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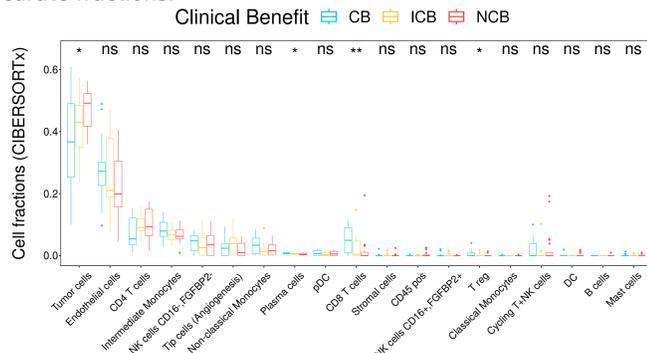
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## Summary

Immune checkpoint blockade (ICB) therapies are now an important tool in the arsenal for the treatment of advanced cancer extending progression-free survival and overall survival. However, only a subset of cancer patients responds to ICB therapies causing an urgent need for novel approaches to better select patients who may benefit from immunotherapy (1). Here, we used metastatic ccRCC samples obtained before ICB treatment and performed cell deconvolution analysis to investigate novel biomarkers of ICB treatment response.

## Tumor composition

We performed cell deconvolution of transcriptomics data (2) to obtain cell type proportions within tumor samples. A single-cell matrix refined by specific markers of cells is used to get more accurate fractions.

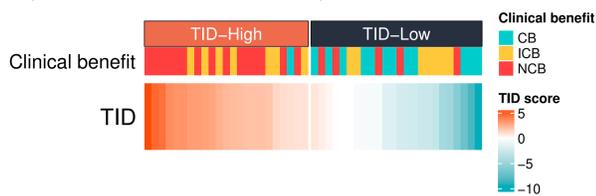


Tumor cells, Endothelial and CD4 T cells are the more abundant cell types in the tumor microenvironment (TME).

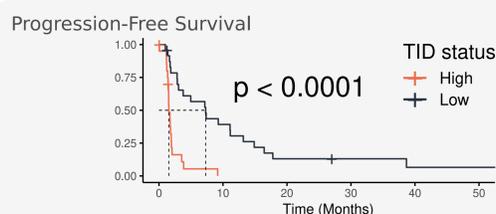
**Tumor, Plasma B, T-CD8 and T-reg cell fractions are different between responders (CB) and non-responders (NCB)**

## Tumor-Immunity Differential score

We leveraged cell proportion information by calculating a score to reflect the balance between the tumor-related and immunity-related parts within the tumor samples (TID).



Clinical response and Progression-Free Survival values were significantly better in the TID-Low group.



**The Tumor-Immunity Differential is strongly associated with immunotherapy treatment response**

## Methods

Cellular deconvolution uses transcriptomics data to predict cell fractions. We used these fractions to divide patients into subtypes associated with immunotherapy treatment outcomes.

### 1 Tumor Biopsy



Kidney tumor (ccRCC)

### 2 Gene quantification (RNA-Seq)

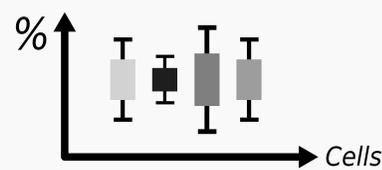
Genes (~20,000)

ABC	TP53	...	ZNF
56	5	...	14

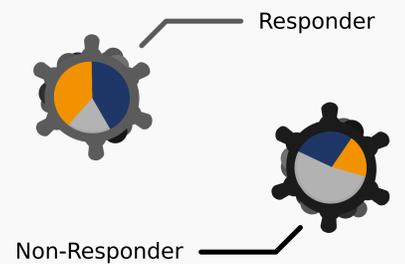
### 3 Cell Deconvolution (CIBERSORTx)

Cell fractions (21 types)

Tumor	Endothelial	...	T-CD8
60%	25%	...	10%

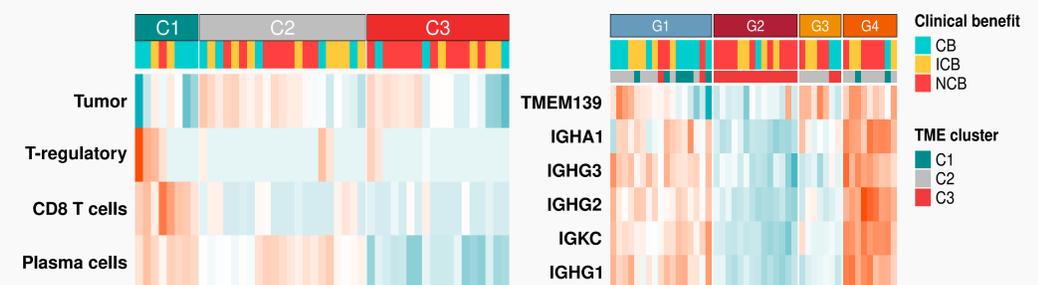


### 4 Treatment response outcome



## Sample clustering

To divide ccRCC samples into different TME profiles, we carried out a clustering based on cell fractions values (C1-C2-C3 clusters). Then, we found 6 genes differentially expressed between these groups including 5 genes related to immunoglobulins.



We observed that the C3-G2 subtypes enriched in bad responders harbored lower values of Plasma B cells and immunoglobulin genes.

**Tumor sample composition and immunoglobulin genes are associated with immunotherapy treatment response.**

## Conclusion

We found that several cell types in the TME of metastatic samples of ccRCC were highly valuable to highlight several TME subtypes (C1-C3) with significant differences in anti-PD-1 (Nivolumab) treatment response, cancer progression and overall survival. Moreover, the TID score was built to predict the treatment response outcome of Nivolumab-treated patients. In addition, differentially expressed genes between C1-C3 TME subtypes revealed 5 genes as single markers of the C3 TME cluster harboring the worst ICB response values. A validation on additional samples of kidney cancer (e.g. by qPCR or RNA-Seq) are scheduled.

## References

- (1) Motzer et al., 2018. Nivolumab plus Ipilimumab versus Sunitinib in Advanced Renal-Cell Carcinoma. The New England journal of medicine.
- (2) Braun et al., 2020. Interplay of somatic alterations and immune infiltration modulates response to PD-1 blockade in advanced clear cell renal cell carcinoma. Nature medicine.