Robust Machine Learning (ML) approach for Screening Microbiome Ecosystem Therapies (MET) Drug Candidates in combination with Immune Checkpoint Inhibitors

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TAKEAWAYS

1. MaaT Pharma gutPrint® developed an **AI Framework** and trained models to grade samples as **"Responder-like"** according to the baseline stool metagenomics of **ICI** treated cancer patients.
2. Improved results were obtained with:
   - **more patient data,** and we assume there is still room for improvement with an increased number of observations
   - **combining** indications.
3. The application of the best performing model (mean LODO AUC = 0.65) to MaaT Pharma's healthy donor cohort or pooled samples (mixes of stools from 4 to 8 healthy donors) classified a large majority of those as "Responder-like" microbiota (especially pooled samples)

INTRODUCTION

- MaaT Pharma, a clinical-stage biotech pioneer in the development of Microbiome Ecosystem Therapies (MET) in oncology, is defining its strategy to customize products in immuno-oncology related indications.
- Correlation between gut microbiome composition with ICI efficacy in cancer therapy was observed (*e.g.* studies listed in table 1), and **FMT proofs of concept** were performed [1, 2].
- Baseline stool metagenomic datasets from **ICI** treated cancer patients were gathered along with their response as defined by Response Evaluation Criteria in Solid Tumors (RECIST) and provided by each source study.
- **Interstudy inconsistencies** (figure 1 and 2) were observed in microbiome signature findings [3]. It is thus critical to tackle this heterogeneity during the development of a **reliable microbiota-based drug candidate screening algorithm**.

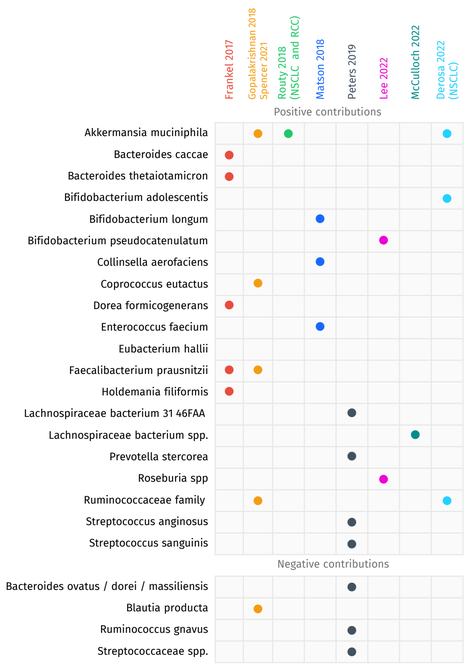

Figure 1: Comparison of species identified in the literature. Only species mentioned in the abstracts of the related papers were gathered.

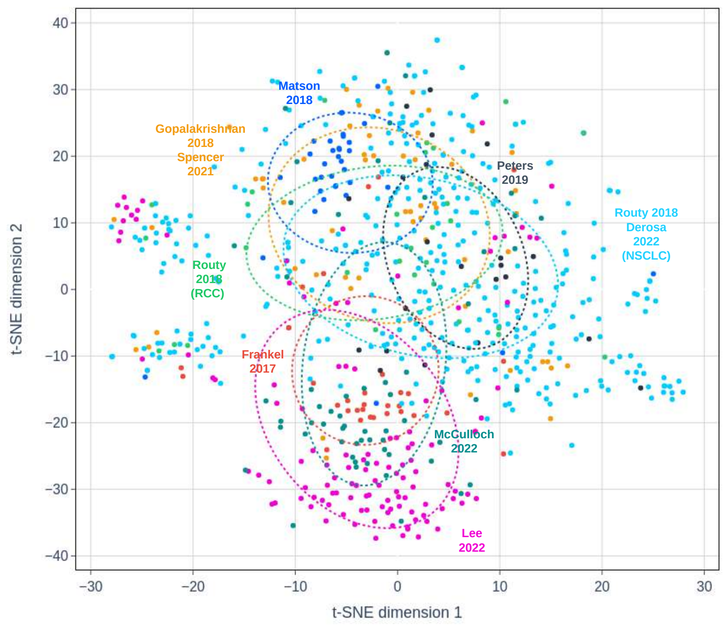

Figure 2: t-SNE of baseline samples from 10 public cohort based on genus, KEGG Pathways and alpha diversity.
Preprocessing steps: relative abundance normalization and standard scaling.

METHODS

As part of **gutPrint®**, MaaT Pharma's bioinformatics and AI platform, a robust machine learning (ML) approach for screening microbiome samples and predict potential responder status to ICIs was developed. The models were trained from microbiota Whole Metagenome Sequencing (WMS) datasets processed by **gutPrint® MgRunner** to provide Functional (KEGG) and Taxonomic (species and genera) information. We relied on the Area Under the ROC Curve (AUC) and precision to assess cross-cohort predictions performances computed though a **Leave-One-Dataset-Out** (LODO) validation strategy.

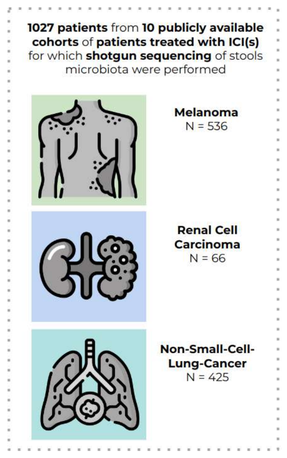

Figure 3: Summary of baseline Whole Metagenome Sequencing (WMS) public datasets

Table 1: Description of baseline WMS public datasets

	Name	Publication reference	Indication	ICI Drug(s)	# patients	Proportion of Responders
2017	**Frankel 2017**	Frankel et al., Neoplasia (2017) 19, 848–855.		Anti-PD1 / Anti-CTLA4	39	0.62
2018	**Gopalakrishnan 2018**	Gopalakrishnan V. et al., Science. 2018;359(6371):97-103.		Anti-PD1	25	0.56
	Routy 2018 (RCC)	Routy B. et al., Science. 2018;359(6371):91-97.		Anti-PD1	66	0.68
	Routy 2018 (NSCLC)			Anti-PD1	87	0.47
	Matson 2018	Matson V. et al., Science. 2018;359(6371):104-108.		Anti-PD1	41	0.38
2019	**Peters 2019**	Peters B.A. et al., Genome Med. 2019;11(1):61.		Anti-PD1 (58,3%) Anti-CTLA4 (8,3%) Anti-PD1/Anti-CTLA4 (33,3%)	27	0.56
2021	**Spencer 2021**	Spencer C. et al., Science. 2021 December 24; 374(6575): 1632–1640.		Anti-PD1 (76,6%) Other ICI (17.2%) Other-systemic (6.2%)	145	0.66
2022	**Lee 2022**	Lee, K. et al., Nature Medicine, February 28, 2022, 1–10.		Anti-PD1 (61%) Anti-CTLA4 (32%) Anti-PD1/Anti-CTLA4 (7%)	165	0.57
	McCulloch 2022	McCulloch, J.A. et al., Nature Medicine, February 28, 2022, 1–12.		Anti-PD1	94	0.54
	Derosa 2022 (NSCLC)	Derosa, L et al., Nature Medicine, February 28: 315–24.		97% Anti-PD1 3% Anti-PD1 + chemo	338	0.52

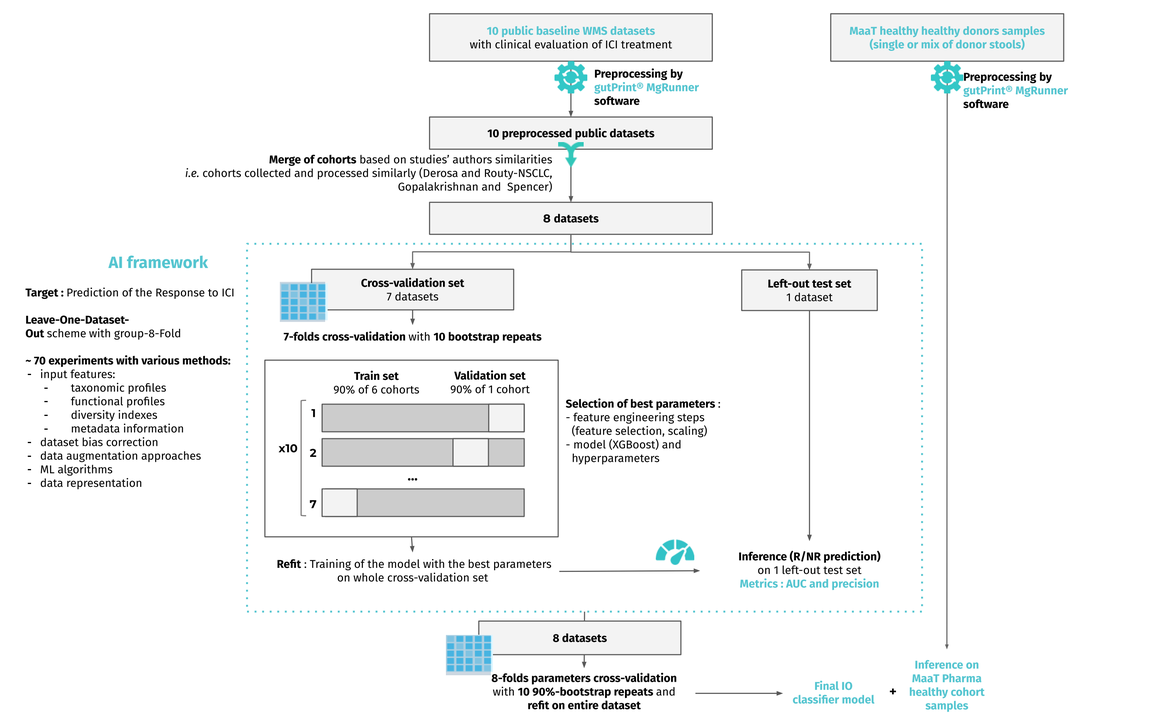

Figure 4: Data processing and AI framework methodologies. All samples were processed by gutPrint® MgRunner to get taxonomic and functional features. The processed datasets were distributed sequentially in the cross-validation set used to select parameters (and then used as the training set), and in the left-out test dataset. This strategy was repeated for each dataset as left-out, and for the 70 designed experiments.

RESULTS

	Routy 2018 - Derosa 2022 (NSCLC)	Routy 2018 (RCC)	Frankel 2017	Gopala-krishnan 2018 - Spencer 2021	Lee 2022	McCulloch 2022	Matson 2018	Peters 2019	ALL MELANOMA	ALL DATASETS
Indication										NSCLC RCC Melanoma
ICI	Anti-PD1	Anti-PD1	Anti-PD1 / Anti-CTLA4	Anti-PD1	Anti-PD1 / Anti-CTLA4	Anti-PD1	Anti-PD1	Anti-PD1 / Anti-CTLA4	Anti-PD1 / Anti-CTLA4	Anti-PD1 / Anti-CTLA4
# patients	381	29	32	57	121	58	36	22	326	736
AUC	0.57	0.71	0.66	0.65	0.66	0.52	0.66	0.74	Mean: 0.65	Mean: 0.65
Precision	0.56	0.81	0.68	0.63	0.66	0.55	0.57	0.67	Mean: 0.63	Mean: 0.65

Table 2 : Best model's performance by dataset and indication

- The best performing experiment provided models based on genera, KEGG Pathways and alpha diversity features as inputs treated with the XGBoost algorithm. AUCs range from 0.52 to 0.74 depending on the left-out cohort (average **AUC** = 0.65) and a precision that ranges between 0.55 and 0.81 (average **precision** = 0.65).
- Those results **outperform** melanoma-centered study with a comparable assessment method [4] for common cohorts (where Matson AUC = 0.61, Gopalakrishnan AUC = 0.56, Frankel AUC = 0.63, and mean Lee datasets AUC = 0.60).

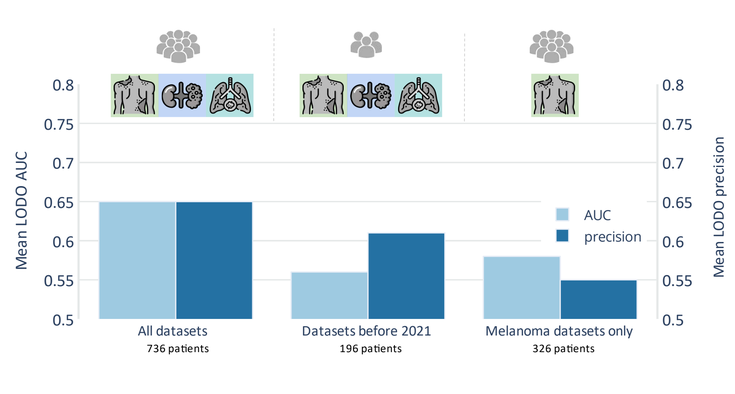

Figure 5 : Best model's results on multiple dataset combinations

Despite the diverse data sources and indications:

- **more datasets** (since 2021) improved the classification performances
- **the multi-indication** approach surpassed the mono-indication (melanoma) training approach for predictions related to melanoma patients.

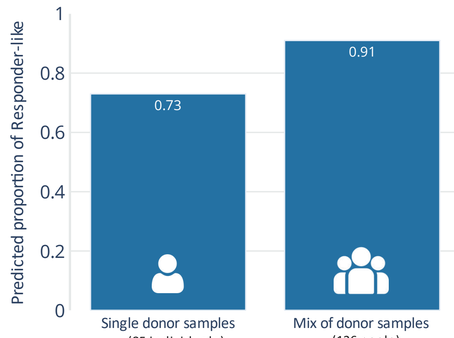

Figure 6 : Predictions for healthy donor stool samples

- Considering the scoring of MaaT pharma healthy cohort samples, 73% of mono-donors and 91% of pools were classified as **"Responder-like"**.

CONCLUSIONS

This study:

- presents a robust methodology to enhance the performances of a multi-cohort-based Machine Learning approach
- shows good to very good predicting performances (0.74 > AUC > 0.65) except for 1 NSCLC (AUC = 0.57) and 1 melanoma (AUC = 0.52) cohort
- highlights the significance of dataset size in ICI microbiota models
- emphasizes the benefice of combining indications to leverage model's performance

Conditioned to the best performing model, the stools (single or pooled) from MaaT Pharma healthy donors harbor a considerable ratio (91%) of "ICI Responder-like", significantly higher than the mono-donor stools (73%) suggesting that pooled ecosystems from healthy donors could better convert ICI-non responders into responders.

Altogether, this work shows evidence of an AI strategy potential to screen and select microbiota-based drug development candidates with the objective to treat solid cancer patients in combination with immunotherapy.

References

[1] D. Davar et al., "Fecal microbiota transplant overcomes resistance to anti–PD-1 therapy in melanoma patients," Science, vol. 371, no. 6529, pp. 595–602, Feb. 2021, doi:10.1126/science.abf3363.

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[3] S. Wojciechowski et al., "Machine learning on the road to unlocking microbiota's potential for boosting immune checkpoint therapy," International Journal of Medical Microbiology, vol. 312, no. 7, p. 151560, Oct. 2022, doi:10.1016/j.ijmm.2022.151560.

[4] K. A. Lee et al., "Cross-cohort gut microbiome associations with immune checkpoint inhibitor response in advanced melanoma," Nat Med, vol. 28, no. 3, pp. 535–544, 2022, doi:10.1038/s41591-022-01695-5.