Predicting immunotherapy response of advanced bladder cancer
a meta-analysis of six independent cohorts

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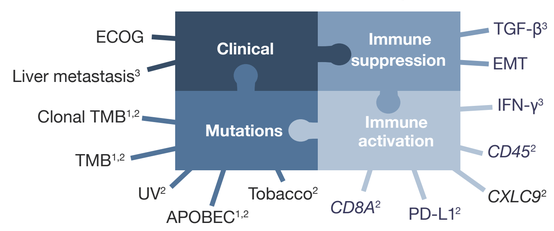
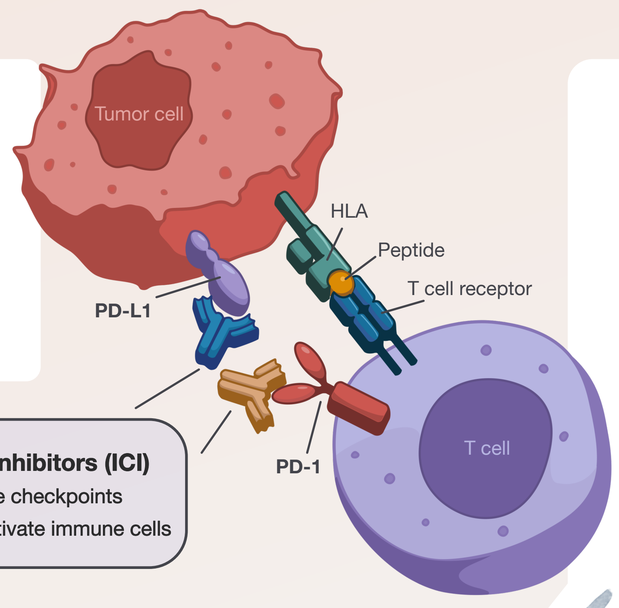
INTRODUCTION

Immune checkpoint inhibitors (ICI) have shown remarkable potential in inducing long-term complete remissions in advanced bladder cancer patients.

However, their effectiveness varies widely among individuals, with **less than 20% of bladder cancer (BLCA) patients responding** to the treatment. This emphasizes the urgent need to understand the underlying factors to better predict clinical response ICI therapy.

Described biomarkers for ICI response in BLCA





Immune checkpoint inhibitors (ICI)
Drugs blocking immune checkpoints that cancer cells use to inactivate immune cells

OUR APPROACH

What did we do?

We integrated multi-omics data from six independent cohorts (N=707) of advanced bladder cancer patients treated with **anti-PD-1/PD-L1** to develop and validate machine learning models for **predicting immunotherapy response**.

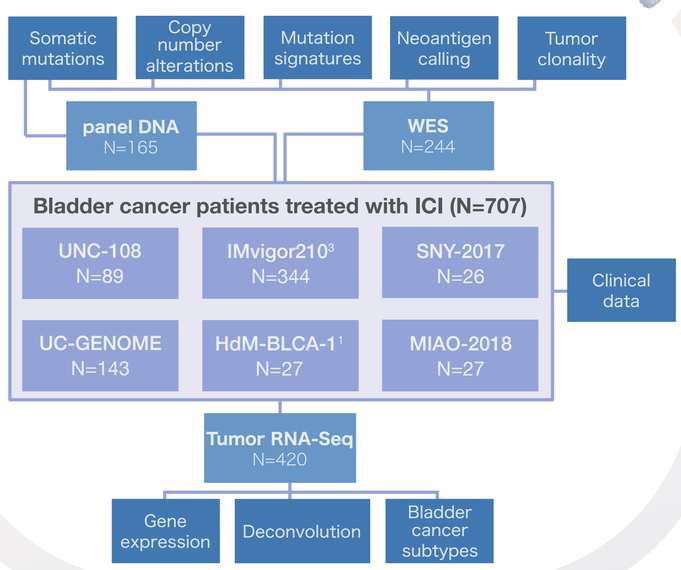
Why is this important?

Known biomarkers are insufficient in separating responding from non-responding patients. Better predictors are needed to allow for a more personalized treatment of metastatic bladder cancer.

What makes our work stand out?

We built the biggest **bladder cancer specific cohort**. Previous pan-cancer studies have failed to build predictive models that were robust enough in independent cohorts.

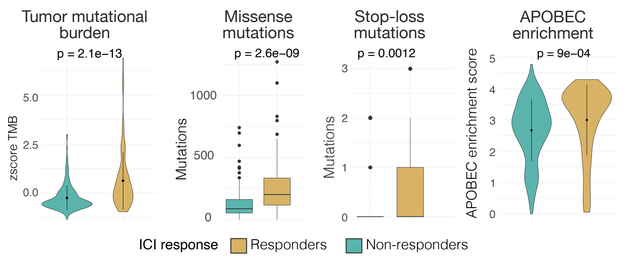
THE DATA



OMICS ANALYSIS

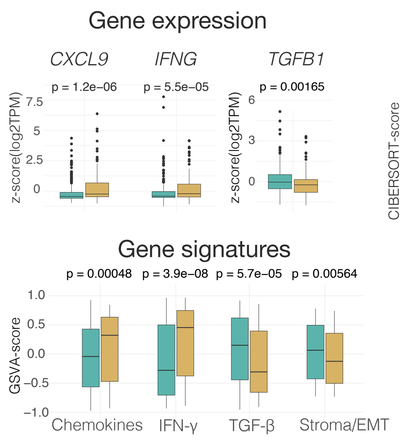
Responders have more somatic mutations

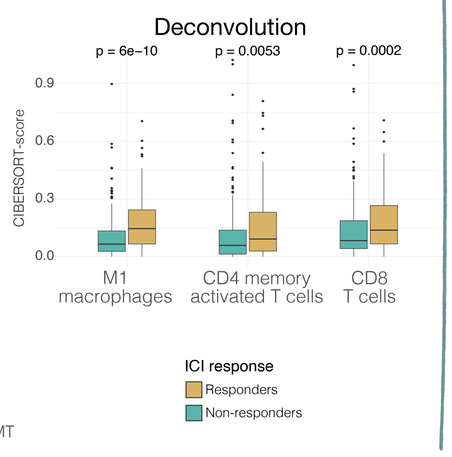
Missense and stop-loss mutations are significantly associated with the response to ICI. Responders are further **enriched in APOBEC-induced mutations** and mutations in the gene *ARHGEF12*.

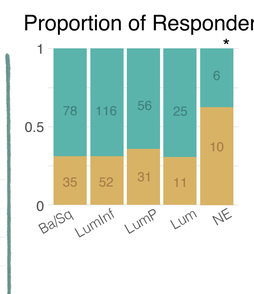


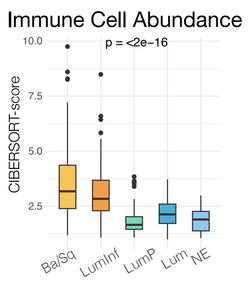
Immune activation markers associated with response

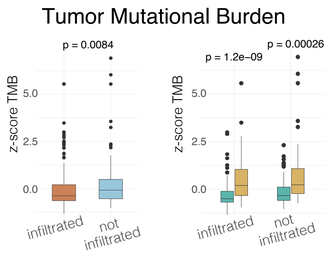
Responders show an enrichment of **pro-inflammatory markers** and have higher **infiltration of immune cells** such as T cells or M1 macrophages, while non-responders show higher values of markers for immune suppression such as TGF-β.

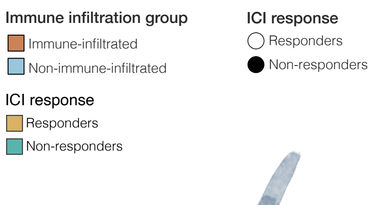






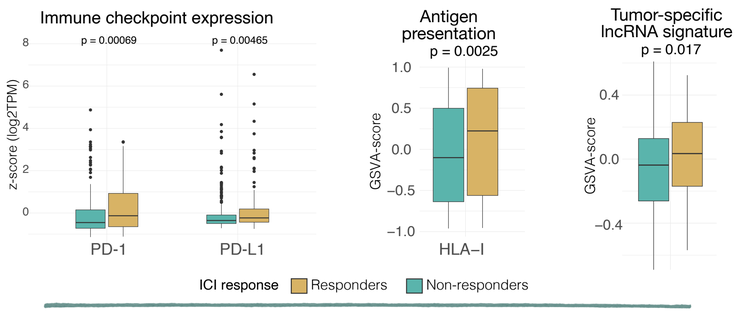






Antigen presentation and Immune checkpoint expression

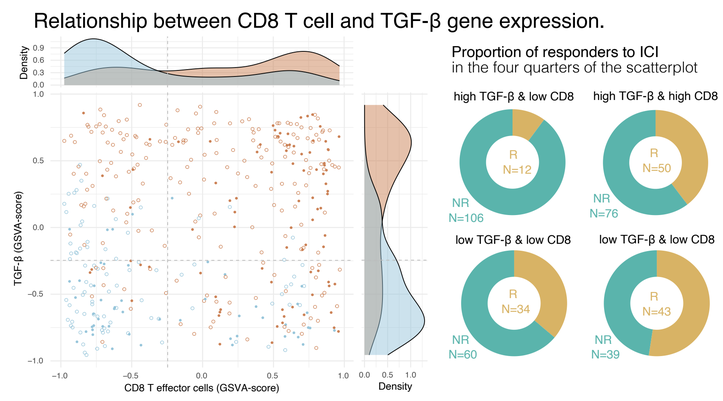
Responders further have high levels of the **immune checkpoint molecules PD-1 and PD-L1** as well as the antigen presenting molecules of the HLA-I group. We further found responders to have a higher expression of tumor-specific long non-coding RNA (lncRNA).



The determinants of response depend on the molecular subtype

Among the five molecular BLCA subtypes, only **neuronal shows a significantly better treatment response** (p value = 0.014). Based on their immune cell infiltration, we analyzed the **immune-infiltrated** (luminal-infiltrated and basal-squamous) and **non-immune-infiltrated** (luminal-papillary, luminal and neuronal) subtypes separately. Immune-infiltrated samples tend to have high CD8+ T cell abundance, and in many cases **also high levels of the TGF-β** signature.

Relationship between CD8 T cell and TGF-β gene expression.



PREDICTION MODELS

PRE-PROCESS
- Remove NAs
- Encoding & scaling

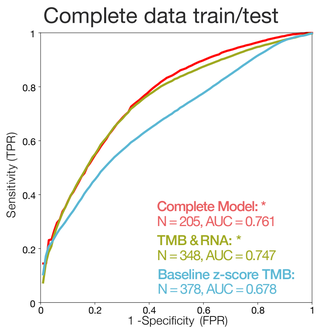
TRAIN & TEST
- Hyperparameter search 15-fold cross-validation for 1000 seeds
- 70/30 Train/test split 1000 seeds
- Bootstrap .632+
- AUCs averaged 1000 seeds

VALIDATION
- External cohort (N=123)

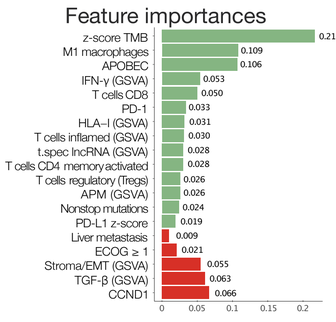
Random Forest Models predicting ICI response

Our complete model performs better than the TMB-only (**AUC=0.761** *vs* 0.678). Variables with a **clear association with response were TMB, M1 macrophages, APOBEC-enrichment, IFN-γ, CD8+ T cells, PD1 and HLA-I**.

Complete data train/test

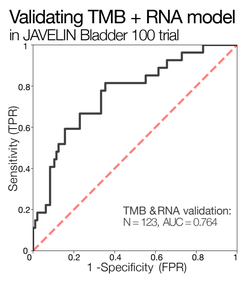


Feature importances



** Complete model: all feautures shown in barplot; TMB&RNA: TMB and RNA-derived feautures. ROC curves are averages of 1000 runs.*

Validating TMB + RNA model in JAVELIN Bladder 100 trial

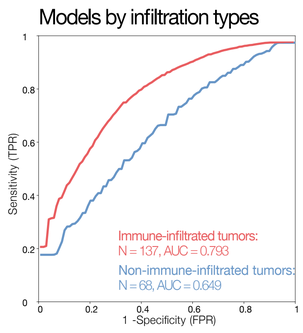


The model achieved an **AUC of 0.764 in the validation run** using an independent BLCA cohort. Furthermore, removing one dataset at a time resulted in models with similar accuracies.

Models by immune-infiltration group

Maximum accuracy was achieved for the immune-infiltrated subgroup while the non-immune-infiltrated model showed low accuracy (AUC=0.793 *vs 0.649*).

Models by infiltration types



** ROC curves are averages of 1000 runs using all variables (complete model).*

In subtype-specific analyses, we found other markers associated to ICI response in non-immune-infiltrated subtypes (PD-L1, antigen presentation machinery, regulatory T cells, ...).

CONCLUSIONS

1. **Tumor mutational burden** (TMB) is the most strongest predictor for ICI response in BLCA. **Pro-inflammatory markers** are non-additive to TMB.

2. We **discovered novel biomarker** associated to ICI response: stop-loss mutations, a long non-coding RNA signature and the inactivation of *ARHGEGEF12*.

3. We build **robust prediction models for ICI response**, incorporating multi-omics data from six cohorts, reaching high accuracy, especially in the immune-infiltrated subtypes.

4. **High immune-infiltrated subtypes do not respond better**. This paradox is likely attributed to lower TMB and immune suppressive mechanisms in these patients.

5. In the non-immune-infiltrated group, we identified **subtype-specific markers** affecting response to ICI. The neuronal subtype, though rare, shows strongest response to immunotherapy.

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[1]Boll LM & Perera-Bel J et al. 2023.The impact of mutational clonality in predicting the response to immune checkpoint inhibitors in advanced urothelial cancer. Scientific Reports. 13(1):15287.
[2]Litchfield K et al. 2021. Meta-analysis of tumor- and T cell-intrinsic mechanisms of sensitization to checkpoint inhibition. Cell. 184(3):596-614.
[3]Mariathasan S et al. 2018. TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. Nature. 554(7693):544-548.