To determine the prognostic and predictive value of the taxonomic composition of the microbiome of patients on the results of treatment of cancer patients

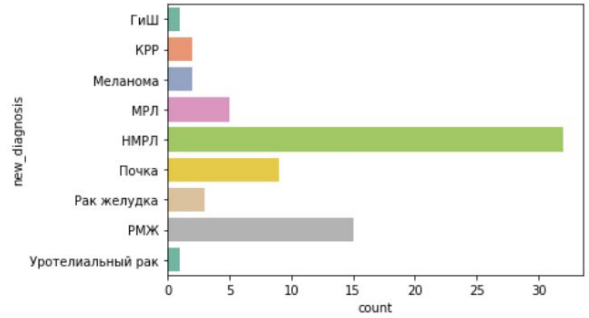
Tasks:

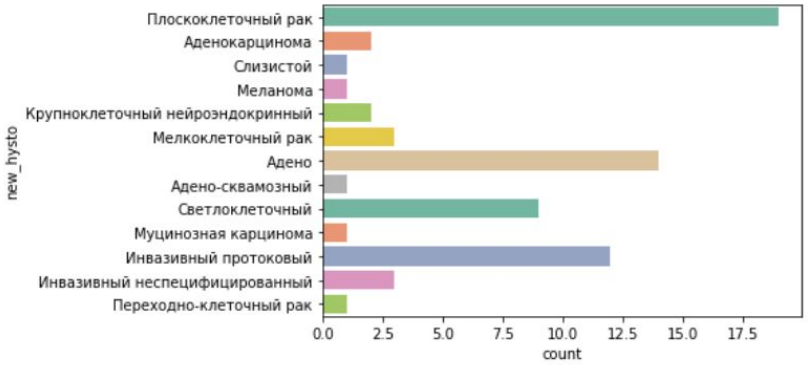
- study the methods of data analysis of sequencing of the 16S rDNA fragment of the microbiome
- evaluate the quality of raw sequencing data
- assess taxonomic diversity (OTU, ASV) of the gut microbiome of patients
- statistical analysis based on patient metadata and patient microbiome characteristics
- to identify correlations between the characteristics of the microbiome and the response to immunotherapy in patients with lung cancer

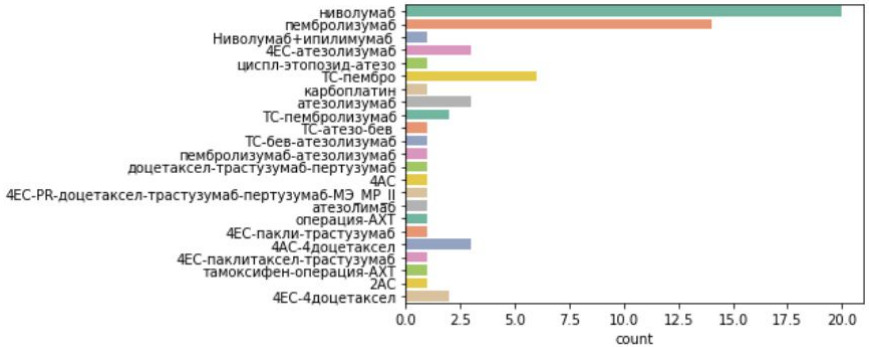
Data

We worked with data of metagenomes of fecal microbiota from 16s of 71 patients.

Patients vary greatly in diagnosis, histology, and therapy received.







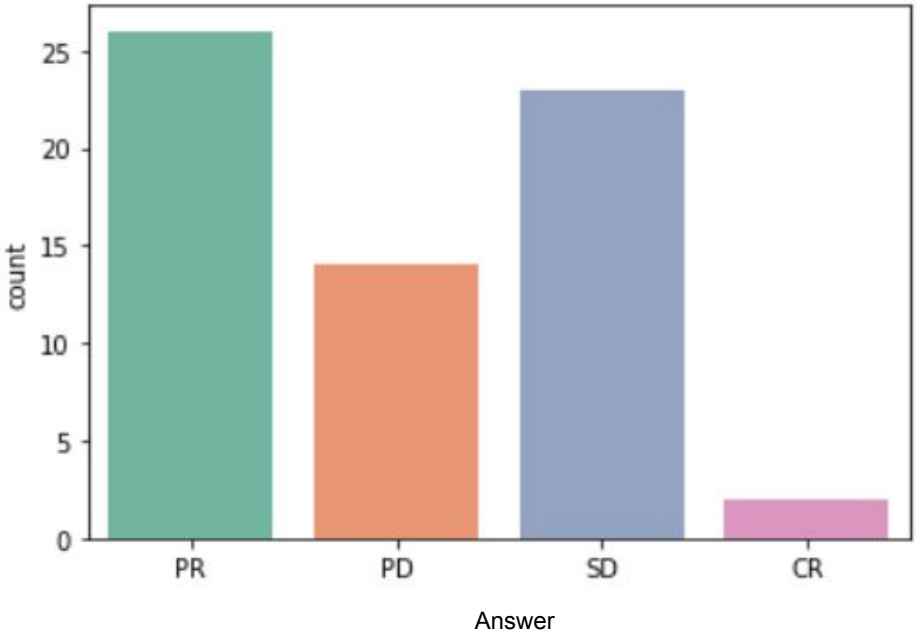
The main predicted class is the objective response of patients to therapy

CR: almost complete recovery;

PR: tumor shrinkage;

PD: enlargement of the tumor or the appearance of new lesions;

SD: no progress.



Pipeline

- analysis of taxonomic diversity (OTU / ASV) of fecal microbiome of patients
  - *dada2, Qiime2*
- normalization of quantitative data of diversity
  - *DESeq2*
- analysis of environmental metrics
  - *Qiime2, ANCOM*
- search for the most influential taxa and metadata, predicting the outcome of therapy using the mos influential predictors
  - *k-NN + LogitBoost, sklearn.linear_model.LogisticRegression, sklearn.ensemble.RandomForestClassifier, skrearn.feature_selection, Logitboost & GradientBoostingClassifier and others.*

DADA2 : Bioinformatics Workflow

'Join paired-end fastq files into merges, denoised, chimera free, inferred sample sequences'

Read in fastq files → Sort F and R reads → Quality Inspection → Filter → Dereplication → Denoising

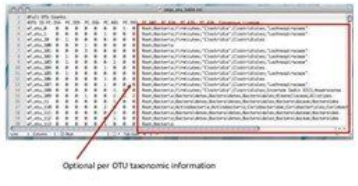
Filter: Using quality scores to trim seq

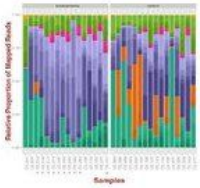
Dereplication: Output unique seq and their abundance ASVs

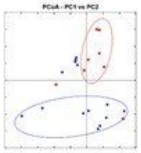
Merge reads → Chimera removal → Assign taxonomy & phylogeny → Construct phyloseq object → Filter → Diversity analysis

Construct phyloseq object: Similar to BIOM file

Filter: Low abundance reads, Rarefaction, Transformation





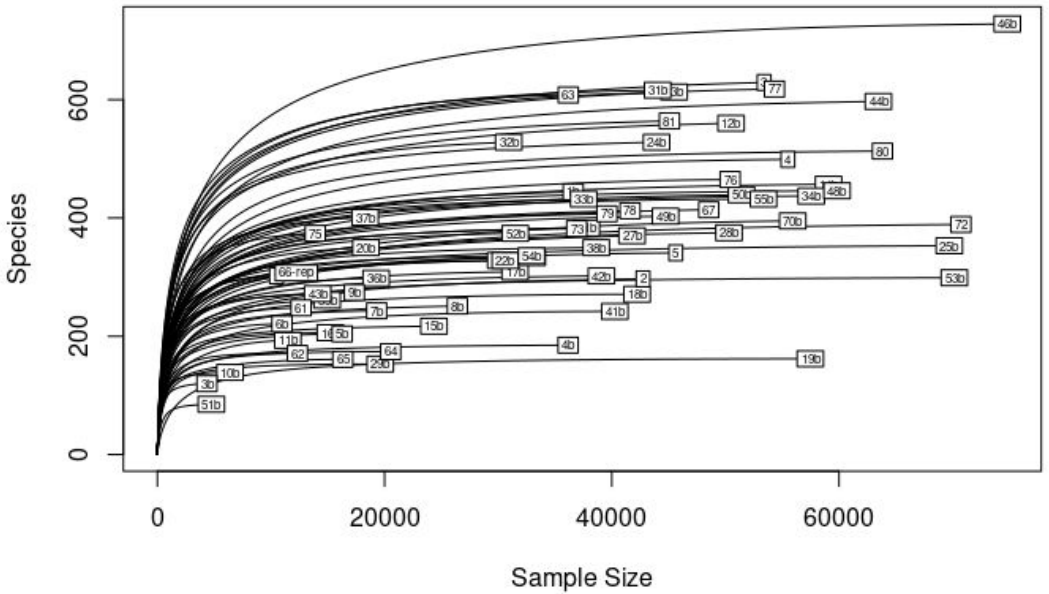


Data normalization

Why it is important: The library sizes for each sample often vary by orders of magnitude, and the counts are overly dispersed. Qiime2 includes vacuum normalization in the pipeline, while dada2 does not.

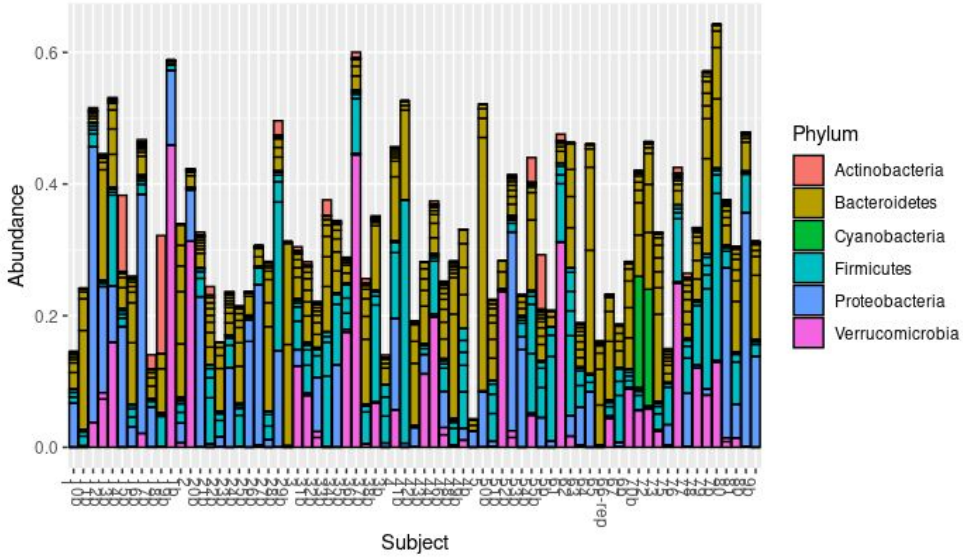
Solution:

- variance-stabilizing normalization using a mixed model such as a negative binomial (Deseq2)
- normalization procedures based on the Poisson-Gamma Blend model provided a systematic improvement in performance over coarse proportions or sparse counts - both of which resulted in a high rate of false positives



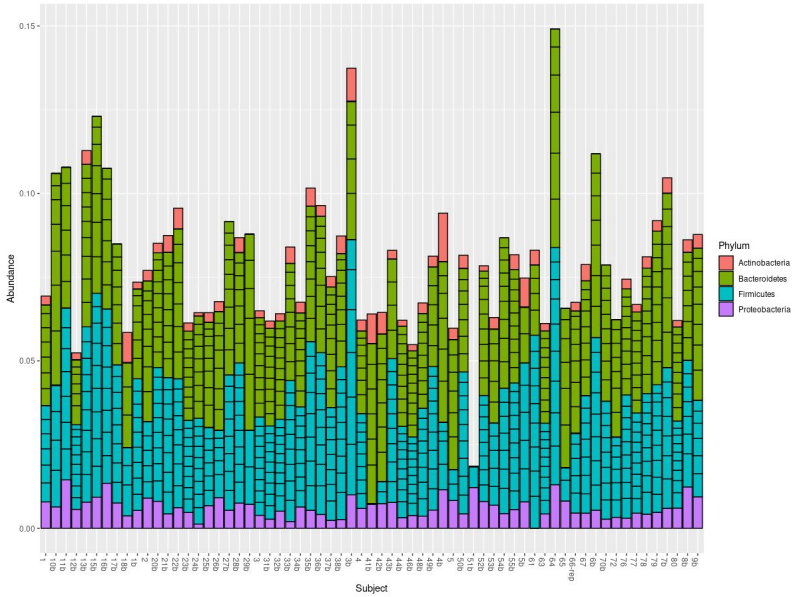
Rarecurve displays the dependency of diversity on the size of the library

Data normalization



Barplot showing the abundance of different phylums for each of the patients. **Unnormalized data**

Barplot showing the abundance of different phylums for each of the patients. **Normalized data**



Environmental metrics used to compare microbiome communities:

Alpha diversity is a criterion for the distribution of taxa in a community**.**

Shannon diversity Index - takes into account the number of species in the sample

Shannon uniformity index - takes into account only the taxonomic composition of the community.

Beta Diversity - comparison of communities between samples

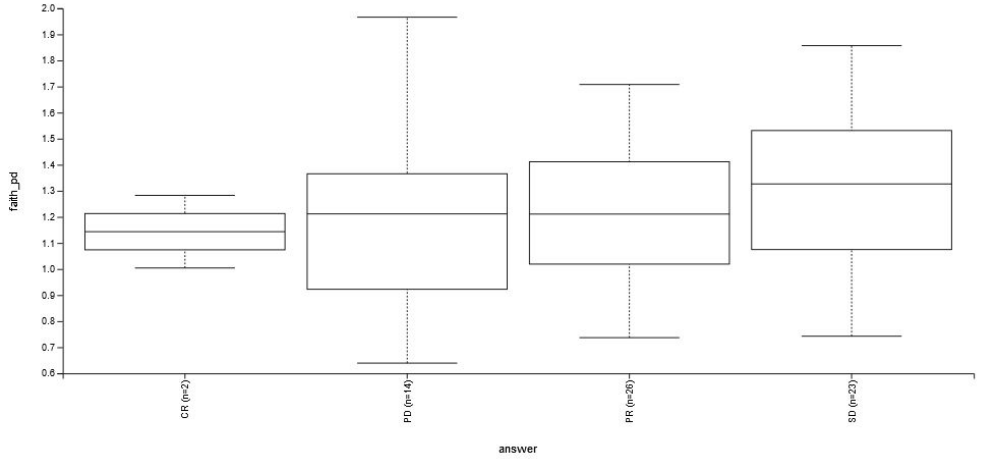
Bray-Curtis distance - includes quantitative data

Jaccard index - based on factorial data on the representation of the taxon in the community

UniFrac is an index based on a phylogenetic tree of samples. **Weighted** (quantitative) and **unweighted** (qualitative) options are used.

Alpha diversity:

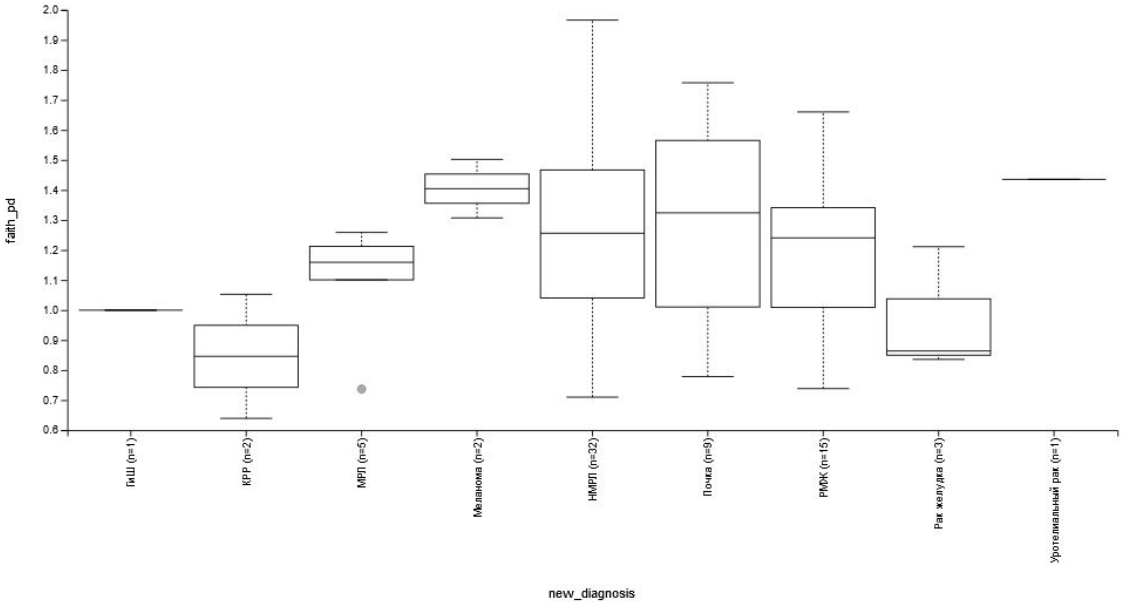
Alpha diversity based on the Shannon diversity index does not differ statistically (based on the Kruskal-Wallis test) among patients with different treatment effects.



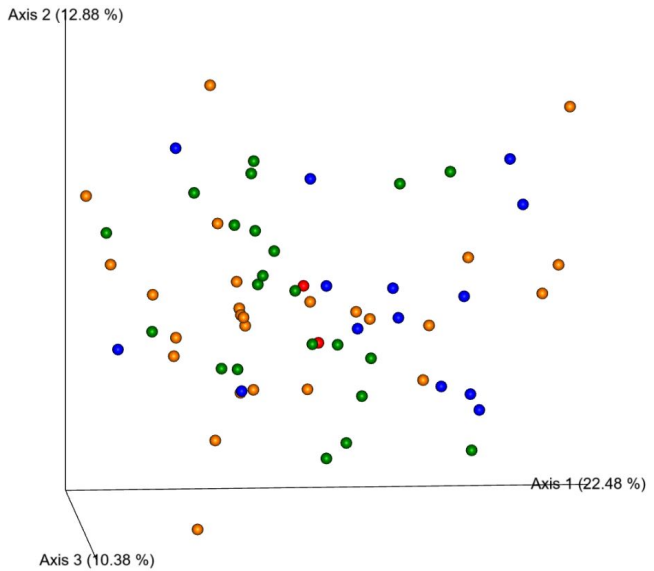
Group 1	Group 2	H	p-value	q-value
CR (n=2)	PD (n=14)	0,025	0,874	0,874
CR (n=2)	PR (n=26)	0,286	0,592	0,874
CR (n=2)	SD (n=23)	0,642	0,423	0,846
PD (n=14)	PR (n=26)	0,065	0,799	0,874
PD (n=14)	SD (n=23)	0,715	0,398	0,846
PR (n=26)	SD (n=23)	1,044	0,307	0,846

However, alpha diversity is highly variable in patients with different types of cancer.

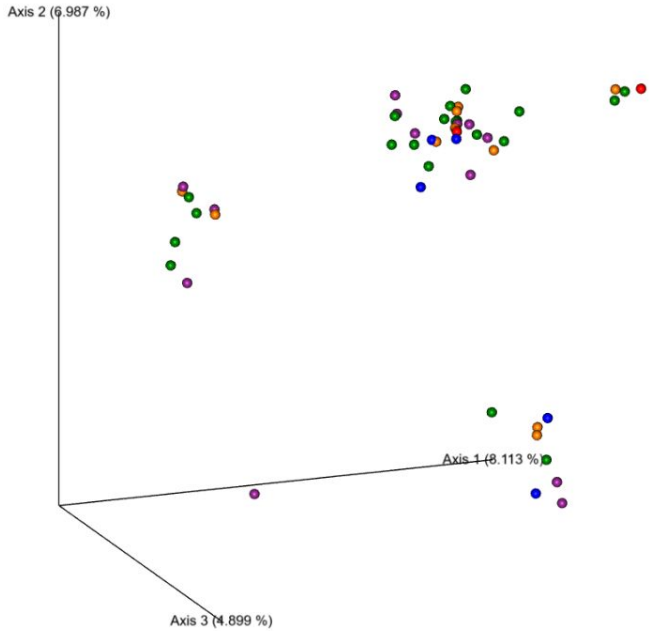
It is possible that these differences are due to the small sample of patients.



Influence of the normalization method on the assessment of community beta diversity:



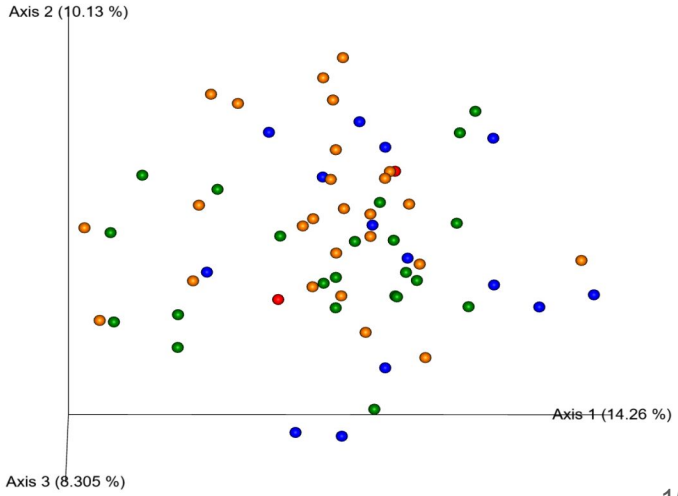
dada2 + Deseq2, negative binomial method



qiime2, dilution method

In terms of beta diversity, there is also no correlation between patients with different disease progression.

Clustering based on the Jaccard index does not identify patient clusters that match metadata. The distance between patients according to the weighted statistics of UniFrac does not reveal significant differences between patients.

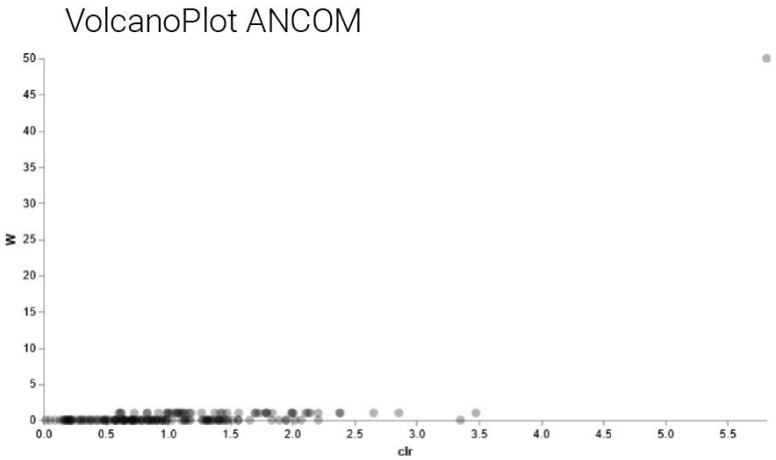


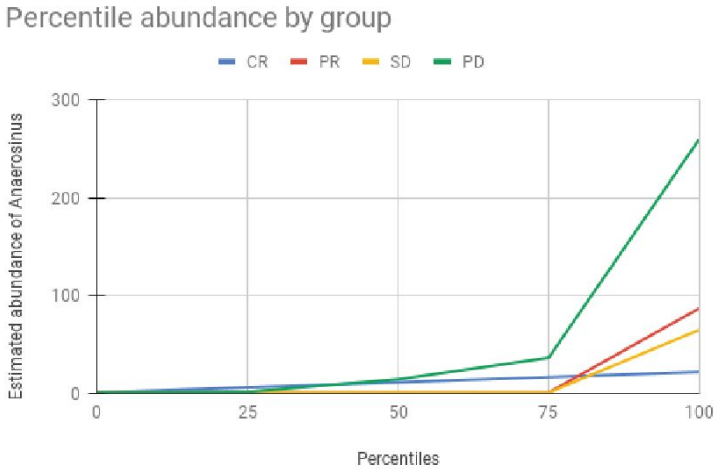
The lack of differences in alpha and beta diversity between patients with different responses to therapy is found in the literature.

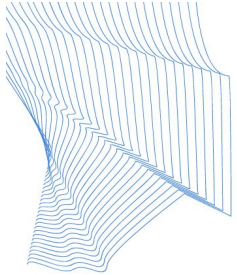
However, statistical tests may fail to identify individual taxa that influence the outcome of the therapy.

Analysis of the differential composition of the microbiome (ANCOM) revealed one taxon that is differentially expressed in patients with different responses to therapy: *Anaerosinus glycerini*.

However, as we can see from the percentile abundance of population accumulation within groups, its distribution is not very uniform and, accordingly, is also not a reliable indicator.



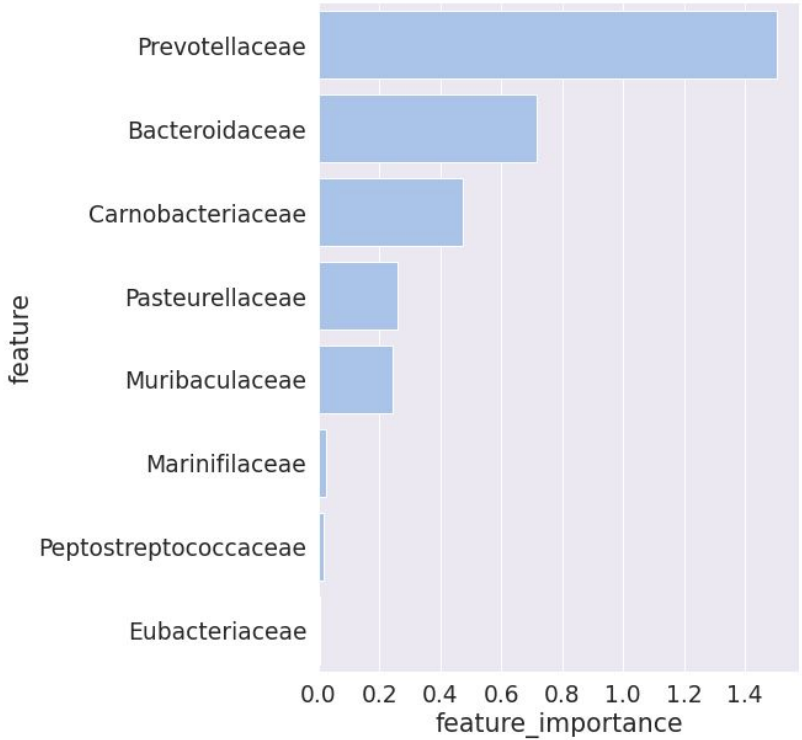




Next, we present the results of predicting the results of therapy by ML methods using different methods of building models.

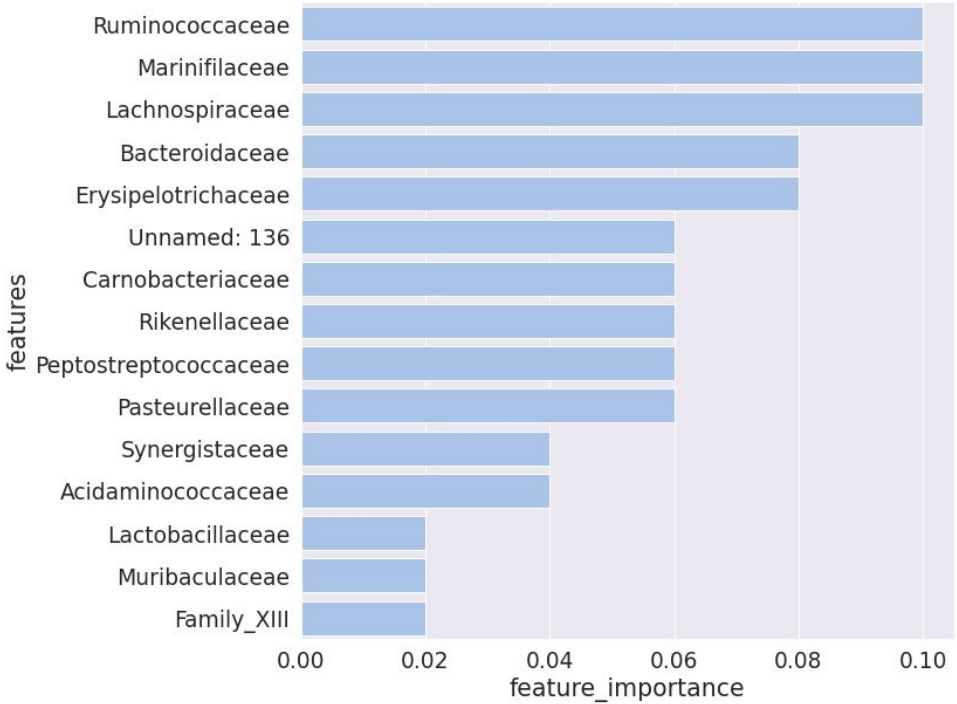
ML with Progress as a dependent variable

LASSO linear regression



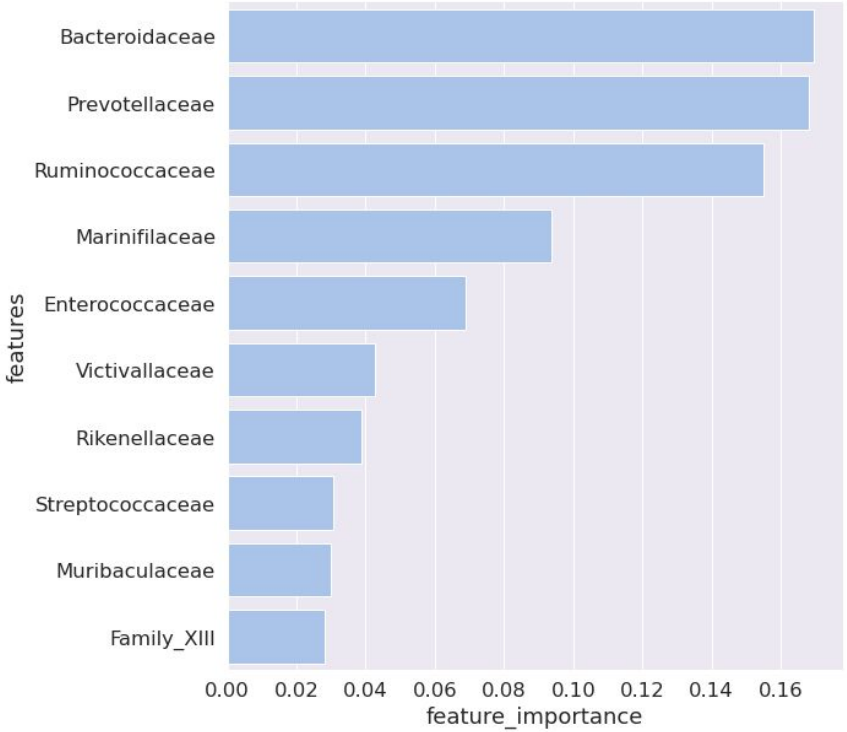
	precision	recall	f1-score	support
есть	0.40	0.40	0.40	5
нет	0.79	0.79	0.79	14
accuracy			0.68	19
macro avg	0.59	0.59	0.59	19
weighted avg	0.68	0.68	0.68	19

Logitboost linear model



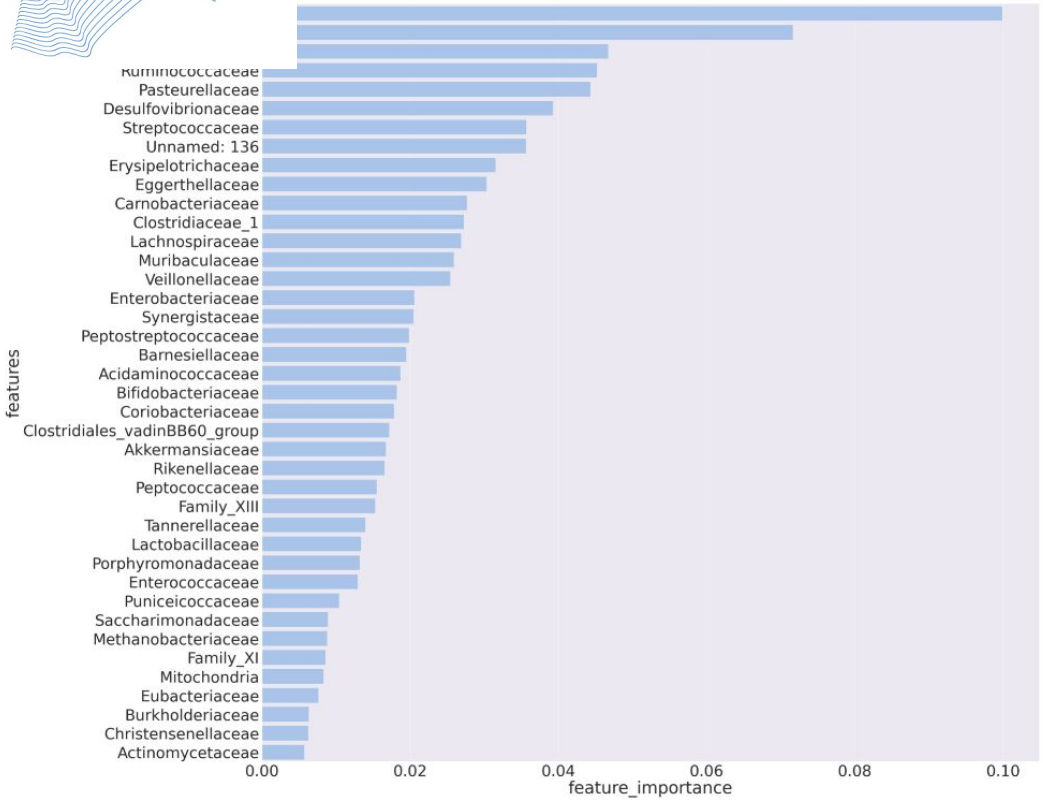
	precision	recall	f1-score	support
есть	0.33	0.20	0.25	5
нет	0.75	0.86	0.80	14
accuracy			0.68	19
macro avg	0.54	0.53	0.52	19
weighted avg	0.64	0.68	0.66	19

GradientBoostingClassifier linear regression



	precision	recall	f1-score	support
есть	0.38	0.60	0.46	5
нет	0.82	0.64	0.72	14
accuracy			0.63	19
macro avg	0.60	0.62	0.59	19
weighted avg	0.70	0.63	0.65	19

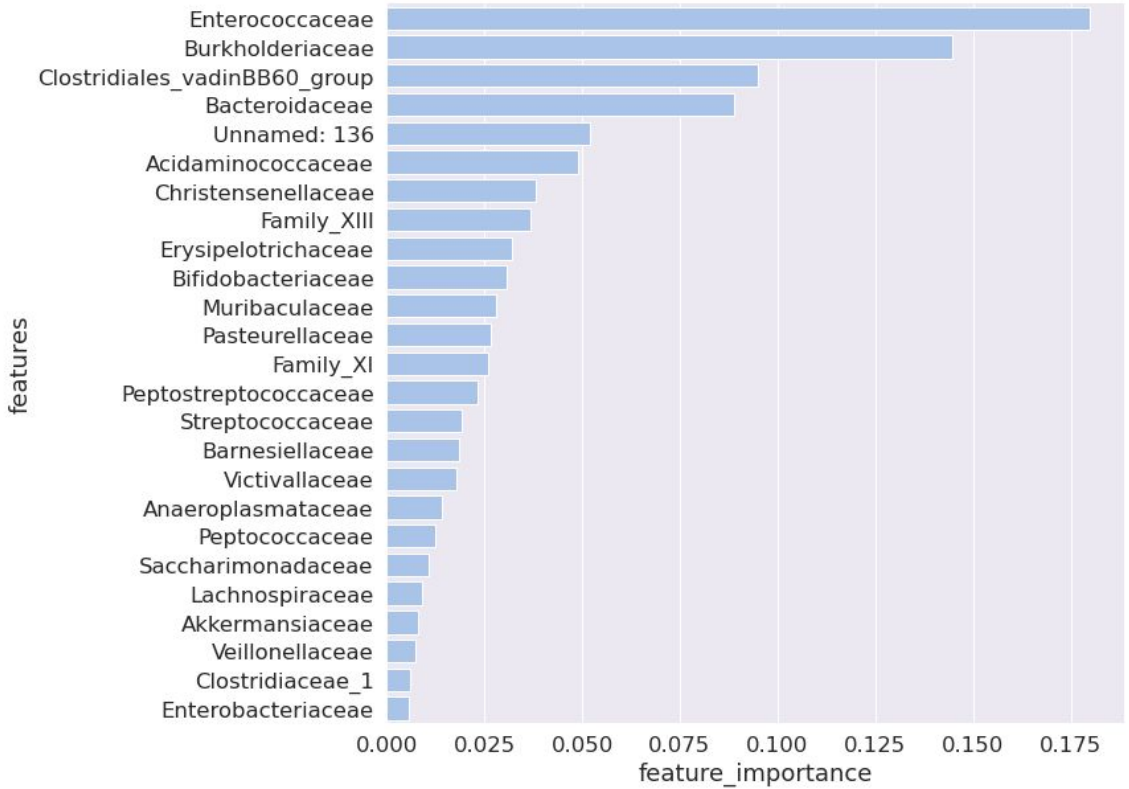
Random forest



	precision	recall	f1-score	support
есть	0.67	0.40	0.50	5
нет	0.81	0.93	0.87	14
accuracy			0.79	19
macro avg	0.74	0.66	0.68	19
weighted avg	0.77	0.79	0.77	19

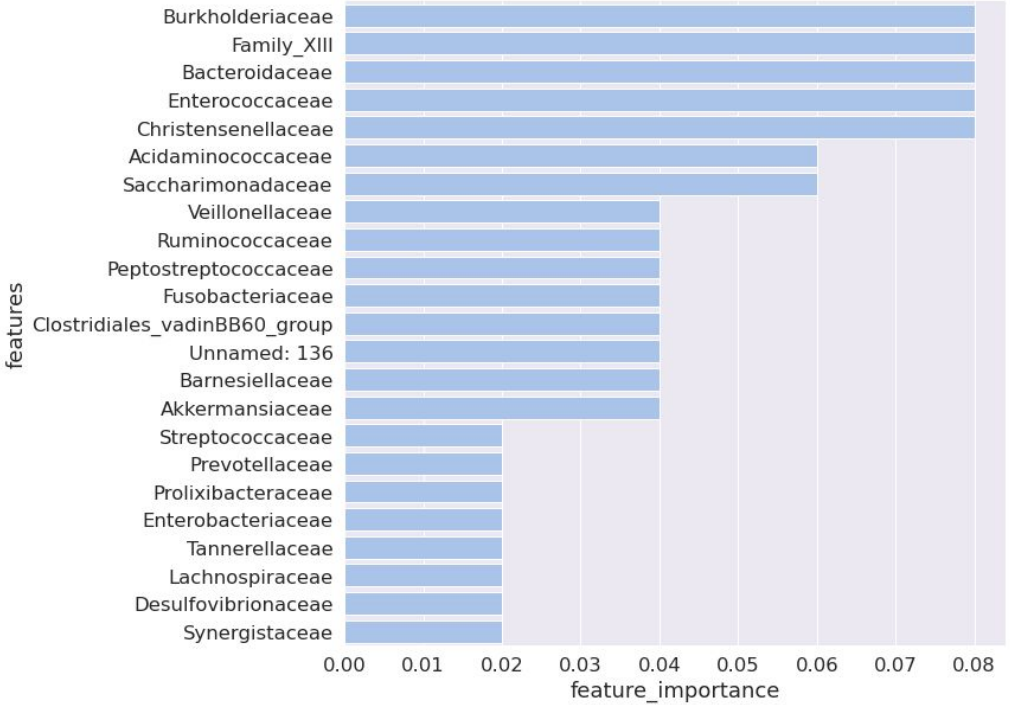
ML with Objective Response as a dependent variable

GradientBoostingClassifier linear regression



	precision	recall	f1-score	support
NR	0.67	0.73	0.70	11
R	0.62	0.56	0.59	9
accuracy			0.65	20
macro avg	0.65	0.64	0.64	20
weighted avg	0.65	0.65	0.65	20

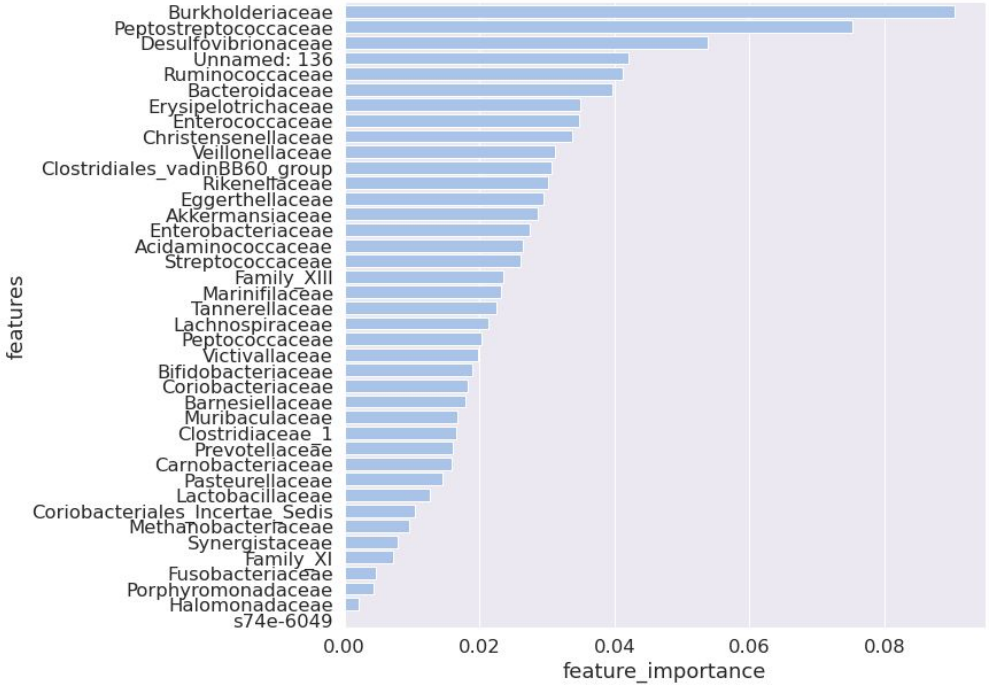
Logitboost linear regression



	precision	recall	f1-score	support
NR	0.75	0.82	0.78	11
R	0.75	0.67	0.71	9
accuracy			0.75	20
macro avg	0.75	0.74	0.74	20
weighted avg	0.75	0.75	0.75	20

NR - no response; R - response

Random forest



	precision	recall	f1-score	support
NR	0.65	1.00	0.79	11
R	1.00	0.33	0.50	9
accuracy			0.70	20
macro avg	0.82	0.67	0.64	20
weighted avg	0.81	0.70	0.66	20

NR - no response; R - response

The use of machine learning methods, however, allowed us to build a model that, with a sufficiently high accuracy, can predict the treatment outcome based on quantitative and qualitative data on the composition of fecal microbiota.

Analysis of the most influential predictors of the model reveals taxa for which correlations with the success of therapy are already known (*Ruminococacceae*, *Carnobacteriaceae, Bacterioaceae*, etc.).

However, the list of predictors does not reproduce when using a different set of methods.

Conclusion

We were unable to identify strong correlations or significant differences between the taxonomic composition of the fecal microbiota of patients and the effectiveness of treatment control points. We believe that the most likely reasons for this are the heterogeneity of patient diagnoses and the use of therapy that blocks different types of receptors.