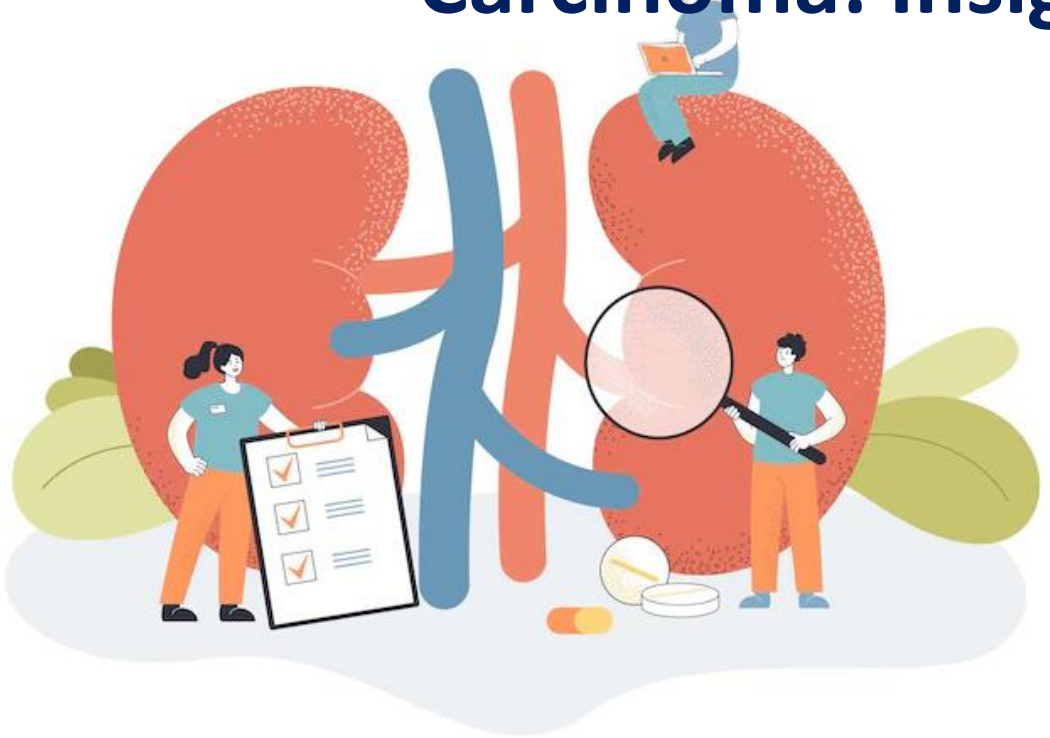
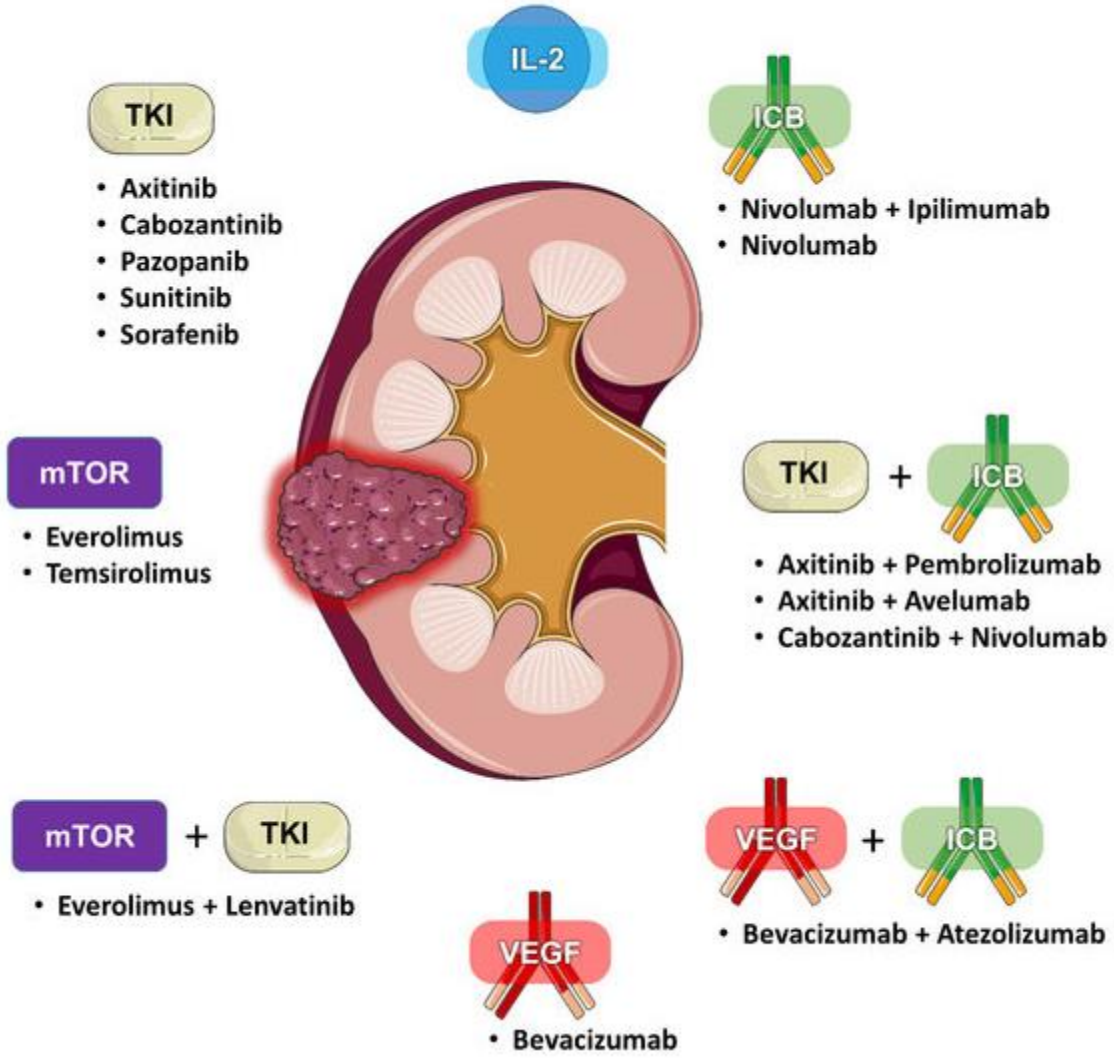
# Epigenetic Determinants of Response to Immune Checkpoint Inhibitors in Clear-Cell Renal Cell Carcinoma: Insights from the BIONIKK Trial



Xiaofan LU, PhD
Team of MALOUF – *Molecular and Translational Oncology,*
Department of Cancer and Functional Genomics, IGBMC

15/03/2025

# Background




## Clear cell renal cell carcinoma (ccRCC)

- Most common subtype of kidney cancer
- Significant treatment challenges when metastatic

Miron et al., 2020 J Pers Med

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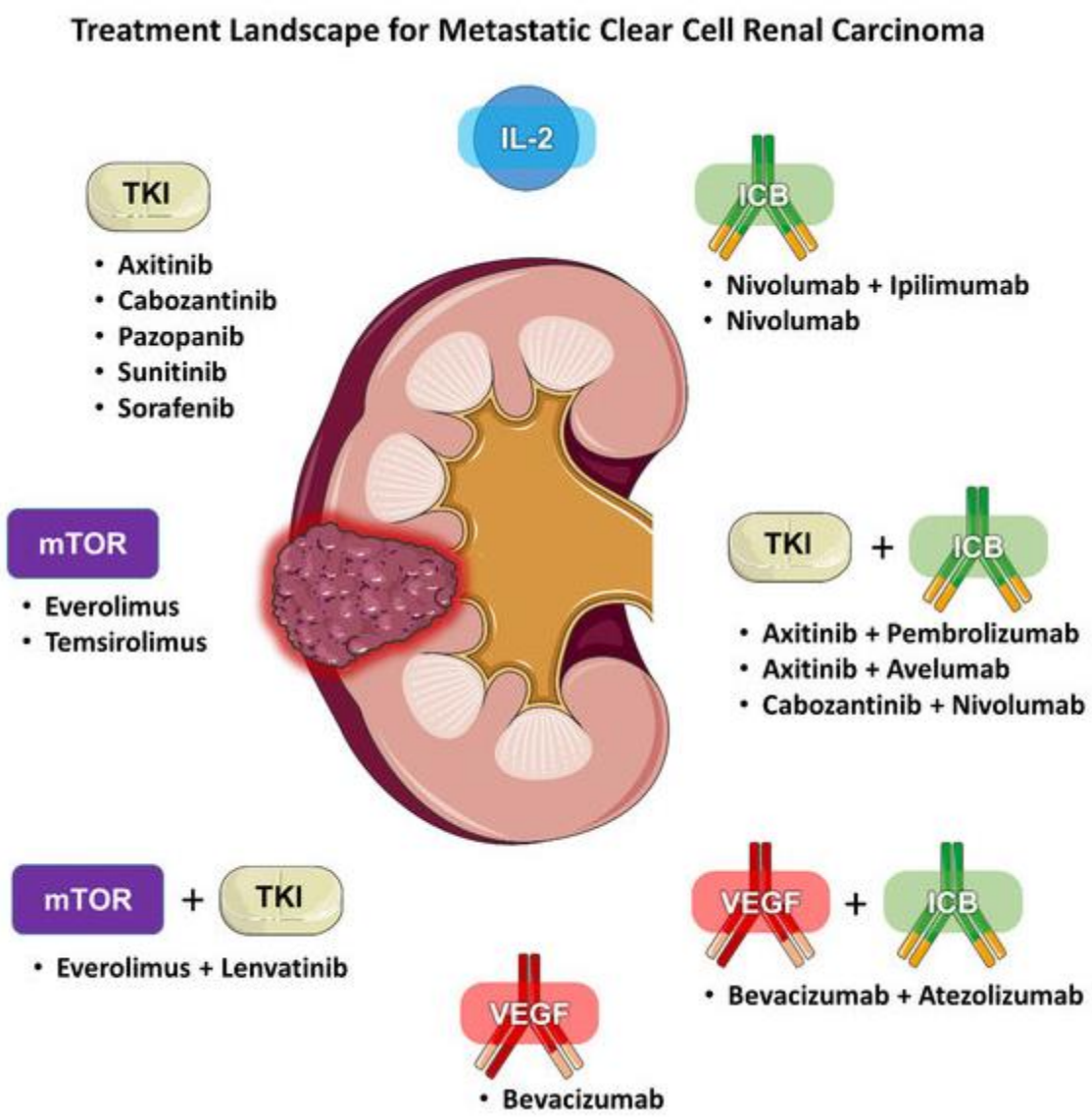


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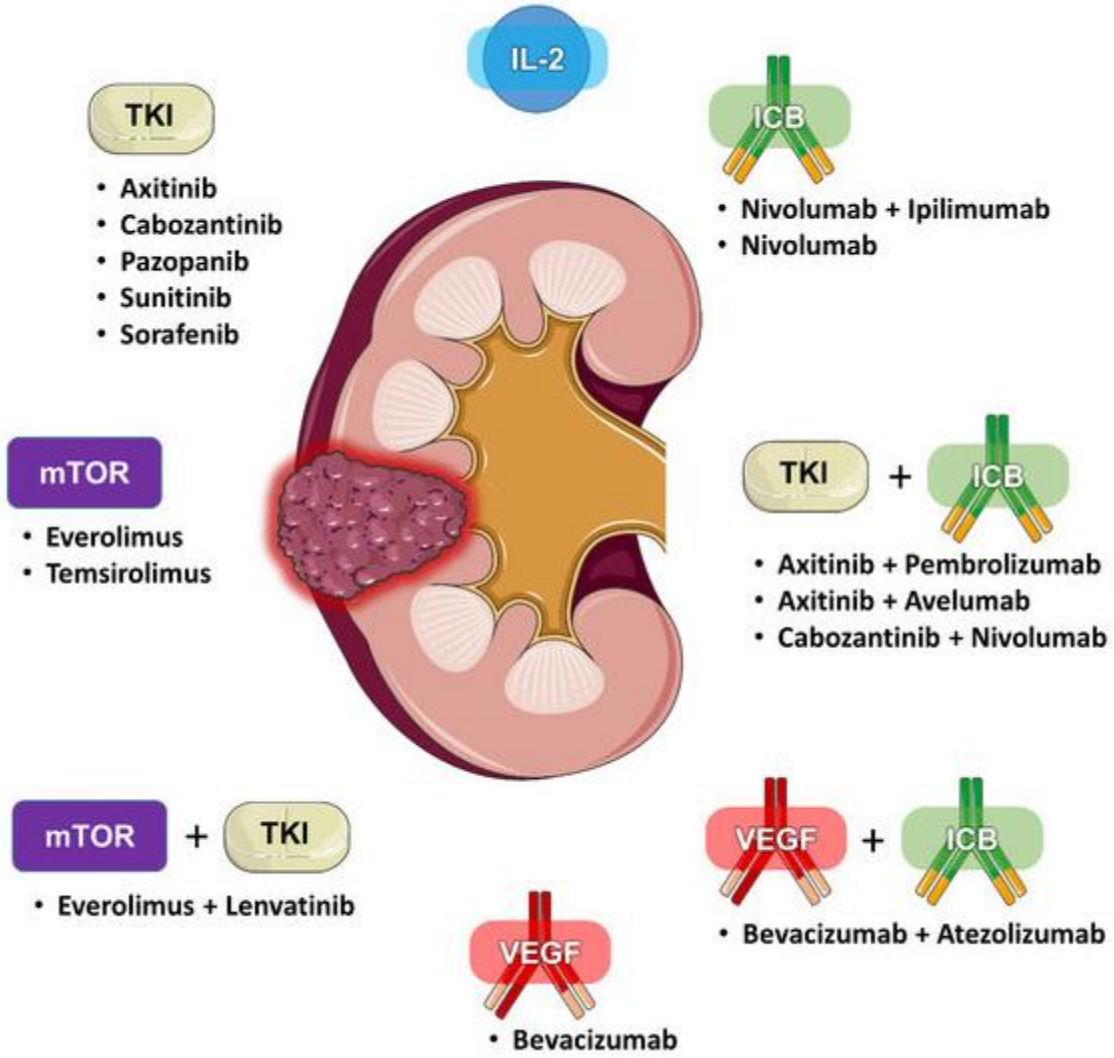


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- Transcriptomic signatures (IMmotion150, JR101)
- Limited correlation with clinical benefit for Ipi/Nivo

Miron et al., 2020 J Pers Med

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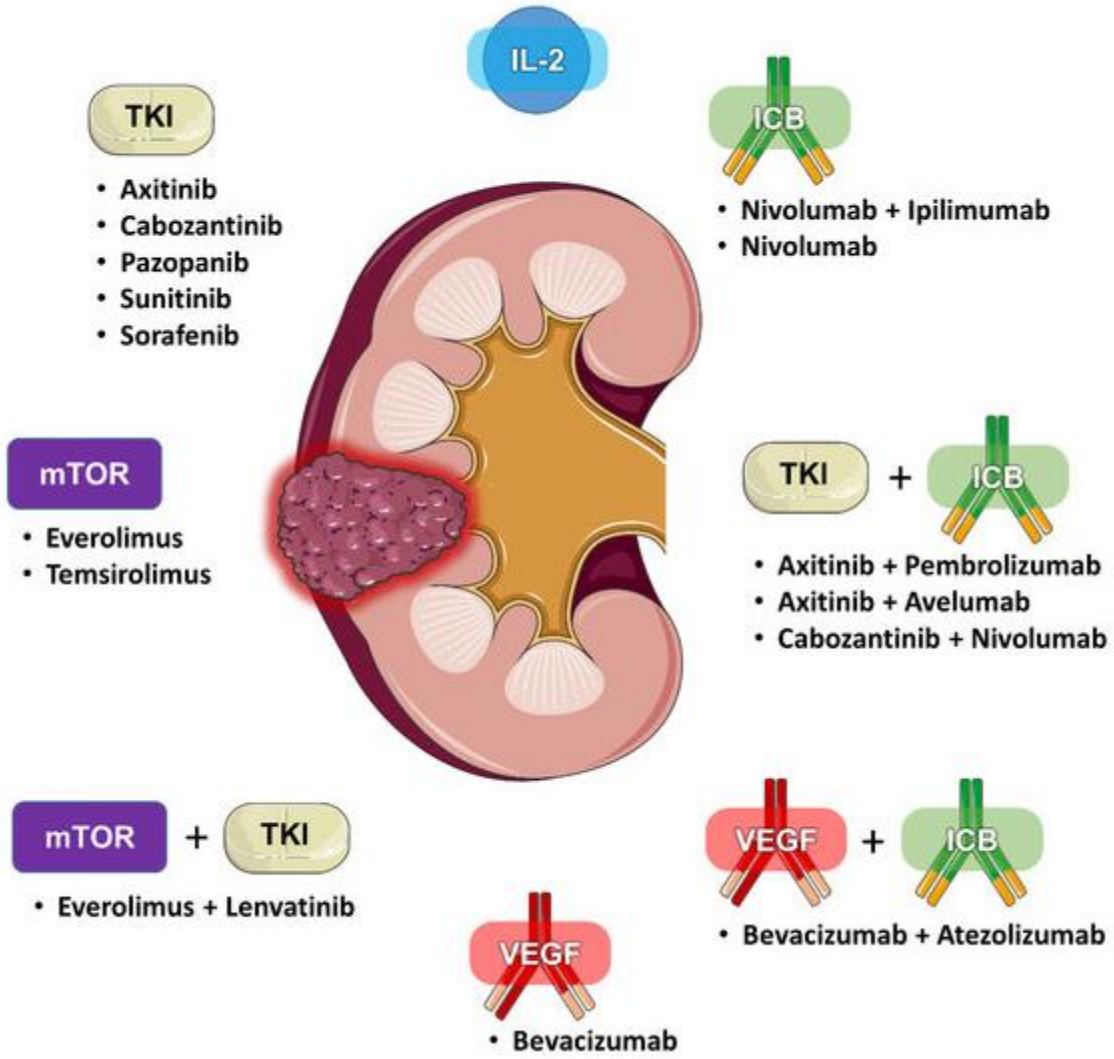


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## Research Gap:

**Reliable biomarkers for immunotherapy response remain an urgent unmet need**

Miron et al., 2020 J Pers Med

# Immunotherapy Biomarkers

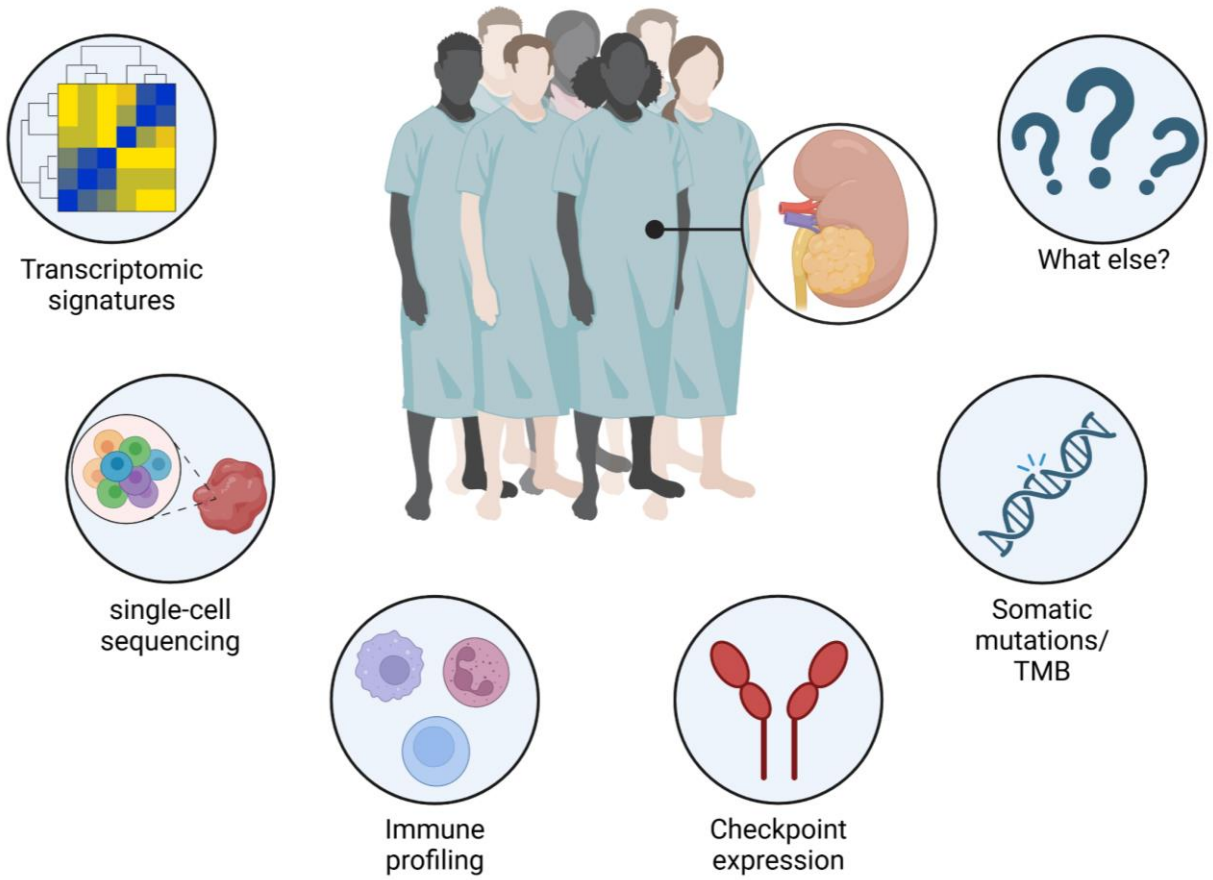
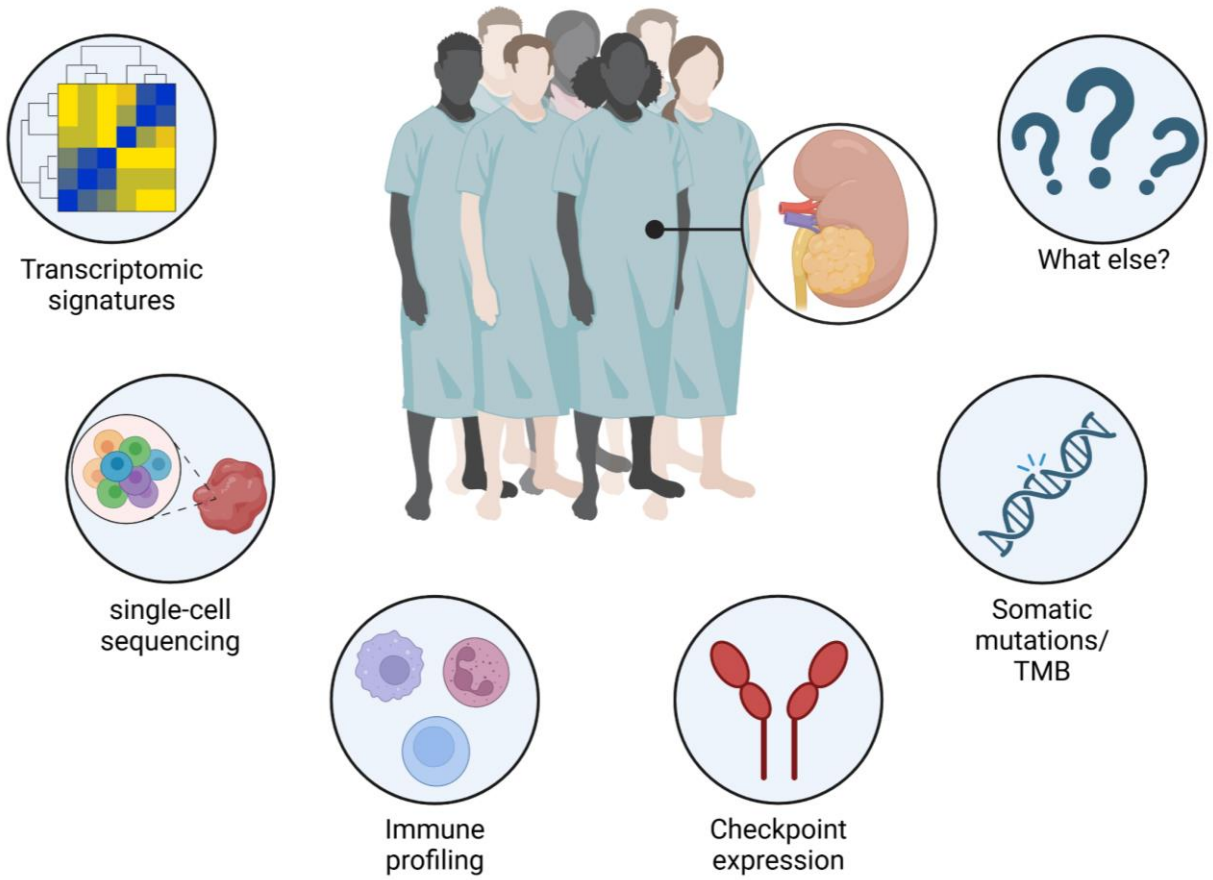



Image created by BioRender at biorender.com

# Immunotherapy Biomarkers

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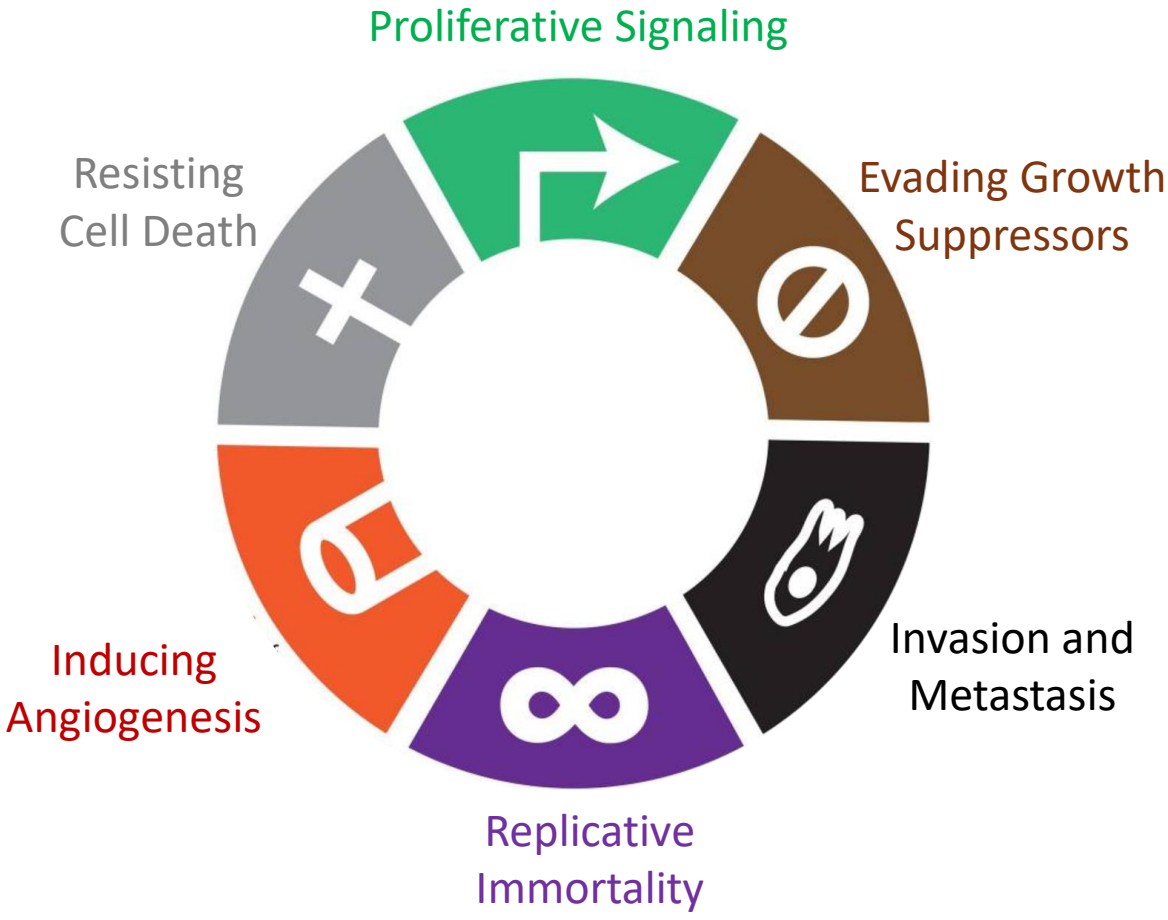
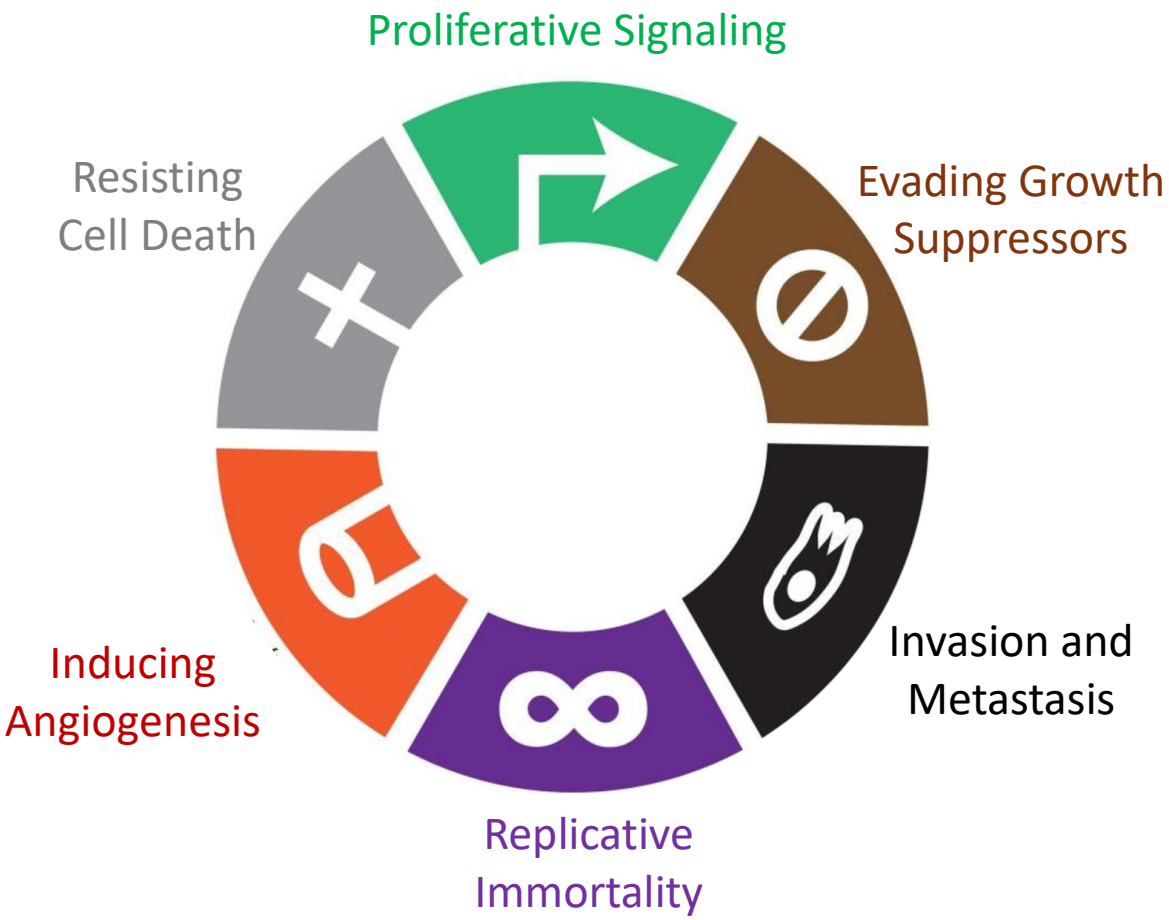



Adjusted from Flavahan et al., 2017 Science

# Cancer Epigenetics




Adjusted from Flavahan et al., 2017 Science

**Types of Epigenetic Changes:**

- DNA methylation (focus of our study)
  - Stable biomarker in clinical samples
  - Quantifiable and reproducible measurement
  - Established technology platforms
  - Strong biological relevance to gene regulation
- Histone modification
- Chromatin remodeling
- Non-coding RNA expression

# What are known?

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-019-12159-9 OPEN

## DNA methylation loss promotes immune evasion of tumours with high mutation and copy number load

Hyunchul Jung[1,10], Hong Sook Kim[2,10], Jeong Yeon Kim[1], Jong-Mu Sun[2], Jin Seok Ahn[2], Myung-Ju Ahn[2], Keunchil Park[2], Manel Esteller[3,4,5,6,7], Se-Hoon Lee[2,8] & Jung Kyoon Choi[1,9]

Mitotic cell division increases tumour mutation burden and copy number load, predictive markers of the clinical benefit of immunotherapy. Cell division correlates also with genomic demethylation involving methylation loss in late-replicating partial methylation domains. Here we find that immunomodulatory pathway genes are concentrated in these domains and transcriptionally repressed in demethylated tumours with CpG island promoter hypermethylation. Global methylation loss correlated with immune evasion signatures independently of mutation burden and aneuploidy. Methylome data of our cohort ($n = 60$) and a published cohort ($n = 81$) in lung cancer and a melanoma cohort ($n = 40$) consistently demonstrated that genomic methylation alterations counteract the contribution of high mutation burden and increase immunotherapeutic resistance. Higher predictive power was observed for methylation loss than mutation burden. We also found that genomic hypomethylation correlates with the immune escape signatures of aneuploid tumours. Hence, DNA methylation alterations implicate epigenetic modulation in precision immunotherapy.

Leukemia (2018) 32:2178–2188
https://doi.org/10.1038/s41375-018-0084-2

ARTICLE

Acute myeloid leukemia

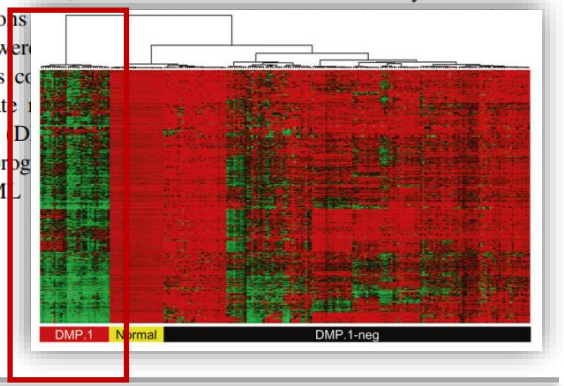
## Demethylator phenotypes in acute myeloid leukemia

Andrew D. Kelly[1] · Jozef Madzo[1] · Priyanka Madireddi[1] · Patricia Kropf[2] · Charly R. Good[1] · Jaroslav Jelinek[1] · Jean-Pierre J. Issa[1,2]

Received: 4 August 2017 / Revised: 29 January 2018 / Accepted: 6 February 2018 / Published online: 7 March 2018


**Abstract**

Acute myeloid leukemia (AML) often harbors mutations in epigenetic regulators, and also has frequent DNA hypermethylation, including the presence of CpG island methylator phenotypes (CIMPs). Although global hypomethylation is well known in cancer, the question of whether distinct demethylator phenotypes (DMPs) exist remains unanswered. Using Illumina 450k arrays for 194 patients from The Cancer Genome Atlas, we identified two distinct DMPs by hierarchical clustering: DMP.1 and DMP.2. DMP.1 cases harbored mutatio
Surprisingly, only 40% of patients with *DNMT3A* mutations we
transformation by this mutation. In contrast, DMP.2 AML was c
suggesting common methylation defects connect these disparate
functioning in immune response (DMP.1) and development
independent 450k data sets (236 additional cases), and found pro
cytogenetics. The existence of DMPs has implications for AM
stratification.




"demethylator"

- DNA hypomethylation correlates with immune evasion/response signatures in multiple cancer types

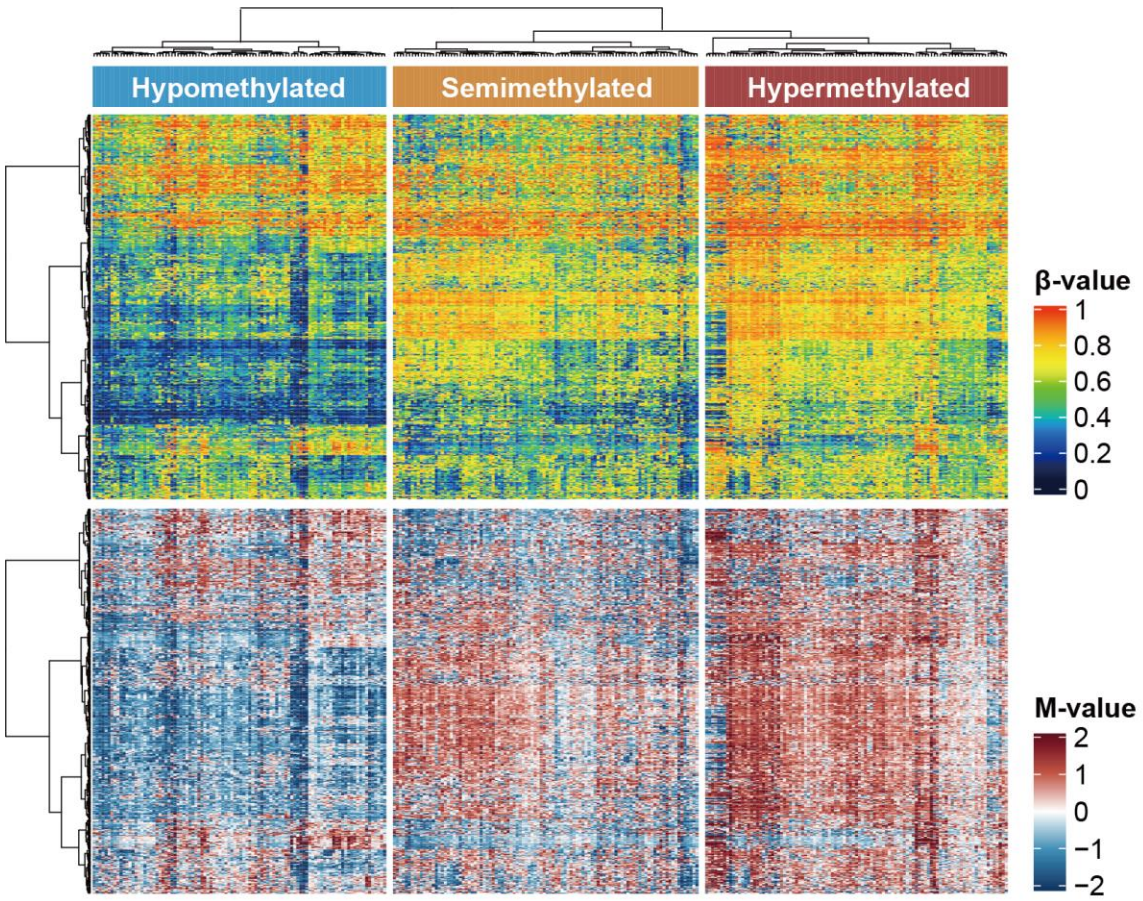
Jung et al., 2019 Nat Commun ;Kelly et al., 2018 Leukemia

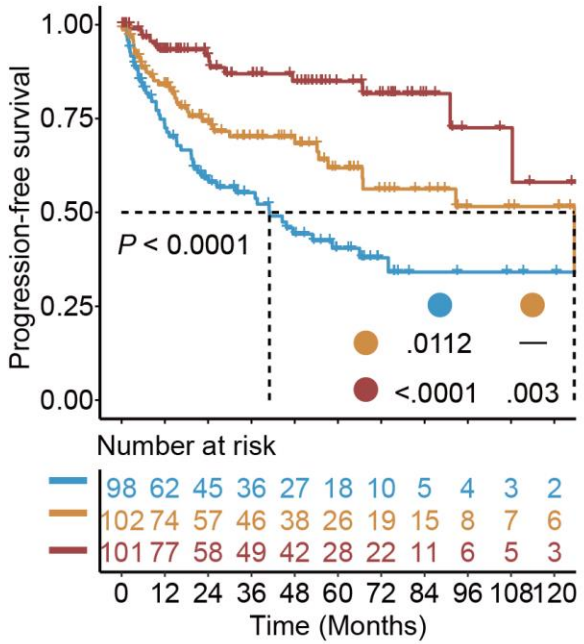
# What are unknown?

- **Epigenetic Heterogeneity in ccRCC**:
  — Do distinct DNA methylation profiles exist?

- **Biomarker Potential**:
  — Can methylation profiles predict immunotherapy efficacy in ccRCC?

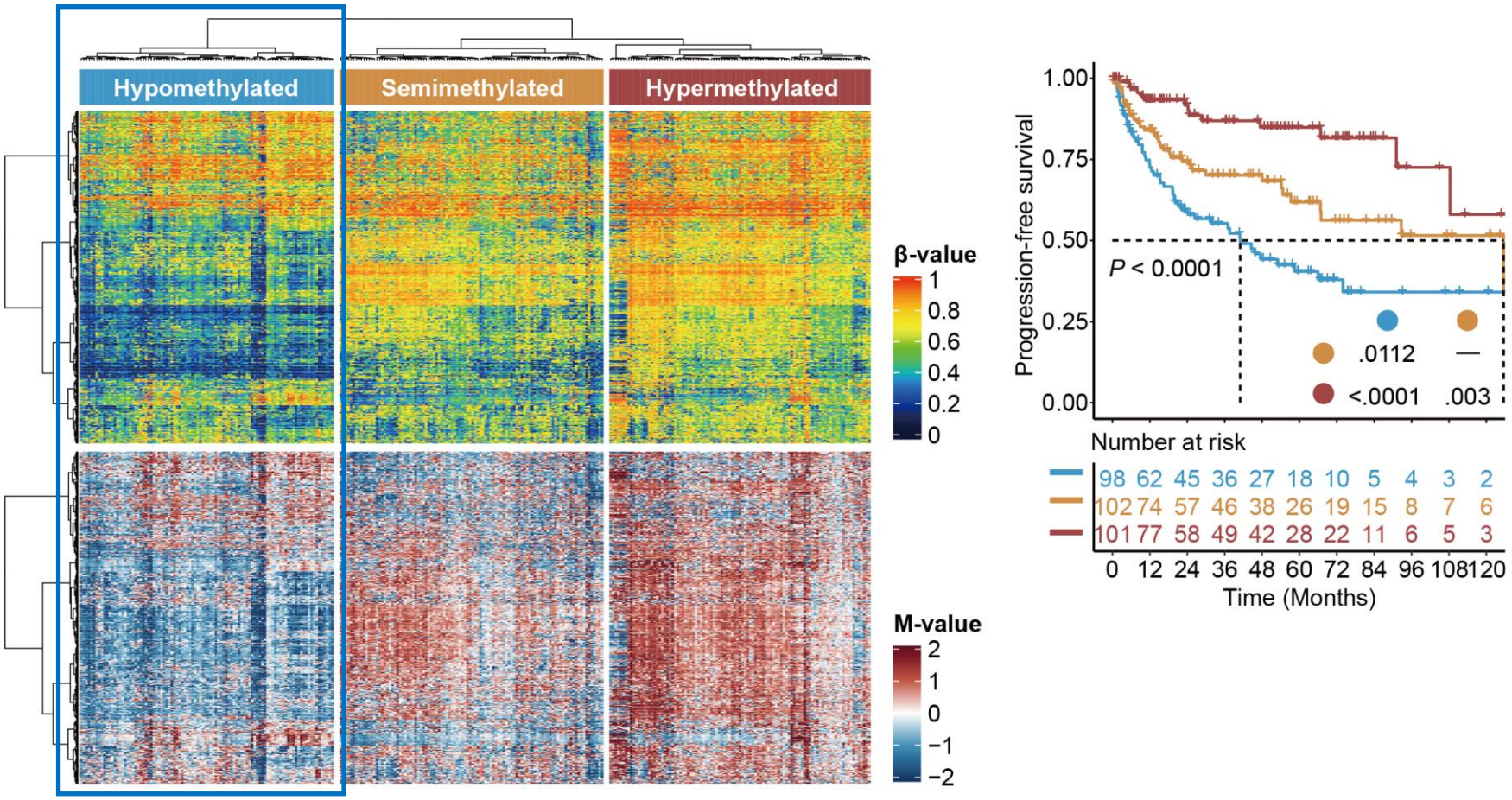
# Identification of demethylator phenotypes in ccRCC




- Global hypomethylation is associated with poor outcome in ccRCC

# Identification of demethylator phenotypes in ccRCC







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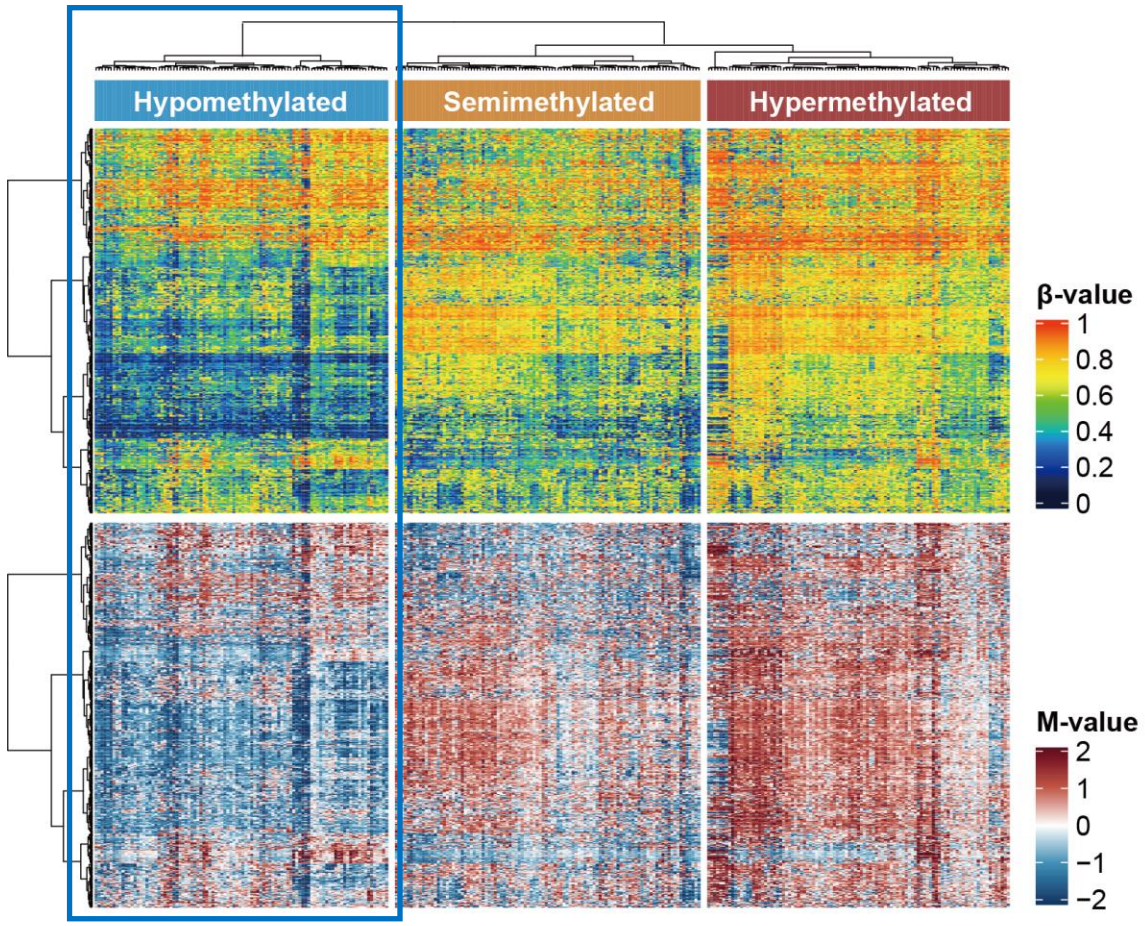
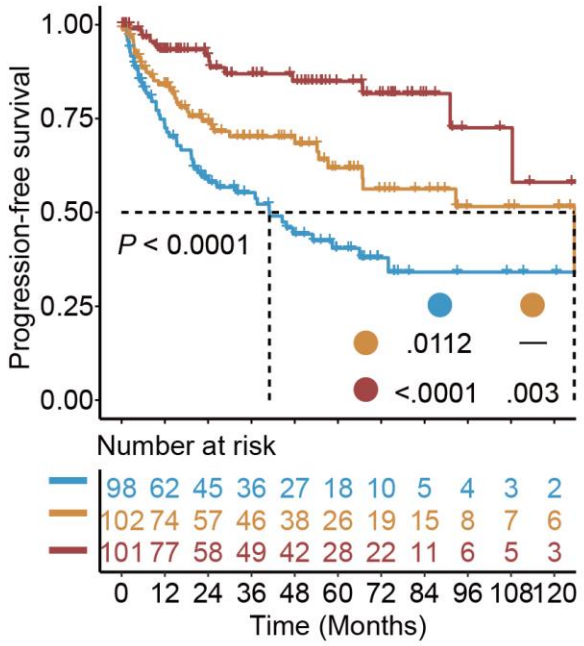
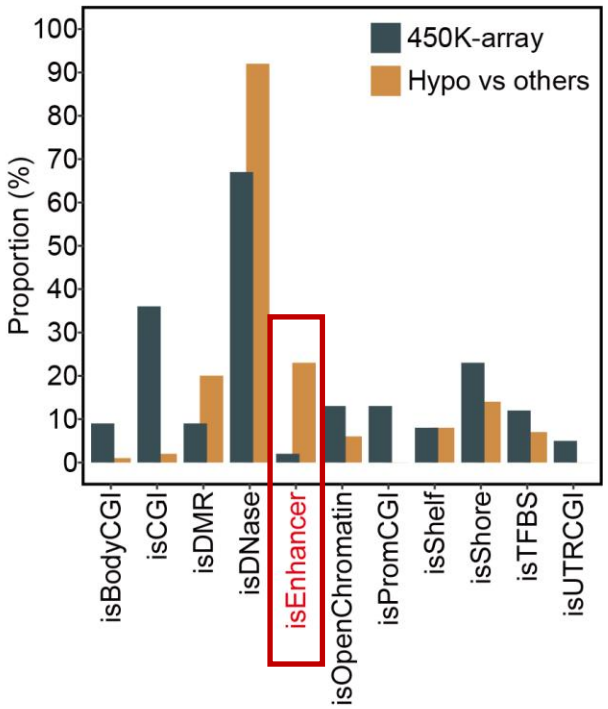
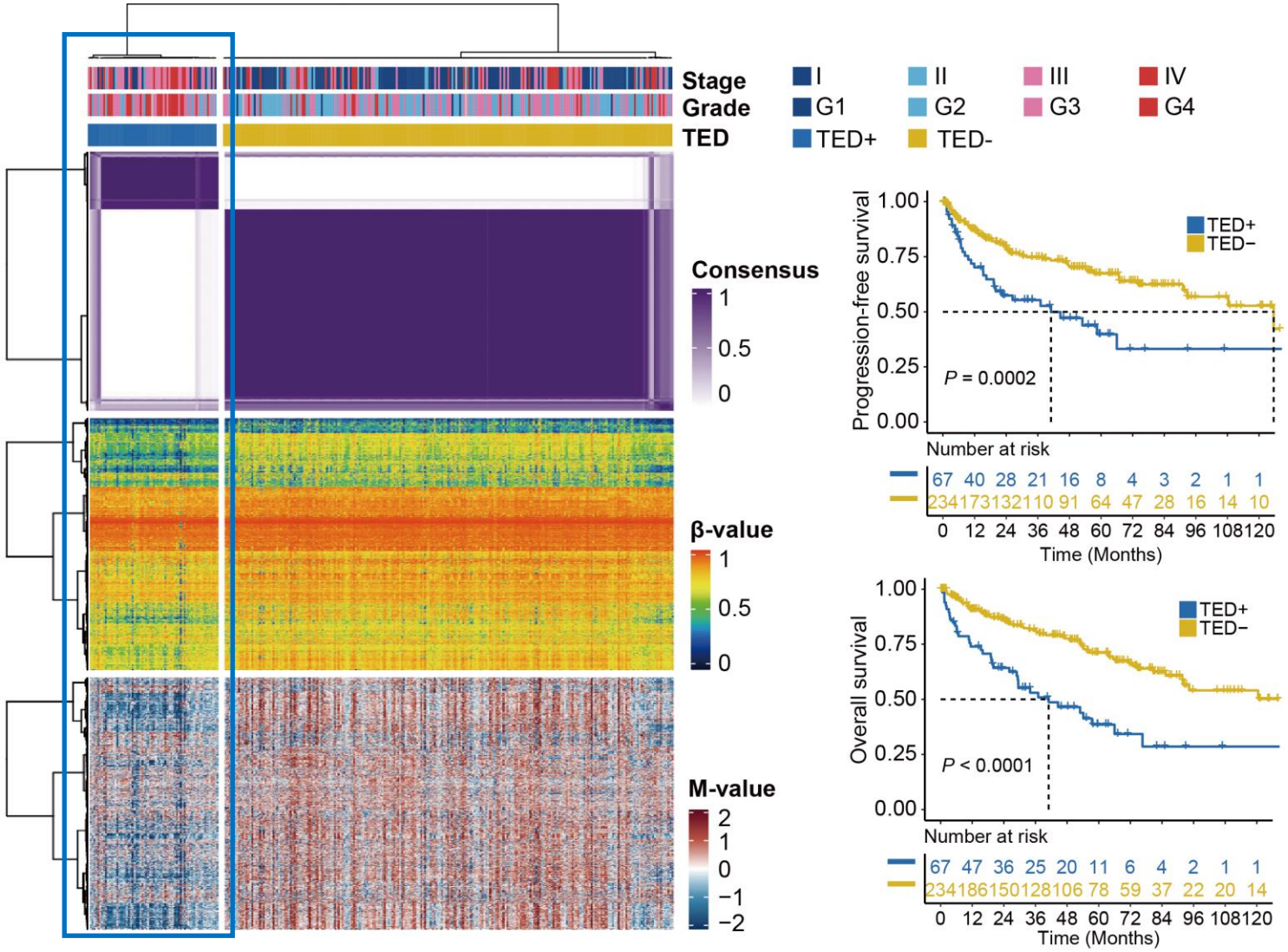


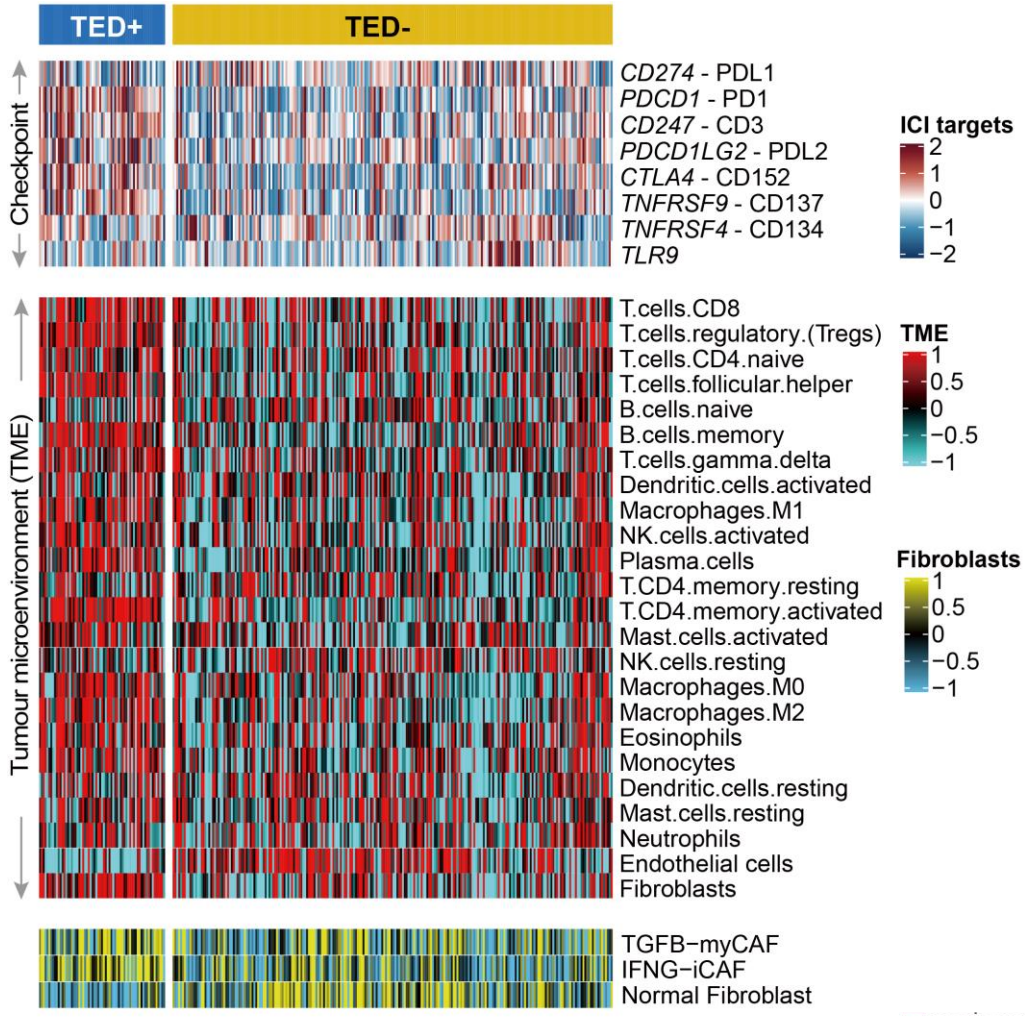




- Global ***hypomethylation*** is associated with ***poor outcome*** in ccRCC
- Demethylation of ***enhancer regions*** is enriched in hypomethylated tumors

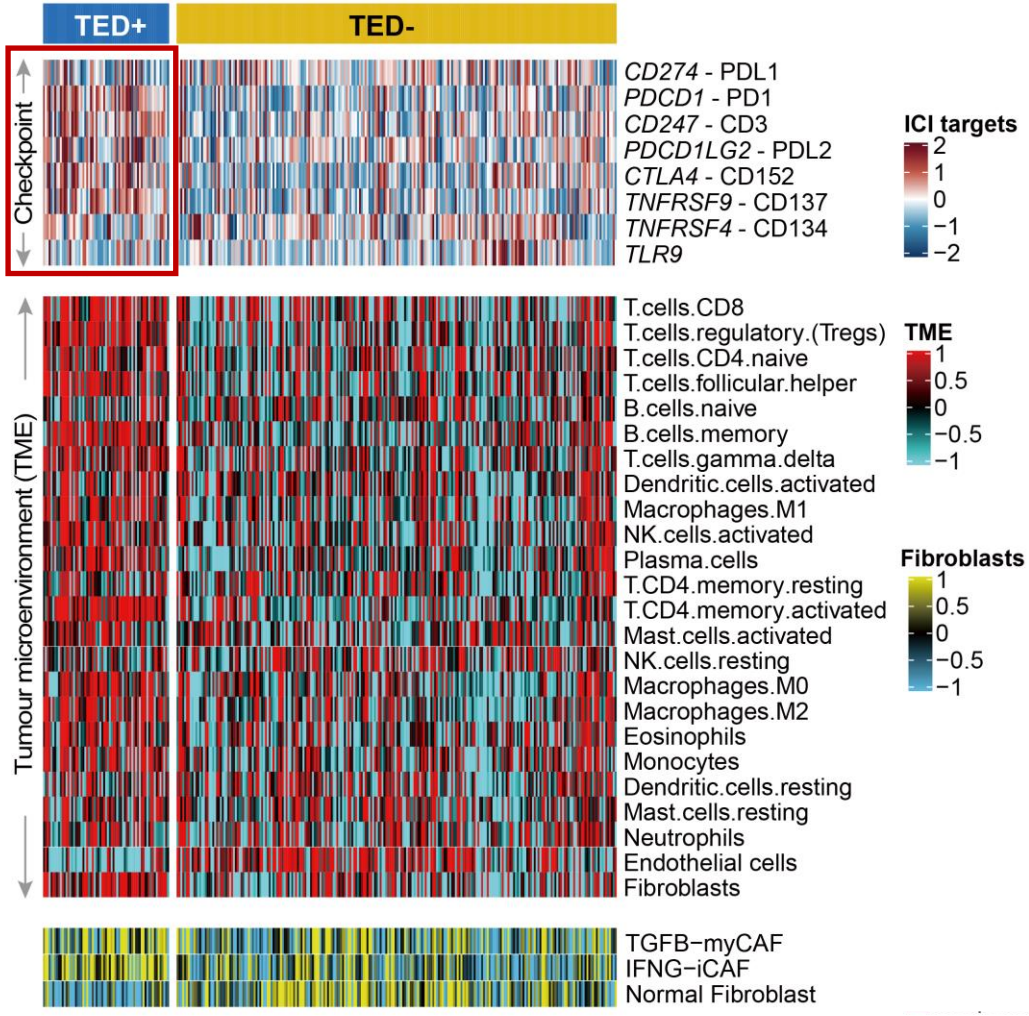



- Re-cluster with ***enhancer probes*** identified **"tumor-associated enhancer demethylator"**, ***TED+***
- *TED+* phenotype converges to tumor aggressiveness




- *TED+* phenotype is charted by a ***unique tumor microenvironment landscape***

**High expression of immune checkpoint genes**

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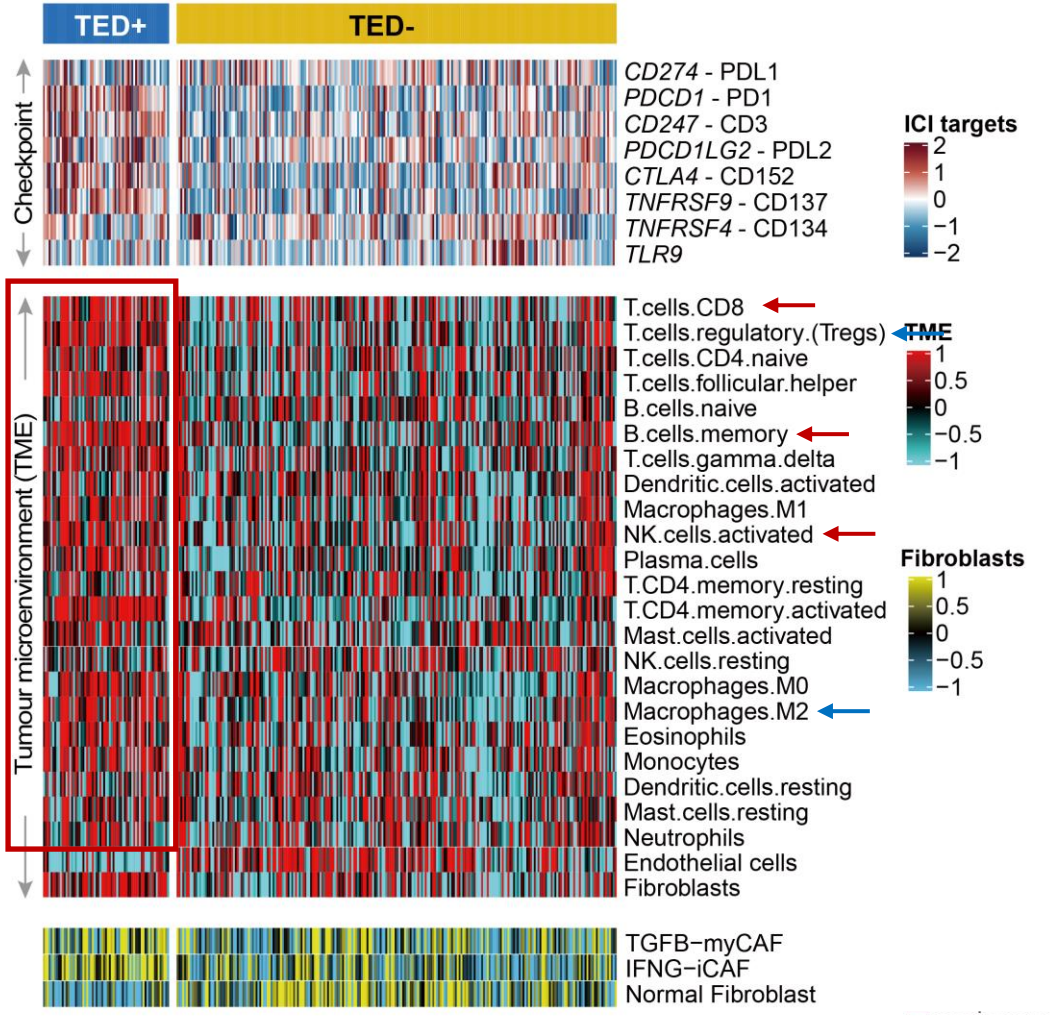
High expression of immune checkpoint genes

Infiltration with both active:
- CD8+,
- memory B,
- NK

and suppressive populations
- Treg
- M2 macrophages

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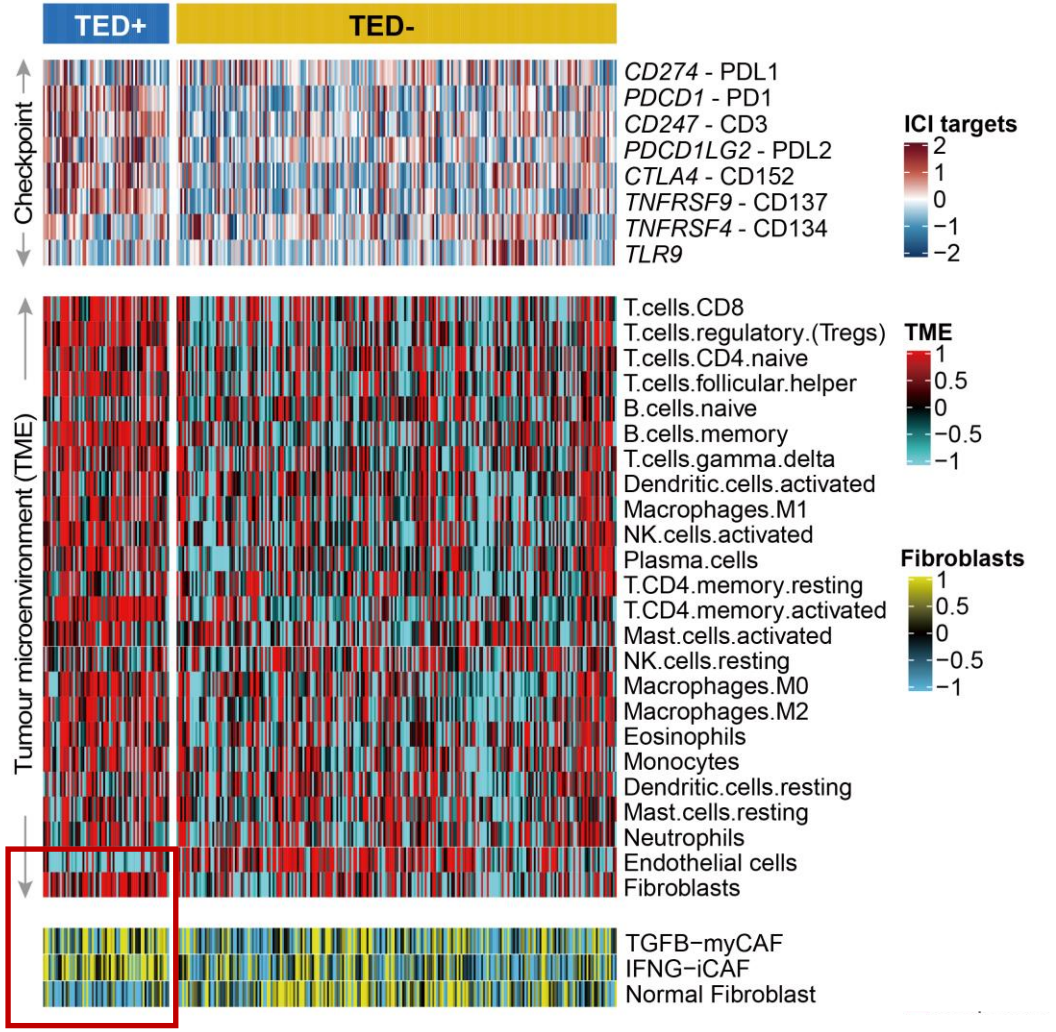
- **Depletion of ECs**
- **Enrichment of CAFs**

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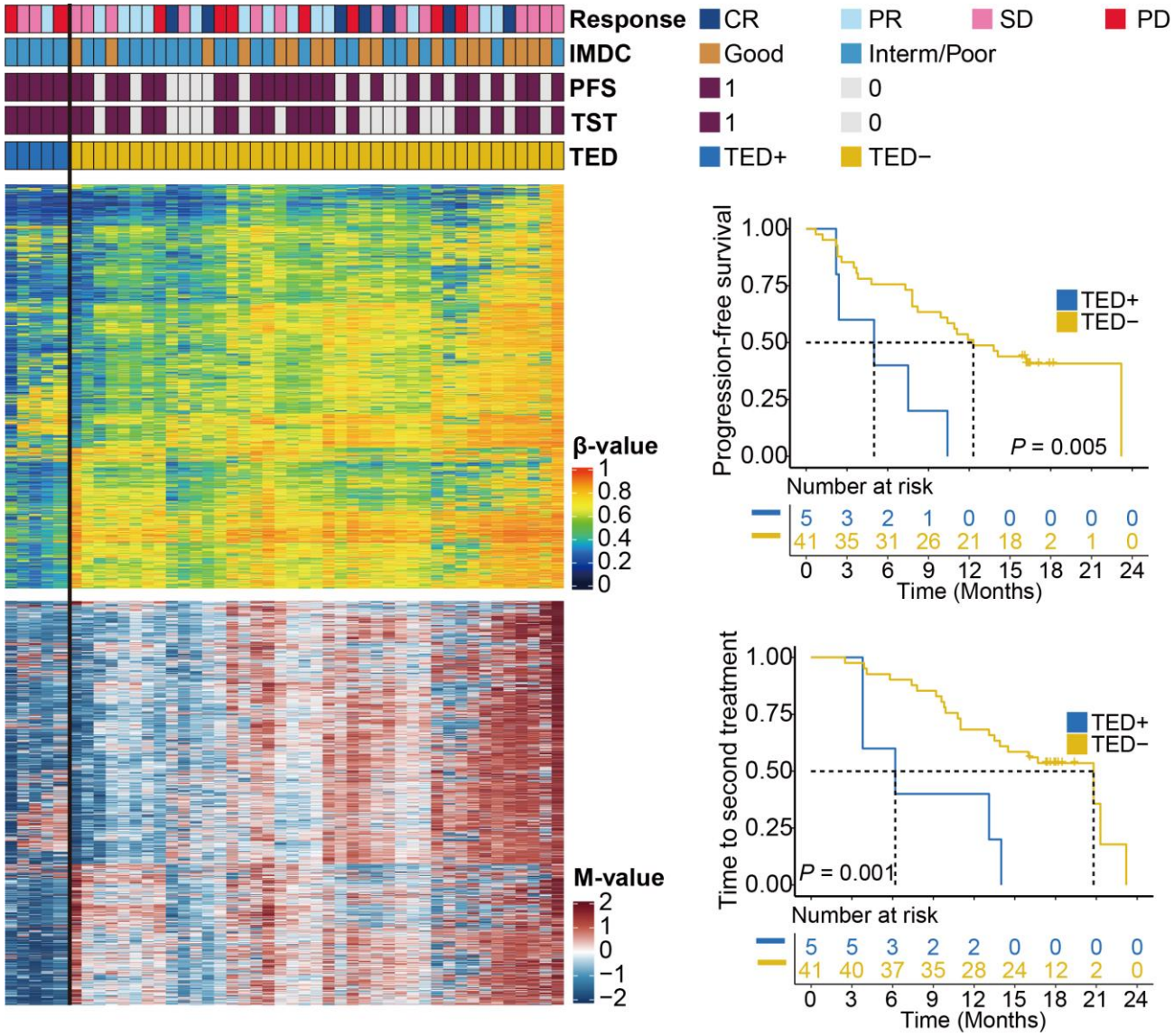
# *TED+* is associated with resistance to Ipi/Nivo in ccRCC

- **BIONIKK trial** where 46 patients with metastatic ccRCC were treated with Ipi/Nivo (IO/IO) and have available materials for EPIC methylation-array profiling



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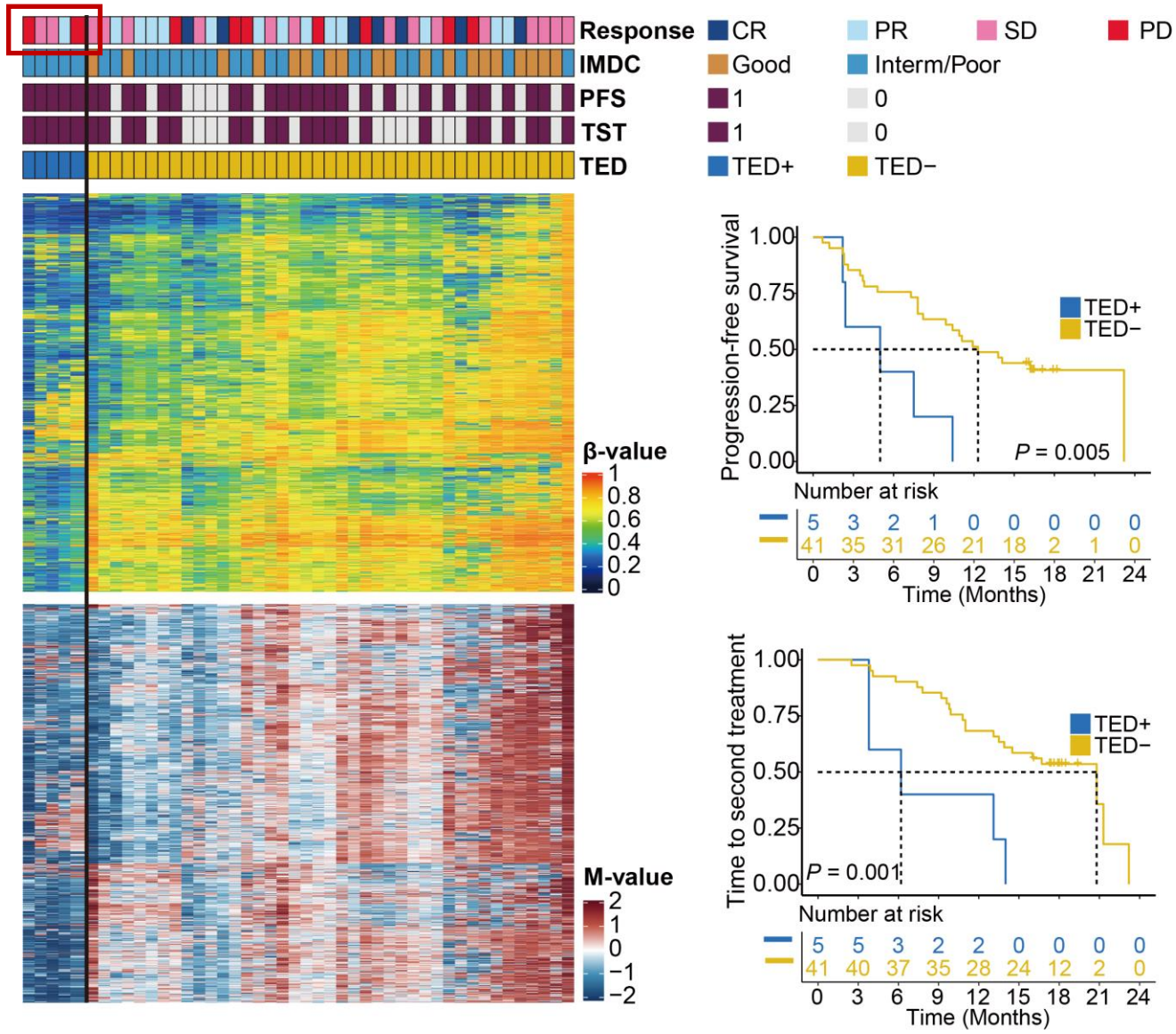
- All of the 5 patients belonging to TED+ experienced tumor progression within 12 months



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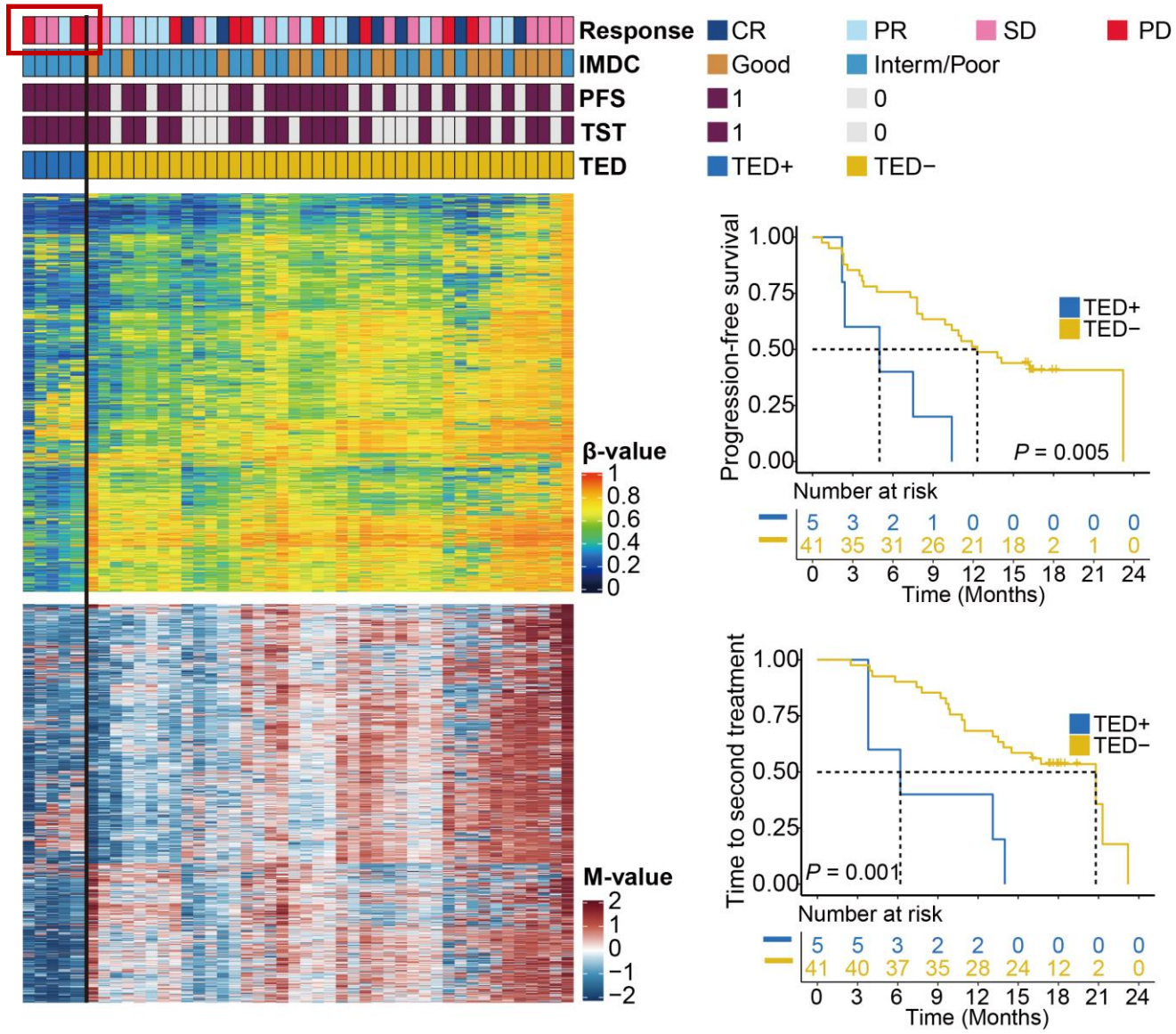
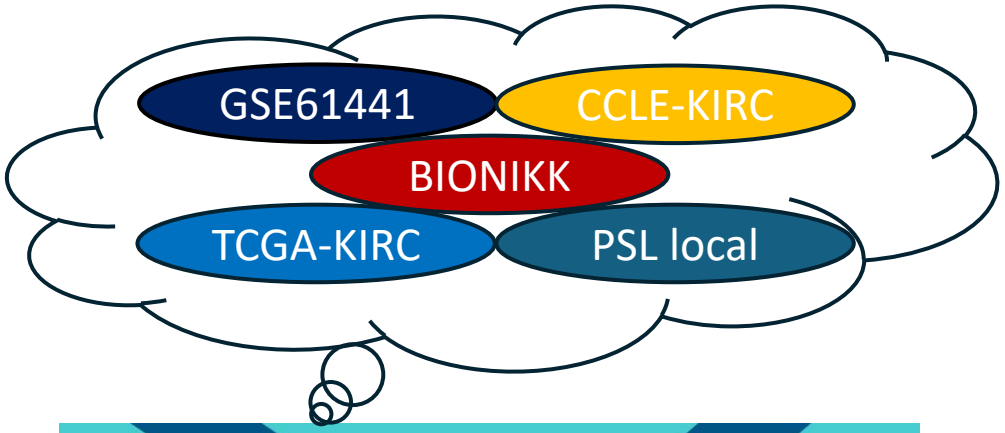
- All of the 5 patients belonging to TED+ experienced tumor progression within 12 months
- *TED* phenotype is ***both prognostic and predictive*** of resistance for patients treated with immunotherapy



# *TED+* is associated with *TET1* demethylation and expression





**A consensus list of hypomethylated probes in *TED+***

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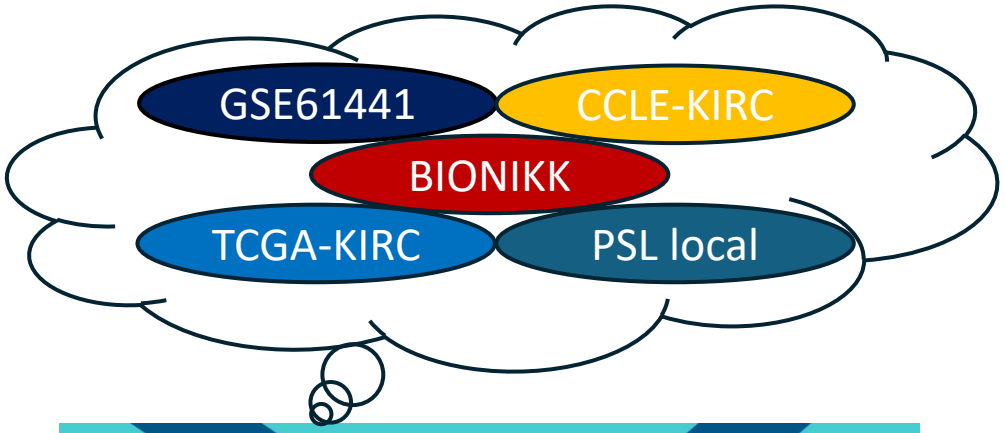



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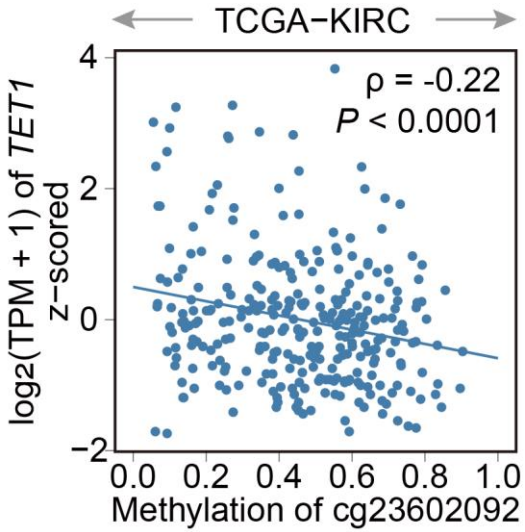


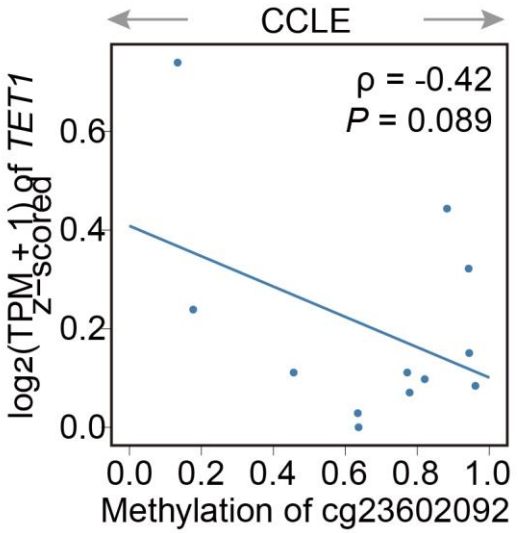

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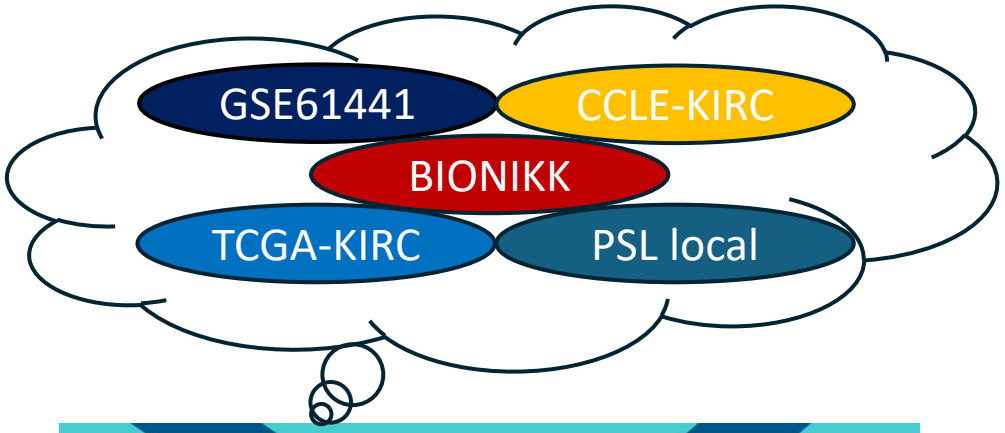



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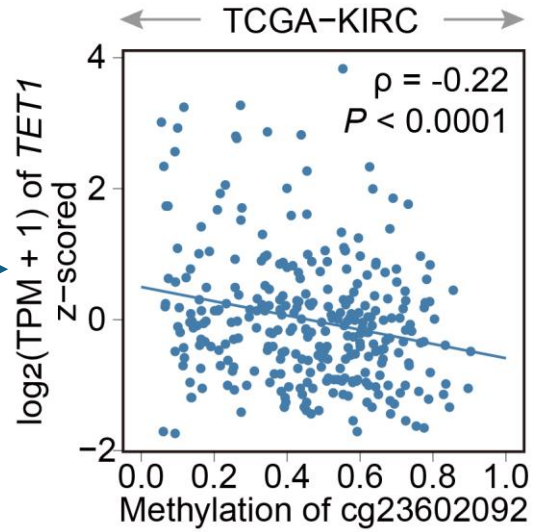
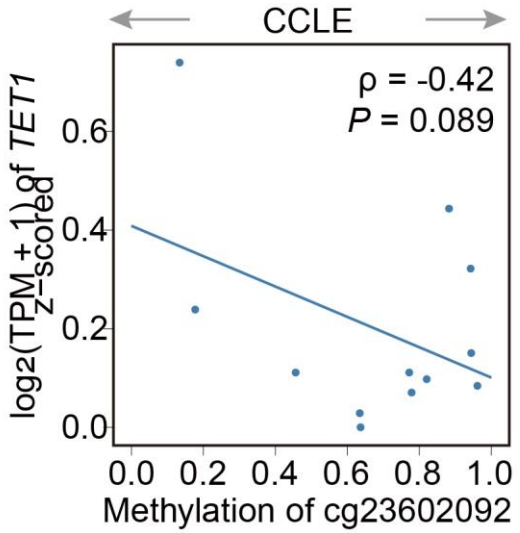



TET1 promoter hypomethylation → TET1 overexpression → TET1-mediated DNA demethylation → Enhancer hypomethylation → TED phenotype

*Converts methylated cytosines into intermediates, leading to loss of methylation*

# Publication

CLINICAL CANCER RESEARCH | TRANSLATIONAL CANCER MECHANISMS AND THERAPY

## An Enhancer Demethylator Phenotype Converged to Immune Dysfunction and Resistance to Immune Checkpoint Inhibitors in Clear-Cell Renal Cell Carcinomas

Xiaofan Lu[1,2], Yann Vano[3], Alexandra Helleux[1], Xiaoping Su[4], Véronique Lindner[5], Guillaume Davidson[1], Roger Mouawad[6], Jean-Philippe Spano[6], Morgan Rouprêt[7], Reza Elaidi[8], Eva Compérat[9], Virginie Verkarre[10], Chengming Sun[11], Christine Chevreau[12], Mostefa Bennamoun[13], Hervé Lang[14], Thibault Tricard[14], Wenxuan Cheng[2], Li Xu[2], Irwin Davidson[1], Fangrong Yan[2], Wolf Herman Fridman[15], Catherine Sautes-Fridman[15], Stéphane Oudard[3], and Gabriel G. Malouf[1,16]

ABSTRACT

**Purpose:** Immune checkpoint inhibitors (ICI) have revolutionized the treatment of patients with clear-cell renal cell carcinomas (ccRCC). Although analyses of transcriptome, genetic alterations, and the tumor microenvironment (TME) have shed light into mechanisms of response and resistance to these agents, the role of epigenetic alterations in this process remains fully unknown.

**Experimental Design:** We investigated the methylome of six ccRCC cohorts as well as one cell line dataset. Of note, we took advantage of the BIONIKK trial aiming to tailor treatments according to Paris Descartes 4-gene expression subgroups, and performed Illumina EPIC profiling for 46 samples related to patients treated with ipilimumab plus nivolumab, and 17 samples related to patients treated with sunitinib.

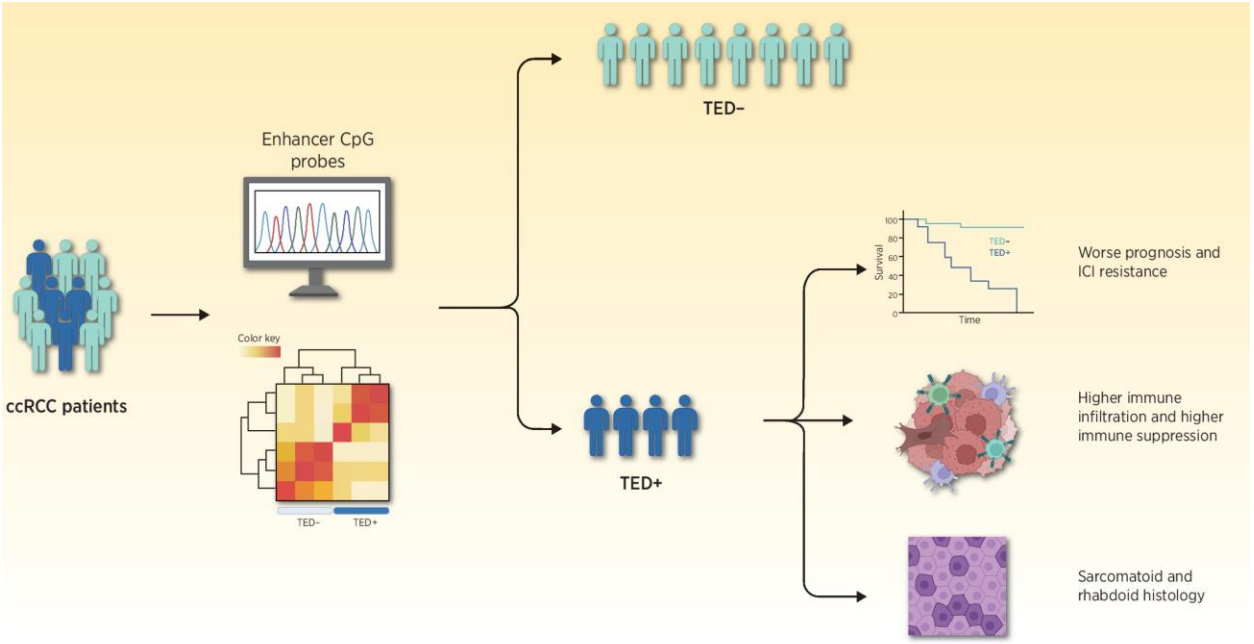
**Results:** A group of tumors associated with enhancer demethylation was discovered, namely *TED*. *TED* was associated with tumors with sarcomatoid differentiation and poor clinical outcome. *TED* harbored *TET1* promoter demethylation, activated the gene expression signature of epithelial–mesenchymal transition and IL6/JAK/STAT3 pathways, and displayed a TME characterized by both immune activation and suppressive populations, fibroblast infiltration, and endothelial depletion. In addition, *TED* was a predictive factor of resistance to the combination of first-line ipilimumab-nivolumab in the BIONIKK clinical trial. Finally, *TED* was associated with activation of specific regulons, which we also found to be predictive of resistance to immunotherapy in an independent cohort.

**Conclusions:** We report on the discovery of a novel epigenetic phenotype associated with resistance to ICIs that may pave the way to better personalizing patients' treatments.

*See related commentary by Zhou and Kim, p. 1170*

## Viewing RCC with a DNA Methylation Lens ENHANCES Understanding of ICI Resistance

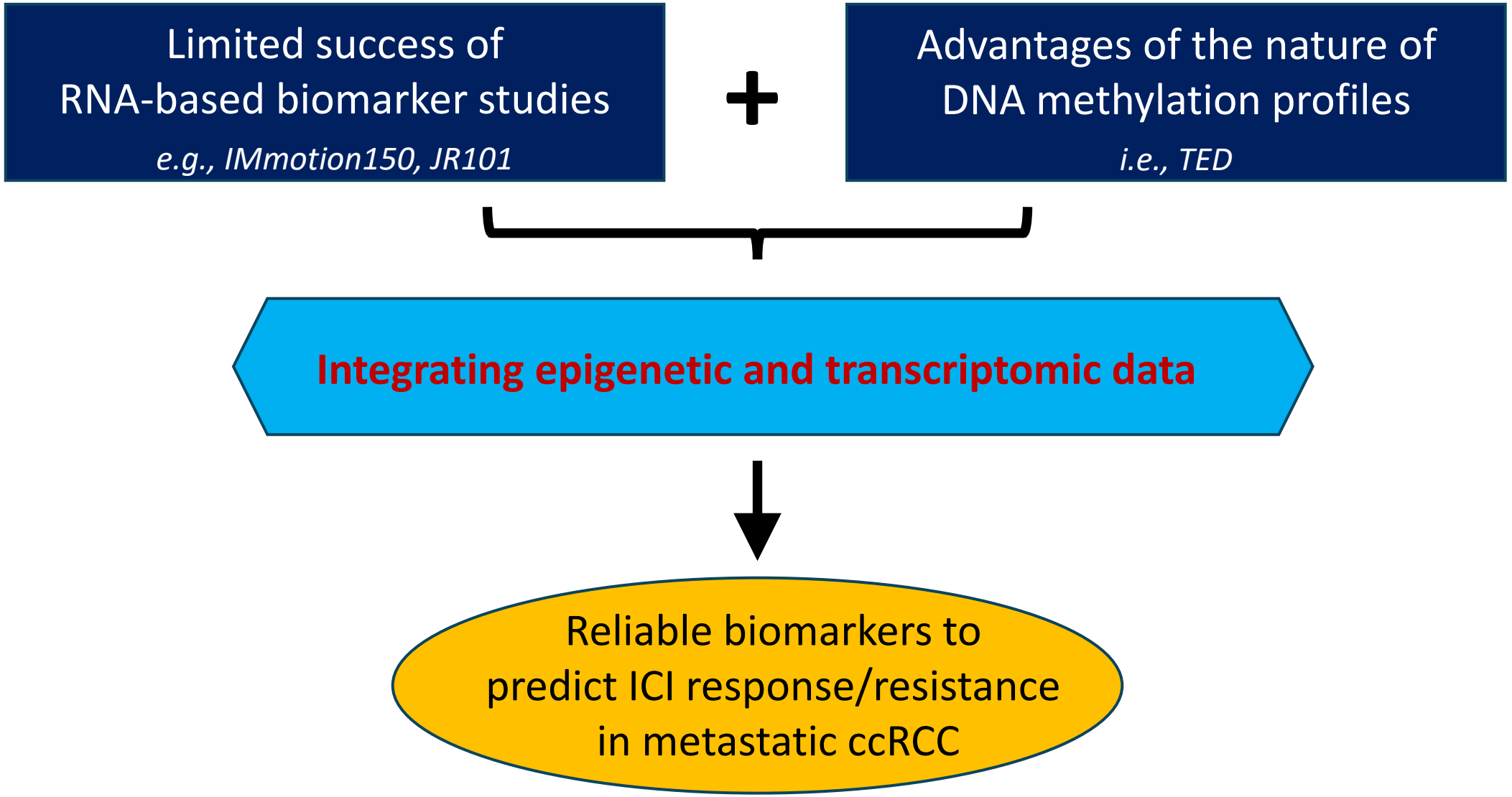
Mi Zhou; William Y. Kim



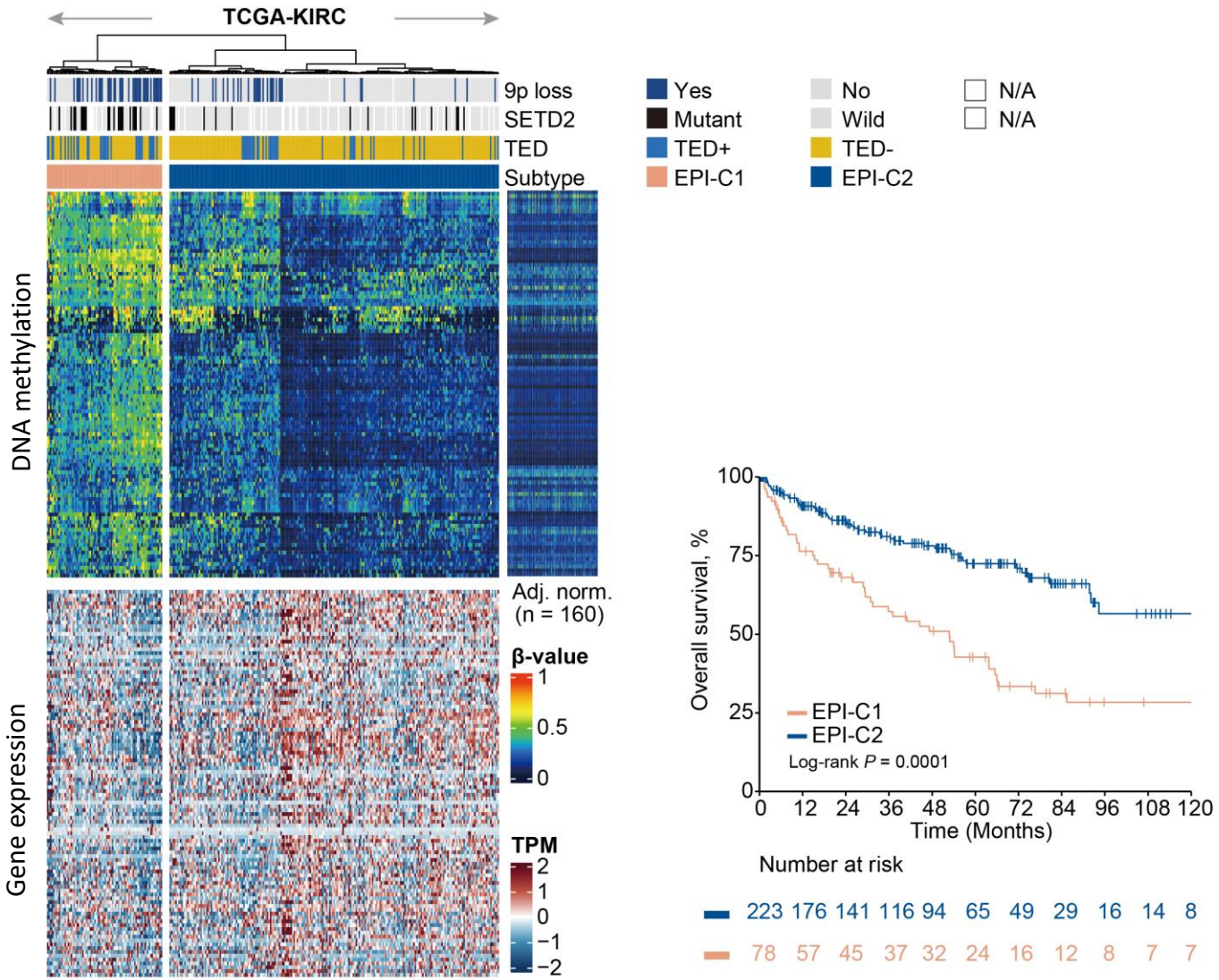

- **CcRCC with an enhancer demethylator phenotype (TED) harbor a worse prognosis and derive less clinical benefit from immunotherapy**

Lu et al., 2022 Clin Cancer Res; Zhou et al., 2022 Clin Cancer Res

# Motivation


Limited success of
RNA-based biomarker studies
e.g., IMmotion150, JR101
+
Advantages of the nature of
DNA methylation profiles
i.e., TED
Integrating epigenetic and transcriptomic data
Reliable biomarkers to
predict ICI response/resistance
in metastatic ccRCC

# Integrative analysis identified an *epigenetic silencing* subtype







- **Integration of DNA methylation and transcriptomic expression to identify probes/genes that were *hypermethylated in promoter CpG islands while repressed in gene expression level*.**

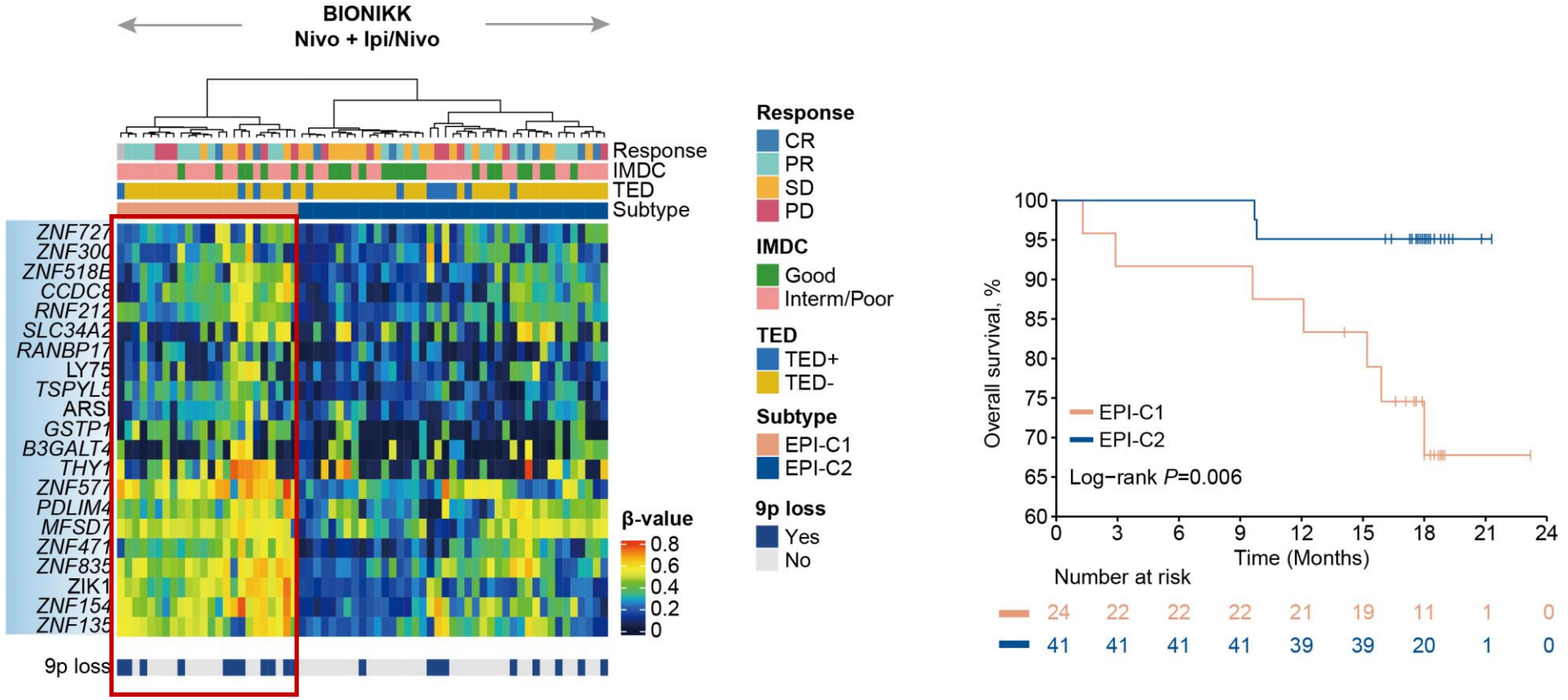
- **Two epigenetic (EPI) subtypes were identified, where EPIC-C1:**
  - **featured 9p loss and *SETD2* mutation**
  - **showed poor outcomes**

# *Epigenetic silencing* contributes to immunotherapy resistance

**48** samples from BIONIKK trial had both DNA methylation and expression data

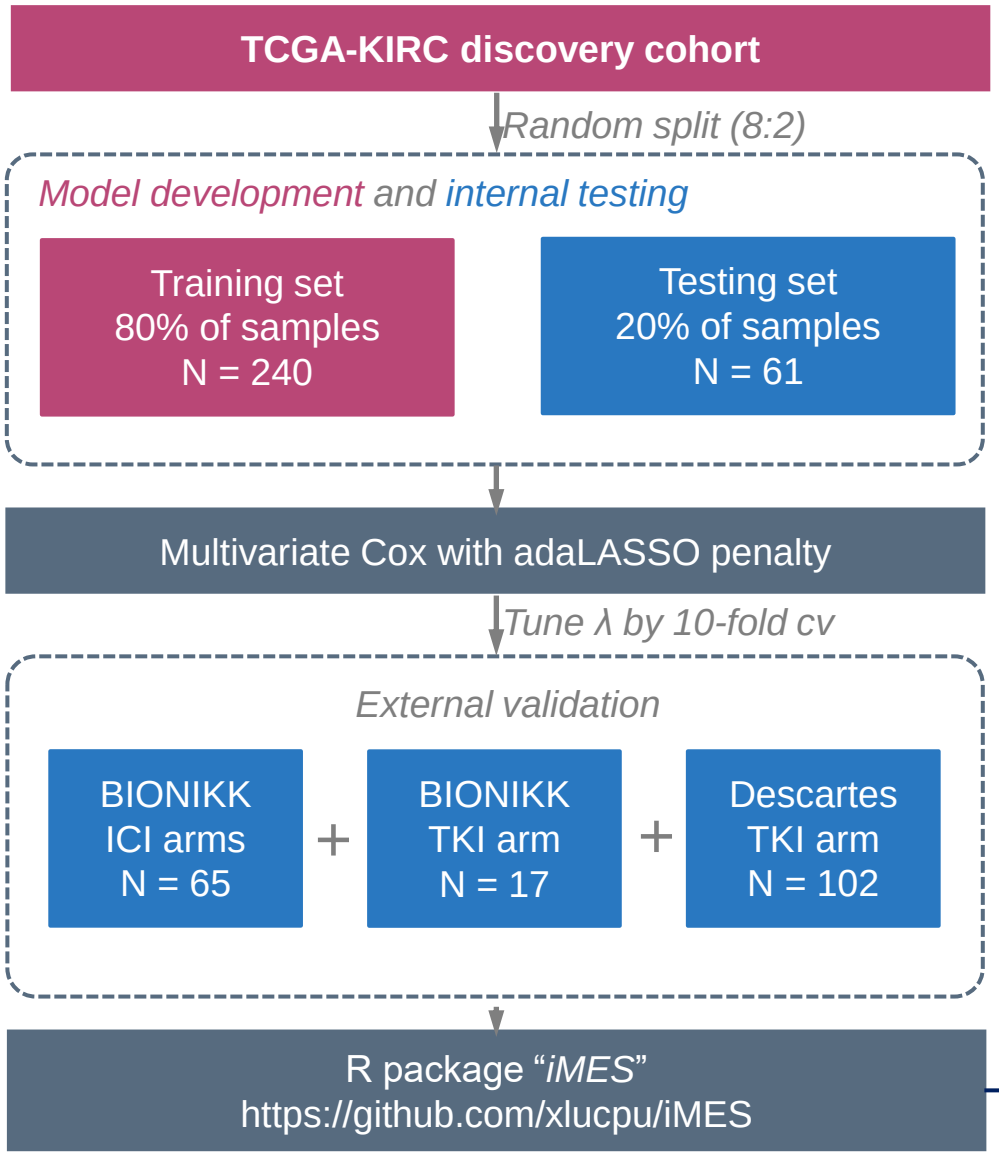
- **12** from the nivolumab (IO) arm
- **27** from the Ipi/Nivo (IO/IO) arm
- **9** from the sunitinib (TKI) arm

# *Epigenetic silencing* contributes to immunotherapy resistance




- 21 genes were consistently silenced by promoter-hypermethylation across different cohorts
- Two EPI subtypes were identified and EPI-C1 converged to 9p loss and poor outcomes

# A methylation index relevant to immunotherapy resistance




## Introduction

This package is designed to compute an Index of Methylation-based Epigenetic Silencing (iMES) using binary DNA methylation data in patients with clear cell renal cell carcinoma. Furthermore, it classifies patients into distinct regulon phenotypes based on transcriptomic expression data.

The primary function, `iMES()`, returns a DataFrame where each row corresponds to a sample name and comprises three columns:

1. 'iMES': raw iMES score
2. 'iMES.mm': min-max normalized iMES score (scaled to a range of 0-10) multiplied by 10
3. 'iMES.group': dichotomized iMES group

The secondary function, `predRegulon()`, assesses the regulon activity status for each sample. It then categorizes samples into either a suppressed or activated regulon phenotype, based on the status count of each regulon within each original group.

Patients with high iMES scores or those categorized in the iMES-high group are potentially at an increased risk of immune evasion and resistance to immune checkpoint inhibitors. The regulon phenotype classified as suppressed is considered to correspond to the iMES-high group, while an activated regulon phenotype is analogous to the iMES-low group.

## Citation

If you use iMES in published research, please cite:

- Lu X, Vano YA, Su X, Helleux A, Lindner V, Mouawad R, Spano JP, Rouprêt M, Compérat E, Verkarre V, Sun CM, Bennamoun M, Lang H, Barthelemy P, Cheng W, Xu L, Davidson I, Yan F, Fridman WH, Sautes-Fridman C, Oudard S, Malouf GG. Silencing of genes by promoter hypermethylation shapes tumor microenvironment and resistance to immunotherapy in clear-cell renal cell carcinomas. *Cell Rep Med*. 2023;4(11):101287. doi: 10.1016/j.xcrm.2023.101287.

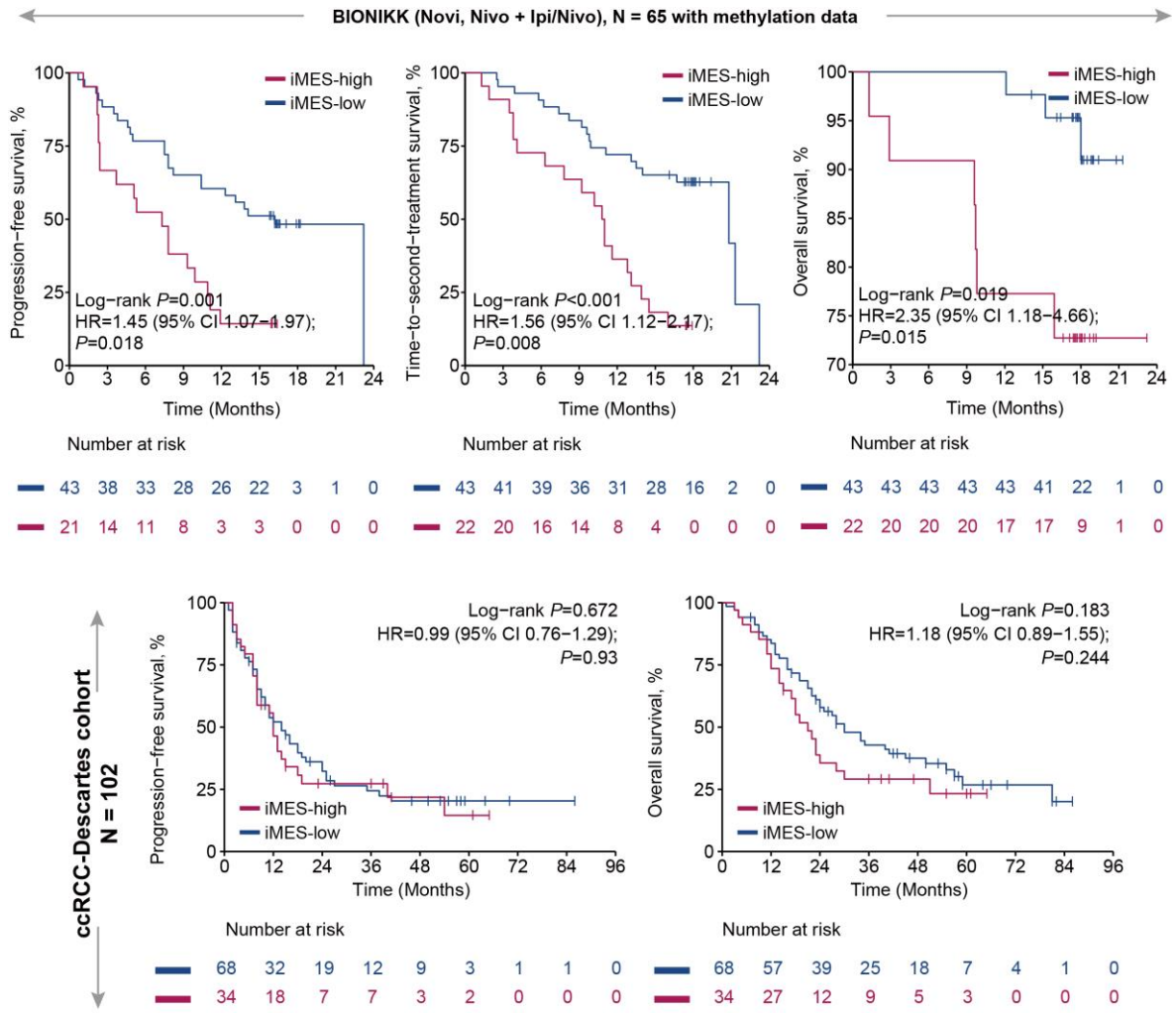
## Installation

You can install the development version of iMES from GitHub with:

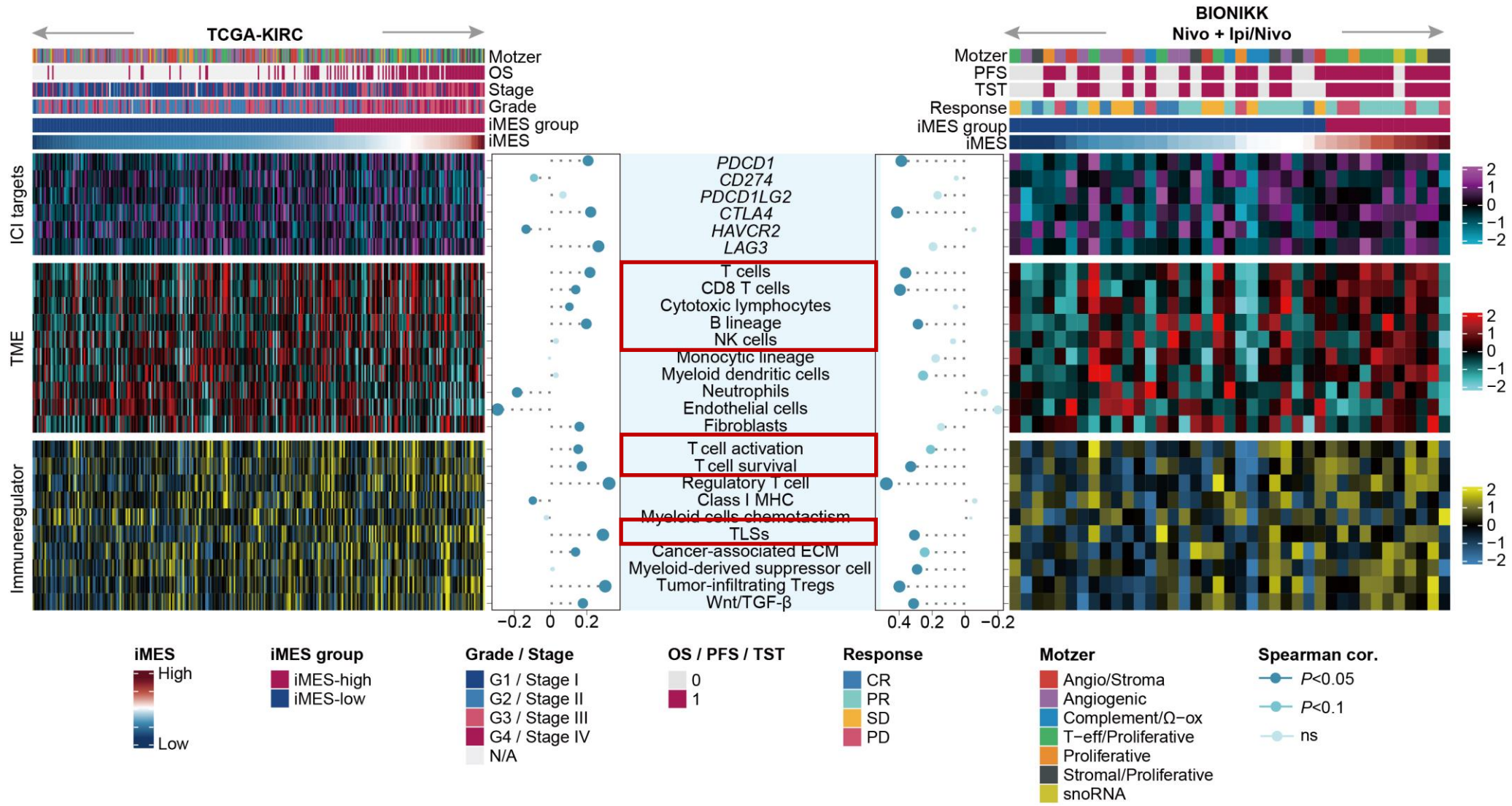
```
# install.packages("devtools")
devtools::install_github("xlucpu/iMES")
```

## Example

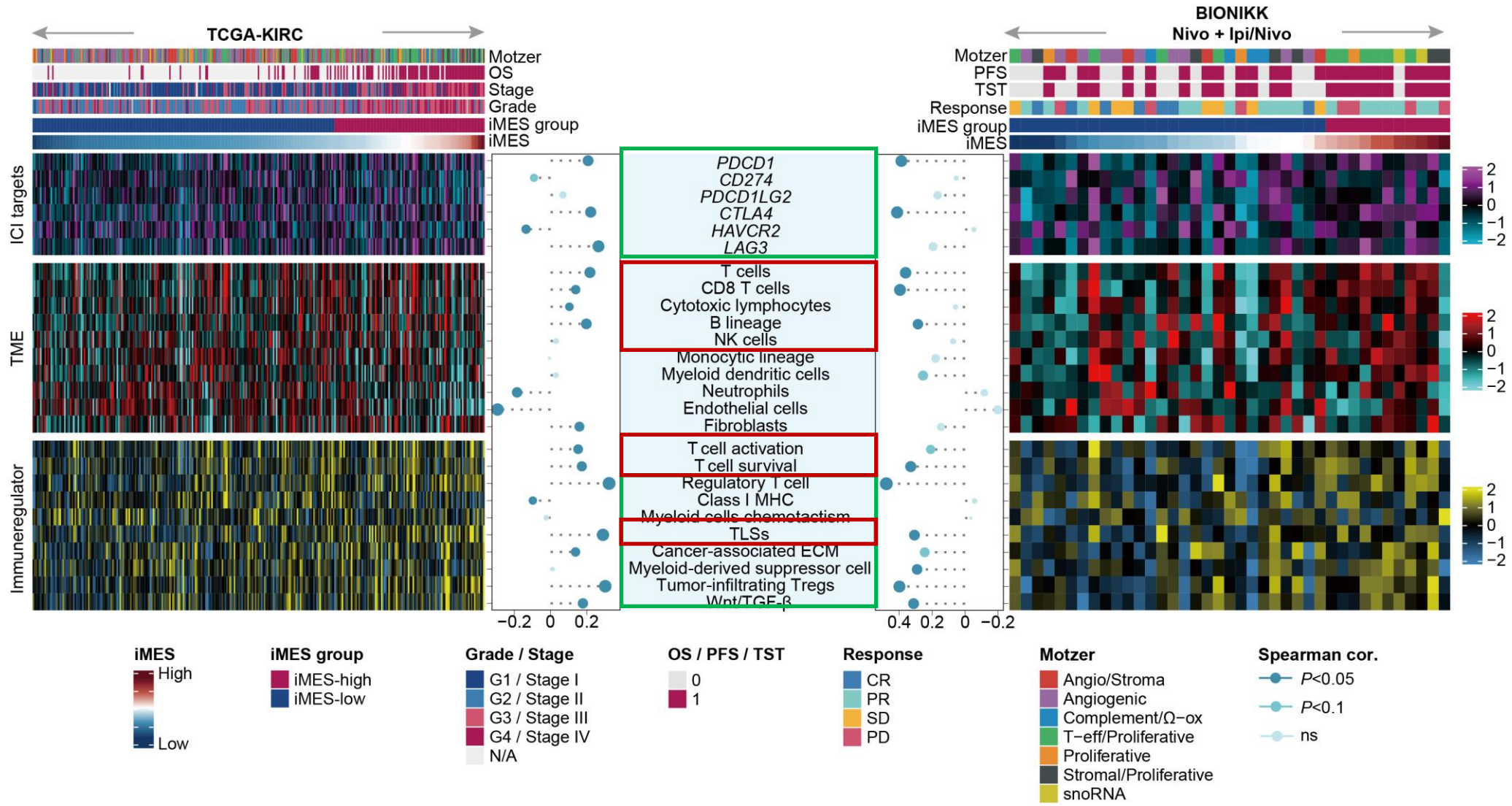
```
## basic example code (not run)
library(iMES)
# Reading DNA methylation beta matrix
methMat <- read.delim("DNA methylation beta matrix.txt",sep = "\t",check.names = F,row.names = 1,header

# Calculate iMES
iMES <- iMES(bmat     = methMat, # a DNA methylation beta matrix with continuous values as input
             methcut  = 0.2, # cut continuous methylation matrix to binary methylation status
             samples  = colnames(methMat)[1:30], # extract the first 30 samples to calculate iMES
             quantile = 3) # dichotomize samples into iMES-high and iMES-low based on a general tertile
```



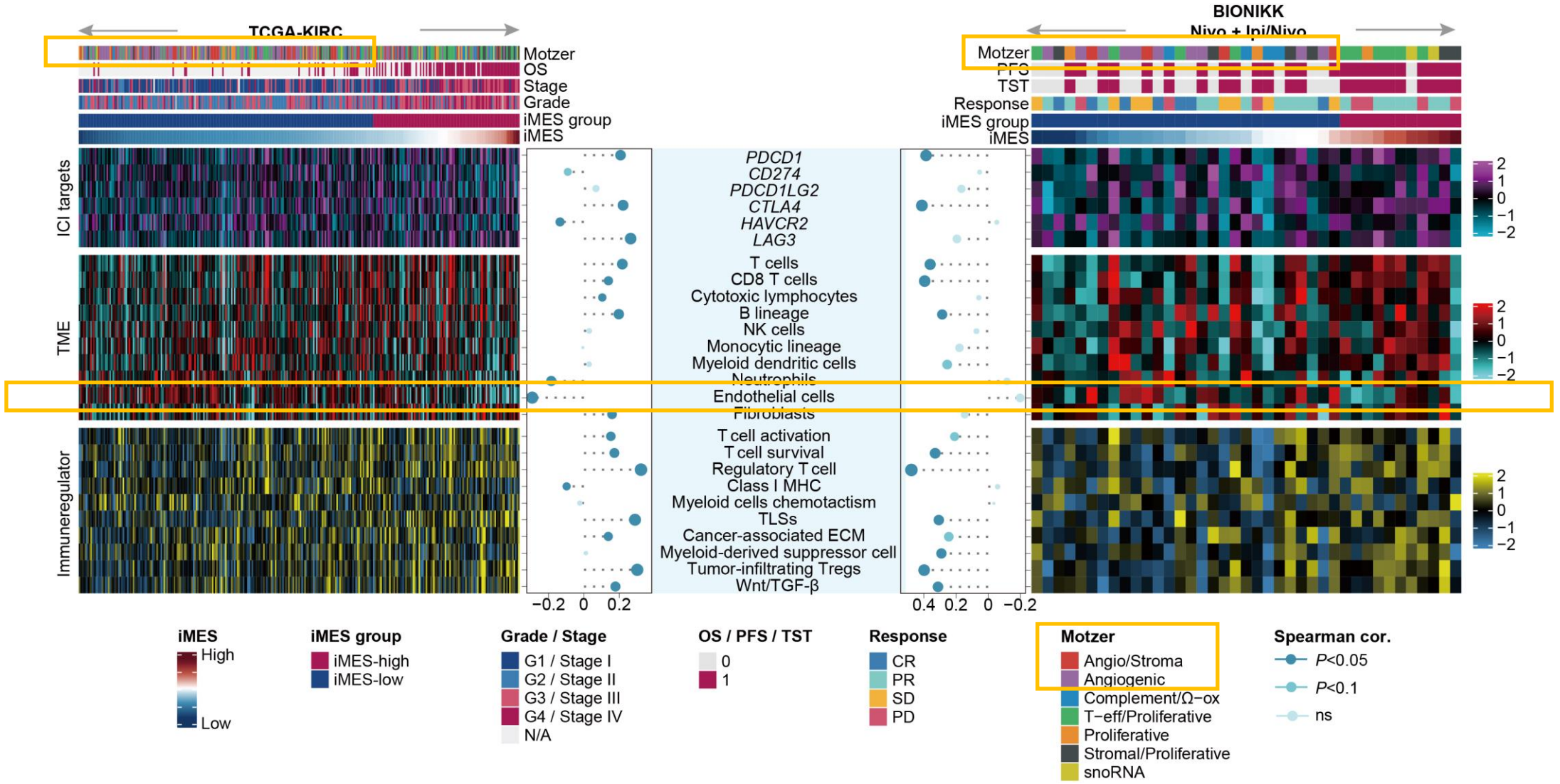

- **iMES** (index of methylation silencing) is a ***prognostic*** factor that tightly associated with patient outcomes
- **iMES** is also a ***predictive*** indicator of resistance for patients with metastatic ccRCC receiving immunotherapy




- **High iMES was related to *high immune infiltration with both activation and suppression signatures***




- **High iMES was related to *high immune infiltration with both activation and suppression signatures***




- **High iMES was related to *high immune infiltration with both activation and suppression signatures***
- **Low iMES was related to *enrichment of epithelial cell, and Motzer's angiogenic and angio/stroma subtypes***
  **— vascularization and tumor angiogenesis might also play a role in immunotherapy response**

# Publication

Cell Reports Medicine

CellPress
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Article

## Silencing of genes by promoter hypermethylation shapes tumor microenvironment and resistance to immunotherapy in clear-cell renal cell carcinomas

Xiaofan Lu,[1,16] Yann-Alexandre Vano,[2,3,16] Xiaoping Su,[4,16] Alexandra Helleux,[1] Véronique Lindner,[5] Roger Mouawad,[6] Jean-Philippe Spano,[6] Morgan Rouprêt,[7] Eva Compérat,[8] Virginie Verkarre,[9] Cheng-Ming Sun,[3] Mostefa Bennamoun,[10] Hervé Lang,[11] Philippe Barthelemy,[12] Wenxuan Cheng,[13] Li Xu,[13] Irwin Davidson,[1] Fangrong Yan,[13] Wolf Hervé Fridman,[3] Catherine Sautes-Fridman,[3] Stéphane Oudard,[3,15,17] and Gabriel G. Malouf[1,14,15,17,18,*]

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## Graphical Abstract

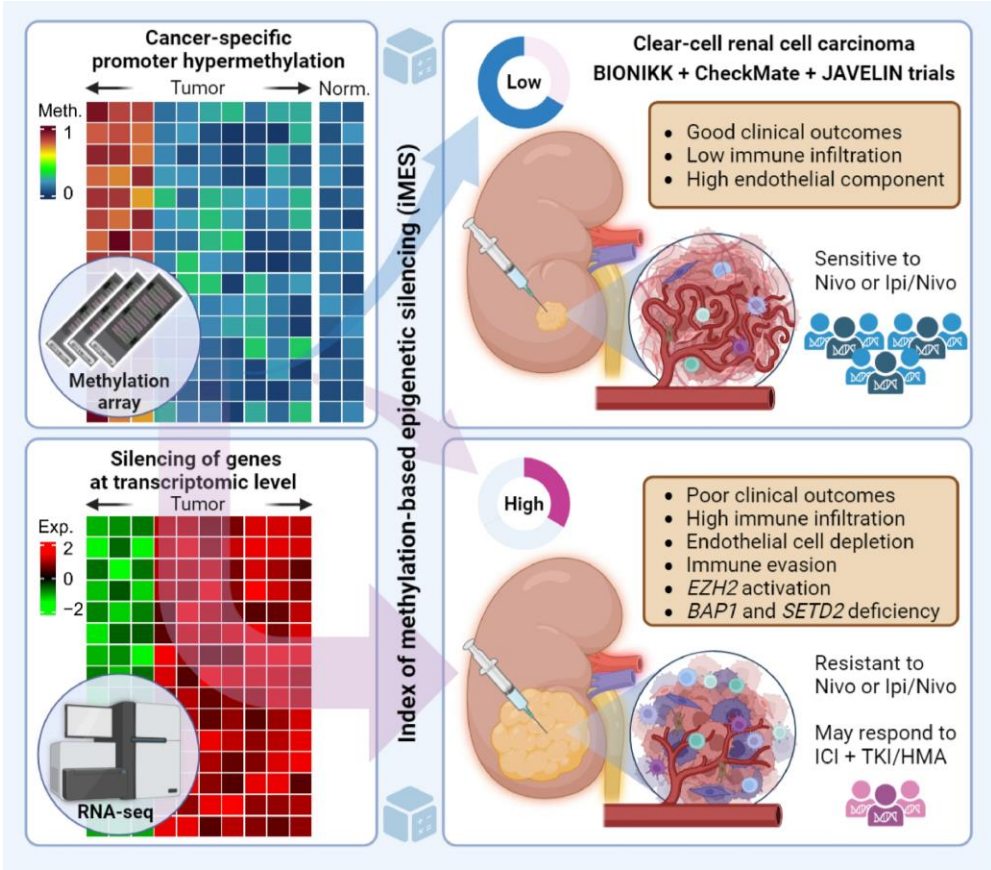
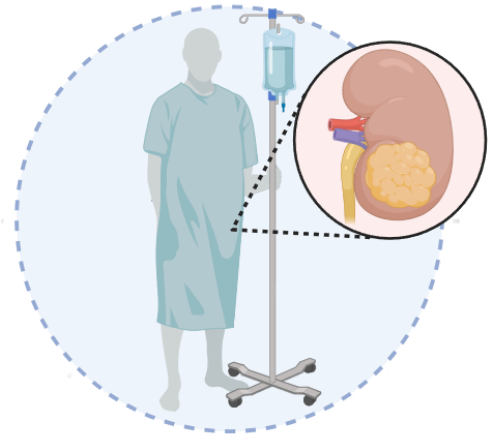



Image created by BioRender at biorender.com

- Gene silencing influences both ccRCC aggressiveness and microenvironment
- iMES reliably predicts ICI treatment outcomes in ccRCC
- iMES and angiogenesis jointly impact response to immunotherapy in ccRCC

Lu et al., 2023 Cell Rep Med

# Summary



**Clear-cell renal cell carcinoma (ccRCC)**

Epigenetic modification

Tolerance to treatment

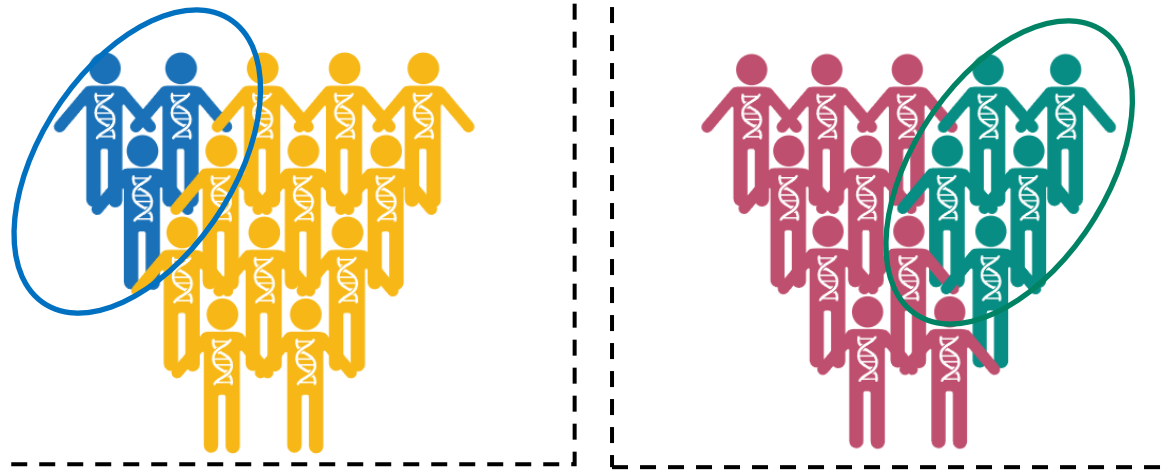
**The role of epigenetic markers as a predictor for immunotherapy efficacy is poorly studies in ccRCC**

## BIONIKK clinical trial (metastatic ccRCC )

**DNA methylation profiles from BIONIKK**

1 *Lu et al., Clin Cancer Res 2022*

2 *Lu et al., Cell Rep Med 2023*



**TED (tumor enhancer demethylator)**

**Epigenetic silencing by promoter hypermethylation** → **iMES (index of methylation-based silencing)**

**Immunotherapy resistance**

Image created by BioRender at biorender.com

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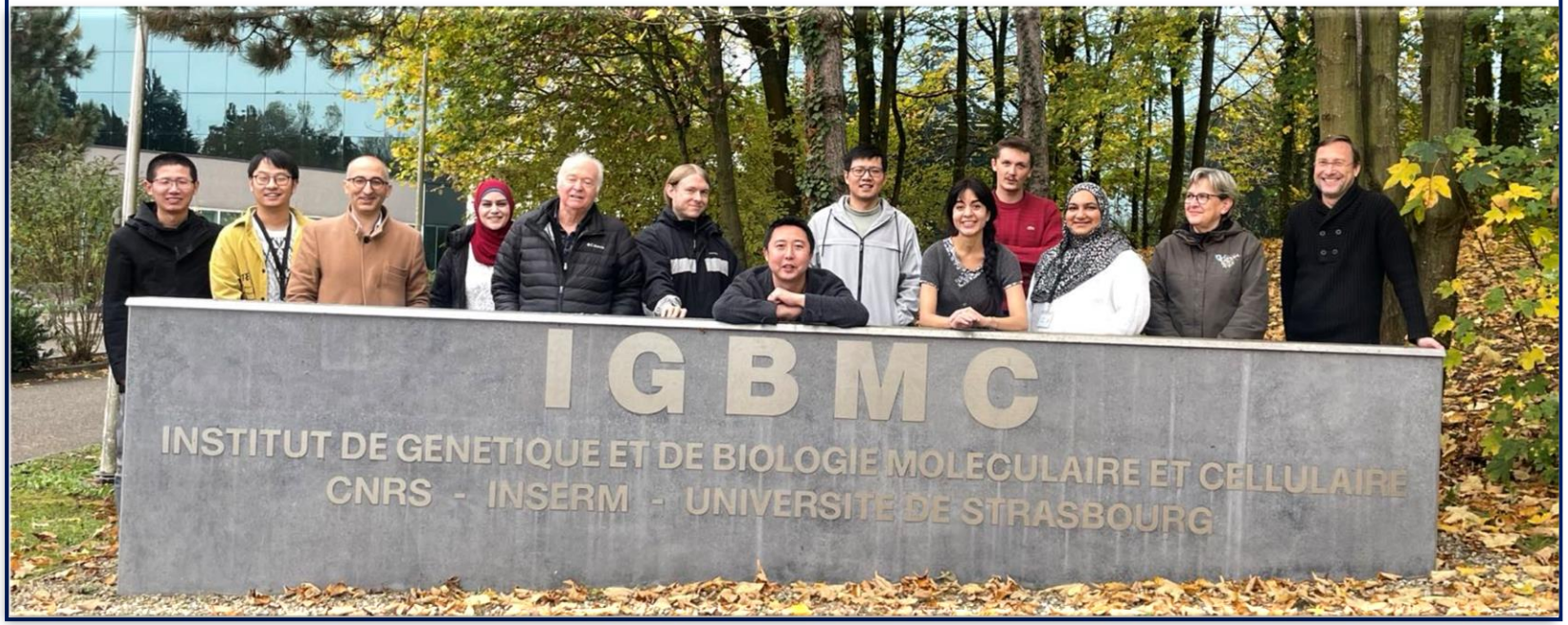


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- Genomeast

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