

UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE MEDICINA



TESIS DOCTORAL

**Molecular taxonomy of renal cell carcinoma and response to
targeted therapies**

**Taxonomía molecular del cáncer renal y respuesta a
tratamientos dirigidos**

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Lucía Carril Ajuria

Directores

Guillermo Antonio de Velasco Oria de Rueda
Eva María Ciruelos Gil
Alejandra Bernardini

Madrid

UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE MEDICINA



TESIS DOCTORAL

**Molecular taxonomy of renal cell carcinoma
and response to targeted therapies**

**Taxonomía molecular del cáncer renal y
respuesta a tratamientos dirigidos**

MEMORIA PARA OPTAR AL GRADO DE DOCTORA

PRESENTADA POR

Lucía Carril Ajuria

DIRECTORES

Dr. Guillermo Antonio de Velasco Oria de Rueda

Dra. Eva María Ciruelos Gil

Dra. Alejandra Bernardini

UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE MEDICINA



TESIS DOCTORAL

**Molecular taxonomy of renal cell carcinoma
and response to targeted therapies**

**Taxonomía molecular del cáncer renal y
respuesta a tratamientos dirigidos**

MEMORIA PARA OPTAR AL GRADO DE DOCTORA

PRESENTADA POR

Lucía Carril Ajuria

DIRECTORES

Dr. Guillermo Antonio de Velasco Oria de Rueda

Dra. Eva María Ciruelos Gil

Dra. Alejandra Bernardini

Programa de Doctorado: Investigación en Ciencias Médico-Quirúrgicas

“The important thing is
to never stop questioning”
Albert Einstein

To Begoña and Jose Manuel, my parents.

ACKNOWLEDGEMENTS

This Ph.D. would have not been carried out without the help of many people to whom I will always be grateful for their accompaniment and support throughout this journey.

I would like to express my most profound gratitude to my Ph.D. director, Dr. Guillermo de Velasco, for having been a true and inspiring mentor.

As early trainee I was attracted to research and Dr. De Velasco gave me the opportunity to start this Ph.D. and thus start my journey into the exciting world of kidney cancer. Thanks for bringing out the best of me, for teaching me the way into research, for your support and sound advice, and for your contagious and continuous motivation. I feel I should also take advantage of this moment and say: “whatever you ask, the answer is always IGNYTA”.

I also could not have undertaken this work without Alejandra Bernardini, Ph.D. co-director, Jesus Paramio, Head of the Molecular Oncology Unit at CIEMAT, and Carolina. Thanks for your valuable assistance and support throughout the doctorate. Thank you for all the “Teams” and insightful conversations. Thank you for your time and patience, and for helping me to understand the complex language of transcriptomics and for introducing me to the fun, and old-school, TMEV.

I would like to extend my sincere thanks to Dr. Eva Ciruelos and Dr. Luis Paz Ares, co-director and tutor respectively of this thesis, but also, outstanding clinicians and researchers whom I have been lucky enough to have as reference and example of excellence.

I have been very fortunate to share this journey with my colleagues Eli, Ana, Maricruz and Paula, whom I am very lucky to call friends. Thank you for always being there, for your advice and support, for the “Andaluzas”, for your sarcastic humour, but above all for your friendship. I always admired you and even today I continue to learn from you.

The Ph.D. did not keep me in Madrid. We both developed ourselves while moving to other countries for professional and personal projects. That’s why I am immensely grateful to Prof. Albiges for believing in me and giving me the opportunity to continue my research in kidney cancer at the Institute Gustave Roussy (Paris), and to Prof. Chaput for welcoming me to her laboratory of Immunomonitoring and thus sowing the seed for my interest in inflammatory circulating biomarkers. Thank you Marie, for teaching me how to handle a pipette and for making me fall in love with the MSD assay, for sharing our interest in research and for our coffees at the “10eme”, and last but not the least, thank you for your friendship. Thanks also to Álvaro, for his support since I arrived in Brussels and, for being almost more worried than me about finishing this Ph.D. thesis.

Thanks must also go to all the Medical Oncology team at the University Hospital of 12 de Octubre (nurses, trainees and seniors), particularly to the Genitourinary Tumours Unit led by Dr. Castellano, for those wonderful years of personal and professional growth, I will always remember them fondly.

To all my friends from Santander, Madrid, Paris, Brussels and elsewhere in Spain and around the world, thank you for your unconditional friendship despite the distance.

Thank you to Alexandra Elbakyan, founder of the Sci-Hub site, you've made the life of researchers much easier.

I thank with love my family, especially my dad, mom, and brother, for their unconditional trust, love, and support, and for their endless patience. Dad, thank you for teaching us to be curious, to make the impossible possible, and to "always try".

Jesus, I cannot thank you enough for all your love and unconditional support. Thank you for all the dreams and projects we share. You are my best companion.

Finally, to all the patients and their families, you are the reason for this project and of our ongoing motivation every day.

SUMMARY

Clear-cell RCC is a unique tumour characterised by high vascularization and immunogenicity. Based on these features, the systemic therapy of metastatic RCC (mRCC) relies on the use of immune checkpoint blockers (ICB) and antiangiogenics (AA). Currently, ICB combinations with either another ICB or AA are the standard of care in first-line setting for advanced clear-cell RCC. ICB can provide long-term responses; however, only a small subset of patients will benefit, and up to 20% of ccRCC patients will be refractory to ICB. Further, a non-negligible percentage of patients will develop severe immune-related adverse events (irAEs). Thus, a better understanding of the molecular mechanisms of response and resistance to ICB is needed to develop a biomarker-based approach for treatment selection.

Unfortunately, to date, there is still no validated biomarker of response to ICB. Unlike other tumours, such as lung cancer or urothelial cancer, the role of PD-L1 as a predictor of response to ICB in advanced ccRCC is still controversial. Similarly, the tumour mutational burden (TMB) has not been shown to be associated with response to ICB. Despite being characterised by high immune infiltration, the degree of CD8⁺ T cell tumour infiltration doesn't seem to be a reliable predictor of response to ICB in ccRCC. Single gene mutations are under evaluation, but no robust association with the ICB response has yet been established. Recently, several translational studies have proposed different gene signatures ("26 JAVELIN Immuno", "IMmotion Angiogenesis", "IMmotion T effector", "IMmotion Myeloid inflammation", "Tumor Inflammation signature") associated with clinical outcomes for ICB and/or AA in ccRCC. However, they all failed to demonstrate a significant association with clinical benefit from nivolumab plus ipilimumab versus sunitinib in treatment naïve RCC patients in the recent biomarker analysis of the CHECKMATE214 trial. As for non-clear cell RCC tumours, there is a paucity of data regarding biomarkers. According to the TCGA analysis, up to 80% of type 1 papillary RCC carry *MET*-gene alteration or a gain of chromosome 7 (which harbors the *MET* gene). This has paved the way for the design of biomarker-driven trials and also for the design of the PAPMET pivotal trial, which made the *MET* inhibitor cabozantinib a new standard of care in first-line setting for advanced papillary RCC.

The hypothesis of the present study is that a better understanding of the molecular and immune landscape of the tumour microenvironment (TME) in mRCC patients treated with ICB will help us identify the underlying mechanisms of resistance and response to ICB. Specifically, we propose that differences in gene expression would translate into differences in tumour immune infiltration composition that would explain not only the clinical outcomes of ICB but also the development of severe immune-related adverse events (irAEs) and the tumour behaviour. In addition, changes in gene expression could also trigger metabolic reprogramming and regulation of protumoral pathways that could also influence the outcomes under ICB.

For this purpose, we retrospectively conducted whole transcriptome characterization of tumour samples from mRCC patients treated with ICB. Tumour samples were obtained prior to the start of ICB. A standardized chart review collected clinical, histological, and radiological information. Personal data was anonymized as per data protection laws. Differential gene expression analysis, gene set enrichment analysis (GSEA), gene set variation analysis (GSVA), Microenvironment Cell Population Counter Method (MCP-counter), ESTIMATE score, and the immunophenoscore (IPS) were used to characterise the TME. The objectives of our study were to identify distinct signalling pathways, molecular and immune subtypes associated with histological subtype, tumour behaviour (metastasis organotropism and IMDC risk group), and response, resistance, and toxicity to ICB.

Out of 92 mRCC patients treated with ICB between 2014 and 2019, 71 patients had available tumour tissue, and only 63 samples met the quality and quantity requirements for analysis. Our results showed that the three main RCC histological subtypes presented distinct gene expression patterns. We observed that ccRCC tumours were enriched in immune response hallmark pathways and presented higher immune and stromal infiltration compared to the other two subtypes. In contrast, chrRCC samples presented the lowest immune infiltration and highest tumour purity. In contrast with previously reported data, chrRCC tumours displayed increased activity of the mTOR pathway, eventually supporting the use of mTOR inhibitors in this population. Although limited by a small sample size, we found that tumours from patients with liver as the only metastatic site were enriched in pro-tumoral, metabolic, and immunosuppressive pathways, but also in inflammatory pathways, compared to tumours from patients with bone or lung as the only metastatic site. In addition, tumours with poor IMDC risk displayed an upregulation of Hallmark pathways involved in the cell cycle and immune response, which could explain the aggressiveness and poor prognosis of these tumours, as well as their increased response to ICB.

Tumour samples were also analysed according to the response to ICB. Differential gene expression revealed an upregulation of *MET* in tumours from non-responders to ICB, suggesting a role for MET signalling in the context of treatment response in mRCC. We also analysed tumours from mRCC patients treated with both ICB and AA, each of which yielded distinct responses. We found that tumours from non-responders to ICB, as well as tumours from patients responding to AA but not to ICB, were enriched in Hallmark pathways involved in metabolic reprogramming, including lipid metabolism and glycolysis, along with the immunosuppressive TGF-beta pathway. As expected, tumours from patients developing severe irAEs were enriched in Hallmark pathways involved in immune response. In addition, the pleiotropic signalling pathways IL2-STAT5 and TNFalpha signalling via NfKappaB were enriched in both non-responders to ICB and in those developing severe irAEs. Finally, immune gene signatures previously reported to be associated with response to ICB failed to show an association with response, toxicity, histological subtype, or tumour behaviour in our dataset.

In conclusion, our study provides insight into the molecular and immune landscape of mRCC TME and its role in tumour behaviour and antitumor response under ICB. We identified distinct molecular and immune patterns associated with histological subtype, tumour behaviour (metastasis organotropism and IMDC group), response, and toxicity to ICB. Particularly, for the first time to our knowledge, we observed the upregulation of both IL2-STAT5 and TNFalpha signalling via NfKappaB in both tumours from non-responders to ICB but responders to AA and in those with severe irAEs. Further research of these two pathways gene sets could lead to the discovery of potential candidate biomarkers of both resistance and severe toxicity from ICB and thus help guide treatment selection in mRCC. Validation of these results on an external dataset is currently ongoing.

RESUMEN

El cáncer renal de células claras (ccRCC) es un tumor peculiar caracterizado por estar muy vascularizado y ser muy inmunogénico. Debido a estas características, el tratamiento sistémico del cáncer renal se basa en el uso de tanto bloqueadores del punto de control inmunológico (ICB) y antiangiogénicos (AA). Actualmente, las combinaciones de ICB, con bien otro ICB o bien un antiangiogénico, son el estándar de tratamiento de primera línea para el ccRCC avanzado. A pesar de que los ICB proporcionan respuestas duraderas, hasta un 20% de pacientes serán desafortunadamente refractarios a los ICB. Además, un porcentaje no despreciable de pacientes desarrollará eventos adversos graves de origen inmunomediado (irAEs). Por ello, resulta necesaria una mayor comprensión de los mecanismos moleculares de respuesta y resistencia a los ICB, de cara al desarrollo de biomarcadores que nos permitan guiar las decisiones terapéuticas.

Desgraciadamente, hasta la fecha, no existe ningún biomarcador de respuesta a ICB validado. A diferencia de otros tumores, como el cáncer de pulmón o el cáncer urotelial, el papel de PD-L1 como predictor de respuesta a ICB en el ccRCC avanzado sigue siendo controvertido. De manera similar, no se ha demostrado que la carga mutacional tumoral (TMB) esté asociada con la respuesta a ICB. A pesar de que el ccRCC se caracteriza por una alta infiltración inmune, el grado de infiltración del tumor por células T CD8+ no parece ser tampoco un predictor fiable de respuesta a ICB en el ccRCC. El valor de las mutaciones genéticas como predictores de respuesta está siendo investigado, pero aún no se ha establecido ninguna asociación sólida con la respuesta a ICB.

Últimamente, varios estudios translacionales han propuesto la asociación de diferentes firmas genéticas (“26JAVELIN Immuno”, “IMmotion Angiogenesis”, “IMmotion T effector”, “IMmotion Myeloid inflammation”, “Tumor Inflammation signature”) con la respuesta a ICB y/o AA en el ccRCC avanzado. Sin embargo, en el análisis post-hoc de biomarcadores realizado en el ensayo CHECKMATE214, ninguna de esas firmas genéticas logró demostrar una asociación significativa con la respuesta a nivolumab más ipilimumab o a sunitinib en pacientes con RCC avanzado vírgenes de tratamiento. En cuanto a los no células claras, la información sobre biomarcadores de respuesta es escasa. Según el análisis del TCGA, hasta el 80% de los carcinomas papilares tipo 1 son portadores de una alteración del gen *MET* o una ganancia del cromosoma 7 (que alberga el gen *MET*). Esto ha allanado el camino para el diseño de ensayos clínicos basados en biomarcadores, y también para el diseño del ensayo pivotal PAPMET que convirtió al inhibidor de MET cabozantinib en un nuevo estándar de tratamiento en primera línea para el carcinoma papilar de células renales avanzado.

La hipótesis del presente estudio es que una mejor comprensión del panorama molecular e inmune del microambiente tumoral (TME) en pacientes con RCC metastásico (mRCC) tratados

con ICB nos ayudará a identificar los mecanismos subyacentes de resistencia y respuesta a los ICB. Específicamente, proponemos que las diferencias en expresión genética se traducirían en diferencias en la composición de la infiltración inmune del tumor que explicarían no solo los resultados clínicos del ICB, sino también el desarrollo de eventos adversos graves de origen inmunomediado (irAE) y el comportamiento del tumor. Además, los cambios en la expresión genética también podrían desencadenar la reprogramación metabólica y la regulación de las vías protumorales que también podrían influir en la respuesta a los ICB.

Para ello, realizamos de manera retrospectiva una caracterización del transcriptoma completo de muestras tumorales de pacientes con mRCC tratados con ICB. Las muestras de los tumores se obtuvieron antes del inicio del ICB. La revisión de historias clínicas estandarizada recopiló información clínica, histológica y radiológica. Los datos personales fueron anonimizados según las leyes de protección de datos. Para caracterizar el TME se realizó análisis diferencial de expresión génica, gene set enrichment analysis (GSEA), gene set variation analysis (GSVA), y se usaron el Microenvironment Cell Population counter method (MCP-counter), el ESTIMATE score y el inmunophenoscore (IPS). El objetivo de nuestro estudio fue la identificación de distintas vías de señalización, subtipos moleculares y subtipos inmunes asociados con el subtipo histológico, el comportamiento del tumor (organotropismo de metástasis y grupo de riesgo según el IMDC) y con la respuesta/resistencia/toxicidad secundaria a los ICB.

De 92 pacientes con mRCC tratados con ICB entre 2014 y 2019, 71 pacientes tenían tejido tumoral disponible y solo 63 muestras cumplieron con los requisitos de calidad y cantidad para el análisis. Nuestros resultados mostraron que los tres subtipos histológicos principales de RCC presentaban distintos patrones de expresión génica. Observamos que los RCC de células claras estaban enriquecidos en las vías de señalización involucradas en la respuesta inmune y presentaban una mayor infiltración inmune y estromal en comparación con los otros dos subtipos. Por el contrario, las muestras de RCC cromóforos presentaron la infiltración inmune más baja y la mayor pureza tumoral. En línea con publicaciones previas, los RCC cromóforos mostraron una mayor actividad de la vía mTOR, lo que eventualmente respaldaría el uso de inhibidores de mTOR en esta población. Aunque limitados por un tamaño de muestra pequeño, los tumores de pacientes con metástasis exclusivamente a nivel hepático, presentaron un enriquecimiento en vías de señalización pro-tumorales, metabólicas e inmunosupresoras, pero también en vías inflamatorias, en comparación con los tumores de pacientes con el pulmón o los huesos como único órgano afecto de metástasis. Además, los tumores de pobre pronóstico según el IMDC mostraron una regulación positiva de las vías de señalización involucradas en el ciclo celular y la respuesta inmune, lo que podría explicar la agresividad y el mal pronóstico de estos tumores, pero también su mayor respuesta a los ICB.

También se analizaron las muestras tumorales según la respuesta a los ICB. La expresión genética diferencial reveló una regulación positiva de *MET* en tumores de pacientes no

respondedores a ICB, lo que sugiere un papel de la señalización de MET en el contexto de la respuesta al tratamiento con ICB en mRCC. También analizamos los tumores de pacientes con mRCC tratados tanto con ICB como con AA, y bajo los cuales habían presentado respuestas opuestas. Descubrimos que los tumores de los pacientes no respondedores a los ICB, así como los de los pacientes respondedores a los AA pero no a los ICB, estaban enriquecidos en las vías Hallmark implicadas en la reprogramación metabólica, incluido el metabolismo lipídico y la glucólisis, así como en la vía de señalización inmunosupresora de TGF-beta. Como era de esperar, los tumores de pacientes con irAE graves presentaron una regulación positiva de las vías de señalización implicadas en la respuesta inmunitaria. Además, observamos que las vías de señalización pleiotrópica IL2-STAT5 y TNFalfa a través de NfKappaB estaban enriquecidas tanto en los tumores de pacientes no respondedores a los ICB como en aquellos que desarrollaron irAE graves. Finalmente, firmas génicas inmunes publicadas previamente y que se habían asociado con beneficio clínico en pacientes tratados con ICB o de AA, no lograron mostrar en nuestro estudio ninguna asociación con la respuesta o toxicidad a los ICB, ni con el subtipo histológico o el comportamiento del tumor.

En conclusión, nuestro estudio proporciona información detallada sobre el panorama molecular e inmunológico del TME en el mRCC y su papel en el comportamiento tumoral y la respuesta antitumoral en pacientes tratados con ICB. Identificamos distintos patrones moleculares e inmunes asociados con el subtipo histológico, el comportamiento del tumor (organotropismo de metástasis y grupo IMDC), la respuesta y la toxicidad secundaria a ICB. En particular, por primera vez hasta donde sabemos, mostramos que las vías Hallmark de señalización IL2-STAT5 y TNFalfa a través de NfKappaB están reguladas positivamente tanto en tumores no respondedores a ICB, pero respondedores a AA, como en tumores de pacientes con irAE graves. Una mayor investigación de los conjuntos de genes correspondientes a estas dos vías podría conducir al descubrimiento de posibles biomarcadores candidatos tanto de resistencia a ICB como de toxicidad grave secundaria a ICB y, por lo tanto, podría ayudar a guiar la selección del tratamiento en el RCC avanzado. Con este fin, actualmente estamos trabajando en la validación de estos resultados en un dataset externo.

CONTENT

ACKNOWLEDGEMENTS	XI
SUMMARY	XIII
RESUMEN	XVII
LIST OF ABBREVIATIONS	XXV
ABSTRACT.....	XXVII
 INTRODUCTION.....	 1
I. Epidemiology of renal cell carcinoma	3
1. Incidence, prevalence, and mortality.....	3
2. Social-demographic characteristics.....	3
3. Genetic risk factors	4
4. Modifiable risk factors	5
II. Histological subtypes and Staging	6
1. Histological subtype.....	6
2. Pathologic prognostic factors	9
III. Systemic treatment of renal cell carcinoma	12
1. The cytokine era	13
2. The targeted therapy era	14
3. The Immuno-Oncology era	15
4. Current systemic treatment recommendations for mRCC	18
5. Treatment toxicity profiles.....	18
IV. Genomic profiling and tumour microenvironment as biomarkers in RCC.....	20
1. Genomic profiling in renal cell carcinoma	20
A. Clear-cell RCC	22
B. Papillary RCC	24
C. Chromophobe RCC	24
2. Tumour microenvironment in ccRCC.....	24
A. TME-related RNA signatures.....	25
B. TME components	27
3. Other clinical-biological biomarkers.....	31

HYPOTHESIS AND OBJECTIVES.....	33
I. Hypothesis	35
II. Objectives	36
General Aim	36
Specific Objectives.....	36
METHODS.....	39
I. Study population, samples acquisition and data collection.....	41
II. Ethical Committee approval	41
III. Tissue preparation	41
IV. RNA extraction and RNA quality evaluation	42
V. Amplification, labelling, hybridization and scanning.....	42
VI. Gene expression and quality control.....	42
VII. Differential gene expression, pathway analysis and immune profile analysis	43
VIII. Quantify the Immunotherapy Response Predictor: Immunophenoscore.....	44
IX. Assessment of transcriptomic signatures.....	44
X. Statistical analysis	45
RESULTS.....	47
I. Clinical and pathological characteristics.....	49
II. Differential gene expression according to renal cell carcinoma histological subtypes.....	51
III. Differential gene expression and metastasis organotropism	62
IV. Differential gene expression and IMDC risk group.....	64
V. To identify distinct molecular signatures associated to response and/or resistance to ICB through gene expression profiling	70
1. Responders versus non-responders	70
2. Differential response to ICB and AA.....	79
VI. To identify distinct molecular signatures associated with the development of severe irAEs through gene expression profiling	85
VII. To assess previously published gene expression signatures in our dataset	89

DISCUSSION	93
I. Differential gene expression according to renal cell carcinoma histological subtypes.....	96
II. Differential gene expression and metastasis organotropism	98
III. Differential gene expression and IMDC risk group.....	100
IV. To identify distinct molecular signatures associated with response and/or resistance to ICB through gene expression profiling.....	101
V. To identify distinct molecular signatures associated with severe irAEs through gene expression profiling	105
VI. Limitations	106
CONCLUSION.....	109
BIBLIOGRAPHY	115
ANNEXES	137
I. Annexe I. Review 1	140
II. Annexe II. Original article 1.....	142
III. Annexe III. Case series 1	144
IV. Annexe IV. Original article 2	146
V. Annexe V. Review 2.....	148
VI. Annexe VI. Original article 3	150
VII. Annexe VII. Original article 4	152

LIST OF FIGURES

Figure 1. Location and cell of origin of RCC histological subtypes.	7
Figure 2. Treatment landscape for mRCC.	12
Figure 3. Evolving treatment landscape in mRCC.	13
Figure 4. Objective response and complete response rate to ICB-combinations from pivotal trials.	16
Figure 5. Systemic treatment recommendations for metastatic ccRCC.	18
Figure 6. Summary of the toxicity of antiangiogenics, mTOR inhibitors, and immune checkpoint blockers.	19
Figure 7. TCGA Pan-kidney cancer analysis.	20
Figure 8. Role of PBRM1 in established ccRCC tumours.	23
Figure 9. The International Metastatic Renal Cell Carcinoma Database Consortium.....	31
Figure 10. Whole gene expression analysis flowchart.....	49
Figure 11. Response rates according to histological subtypes.	51
Figure 12. Differential gene expression according to RCC histological subtypes.	52
Figure 13. Heatmap of all expressed genes.	53
Figure 14. GSEA of Hallmark gene sets associated with metabolic protumoral and inflammatory pathways in clear cell tumours versus papillary tumours.....	55
Figure 15. GSEA of Hallmark gene sets associated with metabolic protumoral and inflammatory pathways in clear cell tumours versus chromophobe tumours.	56
Figure 16. GSEA of Hallmark gene sets associated with metabolic protumoral and inflammatory pathways in chromophobe tumours versus papillary tumours.....	57
Figure 17. Heatmap showing pan-cancer patients grouped by hierarchical clustering using the GSVA enrichment scores for 29 immune-related gene sets and stratified by patient features.	58
Figure 18. Heatmaps of GSVA and MCP-counter analyses.	60
Figure 19. Distribution of stromal score, immune score, ESTIMATEscore, and tumour purity according to histological subtype.	61
Figure 20. GSEA of gene sets associated with metabolic protumoral and inflammatory pathways in patients with liver-only metastases versus those with either lung-only or bone-only metastases.	63
Figure 21. GSEA of Hallmark gene sets enriched or downenriched in tumours of poor IMDC patients versus those from good or intermediate IMDC patients.....	65
Figure 22. GSVA (17 immune pathways) and MCP-counter do not clearly discriminate between IMDC groups.....	67
Figure 23. GSVA (17 immune pathways) and MCP-counter do not clearly discriminate between IMDC groups in clear cell tumours.	68

Figure 24. Differentially expressed genes between responders and non-responders and between responders and progressors.	70
Figure 25. GSEA of Hallmark gene sets enriched/downenriched in tumours of responders to ICB versus non responders to ICB.	71
Figure 26. GSEA of Hallmark gene sets enriched or downenriched in clear cell tumours of responders to ICB versus progressors to ICB	72
Figure 27. (A) GSVA (17 immune pathways) does not clearly discriminate between responders and non responders to ICB.	73
Figure 28. GSVA (17 immune pathways) and MCP counter seem to discriminate between responders and non responders to ICB among clear cell tumours.	74
Figure 29. (A) MCP-counter does not clearly discriminate between responders and non responders to ICB.	76
Figure 30. Distribution of StromalScore, ImmuneScore, ESTIMATE Score and tumour purity according to response type.	77
Figure 31. Distribution of immunophenoscore (IPS) according to response type.	78
Figure 32. GSEA of Hallmark gene sets enriched or downenriched in tumours of patients with non-clinical benefit (non-CB) with ICB but clinical benefit (CB) with AA versus CB with ICB but not with AA	82
Figure 33. GSVA (17 immune pathways) and MCP-counter do not discriminate between those tumours benefiting from ICB but not from AA, and those with the opposite behaviour.	83
Figure 34. Gene set enrichment analysis (GSEA) of Hallmark gene sets enriched or downenriched in tumours of patients with severe irAEs versus those without severe irAEs	86
Figure 35. GSVA (17 immune pathways) and MCP-counter do not discriminate between tumours from patients with and without severe irAEs.	87
Figure 36. GSVA (17 immune pathways) and MCP-counter do not discriminate between tumours from patients with and without severe irAEs among clear cell tumours.	88
Figure 37. GSEA plots of three previously published gene signatures (TIS, 26-JAVELIN and Immunosuppression)	90
Figure 37. Graphical summary of our study and results.	95

LIST OF TABLES

Table 1. Main hereditary kidney cancer syndromes	4
Table 2. WHO 2022 classification of RCC.....	6
Table 3. Types and characteristics of sporadic pRCC.	8
Table 4. ISUP Classification system (72).	9
Table 5. TNM Staging system for Renal Cancer from AJCC/UICC (8 th edition, 2017)	11
Table 6. Key results from main pivotal trials and registries for treatment-naïve mRCC.....	17
Table 7. Clinical and molecular features of the different main histological subtypes of renal cell carcinoma (WHO 2016 classification).	21
Table 8. Main TME-related RNA signatures in mRCC.....	26
Table 9. Prognostic and predictive value of the main TME components in mRCC	29
Table 10. Efficacy results of the main mRCC trials according to PD-L1 expression.	30
Table 11. Previously published gene signatures evaluated in our dataset.....	45
Table 12. Baseline clinical and pathological characteristics and comparison between responders and non-responders.	50
Table 13. Characteristics and clinical outcomes of patients deriving benefit from ICB but not from AA.....	80
Table 14. Characteristics and clinical outcomes of patients deriving benefit from AA but not from ICB.	81

LIST OF ABBREVIATIONS

AA: antiangiogenics	GSEA: Gene set Enrichment Analysis
AEs: adverse events	GSVA: Gene set Variation Analysis
AJCC: American Joint Committee on Cancer	H&E: hematoxiline and eosin
Atezo: atezolizumab	HIC: high Immune cluster
Avelu: avelumab	HIF: hypoxia inducible factor
Axi: axitinib	HLA: human leucocyte antigens
BAP1: BRCA1-associated protein 1	HR: hazard ratio
Beva: bevacizumab	ICB: immune checkpoint blockers
Ca9: carbonic anhydrase-9	IO: immuno-oncology
Cabo: cabozantinib	IDO1: indoleamine 2,3-dioxygenase 1
CB: clinical benefit	IFN: interferon
ccRCC: clear cell Renal cell carcinoma	IHC: immunohistochemical
CDKN2B: cyclin dependent kinase inhibitor 2B	IL-2: interleukin-2
chrRCC: chromophobe Renal cell carcinoma	IMDC: International mRCC Database Consortium
CI: confidence interval	Int: intermediate
CR: complete response	Ipi: ipilimumab
CSS: cancer specific survival	IPS: Immunophenoscore
CTLA-4: cytotoxic T-lymphocyte-associated antigen 4	irAEs: Immune-related adverse events
DDR: DNA damage repair genes	IRB: Institutional Review Board
DEG: differentially expressed genes	IS: Immunosuppression signature
dNLR: derived-neutrophil to lymphocyte ratio	ISUP: International Society of Urological Pathology
EGFR: Epidermal growth factor receptor	KEGG: Kyoto Enciclopedia of Genes and Genomes
ESRD: end-stage renal disease	KPS: Karfnosky Performance status
EZH2: enhancer of zeste homolog 2	LAG3: Lymphocyte Activation gene 3
FABP3: fatty acid binding protein 3	Lenva: lenvatinib
Fav: favourable	HLRCC: Hereditary Leiomyomatosis and Renal cell carcinoma
FC: fold change	LIC: Low Immune cluster
FDR: false discovery rate	LIPI: Lung Immune Prognostic Index
FFPE: formalin-fixed-paraffin-embedded	MCP-counter: Microenvironment Cell Populations-counter
FH: fumarate hydratase	mFUP: Median follow-up
FLCN: folliculin	MHC: Major histocompatibility
GE: gene expression	MIC: Medium Immune cluster
GO: Gene Ontology	

MiT: Microphthalmia transcription factor	RWD: real world data
mOS: median overall survival	SD: stable disease
MSKCC: Memorial Sloan Kettering Cancer Center	SDHD/SDHC: succinate dehydrogenase subunit D/C
mTOR: mammalian target of rapamycin	SETD2: SET Domain containing 2
MVI: microvascular invasion	T-regs: regulatory T cells
N: number of patients	TAM: tumors-associated macrophages
NA: not available	TCA: tricarboxylic acid
Nb: number	TCGA: The Cancer Genome Atlas
NES: normalised enrichment score	TERT: telomerase reverse transcriptase
NGS: next-generation sequencing	TFE3: transcription factor binding to IGHM Enhancer 3
Nivo: nivolumab	TGF-beta: transforming growth factor-beta
NK: natural killer	TIM-3: T cell immunoglobulin and mucin domain-containing protein 3
NR: non-responder	TIS: Tumour inflammation signature
NSCLC: non-small cell lung cancer	TKI: tyrosine-kinase inhibitors
ORR: objective response rate	TLS: tertiary lymphoid structure
PBRM1: protein polybromo-1	TMB: tumour mutational burden
PCA: principal component analysis	TME: tumour microenvironment
PD: progressive disease	TSC1/TSC2: tuberous sclerosis complex subunits 1/2
PD.-L1: programmed death ligand 1	VEGF: vascular endothelial growth factor
PDGFR: platelet-derived growth factor	VEGFR: vascular growth factor receptor
PFS: progression-free survival	VHL: von Hippel-Lindau
pRCC: papillary renal cell carcinoma	WHO: World Health Organization
R: response	USA: United States of America
RCC: renal cell carcinoma	
RECIST: response evaluation criteria of solid tumours	
RMA: robust multiarray average	
RNAseq: RNA sequencing	

ABSTRACT

Title: Molecular taxonomy of renal cell carcinoma (RCC) and response to targeted therapies

Keywords: kidney cancer; immune checkpoint blockers; transcriptomics; gene expression; biomarkers.

Background: Immune checkpoint blocker (ICB) combination therapy, either in combination with another ICB or with an antiangiogenic (AA) is the standard of care in a first-line setting for metastatic RCC (mRCC) patients. Although ICB provide durable responses, a significant subset will still be refractory to ICB. Unfortunately, despite great efforts, there is still a lack of reliable biomarkers to help guide treatment selection in mRCC. We hypothesised that a better understanding of the molecular and immune features of the tumour microenvironment of mRCC patients treated with ICB would help us identify the underlying mechanisms of resistance and response to ICB. Thus, our aim was to provide a better understanding of the immune landscape of the TME of mRCC and of the underlying mechanisms of resistance to ICB.

Methods: We retrospectively conducted whole transcriptome characterization of tumour samples from mRCC patients treated with ICB. Tumour samples were obtained prior to the start of ICB. A standardised chart review collected clinical, histological, and radiological information. Personal data was anonymized as per data protection laws. Differential gene expression analysis, gene set enrichment analysis (GSEA), gene set variation analysis (GSVA), Microenvironment Cell Population Counter Method (MCP-counter), ESTIMATE score, and the immunophenoscore (IPS) were used to characterise the TME. The objectives of our study were to identify distinct signalling pathways, molecular and immune subtypes associated with histological subtype, tumour behaviour (metastasis organotropism and IMDC risk group), and response, resistance, and toxicity to ICB.

Results: Out of 92 mRCC patients treated with ICB between 2014 and 2019, 71 had available tumour tissue, and only 63 samples were included for analysis. Clear cell tumours were enriched in immune response Hallmark

pathways and presented higher immune and stromal infiltration. In contrast, chrRCC samples presented the lowest immune infiltration and highest tumour purity and displayed increased activity of the mTOR pathway. Tumours from patients with liver-only metastasis were enriched in pro-tumoral, metabolic, and immunosuppressive pathways, but also in inflammatory pathways. Poor IMDC tumours displayed an upregulation of pathways involved in the cell cycle and immune response. Differential gene expression revealed an upregulation of *MET* in tumours from non-responders to ICB. Tumours from non-responders to ICB, as well as tumours from patients responding to AA but not to ICB, were enriched in Hallmark pathways involved in metabolic reprogramming along with the immunosuppressive TGF-beta pathway. Tumours from patients with severe irAEs were enriched in immune response pathways. IL2-STAT5 and TNFalpha signalling via NfKappaB were enriched in non-responders to ICB and in those developing severe irAEs. Immune gene signatures previously reported to be associated with response to ICB failed to show an association with response, toxicity, histological subtype, or tumour behaviour in our dataset.

Conclusion: We identified distinct molecular / immune patterns associated with histological type, tumour behaviour, response, and toxicity to ICB. IL2-STAT5 and TNFalpha signalling via NfKappaB pathways were upregulated in tumours from non-responders to ICB but responders to AA and from those with severe irAEs. Validation of these results on an external dataset is currently ongoing.

INTRODUCTION



I. EPIDEMIOLOGY OF RENAL CELL CARCINOMA

1. Incidence, prevalence, and mortality

Renal cancer ranks twelfth in the ranking of solid tumours, with 400.000 new cases in 2018 worldwide. The incidence of renal cancer, unlike other tumours, continues to rise due to the increased development of tumour screening and registry programmes (1). This is the reason why the incidence of cancer is relatively higher in the more developed countries, especially in Europe and the USA. On the other hand, an earlier diagnosis has reduced cancer-related mortality in many European countries (2). In Spain, the estimate of new renal cancer cases in 2020 is estimated at around 280,000 (3). Specifically, kidney cancer ranks ninth in incidence with 7300 new cases in 2020 and seventh in prevalence with $\approx$ 21.500 cases in 2018 (3,4).

In terms of mortality, kidney cancer constitutes the second most lethal genitourinary tumour, behind bladder cancer, occupying the eleventh place in the mortality rank among all solid tumours in 2018, with 2131 deaths that year (4).

2. Social-demographic characteristics

- **Sex and age:** The incidence of kidney cancer is higher in men than women (2:1). This difference is stable by age over time and across countries worldwide, suggesting that it is intrinsic to sex (5,6). The risk of developing kidney cancer increases with age, especially over 75 years (5,7).
- **Geography and ethnicity:** The incidence of renal cell carcinoma (RCC) varies greatly between countries and continents. In general, countries with a lower incidence of RCC are located in Africa and Asia (1/100.000 inhabitants) and those with a higher incidence (>10/100.000 inhabitants) in Europe, North America, and Australia (7), between the years 2003 and 2007.

3. Genetic risk factors

Around 5% of kidney cancers have a hereditary predisposition. To date, more than 10 hereditary kidney cancer syndromes have been reported, and in general, they are characterised by mutations in genes related to metabolism and epigenetic regulation(8) (Table 1).

Syndrome	Gene	RCC subtype
Von Hippel-Lindau	<i>VHL</i>	Clear cell RCC
Birt-Hogg-Dubé	<i>FLCN</i>	Chromophobe RCC, oncocytoma or an hybrid of both, clear cell
Hereditary papillary RCC	<i>MET</i>	Papillary RCC
Reed syndrome or hereditary leiomyomatosis (HLRCC)	<i>FH</i>	Papillary RCC
Paraganglioma-Hereditary feocromocytome	<i>SDHBM SDHD, SDHC</i>	Clear cell RCC, oncocytic neoplasm, cromophobe
Cowden syndrome	<i>PTEN</i>	Clear cell RCC, papillary or chromophobe
Tuberose sclerosis	<i>TSC1</i> y <i>TSC2</i>	Clear cell RCC, angiomyolipomes
3p chromosome translocation	Includes loss of : <i>VHL</i> , <i>BAP1</i> , <i>PBRM1</i> y <i>SETD2</i>	Clear cell RCC
BAP1-related tumor predisposition syndrome	<i>BAP1</i>	Clear cell RCC
Other reported germline mutations : <i>PBRM1</i> , <i>CDKN2B</i>		

Table 1. Main hereditary kidney cancer syndromes.

Note. Adapted from “Hereditary kidney cancer syndromes: Genetic disorders driven by alterations in metabolism and epigenome regulation” by Hasumi H, Yao M. 2018. Cancer Sci. 109(3):581–6.

4. Modifiable risk factors

Smoking, obesity, and hypertension are the main risk factors associated with a higher chance of developing kidney cancer. Smoking increases the risk of kidney cancer in both sexes, and the risk of developing kidney cancer is strongly associated with the dose (9,10). Obesity is a well-established risk factor for kidney cancer. It has been estimated that overweight and obese people have an increased risk of RCC of 28% and 77%, respectively (11). However, despite its association with RCC, it seems that metastatic RCC (mRCC) patients with a high body mass index may present a more indolent behaviour (12). Hypertension is considered an independent risk factor for RCC (13). The biological rationale for this association could be that hypertensive patients could be subjected to chronic hypoxia secondary to hypoxia-inducible factors (HIF) which in turn promote proliferation and angiogenesis (14).

Data is limited, but it seems physical activity and a diet rich in vegetables and fruits may have a protective effect, while red meat consumption seems to increase the risk of RCC (15–18). In addition, based on different meta-analyses and cohort studies, it seems moderate alcohol consumption has been associated with a lower risk of kidney cancer (19–22). Currently, it is not clear the mechanism by which exposure to alcohol can act as a protective factor; however, one of the hypotheses could be the increase in insulin sensitivity observed with alcohol consumption, as hyperinsulinemia and insulin resistance may increase the risk of RCC (23–25).

Finally, although RCC is not considered an occupational disease, some specific jobs and industrial products have been linked to an increased risk of RCC, such as trichloroethylene (26).

II. HISTOLOGICAL SUBTYPES AND STAGING

1. Histological subtype.

Renal cell carcinoma is histologically heterogenous (27). Histological classification is key due to its prognostic and therapeutic implications. The histological classification of kidney tumours has greatly evolved from the first WHO and Mainz classification in 1986 to the current WHO classification (World Health Organization) of 2022, where multiple new categories were added (28). According to this classification, renal cell tumours can be classified into different types according to their histology and molecular characteristics. (Table 2 and Figure 1).

WHO 2022 Classification of renal cell carcinoma	
Clear cell RCC	Succinate dehydrogenase-deficient RCC
Papillary RCC	ALK-rearranged RCC
Chromophobe RCC	Medullary carcinoma, NOS
Collecting duct carcinoma	SMARCB1-deficient medullary-like RCC
Mucinous tubular and spindle cell carcinoma	SMARCB1-deficient undifferentiated RCC, NOS
Multicentric cystic renal neoplasia with low malignant potential	SMARCB1-deficient dedifferentiated RCC, of other specific subtypes
Tubulocystic RCC	Clear cell papillary RCC
Acquired cystic disease-associated RCC	Renal cell carcinoma, unclassified
TFE3-rearranged RCC	Papillary adenoma
TFEB-altered RCC	Oncocytoma
ELOC (formerly TCEB1) mutated RCC	Other oncocytic tumours of the kidney
Fumarate hydratase-deficient RCC	Eosinophilic solid and cystic RCC
Hereditary leiomyomatosis and RCC syndrome-associated RCC	

Table 2. WHO 2022 classification of RCC

Note. Adapted from “The 2022 WHO Classification of Tumours of the Urinary System and Male Genital Organs—Part A: Renal, Penile, and Testicular Tumours” by Moch H, Amin MB, Berney DM, Compérat EM, Gill AJ, Hartmann A, et al. European Urology. 2022 Nov;82(5):458–68.

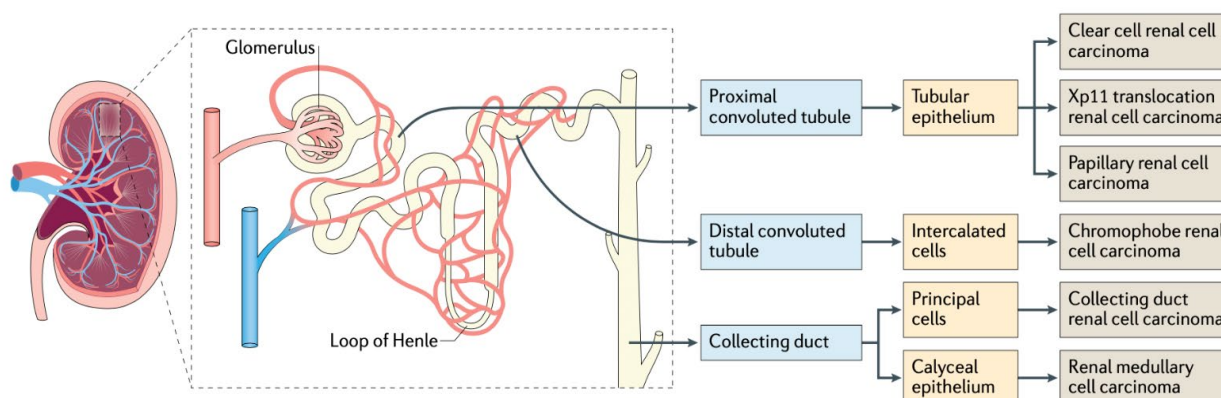


Figure 1. Location and cell of origin of RCC histological subtypes.

Note. From “Genomic profiling in renal cell carcinoma” by Dizman N, Philip EJ, Pal SK. Nat Rev Nephrol. 2020 Aug;16(8):435–51.

Clear cell renal cell carcinoma (ccRCC) is the most frequent subtype, accounting for 70-80% of malignant kidney tumours. The term “clear cell” refers to its morphology under a microscope, with a clear cytoplasm that does not stain for haematoxylin-eosin and is delimited by a marked membrane (29). In 90% of cases, they present an alteration of the von Hippel-Lindau suppressor gene located on chromosome 3, which activates pro-angiogenic signalling pathways (30). Up to 95% of ccRCCs are sporadic, but up to 5% can be associated with hereditary syndromes such as VHL syndrome or tuberous sclerosis (Table 1). Most ccRCCs are solitary and have a cortical localization (29). From an immunohistochemical (IHC) point of view, they are characterised by expressing cytokeratins of low molecular weight (8,18,19) and vimentin (31).

Papillary RCC (pRCC) is the second most frequent histological subtype, accounting for 10-15% of RCCs (27,30,32) (Table 3). Most pRCCs are sporadic, but cases associated with hereditary syndromes have been reported, such as hereditary leiomyomatosis and RCC syndrome (HLRCC). This syndrome is characterised by the development of multiple uterine and cutaneous leiomyomas, as well as the development of an aggressive form of RCC (33,34). This syndrome is caused by a germline mutation of the fumarate hydratase (*FH*) gene that codes for a Krebs cycle enzyme (35,36). Although this type of RCC has traditionally been considered a pRCC, we now know that it can present in the shape of different histological subtypes (30–32,37). In this sense, since the last update of the WHO histological classification in 2016, this disease is now considered as “HLRCC associated RCC” (27).

Types and characteristics of sporadic pRCC		
	Type 1 pRCC	Type 2 pRCC
Prognosis	Better	Worse
Incidence	More frequent	Less frequent
Morphology	Little basophil cytoplasm Rounded core Little apparent nucleolus Arranged in the hyaline-vascular axes of the papillae. Histiocytes accumulation Psammoma bodies	Abundant and eosinophilic cytoplasm Voluminous nucleus Prominent nucleolus Irregular papillae Large cellules
Nuclear grade	Low	High
TNM	Less advanced disease	More advanced disease
Immunohistochemistry	CD10, keratine 7, p504S MUC1	CD10, keratine 7, p504S e-cadherine
Molecular alterations	<i>MET</i> alterations / <i>EGFR</i> mutations	<i>SETD2</i> or <i>CDKN2A</i> mutations/ <i>TFE3</i> fusions

Table 3. Types and characteristics of sporadic pRCC.

Chromophobe RCC (chrRCC) is the third most frequent subtype, accounting for around 5% of all RCCs (29). Most are sporadic, but hereditary forms are also seen, such as the Birt-Hogg-Dubé associated chrRCC (38). In general, this tumour has an indolent behaviour with slow local growth and is characterised by a favourable prognosis, with an 80%–90% specific 10-year cancer survival (32). However, it can sometimes present a sarcomatoid transformation of greater aggressiveness. Histologically, it is characterised by large cells with transparent cytoplasm, marked membranes, and binucleated, irregular, and hyperchromatic nuclei (29,39). The tumour suppressor protein *TP53* is mutated in 32% of chrRCC tumours, and the mTOR (mammalian target of rapamycin) signalling pathway is frequently altered in these tumours (39).

The collecting duct carcinoma is a rare histological subtype (1%) that derives from the main cells of the distal segment of the Bellini collector ducts in the renal marrow (40). This tumour is characterised by aggressive behaviour and a poor prognosis (40).

The multilocular cystic RCC variant accounts for 4% of RCCs and has indolent behaviour with an excellent prognosis (41).

In addition to all the histological subtypes included in Table 2, approximately 5% of renal tumours do not meet criteria for a specific category and are labelled as unclassifiable RCCs. There is another group of tumours in which the epithelial element is not recognisable, which are usually high-grade or sarcomatoid carcinomas and have a poor prognosis.

2. Pathologic prognostic factors

During the 2012 ISUP conference, a consensus was reached on the factors contributing to the poor prognosis of adult renal tumours.

- Histological subtype (Tabla 2)
- Tumour grade (Tabla 4). Until recently, the Fuhrman classification system was the tumour grading system used in clinical practice. This system, developed in 1982, defines four grades based on nuclear characteristics, being able to identify three different prognostic groups (grade 1, grade 2-3, and grade 4)(42,43). However, in 2012 the International Society of Urological Pathology proposed a new grading system (the ISUP grading system) based on nucleolar prominence and the presence of rhabdoid and/or sarcomatoid differentiation (Table 4)(30,44). The use of this grading system is limited to ccRCC and pRCC, as its prognostic role is not yet validated in other types of tumours (45).

ISUP classification system	
Grade 1	Nucleoli absent or inconspicuous and basophilic at 40x (rare)
Grade 2	Nucleoli not prominent at 10x but visible and eosinophilic at 40x (40% of tumours)
Grade 3	Nucleoli conspicuous and eosinophilic at 10x (30 - 40% of tumours)
Grade 4	Extreme nuclear pleomorphism, multinucleated cells, rhabdoid/sarcomatoid differentiation (15% of tumours)

Table 4. ISUP Classification system (72).

Note. Adapted from “The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs-Part A: Renal, Penile, and Testicular Tumours” by Moch H, Cubilla AL, Humphrey PA, Reuter VE, and Ulbright TM. 2016. Eur Urol. 70(1):93–105.

- Sarcomatoid differentiation. RCC with sarcomatoid differentiation is not considered a specific RCC subtype because the presence of sarcomatoid areas can be found in all the different histological subtypes (44). However, it is important to report changes or the presence of sarcomatoid dedifferentiation, as it has a poor prognosis and also has therapeutic implications (44,46).
- Rhabdoid differentiation. It is characterised by neoplastic cells that morphologically resemble rhabdomyoblasts but differ from these by their ultrastructural and immunophenotypic characteristics (44,47). Just as sarcomatoid differentiation does not constitute a specific histologic subtype of RCC, rhabdoid differentiation represents a pathway of RCC decriminalisation towards a more aggressive and high-grade biological pattern (44).

- Microvascular invasion (MVI). It is defined as the presence of a tumour in the small vessels within the renal parenchyma (44). MVI is considered an independent predictor of tumour recurrence after surgery, but there is not enough evidence to confirm its independent prognostic value in nonmetastatic RCC (44,48,49).
- Tumour necrosis. It is described in up to 27-31% of cases, and it seems to be related to the histologic subtype. Thus, it is observed in 27-32% of ccRCC, 32-40% of pRCC, and 3-14% chrRCC (44,50-53). Tumour necrosis seems to have a prognosis value in ccRCC; however, for the moment, it has not been validated in other histologic subtypes (53).
- pTNM staging system. This classification system was developed and updated by the American Joint Committee on Cancer/International Union Against Cancer (AJCC/UICC) (Table 5). “T” refers to tumour, “N” refers to lymph nodes, and “M” refers to distant metastasis (Table 5). The term stage requires the combination of three elements (T,N,M) that have a prognostic value. pTNM alludes to the “pathologic” classification of TNM, which is the clinical stage supplemented by intraoperative findings and pathological, macroscopic, and microscopic evaluation of the surgical piece or biopsy (54).

TNM Staging System for Renal Cancer from AJCC/UICC(8th edition, 2017)	
T	Primary tumour
Tx	Primary tumour can not be assessed
T0	No evidence of the primary tumour
T1	Tumour ≤7cm in greatest dimension, limited to the kidney
T1a	Tumour ≤4cm in greatest dimension, limited to the kidney
T1b	Tumour >4 cm but ≤7cm in greatest dimension, limited to the kidney
T2	Tumour >7cm in greatest dimension, limited to the kidney
T2a	Tumour >7 cm but ≤10cm in greatest dimension, limited to the kidney
T2b	Tumour >10cm in greatest dimension, limited to the kidney
T3	Tumour extends into major veins or perinephric tissues but not into the ipsilateral adrenal gland and not beyond Gerota fascia
T3a	Tumour extends into the renal vein or its segmental branches, or invades the pelvicalyceal system, or invades perirenal and/or renal sinus fat but not beyond Gerota's fascia
T3b	Tumour extends into the vena cava and below the diaphragm
T3c	Tumour extends into the vena cava above the diaphragm or invades the wall of the vena cava
T4	Tumour invades beyond the Gerota's fascia (including contiguous extension into ipsilateral adrenal gland)
N	Regional lymph nodes (RLN)
Nx	RLN can not be assessed
N0	No regional lymph node metastasis
N1	Metastasis in RLN
M	Distant metastasis
M0	No distant metastasis
M1	Distant metastasis

Stage grouping			
	T	N	M
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T1-T2	N1	M0
	T3	Nx, N0, N1	M0
Stage IV	T4	Any N	M0
	Any T	Any N	M1

Table 5. TNM Staging system for Renal Cancer from AJCC/UICC (8th edition, 2017)

Note. Adapted from "The AJCC Cancer Staging manual", 8th edition (2017).

III. SYSTEMIC TREATMENT OF RENAL CELL CARCINOMA

Approximately one out of every three RCC patients will not be candidates for surgery at diagnosis due to locally advanced or metastatic disease. In addition, 30% of RCC patients with localised disease treated with nephrectomy with a curative intent will relapse in distant sites and will therefore require systemic treatment (55,56).

RCC is a chemo-resistant tumour where, in addition, hormonotherapy has no activity (57). RCC is characterised, however, by high vascularization and immunogenicity. Based on these features, the systemic treatment of RCC is based on antiangiogenics (AA) and immunotherapy, consisting of cytokine administration, formerly and now immune checkpoint blockers (ICB) (58) (Figure 2 and 3).

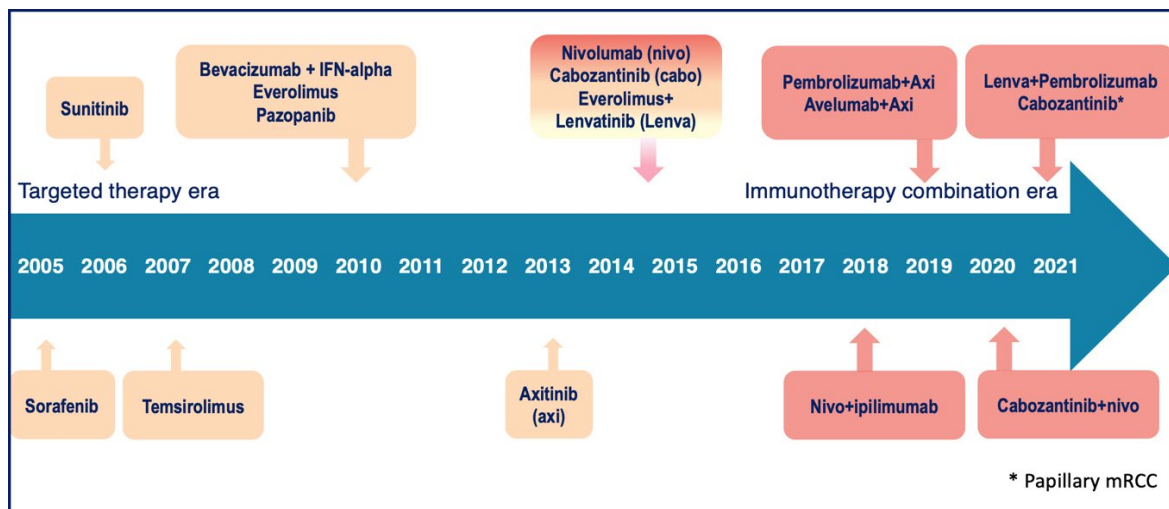


Figure 2. Treatment landscape for mRCC.

Abbreviations: IFN: interferón; Nivo: nivolumab; Cabo: cabozantinib; Lenva: lenvatinib; axi: axitinib.

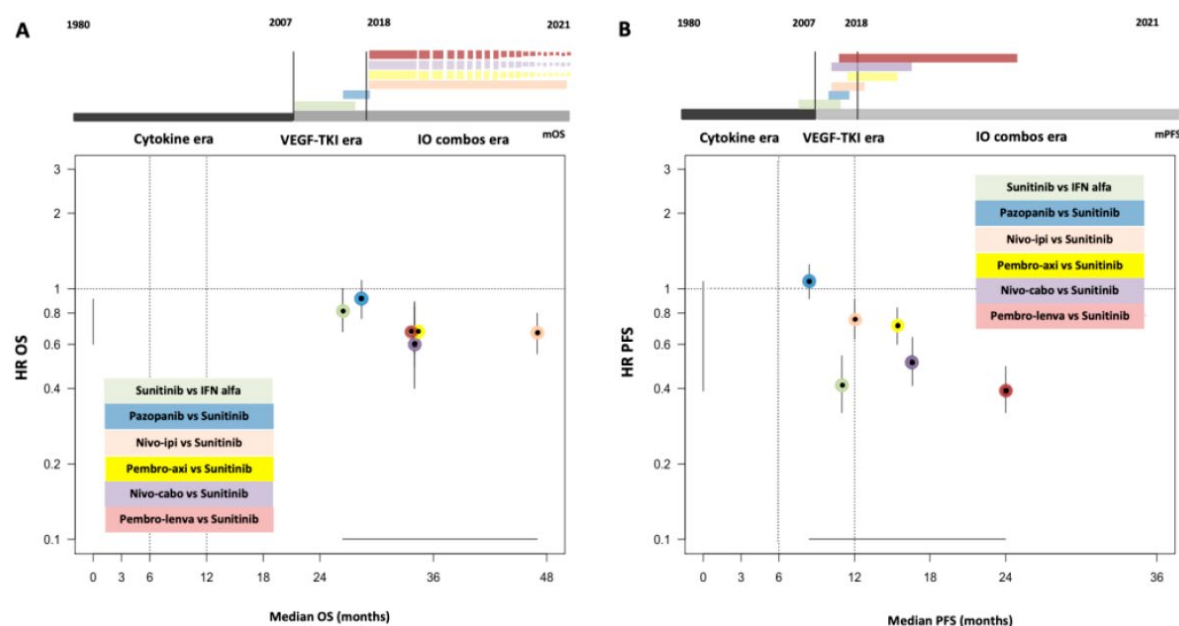


Figure 3. Evolving treatment landscape in mRCC.

These two panels summarize the impact of each treatment on progression-free survival (PFS) (A) and overall survival (OS) (B). The Javelin 101 and the IMmotion151 have not been included in this figure due to still lack of significant survival advantage. *: not reached; longer follow up needed. VEGF-TKI: vascular endothelial growth factor-tyrosine kinase inhibitors. IO: immune-oncology therapy. Pembro-axi: pembrolizumab-axitinib. Nivo-ipi: nivolumab-ipilimumab. Nivo-cabo: nivolumab-cabozantinib. Pembro-lenva: pembrolizumab-lenvatinib. HR: hazard ratio.

1. The cytokine era

Long considered an immunogenic tumour, immunotherapy has been the cornerstone of mRCC for decades. Immunotherapy was first introduced in the 1980s and 1990s in the form of cytokines such as interleukin 2 (IL-2), interferon alpha, or a combination of both. However, the efficacy of this traditional immunotherapy was limited, achieving objective response rates (ORR) of 5-20% and a median overall survival (mOS) of 7-12 months for mRCC with no negligible toxicity (59–64). (Figure 3). Nevertheless, up to 10% could achieve complete remission with high-dose IL-2, and therefore it became the preferred regimen for mRCC, even though its indication was limited due to its severe toxicity (65). Currently, due to the development of other more effective and better tolerated therapies, the use of cytokines has been left behind.

2. The targeted therapy era

A better understanding of the biology of the RCC allowed us to identify potential novel therapeutic targets involved in angiogenesis, such as the vascular endothelial growth factor (VEGF), its receptor (VEGFR), and the platelet-derived growth factor (PDGFR), and in cell proliferation, such as the mTOR pathway (Figure 3 and Table 6).

Consequently, the first pivotal trials of targeted therapy agents, mainly antiangiogenics and mTOR inhibitors, were carried out after 2005 (Figure 3 and Table 6). Thus, in 2007 the first antiangiogenic (AA) tyrosine kinase inhibitors (TKI) were approved. In 2007 sunitinib was approved in treatment-naïve mRCC after improving compared to interferon alpha (IFN alpha) PFS (11.0 versus 5.0 months; HR 0.42, 95% CI. 0.32-0.54) and OS (26.4 versus 21.8 months; HR 0.82, 95% CI. 0.67-1.00)(66). The same year, sorafenib was approved in a second-line setting, according to the results of the TARGET trial, which showed an improvement in both PFS and OS compared to placebo (66,67). The use of combined bevacizumab and IFN alpha was also assessed in two randomised phase III trials, demonstrating benefit in PFS compared to interferon alfa but not in terms of OS (68,69).

Not long after, the first mTOR inhibitor, temsirolimus, was approved based on the results of the Global ARCC trial, where it showed superiority compared to interferon alfa monotherapy, both in terms of OS and PFS (68–70). At the same time, everolimus, another mTOR inhibitor, was approved in VEGF-refractory patients based on the results of the phase III RECORD-1 trial (71). During 2010–2014, other multitargeted TKIs targeting VEGF joined the therapeutic arsenal of mRCC, such as pazopanib in the first-line setting and axitinib in the second-line setting (72–74).

More recently, during the transition into “the immuno-oncology (IO) era” a novel oral potent multikinase inhibitor targeting VEGFR, AXL, and MET, cabozantinib, was incorporated into the treatment landscape of RCC in a second-line setting based on the results of the phase 3 METEOR trial (75). Furthermore, in 2015, cabozantinib was also approved as first-line treatment for intermediate-poor-risk patients, according to the International mRCC Database Consortium criteria (IMDC), based on the results of the phase 2 randomised CABOSUN trial. In this trial, cabozantinib showed a significant increased PFS (8.2 vs 5.6 months; HR 0.66, 95% CI 0.46-0.95) and ORR (33 vs 12%) compared to sunitinib in intermediate-poor-risk treatment-naïve mRCC patients, with a manageable and similar toxicity profile to other AA (76). At that time, another novel multikinase inhibitor targeting VEGFR, FGFR, PDGFR, RET, and c-KIT, lenvatinib, was incorporated into the second-line setting in combination with everolimus, based on its superiority in terms of both PFS and ORR compared to everolimus alone (75).

3. The Immuno-Oncology era

The treatment landscape of advanced RCC switched again after 2015 with the development and incorporation of ICB as a standard of care, both in treatment-naïve and previously treated mRCC patients (58) (Figure 3 and Table 6). Firstly, in 2015 nivolumab, an anti-PD-1 monoclonal antibody, was approved as a second or further treatment line in mRCC patients previously treated with an antiangiogenic based on the results of the randomised phase 3 Checkmate 025 trial. This trial demonstrated the superiority of nivolumab over everolimus in terms of OS (25.0 vs 19.6 months, HR 0.73, 95% CI 0.57-0.93) and ORR (25 vs 5%), regardless of PD-L1 expression, and a more tolerable toxicity profile (77).

Subsequently, based on the non-overlapping effects of anti-CTLA-4 and anti-PD-1/PD-L1, the development of double checkpoint inhibition was proposed to further improve the anti-tumour response. Anti-CTLA-4 antibodies induce predominantly memory T cell proliferation, while anti-PD-1/PD-L1 antibodies induce greater cytotoxicity and natural killer (NK) proliferation, providing the rationale for the combination of these agents (78). The Checkmate 214 led to the approval in 2018 of nivo-ipi in a front-line setting for intermediate-to-poor-risk mRCC patients after demonstrating superior OS, PFS, and ORR compared to sunitinib in this population, with updated results showing a mOS of 47 versus 26.6 months in the sunitinib arm (HR 0.66, 95% CI 0.55-0.80), a PFS of 12 versus 8.3 months for sunitinib (HR 0.76, 95% CI 0.63-0.91), and a 65% ORR versus 50% in the sunitinib arm, with a 10% CR versus 1% for sunitinib (79,80) (Table 6 and Figure 4).

Further, based on the presumed interplay of the immune system and the angiogenesis in carcinogenesis, clinical trials investigating the combination ICB with AA were also conducted (81). The KEYNOTE-426 compared pembro-axi versus sunitinib in treatment-naïve mRCC patients, demonstrating the superiority of this ICB-AA combination over sunitinib in both coprimary endpoints: PFS (15.4 vs 11.1 months; HR 0.71, 95% CI 0.60-0.84) and OS (NR vs 35.7 months; HR 0.68, 95% CI 0.55-0.85), as well as a superiority in ORR (60 vs 40%), regardless of PD-L1 expression (82,83) (Figure 4 and Table 6). Combinations of atezolizumab-bevacizumab (atezo-beva) or axitinib-avelumab (axi-avelu) did not show improvement in OS.

More recently, the nivolumab-cabozantinib (nivo-cabo) combination has become a new front-line treatment option for treatment-naïve mRCC based on its superiority over sunitinib in terms of both PFS, OS, and ORR, regardless of IMDC risk group and PD-L1 expression (84) (Figure 4 and Table 6). Finally, the three-arm phase III CLEAR study demonstrated the superiority of pembrolizumab-lenvatinib (pembro-lenva) versus sunitinib in both terms of PFS, OS, and ORR, achieving a complete response rate (CR) of 16% (85) (Figure 4 and Table 6).

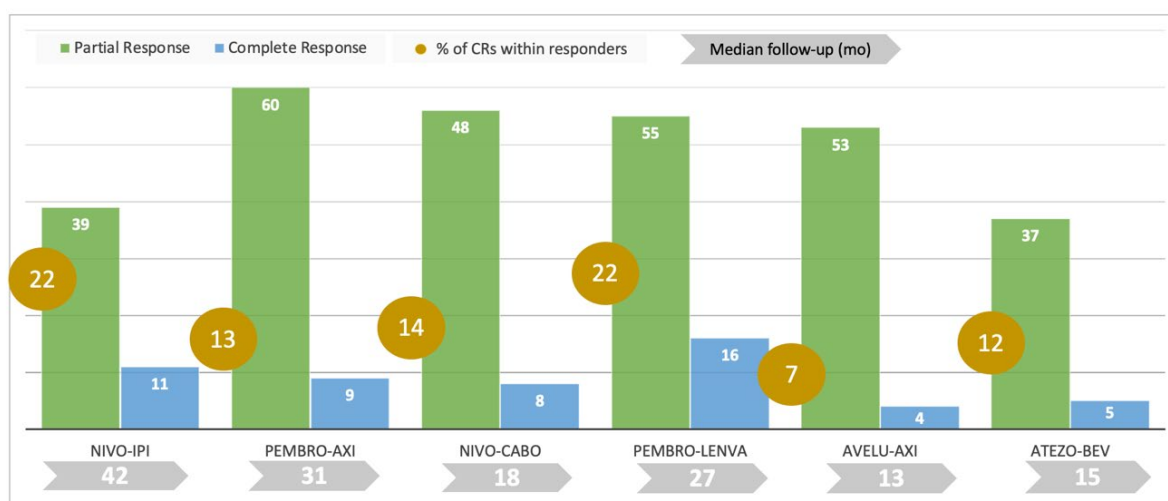


Figure 4. Objective response and complete response rate to ICB-combinations from pivotal trials.

Abbreviations: NIVO-IPI: nivolumab-ipilimumab; PEMBRO-AXI: pembrolizumab-axitinib; NIVO-CABO: nivolumab-cabozantinib; PEMBRO-LENVA: pembrolizumab-lenvatinib; AVELU-AXI: avelumab-axitinib; ATEZO-BEV: atezolizumab-axitinib; CR: complete response; mo: months. Adapted from “Complete response and Renal cell carcinoma in the Immunotherapy era: The paradox of good news” by Zambrana F, Carril-Ajuria L, Gómez de Liaño A, Martínez Chanza N, Manneh R, Castellano D, et al. 2021. Cancer Treatment Reviews.

Trials	Phase	Sample size	Treatment arms	Year	mPFS (m) HR (CI)	mOS (m) HR (CI)	ORR (%) P value	CR (%)	G ≥3 AEs	Risk groups (%)			mFU (m)
										FAV	INT	POOR	
CHECKMATE 214 (113)	3	1096	Nivo-ipi vs Sunitinib	2018	12.4 vs 12.3 0.88 (0.75-1.04)	NR vs 38.4 0.72 (0.61-0.86)	39 vs 33 0.02	11 vs 2	46 vs 63	23	62	17	42
KEYNOTE 426 (117)	3	861	Pembro-Axi vs Sunitinib	2018	15.4 vs 11.1 0.71 (0.60-0.84)	NR vs 35.7 0.68 (0.55-0.85)	60 vs 40 <0.0001	9 vs 3	76 vs 71	32	55	13	30.6
CHECKMATE 214 (120)	3	651	Nivo-cabo vs Sunitinib	2020	16.6 vs 8.3 0.51 (0.40-0.89)	NR vs NR 0.60 (0.40-0.89)	56 vs 27 <0.0001	8 vs 5	61 vs 51	23	58	19	18.1
CLEAR (121)	3	1069	Pembro-lenva Evero-lenva Sunitinib	2021	24(PL)vs 15(EL) vs 9(S) HR1: 0.35 (0.32-0.49) HR2: 0.65 (0.53-0.86)	NR(PL)vsNR(EL)vs NR(S) HR1: 0.66 (0.49-0.88) HR2: 1.15 (0.88-1.50)	71(PL)vs 54(EL) vs 36(S) 1.15 (0.88-1.50)	16 (PL) 10 (EL) 4 (S)	72 (PL) 73 (EL) 54 (S)	NA	NA	NA	27
JAVELIN Renal 101 (118)	3	886	Avelu-Axi vs Sunitinib	2019	13.8 vs 7.0 0.69 (0.57-0.82)	Immature 0.80	53 vs 27 <0.001	3.8 vs 2	71 vs 72	20	65	15	13
IMMOTION 151 (114)	3	915	Atezo-Beva vs Sunitinib	2019	15.0 vs 7.7 ** 0.74 (0.57-0.96)	33.6 vs 34.9 0.93 (0.76-1.14)	37 vs 33	5 vs 2	40 vs 54	20	69	12	15
NCT00083889 (97)	3	750	Sunitinib vs IFN alpha	2009	11.0 vs 5.0 0.42 (0.32-0.54)	26.4 vs 21.8 0.82 (0.67-1.0)	47 vs 12 <0.001	3 vs 1	NA	35	56	6	NA
COMPARZ (104)	3 non-inferiority	1110	Pazopanib vs Sunitinib	2013	8.4 vs 9.5 1.05 (0.90-1.22)	28.4 vs 29.3 0.91 (0.76-1.08)	31 vs 25 0.03	<1 vs <1	NA	25	55	18	NA
PROCLAIM registry* (94)	Retrospective	810	High dose interleukin-2	2019	NA	Favor risk: 63.3 Inter risk: 43.4 Poor risk: 14	24	5.4	<1	31	59	10	23.4

Table 6. Key results from main pivotal trials and registries for treatment-naïve mRCC.

Abbreviations: N: number of patients included. mPFS: median progression free survival. mOS: median overall survival. mFU: median follow up. ORR: overall response rate. CI: confidence interval. RWD: real world data. HR: hazard ratio. HR1: HR pembro-lenva vs sunitinib. HR2: evero-lenva vs sunitinib. NR: not reached. Nivo-ipi: nivolumab-iplimumab. Pembro-axi: pembrolizumab-axitinib. Nivo-cabo: nivolumab-cabozantinib. Avelu-axi: avelumab-axitinib. Atezo-beva: atezolizumab-bevacizumab. Pazo: pazopanib. INF: interferon. G ³ AEs : grade ³ adverse events. FAV: favorable. INT: intermediate. *89% treatment naïve. ** PD-L1 positive population.

4. Current systemic treatment recommendations for mRCC

Immunotherapy has become the new backbone of systemic therapy for RCC. The use of ICB combinations in frontline settings led to an OS improvement and a dramatic increase in CR (Figure 4). Thus, ICB combinations are strongly recommended by all treatment guidelines in first-line settings. Nivo-cabo, pembro-lenva, and pembro-axi are recommended for all risk groups, while nivo-ipi is only recommended in intermediate-to-poor-risk patients (Figure 5). In those cases in which ICB therapy cannot be given due to unavailability or intolerance, sunitinib and pazopanib remain options, regardless of risk group, and cabozantinib can be an additional option in intermediate-to-poor-risk patients. Regarding second-line therapy, patients who have received ICB combinations should be offered any AA that has not been given in combination with ICB, and those previously treated with AA monotherapy could be offered nivolumab monotherapy or cabozantinib (Figure 4).

First-line therapy		
	Standard of care	Alternative*
IMDC favourable risk	Nivolumab/cabozantinib Pembrolizumab/Lenvatinib Pembrolizumab/axitinib	Pazopanib*
IMDC intermediate and poor risk	Nivolumab/cabozantinib Pembrolizumab/Lenvatinib Pembrolizumab/axitinib Nivolumab/ipilimumab	Cabozantinib Sunitinib Pazopanib
Later-line therapy		
	Standard of care	Alternative
Prior IO	Any VEGF-targeted therapy that has not been used previously in combination with IO	
Prior TKI	Nivolumab Cabozantinib	Axitinib

Figure 5. Systemic treatment recommendations for metastatic ccRCC.

Note. Adapted from “European Association of Urology Guidelines on Renal Cell Carcinoma: The 2022 Update” by Ljungberg B, Albiges L, Abu-Ghanem Y, Bedke J, Capitanio U, Dabestani S, et al. European Urology. 2022 Mar;S0302283822016761.

5. Treatment toxicity profiles

Each family of drugs (AA, mTOR inhibitors and ICB) is associated with a unique toxicity profile. The main associated toxicities for each family of drugs are described in Figure 6. Class-effect toxicities observed with AA include fatigue, asthenia, diarrhoea, nausea, anorexia, rash, hand-foot syndrome, or hypertension. Class-effect toxicities observed with mTOR inhibitors include stomatitis, mucositis, hyperglycemia, hypertriglyceridemia, fatigue, anorexia, pneumonitis, or diarrhoea. Specifically, ICBs are generally better tolerated and are characterised by a late onset of adverse events, which generally occur between 8-12 weeks from the start of treatment, with colitis and skin toxicity being the most frequent and the ones with the earliest onset. AA tyrosine kinase inhibitors and mTOR inhibitor-related toxicities are usually managed with dose interruption, dose reduction, or schema adaptation, along with medical-oriented treatment, according to the severity of the adverse events. In contrast, although the toxicity managing varies according to organ system affected, immune-related adverse events are usually managed with medical treatment if necessary, for grade 1-2 toxicities; dose interruption and start of systemic corticosteroids treatment may be considered in grade 2 toxicities with treatment resumption when toxicities revert to grade 1 or less, and grade 3 toxicities usually warrant treatment cessation and high-dose corticosteroids that should be tapered over 4-6 weeks. Grade 4 toxicities, except for endocrinopathies, will warrant permanent discontinuation of ICB.

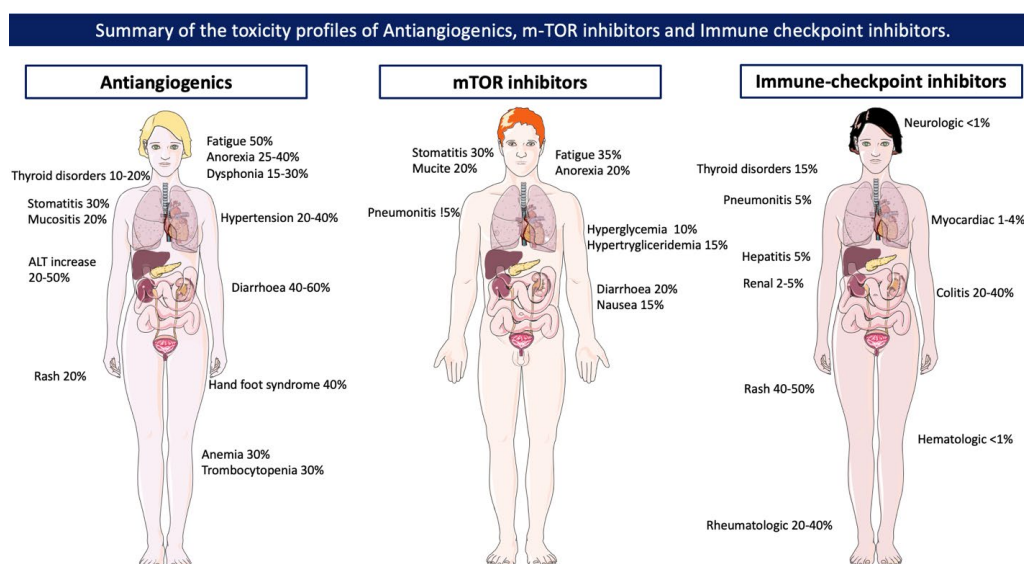


Figure 6. Summary of the toxicity of antiangiogenics, mTOR inhibitors, and immune checkpoint blockers.

In this new therapeutic context, with such a wide range of treatments that have greatly improved survival outcomes, there is a need to update scoring systems and identify biomarkers that will guide treatment decisions, improve survival outcomes, and avoid unnecessary toxicities.

IV. GENOMIC PROFILING AND TUMOUR MICROENVIRONMENT AS BIOMARKERS IN RCC

1. Genomic profiling in renal cell carcinoma

RCC is not only a heterogeneous disease from a histological point of view, but also from a molecular perspective, with different patterns of genetic alterations associated to each histological subtype (Table 7 and Figure 7).

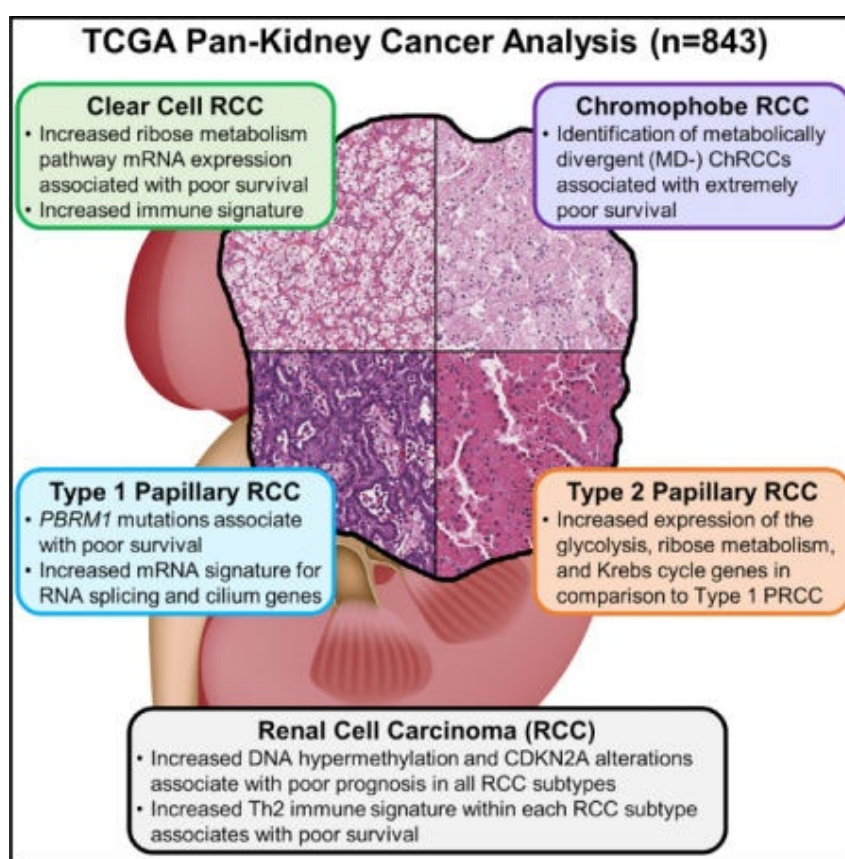


Figure 7. TCGA Pan-kidney cancer analysis.

Note. From “The Cancer Genome Atlas Comprehensive Molecular Characterization of Renal Cell Carcinoma” by Ricketts CJ, De Cubas AA, Fan H, Smith CC, Lang M, Reznik E, et al. Cell Reports. 2018 Apr;23(1):313-326.e5.

Subtype	Clinical features	Molecular/biologic
Major subtypes		
Clear cell	75% of RCC-heterogeneous biologic behaviour	Loss of VHL in the majority
Papillary type I	Slow growing/less likely to metastasize	<i>MET</i> alterations, chromosomal gains
Papillary type II	Often aggressive course, but some oncoytic; multiple molecular entities	3 molecular entities: <i>CDKN2A</i> silencing; <i>SETD2</i> , <i>BAP1</i> , <i>PBRM1</i> mutations; increased NRF2-antioxidant response pathway; CIMP phenotype- <i>FH</i> mutation-see below; <i>TFE3</i> fusions-see below.
Chromophobe	Indolent; rare metastases, but if so, often liver, often oligometastases	Impairment of gamma-glutamyltransferase 1 activity
Translocation	Pediatric; young adults; 40% lymph node involvement; range of intermediate to poor risk	MiT family translocations; previously t(6;11); Xp11; nuclear <i>TFE3</i> fusions
Medullary	Sickle trait; aggressive; widely metastatic, chemotherapy	Loss of SMARCB1 (chromatin remodeler and tumour suppressor)
Collecting duct	Aggressive; chemotherapy, some targeted therapies	IHC-PAX8 and integrase interactor-1 (INI-1); unique transcriptomic profile-metabolic shift-impaired oxidoreductase activity, pyruvate metabolism, and TCA cycle.
2016 New classifications		
HLRCC	Hereditary/poor prognosis	Fumarate hydratase mutation
SDH deficient	Hereditary/poor prognosis	Succinate dehydrogenase deficient-dysfunction of mitochondrial complex II
Tubulocystic	Indolent/oncocytoma-like; very rarely metastatic present at lower grade and stage	Downregulated non-coding miRNA expressions compared with papillary; ongoing research
Acquired cystic	Often indolent, arise in ESRD; calcium oxalate crystal deposition common	Fewer unfavorable pathological features than other RCCs
Clear cell papillary	Low-grade clear cells arranged in papillae	Coexpression of CA9, HIF-alfa, GLUT-1; absence of <i>VHL</i> gene alterations.

Table 7. Clinical and molecular features of the different main histological subtypes of renal cell carcinoma (WHO 2016 classification).

Ca9: carbonic anhydrase-9; ESRD: end-stage renal disease; HIF: hypoxia inducible factor; HLRCC: hereditary leiomyomatosis and RCC syndrome associated RCC; IHC: immunohistochemistry; RCC: renal cell carcinoma; SDH: succinate dehydrogenase-deficient RCC; TCA: tricarboxylic acid; *VHL*: von Hippel-Lindau.

Note. Adapted from “The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs—Part A: Renal, Penile, and Testicular Tumours” by Moch H, Cubilla AL, Humphrey PA, Reuter VE, Ulbright TM. 2016. European Urology. 70(1):93–105.

A. Clear-cell RCC

The somatic genomic landscape of ccRCC tumours is characterised by *VHL* inactivation through the loss of the chromosome arm 3p (encompassing the genes *VHL*, *PBRM1*, *BAP1*, and *SETD2*), which is found altered in ≈90% of the cases. However, even if *VHL* biallelic inactivation is a critical founder event, it is not sufficient for tumour development, and additional mutations and epigenetic changes are necessary. *VHL* is an E3 ligase substrate that labels the transcription factors hypoxia-inducible factor (HIF) 1α and HIF2α with ubiquitin for proteosomal degradation. The accumulation of HIF triggers the uncontrolled expression of angiogenesis-promoting genes and gives rise to highly vascularized tumours (86).

Recent advances in next-generation sequencing (NGS) and their implementation in large cohorts of cancer patients (e.g. The Cancer Genome Atlas (TCGA) projects), have led to the identification of additional genes frequently mutated in ccRCC, such as *PBRM1* (≈40–50%), *SETD2* (12%), *BAP1* (10%), and *KDM5C* (5%) (86). These are genes coding for proteins participating in the regulation of chromatin remodelling and histone methylation. Although *VHL* is the initiating event in ccRCC, the acquisition of additional mutations is a common characteristic during ccRCC tumour development and metastasis (Figure 8).

Genomic alteration and clinical outcomes in metastatic ccRCC:

- ***PBRM1***, the second most commonly mutated gene in ccRCC, seems to play a relevant role in shaping tumour aggressiveness and pathway dependencies (Figure 8). The gene *PBRM1* encodes the bromodomain-containing protein BAF180 that serves as the DNA-targeting subunit of the pBAF SWI/SNF complex (87). Despite the efforts made to investigate the potential association between *PBRM1* status and clinical outcomes, the potential prognostic and/or predictive value of *PBRM1* still remains unclear. Overall, reported results suggest that *PBRM1* status has a different role in localised and advanced disease, constituting a poor prognostic factor in localised disease and a good prognostic factor in advanced disease (86). In addition, it seems that *PBRM1* may also have a predictive value for response to immunotherapy; however, further studies are needed to confirm this (86,88).

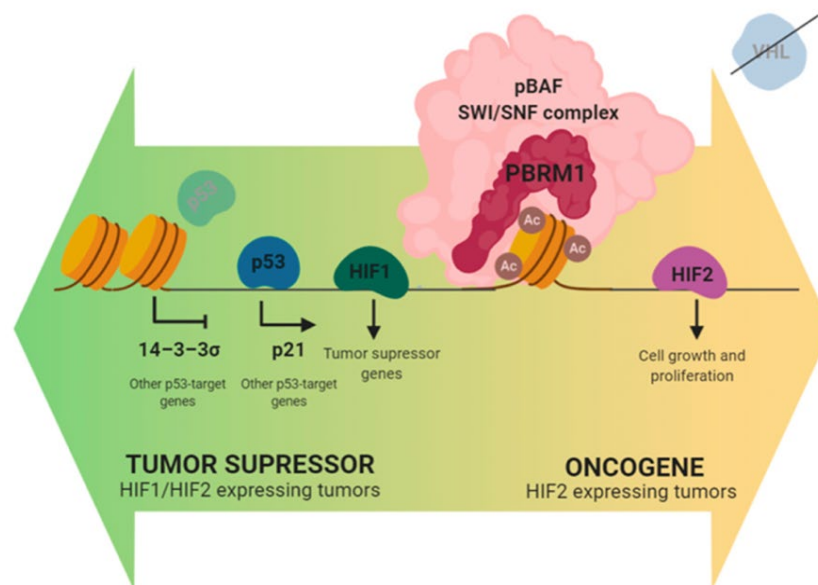


Figure 8. Role of PBRM1 in established ccRCC tumours.

Note. Adapted from "Prognostic and Predictive Value of PBRM1 in Clear Cell Renal Cell Carcinoma" by Carril-Ajuria L, Santos M, Roldán-Romero JM, Rodríguez-Antona C, de Velasco G. *Cancers*. 2019 Dec 19;12(1):16.

- **BAP1** is a two-hit tumour suppressor gene located, similarly to *PBRM1*, in the short arm of chromosome 3. The frequency of *BAP1* mutations in ccRCC ranges from 5-16% (89–91). *BAP1* inactivation is associated with the presence of metastasis at diagnosis, the presence of necrosis, and worse survival outcomes in localised ccRCC (90,92–95). In addition, in the metastatic setting, the genomic analysis conducted by Voss and colleagues in patients from the COMPARZ and RECORD-3 trials found that *BAP1* mutations were associated with worse PFS and OS in treatment-naïve metastatic ccRCC patients treated with VEGF-targeted therapy compared to those with non-mutated *BAP1* (91). Data on the predictive role of *BAP1* in the context of immunotherapy is still lacking.
- **SETD2** is a chromatin regulator and seems to contribute to microsatellite instability (89,96). *PBRM1* and *BAP1* are also located on chromosome 3p. In contrast to *BAP1* and *PBRM1*, which are generally mutually exclusive, *SETD2* mutations are often observed in patients with *PBRM1* mutations (97). Similarly to *BAP1*, an analysis of 421 primary ccRCC patients from the TCGA cohort showed that the *SETD2* mutation was associated with a higher rate of disease recurrence and worse cancer-specific survival (CSS) (95). However, data from the COMPARZ and RECORD-3 trials have failed to demonstrate an association between *SETD2* mutation status and survival outcomes (91).
- **EZH2**: The histone methyl transferase enzyme is encoded by the *EZH2* gene and mediates epigenetic gene silencing of various genes, including tumour suppressor genes (98). High *EZH2* expression is associated with a poor prognosis.

- **DNA damage repair genes** (DDR genes): DDR gene alterations increase tumour-infiltrating lymphocytes, genomic instability, and tumour mutational burden (TMB) which enhances anti-PD1 responsiveness (99–102). Several studies, including urothelial carcinoma, suggest that DDR gene alterations can be predictive of response to ICB (101,102). However, only two studies have evaluated the impact of DDR alteration and response to ICB in RCC, with conflicting results (103,104).

B. Papillary RCC

Papillary RCC exhibits higher overall mutation rates than ccRCC (89,105). Although the rate of chromatin regulator mutations is comparable between both histologic subtypes, in contrast to ccRCC, PBRM1 mutations seem to be associated with worse OS in pRCC (105). Based on these genomic features, several studies have demonstrated the antitumor activity of different multikinase inhibitors targeting MET in pRCC, irrespective of *MET* mutation status, although patients with MET alterations had better outcomes (106–112). Owing to the results of the phase 2 randomised PAPMET trial comparing sunitinib, cabozantinib, savolitinib, and crizotinib in pRCC, which demonstrated the superiority of cabozantinib in terms of PFS, cabozantinib stands as one of the preferred options for this RCC subtype (106).

C. Chromophobe RCC

Genomic data from the TCGA cohort show that overall mutation rates and chromatin regulator gene mutations are lower in chrRCC than in other histologic subtypes (39). Chromophobe RCC is enriched, however, by mutations of two suppressor genes, *TP53* and *PTEN*, the latter of which is associated with worse survival outcomes. As both alterations are more frequent in metastatic than localised chrRCC, it seems they might have a role in metastatic progression. In addition, TERT rearrangements have been identified in chrRCC, suggesting they could drive chrRCC development (39). To date, there is still no evidence of potential predictive markers of response to systemic therapy in this rare type of RCC. However, genomic alterations involved in the mTOR pathway were identified in 23% of chrRCC, suggesting the potential benefit of mTOR therapies in this RCC subtype.

2. Tumour microenvironment in ccRCC

The tumour microenvironment (TME) is a dynamic and heterogenous ecosystem composed of different cellular components, including tumour cells, immune cells, endothelial cells, adipose cells, stromal cells, and extracellular matrix components (113). This ecosystem modulates tumour

development by promoting tumour growth, tumour progression, and metastatic growth. In order to understand the mechanisms of resistance to ICB and identify potential biomarkers of response and resistance, Chevrier et al. reported in 2017 an in-depth immune atlas of the TME of RCC (114). Using mass cytometry, they performed an in-depth immune profiling of samples from 73 ccRCC patients and five healthy controls. T cells were the most frequent immune cell population (50%) in ccRCC tumours, followed by myeloid cells (31%), natural killer cells (9%) and B cells (4%) (114).

A. TME-related RNA signatures

Gene signatures have emerged as promising potential biomarkers of response or resistance to targeted therapy. In 2015, Beuselinck and colleagues developed a 35-gene signature able to classify metastatic ccRCC patients in four different groups with distinct biological features: ccrcc1 = “c-myc-up”, ccrcc2 = “classical”, ccrcc3 = “normal-like”, and ccrcc4 = “c-myc-up and immune-up”, representing, respectively, 33%, 41%, 11% and 15% of patients, and that were associated with different responses to sunitinib (115).

With the incorporation of ICB as a standard of care across different tumour types, an 18-gene signature containing genes related to antigen presentation, chemokine expression, cytotoxic activity, and adaptive immune resistance was validated using samples of patients treated with anti-PD1 therapy (pembro) across different tumour types (116,117). This tumour inflammation signature (TIS) was later applied to tumour gene expression data from the TCGA, including a wide range of immunotherapy-naïve tumour types (116). The results showed that responders to ICB therapy, including RCC patients, had higher TIS scores.

In the context of ICB combination therapy, three main TME-related RNA signatures have been developed to help treatment-decision-making in mRCC (Table 8).

Correlative studies of the phase 2 IMmotion 150 (atezo-beva vs sunitinib or atezo) identified an angiogenesis high signature that was more frequent in the favourable-risk group and correlated with PFS benefit to sunitinib, while the T-effector high signature was more frequent in the intermediate/poor risk group and was associated with PFS benefit to atezo-beva (118). The role of these gene signatures was further confirmed in a comprehensive analysis of 823 tumours of advanced RCC treated with atezo-beva versus sunitinib within the IMmotion 151 trial (119). Interestingly, angiogenic-enrich clusters presented a better prognosis across treatment arms, while immune and cell-cycle-enrich clusters presented a significantly improved ORR with atezo-beva compared to sunitinib. Importantly, responders to atezo-beva were enriched in genes associated with proliferation and immune pathways, while genes associated with the VEGF pathway were enriched in responders to sunitinib (119).

Study and reference	Study design	Nb of patients	Signatures	Genes involved in the signature	Treatments	Biological Material	Study method	Predictive value of Response	
								AA	ICB
IMmotion 150 McDermott et al. (118)	Randomized ph2 and ph3 prospective studies	300 (IMmotion 150)	Angiogenesis T effector Myeloid infl	Angio: <i>VEGFA, KDR, ESM1, PECAM1, NGPTL4</i> , and <i>CD34</i> . T eff: <i>CD8A, EOMES, PRF1, IFNG</i> , and <i>CD274</i> .	Atezo-beva vs Sunitinib (Atezo-beva vs atezo ph2)	FFPE	RNAseq	YES Improved PFS with Sunitinib for Angio ^{high}	YES Improved PFS with Atezo-beva for Angio ^{low} and Teff ^{high}
IMmotion 151 Motzer et al. (119)		851 (IMmotion 151)		Myeloid: <i>IL-6, CXCL1, CXCL2, CXCL3, CXCL8</i> , and <i>PTGS2</i>					
JAVELIN Renal 101 (120)	Randomized ph3 prospective study	886	JAVELIN immuno	T-cell receptor T-cell act, dif, prol NK-mediated cytoto Chemokine Other immune-response genes	Avelu-axi vs Sunitinib	FFPE	RNAseq	NO	YES Improved PFS with avelu-axi for JAVELIN ^{high}

Table 8. Main TME-related RNA signatures in mRCC.

Abbreviations. Ph: phase; Infl: inflammation; Angio: angiogenesis; T eff: T effector; act: activation; dif: differentiation; prol: proliferation; cyto: cytotoxicity; atezo-beva: atezolizumab-bevacizumab; Nb: number; Avelu-axi: avelumab-axitinib; FFPE: formalin-fixed paraffin-embedded; RNAseq: RNA sequencing; PFS: progression-free survival; Angio^{high}: high angiogenesis signature; Angio^{low}: low angiogenesis signature; T eff^{high}: high T effector signature; JAVELIN^{high}: high JAVELIN signature; AA: antiangiogenics; and ICB: immune-checkpoint blocker.

Similarly, higher CD8+ T-cells in the TME correlated with an increased response to avelu-axi in the JAVELIN Renal 101. In addition, the angiogenesis signature was significantly associated with prolonged PFS with sunitinib, while the immune signature was associated with PFS benefit with avelu-axi (120).

Unfortunately, the biomarker analysis from CheckMate 214 (nivo-ipi vs sunitinib) evaluating six different gene expression signatures, including the IMmotion 150 T-effector^{high} and the JAVELIN Renal 101 immuno signatures, among others, did not show any associations between survival and immune-related gene expression signatures (121). It should be noted, however, that these signatures were developed in patients receiving ICB-AA and not double ICB, which could limit their applicability to the CheckMate 214.

B. TME components

- **CD8+ T cells.** Although tumour infiltrating CD8+ T cells are usually associated with good prognosis in most tumour types, CD8+ T cell infiltration is associated with poor prognosis in mRCC (122). They have also been investigated as a potential predictive biomarker in mRCC, however the results are controversial (Table 9). In a retrospective analysis of CD8+ T cell infiltration patterns in baseline tumour samples from either patients treated with anti-PD-1 or mTOR inhibition, the density of CD8+ T-cells in the tumour centre and the tumour margin was not able to find an association between infiltration phenotype and clinical benefit (123). Conversely, in the JAVELIN Renal 101, increased infiltrating CD8+ T-cells were associated with improved PFS in the avelumab-axitinib arm but not in the sunitinib arm.
- **Regulatory T cells (T-regs).** Analysis of the TCGA showed that patients with worse clinical outcomes were enriched in T-regs. To date, there is insufficient data to suggest a predictive role for T-regs in mRCC (124) (Table 9).
- **Tumour-associated macrophages (TAM).** TAM can be divided into two groups: M1-type producing inflammatory cytokines and M2-type expressing PD-1 ligands and anti-inflammatory cytokines and recruiting T-regs to the tumour site (125). Tumour cells can switch the potential phenotype of macrophages into TAM, which is characterised as the M2d subtype. Interestingly, in the analysis conducted by Voss et al. M2 macrophages were associated with favourable outcomes in mRCC treated with ICB, while no association was found with outcomes in AA (126). In the NIVOREN study, CD163 (M2) with higher densities in the invasive margin were associated with better PFS but not OS in mRCC patients treated with nivolumab (127) (Table 9).

- **B cells and tertiary lymphoid** structures Infiltrating B cells have been detected in different solid tumours, including mRCC (114,128). There is increasing evidence that tumour-infiltrating B cells, especially those present with T cells in organised lymphoid aggregates called tertiary lymphoid structures (TLS), correlate with a better prognosis (128,129). Five recently reported studies have shown an association of B cells and TLS with clinical outcomes in cancer patients, including mRCC, treated with ICI (130–134). More recently, in the NIVOREN study, no significant differences in OS/PFS were seen in mRCC patients receiving nivolumab according to the density of TLS, although high-TLS tumours presented a higher chance of response to nivolumab (135) (Table 9).
- **PD-L1.** PD-L1 expression by IHC correlates with overall survival both in localised and metastatic settings. In the localised setting, positive PD-L1 expression correlates with aggressive pathological features as well as with worse clinical outcomes, including PFS and OS (136,137). In the metastatic setting, PD-L1 expression has consistently been associated with a worse prognosis (Table 9). This has been confirmed in a recently published meta-analysis including 1323 mRCC patients (138,139). However, the role of PD-L1 expression as a predictor of response to AA or ICB remains unclear. The pivotal trials of both ICB-based therapy and AA therapy did not find significant differences in the benefit of the experimental arm versus the control arm between the negative and the positive PD-L1 subgroups (Table 10)(77,83,140–144). However, in an exploratory analysis of the CheckMate 214, positive PD-L1 patients presented a significantly superior mPFS (22.8 months) in the nivo-ipi arm compared to the sunitinib arm (5.9 months), whereas no significant difference in PFS was seen in the PD-L1 negative population (140). It also seemed to enhance the survival benefit provided by nivo-ipi compared to sunitinib, as well as the benefit in ORR (140) (Table 9 and 10). More robust data is still needed to demonstrate and validate PD-L1 expression by IHC as a biomarker of response to ICB.
- **LAG3 and TIM-3.** LAG-3 and TIM-3 are co-inhibitory receptors identified on exhausted CD8+ T cells. According to recent results, the combination of PD-L1 expression with other immune checkpoints responsible for the exhausted phenotype of T-cells, such as TIM-3, LAG3, and PD-1, could improve the ability of PD-L1 expression to predict response to ICB (145) (Table 9).

TME Element	Status	Prognostic role	Predictive value of response to ICB
Cells			
CD8+ T cells (122)	High density	Poor	Insufficient data
Regulatory CD4+ T cells (146)	High density	Poor	No
Tumour-associated Macrophages (147)	High density	Poor	Insufficient data
B cells and Tertiary lymphoid structures (TLS) (129,130,135)	High density	Good	Insufficient data
Immune checkpoints			
PD-L1	Overexpression	Poor	No**
LAG3	Overexpression	Poor*	Insufficient data
TIM3	Overexpression	Poor	Insufficient data

Table 9. Prognostic and predictive value of the main TME components in mRCC

Legends: TIM-3 associated with poor clinical outcome in RCC when co-expressed with PD-1+ and CD8+ on T cells. ** Discordant data have been identified in Checkmate 214. Abbreviations: ICB: Immune checkpoint blockers. ccRCC: clear cell renal cell carcinoma. LAG3: lymphocyte-activation gene 3. TIM3: T-cell immunoglobulin and mucin domain-containing protein-3.

Note. Adapted from “Tumor Microenvironment Features as Predictive Biomarkers of Response to Immune Checkpoint Inhibitors (ICI) in Metastatic Clear Cell Renal Cell Carcinoma (mccRCC)” by Simonaggio A, Epailard N, Pobel C, Moreira M, Oudard S, Vano YA. Cancers. 2021 Jan 10;13(2):231.

TRIALS	N _{PD-L1} /N _{Total}	Technique	Positivity	PD-L1+ (%)	Efficacy Endpoint	PD-L1 +	PD-L1-
CHECKMATE 025 (54) Nivolumab vs Everolimus	776/821	Dako PD-L1 IHC 28-8 pharmDx	≥1% (TC)	24.0	OS (m)	N: 21.8 vs E: 18.8 (HR 0.70)	N: 27.4 vs E: 21.2 (HR 0.70)
CHECKMATE 214(4) Nivolumab vs Sunitinib	776/845*	Dako PD-L1 IHC 28-8 pharmDx	≥1% (TC)	27.5	OS (m)	N/I: NR vs S: NR (HR 0.45)	N/I: NR vs S: 19.6 (HR 0.73)
COMPARZ trial(52) Pazopanib vs Sunitinib	453/1100	Anti-PD-L1 mouse IgG AB (clone 5H1)	High >125 High > 55 (TC)	3 (>125) 13 (>55)	OS (m)	P: 5.1 vs S: 8.9	P: 31.6 vs S: 27.4
CABOSUN trial(53) Cabo vs Sunitinib	110/157	HALO platform multiplex-IHC v1.2	≥1% (TC)	23	OS (m)	C: 18.1 vs S: 21.0 (HR 0.85)	C: 30.3m vs 22.4 (HR 0.71)
METEOR trial(53) Cabo vs Everolimus	306/658	HALO platform multiplex-IHC v1.2	≥1% (TC)	29	OS (m)	C: 18.4 vs E: 13.9 (HR 0.82)	C:NR vs 18.4 (HR 0.58)
KEYNOTE 426(10) Pembrolizumab vs Sunitinib	822/861	PD-L1 IHC 22C3 pharmDx	CP5≥1*	60.5	OS (m)	HR: 0.54	HR: 0.59
JAVELIN Renal 101(34) Avelumab vs Sunitinib	804/886	Ventana PD-L1 (SP263)	>1% (IC)	63	PFS (m) OS (m)	A+A: 13.3 vs S: 8.2 (HR: 0.62) HR: 0.83	A+A: 13.3 vs S: 7.0 (HR: 0.69) HR: 0.93
IMMOTION 151(35) Atezolizumab vs Sunitinib	NA/915	Ventana PD-L1 (SP142)	>1% (IC)	39.6	PFS (m) OS (m)	A+B: 11.2 vs S:7.7 (HR: 0.74) HR: 0.84	A+B: 11.2 vs S: 8.4 (HR: 0.83) HR: 0.93

Table 10. Efficacy results of the main mRCC trials according to PD-L1 expression.

Abbreviations: N, number of patients; nivolumab; ipi or I, ipilimumab; cabo or C, cabozantinib; pembrolizumab; axi, axitinib; atezo, atezolizumab; beva, bevacizumab; avelu, avelumab; TC, tumor center; IC, immune cells; m, months; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; A+A, avelumab+axitinib; A+B, atezolizumab+bevacizumab.

3. Other clinical-biological biomarkers

Despite the advances in genomic profiling and a better understanding of the TME in mRCC, there is still no validated biomarker of response to guide systemic therapy. Thus, for now, the IMDC remains the only validated prognostic score for patients treated with targeted therapy independent of treatment line, and therefore it is used as a stratification criteria in clinical trials (Figure 9). Moreover, according to the results of CheckMate 214, it is currently used to guide front-line treatment selection (58,148). Although this classification is consistently used to stratify patients in clinical trials, its role in the “ICB era” still needs to be properly defined.

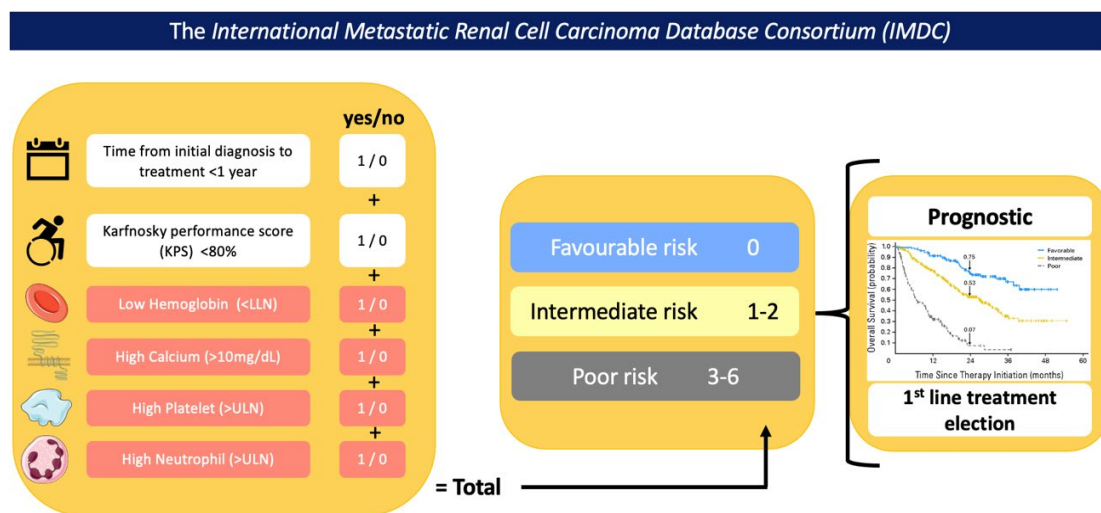


Figure 9. The International Metastatic Renal Cell Carcinoma Database Consortium.

Recently, the IMDC reported the results of a retrospective analysis of 687 patients treated with ICB, showing that the IMDC risk groups were able to stratify patients according to OS in the second through the fourth line of therapy but not in the first line. (149).

Inflammatory markers such as the derived neutrophil to lymphocyte ratio (dNLR) or the Lung Immune Prognostic Index (LIPI, defined by lactate dehydrogenase (LDH) and dNLR) have been associated with a worse prognosis in mRCC (150–153). However, in a post-hoc analysis of the CheckMate 214 LIPI, it failed to demonstrate a predictive role (151).

The location and number of metastatic sites have also been evaluated. In CheckMate 214, responders to nivo-ipi had fewer metastatic sites, fewer bone metastases, and less kidney involvement (154).

HYPOTHESIS AND OBJECTIVES



I. HYPOTHESIS

Currently, there are two different types of systemic therapies that shape the treatment of mRCC: AA and ICB. Immune checkpoint blockers have the potential to provide sustained responses and long-term survival for different solid tumours, including mRCC. However, only a subset of patients will benefit from these treatments, and currently there is still no validated biomarker of response to ICB to help guide treatment selection in mRCC.

Despite its success in different tumour types and its capacity to enrich for response with double ICB in mRCC, the predictive role of PD-L1 in mRCC is still unclear (155). Similarly, there is no evidence for the value of other potential biomarkers of response, such as TMB, tumour indel burden, or human leukocyte antigen zygosity, as predictors of response to ICB in mRCC (118,120,123). In addition, data on the role of infiltrating cytotoxic T cells as predictors of response in mRCC are controversial (120,123). Several gene expression studies have identified molecular subtypes and TME-related RNA signatures associated with response to ICB and AA in mRCC, but they still need to be validated in external cohorts (118,120).

We hypothesized that using whole transcriptome characterization of the TME of mRCC patients treated with ICB we would be able to understand the underlying mechanisms of resistance and response to ICB. Specifically, we propose that differences in gene expression would translate into differences in tumour immune infiltration composition that would explain not only the clinical outcomes to ICB, but also the development of severe immune related adverse events (irAEs) and the tumour behaviour. In addition, changes in gene expression could also trigger metabolic reprogramming and regulation of protumoral pathways that could also influence the outcomes under ICB.

II. OBJECTIVES

General Aim

To carry out gene expression profiling of mRCC tumours treated with ICB in order to identify distinct signalling pathways or molecular subtypes associated with tumour behaviour and response, resistance, or toxicity to ICB.

Specific Objectives

Objective 1.

To perform molecular characterization of RCC histological subtypes through gene expression profiling using gene set enrichment analysis (GSEA), pathway, and immune profile analysis

Objective 2.

To identify molecular signatures that distinguish metastasis organotropism through gene expression profiling using GSEA, pathway, and immune profile analysis in mRCC tumours treated with ICB.

Objective 3.

To identify distinct molecular signatures associated with IMDC risk groups through gene expression profiling using GSEA, pathway, and immune profile analysis in mRCC tumours treated with ICB.

Objective 4.

To identify distinct molecular signatures associated with response and/or resistance to ICB through gene expression profiling using GSEA, pathway, and immune profile analysis in mRCC tumours treated with ICB.

Objective 5.

To identify distinct molecular signatures associated with the development of severe irAEs through gene expression profiling using GSEA, pathway, and immune profile analysis in mRCC tumours treated with ICB.

Objective 6.

To evaluate the predictive value of previously published gene expression signatures (Tumour inflammation signature, JAVELIN Renal 101 and Immunosuppression signature) in mRCC tumours treated with ICB.

METHODS



I. STUDY POPULATION, SAMPLES ACQUISITION AND DATA COLLECTION

This single-centre retrospective study included non-consecutive patients over the age of 18 years with a histologic confirmed diagnosis of mRCC, who had received ICB therapy among other treatments, irrespective of treatment line, and with available tumour tissue. Formalin-fixed-paraffin-embedded (FFPE) tissue samples were collected under Institutional Review Board (IRB)-approved protocols at the University Hospital 12 de Octubre. A central pathological review was performed at the local anatomical pathology department.

A standardised chart review collected the date of diagnosis, age at diagnosis, gender, date of nephrectomy, date of first metastasis, prognostic factors according to the IMDC, type of metastatic site at initiation of systemic treatment, the different treatment lines, and the clinical outcomes and toxicity within each therapy line. Personal data was anonymized as per data protection laws. All patients had regular computed tomography (CT) scanner evaluations every two or three months, based on local practice. The response to treatment was determined locally by the Response Evaluation Criteria for Solid Tumours (RECIST).

II. ETHICAL COMMITTEE APPROVAL

This study was approved by the Ethics Committee for Human Research with Medicinal Products of the University Hospital 12 de Octubre (Nº CEIm: 18/055) and conducted in accordance with the International Conference on Harmonization and the Declaration of Helsinki.

III. TISSUE PREPARATION

The archival FFPE RCC tissue sections and FFPE blocks were stored at room temperature. Five μm tumour sections were obtained from each tumour block using a microtome. At least two sections at the beginning and end of sectioning were processed on slides and stained with haematoxylin and eosin (H&E) to monitor the histological subtype and estimate the percentage of tumour cells. Blocks with at least 50% of tumour content were selected for inclusion in the study. If tumour content was less than 50%, sections were subjected to macrodissection following the H&E-stained sections. Adjacent serially unstained sections were then submitted for RNA extraction.

IV. RNA EXTRACTION AND RNA QUALITY EVALUATION

Total RNA was extracted from five recently cut 10 µm FFPE section using the RNA Storm RNA isolation kit for FFPE tissue samples (Cell Data) according to the manufacturer's protocol. The yield and purity of RNA were determined using the RNA High Sensitivity Kit in Qubit (Thermo Fisher) and a NanoDrop 1000 Spectrophotometer (Thermo Scientific), respectively.

V. AMPLIFICATION, LABELLING, HYBRIDIZATION AND SCANNING

cDNA from 30 ng of total RNA was generated, fragmented, amplified (6 cycles), biotinylated, and hybridised to the Clariom D Human Arrays (Affymetrix) according to the instructions of GeneChip™WT Pico Reagent Kit (Thermo Scientific) manual. Arrays were washed and stained on a GeneChip Fluidics Station 450 (Affymetrix); scanning was carried out with the GeneChip Scanner 3000 7 G (Thermo Fisher) and image analysis was performed with the GeneChip Command Console Scan Control (Affymetrix). Each of these steps was conducted according to the respective manufacturer's manual.

VI. GENE EXPRESSION AND QUALITY CONTROL

Quality control, background adjustment, quantile normalisation, and summarization were performed with the robust multiarray average (RMA) algorithm using the Transcriptome Analysis Console (TAC) version 4.0 and the Oligo R package. Array quality parameters were explored in order to ensure array quality. Distance between arrays and the arrays intensity distribution from the array QualityMetrics R package, hybridization controls, and Area Under the Curve determinations from TAC analysis were used as threshold quality metrics to exclude arrays from analysis. Outlier samples were excluded from the analysis.

VII. DIFFERENTIAL GENE EXPRESSION, PATHWAY ANALYSIS AND IMMUNE PROFILE ANALYSIS

The features of the expression set were filtered by nsFilter, which includes varFilter and featureFilter from the geneFilter R package. This filter strategy allowed us to keep 50% more variable features in the expression set and filter out uninformative features such as control probe sets.

To identify Differentially Expressed Genes (DEG) (upregulated and downregulated) we performed a statistical comparison using the Limma and Oligo R package implemented in the R version 4,0,2, which estimated the fold change between sample groups by fitting probe set microarrays data to a linear model and using an empirical Bayes method to moderate the standard errors of the estimated log-fold changes for each probe set (156). DEG were those with adjusted p-value <0,05 and log fold change (FC)>1. Gene Set Enrichment Analysis (GSEA) uses lists of genes to identify enrichment in specific cellular functions and pathways between groups of samples (157).

To make a cluster analysis separating samples on the basis of the TME features a total of 29-immune and angiogenesis related gene sets were selected. Gene Set Variation Analysis from R package GSVA was performed to obtain the immune profile of the RCC samples. This function performs a non-parametric, unsupervised analysis for estimating variations of the given gene sets through the samples in the expression matrix, returning an enrichment score for each sample. Three categories, characterized by different immune and inflammatory enrichments, were defined and named as “Low Immune-Cluster (LIC)”, “Medium ImmuneCluster (MIC)” and “High ImmuneCluster (HIC)”. Gene Set Variation Analysis was also performed using only immune-related gene sets (17 gene sets), covering both innate and adaptive responses.

To estimate the presence of immune cell populations in the tissues, two different tools were used.

First, the R package Microenvironment Cell Populations-counter (MCP-counter) deconvolution method was used to estimate the infiltration of different immune cells (T cells, cytotoxic T cells, CD8 T cells, Natural Killer cells, B lineage, monocytic lineage, myeloid dendritic cells, neutrophils, endothelial cells and fibroblasts) in the tumoral tissue. This is a method for quantification of immune cell's relative abundances in heterogeneous tissues using marker genes optimized for interrogating microarray data.

Second, the R package ESTIMATE (Estimation of Stromal and Immune cells in Malignant Tumour tissues using Expression data) was used to evaluate the immune infiltration (ImmuneScore), overall stromal content (StromalScore) and combined score (ESTIMATEScore), that estimates the tumour purity (158).

VIII. QUANTIFY THE IMMUNOTHERAPY RESPONSE PREDICTOR: IMMUNOPHENOSCORE

The superior immune response molecular marker, Immunophenoscore (IPS) (159), was used to characterise the intratumoral immune landscapes. The scoring scheme was created from a panel of immune-related genes belonging to the four clusters: major histocompatibility complex (MHC)-related molecules, checkpoints or immunomodulators, effector cells, and suppressor cells. For each class, a sample-wise Z score from gene expression data was extracted and calculated. The weighted averaged Z score was then calculated by averaging the Z scores within the respective category, leading to four values and the sum of the weighted averaged Z scores of the four categories. It also generates a z-score summarizing these four categories. The higher the z-score of IPS, the more immunogenic the sample.

IX. ASSESSMENT OF TRANSCRIPTOMIC SIGNATURES

We evaluated if previously published gene signatures (Table 11) were able to discriminate samples in our cohort according to response, toxicity and IMDC prognostic group. Among previously published gene signatures, only those that met with the stipulated gene signature quality criteria, that is, those with at least ten related functional genes (Tumour Inflammation Signature (TIS) and 26-JAVELIN Immuno), were tested in our dataset.

18_TIS (116)	26-JAVELIN Immuno (120)
<i>PSMB10</i>	<i>CD3G</i>
<i>HLA-DQA1</i>	<i>CD3E</i>
<i>HLA-DRB1</i>	<i>CD8B</i>
<i>CMKLR1</i>	<i>THEMIS</i>
<i>HLA-E</i>	<i>TRAT1</i>
<i>NKG7</i>	<i>GRAP2</i>
<i>CD8A</i>	<i>CD247</i>
<i>CCL5</i>	<i>CD2</i>
<i>CXCL9</i>	<i>CD96</i>
<i>CD27</i>	<i>PRF1</i>
<i>CXCR6</i>	<i>CD6</i>
<i>IDO1</i>	<i>IL7R</i>
<i>STAT1</i>	<i>ITK</i>
<i>TIGIT</i>	<i>GPR18</i>
<i>LAG3</i>	<i>EOMES</i>
<i>CD274</i>	<i>SIT1</i>
<i>PDCD1LG2</i>	<i>NLRC3</i>
<i>CD276</i>	<i>CD244</i>
	<i>KLRD1</i>
	<i>SH2D1A</i>
	<i>CCL5</i>
	<i>XCL2</i>
	<i>CST7</i>
	<i>GFI1</i>
	<i>KCNA3</i>
	<i>PSTPIP1</i>

Table 11. Previously published gene signatures evaluated in our dataset.

X. STATISTICAL ANALYSIS

A descriptive analysis was used to summarize the baseline characteristics of the study population and of each cohort (responders and non-responders to ICB). Categorical variables were described as absolute frequency (%), while numerical variables were described as mean $\pm$ standard deviation, for variables normally distributed (Shapiro test, $p > 0.05$), or as median (IQR) for variable not normally distributed. Associations between the responders/non-responders and clinical factors were analysed using χ^2 or Fisher's exact tests for categorical variables and the T-test or the Mann-Whitney U test for numerical variables.

RESULTS



I. CLINICAL AND PATHOLOGICAL CHARACTERISTICS

Ninety-two metastatic RCC patients treated with ICB, regardless treatment line, between 2014 and 2019 were identified at the Oncology Department of the University Hospital 12 de Octubre. From these patients, only 71 patients had available tumour tissue, and seven samples were discarded due to low tumour content or low RNA quantity or quality. In addition, two samples corresponded to the same patient and thus one of them was discarded, leaving a total of 63 samples for analysis (Figure 10).

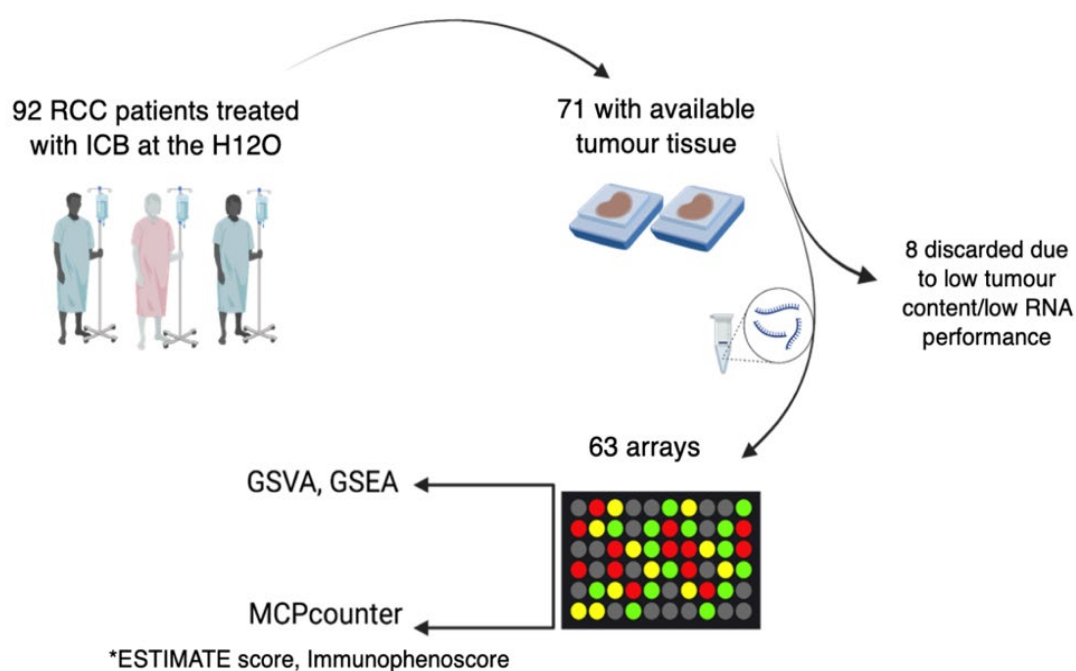


Figure 10. Whole gene expression analysis flowchart.

The main clinical and pathological characteristics of the patients included in the analysis and the comparison between the two groups of patients (responders and non-responders to ICB) are presented in Table 12.

Non-responder patients to ICB were significantly enriched in bone metastases (41.3%) compared to responder patients (0%). Likewise, brain metastases were only seen in the non-responder group. Immune-checkpoint inhibitor therapy as a front-line treatment was more frequent in the responder group (35.3%) than in the non-responder group (19.6%). Previous treatment with AA was more frequent in the non-responder group (45.7% vs 11.8%). No significant differences were observed between the two groups according to the IMDC risk group; however, the responder group presented a higher rate of intermediate patients while the non-responder group presented a higher rate of good prognosis patients (Table 12).

	Overall population N=63	Non-responders ICB N= 46	Responders- ICB N=17	p-value
Age at Diagnosis, media (range), years	60 (30-84)	60 (30-84)	63 (40-81)	0.624
Age at diagnosis >60, n (%)	27 (42.2)	19 (41.3)	8 (47.1)	0.777
Male, n (%)	42 (65.6)	31 (67.4)	11 (64.7)	1.000
IMDC, n (%)				
- Good	20 (38.5)	16 (42.1)	4 (30.7)	0.868
- Intermediate	27 (52.9)	19 (50.0)	8 (61.5)	
- Poor	4 (7.7)	3 (7.9)	1 (7.7)	
Missing	12	8	4	
KPS<80, n (%)	6 (9.4)	4 (8.7)	2 (11.8)	0.657
Metastases at diagnosis, n (%)	24 (42.8)	19 (41.3)	5 (35.7)	0.354
Missing	7	0	3	
Nephrectomy, n (%)	61 (96.8)	45 (97.8)	16 (94.1)	0.470
ccRCC, n (%)	44 (69.8)	31 (67.4)	13 (76.5)	0.757
Fhurman grade 3-4, n (%)	25 (51.0)	17 (37.0)	8 (47.1)	0.321
Missing	14	9	5	
Sarcomatoid features, n (%)	3 (4.8)	2 (4.3)	1 (6.25)	1.000
Missing	1	0	1	
Treatment-naïve at ICB start, n (%)	15 (23.8)	9 (19.6)	6 (35.3)	0.317
Nb of previous treatment lines to ICB, n (%)				
- 0	15 (23.8)	9 (19.6)	6 (35.3)	0.148
- 1-2	27 (42.9)	23 (50)	4 (23.5)	
- >2	21 (33.3)	14 (50)	7 (41.2)	
Previous type of therapy, n (%)				
- None	16 (25.4)	9 (19.6)	7 (41.2)	0.043
- Anti-VEGF only	23 (36.5)	21 (45.7)	2 (11.8)	
- Anti-VEGF and mTOR inhibitor	23 (36.5)	15 (32.6)	8 (47.0)	
- mTOR inhibitor only	1 (1.6)	1 (2.1)	0 (0.0)	
Brain metastasis before ICB, n (%)	2 (3.2)	2 (4.3)	0 (0.0)	0.600
Bone metastasis before ICB, n (%)	19 (30.2)	19 (41.3)	0 (0.0)	0.001

Table 12. Baseline clinical and pathological characteristics and comparison between responders and non-responders.

Abbreviations: ICB, immune checkpoint blockers; IMDC, International Metastatic RCC Data Consortium; KPS, Karnofsky Performance Status; ccRCC, clear cell renal cell carcinoma; Nb, number; VEGF, vascular endothelial growth factor; mTOR, mammalian target of rapamycin.

II. DIFFERENTIAL GENE EXPRESSION ACCORDING TO RENAL CELL CARCINOMA HISTOLOGICAL SUBTYPES

The dataset of the present study contained 63 samples: 44 ccRCC, 10 chrRCC, and 8 pRCC; histological subtype was unknown for one tumour sample. (Figure 11).

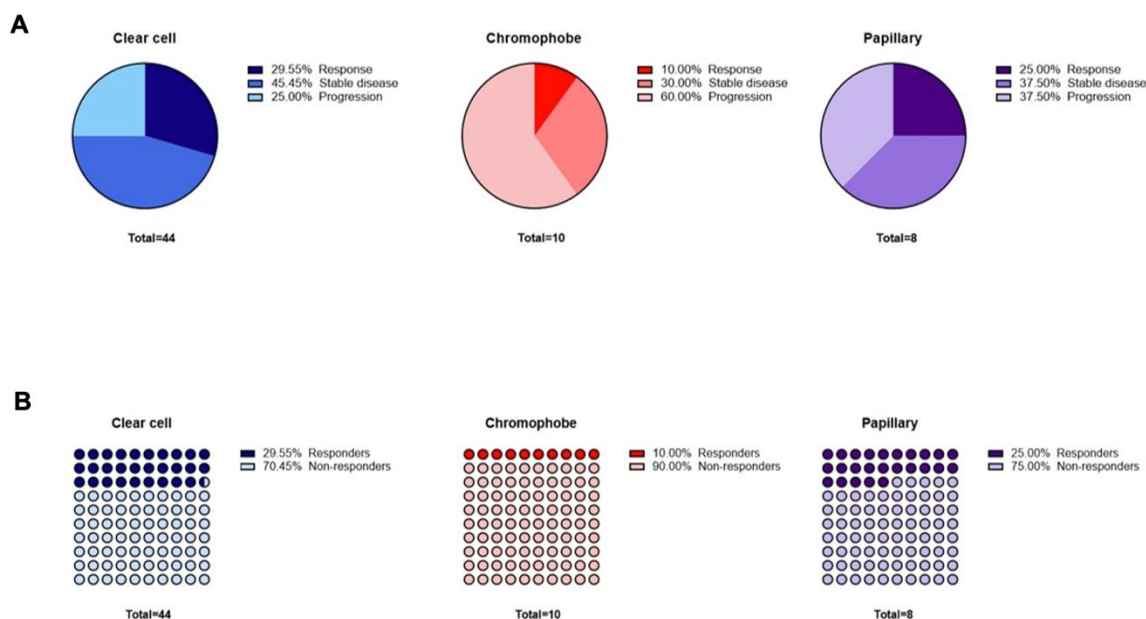


Figure 11. Response rates according to histological subtypes.

(A) Rates of response, stable disease, and progression according to histological subtype. (B) Response rate according to histological subtypes.

Each array was analysed with Affy and Limma packages of R software, RMA method was used to pre-process the dataset, then by a Bayes method differentially expressed genes with p value $<0,05$ and $\log$ fold change ($\log|FC|$) >1 criteria were obtained from the dataset. We identified 3 DEGs between ccRCC and pRCC samples, 137 DEGs between ccRCC and chrRCC samples, and 62 DEGs between chrRCC and pRCC samples (figure 12B). The TAC software (Affymetrix) was used to produce a PCA plot (Fig 12A) and the gplot package (R software) was used to produce volcano plots of the DEGs between different RCC histological subtypes (Figures 12C).

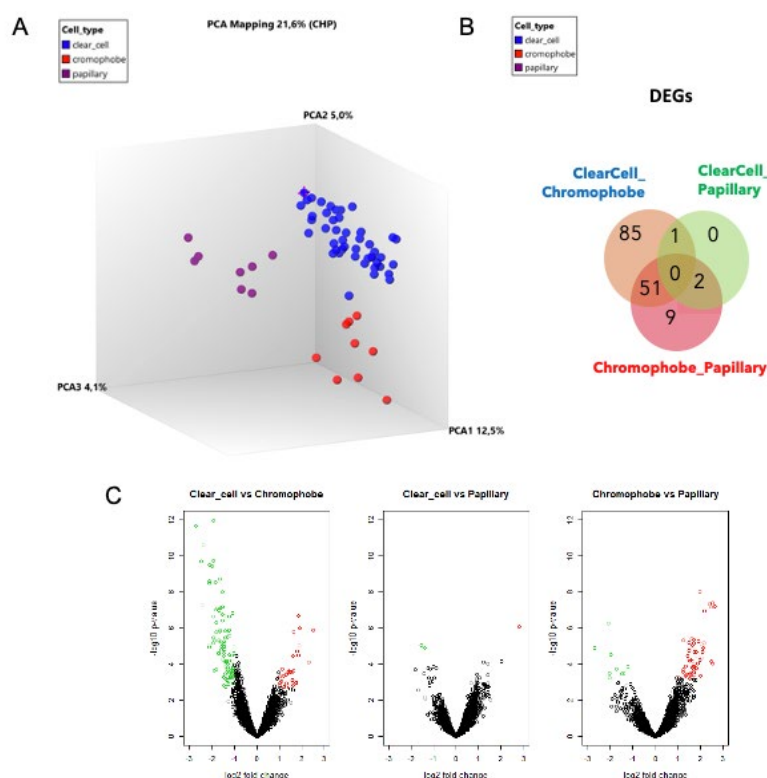


Figure 12. Differential gene expression according to RCC histological subtypes.

(A) Principal component analysis (PCA) of the gene expression profiles in clear cell, chromophobe, and papillary RCC patients. The three-dimensional projection of the three principal components in the figure, which captures 21.6 of the total variance, shows clear separation of the three groups. (B) Venn diagram showing DEGs in the three histological subtypes. (C) Volcano plot of all DEG between the 3 different subtypes, showing the significantly upregulated genes (red dots) and the downregulated genes (green dots).

An unsupervised hierarchical clustering analysis of all probe set (gene) expressions obtained from GE array analysis clustered samples according to the three RCC histological subtypes (Figure 13).

As expected, the principal component analysis (PCA) revealed the heterogeneity between the three main histological subgroups, ccRCC, chrRCC and pRCC, but a relatively less variability was shown within each histological subtype (Figure 12A). Samples corresponding to the papillary subtype were particularly different from the clear cell subtype.

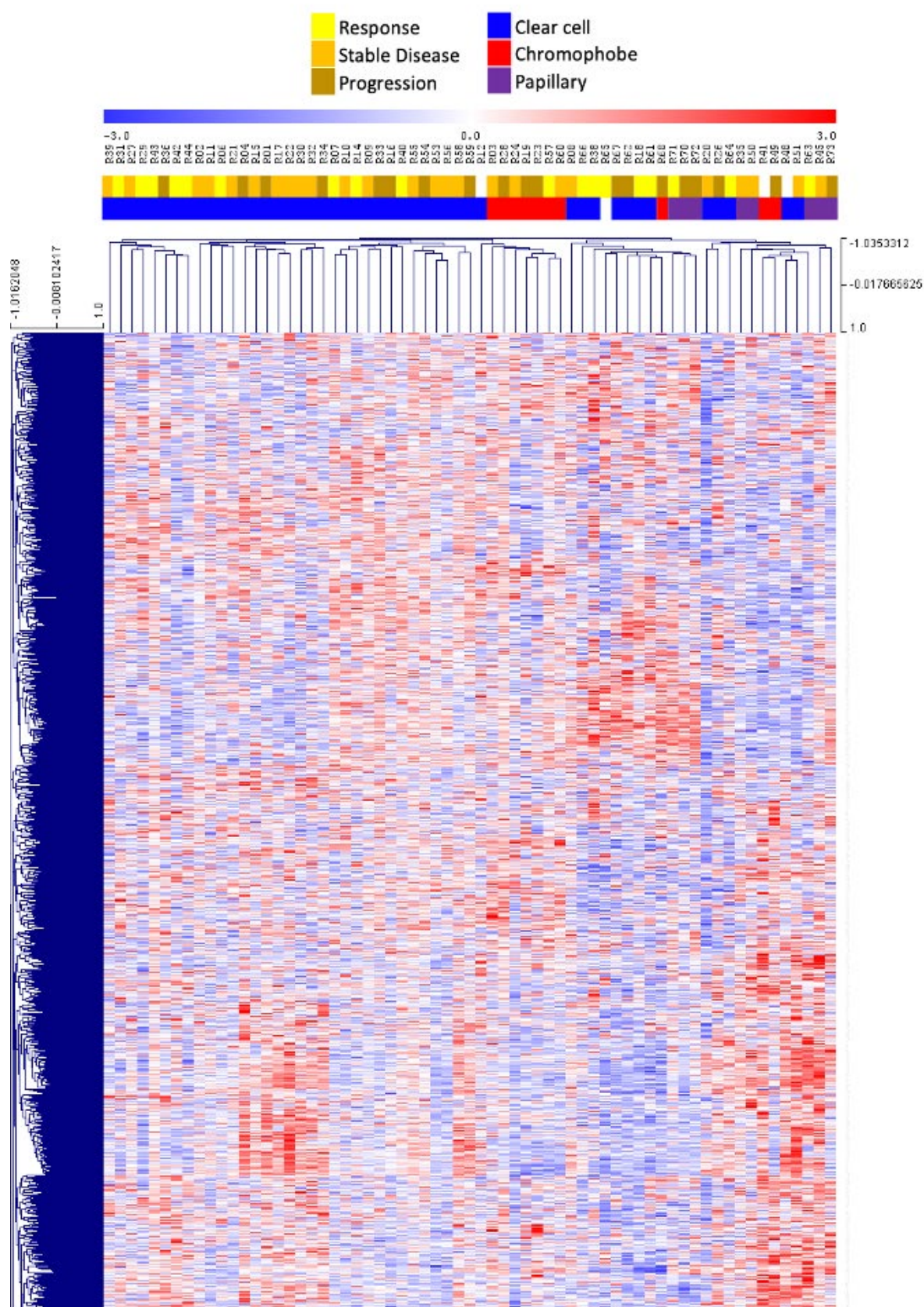


Figure 13. Heatmap of all expressed genes.

The colour scale bar shows the relative gene expression changes; red denotes upregulation and blue denotes downregulation. Histological subtypes and types of responses are represented by different colours that can be found on the legend.

We next performed GSEA on the 63 samples. Comparisons of the HALLMARK pathways dataset activity indicated that inflammatory pathways (i.e. TNFalpha signalling via NFkB, NES=2.38 or Allograft rejection, NES=2.06; FDR q-value <0.001), and protumoral pathways (i.e. Epithelial mesenchymal transition (EMT), NES=2.37, or Hypoxia, NES=2.30, FDR q-value <0.001), were significantly enriched in clear cell tumours versus both papillary (Figure 14) and chromophobe (Figure 15) tumours. In contrast, metabolic pathways involved in oxidative phosphorylation (NES=2.63, FDR q-value<0.001) and lipid metabolism (i.e Fatty acid metabolism, NES=2.13, or Adipogenesis, NES=2.11; FDR q-value<0.001) were significantly enriched in chromophobe tumours compared to papillary tumours (Figure 16).

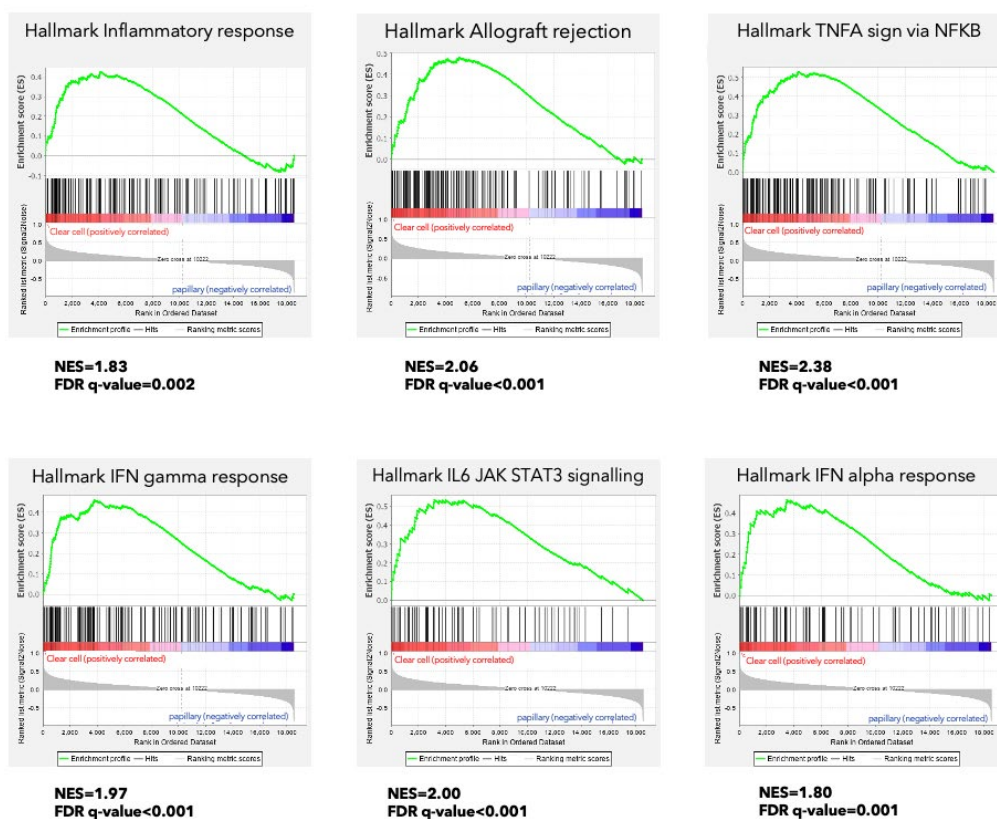
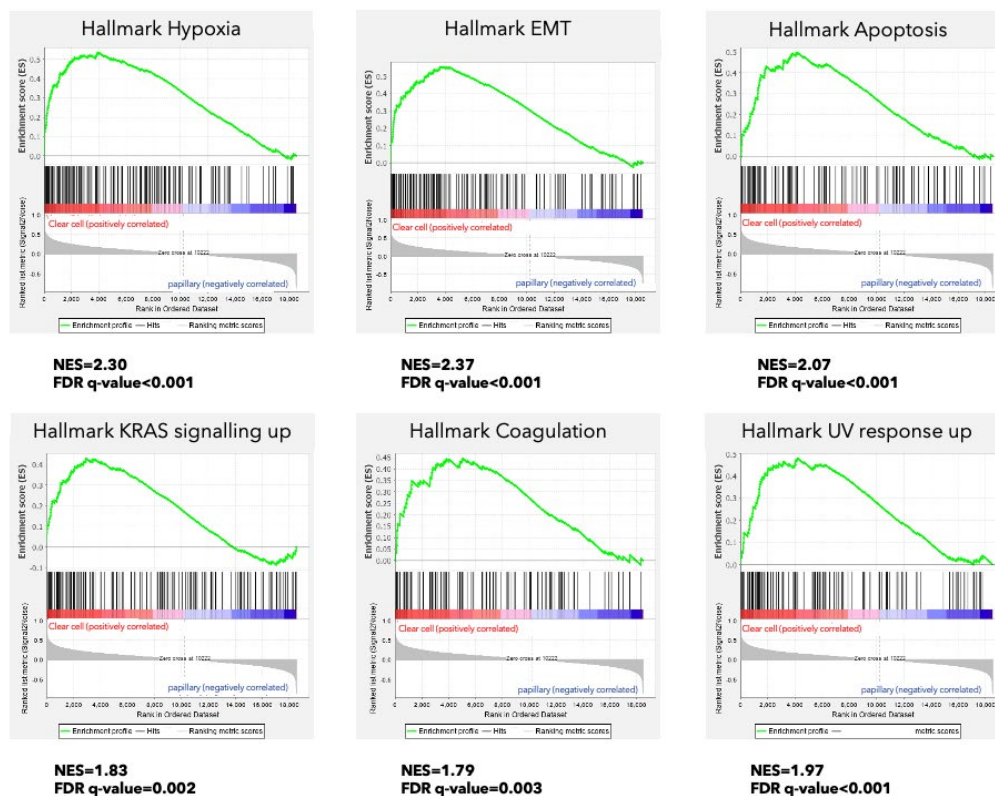
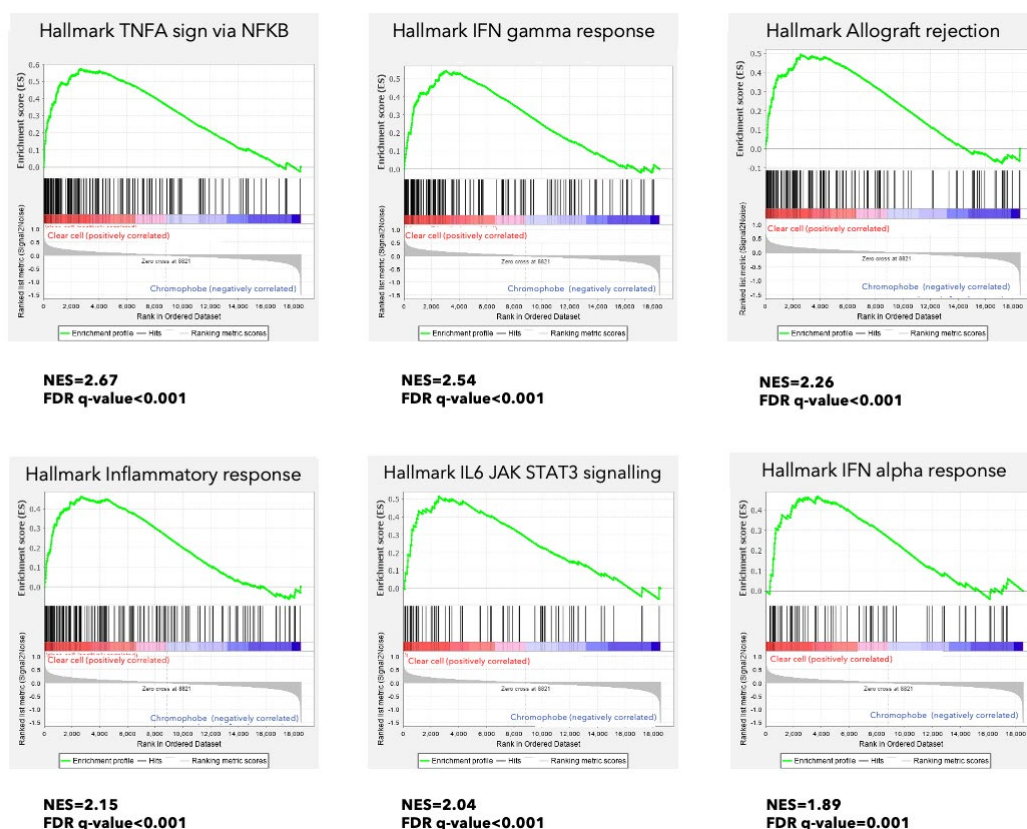
B**A**

Figure 14. GSEA of Hallmark gene sets associated with metabolic protumoral and inflammatory pathways in clear cell tumours versus papillary tumours.

A



B

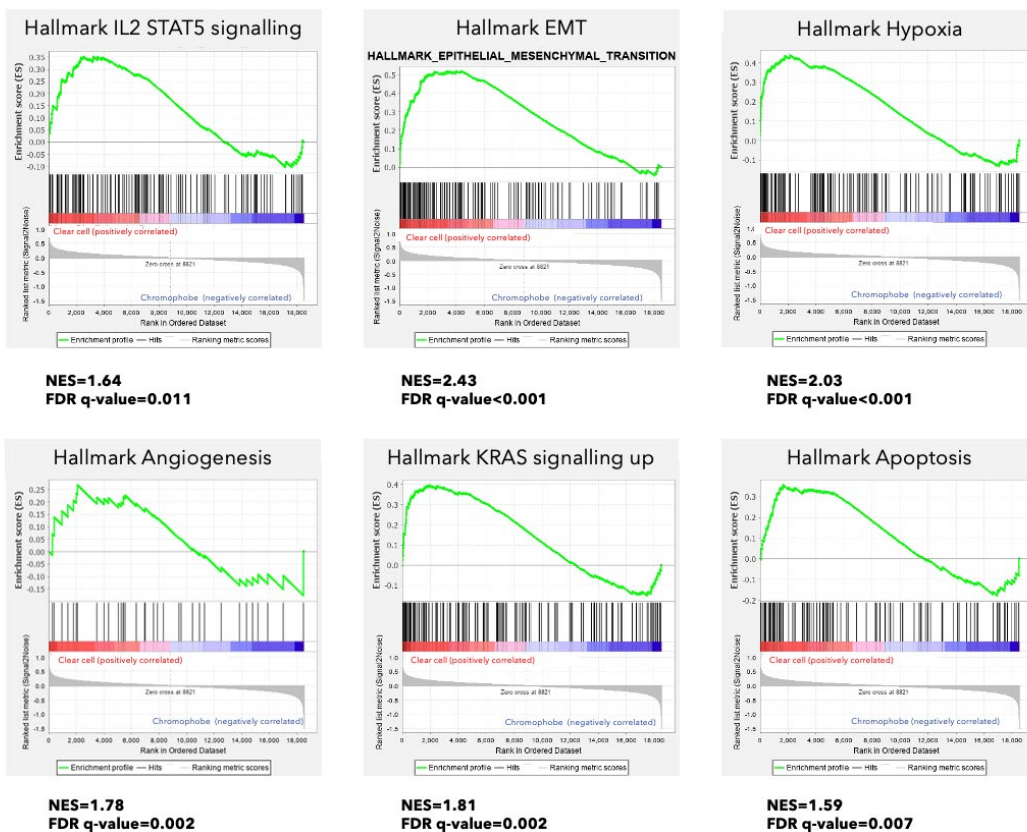
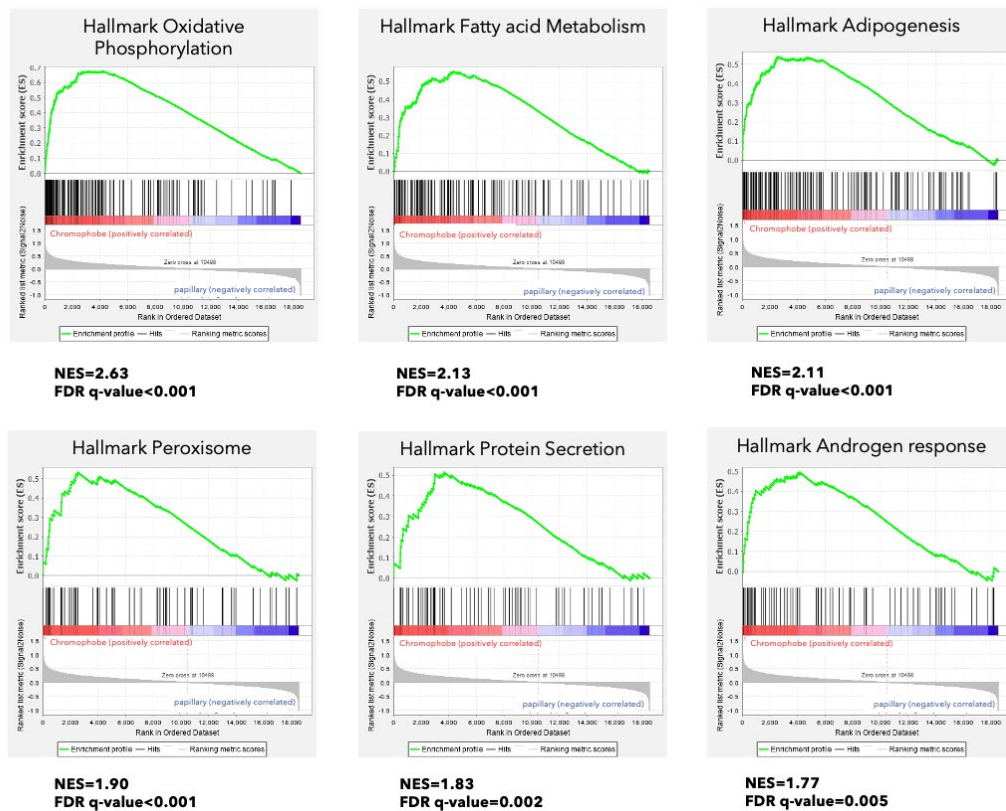


Figure 15. GSEA of Hallmark gene sets associated with metabolic protumoral and inflammatory pathways in clear cell tumours versus chromophobe tumours.

A



B

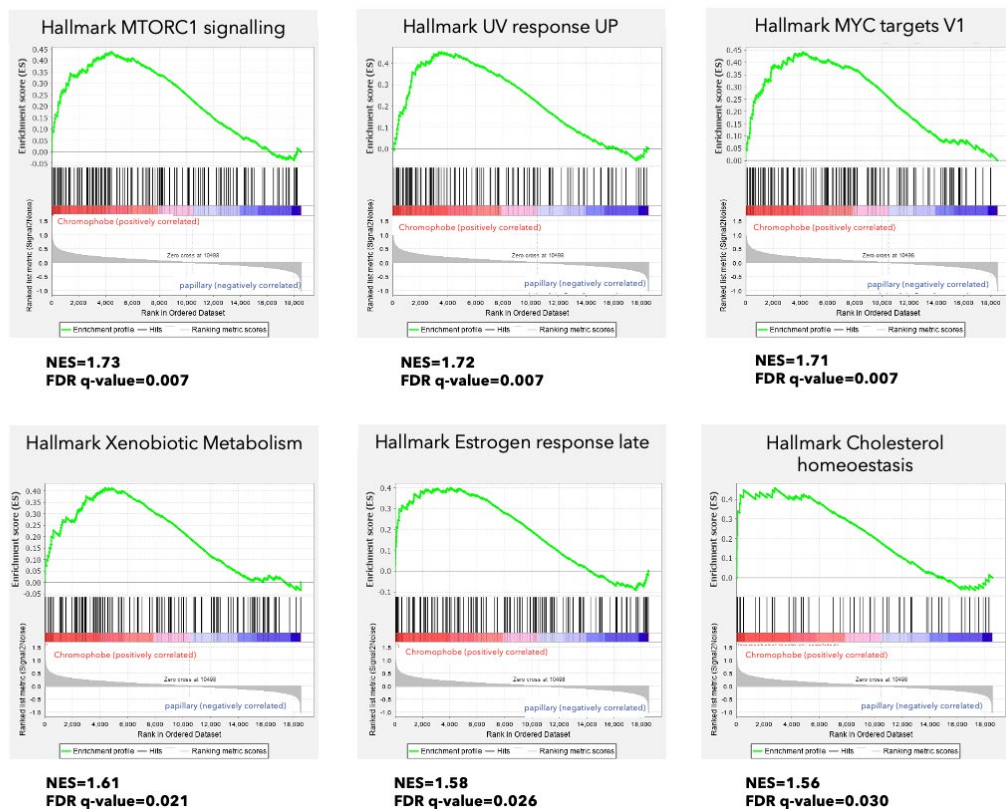


Figure 16. GSEA of Hallmark gene sets associated with metabolic protumoral and inflammatory pathways in chromophobe tumours versus papillary tumours.

Building on GSEA results, we performed hierarchical clustering using the GSVA enrichment scores for 29 immune-related gene sets. Patients were clustered in three major groups defined as 29 low immune cluster (29LIC), 29 medium immune cluster (29MIC), and 29 high immune cluster (29HIC) (Figure 17).

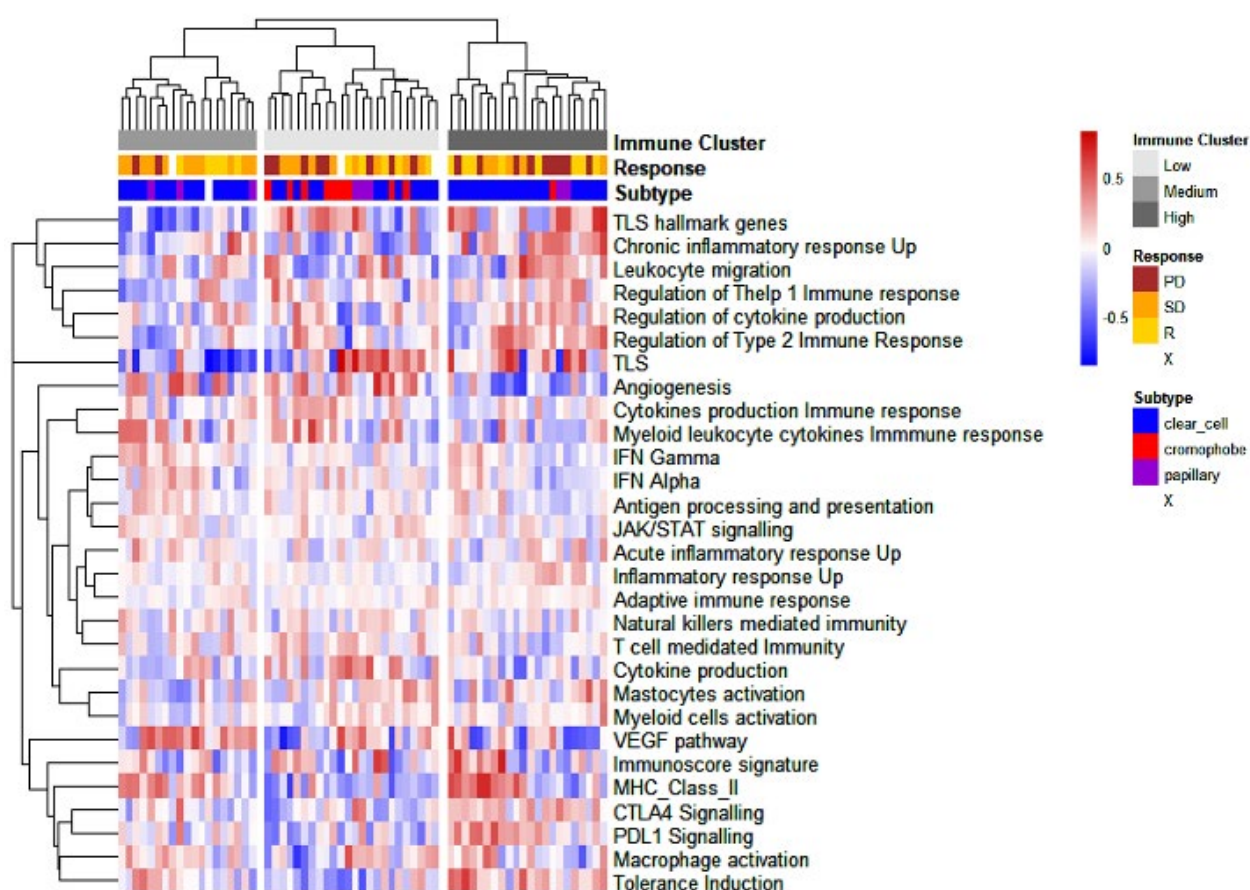


Figure 17. Heatmap showing pan-cancer patients grouped by hierarchical clustering using the GSVA enrichment scores for 29 immune-related gene sets and stratified by patient features.

Abbreviations: TLS, tertiary lymphoid structures; IFN, interferon gamma; MHC, major histocompatibility complex; CTLA4, cytotoxic T-lymphocyte-associated protein 4; PDL1, programmed death-ligand 1; PD, progressive disease; SD, stable disease; R, response; X, unknown.

As shown in Figure 17, 29HIC and 29MIC were enriched in ccRCC tumours, while in the 29LIC, the three different histological subtypes (ccRCC, pRCC and crRCC) were equally present. Curiously, the non-clear cell tumours, crRCC and pRCC, that clustered into the 29LIC were in general non-responders (stable disease or progressors) to ICB in our cohort (Figure 17).

We also performed unsupervised hierarchical clustering using GSVA enrichment scores for 17 immune pathways from the Hallmark, Reactome, KEGG, Biocarta, and Gene Ontology (GO) datasets. Patients were divided into four immune clusters (Figure 18A): cluster 1 (immune high,

but myeloid high and T helper 1 type low), cluster 2 (T helper 1 type high and myeloid low), cluster 3 (immune low, but cytokine activation high), and cluster 4 (immune low). Interestingly, cluster 2, enriched in T helper 1 type, CTLA-4, and PD-L1 signalling pathways and downregulated in the myeloid pathway, consisted of only clear cell renal carcinoma samples. Cluster 1, enriched in immune-related pathways but myeloid high and T helper 1 type low, was also enriched in clear cell tumours. The three histological subtypes were evenly distributed among the other two immune clusters.

Furthermore, unsupervised hierarchical clustering analysis of the MCP-counter identified four immune cell clusters: “cytotoxic effector”, “mixed inflamed”, “stromal myeloid”, and immune low (Figure 18B). The names were given according to the different proportions of immune cells. The three different histological subtypes were present in the four immune cell clusters; however, the “stromal myeloid” cluster seemed to be enriched in clear cell renal cell carcinoma samples compared to the other two histologies. The GSVA immune clusters did not align with the immune cell clustering. However, the “cytotoxic effector” immune cell cluster was characterised by a lack of cluster 1 samples (T helper 1 type low and myeloid high) samples.

We next calculated and compared the ImmuneScore, StromalScore, ESTIMATE score and tumour purity according to the RCC histological subtype using the ESTIMATE algorithm. The ESTIMATE algorithm is a tool for predicting tumour purity and the presence of infiltrating stromal and immune cells in tumours using gene expression data (158). The stromal and immune scores capture, respectively, the presence of stroma and immune cells in the tumour tissue. The estimated score is the combination of the stromal and immune scores, that is, of non-tumour cells, and thus infers the tumour purity. A high estimate score indicated less tumour content. Significant differences in stromal infiltration, immune infiltration, and tumour purity were observed among the three subtypes (Figure 19). Clear cell RCC samples presented a higher immune and stromal infiltration (p value < 0.01), and an inferior tumour purity than the other two subtypes, which could explain their greater response to ICB. Chromophobe RCC samples had the highest tumour purity and the lowest Immune and Stromal scores (Figure 19).

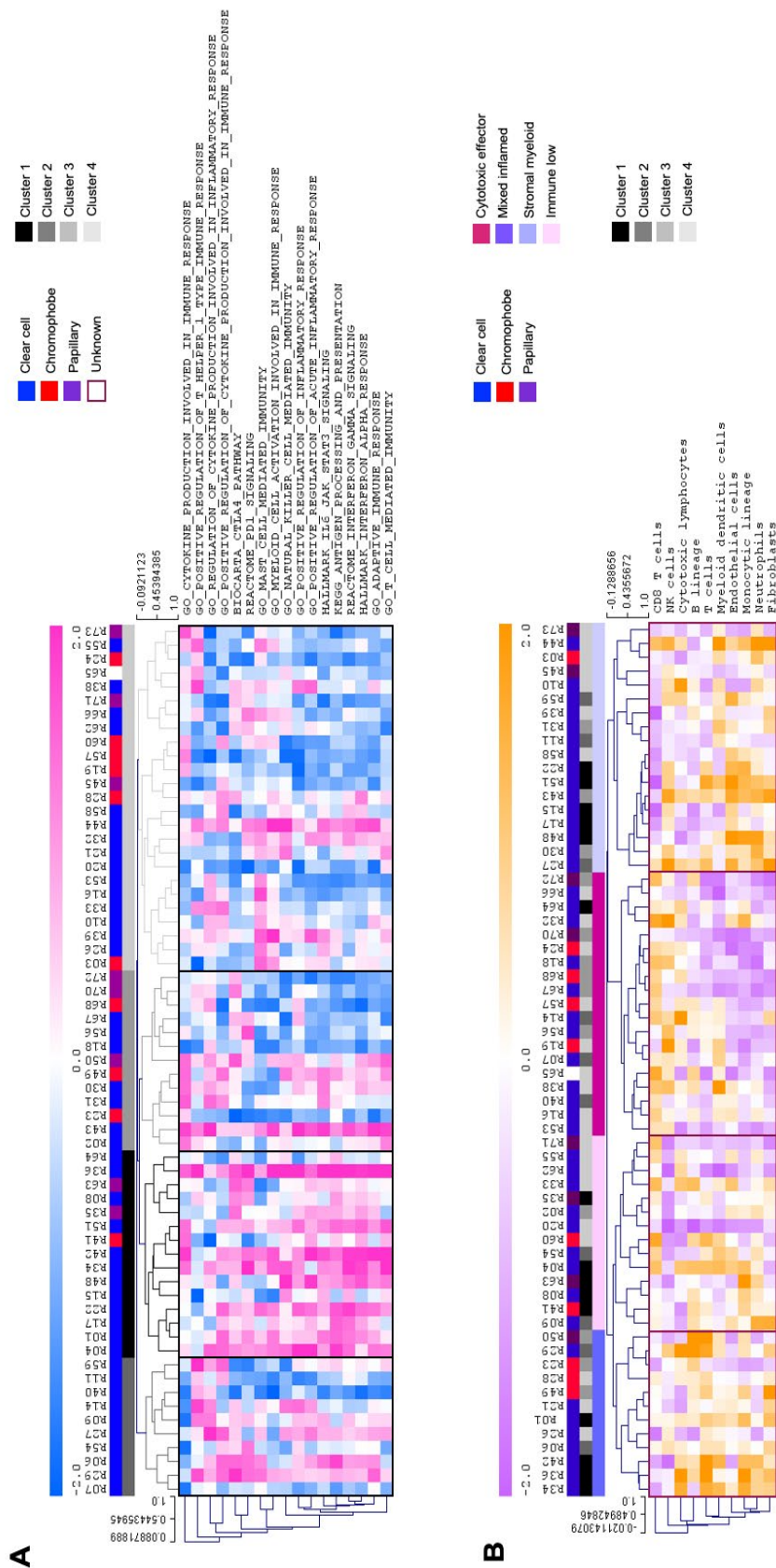


Figure 18. Heatmaps of GSEA and MCP-counter analyses.

(A) Heatmap showing tumours grouped by hierarchical clustering using the GSEA enrichment scores for 17 immune pathways from the Hallmark, Reactome, KEGG, Biocarta and Gene Ontology (GO) datasets. Patients were divided into four immune clusters: cluster 1 (T helper 1 low and myeloid high), cluster 2 (T helper 1 high and myeloid low), cluster 3 (cytokine activation), and cluster 4 (immune low). (B) Heatmap showing the clustering of tumours on MCP-counter scores into four groups: Cytotoxic effector, mixed inflamed, stromal-myeloid and immune low

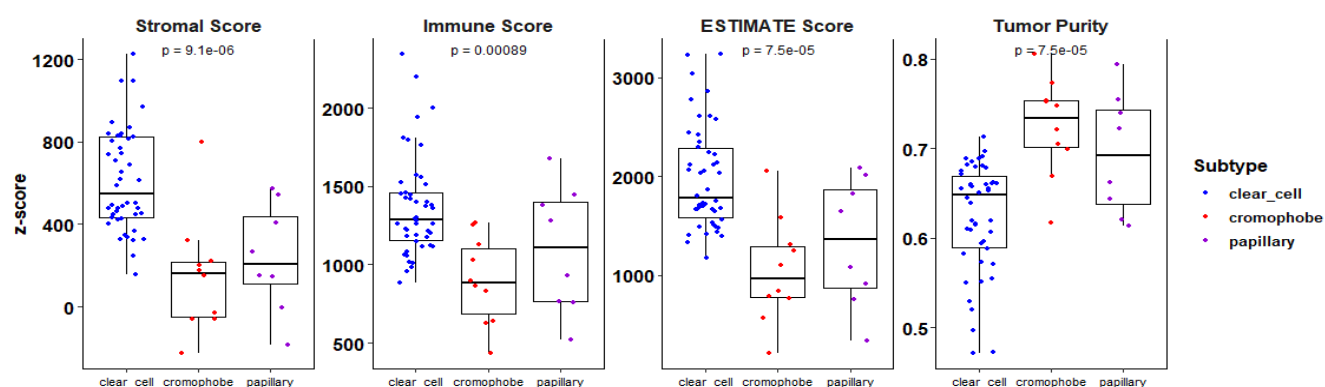


Figure 19. Distribution of stromal score, immune score, ESTIMATE score, and tumour purity according to histological subtype.

Boxplot representation of these scores in clear cell (blue), chromophobe (red) or papillary (violet) RCC patients.

These results underscore the gene expression heterogeneity among the three main RCC histological subtypes (clear cell, papillary and chromophobe). GSEA and GSVA analysis suggest that inflammatory response-related pathways are significantly enriched in clear cell tumours, compared to papillary and chromophobe tumours. Further, the results of the MCP counter and ESTIMATE score show that clear cell tumours are enriched in immune and stromal infiltration compared to non-clear cell tumours. These transcriptomic variations could explain the different response to ICB between clear cell and non-clear cell RCC tumours.

III. DIFFERENTIAL GENE EXPRESSION AND METASTASIS ORGANOTROPISM

We performed GSEA of 35 tumours of patients identified as lung-only (28), liver-only (3) or bone-only metastases (4). Given the small sample size, despite the molecular differences according to histological subtype, clear cell and non-clear cell patients were included in this analysis. Comparisons of the HALLMARK pathways dataset activity indicated that patients with liver as only site of metastasis in first line setting were significantly enriched compared to lung-only and bone-only patients in metabolic pathways including fatty acid metabolism, adipogenesis and oxidative phosphorylation (FDR q value <0.001, Figure 20), but also other protumoral pathways such as hypoxia or mTORC1 signalling (FDR q value <0.001, Figure 20). TGF-beta signalling and inflammatory pathways such as the inflammatory response and the TNF-alpha signalling via NfKappaB were also upregulated in these patients (FDR q value <0.001, Figure 20). Patients with lung or bone as only site of metastasis did not show a significant enrichment or under-enrichment in any pathway compared to the other two groups (data not shown).

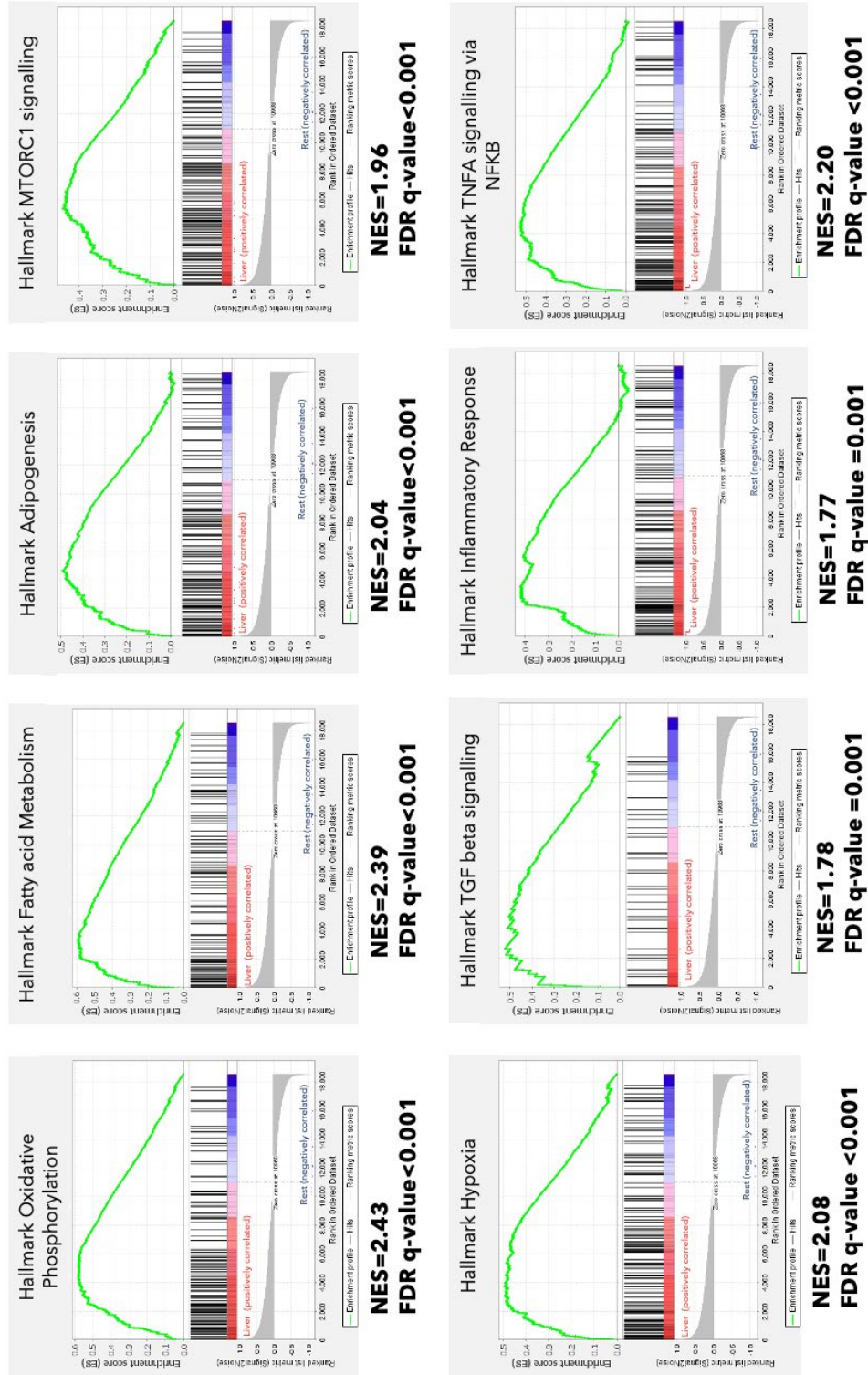


Figure 20. GSEA of gene sets associated with metabolic protumoral and inflammatory pathways in patients with liver-only metastases versus those with either lung-only or bone-only metastases.

IV. DIFFERENTIAL GENE EXPRESSION AND IMDC RISK GROUP

We performed GSEA of 51 RCC patients with available IMDC information in first line setting, including 20 good, 27 intermediate and four poor prognostic patients. Gene set enrichment analysis using the following four datasets: Hallmarks, Biocarta, Immunologic signatures, and KEGG, did not show a significant enrichment or under enrichment between the IMDC groups. Thus, aware of the molecular differences between the different histological subtypes, we next performed GSEA in only clear cell tumours (n=36). Although limited by the small sample size and the few tumours included in the poor prognostic group (2 IMDC poor, 19 IMDC intermediate, and 15 IMDC good), comparisons of the HALLMARK pathway datasets activity showed that poor IMDC patients were enriched, compared to intermediate and good IMDC risk patients, in protumoral pathways including epithelial-mesenchymal transition (EMT) (FDR q value <0.001), mitotic spindle (FDR q value <0.001), G2M checkpoints (FDR q value <0.001), MYC target (FDR q value <0.001), and glycolysis (FDR q value = 0.015), and immunosuppression pathways such as TGF beta signalling (FDR q value < 0.001) and IL2-STAT5 signalling (FDR q value = 0.039) (Figure 21). However, poor IMDC patients were also enriched in pathways of immune activation including Allograft rejection (FDR q value =0.001), Interferon alpha response (FDR q value <0.001), Interferon gamma response (FDR q value <0.001), TNF alpha signalling via NFkappaB (FDR q value <0.001), and Inflammatory response (FDR q value =0.003) (Figure 21).

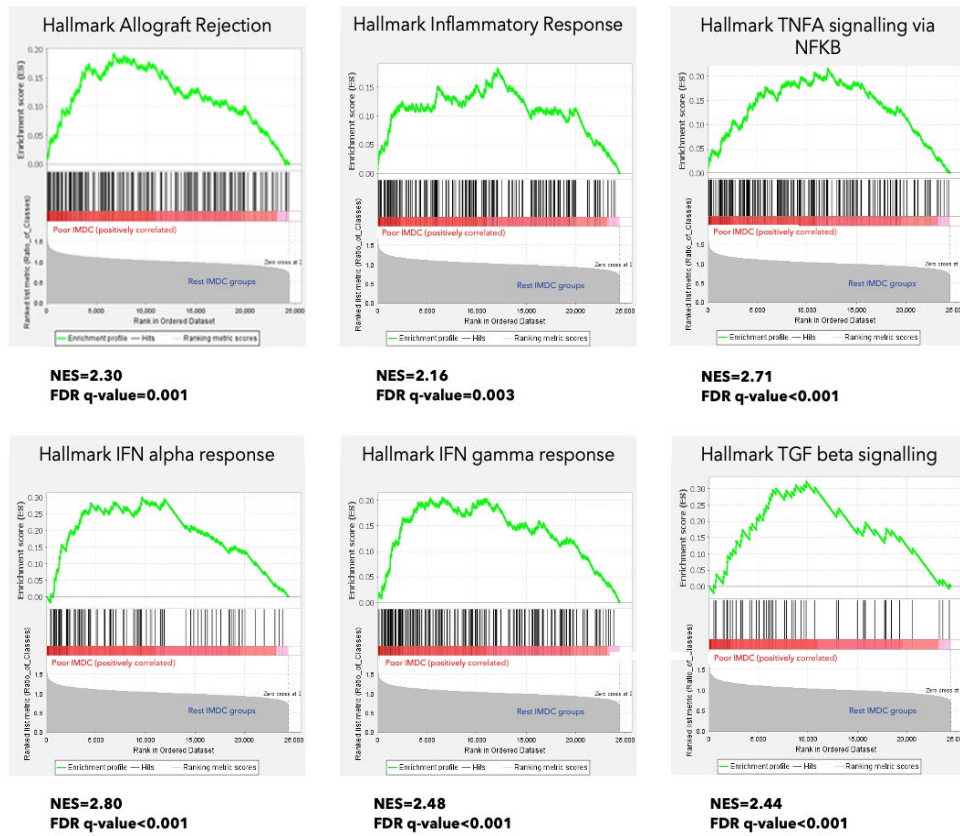
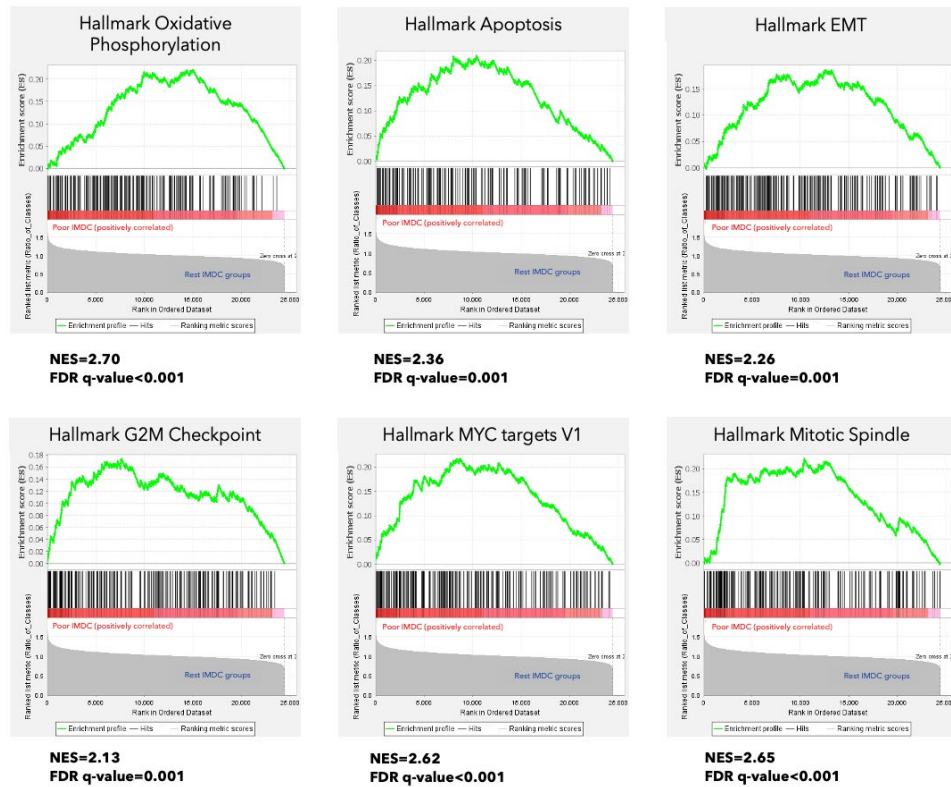
A**B**

Figure 21. GSEA of Hallmark gene sets enriched or downenriched in tumours of poor IMDC patients versus those from good or intermediate IMDC patients.

Based on these results, we performed unsupervised hierarchical clustering using GSVA enrichment scores for 17 immune pathways from the Hallmark, Reactome, KEGG, Biocarta and Gene Ontology (GO) datasets. Patients were divided into four immune clusters (Figure 22A). Cluster 1 (T helper 1 type low, myeloid high, and immune checkpoints high), cluster 2 (T helper 1 type high, myeloid high, and immune checkpoints low), cluster 3 (immune low, but myeloid low and cytokine activation high), and cluster 4 (immune low, but PD-L1 signalling and T helper 1 type high). The three IMDC groups were dispersed among the four immune clusters, however it seemed that clusters 1 and 2 were enriched in intermediate and poor IMDC risk patients; few favourable risk patients were found in these two clusters. (Figure 22A). GSVA on only clear cell tumours divided them into four immune clusters (Figure 23A); IMDC groups were dispersed again equally between the four clusters.

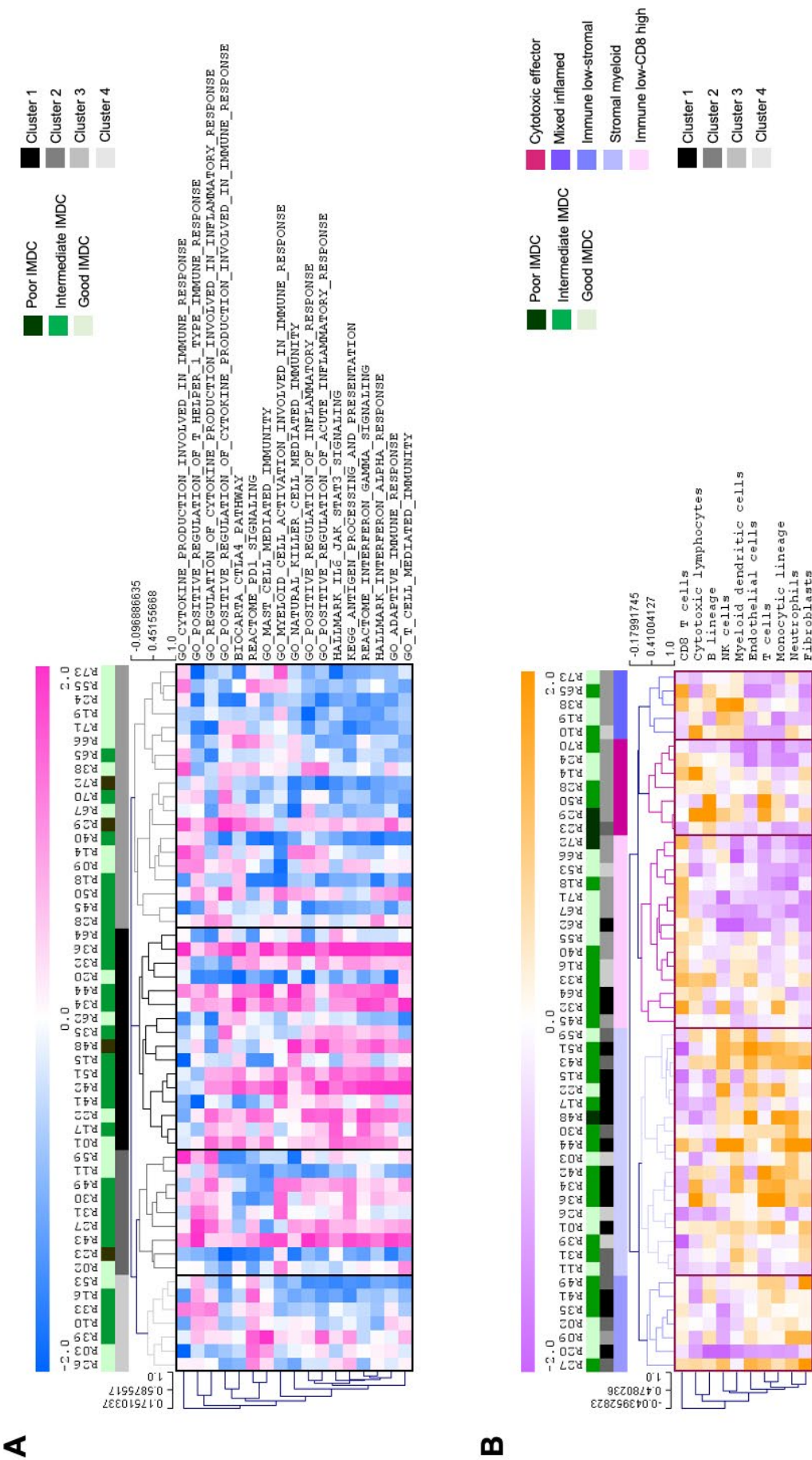


Figure 22. GSVA (17 immune pathways) and MCP-counter do not clearly discriminate between IMDC groups.

(A) Heatmap showing tumours with available IMDC information grouped by hierarchical clustering using GSVA enrichment scores for 17 immune pathways from the Hallmark, Reactome, KEGG, Biocarta and Gene Ontology (GO) datasets. Tumours were divided into four immune clusters: Cluster 1 (T helper 1 type low, myeloid high, and immune checkpoints high), cluster 2 (T helper 1 type high, myeloid high, and immune checkpoints low), cluster 3 (immune low, but myeloid low and cytokine activation high), and cluster 4 (immune low, but PDL1 signalling and T helper 1 type high). (B) Heatmap showing the clustering of tumours on MCP-counter scores into five groups: “cytotoxic effector”, “mixed inflamed”, “immune low-stromal”, “stromal myeloid”, and “immune low-CD8 high”. IMDC risk groups did not form clearly separated clusters.

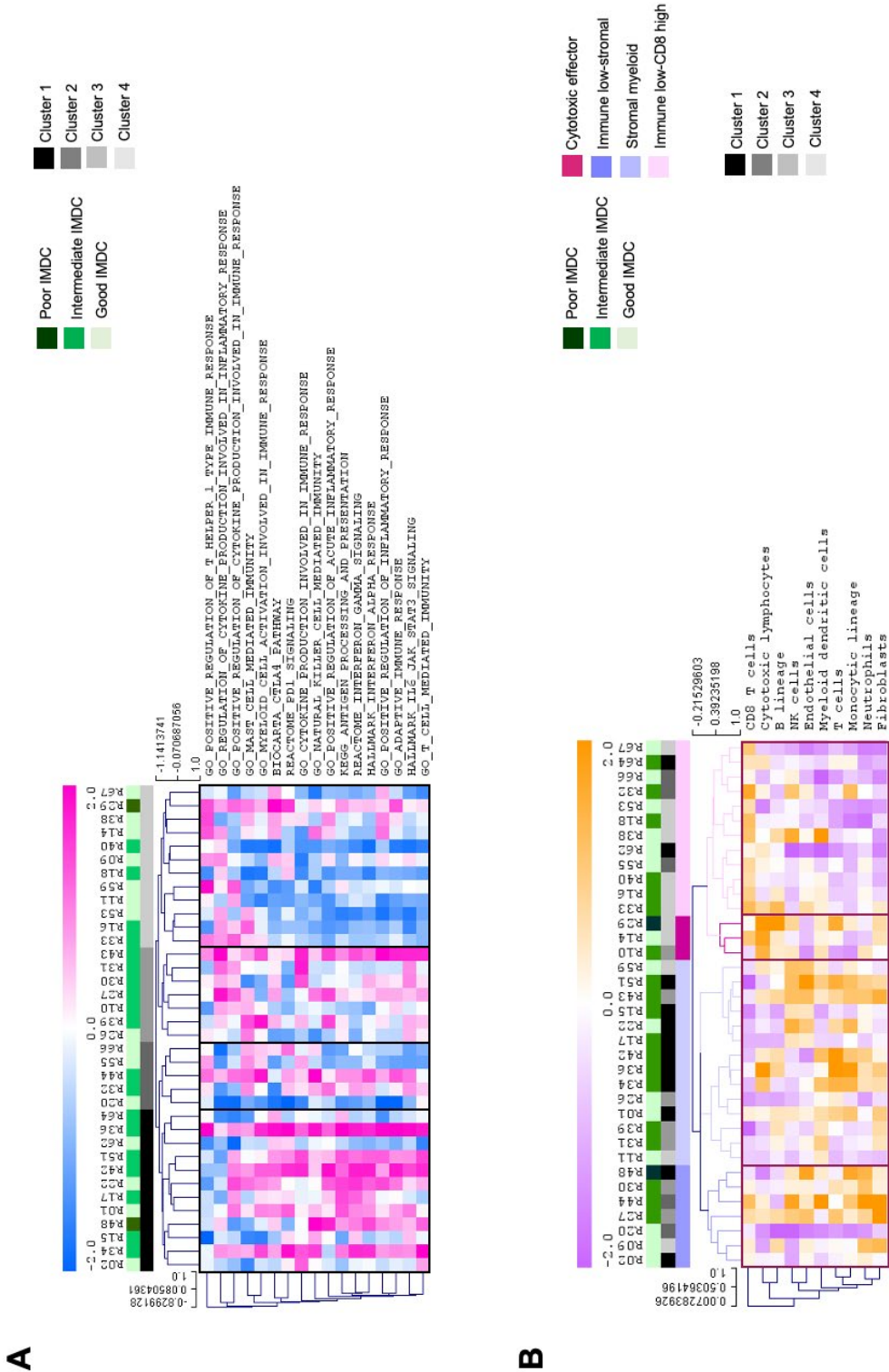


Figure 23. GSEA (17 immune pathways) and MCP-counter do not clearly discriminate between IMDC groups in clear cell tumours.

(A) Heatmap showing clear cell tumours with available IMDC information grouped by hierarchical clustering using GSEA enrichment scores for 17 immune pathways from the Hallmark, Reactome, KEGG, Biocarta and Gene Ontology (GO) datasets. Tumours were divided into four immune clusters: Cluster 1 (T helper 1 type low, myeloid high, and immune checkpoints high), cluster 2 (T helper 1 type high, myeloid high, and immune checkpoints low), cluster 3 (immune low, but myeloid low and cytokine activation high), and cluster 4 (immune low, but PDL1 signalling and T helper 1 type high). (B) Heatmap showing the clustering of clear cell tumours on MCP-counter scores into five groups: “cytotoxic effector”, “mixed inflamed”, “immune low-stromal”, “stromal myeloid”, and “immune low-CD8 high”. IMDC risk groups did not form clearly separated clusters.

Unsupervised hierarchical clustering analysis of the MCP-counter grouped tumour into five immune cell clusters: “cytotoxic effector”, “mixed inflamed”, “immune low-stromal”, “stromal myeloid”, and “immune low-CD8 high”. IMDC risk groups did not form clearly separated clusters (Figure 22B). We further focused on clear cell tumours (Figure 23B). Clear cell tumours were grouped into four immune cell clusters: “cytotoxic effector”, “immune low-stromal”, “stromal myeloid”, and “immune low-CD8 high”. Again, samples did not group according to IMDC group.

Despite the small sample size, with only four poor IMDC risk RCC tumours in our cohort, the GSEA results suggest that, although enriched in protumoral and immunosuppressive pathways, poor IMDC risk RCC tumours are also characterized by an upregulation of pro-immune pathways. This is in line with the GSVA results showing a predominance of intermediate and poor IMDC samples in clusters 1 (T helper 1 type low, myeloid high, and immune checkpoints high) and 2 (T helper 1 type high, myeloid high, and immune checkpoints low), compared to clusters 3 (immune low, but myeloid low and cytokine activation high) and 4 (immune low, but PDL1 signalling and T helper 1 type high) that were enriched in favourable risk samples.

V. TO IDENTIFY DISTINCT MOLECULAR SIGNATURES ASSOCIATED TO RESPONSE AND/OR RESISTANCE TO ICB THROUGH GENE EXPRESSION PROFILING

1. Responders versus non-responders

To investigate differences in gene expression profiles between responders and non-responders to ICB, we first performed a DEG analysis using microarray data from ccRCC patients only. Using a FDR <0.1, no significant differences in DEG were observed. Thus, we attempted another analysis using raw p values ($p < 0.05$) instead of FDR <0.1. Between non-responders (progressors plus stable disease) (46) and responders (17), there were 21 DEGs with significant differences (p value <0.05), including *MET* and *FABP3* (Figure 24).

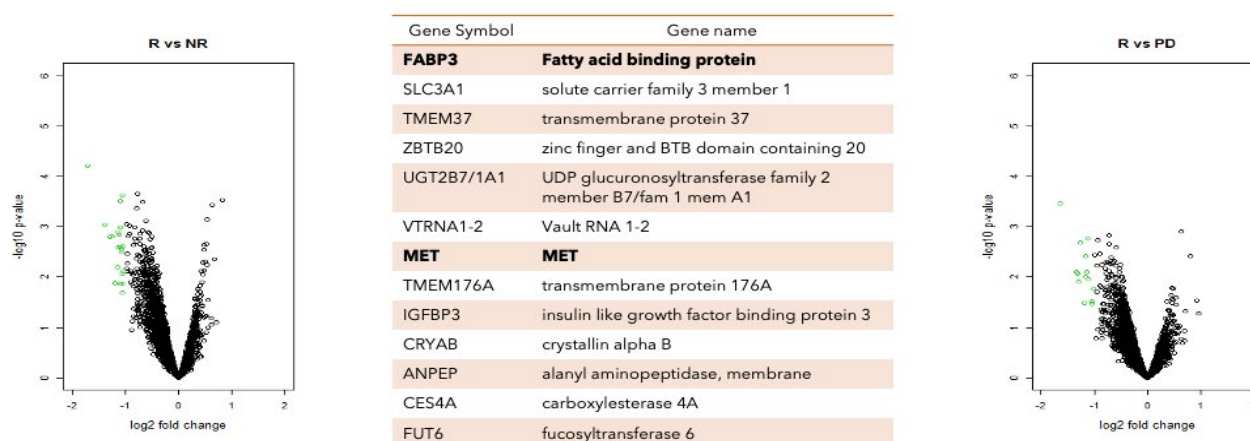


Figure 24. Differentially expressed genes between responders and non-responders and between responders and progressors.

Shared DEGs with significant differences (p value <0.05) for the two comparisons: responders (R) versus non-responders (NR), and responders (R) versus progressors (PD).

Regarding the pathway and process enrichment analysis, responder patients, compared to non-responders to ICB, presented a downregulation of Hallmark pathways involved in metabolism such as Oxidative phosphorylation (FDR q value <0.001), Adipogenesis (FDR q value < 0.005), Glycolysis (FDR q value < 0.005), Fatty acid metabolism (FDR q value < 0.01) or the peroxisome pathway (FDR q value < 0.05), protumoral pathways such as Hypoxia (FDR q value < 0.005), MYC target (FDR q value < 0.005), or MTORC1 signalling (FDR q value < 0.01), immunosuppression pathways such as TGF beta (FDR q value < 0.025) and IL2-STAT5 signalling (FDR q value < 0.05), and reactive oxygen species pathways (FDR q value < 0.05) (Figure 25).

We also compared responders (n=14) versus progressors (n=11) to ICB using only ccRCC tumours. With an FDR p value <0.001 the HALLMARK gene sets of the glycolysis, hypoxia, and adipogenesis pathways were significantly downregulated in responders compared to progressors. The oxidative phosphorylation (FDR q value < 0.01), peroxisome (FDR q value < 0.025), TGF beta (FDR q value < 0.01) and IL2-STAT5 (FDR q value < 0.05) signalling were also downregulated in responders compared to progressors (Figure 26).

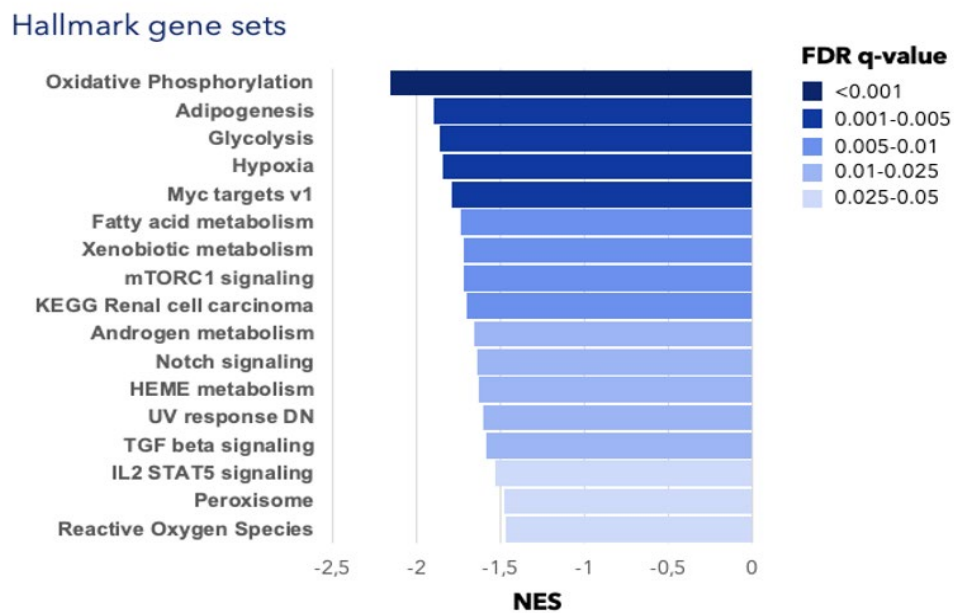


Figure 25. GSEA of Hallmark gene sets enriched/downenriched in tumours of responders to ICB versus non responders to ICB (FDR q-value< 0.05).

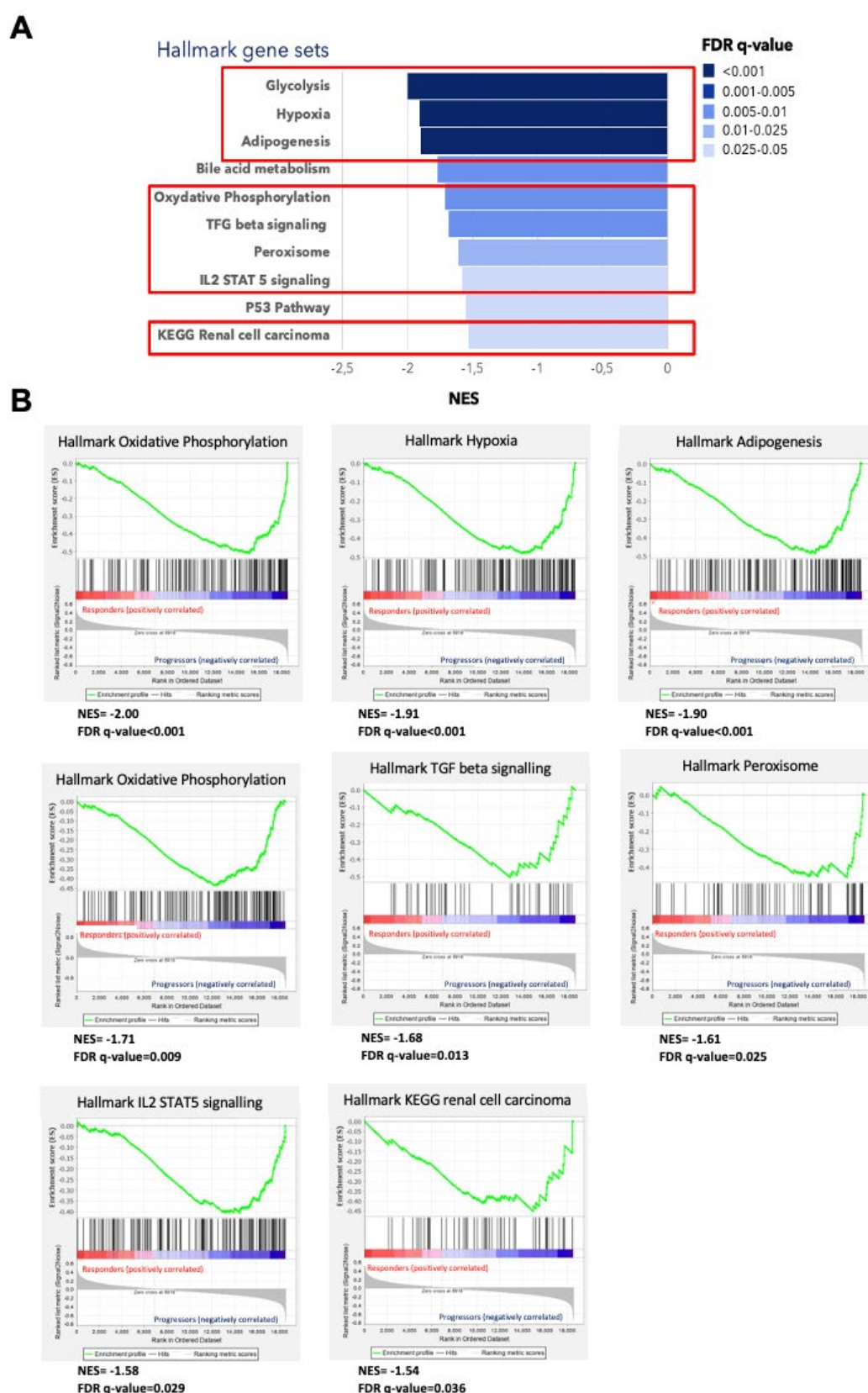


Figure 26. GSEA of Hallmark gene sets enriched or downenriched in clear cell tumours of responders to ICB versus progressors to ICB (FDR q-value< 0.05).

The results are presented as bar plot (A) and as running score and preranked list of GSEA result (B). In red, pathways that were downenriched in both the GSEA, including all tumours, and the GSEA, including only clear cell tumour.

To investigate the presence of immune heterogeneity between responders and non-responders, we performed unsupervised hierarchical clustering using GSVA enrichment scores for 17 immune pathways from the Hallmark, Reactome, KEGG, Biocarta and GO datasets (Figure 27A). Patients clustered into four immune clusters: cluster 1 (T helper 1 type low and myeloid high), cluster 2 (T helper 1 type high and myeloid low), cluster 3 (immune low, but cytokine activation), and cluster 4 (immune low). Responder and non-responder tumours were distributed evenly among the four immune clusters (Figure 27A). When evaluating only ccRCC, samples clustered again into four immune groups. This time most of ccRCC responder samples clustered together in cluster 4 characterised by being immune low, but immune checkpoints and T helper 1 type high (Figure 28A).

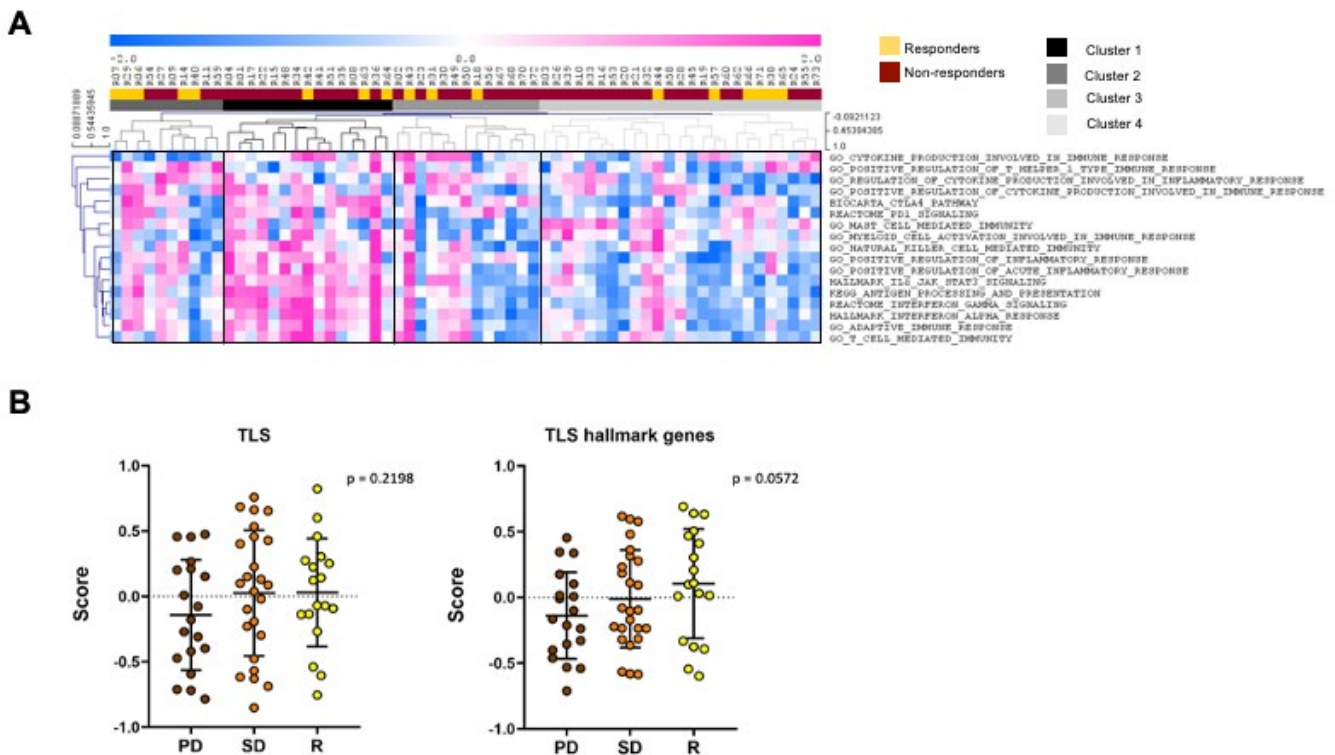


Figure 27. (A) GSVA (17 immune pathways) does not clearly discriminate between responders and non responders to ICB.

Heatmap showing tumours grouped by hierarchical clustering using the GSVA enrichment scores for 17 immune pathways from the Hallmark, Reactome, KEGG, Biocarta and Gene Ontology (GO) datasets. Tumours were divided into four immune clusters: cluster 1 (T helper 1 type low and myeloid high), cluster 2 (T helper 1 type high and myeloid low), cluster 3 (immune low, but cytokine activation), and cluster 4 (immune low). Responder and non-responder tumours were distributed among the four immune clusters. **(B) Scatterplots showing TLS gene set enrichment scores according to response.** TLS gene set: CD79B, EIF1AY, PTGDS, RBP5, CCR6, SKAP1, LAT, CETP, CD1D, and TLS hallmark genes: CCL19, CCL21, CXCL13, CCR7, CXCR5, SELL, LAMP3. No differences in TLS enrichments score or TLS Hallmark genes enrichment score was observed according to response ($p = 0.2198$ and $p = 0.0572$, respectively).

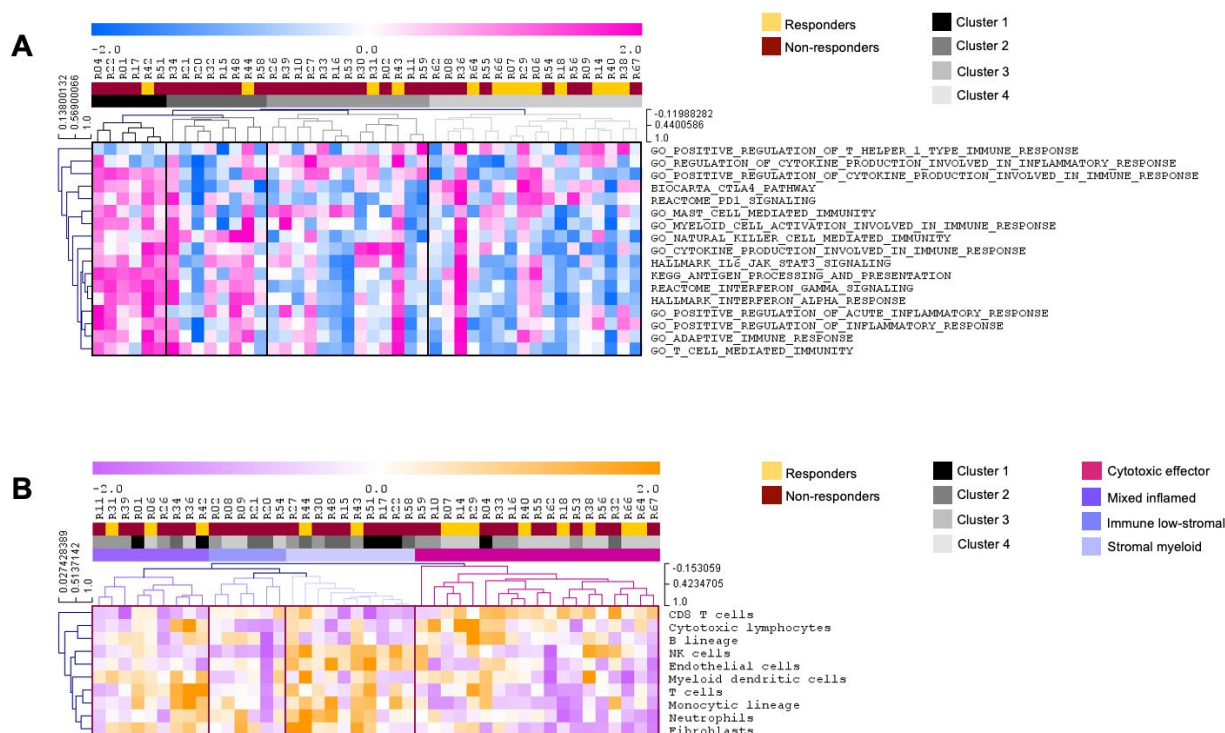


Figure 28. GSVA (17 immune pathways) and MCP counter seem to discriminate between responders and non responders to ICB among clear cell tumours.

(A) Heatmap of clear cell tumours grouped by hierarchical clustering using GSVA enrichment scores of the 17 immune pathways. Clear cell tumours were divided into four immune clusters: cluster 1 (T helper 1 type low and myeloid high), cluster 2 (acute inflammatory response), cluster 3 (immune low-cytokine activation), and cluster 4 (immune low, but immune checkpoints and T helper 1 type high). Responders grouped separately in cluster 4. (B) Heatmap of immune-cell profiling using the MCP-counter showed that tumours clustered into four different immune clusters: “cytotoxic effector”, “mixed inflamed”, “immune low-stromal”, and “stromal-myeloid”. A higher proportion of responders were observed in the cytotoxic effector immune cell cluster.

In the GSVA-derived immune clustering heatmap (29 immune-related gene sets) previously presented on Figure 17 we saw that patients “immune high” are associated with a downregulation of angiogenic gene sets (Angio low), while patients “immune low”, according to the 29GSVA, clusters are associated with an upregulation of angiogenic gene sets (Angio high). Interestingly, if we look at the responders that are “immune low” they all happen to present an enrichment in TLS-related gene sets (Figure 17). Based on these results and recent data supporting the potential role of TLS as a predictor of benefit from ICB, we then compared the enrichment score of the TLS gene set and the TLS hallmark gene set between patients with either progressive disease (PD), response (R), or stable disease (SD). A non-significant trend for a higher TLS score was observed in responders to ICB (Figure 27B).

We next explored the TME using the MCP-counter method. The TME is composed of immune cells, inflammatory mediators, endothelial cells, extracellular matrix, and mesenchymal cells. Unsupervised hierarchical clustering grouped patients into three main immune clusters according to the abundance of 10 immune cell populations (Figure 29A and 29C). The “cytotoxic effector” immune cell cluster was enriched in responder tumours. Responder tumours were enriched in cytotoxic lymphocytes, CD8 T cells, and other effector cells. Although both responders and non-responders were included in the “mixed-inflamed” cluster, by studying individual GE in specific progressive samples, we found that R34, R36, and R01 presented an upregulation of genes related to exhausted T-cell biomarkers, T-regulators, inhibitory signals, and a downregulation of genes related to immune activation signals (Figure 29B), associating specific non-responder samples with an immune silent phenotype. As seen across histological subtypes, when using only ccRCC samples, the “cytotoxic effector” group was enriched in ccRCC responders compared the other three clusters.

We also calculated and compared the ImmuneScore, StromalScore, ESTIMATE score and tumour purity according to treatment response using the ESTIMATE algorithm. This analysis included all RCC subtypes. No significant differences were seen in stromal or immune infiltration according to the type of response (Figure 30).

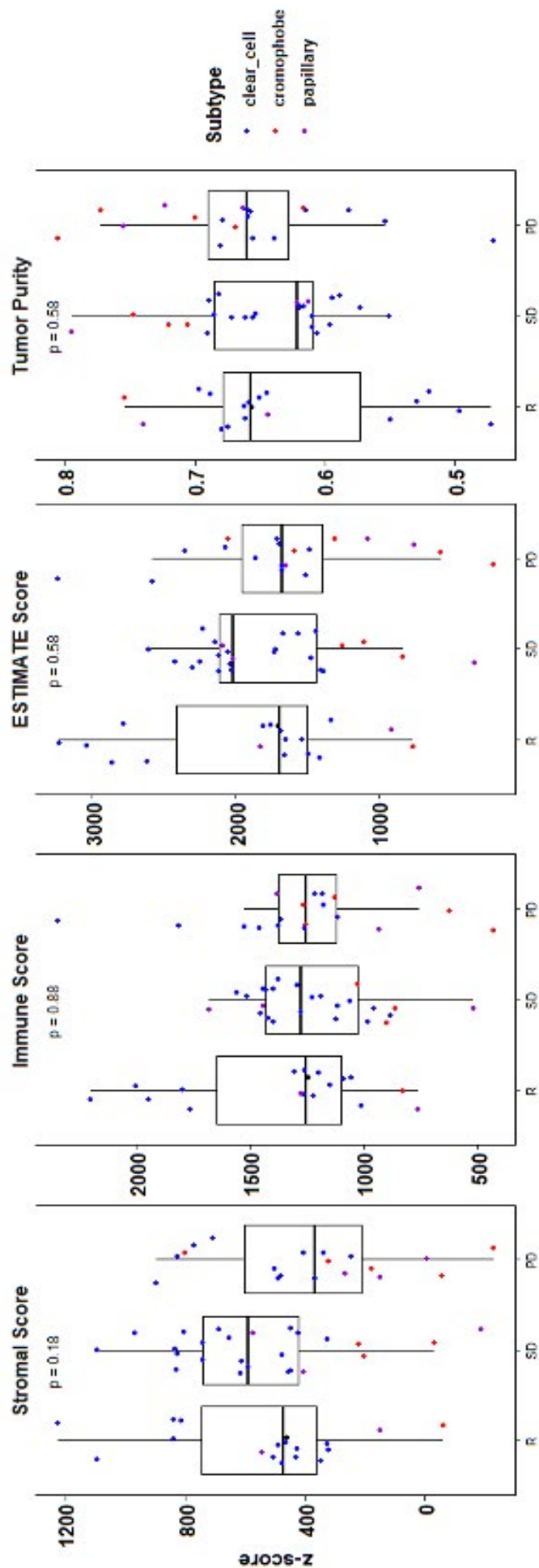


Figure 30. Distribution of StromalScore, ImmuneScore, ESTIMATE Score and tumour purity according to response type.

Boxplot representation of these scores in patients showing response (R), stable disease (SD) or progressive disease (PD) under immunotherapy Blue dots correspond to ccRCC, red dots to chrRCC, and violet dots to pRCC patients.

The Immunophenoscore (IPS) is used to determine tumour immunogenicity and predict response to ICB therapy across different tumour types. In our study, we did not find a significant association between the IPS and the response to ICB (Figure 31). No significant differences were found in MHC (major histocompatibility complex), effector cells, or suppressor cell checkpoints among the different response groups (Figure 31).

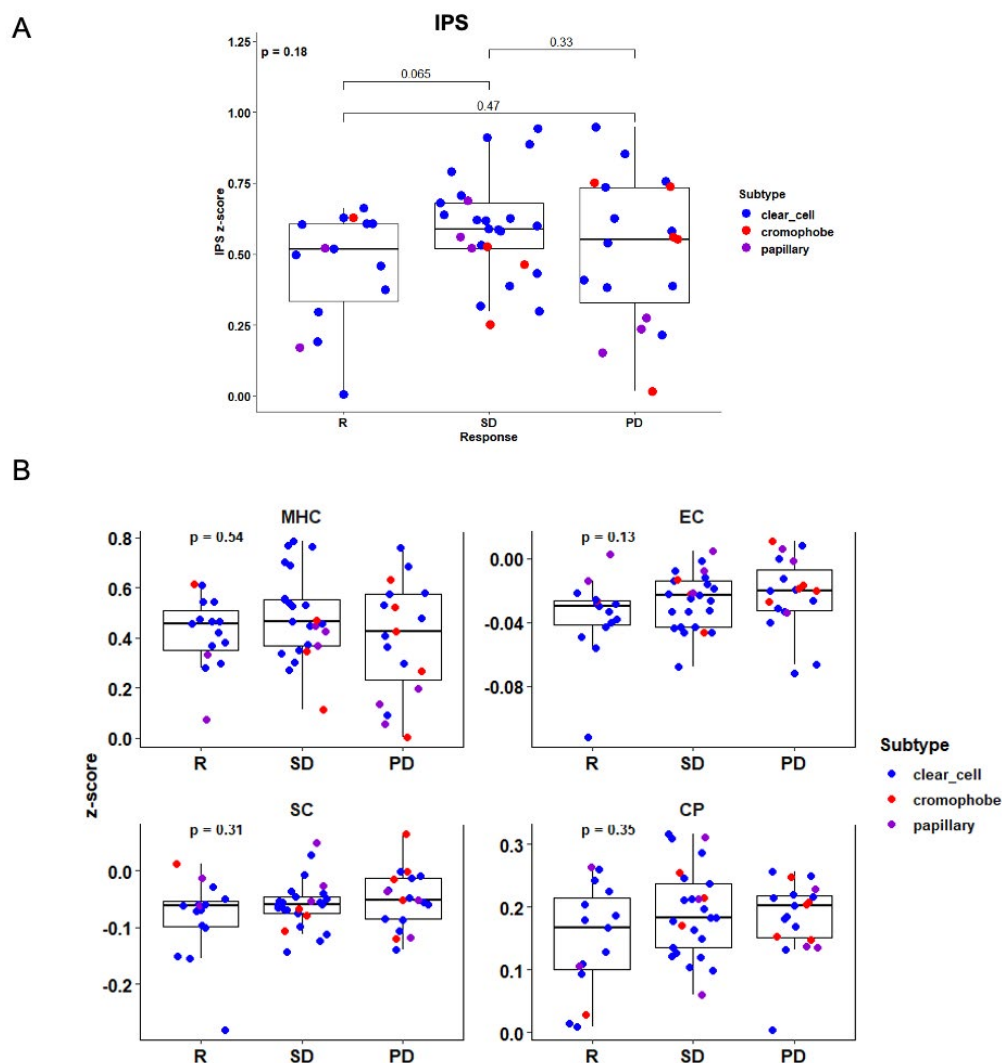


Figure 31. Distribution of immunophenoscore (IPS) according to response type.

(A) Boxplot representation of IPS in patients showing response (R), stable disease (SD) or progressive disease (PD) after immunotherapy (B) Boxplot representation of immune-related genes corresponding to the antigen presentation cluster (MHC), effector cell cluster (EC), suppressor cell cluster (SC) and checkpoint cluster (CP), in patients showing R, SD, or PD under immunotherapy. Blue dots correspond to clear-cell RCC, red dots to papillary RCC, and violet dots to chromophobe RCC patients.

2. Differential response to ICB and AA

Next, we performed GSEA in tumour specimens from an hyperselected subgroup of patients (n=20) characterized by having received, each of them, both ICB and antiangiogenic (AA) therapies, regardless of the sequence, and also by having responded significantly different to each treatment type. Tumours were thus divided into two groups: clinical benefit (CB) with ICB but non-CB with AA (n=6) versus non-CB with ICB but CB with AA (n=14) (Tables 13 and 14). Comparisons between these two groups demonstrated that patients without CB under ICB but with CB under AA presented a significant upregulation of protumoral HALLMARK pathways such as hypoxia, mTORC1, KRAS signalling and P53, metabolic pathways such as glycolysis and lipid metabolism pathways, and immunosuppression pathways such as IL2-STAT5 and TGF beta signalling (FDR q value <0.025) (Figure 32). The pleiotropic TNFalpha signalling via NfKappaB pathway was also upregulated in this group of patients (FDR q value <0.025). We also performed GSEA according to differential response to ICB and AA in only clear-cell patients, but no significant enrichment or under-enrichment was observed.

	Sex	Age at Dx	Age at Mets	Nx	Histological subtype	IMDC	Time from metastatic sites Dx to 1 st line <1y	TAA	Therapy line	BR	If no PD as BR, TTF (mo)	ICB	Therapy line	BR	If no PD as BR, TTF (mo)	
R07	Female	59	59	yes	clear cell	NA	no	Lung	Pazopanib	1st	PD	Nivolumab	3rd	CR	67 ^A	
R39	Male	76	76	yes	clear cell	intermediate	no	Lung, bone and lymph nodes	Pazopanib	2nd	PD	Atezolizumab+ bevacizumab	1st	SD	10 ^B	
R57	Male	61	70	yes	chromophobe	NA	no	Lymph nodes and ipsilateral kidney	Sunitinib	1st	NA	12	anti-PD1	3rd	PR	43 ^A
R63	Male	47	47	yes	papillary	NA	yes	Lung and peritoneal	Sunitinib	1st	SD	3	Nivolumab	2nd	PR	26
R65	Male	59	59	yes	clear cell	intermediate	yes	Lung and supra-infradiaphragmatic lymph nodes	Sunitinib	1st	PD	Nivolumab	2nd	PR	17 ^A	
R66	Male	47	49	yes	clear cell	good	no	Lung, liver and mediastinal lymph nodes	Sunitinib	1st	PD	Nivolumab	4th	PR	33 ^A	

Table 13. Characteristics and clinical outcomes of patients deriving benefit from ICB but not from AA.

^A Last FUP; ^B stop at 8 mo due to grade 2 nephritis. Abbreviations: Dx, diagnosis; IMDC, International mRCC Database Consortium; y, year; AA, antiangiogenics; BR, best response; TTF, time to treatment failure; PD, progression of disease; SD, stable disease; PR, partial response; NA, not available; CR, complete response; ICB, immune checkpoint blockers.

Sex	Age at Dx	Age at Mets	Nx	Histological subtype	IMDC	Time from Dx to 1 st line <1y	metastatic sites first line	AA	Therapy line	BR	If no PD as BR, TTF (mo)	ICB	Therapy lin#e	BR	If no PD as BR, TTF (mo)
R01	Male	57	68	Yes	clear cell	good	No	Mediastinal lymph nodes	Sunitinib	1st	PR	18	Nivolumab	3rd	PD
R04	Male	80	81	Yes	clear cell	NA	Yes	Liver and contralateral kidney	Pazopanib	1st	SD	49	Nivolumab	5th	PD
R16	Male	57	62	Yes	clear cell	intermediate	No	Lung and pancreas	Sunitinib	1st	PR	17	Nivolumab+ Ipilimumab	2nd	PD
R19	Male	37	39	Yes	chromophobe	good	No	Lung, bone and soft tissue	Sunitinib	1st	PR	18	Nivolumab	2nd	PD
R20	Male	76	78	Yes	clear cell	good	No	Lung and lymph nodes	Pazopanib	1st	SD	20	Nivolumab	2nd	SD
R28	Male	56	57	Yes	chromophobe	intermediate	Yes	Liver, bone and soft tissue	Sunitinib	1st	SD	6	Nivolumab	4th	PD
R30	Male	51	51	Yes	clear cell	intermediate	Yes	Lung and lymph nodes	Sunitinib	1st	SD	22	anti-PDL1/2/ VISTA	4th	SD
R32	Male	74	75	Yes	clear cell	intermediate	no	Lung, liver and adrenal gland	Sunitinib	1st	SD	18	Nivolumab	2nd	SD
R36	Male	65	65	Yes	clear cell	intermediate	Yes	Lung and liver	Sunitinib	2nd	CR	43	Nivolumab+ Ipilimumab	1st	PD
R41	Female	73	73	Yes	chromophobe	intermediate	Yes	Bone and lymph nodes	Pazopanib	1st	PR	8	Nivolumab	3rd	PD
R54	Female	66	76	Yes	clear cell	NA	No	Lung and pancreas	Pazopanib	3rd	SD	23	Nivolumab	4th	PD
R59	Female	58	69	Yes	clear cell	good	No	Lung and contralateral kidney	Sunitinib	1st	PR	29	Nivolumab	3rd	PD
R67	Female	56	57	Yes	clear cell	good	No	Lung and liver	Sunitinib	1st	PR	4	Nivolumab	3rd	PD
R70	Male	63	64	Yes	papillary	intermediate	Yes	Lung and bone	Axitinib	3rd	SD	48	Nivolumab	4th	PD

Table 14. Characteristics and clinical outcomes of patients deriving benefit from AA but not from ICB.

Abbreviations: Dx, diagnosis; Mets, metastasis; IMDC, International mRCC Database Consortium; y, year; AA, antiangiogenics; BR, best response; TTF, time to treatment failure; PD, progression of disease; SD, stable disease; PR, partial response; NA, not available; CR, complete response; ICB, immune checkpoint blockers.

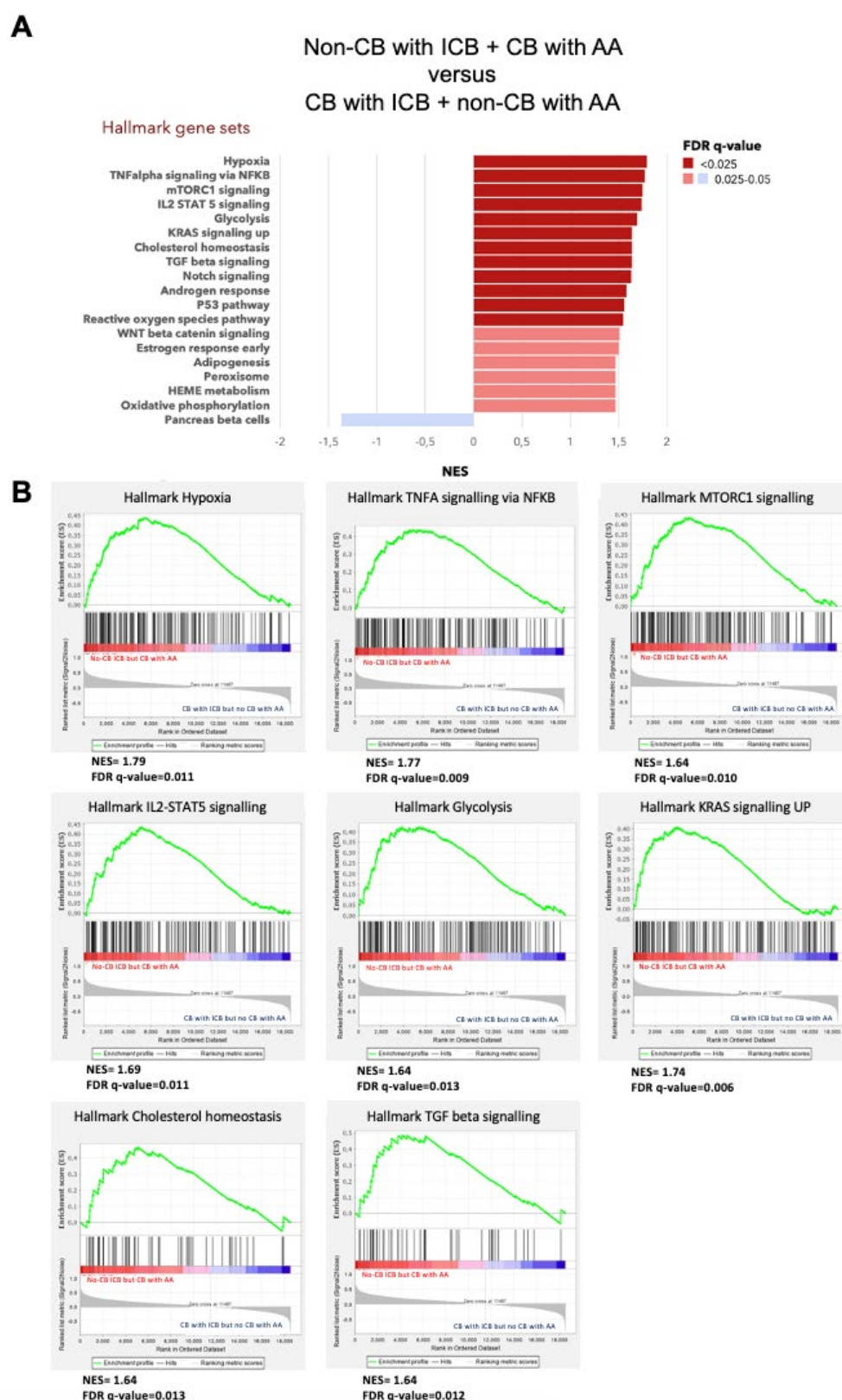


Figure 32. GSEA of Hallmark gene sets enriched or downenriched in tumours of patients with non-clinical benefit (non-CB) with ICB but clinical benefit (CB) with AA versus CB with ICB but not with AA (FDR q-value < 0.05).

The results are presented as bar plot (A) and as running score and preranked list of GSEA result (B).

Following the previous steps, we performed unsupervised hierarchical clustering using GSVA enrichment scores for 17 immune pathways from the Hallmark, Reactome, KEGG, Biocarta and GO datasets of the 20 patients presenting differential response to ICB and AA (Figure 33A). GSVA identified three immune clusters. These immune clusters failed to discriminate the tumours with differential response to ICB and AA (non-CB with ICB but CB with AA versus CB with ICB but not with AA) (Figure 33A).

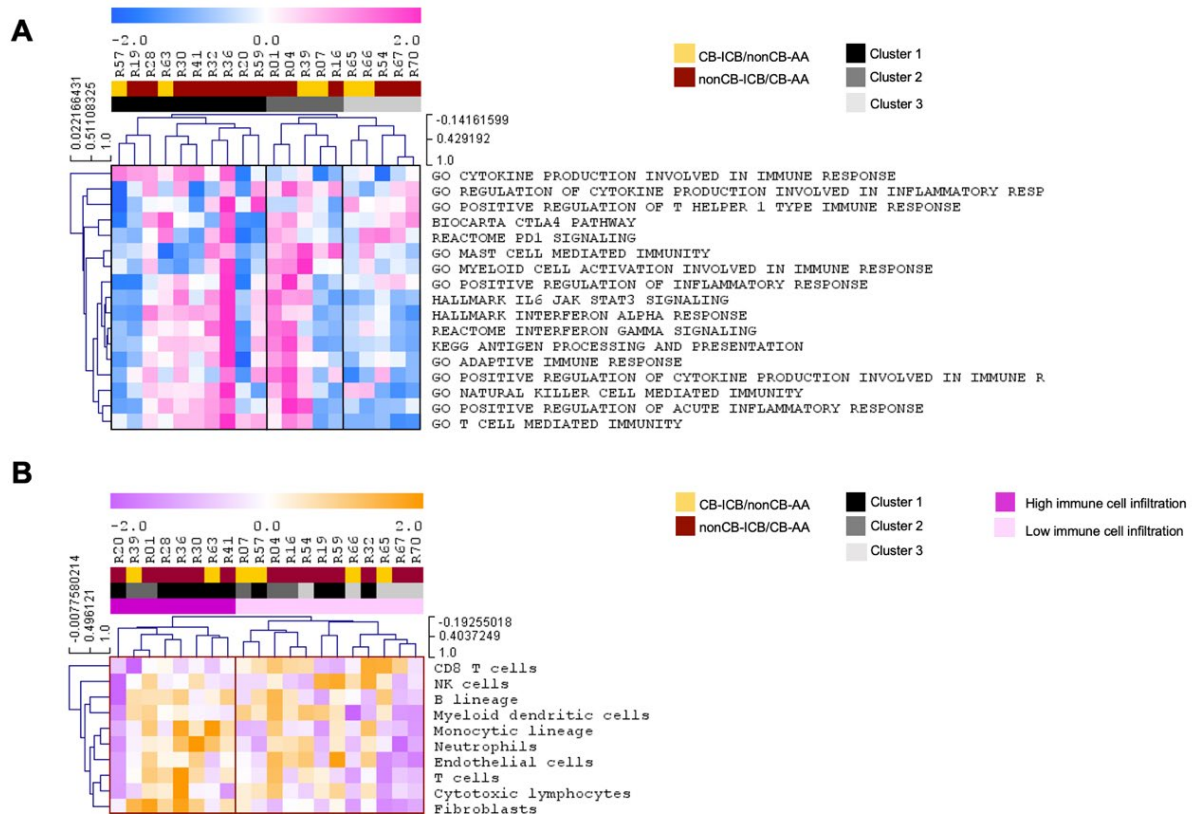


Figure 33. GSVA (17 immune pathways) and MCP-counter do not discriminate between those tumours benefiting from ICB but not from AA, and those with the opposite behaviour.

(A) Heatmap showing tumours grouped by hierarchical clustering using the GSVA (using 17 immune pathways from the Hallmark, Reactome, KEGG, Biocarta and Gene Ontology (GO) datasets) for patients with differential response to ICB and AA (CB with ICB but not with AA and CB with AA but not with ICB). Tumours were divided into three immune clusters: 17-Immune cluster high, highest activity in immune pathways, 17-Immune cluster intermediate, and 17-Immune cluster low, lowest activity in immune pathways. (B) Heatmap of immune-cell profiling using the MCP-counter showing separate immune clusters in tumours with CB with ICB but not from AA versus those with CB from AA but not from ICB.

The immune cell estimation using the MCP-counter method was able to group the samples into two immune cell clusters (Figure 33B). These immune cell clusters failed to discriminate the tumours with differential response to ICB and AA (non-CB with ICB but CB with AA versus CB with ICB but not with AA). However, we noticed that patients with CB from ICB but not from AA were frequently enriched in either B cells, CD8 T cells, or natural killer cells (Figure 33B).

In summary, we found that the TME of tumours from responders to ICB was enriched in cytotoxic T cells and other effector immune cells and was also characterised by a downregulation of immunosuppressive, metabolic, and protumoral pathways compared to tumours from non-responder patients. Similarly, tumors without CB from ICB but deriving CB from AA were enriched in immunosuppressive, metabolic and protumoral pathways compared to those deriving CB from ICB but not from AA. In addition, a trend for higher TLS scores in responder tumours was observed in our cohort.

VI. TO IDENTIFY DISTINCT MOLECULAR SIGNATURES ASSOCIATED WITH THE DEVELOPMENT OF SEVERE irAEs THROUGH GENE EXPRESSION PROFILING

To investigate differences in gene expression profiles between patients with or without irAEs, we first performed a DEG analysis using microarray data from 61 patients with available toxicity data, including clear and no-clear cell RCC. Using a FDR <0.1, we did not find any DEGs between tumours from RCC patients who developed severe irAEs and those who didn't.

Pathway enrichment analysis showed that among the top six gene sets (ranked by NES) upregulated in the severe irAEs group versus the non-severe irAEs group, there were four proimmune pathways upregulated: allograft rejection, inflammatory response, TNFalpha signalling via NFkB, and interferon gamma response (FDR q-value < 0.001) (Figure 34).

Following the previous steps, we next performed unsupervised hierarchical clustering using GSVA enrichment scores for 17 immune pathways from the Hallmark, Reactome, KEGG, Biocarta, and GO datasets. As previously shown, samples clustered into four immune clusters: cluster 1 (T helper 1 type low and myeloid high), cluster 2 (T helper high and myeloid low), cluster 3 (immune low but cytokine activation), and cluster 4 (immune low) (Figure 35A). Tumours from patients with severe irAEs did not group separately from those who did not present severe irAE, although cluster 2 included a higher proportion of tumours from patients with severe irAEs compared to the other three clusters. GSVA, focusing on clear cell tumours only, did not show additional findings (figure 36A).

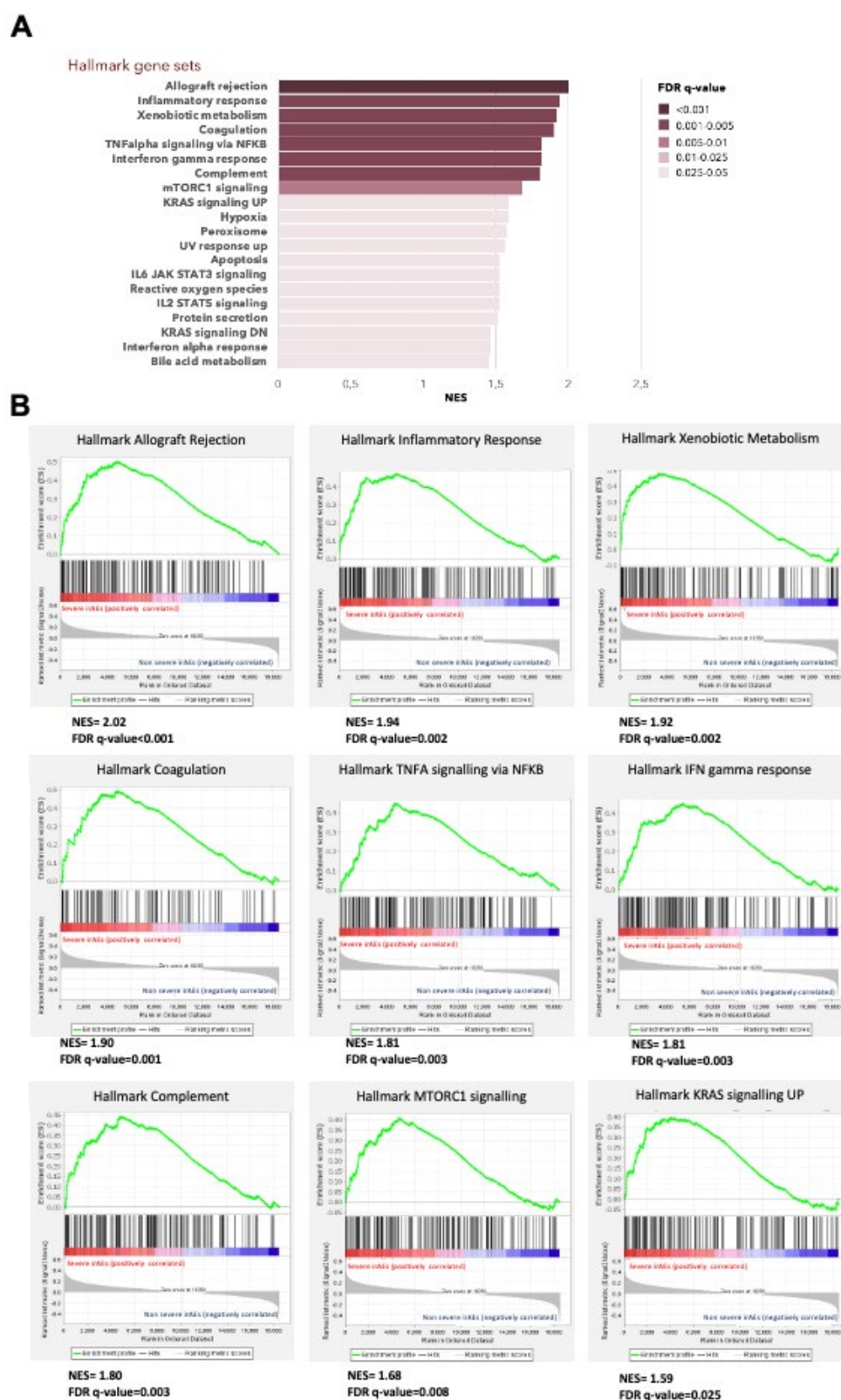


Figure 34. Gene set enrichment analysis (GSEA) of Hallmark gene sets enriched or downenriched in tumours of patients with severe irAEs versus those without severe irAEs (FDR q-value < 0.05).

The results are presented as bar plot (A) and as running score and preranked list of GSEA result (B).

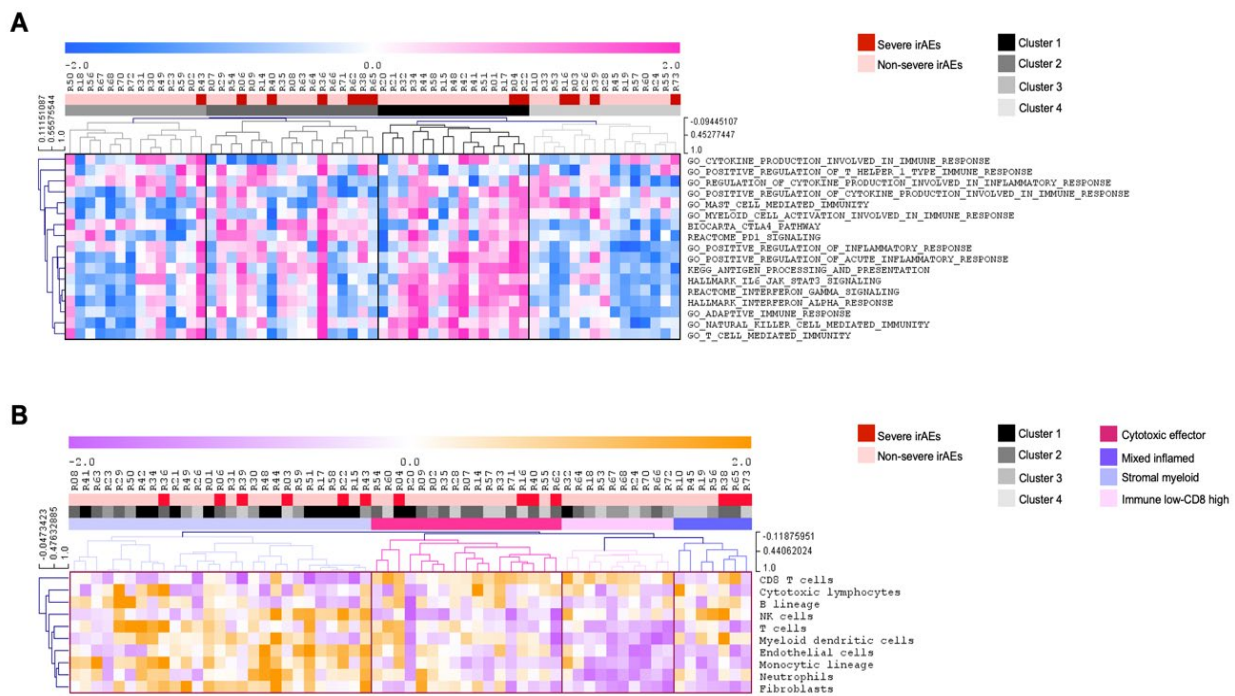


Figure 35. GSVA (17 immune pathways) and MCP-counter do not discriminate between tumours from patients with and without severe irAEs.

(A) Heatmap showing tumours grouped by hierarchical clustering using the GSVA Enrichment scores for 17 immune pathways from the Hallmark, Reactome, KEGG, Biocarta and Gene Ontology (GO) datasets. Patients were divided into four immune clusters: cluster 1 (T helper 1 type low and myeloid high), cluster 2 (T helper high and myeloid low), cluster 3 (immune low but cytokine activation), and cluster 4 (immune low). Tumours from patients with severe irAEs did not group separately from those who did not present severe irAE. (B) Heatmap of immune cell estimation using the MCP-counter showed that tumours clustered into four different immune clusters: “cytotoxic effector”, “mixed inflamed”, “stromal myeloid”, and “immune low-CD8 high”, but tumours from patients with severe irAEs and those without severe irAEs did not cluster separately.

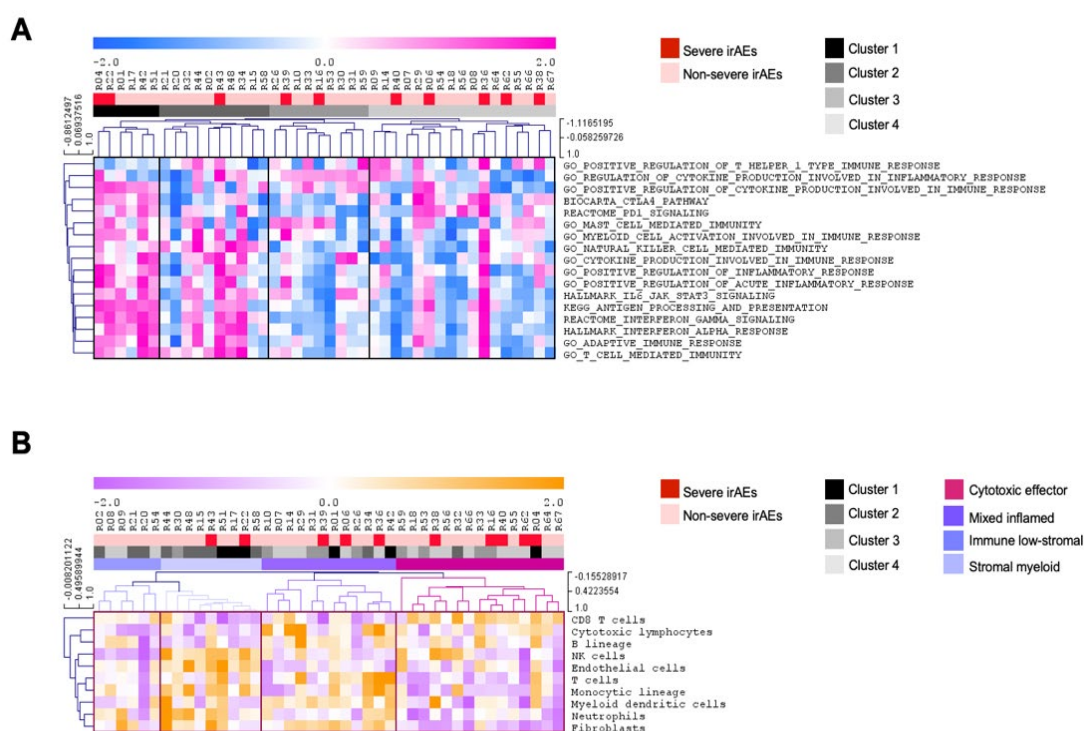


Figure 36. GSVA (17 immune pathways) and MCP-counter do not discriminate between tumours from patients with and without severe irAEs among clear cell tumours.

(A) Heatmap showing clear cell tumours grouped by hierarchical clustering using the GSVA enrichment scores for 17 immune pathways from the Hallmark, Reactome, KEGG, BioCarta, and Gene Ontology (GO) datasets. Patients were divided into four immune clusters: cluster 1 (T helper 1 type low and myeloid high), cluster 2 (acute inflammatory response), cluster 3 (immune low), and cluster 4 (immune low but IC high). Tumours from patients with severe irAEs did not group separately from those who did not present severe irAE. (B) A heatmap of immune cell estimation using the MCP-counter showed that tumours clustered into four different immune clusters: “cytotoxic effector”, “mixed inflamed”, “stromal myeloid”, and “immune low-CD8 high”. Although the MCP counter failed to clearly discriminate between those with or without severe irAEs, the “immune low-stromal” cluster was characterise by the absence of tumors with severe irAEs.

As previously observed, immune cell estimation using the MCP-counter clustered the tumour samples into four immune cell groups (“cytotoxic effector”, “mixed inflamed”, “stromal myeloid”, and “immune low-CD8 high”). However, tumours from patients with severe irAEs did not cluster together and were evenly distributed among the four immune clusters (35B). MCP-counter analyses using clear cell tumours only clustered the samples again into four immune cell groups: (“cytotoxic effector”, “mixed inflamed”, “immune low-stromal”, and “stromal myeloid” (Figure 36B). Although ccRCC tumour samples were not grouped separately according to the severity of irAEs, none of the samples from patients with severe irAEs were included in the “immune-low-stromal” group, and the highest proportion of severe irAEs was observed in the cytotoxic effector group.

Thus, the results of our analysis showed an upregulation of inflammatory pathways in tumour samples from patients developing severe irAEs compared to those without severe irAEs. Immune clusters using GSVA and the MCP counter method failed to clearly discriminate between the two groups of samples.

VII. TO ASSESS PREVIOUSLY PUBLISHED GENE EXPRESSION SIGNATURES IN OUR DATASET

We evaluated if previously published immune gene signatures (JAVELIN Renal 101 Immuno signature (26-JAVELIN) and the tumour inflammation signature (TIS)) were able to discriminate in our cohort according to response, toxicity and IMDC prognostic group. The 26-JAVELIN gene expression signature comprises regulators of both adaptive and innate immune response and has been associated with improved PFS in mRCC patients treated with avelu-axi within the JAVELIN Renal 101 trial (120). The TIS quantifies an activate but suppressed adaptive immune response and has been associated with benefit from ICB across different solid tumours (116).

First, we assessed if these two gene signatures were able to discriminate between responders and non-responders to ICB in clear cell tumours (n=46). None of these gene expression signatures were significantly enriched or down-enriched in responders compared to non-responders to ICB (Figure 37A). However, a non-significant trend for enrichment in the 26-JAVELIN in responders was observed.

We also evaluated these two signatures in patients displaying a differential response to ICB and AA. The TIS was significantly downregulated in responders to ICB, but not to AA, compared to non-responders to ICB (Figure 37B). The 26-JAVELIN was not able to discriminate between both groups (Figure 37B).

Finally, we also investigated the association of these immune gene signatures with the development of severe irAEs and according to IMDC groups. None of these signatures showed a significant difference in enrichment or down-enrichment according to the presence or not of severe irAEs (Figure 37C). Although no significant differences were observed according to IMDC groups, favourable patients, compared to intermediate/poor patients, seemed to present a down-enrichment of the TIS (Figure 37D).

In summary, in this real-world cohort, among previously reported immune gene signatures (26-JAVELIN and TIS) only the TIS was able to discriminate those with CB from ICB but not from AA versus those without CB from ICB but benefit from AA. The 26-JAVELIN was not able to discriminate tumours from those with or without severe irAEs.

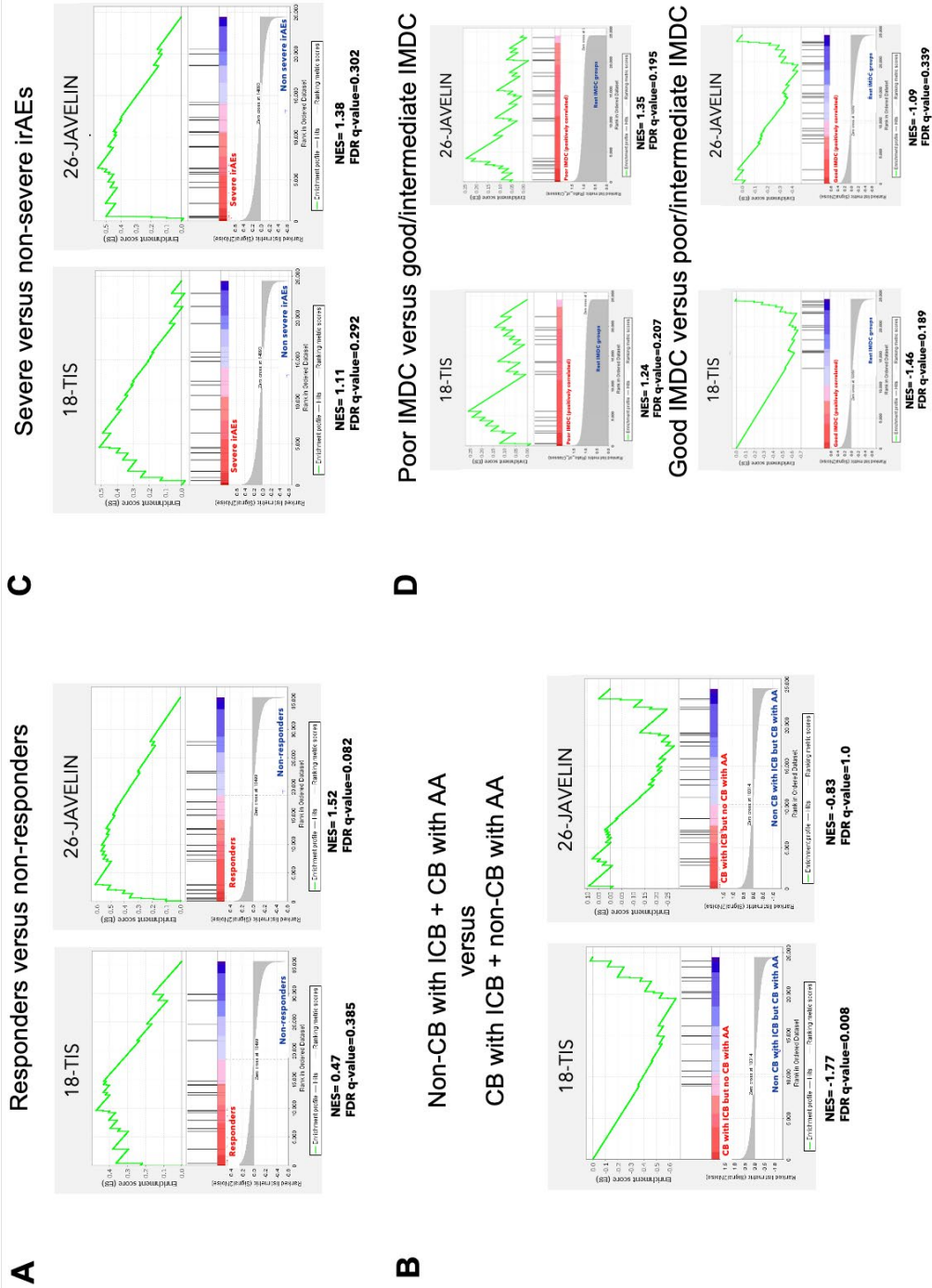
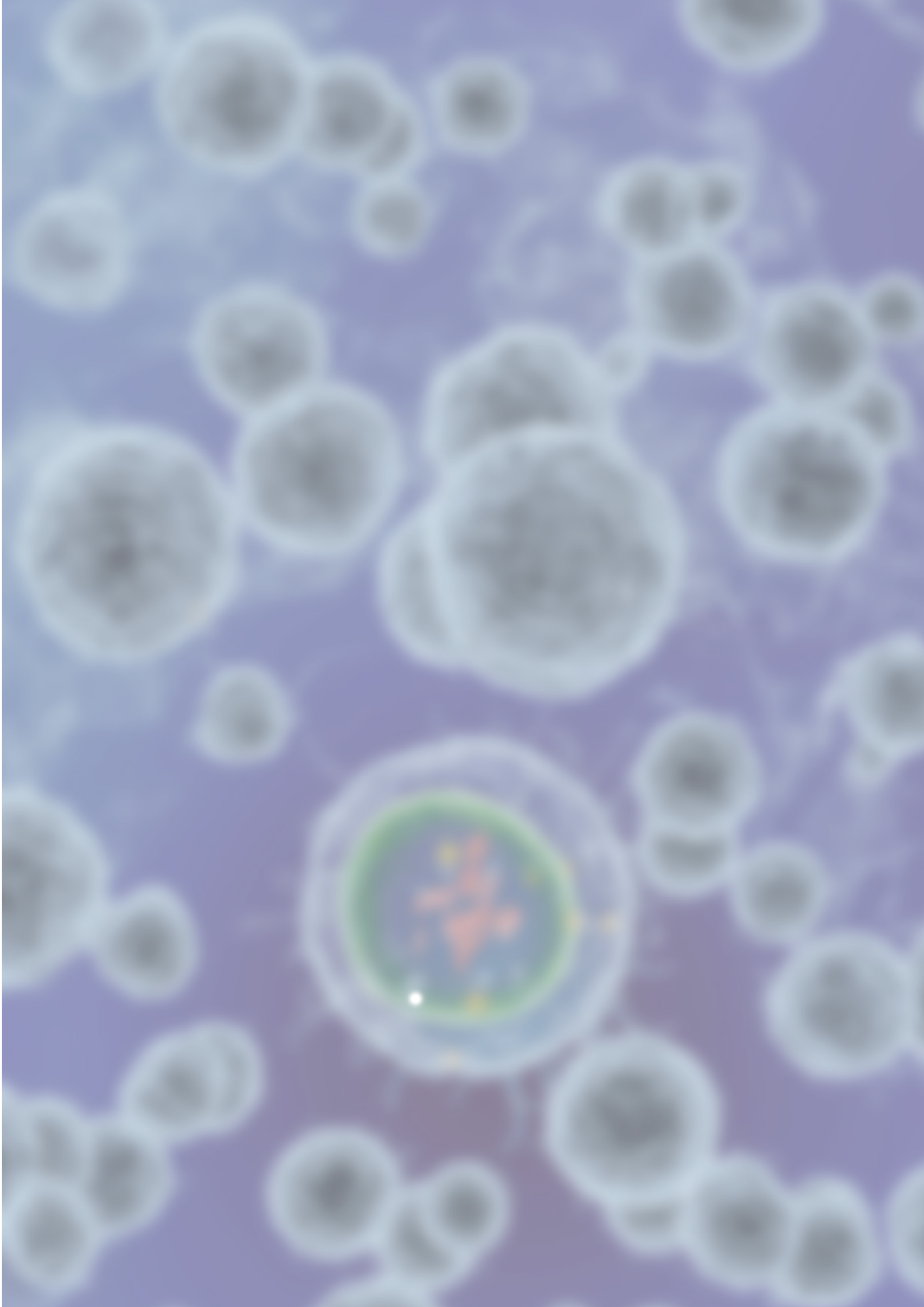


Figure 37. GSEA plots of three previously published gene signatures (TIS, 26-JAVELIN and Immunosuppression) (FDR q-value< 0.05).

(A) between responders and non-responders to ICB, (B) non-CB with ICB but CB with AA and CB with ICB but not with AA, (C) severe and non-severe irAEs, (D) according to IMDC risk group (on top, Poor IMDC versus rest of samples, below, good IMDC versus rest of samples) (D).



DISCUSSION



DISCUSSION

In the present study, we performed whole transcriptome characterization of primary tumour samples from RCC patients treated with ICB to understand molecular and immune features of the TME and its association with histological subtypes, tumour behaviour (IMDC risk groups and metastasis organotropism), clinical outcomes to ICB, and toxicity (see the graphical summary below, Figure 38). While we conducted an assessment of immune gene expression signatures within our dataset, it's important to note that we did not validate any additional gene expression signatures from external studies. This observation highlights a potential limitation in terms of the external validity of such signatures from other sources.

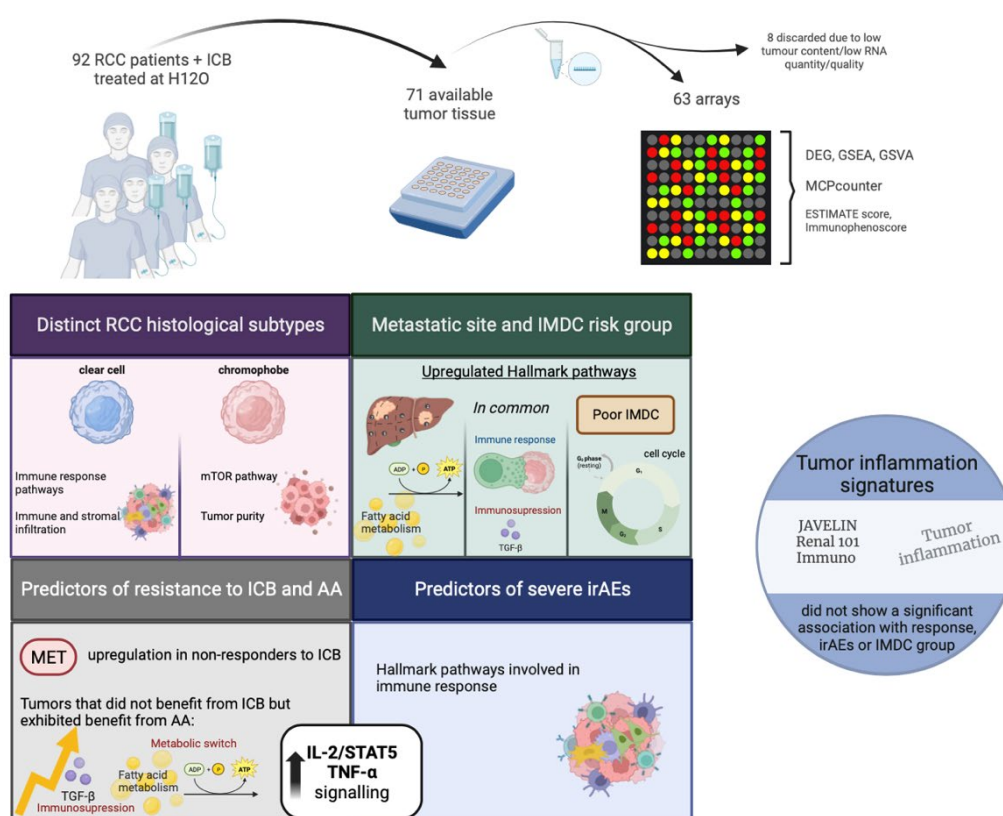


Figure 37. Graphical summary of our study and results.

I. DIFFERENTIAL GENE EXPRESSION ACCORDING TO RENAL CELL CARCINOMA HISTOLOGICAL SUBTYPES

Renal cell carcinoma is not a single disease. Renal cell carcinoma is a heterogeneous disease comprising different histological subtypes with different clinical behaviour and response to systemic therapies (160). The first of our aims was to describe and confirm the molecular heterogeneity between the main three histological RCC subtypes (ccRCC, pRCC, and chrRCC). Transcriptome analysis performed in this study showed significant differences in gene expression among the three histological subtypes, and particularly between the ccRCC and chrRCC subtypes (Figure 12). These results are aligned with previous studies from The Cancer Genome Atlas (TCGA) (39,89,124,161). A greater difference in gene expression was observed between ccRCC and chrRCC tumours, compared to pRCC tumours, which supports the theory that the molecular differences between the three histologies could be due to the different cell types of each nephron site (162). Papillary RCC tumours present an expression profile more similar to the proximal nephron, just as ccRCC tumours, while chrRCC's expression profile is more similar to the distal nephron (39,162,163),

Additionally, our analysis identified pathways exhibiting differential activity among RCC subtypes that were also observed in our cohort. As is widely recognised, clear cell tumours are highly immunogenic and display one of the highest scores in immune infiltration (164). In our cohort, ccRCC tumours demonstrated a significant enrichment in pathways related to immune response compared to pRCC and chrRCC tumours (Figure 13 and 14).

Chromophobe RCC tumours displayed the lowest immune infiltration and highest tumour purity in our study (Figure 20), which could explain the lack of response to ICB in advanced chrRCC patients. Recently, Labaki et al. evaluated the TME of a small cohort of oncocytic neoplasms (n=5), including chrRCC tumours, with matched normal kidney samples using single-cell RNA-seq (165). In accordance with our results, they found that renal oncocytic tumours exhibited a low immune infiltration, a non-exhausted immune phenotype, and a lack of clonally expanded tumour-specific T-cells (165).

Reviewing the GSEA-related molecular pathways, our study showed that EMT and hypoxia pathways were also enriched in ccRCC tumours relative to the other two subtypes (Figure 13 and 14). This finding aligns with previous research by Chen et al. where the most aggressive subtype, the CC-e.3, exhibited the highest upregulation of these pathways (163).

Metabolic reprogramming is one of the updated hallmarks of cancer (166). Metabolic reprogramming allows cancer cells meet their energy need for proliferation and survival (166). Previous studies from the TCGA, suggest an upregulation of oxidative phosphorylation and an increased mitochondrial biosynthesis in chrRCC tumours compared to the other two histological

subtypes, ccRCC and pRCC, that present a glycolytic phenotype (39,89,167,168). Accordingly, the oxidative phosphorylation pathway was the top one enriched in chrRCC tumours compared to pRCC tumours (Figure 16). Recent research supports the dysregulation of lipid metabolism in ccRCC tumorigenesis (169). Fatty acids (FA) are key for the lipid barrier but also as a source of energy (170). In ccRCC lipid metabolism is characterised by increased synthesis and utilization and decreased oxidation (171). Indeed, the presence of intracellular accumulation of lipid droplets in ccRCC and the “obesity paradox”, by which obesity is associated with a higher risk of ccRCC but however with a better prognosis in the advance setting and under treatment with ICB, support an important role of lipid metabolism in ccRCC (172). Unexpectedly, in our series ccRCC tumours did not show an enrichment in FA metabolism related pathways compared to the other two subtypes. And, surprisingly, chrRCC tumours exhibited however an upregulation of pathways involved FA metabolism, adipogenesis, and cholesterol, compared to pRCC (Figure 15). Previous research by Pandey et al. seemed to also suggest an upregulation of FA synthesis pathways in chrRCC tumours compared to the other two subtypes (167). Further research, particularly in chrRCC and pRCC, is needed to better understand the underlying lipid metabolic alterations in the different RCC subtypes. AMPK-mTOR signalling dysregulation has been associated with the aggressive chrRCC phenotype with poor clinical outcomes, leading to poor clinical outcomes (39,167,173). However, some mutations, such as *TSC2*, have been linked to a response to mTOR inhibitors (174,175). In our study, chrRCC tumours presented an upregulation of the mTOR pathway compared to the other two subtypes, eventually supporting the use of mTOR inhibitors in this population (Figure 15).

II. DIFFERENTIAL GENE EXPRESSION AND METASTASIS ORGANOTROPISM

The clinical behaviour of RCC exemplifies a paradigm of heterogeneity, not only among different histological subtypes but also within the same subtype and intratumoral level (160). Intriguingly, despite the underlying molecular and biological differences, the main subtypes of RCC (clear cell, papillary and chromophobe) share lymph nodes, lung, bone, liver, and adrenal as the five most frequent sites of metastases (160). This observation has been validated in our series. The site of metastases has been shown to have a significant impact on clinical outcomes and treatment (176). Several studies have shown that the sites of metastasis are associated with clinical outcomes in mRCC, regardless of histological subtype (160,177–179). Advanced RCCs with endocrine organ involvement have an excellent prognosis, while brain, bone, or liver metastases are associated with a poor prognosis compared to lung or lymph node metastasis (160,177,178,180,181). Thus, the site of metastases may reflect different clinical behaviours and, thus, a different underlying biology. Nguyen et al. conducted a comprehensive genomic prospective characterization of 25000 patients with different solid tumours and found that the genomic landscape of metastasis differed according to their target organ (182).

To provide more insight to the mechanisms underlying organ-specific metastases, we performed transcriptomic characterization in patients characterized by having only one metastatic site in first line setting: lung-only (28), liver-only (3), bone-only (9). We did not find any Hallmark pathway to be significantly enriched in lung-only or bone-only tumours in our series. Tumour samples from RCC patients with liver as only site of metastases presented however a significant increase activity in metabolic pathways such as oxidative phosphorylation and fatty acid (FA) metabolism but also in pathways involved in inflammatory response and immunosuppression, and in oncogenic pathways such as hypoxia and the mTOR pathway, compared to those with lung or bone as only site of metastasis in first line setting (Figure 20). Using pathway enrichment analysis to compare liver tumour samples metastasized from breast and colon cancer with liver control tissue samples, Zhang and colleagues identified several KEGG signalling pathways and GO biologic processes specific to liver metastases (183). Interestingly, consistent with our results, a major part of signalling pathways and biologic processes upregulated in liver metastases in this study were involved in metabolism, particularly in glucose and lipid metabolism, and in the hypoxia pathway (183). In addition, cell cycle and FoxO signalling pathways were also dysregulated in liver metastases in this study, which together with our findings support a key role of the mTOR pathway promoting both tumour cells proliferation and a metabolic switch (183,184). Although, as described in the following sections, FA metabolism seems to play a role in the response to ICB in RCC patients and alterations in FA metabolism can be found in both cancer and in immune cells, it should be noted that the liver itself plays a key role in the regulation of FA metabolism, and this could also explain our findings (185). We did not find other studies in the literature

reporting on the activity of immune pathways according to primary metastatic site. However, our results, showing an enrichment of primary tumours of patients with liver only metastases, in both immune response pathways and immunosuppression pathways, are not surprising given that mRCC with liver metastases are usually poor IMDC prognostic tumours, characterized by tumoral and systemic inflammation, but also peripheric and tumoral immunosuppression that may condition inferior outcomes to ICB (186,187).

Our study is limited by the absence of matched metastatic biopsies that would have provided a better understanding of the molecular evolutionary process underlying tumour progression and dissemination. Results from the landmark study led by Turajlic and colleagues of matched primary and metastatic biopsies from 100 ccRCC showed that primary tumours, compared to their matched metastatic samples, present more clonal heterogeneity and more frequent driver events. In addition, very few de novo driver events were observed in metastases, suggesting that the primary tumour harboured a driver diversity that allowed for the selection of metastases-competent populations (176).

III. DIFFERENTIAL GENE EXPRESSION AND IMDC RISK GROUP

The IMDC risk score is a clinical prognostic model with demonstrated predictive value in the context of ICB (149,188). This prognostic model includes two clinical variables (time from diagnoses to start of first line treatment < one year, and KPS <80%) and four laboratory factors (hypercalcemia, anaemia, thrombocytosis, and neutrophilia), a reflection of systemic inflammation (148). In the CheckMate 214 trial, the superiority of nivolumab-ipilimumab compared to sunitinib was only observed in intermediate/poor IMDC risk patients and in the intention-to-treat population; sunitinib remained superior in those with a favourable prognosis (189). Moreover, the results of the COSMIC313 showed no benefit from adding cabozantinib to nivo-ipi in poor IMDC-risk patients, as opposed to intermediate IMDC-RCC patients (190). These differences in clinical outcomes and response to ICB, AA, or ICB+AA according to IMDC groups could be justified by differences in tumour biology and immune microenvironment among the three IMDC groups. Indeed, in the comprehensive multi-omics analysis performed within the IMmotion151, intermediate and poor IMDC and MSKCC tumours were enriched in T-effector/proliferative, proliferative, stromal and complement/omega-oxidation clusters, while favourable-risk patients presented an enrichment in angiogenic and angio/stromal clusters (119). Similarly, in the gene-expression-based subgrouping by Beuselinck et al., ccr4cc (cmyp up/immune up) was enriched in intermediate and poor IMDC/MSKCC risk patients and presented a poor response to sunitinib compared to the other three molecular clusters (115). Verbiest and colleagues confirmed that favourable IMDC risk patients were enriched for the ccr2cc (classical) subtype and angio-high tumours, while poor IMDC risk tumours were enriched for the ccr4cc subtype and angio-low tumours (191). This is consistent with the association between higher scores of a T-effector gene expression signature and increased benefit from ICB-based therapy and between higher expression of an angiogenesis gene signature and increased benefit from sunitinib in the JAVELINRenal101 and IMmotion150 trials (118,120). In line with the previously mentioned data, in our study we observed not only an upregulation of proimmune and protumoral pathways involved in the cell cycle in poor IMDC tumours, which is consistent with the findings of previously mentioned molecular classifications, but also an upregulation of immunosuppression pathways (115,119) (Figure 21). Both the “Teffector/proliferative” and “proliferative” clusters from the IMmotion151, as well as the “cmyp up/immune up” cluster from Beuselinck and colleagues, that were enriched in intermediate and poor IMDC risk patients, presented a higher expression of T-effector and cell cycle pathways, which were also found to be significantly enriched in poor IMDC clear cell tumours in our study.

Thus, although limited by a small sample size that should be increased, our results suggest and raise the hypothesis that poor IMDC-risk ccRCC tumours are not only characterised by a proliferative and inflamed TME, which confers both a poor prognosis and an increased response to ICB, but also by a higher immunosuppression that could be responsible for the significant subset of poor-risk patients that are still primary resistant to ICB. These results will be further evaluated in an external, and larger, database.

IV. TO IDENTIFY DISTINCT MOLECULAR SIGNATURES ASSOCIATED WITH RESPONSE AND/OR RESISTANCE TO ICB THROUGH GENE EXPRESSION PROFILING

Several studies have made attempts to develop immune prediction signatures, but no trial has included patients treated with both sequential ICB and AA treatments. Despite the bias of retrospective studies, we were able to include a cohort to help us identify biomarkers of response applicable in routine clinical practice.

The results of GSEA performed in pre-treatment RCC tumour samples in our study suggest that specific biological processes may be associated with resistance to ICB in mRCC. Metabolic-related Hallmark gene sets, including glycolysis, oxidative phosphorylation, fatty acid (FA) metabolism, and adipogenesis, were particularly down-enriched in tumours from responder patients to ICB (Figure 25). When we compared tumours from patients without clinical benefit from ICB but with clinical benefit from AA versus those with clinical benefit from ICB but nonclinical benefit from AA, these metabolic pathways were enriched again in those without clinical benefit from ICB but with clinical benefit from AA (Figure 32).

Inflammation signatures previously reported were not shown to be associated with clinical outcomes for nivo+ipi in the CheckMate 214 (116,118,192). The molecular classification of Beuselinck et al., initially developed in aRCC patients treated with sunitinib, was used in the randomised biomarker-driven BIONIKK trial, showing that the response to nivolumab alone, nivo+ipi, or antiangiogenic varied according to the molecular subgroup, that is, according to the composition of the TME (193). However, it was far from good enough to be applied to clinical practice. The addition of ipilimumab to nivolumab in ccr4cc (“immune-high”) patients did not increase the response rate, while it did increase response rates in patients ccr1cc (“immune low”), on the other hand, sunitinib was superior to nivo+ipi in ccr2cc (“angio-high”) patients, while nivo+ipi was superior in terms of response rate to sunitinib in ccr3cc (“normal-like”) patients (193). Thus, understanding the TME seems key to guiding treatment selection, but gene expression signatures are still not ready for prime time.

In our study, we performed gene expression analysis of primary tumour samples from a real-world cohort of aRCC patients treated with ICB, regardless of therapy line, and compared responders and non-responders to ICB. We identified 21 DEGs between clear cell responders and non-responders, among which were especially relevant *MET* and *FABP3* (Figure 24). These two genes were upregulated in non-responders compared to responders to ICB, but also in progressors compared to responders to ICB.

The proto-oncogene *MET* encodes the c-MET protein, also called the tyrosine-protein kinase MET (194). Activation of c-MET triggers a whole spectrum of biological activities that promote

proliferation, angiogenesis, and metastasis (194). Thus, it is not surprising that MET is dysregulated in many types of solid tumours, including RCC, and that is associated with a poor prognosis (194,195). From a biological perspective, c-MET signalling does not only promote tumorigenesis but also immunosuppression through different mechanisms, including suppression of CD8+ T cell antitumor responses by c-MET+ neutrophils and upregulation of the immunosuppressive enzyme IDO1, promotion of TGF-beta synthesis, and upregulation of PD-L1 in tumour cells (194,196–198). Consistent with these observations, in our study, tumours from non-responders and progressors to ICB presented a higher expression of *MET*. Thus, our results further support the rationale for the combination of cabozantinib (a tyrosine-kinase inhibitor with activity against MET, VEGFR2, FLT3, c-KIT, RET and AXL) with nivolumab in advanced RCC (194,199).

The gene *FABP3* encodes Fatty Acid Binding Protein 3 (FABP3) which is a member of the PPAR signalling pathway. FABPs are a family of lipid-binding proteins involved in fatty acid uptake, transport, and cell metabolism. FABP3 has been reported to be involved in tumorigenesis by providing an energy source for the rapidly proliferating tumour cells. FABP3 has also been reported to be a prognostic marker in NSCLC, breast, and ovarian cancers. However, to date, there is a lack of data regarding FABP3 and RCC.

Oxidative phosphorylation is used by normal cells as an energy source; however, in order to satisfy the biosynthesis and energy requirements, highly proliferating cells, such as cancer cells, need to reprogram metabolism, increasing glycolysis and FA metabolism (200). Metabolic reprogramming through anaerobic glycolysis has been reported to reduce the antitumor immune response by increasing the competition for glucose in the TME between cancer and immune cells and by producing immunosuppressive metabolites such as lactate (201). However, there is also data suggesting that tumour glycolysis could increase tumour immunity and increase tumour PD-L1 expression in different solid tumours, including pRCC (202). In the Checkmate214, enrichment in the Hallmark Glycolysis gene set was associated with a poor prognosis in mRCC patients, regardless of treatment type (192).

Strikingly, our investigation illustrated a noteworthy revelation: an enrichment of glycolysis within the TME not only in patients unresponsive to ICB but also in those lacking clinical benefit from ICB while currently benefiting from AA (Figure 25, 26 and 32). This intriguing observation led to the hypothesis that increased glycolysis activity within the TME might serve as an indicator of resistance to ICB and as a predictive marker for favourable responses to AA therapy. Furthermore, we brought into focus the intriguing debate surrounding the prognostic and predictive roles of FA metabolism in patients with mRCC receiving ICB.

As with glycolysis, several studies have evaluated the potential prognostic and predictive role of lipid metabolism in mRCC patients treated with ICB with controversial results (119,192,203,204). Our results are in line with the negative correlation observed in Checkmate 009 between this

gene set and response to nivolumab, where most patients had received prior AA therapy, and with the negative association of fatty acid metabolism and survival in ccRCC reported by Zhao et al. previously (203,204). The CheckMate 214 revealed however an unexpected twist: the Hallmark FA metabolism gene set appeared to be associated with a favourable prognosis, irrespective of therapy type (AA or double ICB). This apparent paradox underscores the intricacies of cancer biology and the dynamic interplay between metabolic pathways, gene signatures, and clinical outcomes, which manifest uniquely across diverse patient cohorts and study settings (121). In IMmotion 151, cluster 2 was enriched in angiogenesis and FA oxidation pathways, and cluster 5 was enriched in cell cycle and FA synthesis pathways (119). Non-significant differences between atezo-beva or sunitinib were observed in cluster 2, characterised by increased activity of the FA oxidation pathway, while atezo-beva was significantly superior to sunitinib in cluster 5, characterised by increased activity of the FA synthesis pathway (119). The different results obtained in these cohorts could be explained by the type of fatty acid metabolism, the history of previous AA therapy or not, and the type of ICB therapy, single or double, either with another ICB or with an AA.

Of note, both glycolysis and FA metabolism have been reported to be upregulated in CD8-positive T cells in mRCC (205). Thus, in the absence of single-cell RNAseq that helps us determine if the transcriptional programmes are activated in tumours and/or in tumour-infiltrating immune cells, these findings should be interpreted with caution.

Several immune-related pathway gene sets were also associated with the absence of response to ICB but however benefit from AA (Figure 33). As expected, and in line with previous studies performed in other cancer types treated with ICB, the immunosuppressive TGF beta pathway was enriched in patients unresponsive to ICB and in those deriving clinical benefit from AA but not from ICB (206–208). In the study by Chen and colleagues, they were able to identify a novel immune class in head and neck squamous carcinoma, with two subclasses characterised by active or exhausted immune responses associated with favourable and poor prognosis, respectively (206). The exhausted immune subtype was characterised by an M2 macrophage signature, activated stroma, and WNT/TGF beta pathway activation (206). Although the TGF beta signalling pathway has not been previously reported to be associated with clinical outcomes for ICB in mRCC, it has been found to be associated with improved PFS for sunitinib in mRCC patients, which is consistent with our results. In contrast to the previously reported associations between IL2-STAT5 activation and improved PFS and between TNFalpha signalling via NFkBeta activation and improved OS in mRCC patients treated with nivo+ipi within the Checkmate214 trial, in our study, IL2-STAT5 and TNFalpha signalling via NFkBeta pathways were enriched in patients not responding to ICB but however benefiting from AA (Figure 33). The differences in the results obtained for the IL2-STAT5 pathway between the Checkmate214 study and our cohort could be explained by the divergent effects of IL2-STAT5 signalling in effector and regulatory CD4+ T cell populations (209). The pleiotropic effects of TNF alpha could also justify the differences between

our results and Checkmate 214. However, it should be noted that, in line with our results, data from preclinical studies and from a small phase 1b clinical trial in melanoma patients suggest that TNF blockers could indeed enhance the efficacy of ICB through the increase of circulating T-1 helper cells and IFN gamma concentration (210,211). In our study, upregulation of the hypoxia and KRAS pathways were associated with the absence of a response to ICB but benefit from AA (Figure 33). These results contrast again with the biomarker analyses from Checkmate214, which found an association between activation of these two pathways and improved outcomes in mRCC patients treated with nivo+ipi, and, with the negative correlation between KRAS signalling activity and PD-L1 expression reported by Denize and colleagues (212). Finally, consistent with recent studies supporting the predictive role of TLS in response to ICB across different tumour types, including mRCC, a trend for higher TLS gene set enrichment scores was observed in responders to ICB in our study (130–132,213) (Figure 27B). Statistical significance was however not reached, probably due to sample size and heterogeneity in treatment lines and ICB types, single and combinations. Further investigation and validation of these gene sets associated with the absence of clinical benefit from ICB but, however, benefit from AA would provide more insight and help develop a reliable predictor of response to systemic therapy to guide treatment selection and sequence in mRCC.

The presence of an immunosuppressive TME, constituted of immunosuppressive cells such as myeloid-derived suppressor cells, neutrophils, endothelial, macrophages or cancer-associated fibroblasts, has been associated with worse clinical outcomes to nivolumab in mRCC patients (214). In our study, no significant associations between individual immune cell subtypes and response to ICB was observed. However, out of the four patient subgroups derived by unsupervised clustering, the “cytotoxic effector” subgroup, characterized by higher levels of CD8+ T cells, NK cells and cytotoxic lymphocytes, and a lower level of stromal and immunosuppressive cells, was enriched in responder patients, compared to the other three subgroups (Figure 29A and 29C). These results are consistent with the association of the IMmotion 150 T effector and the JAVELIN Renal Immuno signatures with improved clinical outcomes under ICB therapy (118,120). Further investigation of these immune subgroups in a larger and homogeneous cohort of mRCC patients treated with ICB may help confirm a significant association between the “cytotoxic effector” subgroup and response to ICB.

V. TO IDENTIFY DISTINCT MOLECULAR SIGNATURES ASSOCIATED WITH SEVERE IRAES THROUGH GENE EXPRESSION PROFILING

Different candidate predictors of irAEs have been proposed, including circulating blood counts, circulating cytokines, serum proteins, HLA genotypes, intestinal microbiota, or microRNA and blood gene expression profiling (215). However, although some of these markers (i.e. circulating blood counts) are accessible in clinical practice, prospective validation in large cohorts is needed before incorporation into routine clinical practice. Of note, most of these studies were retrospective and focused on very specific populations, organ-specific toxicities or specific ICB therapies. In addition, there is a lack of studies investigating potential predictors of irAEs in mRCC patients and a lack of studies investigating potential predictors of irAEs using tumour gene expression profiling. In our study, we aimed to identify potential predictors of severe irAEs by performing gene expression profiling on primary RCC tumour tissue of patients treated with ICB regardless treatment line.

We did not find any DEGs between tumours from patients developing severe irAEs and those who did not. However, as it would be expected, upregulation of immune-related pathways gene sets associated with increased immune response (allograft rejection, inflammatory response, interferon gamma response) was associated with the development of irAEs (Figure 34). Curiously, gene sets of immune pathways with ambivalent and pleiotropic effects such as IL2-STAT5 or TNFalpha signalling via NfKappaB pathways, which were found in our dataset to be associated with the absence of benefit from ICB, but however benefit from AA, were also upregulated in tumours from patients with severe irAE (Figure 34). Although these results could be explained by a small sample size, they also raise the hypothesis that these two immune-related pathway gene sets could behave as predictors of resistance to ICB or response to AA and, at the same time, as predictors of severe irAEs and could thus help guide treatment selection in mRCC. Further evaluation of these two gene sets will be performed on an external and larger dataset.

Although no significant differences in the rate of severe irAEs were observed among any of the four immune clusters derived by GSVA unsupervised clustering, cluster 2, characterised by higher activity of the T helper 1 type pathway and lower activity of the myeloid pathway, presented a higher rate of severe irAEs (Figure 35A). Moreover, immune cell estimation using the MCP counter on ccRCC samples showed that the so-called “immune-low-stromal” group was characterised by an absence of severe irAEs, while the “cytotoxic effector” group had the highest proportion of severe irAEs. These results support the key role of the TME immune cell composition in the development of severe irAEs (Figure 35B). Further investigation in a larger dataset may find an association between a specific GSVA-derived or MCP-counter-derived subgroup and a higher rate of severe irAEs.

VI. LIMITATIONS

Our study presents several limitations that must be considered when interpreting our findings. Firstly, it is important to acknowledge that this study is conducted at a single centre, using a retrospective design (potential selection bias), and involving a small sample size.

Additionally, the heterogeneity of the tumour samples in terms of histological subtype, type of ICB therapy (monotherapy or combination therapy) as well as the treatment line of ICB therapy, poses a significant limitation. To avoid the impact of histological subtype heterogeneity, gene expression analyses were performed for all the comparisons both across histological subtypes and in clear cell samples only, although the latter were limited by the small sample size. Our study analysed samples from primary tumours or metastasis of mRCC patients treated with ICB between 2014 and 2019 and thus obtaining a homogenous ICB-treated population with a sufficient sample size was not possible. Patients treated with frontline ICB combination were treated within clinical trials, and the only ICB treatments given without clinical trial were those given in second or posterior treatment lines. This limitation led us to another important limitation, FFPE samples evaluated in our study corresponded mostly to primary tumours, and from metastases biopsied at diagnosis, and thus the impact of previous targeted therapies on the TME was surely missed. In addition, samples from metastases, come from organs different to the kidney and thus with different gene expression patterns. The small sample size prevented us however from analysing separately tumours samples treated with ICB in front line setting from those receiving ICB therapy in second or later lines. Having matched samples of the primary tumour and of the metastasis, as well as samples prior to ICB treatment and at progression, would have provided more insight to the understanding of the mechanisms underlying the response and resistance to ICB.

Treatment regimen heterogeneity was another limitation of our study. The diverse nature of ICB regimens and combinations used in clinical practice may introduce heterogeneity in treatment responses, making it challenging to attribute observed outcomes to a specific therapeutic agent or combination. Further, the study's follow-up duration may be insufficient to assess long-term treatment outcomes, including late-onset responses, durability of responses, and potential late toxicities associated with ICB. The influence of potential co-medications was not assessed.

Another limitation was the use of RNA microarrays compared to single cell RNA sequencing (scRNAseq) or spatial transcriptome. RNA microarrays provided us with an overview of the average differences in gene expression; however, the use of scRNAseq or spatial transcriptome would have enabled us to identify if the differences, for example, in the activity of certain biological processes, were linked to specific cell subtypes. Also, the variability in the quality and handling of RNA samples and microarray data may introduce measurement error and data inconsistencies.

Finally, our study lacked functional validation. Our study relies on gene expression data, which provides insights into potential mechanisms of resistance and response to ICB but does not confirm functional changes at the protein or cellular levels, warranting further validation studies. No other type of potential immune tissue or blood-based biomarkers were explored in our study.

CONCLUSION



CONCLUSION

Conclusion 1: Distinct RCC Histological subtypes

- a. The three main histological RCC subtypes (ccRCC, pRCC, and chrRCC) presented distinct gene expression patterns.
- b. Specifically, ccRCC tumours presented an enrichment in Hallmark pathways involved in immune response, as well as higher immune and stromal infiltration.
- c. Chromophobe RCC tumours presented the lowest immune infiltration and highest tumour purity, while also displaying increased activity of the mTOR pathway.

Conclusion 2: Metastatic Sites and Gene set analysis

- a. Gene set enrichment analysis comparing primary tumours from patients with exclusive liver, lung, or bone as site of metastases, unveiled unique patterns.
- b. Tumours from patients with exclusive liver metastases versus those with exclusive lung or bone metastases were enriched in hallmark pathways involved in both protumoral and metabolic pathways and immunosuppressor pathways, but also in pathways involved in immune response, which would explain the aggressiveness of these tumours but could also suggest an increased response to ICB.

Conclusion 3: Transcriptomic analysis and IMDC

- a. Poor IMDC tumours were enriched in hallmark pathways involved both in the cell cycle and immune response pathways, which could explain the aggressiveness of these tumours but also their increased response to ICB.

Conclusion 4: Predictor of response to ICB and AA

- a. The study represents a pioneering effort in analysing tumours from mRCC patients treated with both ICB and AA, each of which yielded distinct responses.
- b. Hallmark pathways involved in metabolic reprogramming, including glycolysis and lipid metabolism pathways, along with the immunosuppressive TGF-beta pathway and protumoral pathways, were enriched in tumours that did not benefit from ICB but nevertheless benefited from AA.
- c. IL2-STAT5 and TNFalpha signalling via NfKappaB were also enriched in tumours not benefiting from ICB, but however benefiting from AA.

Conclusion 5: Transcriptomic and irAEs

- a. Hallmark pathways involved in immune response were upregulated in tumours of patients developing severe irAEs.
- b. Additionally, IL2-STAT5 and TNFalpha signalling via NfKappaB were also enriched in the tumours of patients with severe irAEs.

Conclusion 6: Tumour Inflammation Signatures

- a. The TIS was significantly downregulated in tumours deriving clinical benefit from ICB but not from AA. No other significant association with development of severe irAEs or IMDC risk group were observed.
- b. The 26-JAVELIN did not show a significant association with response to ICB, the development of severe irAEs, or the IMDC risk group in our study.

*"I haven't failed.
I've just found 10.000 ways that won't work"*

Thomas Edison

BIBLIOGRAPHY



BIBLIOGRAPHY

1. Capitanio U, Montorsi F. Renal cancer. *Lancet Lond Engl*. 27 de febrero de 2016;387(10021):894-906.
2. Levi F, Ferlay J, Galeone C, Lucchini F, Negri E, Boyle P, et al. The changing pattern of kidney cancer incidence and mortality in Europe. *BJU Int*. abril de 2008;101(8):949-58.
3. Estimación de la incidencia de cáncer en España. :21.
4. Cifras_del_cancer_2020.pdf [Internet]. [citado 9 de junio de 2020]. Disponible en: https://seom.org/seomcms/images/stories/recursos/Cifras_del_cancer_2020.pdf
5. Scelo G, Larose TL. Epidemiology and Risk Factors for Kidney Cancer. *J Clin Oncol Off J Am Soc Clin Oncol*. 29 de octubre de 2018;JCO2018791905.
6. Scelo G, Li P, Chanudet E, Muller DC. Variability of Sex Disparities in Cancer Incidence over 30 Years: The Striking Case of Kidney Cancer. *Eur Urol Focus*. 2018;4(4):586-90.
7. Znaor A, Lortet-Tieulent J, Laversanne M, Jemal A, Bray F. International Variations and Trends in Renal Cell Carcinoma Incidence and Mortality. *Eur Urol*. marzo de 2015;67(3):519-30.
8. Hasumi H, Yao M. Hereditary kidney cancer syndromes: Genetic disorders driven by alterations in metabolism and epigenome regulation. *Cancer Sci*. marzo de 2018;109(3):581-6.
9. Humans IWG on the E of CR to. TOBACCO SMOKING [Internet]. Personal Habits and Indoor Combustions. International Agency for Research on Cancer; 2012 [citado 10 de junio de 2020]. Disponible en: <https://www.ncbi.nlm.nih.gov/books/NBK304395/>
10. Hunt JD, van der Hel OL, McMillan GP, Boffetta P, Brennan P. Renal cell carcinoma in relation to cigarette smoking: meta-analysis of 24 studies. *Int J Cancer*. 10 de marzo de 2005;114(1):101-8.
11. Arnold M, Pandeya N, Byrnes G, Renehan PAG, Stevens GA, Ezzati PM, et al. Global burden of cancer attributable to high body-mass index in 2012: a population-based study. *Lancet Oncol*. enero de 2015;16(1):36-46.
12. Albiges L, Hakimi AA, Xie W, McKay RR, Simantov R, Lin X, et al. Body Mass Index and Metastatic Renal Cell Carcinoma: Clinical and Biological Correlations. *J Clin Oncol Off J Am Soc Clin Oncol*. 20 de octubre de 2016;34(30):3655-63.
13. Hidayat K, Du X, Zou SY, Shi BM. Blood pressure and kidney cancer risk: meta-analysis of prospective studies. *J Hypertens*. 2017;35(7):1333-44.

14. Sharifi N, Farrar WL. Perturbations in hypoxia detection: a shared link between hereditary and sporadic tumor formation? *Med Hypotheses*. 2006;66(4):732-5.
15. DellaValle CT, Daniel CR, Aschebrook-Kilfoy B, Hollenbeck AR, Cross AJ, Sinha R, et al. Dietary intake of nitrate and nitrite and risk of renal cell carcinoma in the NIH-AARP Diet and Health Study. *Br J Cancer*. enero de 2013;108(1):205-12.
16. Rohrmann S, Linseisen J, Overvad K, Lund Würtz AM, Roswall N, Tjønneland A, et al. Meat and fish consumption and the risk of renal cell carcinoma in the European prospective investigation into cancer and nutrition: Meat and Fish Consumption and the Risk of Renal Cell Carcinoma. *Int J Cancer*. 1 de marzo de 2015;136(5):E423-31.
17. Weikert S, Boeing H, Pischon T, Olsen A, Tjønneland A, Overvad K, et al. Fruits and vegetables and renal cell carcinoma: Findings from the European prospective investigation into cancer and nutrition (EPIC). *Int J Cancer*. 15 de junio de 2006;118(12):3133-9.
18. van Dijk BAC, Schouten LJ, Kiemeneij LALM, Goldbohm RA, van den Brandt PA. Vegetable and fruit consumption and risk of renal cell carcinoma: Results from the Netherlands cohort study. *Int J Cancer*. 20 de noviembre de 2005;117(4):648-54.
19. Karami S, Daugherty SE, Purdue MP. A prospective study of alcohol consumption and renal cell carcinoma risk: Alcohol intake and kidney cancer. *Int J Cancer*. 1 de julio de 2015;137(1):238-42.
20. Lee JE, Hunter DJ, Spiegelman D, Adami HO, Albanes D, Bernstein L, et al. Alcohol intake and renal cell cancer in a pooled analysis of 12 prospective studies. *J Natl Cancer Inst*. 16 de mayo de 2007;99(10):801-10.
21. Song DY, Song S, Song Y, Lee JE. Alcohol intake and renal cell cancer risk: a meta-analysis. *Br J Cancer*. 22 de mayo de 2012;106(11):1881-90.
22. Wozniak MB, Brennan P, Brenner DR, Overvad K, Olsen A, Tjønneland A, et al. Alcohol consumption and the risk of renal cancers in the European prospective investigation into cancer and nutrition (EPIC). *Int J Cancer*. 15 de octubre de 2015;137(8):1953-66.
23. Koppes LLJ, Dekker JM, Hendriks HFJ, Bouter LM, Heine RJ. Moderate alcohol consumption lowers the risk of type 2 diabetes: a meta-analysis of prospective observational studies. *Diabetes Care*. marzo de 2005;28(3):719-25.
24. Graff RE, Sanchez A, Tobias DK, Rodríguez D, Barrisford GW, Blute ML, et al. Type 2 Diabetes in Relation to the Risk of Renal Cell Carcinoma Among Men and Women in Two Large Prospective Cohort Studies. *Diabetes Care*. 2018;41(7):1432-7.
25. Bao C, Yang X, Xu W, Luo H, Xu Z, Su C, et al. Diabetes mellitus and incidence and mortality of kidney cancer: a meta-analysis. *J Diabetes Complications*. agosto de 2013;27(4):357-64.

26. Occupational Trichloroethylene Exposure and Renal Carcinoma Risk: Evidence of Genetic Susceptibility by Reductive Metabolism Gene Variants | Cancer Research [Internet]. [citado 16 de junio de 2020]. Disponible en: <https://cancerres.aacrjournals.org/content/70/16/6527>
27. Moch H, Cubilla AL, Humphrey PA, Reuter VE, Ulbright TM. The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs-Part A: Renal, Penile, and Testicular Tumours. *Eur Urol*. 2016;70(1):93-105.
28. Moch H, Amin MB, Berney DM, Comp  rat EM, Gill AJ, Hartmann A, et al. The 2022 World Health Organization Classification of Tumours of the Urinary System and Male Genital Organs—Part A: Renal, Penile, and Testicular Tumours. *Eur Urol*. noviembre de 2022;82(5):458-68.
29. Lopez-Beltran A, Scarpelli M, Montironi R, Kirkali Z. 2004 WHO Classification of the Renal Tumors of the Adults. *Eur Urol*. 1 de mayo de 2006;49(5):798-805.
30. Warren AY, Harrison D. WHO/ISUP classification, grading and pathological staging of renal cell carcinoma: standards and controversies. *World J Urol*. diciembre de 2018;36(12):1913-26.
31. Zhou M, Roma A, Magi-Galluzzi C. The usefulness of immunohistochemical markers in the differential diagnosis of renal neoplasms. *Clin Lab Med*. junio de 2005;25(2):247-57.
32. Network TCGAR. <https://doi.org/10.1056/NEJMoa1505917>. Massachusetts Medical Society; 2016 [citado 26 de junio de 2020]. Comprehensive Molecular Characterization of Papillary Renal-Cell Carcinoma. Disponible en: <https://www.nejm.org/doi/10.1056/NEJMoa1505917>
33. Muller M, Ferlicot S, Guillaud-Bataille M, Le Teuff G, Genestie C, Deveaux S, et al. Reassessing the clinical spectrum associated with hereditary leiomyomatosis and renal cell carcinoma syndrome in French *FH* mutation carriers: MULLER et al. *Clin Genet*. diciembre de 2017;92(6):606-15.
34. Linehan WM, Rouault TA. Molecular pathways: Fumarate hydratase-deficient kidney cancer--targeting the Warburg effect in cancer. *Clin Cancer Res Off J Am Assoc Cancer Res*. 1 de julio de 2013;19(13):3345-52.
35. Menko FH, Maher ER, Schmidt LS, Middleton LA, Aittom  ki K, Tomlinson I, et al. Hereditary leiomyomatosis and renal cell cancer (HLRCC): renal cancer risk, surveillance and treatment. *Fam Cancer*. diciembre de 2014;13(4):637-44.
36. The Multiple Leiomyoma Consortium. Germline mutations in *FH* predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. *Nat Genet*. abril de 2002;30(4):406-10.

37. Muller M, Guillaud-Bataille M, Salleron J, Genestie C, Deveaux S, Slama A, et al. Pattern multiplicity and fumarate hydratase (FH)/S-(2-succino)-cysteine (2SC) staining but not eosinophilic nucleoli with perinucleolar halos differentiate hereditary leiomyomatosis and renal cell carcinoma-associated renal cell carcinomas from kidney tumors without FH gene alteration. *Mod Pathol Off J U S Can Acad Pathol Inc.* 2018;31(6):974-83.
38. Furuya M, Hasumi H, Yao M, Nagashima Y. Birt-Hogg-Dubé syndrome-associated renal cell carcinoma: Histopathological features and diagnostic conundrum. *Cancer Sci.* enero de 2020;111(1):15-22.
39. Davis CF, Ricketts CJ, Wang M, Yang L, Cherniack AD, Shen H, et al. The Somatic Genomic Landscape of Chromophobe Renal Cell Carcinoma. *Cancer Cell.* septiembre de 2014;26(3):319-30.
40. Pagani F, Colecchia M, Sepe P, Apollonio G, Claps M, Verzoni E, et al. Collecting ducts carcinoma: An orphan disease. Literature overview and future perspectives. *Cancer Treat Rev.* septiembre de 2019;79:101891.
41. Wahal SP, Mardi K. Multilocular Cystic Renal Cell Carcinoma: A Rare Entity with Review of Literature. *J Lab Physicians.* enero de 2014;6(01):050-2.
42. Delahunt B, Eble JN, Egevad L, Samaratunga H. Grading of renal cell carcinoma. *Histopathology.* enero de 2019;74(1):4-17.
43. Fuhrman SA, Lasky LC, Limas C. Prognostic significance of morphologic parameters in renal cell carcinoma. *Am J Surg Pathol.* octubre de 1982;6(7):655-63.
44. Delahunt B, Cheville JC, Martignoni G, Humphrey PA, Magi-Galluzzi C, McKenney J, et al. The International Society of Urological Pathology (ISUP) grading system for renal cell carcinoma and other prognostic parameters. *Am J Surg Pathol.* octubre de 2013;37(10):1490-504.
45. Delahunt B, Srigley JR, Montironi R, Egevad L. Advances in Renal Neoplasia. *Urology.* mayo de 2014;83(5):969-74.
46. Shuch B, Bratslavsky G, Linehan WM, Srinivasan R. Sarcomatoid Renal Cell Carcinoma: A Comprehensive Review of the Biology and Current Treatment Strategies. *The Oncologist.* enero de 2012;17(1):46-54.
47. Gökden N, Nappi O, Swanson PE, Pfeifer JD, Vollmer RT, Wick MR, et al. Renal cell carcinoma with rhabdoid features. *Am J Surg Pathol.* octubre de 2000;24(10):1329-38.
48. Lang H, Lindner V, Saussine C, Havel D, Faure F, Jacqmin D. Microscopic venous invasion: a prognostic factor in renal cell carcinoma. *Eur Urol.* noviembre de 2000;38(5):600-5.
49. Sanli O, Zorba OU, Erdem S, Tezer M, Kilicar-Slan I, Esen T, et al. Microscopic venous invasion is associated with disease free and cancer free survival in renal cell carcinoma. *Minerva Urol E Nefrol Ital J Urol Nephrol.* diciembre de 2010;62(4):347-53.

50. Isbarn H, Patard JJ, Lughezzani G, Rioux-Leclercq N, Crépel M, Cindolo L, et al. Limited Prognostic Value of Tumor Necrosis in Patients With Renal Cell Carcinoma. *Urology*. 1 de junio de 2010;75(6):1378-84.
51. Katz MD, Serrano MF, Grubb RL, Skolarus TA, Gao F, Humphrey PA, et al. Percent microscopic tumor necrosis and survival after curative surgery for renal cell carcinoma. *J Urol*. marzo de 2010;183(3):909-14.
52. Lee SE, Byun SS, Oh JK, Lee SC, Chang IH, Choe G, et al. Significance of macroscopic tumor necrosis as a prognostic indicator for renal cell carcinoma. *J Urol*. octubre de 2006;176(4 Pt 1):1332-7; discussion 1337-1338.
53. Pichler M, Hutterer GC, Chromecki TF, Jesche J, Kampel-Kettner K, Rehak P, et al. Histologic tumor necrosis is an independent prognostic indicator for clear cell and papillary renal cell carcinoma. *Am J Clin Pathol*. febrero de 2012;137(2):283-9.
54. Brierley J, Gospodarowicz MK, Wittekind C, editores. *TNM classification of malignant tumours*. Eighth edition. Chichester, West Sussex, UK ; Hoboken, NJ: John Wiley & Sons, Inc; 2017.
55. SEER [Internet]. [citado 29 de junio de 2020]. SEER Cancer Statistics Review, 1975-2017. Disponible en: https://seer.cancer.gov/csr/1975_2017/index.html
56. Janzen NK, Kim HL, Figlin RA, Belldegrun AS. Surveillance after radical or partial nephrectomy for localized renal cell carcinoma and management of recurrent disease. *Urol Clin North Am*. noviembre de 2003;30(4):843-52.
57. Choueiri TK, Motzer RJ. <http://dx.doi.org/10.1056/NEJMra1601333>. Massachusetts Medical Society; 2017 [citado 29 de junio de 2020]. Systemic Therapy for Metastatic Renal-Cell Carcinoma. Disponible en: <https://www.nejm.org/doi/10.1056/NEJMra1601333>
58. Albiges L, Powles T, Staehler M, Bensalah K, Giles RH, Hora M, et al. Updated European Association of Urology Guidelines on Renal Cell Carcinoma: Immune Checkpoint Inhibition Is the New Backbone in First-line Treatment of Metastatic Clear-cell Renal Cell Carcinoma. *Eur Urol*. agosto de 2019;76(2):151-6.
59. Negrier S, Escudier B, Lasset C, Douillard JY, Savary J, Chevreau C, et al. Recombinant human interleukin-2, recombinant human interferon alfa-2a, or both in metastatic renal-cell carcinoma. Groupe Français d'Immunothérapie. *N Engl J Med*. 30 de abril de 1998;338(18):1272-8.
60. Fisher RI, Rosenberg SA, Fyfe G. Long-term survival update for high-dose recombinant interleukin-2 in patients with renal cell carcinoma. *Cancer J Sci Am*. febrero de 2000;6 Suppl 1:S55-57.

61. McDermott DF, Regan MM, Clark JI, Flaherty LE, Weiss GR, Logan TF, et al. Randomized phase III trial of high-dose interleukin-2 versus subcutaneous interleukin-2 and interferon in patients with metastatic renal cell carcinoma. *J Clin Oncol Off J Am Soc Clin Oncol*. 1 de enero de 2005;23(1):133-41.
62. Yang JC, Sherry RM, Steinberg SM, Topalian SL, Schwartzentruber DJ, Hwu P, et al. Randomized study of high-dose and low-dose interleukin-2 in patients with metastatic renal cancer. *J Clin Oncol Off J Am Soc Clin Oncol*. 15 de agosto de 2003;21(16):3127-32.
63. Aass N, De Mulder PHM, Mickisch GHJ, Mulders P, van Oosterom AT, van Poppel H, et al. Randomized phase II/III trial of interferon Alfa-2a with and without 13-cis-retinoic acid in patients with progressive metastatic renal cell Carcinoma: the European Organisation for Research and Treatment of Cancer Genito-Urinary Tract Cancer Group (EORTC 30951). *J Clin Oncol Off J Am Soc Clin Oncol*. 20 de junio de 2005;23(18):4172-8.
64. Fishman M, Dutcher JP, Clark JI, Alva A, Miletello GP, Curti B, et al. Overall survival by clinical risk category for high dose interleukin-2 (HD IL-2) treated patients with metastatic renal cell cancer (mRCC): data from the PROCLAIMSM registry. *J Immunother Cancer*. 27 de marzo de 2019;7(1):84.
65. Floros T, Tarhini AA. Anticancer Cytokines: Biology and Clinical Effects of Interferon- α 2, Interleukin (IL)-2, IL-15, IL-21, and IL-12. *Semin Oncol*. agosto de 2015;42(4):539-48.
66. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Oudard S, et al. Overall Survival and Updated Results for Sunitinib Compared With Interferon Alfa in Patients With Metastatic Renal Cell Carcinoma. *J Clin Oncol*. 1 de agosto de 2009;27(22):3584-90.
67. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Staehler M, et al. Sorafenib for Treatment of Renal Cell Carcinoma: Final Efficacy and Safety Results of the Phase III Treatment Approaches in Renal Cancer Global Evaluation Trial. *J Clin Oncol* [Internet]. 18 de mayo de 2009 [citado 30 de junio de 2020]; Disponible en: <https://ascopubs-org.m-hdoct.a17.csinet.es/doi/pdf/10.1200/JCO.2008.19.5511>
68. Escudier B, Bellmunt J, Négrier S, Bajetta E, Melichar B, Bracarda S, et al. Phase III Trial of Bevacizumab Plus Interferon Alfa-2a in Patients With Metastatic Renal Cell Carcinoma (AVOREN): Final Analysis of Overall Survival. *J Clin Oncol*. 1 de mayo de 2010;28(13):2144-50.
69. Rini BI, Halabi S, Rosenberg JE, Stadler WM, Vaena DA, Archer L, et al. Phase III Trial of Bevacizumab Plus Interferon Alfa Versus Interferon Alfa Monotherapy in Patients With Metastatic Renal Cell Carcinoma: Final Results of CALGB 90206. *J Clin Oncol*. 1 de mayo de 2010;28(13):2137-43.
70. Hudes G, Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, et al. Temsirolimus, Interferon Alfa, or Both for Advanced Renal-Cell Carcinoma. *N Engl J Med*. 31 de mayo de 2007;356(22):2271-81.

71. Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. *The Lancet*. agosto de 2008;372(9637):449-56.
72. Motzer RJ, Hutson TE, Cella D, Reeves J, Hawkins R, Guo J, et al. Pazopanib versus Sunitinib in Metastatic Renal-Cell Carcinoma. *N Engl J Med*. 22 de agosto de 2013;369(8):722-31.
73. Sternberg CN, Davis ID, Mardiak J, Szczylik C, Lee E, Wagstaff J, et al. Pazopanib in Locally Advanced or Metastatic Renal Cell Carcinoma: Results of a Randomized Phase III Trial. *J Clin Oncol*. 20 de febrero de 2010;28(6):1061-8.
74. Rini BI, Escudier B, Tomczak P, Kaprin A, Szczylik C, Hutson TE, et al. Comparative effectiveness of axitinib versus sorafenib in advanced renal cell carcinoma (AXIS): a randomised phase 3 trial. *The Lancet*. diciembre de 2011;378(9807):1931-9.
75. Motzer RJ, Hutson TE, Glen H, Michaelson MD, Molina A, Eisen T, et al. Lenvatinib, everolimus, and the combination in patients with metastatic renal cell carcinoma: a randomised, phase 2, open-label, multicentre trial. *Lancet Oncol*. noviembre de 2015;16(15):1473-82.
76. Choueiri TK, Hessel C, Halabi S, Sanford B, Michaelson MD, Hahn O, et al. Cabozantinib versus sunitinib as initial therapy for metastatic renal cell carcinoma of intermediate or poor risk (Alliance A031203 CABOSUN randomised trial): Progression-free survival by independent review and overall survival update. *Eur J Cancer*. mayo de 2018;94:115-25.
77. Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, et al. Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. *N Engl J Med*. 5 de noviembre de 2015;373(19):1803-13.
78. Das R, Verma R, Sznol M, Boddupalli CS, Gettinger SN, Kluger H, et al. Combination Therapy with Anti-CTLA-4 and Anti-PD-1 Leads to Distinct Immunologic Changes In Vivo. *J Immunol*. 1 de febrero de 2015;194(3):950-9.
79. Albiges L, Tannir N, Burotto M, McDermott DF, Plimack ER, Barthélémy P, et al. 711P Nivolumab + ipilimumab (N+I) vs sunitinib (S) for first-line treatment of advanced renal cell carcinoma (aRCC) in CheckMate 214: 4-year follow-up and subgroup analysis of patients (pts) without nephrectomy. *Ann Oncol*. 1 de septiembre de 2020;31:S559-60.
80. Motzer RJ, Tannir NM, McDermott DF, Arén Frontera O, Melichar B, Choueiri TK, et al. Nivolumab plus Ipilimumab versus Sunitinib in Advanced Renal-Cell Carcinoma. *N Engl J Med*. 5 de abril de 2018;378(14):1277-90.
81. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 4 de marzo de 2011;144(5):646-74.

82. Atkins MB, Plimack ER, Puzanov I, Fishman MN, McDermott DF, Cho DC, et al. Axitinib in combination with pembrolizumab in patients with advanced renal cell cancer: a non-randomised, open-label, dose-finding, and dose-expansion phase 1b trial. *Lancet Oncol.* 2018;19(3):405-15.
83. Rini BI, Plimack ER, Stus V, Gafanov R, Hawkins R, Nosov D, et al. Pembrolizumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma. *N Engl J Med.* 21 de 2019;380(12):1116-27.
84. Choueiri TK, Powles T, Burotto M, Bourslon MT, Zurawski B, Juárez VMO, et al. 696O_PR Nivolumab + cabozantinib vs sunitinib in first-line treatment for advanced renal cell carcinoma: First results from the randomized phase III CheckMate 9ER trial. *Ann Oncol.* 1 de septiembre de 2020;31:S1159.
85. Motzer R, Alekseev B, Rha SY, Porta C, Eto M, Powles T, et al. Lenvatinib plus Pembrolizumab or Everolimus for Advanced Renal Cell Carcinoma. *N Engl J Med.* 8 de abril de 2021;384(14):1289-300.
86. L CA, M S, Jm RR, C RA, G de V. Prognostic and Predictive Value of PBRM1 in Clear Cell Renal Cell Carcinoma. *Cancers [Internet].* 19 de diciembre de 2019 [citado 11 de julio de 2021];12(1). Disponible en: <https://pubmed.ncbi.nlm.nih.gov/31861590/>
87. Chowdhury B, Porter EG, Stewart JC, Ferreira CR, Schipma MJ, Dykhuizen EC. PBRM1 Regulates the Expression of Genes Involved in Metabolism and Cell Adhesion in Renal Clear Cell Carcinoma. Kyprianou N, editor. *PLOS ONE.* 21 de abril de 2016;11(4):e0153718.
88. Dizman N, Philip EJ, Pal SK. Genomic profiling in renal cell carcinoma. *Nat Rev Nephrol.* agosto de 2020;16(8):435-51.
89. Ricketts CJ, De Cubas AA, Fan H, Smith CC, Lang M, Reznik E, et al. The Cancer Genome Atlas Comprehensive Molecular Characterization of Renal Cell Carcinoma. *Cell Rep.* abril de 2018;23(1):313-326.e5.
90. Gossage L, Murtaza M, Slatter AF, Lichtenstein CP, Warren A, Haynes B, et al. Clinical and pathological impact of *VHL*, *PBRM1*, *BAP1*, *SETD2*, *KDM6A*, and *JARID1c* in clear cell renal cell carcinoma: *VHL*, *PBRM1*, *BAP1*, *JARID1c*, *SETD2*, & *KDM6a* in CCRCC. *Genes Chromosomes Cancer.* enero de 2014;53(1):38-51.
91. Voss MH, Reising A, Cheng Y, Patel P, Marker M, Kuo F, et al. Genomically annotated risk model for advanced renal-cell carcinoma: a retrospective cohort study. *Lancet Oncol.* diciembre de 2018;19(12):1688-98.
92. Peña-Llopis S, Vega-Rubín-de-Celis S, Liao A, Leng N, Pavía-Jiménez A, Wang S, et al. *BAP1* loss defines a new class of renal cell carcinoma. *Nat Genet.* julio de 2012;44(7):751-9.
93. Kapur P, Peña-Llopis S, Christie A, Zhrebker L, Pavía-Jiménez A, Rathmell WK, et al. Effects on survival of *BAP1* and *PBRM1* mutations in sporadic clear-cell renal-cell carcinoma: a retrospective analysis with independent validation. *Lancet Oncol.* febrero de 2013;14(2):159-67.

94. Joseph RW, Kapur P, Serie DJ, Eckel-Passow JE, Parasramka M, Ho T, et al. Loss of BAP1 protein expression is an independent marker of poor prognosis in patients with low-risk clear cell renal cell carcinoma: BAP1 IHC in ccRCC Associated With Poor Prognosis. *Cancer*. 1 de abril de 2014;120(7):1059-67.
95. Hakimi AA, Ostrovnaya I, Reva B, Schultz N, Chen YB, Gonen M, et al. Adverse Outcomes in Clear Cell Renal Cell Carcinoma with Mutations of 3p21 Epigenetic Regulators *BAP1* and *SETD2* : A Report by MSKCC and the KIRC TCGA Research Network. *Clin Cancer Res*. 15 de junio de 2013;19(12):3259-67.
96. Li F, Mao G, Tong D, Huang J, Gu L, Yang W, et al. The Histone Mark H3K36me3 Regulates Human DNA Mismatch Repair through Its Interaction with MutS α . *Cell*. abril de 2013;153(3):590-600.
97. Peña-Llopis S, Christie A, Xie XJ, Brugarolas J. Cooperation and Antagonism among Cancer Genes: The Renal Cancer Paradigm. *Cancer Res*. 15 de julio de 2013;73(14):4173-9.
98. Jenuwein T, Allis CD. Translating the Histone Code. *Science*. 10 de agosto de 2001;293(5532):1074-80.
99. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma. *N Engl J Med*. 4 de diciembre de 2014;371(23):2189-99.
100. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science*. 3 de abril de 2015;348(6230):124-8.
101. Teo MY, Seier K, Ostrovnaya I, Regazzi AM, Kania BE, Moran MM, et al. Alterations in DNA Damage Response and Repair Genes as Potential Marker of Clinical Benefit From PD-1/PD-L1 Blockade in Advanced Urothelial Cancers. *J Clin Oncol*. 10 de junio de 2018;36(17):1685-94.
102. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med*. 25 de junio de 2015;372(26):2509-20.
103. Ged Y, Chaim J, Knezevic A, Carlo MI, Foster A, Feldman DR, et al. Alterations in DNA damage repair (DDR) genes and outcomes to systemic therapy in 225 immune-oncology (IO) versus tyrosine kinase inhibitor (TKI) treated metastatic clear cell renal cell carcinoma (mccRCC) patients (pts). *J Clin Oncol*. 1 de marzo de 2019;37(7_suppl):551-551.
104. Labriola MK, Zhu J, Gupta R, McCall S, Jackson J, Kong EF, et al. Characterization of tumor mutation burden, PD-L1 and DNA repair genes to assess relationship to immune checkpoint inhibitors response in metastatic renal cell carcinoma. *J Immunother Cancer*. marzo de 2020;8(1):e000319.

105. Pal SK, Ali SM, Yakirevich E, Geynisman DM, Karam JA, Elvin JA, et al. Characterization of Clinical Cases of Advanced Papillary Renal Cell Carcinoma via Comprehensive Genomic Profiling. *Eur Urol.* enero de 2018;73(1):71-8.
106. Pal SK, Tangen C, Thompson IM, Balzer-Haas N, George DJ, Heng DYC, et al. A comparison of sunitinib with cabozantinib, crizotinib, and savolitinib for treatment of advanced papillary renal cell carcinoma: a randomised, open-label, phase 2 trial. *Lancet Lond Engl.* 20 de febrero de 2021;397(10275):695-703.
107. Choueiri TK, Heng DYC, Lee JL, Cancel M, Verheijen RB, Mellemaard A, et al. Efficacy of Savolitinib vs Sunitinib in Patients With MET-Driven Papillary Renal Cell Carcinoma: The SAVOIR Phase 3 Randomized Clinical Trial. *JAMA Oncol* [Internet]. 29 de mayo de 2020 [citado 1 de junio de 2020]; Disponible en: <https://jamanetwork.com/journals/jamaoncology/fullarticle/2766797>
108. Schöffski P, Wozniak A, Escudier B, Rutkowski P, Anthoney A, Bauer S, et al. Crizotinib achieves long-lasting disease control in advanced papillary renal-cell carcinoma type 1 patients with MET mutations or amplification. EORTC 90101 CREATE trial. *Eur J Cancer.* diciembre de 2017;87:147-63.
109. Martinez Chanza N, Bossé D, Bilen MA, Geynisman DM, Balakrishnan A, Jain RK, et al. Cabozantinib (Cabo) in advanced non-clear cell renal cell carcinoma (nccRCC): A retrospective multicenter analysis. *J Clin Oncol.* 20 de mayo de 2018;36(15_suppl):4579-4579.
110. Choueiri TK, Plimack E, Arkenau HT, Jonasch E, Heng DYC, Powles T, et al. Biomarker-Based Phase II Trial of Savolitinib in Patients With Advanced Papillary Renal Cell Cancer. *J Clin Oncol.* 10 de septiembre de 2017;35(26):2993-3001.
111. Twardowski PW, Tangen CM, Wu X, Plets MR, Plimack ER, Agarwal N, et al. Parallel (Randomized) Phase II Evaluation of Tivantinib (ARQ197) and Tivantinib in Combination with Erlotinib in Papillary Renal Cell Carcinoma: SWOG S1107. *Kidney Cancer.* 27 de noviembre de 2017;1(2):123-32.
112. Choueiri TK, Vaishampayan U, Rosenberg JE, Logan TF, Harzstark AL, Bukowski RM, et al. Phase II and biomarker study of the dual MET/VEGFR2 inhibitor foretinib in patients with papillary renal cell carcinoma. *J Clin Oncol Off J Am Soc Clin Oncol.* 10 de enero de 2013;31(2):181-6.
113. Heidegger I, Pircher A, Pichler R. Targeting the Tumor Microenvironment in Renal Cell Cancer Biology and Therapy. *Front Oncol.* 14 de junio de 2019;9:490.
114. Chevrier S, Levine JH, Zanotelli VRT, Silina K, Schulz D, Bacac M, et al. An Immune Atlas of Clear Cell Renal Cell Carcinoma. *Cell.* 4 de mayo de 2017;169(4):736-749.e18.

115. Beuselinck B, Job S, Becht E, Karadimou A, Verkarre V, Couchy G, et al. Molecular Subtypes of Clear Cell Renal Cell Carcinoma Are Associated with Sunitinib Response in the Metastatic Setting. *Clin Cancer Res*. 15 de marzo de 2015;21(6):1329-39.
116. Danaher P, Warren S, Lu R, Samayoa J, Sullivan A, Pekker I, et al. Pan-cancer adaptive immune resistance as defined by the Tumor Inflammation Signature (TIS): results from The Cancer Genome Atlas (TCGA). *J Immunother Cancer*. diciembre de 2018;6(1):63.
117. Ayers M, Lunceford J, Nebozhyn M, Murphy E, Loboda A, Kaufman DR, et al. IFN- γ -related mRNA profile predicts clinical response to PD-1 blockade. *J Clin Invest*. 26 de junio de 2017;127(8):2930-40.
118. McDermott DF, Huseni MA, Atkins MB, Motzer RJ, Rini BI, Escudier B, et al. Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma. *Nat Med*. junio de 2018;24(6):749-57.
119. Motzer RJ, Banchereau R, Hamidi H, Powles T, McDermott D, Atkins MB, et al. Molecular Subsets in Renal Cancer Determine Outcome to Checkpoint and Angiogenesis Blockade. *Cancer Cell*. noviembre de 2020;S1535610820305420.
120. Motzer RJ, Robbins PB, Powles T, Albiges L, Haanen JB, Larkin J, et al. Avelumab plus axitinib versus sunitinib in advanced renal cell carcinoma: biomarker analysis of the phase 3 JAVELIN Renal 101 trial. *Nat Med*. noviembre de 2020;26(11):1733-41.
121. Motzer RJ, Choueiri TK, McDermott DF, Powles T, Yao J, Ammar R, et al. Biomarker analyses from the phase III CheckMate 214 trial of nivolumab plus ipilimumab (N+I) or sunitinib (S) in advanced renal cell carcinoma (aRCC). *J Clin Oncol*. 20 de mayo de 2020;38(15_suppl):5009-5009.
122. Fridman WH, Zitvogel L, Sautès-Fridman C, Kroemer G. The immune contexture in cancer prognosis and treatment. *Nat Rev Clin Oncol*. diciembre de 2017;14(12):717-34.
123. Braun DA, Hou Y, Bakouny Z, Ficial M, Sant' Angelo M, Forman J, et al. Interplay of somatic alterations and immune infiltration modulates response to PD-1 blockade in advanced clear cell renal cell carcinoma. *Nat Med*. 2020;26(6):909-18.
124. The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. *Nature*. julio de 2013;499(7456):43-9.
125. Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. *Nature*. abril de 2013;496(7446):445-55.
126. Voss MH, Buros Novik J, Hellmann MD, Ball M, Hakimi AA, Miao D, et al. Correlation of degree of tumor immune infiltration and insertion-and-deletion (indel) burden with outcome on programmed death 1 (PD1) therapy in advanced renal cell cancer (RCC). *J Clin Oncol*. 20 de mayo de 2018;36(15_suppl):4518-4518.

127. Terry S, Dalban C, Rioux-Leclercq N, Adam J, Meylan M, Buart S, et al. Association of AXL and PD-L1 Expression with Clinical Outcomes in Patients with Advanced Renal Cell Carcinoma Treated with PD-1 Blockade. *Clin Cancer Res*. 15 de diciembre de 2021;27(24):6749-60.
128. Largeot A, Pagano G, Gonder S, Moussay E, Paggetti J. The B-side of Cancer Immunity: The Underrated Tune. *Cells*. 13 de mayo de 2019;8(5).
129. Sautès-Fridman C, Verneau J, Sun CM, Moreira M, Chen TWW, Meylan M, et al. Tertiary Lymphoid Structures and B cells: Clinical impact and therapeutic modulation in cancer. *Semin Immunol*. abril de 2020;48:101406.
130. Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, et al. B cells and tertiary lymphoid structures promote immunotherapy response. *Nature*. enero de 2020;577(7791):549-55.
131. Cabrita R, Lauss M, Sanna A, Donia M, Skaarup Larsen M, Mitra S, et al. Tertiary lymphoid structures improve immunotherapy and survival in melanoma. *Nature*. enero de 2020;577(7791):561-5.
132. Petitprez F, de Reyniès A, Keung EZ, Chen TWW, Sun CM, Calderaro J, et al. B cells are associated with survival and immunotherapy response in sarcoma. *Nature*. enero de 2020;577(7791):556-60.
133. Vanhersecke L, Brunet M, Guégan JP, Rey C, Bougouin A, Cousin S, et al. Mature tertiary lymphoid structures predict immune checkpoint inhibitor efficacy in solid tumors independently of PD-L1 expression. *Nat Cancer*. agosto de 2021;2(8):794-802.
134. Gao J, Navai N, Alhalabi O, Siefker-Radtke A, Campbell MT, Tidwell RS, et al. Neoadjuvant PD-L1 plus CTLA-4 blockade in patients with cisplatin-ineligible operable high-risk urothelial carcinoma. *Nat Med*. diciembre de 2020;26(12):1845-51.
135. Carril-Ajuria L, Desnoyer A, Meylan M, Dalban C, Naigeon M, Cassard L, et al. Baseline circulating unswitched memory B cells and B-cell related soluble factors are associated with overall survival in patients with clear cell renal cell carcinoma treated with nivolumab within the NIVOREN GETUG-AFU 26 study. *J Immunother Cancer*. mayo de 2022;10(5):e004885.
136. Chipollini J, da Costa WH, Werneck da Cunha I, de Almeida E Paula F, Guilherme O Salles P, Azizi M, et al. Prognostic value of PD-L1 expression for surgically treated localized renal cell carcinoma: implications for risk stratification and adjuvant therapies. *Ther Adv Urol*. diciembre de 2019;11:1756287219882600.
137. Shen M, Chen G, Xie Q, Li X, Xu H, Wang H, et al. Association between PD-L1 Expression and the Prognosis and Clinicopathologic Features of Renal Cell Carcinoma: A Systematic Review and Meta-Analysis. *Urol Int*. 2020;104(7-8):533-41.
138. Kahlmeyer A, Stöhr CG, Hartmann A, Goebell PJ, Wullich B, Wach S, et al. Expression of PD-1 and CTLA-4 Are Negative Prognostic Markers in Renal Cell Carcinoma. *J Clin Med* [Internet].

- 24 de mayo de 2019 [citado 12 de enero de 2021];8(5). Disponible en: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6572544/>
139. Iacovelli R, Nolè F, Verri E, Renne G, Paglino C, Santoni M, et al. Prognostic Role of PD-L1 Expression in Renal Cell Carcinoma. A Systematic Review and Meta-Analysis. *Target Oncol.* abril de 2016;11(2):143-8.
140. Motzer RJ, Tannir NM, McDermott DF, Arén Frontera O, Melichar B, Choueiri TK, et al. Nivolumab plus Ipilimumab versus Sunitinib in Advanced Renal-Cell Carcinoma. *N Engl J Med.* 5 de abril de 2018;378(14):1277-90.
141. Motzer RJ, Penkov K, Haanen J, Rini B, Albiges L, Campbell MT, et al. Avelumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma. *N Engl J Med.* 21 de marzo de 2019;380(12):1103-15.
142. Choueiri TK, Figueroa DJ, Fay AP, Signoretti S, Liu Y, Gagnon R, et al. Correlation of PD-L1 Tumor Expression and Treatment Outcomes in Patients with Renal Cell Carcinoma Receiving Sunitinib or Pazopanib: Results from COMPARZ, a Randomized Controlled Trial. *Clin Cancer Res.* 1 de marzo de 2015;21(5):1071-7.
143. Flaifel A, Xie W, Braun DA, Ficial M, Bakouny Z, Nassar AH, et al. PD-L1 Expression and Clinical Outcomes to Cabozantinib, Everolimus, and Sunitinib in Patients with Metastatic Renal Cell Carcinoma: Analysis of the Randomized Clinical Trials METEOR and CABOSUN. *Clin Cancer Res.* 15 de octubre de 2019;25(20):6080-8.
144. Rini BI, Powles T, Atkins MB, Escudier B, McDermott DF, Suarez C, et al. Atezolizumab plus bevacizumab versus sunitinib in patients with previously untreated metastatic renal cell carcinoma (IMmotion151): a multicentre, open-label, phase 3, randomised controlled trial. *The Lancet.* 15 de junio de 2019;393(10189):2404-15.
145. Pignon JC, Jegede O, Shukla SA, Braun DA, Horak CE, Wind-Rotolo M, et al. irRECIST for the Evaluation of Candidate Biomarkers of Response to Nivolumab in Metastatic Clear Cell Renal Cell Carcinoma: Analysis of a Phase II Prospective Clinical Trial. *Clin Cancer Res.* 1 de abril de 2019;25(7):2174-84.
146. Vignali DAA, Collison LW, Workman CJ. How regulatory T cells work. *Nat Rev Immunol.* julio de 2008;8(7):523-32.
147. Ries CH, Cannarile MA, Hoves S, Benz J, Wartha K, Runza V, et al. Targeting Tumor-Associated Macrophages with Anti-CSF-1R Antibody Reveals a Strategy for Cancer Therapy. *Cancer Cell.* junio de 2014;25(6):846-59.
148. Heng DYC, Xie W, Regan MM, Warren MA, Golshayan AR, Sahi C, et al. Prognostic factors for overall survival in patients with metastatic renal cell carcinoma treated with vascular endothelial growth factor-targeted agents: results from a large, multicenter study. *J Clin Oncol Off J Am Soc Clin Oncol.* 1 de diciembre de 2009;27(34):5794-9.

149. Yip SM, Wells C, Moreira R, Wong A, Srinivas S, Beuselinck B, et al. Checkpoint inhibitors in patients with metastatic renal cell carcinoma: Results from the International Metastatic Renal Cell Carcinoma Database Consortium. *Cancer*. 15 de septiembre de 2018;124(18):3677-83.
150. RebuZZi SE, Signori A, Banna GL, Maruzzo M, De Giorgi U, Pedrazzoli P, et al. Inflammatory indices and clinical factors in metastatic renal cell carcinoma patients treated with nivolumab: the development of a novel prognostic score (Meet-URO 15 study). *Ther Adv Med Oncol*. enero de 2021;13:175883592110196.
151. Carril-Ajuria L, Motzer RJ, Tannir NM, McDermott DF, Hammers HJ, Plimack ER, et al. Prognostic value of the lung immune prognostic index in patients with untreated advanced renal cell carcinoma (aRCC) receiving nivolumab plus ipilimumab (N+I) or sunitinib (SUN) in the CheckMate 214 trial. *J Clin Oncol*. 1 de junio de 2022;40(16_suppl):4538-4538.
152. Meyers, Stukalin, Vallerand, Lewinson, Suo, Dean, et al. The Lung Immune Prognostic Index Discriminates Survival Outcomes in Patients with Solid Tumors Treated with Immune Checkpoint Inhibitors. *Cancers*. 2 de noviembre de 2019;11(11):1713.
153. Lavaud P, Dalban C, Negrier S, Chevreau C, Gravis G, Oudard S, et al. Validation of the lung immune prognostic index (LIPI) in patients with metastatic renal cell carcinoma treated with nivolumab in the GETUG-AFU 26 NIVOREN trial. *J Clin Oncol*. 20 de febrero de 2020;38(6_suppl):735-735.
154. Nivolumab plus ipilimumab versus sunitinib in first-line treatment for advanced renal cell carcinoma: extended follow-up of efficacy and safety results from a randomised, controlled, phase 3 trial - The Lancet Oncology [Internet]. [citado 12 de enero de 2021]. Disponible en: [https://www.thelancet.com/journals/lanonc/article/PIIS1470-2045\(19\)30413-9/fulltext](https://www.thelancet.com/journals/lanonc/article/PIIS1470-2045(19)30413-9/fulltext)
155. Albiges L, Flippot R, Escudier B. Immune Checkpoint Inhibitors in Metastatic Clear-cell Renal Cell Carcinoma: Is PD-L1 Expression Useful? *Eur Urol*. junio de 2021;79(6):793-5.
156. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 20 de abril de 2015;43(7):e47-e47.
157. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci*. 25 de octubre de 2005;102(43):15545-50.
158. Yoshihara K, Shahmoradgoli M, Martínez E, Vegesna R, Kim H, Torres-Garcia W, et al. Inferring tumour purity and stromal and immune cell admixture from expression data. *Nat Commun*. 11 de octubre de 2013;4(1):2612.
159. Charoentong P, Finotello F, Angelova M, Mayer C, Efremova M, Rieder D, et al. Pan-cancer Immunogenomic Analyses Reveal Genotype-Immunophenotype Relationships and Predictors of Response to Checkpoint Blockade. *Cell Rep*. enero de 2017;18(1):248-62.

160. Dudani S, De Velasco G, Wells JC, Gan CL, Donskov F, Porta C, et al. Evaluation of Clear Cell, Papillary, and Chromophobe Renal Cell Carcinoma Metastasis Sites and Association With Survival. *JAMA Netw Open*. 21 de enero de 2021;4(1):e2021869.
161. The Cancer Genome Atlas Research Network. Comprehensive Molecular Characterization of Papillary Renal-Cell Carcinoma. *N Engl J Med*. 14 de enero de 2016;374(2):135-45.
162. Cheval L, Pierrat F, Rajerison R, Piquemal D, Doucet A. Of Mice and Men: Divergence of Gene Expression Patterns in Kidney. *Laudet V, editor. PLoS ONE*. 3 de octubre de 2012;7(10):e46876.
163. Chen F, Zhang Y, Şenbabaoğlu Y, Ciriello G, Yang L, Reznik E, et al. Multilevel Genomics-Based Taxonomy of Renal Cell Carcinoma. *Cell Rep*. marzo de 2016;14(10):2476-89.
164. Şenbabaoğlu Y, Gejman RS, Winer AG, Liu M, Van Allen EM, de Velasco G, et al. Tumor immune microenvironment characterization in clear cell renal cell carcinoma identifies prognostic and immunotherapeutically relevant messenger RNA signatures. *Genome Biol*. diciembre de 2016;17(1):231.
165. Labaki C, Zhang L, Hou Y, Bi K, Hobeika C, Bakouny Z, et al. Molecular characterization of the tumor microenvironment in chromophobe renal cell carcinoma (ChRCC) and related oncocytic neoplasms. *J Clin Oncol*. 1 de junio de 2022;40(16_suppl):4549-4549.
166. Yoshida GJ. Metabolic reprogramming: the emerging concept and associated therapeutic strategies. *J Exp Clin Cancer Res*. diciembre de 2015;34(1):111.
167. Pandey N, Lanke V, Vinod PK. Network-based metabolic characterization of renal cell carcinoma. *Sci Rep*. 6 de abril de 2020;10(1):5955.
168. Zhang L, Henske EP. Chromophobe renal cell carcinoma: New genetic and metabolic insights. *Urol Oncol Semin Orig Investig*. agosto de 2020;38(8):678-81.
169. Heravi G, Yazdanpanah O, Podgorski I, Matherly LH, Liu W. Lipid metabolism reprogramming in renal cell carcinoma. *Cancer Metastasis Rev*. marzo de 2022;41(1):17-31.
170. Long J, Zhang CJ, Zhu N, Du K, Yin YF, Tan X, et al. Lipid metabolism and carcinogenesis, cancer development. *Am J Cancer Res*. 2018;8(5):778-91.
171. Saito K, Arai E, Maekawa K, Ishikawa M, Fujimoto H, Taguchi R, et al. Lipidomic Signatures and Associated Transcriptomic Profiles of Clear Cell Renal Cell Carcinoma. *Sci Rep*. 30 de junio de 2016;6(1):28932.
172. Stone L. Improving understanding of the obesity paradox in ccRCC. *Nat Rev Urol*. febrero de 2020;17(2):63-63.
173. Roldan-Romero JM, Santos M, Lanillos J, Caleiras E, Anguera G, Maroto P, et al. Molecular characterization of chromophobe renal cell carcinoma reveals mTOR pathway alterations in patients with poor outcome. *Mod Pathol*. diciembre de 2020;33(12):2580-90.

174. Kwiatkowski DJ, Choueiri TK, Fay AP, Rini BI, Thorner AR, De Velasco G, et al. Mutations in TSC1, TSC2, and MTOR Are Associated with Response to Rapalogs in Patients with Metastatic Renal Cell Carcinoma. *Clin Cancer Res*. 15 de mayo de 2016;22(10):2445-52.
175. Roldan-Romero JM, Beuselinck B, Santos M, Rodriguez-Moreno JF, Lanillos J, Calsina B, et al. PTEN expression and mutations in *TSC1*, *TSC2* and *MTOR* are associated with response to rapalogs in patients with renal cell carcinoma. *Int J Cancer*. marzo de 2020;146(5):1435-44.
176. Turajlic S, Xu H, Litchfield K, Rowan A, Chambers T, Lopez JI, et al. Tracking Cancer Evolution Reveals Constrained Routes to Metastases: TRACERx Renal. *Cell*. abril de 2018;173(3):581-594.e12.
177. Beuselinck B, Oudard S, Rixe O, Wolter P, Blesius A, Ayllon J, et al. Negative impact of bone metastasis on outcome in clear-cell renal cell carcinoma treated with sunitinib. *Ann Oncol*. abril de 2011;22(4):794-800.
178. Gravis G, Chanez B, Derosa L, Beuselinck B, Barthelemy P, Laguerre B, et al. Effect of glandular metastases on overall survival of patients with metastatic clear cell renal cell carcinoma in the antiangiogenic therapy era. *Urol Oncol Semin Orig Investig*. abril de 2016;34(4):167.e17-167.e23.
179. Bianchi M, Sun M, Jeldres C, Shariat SF, Trinh QD, Briganti A, et al. Distribution of metastatic sites in renal cell carcinoma: a population-based analysis. *Ann Oncol*. abril de 2012;23(4):973-80.
180. Grassi P, Doucet L, Giglione P, Grünwald V, Melichar B, Galli L, et al. Clinical Impact of Pancreatic Metastases from Renal Cell Carcinoma: A Multicenter Retrospective Analysis. Lee JW, editor. *PLOS ONE*. 11 de abril de 2016;11(4):e0151662.
181. Gong J, Maia MC, Dizman N, Govindarajan A, Pal SK. Metastasis in renal cell carcinoma: Biology and implications for therapy. *Asian J Urol*. octubre de 2016;3(4):286-92.
182. Nguyen B, Fong C, Luthra A, Smith SA, DiNatale RG, Nandakumar S, et al. Genomic characterization of metastatic patterns from prospective clinical sequencing of 25,000 patients. *Cell*. febrero de 2022;185(3):563-575.e11.
183. Zhang L, Fan M, Napolitano F, Gao X, Xu Y, Li L. Transcriptomic analysis identifies organ-specific metastasis genes and pathways across different primary sites. *J Transl Med*. diciembre de 2021;19(1):31.
184. Saxton RA, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. *Cell*. marzo de 2017;168(6):960-76.
185. Ma Y, Nenkov M, Chen Y, Press AT, Kaemmerer E, Gassler N. Fatty acid metabolism and acyl-CoA synthetases in the *liver-gut axis*. *World J Hepatol*. 27 de noviembre de 2021;13(11):1512-33.

186. Donskov F, Von Der Maase H. Impact of Immune Parameters on Long-Term Survival in Metastatic Renal Cell Carcinoma. *J Clin Oncol*. 1 de mayo de 2006;24(13):1997-2005.
187. Di Nunno V, Mollica V, Schiavina R, Nobili E, Fiorentino M, Brunocilla E, et al. Improving IMDC Prognostic Prediction Through Evaluation of Initial Site of Metastasis in Patients With Metastatic Renal Cell Carcinoma. *Clin Genitourin Cancer*. abril de 2020;18(2):e83-90.
188. Ernst MS, Navani V, Wells JC, Donskov F, Basappa N, Labaki C, et al. Outcomes for International Metastatic Renal Cell Carcinoma Database Consortium Prognostic Groups in Contemporary First-line Combination Therapies for Metastatic Renal Cell Carcinoma. *Eur Urol*. julio de 2023;84(1):109-16.
189. Motzer RJ, McDermott DF, Escudier B, Burotto M, Choueiri TK, Hammers HJ, et al. Conditional survival and long-term efficacy with nivolumab plus ipilimumab versus sunitinib in patients with advanced renal cell carcinoma. *Cancer*. junio de 2022;128(11):2085-97.
190. Choueiri TK, Powles T, Albiges L, Burotto M, Szczylik C, Zurawski B, et al. Cabozantinib plus Nivolumab and Ipilimumab in Renal-Cell Carcinoma. *N Engl J Med*. 11 de mayo de 2023;388(19):1767-78.
191. Verbiest A, Renders I, Caruso S, Couchy G, Job S, Laenen A, et al. Clear-cell Renal Cell Carcinoma: Molecular Characterization of IMDC Risk Groups and Sarcomatoid Tumors. *Clin Genitourin Cancer*. octubre de 2019;17(5):e981-94.
192. Motzer RJ, Choueiri TK, McDermott DF, Powles T, Vano YA, Gupta S, et al. Biomarker analysis from CheckMate 214: nivolumab plus ipilimumab versus sunitinib in renal cell carcinoma. *J Immunother Cancer*. marzo de 2022;10(3):e004316.
193. Vano YA, Elaidi R, Bennamoun M, Chevreau C, Borchellini D, Pannier D, et al. Nivolumab, nivolumab–ipilimumab, and VEGFR-tyrosine kinase inhibitors as first-line treatment for metastatic clear-cell renal cell carcinoma (BIONIKK): a biomarker-driven, open-label, non-comparative, randomised, phase 2 trial. *Lancet Oncol*. mayo de 2022;23(5):612-24.
194. Lindner AK, Pichler M, Thurnher M, Pichler R. Targeting c-Met to Improve Immune Checkpoint Inhibition in Metastatic Renal Cell Carcinoma. *Eur Urol*. enero de 2022;81(1):1-2.
195. Lalani AKA, Gray KP, Albiges L, Callea M, Pignon JC, Pal S, et al. Differential expression of c-Met between primary and metastatic sites in clear-cell renal cell carcinoma and its association with PD-L1 expression. *Oncotarget*. 28 de noviembre de 2017;8(61):103428-36.
196. Finisguerra V, Di Conza G, Di Matteo M, Serneels J, Costa S, Thompson AAR, et al. MET is required for the recruitment of anti-tumoural neutrophils. *Nature*. junio de 2015;522(7556):349-53.
197. Glodde N, Bald T, Van Den Boorn-Konijnenberg D, Nakamura K, O'Donnell JS, Szczepanski S, et al. Reactive Neutrophil Responses Dependent on the Receptor Tyrosine Kinase c-MET Limit Cancer Immunotherapy. *Immunity*. octubre de 2017;47(4):789-802.e9.

198. Munn DH, Sharma MD, Baban B, Harding HP, Zhang Y, Ron D, et al. GCN2 Kinase in T Cells Mediates Proliferative Arrest and Anergy Induction in Response to Indoleamine 2,3-Dioxygenase. *Immunity*. mayo de 2005;22(5):633-42.
199. Choueiri TK, Powles T, Burotto M, Escudier B, Bours MT, Zurawski B, et al. Nivolumab plus Cabozantinib versus Sunitinib for Advanced Renal-Cell Carcinoma. *N Engl J Med*. 4 de marzo de 2021;384(9):829-41.
200. Xiao C, Tian H, Zheng Y, Yang Z, Li S, Fan T, et al. Glycolysis in tumor microenvironment as a target to improve cancer immunotherapy. *Front Cell Dev Biol*. 2022;10:1013885.
201. Cerezo M, Rocchi S. Cancer cell metabolic reprogramming: a keystone for the response to immunotherapy. *Cell Death Dis*. 11 de noviembre de 2020;11(11):964.
202. Jiang Z, Liu Z, Li M, Chen C, Wang X. Increased glycolysis correlates with elevated immune activity in tumor immune microenvironment. *EBioMedicine*. abril de 2019;42:431-42.
203. Ross-Macdonald P, Walsh AM, Chasalow SD, Ammar R, Papillon-Cavanagh S, Szabo PM, et al. Molecular correlates of response to nivolumab at baseline and on treatment in patients with RCC. *J Immunother Cancer*. marzo de 2021;9(3):e001506.
204. Zhao Z, Liu Y, Liu Q, Wu F, Liu X, Qu H, et al. The mRNA Expression Signature and Prognostic Analysis of Multiple Fatty Acid Metabolic Enzymes in Clear Cell Renal Cell Carcinoma. *J Cancer*. 2019;10(26):6599-607.
205. Beckermann KE, Hongo R, Ye X, Young K, Carbonell K, Healey DCC, et al. CD28 costimulation drives tumor-infiltrating T cell glycolysis to promote inflammation. *JCI Insight*. 20 de agosto de 2020;5(16):e138729, 138729.
206. Chen YP, Wang YQ, Lv JW, Li YQ, Chua MLK, Le QT, et al. Identification and validation of novel microenvironment-based immune molecular subgroups of head and neck squamous cell carcinoma: implications for immunotherapy. *Ann Oncol Off J Eur Soc Med Oncol*. 1 de enero de 2019;30(1):68-75.
207. Zou R, Jiang Q, Jin T, Chen M, Yao L, Ding H. Pan-cancer analyses and molecular subtypes based on the cancer-associated fibroblast landscape and tumor microenvironment infiltration characterization reveal clinical outcome and immunotherapy response in epithelial ovarian cancer. *Front Immunol*. 2022;13:956224.
208. Wang Y, Chen L, Yu M, Fang Y, Qian K, Wang G, et al. Immune-related signature predicts the prognosis and immunotherapy benefit in bladder cancer. *Cancer Med*. octubre de 2020;9(20):7729-41.
209. Jones DM, Read KA, Oestreich KJ. Dynamic Roles for IL-2–STAT5 Signaling in Effector and Regulatory CD4+ T Cell Populations. *J Immunol*. 1 de octubre de 2020;205(7):1721-30.

210. Montfort A, Filleron T, Virazels M, Dufau C, Milhès J, Pagès C, et al. Combining Nivolumab and Ipilimumab with Infliximab or Certolizumab in Patients with Advanced Melanoma: First Results of a Phase Ib Clinical Trial. *Clin Cancer Res.* 15 de febrero de 2021;27(4):1037-47.
211. Lai X, Hao W, Friedman A. TNF- α inhibitor reduces drug-resistance to anti-PD-1: A mathematical model. *PloS One.* 2020;15(4):e0231499.
212. Denize T, Hou Y, Pignon JC, Walton E, West DJ, Freeman GJ, et al. Transcriptomic Correlates of Tumor Cell PD-L1 Expression and Response to Nivolumab Monotherapy in Metastatic Clear Cell Renal Cell Carcinoma. *Clin Cancer Res.* 15 de septiembre de 2022;28(18):4045-55.
213. Groeneveld CS, Fontugne J, Cabel L, Bernard-Pierrot I, Radvanyi F, Allory Y, et al. Tertiary lymphoid structures marker CXCL13 is associated with better survival for patients with advanced-stage bladder cancer treated with immunotherapy. *Eur J Cancer.* mayo de 2021;148:181-9.
214. Meylan M, Beuselinck B, Dalban C, Vano Y, Rioux Leclercq N, Sautès-Fridman C, et al. 7000 Kidney ccRCC immune classification (KIC) enhances the predictive value of T effector (Teff) and angiogenesis (Angio) signatures in response to nivolumab (N). *Ann Oncol.* septiembre de 2020;31:S553.
215. Chennamadhavuni A, Abushahin L, Jin N, Presley CJ, Manne A. Risk Factors and Biomarkers for Immune-Related Adverse Events: A Practical Guide to Identifying High-Risk Patients and Rechallenging Immune Checkpoint Inhibitors. *Front Immunol.* 26 de abril de 2022;13:779691.

ANNEXES



ANNEXES

Manuscripts related to kidney cancer published during the PhD as first, co-first, or second author are included below.

I. ANNEXE I. REVIEW 1

Prognostic and Predictive Value of PBRM1 in Clear Cell Renal Cell Carcinoma, Cancers, 2019.

First author.



Review

Prognostic and Predictive Value of *PBRM1* in Clear Cell Renal Cell Carcinoma

Lucía Carril-Ajuria ¹, María Santos ², Juan María Roldán-Romero ²,
Cristina Rodríguez-Antona ^{2,3,*} and Guillermo de Velasco ^{1,*}

¹ Department of Medical Oncology, University Hospital 12 de Octubre, 28041 Madrid, Spain; luciacarril@hotmail.com

² Hereditary Endocrine Cancer Group, Human Cancer Genetics Programme, Spanish National Cancer Research Centre (CNIO), 28029 Madrid, Spain; msantosr@cnio.es (M.S.); jmroldan@cnio.es (J.M.R.-R.)

³ Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), 28029 Madrid, Spain

* Correspondence: crodriguez@cnio.es (C.R.-A.); gdevelasco.gdv@gmail.com (G.d.V.)

Received: 30 November 2019; Accepted: 15 December 2019; Published: 19 December 2019



Abstract: Renal cell carcinoma (RCC) is the most frequent kidney solid tumor, the clear cell RCC (ccRCC) being the major histological subtype. The probability of recurrence and the clinical behavior of ccRCC will greatly depend on the different clinical and histopathological features, already incorporated to different scoring systems, and on the genomic landscape of the tumor. In this sense, ccRCC has for a long time been known to be associated to the biallelic inactivation of Von Hippel-Lindau (*VHL*) gene which causes aberrant hypoxia inducible factor (HIF) accumulation. Recently, next generation-sequencing technologies have provided the bases for an in-depth molecular characterization of ccRCC, identifying additional recurrently mutated genes, such as *PBRM1* (~40–50%), *SETD2* (~12%), or *BAP1* (~10%). *PBRM1*, the second most common mutated gene in ccRCC after *VHL*, is a component of the SWI/SNF chromatin remodeling complex. Different studies have investigated the biological consequences and the potential role of *PBRM1* alterations in RCC prognosis and as a drug response modulator, although some results are contradictory. In the present article, we review the current evidence on *PBRM1* as potential prognostic and predictive marker in both localized and metastatic RCC.

Keywords: polybromo-1; *PBRM1*; renal cell carcinoma; biomarker; prognosis; predictive role

1. Introduction

Kidney cancer is the 12th most frequent solid tumor worldwide with around 400,000 new cases and 175,000 deaths from renal cell carcinoma (RCC) estimated in 2018 [1]. RCC accounts for almost 90% of all kidney tumors, the clear cell (ccRCC) being the most common histologic subtype [2].

Approximately 70% of the RCC will present with localized disease, but around one third of them will relapse after surgical excision [3,4]. However, the probability of recurrence will depend greatly on different clinical and histopathological features, which have been incorporated to up to 20 different scoring systems characterized by substantial heterogeneity [5–7].

Regarding the molecular characteristics of RCC, the inactivation of the Von Hippel-Lindau (*VHL*) gene is by far the most common oncogenic driver event in ccRCC. *VHL* is an E3 ligase substrate that labels the transcription factors hypoxia inducible factor (HIF) 1α and HIF2α with ubiquitin for proteosomal degradation. The accumulation of HIF triggers the uncontrolled expression of angiogenesis promoting genes and gives rise to highly vascularized tumors [8].

Recent advances in next-generation sequencing (NGS) and their implementation in large cohort of cancer patients (e.g., The Cancer Genome Atlas (TCGA) projects), have led to the identification of

II. ANNEXE II. ORIGINAL ARTICLE 1

Systemic Analysis and Review of Nivolumab-Ipilimumab combination as rescue strategy in Renal Cell carcinoma after treatment with anti-PD-1/PD-L1 therapy, Clinical Genitourinary cancers, 2021.

First author.

Review



Systemic Analysis and Review of Nivolumab-ipilimumab Combination as a Rescue Strategy for Renal Cell Carcinoma After Treatment With Anti-PD-1/PD-L1 Therapy

Lucia Carril-Ajuria,¹ David Lora,^{2,3} Alberto Carretero-González,¹ Maricruz Martín-Soberón,¹ Patricia Rioja-Viera,⁴ Daniel Castellano,¹ Guillermo de Velasco¹

Abstract

Nivolumab-ipilimumab has become the standard of care in the frontline setting for intermediate-/poor-risk metastatic renal cell carcinoma (mRCC). This regimen is associated with survival improvement but significant toxicity. Anti-programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) monotherapy may provide response and offers a better safety profile. In this context, nivolumab-ipilimumab has been postulated as a rescue treatment after anti-PD-1/PD-L1 therapy. Recent retrospective data has shown positive results, and several nonrandomized clinical trials (NRCTs) have evaluated this strategy. Therefore, we performed a meta-analysis of available NRCTs to clarify the efficacy and safety of salvage nivolumab-ipilimumab in mRCC after prior anti-PD-1/PD-L1 monotherapy. We searched PubMed, Medline, Embase, and the Cochrane Central Register of Controlled Trials to identify clinical trials investigating the efficacy and safety of salvage nivolumab-ipilimumab after prior anti-PD-1/PD-L1 in patients with mRCC. Only phase II NRCTs were available for the analysis. The pooled effect of single proportions with a 95% confidence interval (CI) was used as the measure of effect (overall response rate [ORR] and incidence of grade ≥ 3 adverse events). Four studies accounting for 237 patients were included. All patients received prior anti-PD-1/PD-L1 monotherapy. The pooled ORR of salvage nivolumab-ipilimumab after prior anti-PD-1/PD-L1 failure was 10.0% (95% CI, 6%-14%; $I^2 = 41\%$; $P = .17$). The incidence of grade ≥ 3 irAEs was 27.0% (95% CI, 20%-35%; $I^2 = 0\%$; $P = .56$). The results of this analysis suggest that the use of salvage nivolumab-ipilimumab in mRCC after prior anti-PD-1/PD-L1 has limited activity with a 10% ORR, and a non-negligible toxicity with 1 of 4 patients developing grade ≥ 3 immune-related adverse events.

Clinical Genitourinary Cancer, Vol. 19, No. 2, 95-102 © 2020 Elsevier Inc. All rights reserved.

Keywords: Immune checkpoint blockade, Immunotherapy, Kidney cancer, Meta-analysis, Rechallenge

Introduction

The treatment landscape for metastatic renal cell carcinoma (mRCC) has evolved greatly with the recent incorporation of immune checkpoint blockers (ICBs). Nivolumab is approved as second-line treatment post-antiangiogenic (AA) therapy and is widely used in mRCC.¹ Anti-programmed cell death 1 (anti-PD-1) monotherapy has also been assessed in the first-line setting, in the phase II Keynote 427 trial, showing promising results with one-third of the patients responding and with an excellent safety profile.² However, the activity of anti-PD-1 therapy is still limited, with approximately 20% to 30% of patients presenting with progression as the best response.^{1,2} Recently, based on the results of 2 phase III trials, the Checkmate (CM) 214 and the Keynote 426

¹Medical Oncology Department

²CIBERESP, Instituto i+12, University Hospital 12 de Octubre, Madrid, Spain

³Facultad de Estudios Estadísticos, Universidad Complutense de Madrid (UCM), Madrid, Spain

⁴Medical Oncology Department, Instituto Nacional de Enfermedades Neoplásicas, Lima, Peru

Submitted: Jul 16, 2020; Revised: Oct 6, 2020; Accepted: Oct 10, 2020; Epub: Oct 15, 2020

Address for correspondence: Guillermo de Velasco, MD, PhD, Medical Oncology Department, University Hospital 12 de Octubre, 28041 Madrid, Spain
E-mail contact: gdelvasco.gdv@gmail.com

III. ANNEXE III. CASE SERIES 1

A case series of advanced renal cell carcinoma patients treated with neoadjuvant cabozantinib prior to cytoreductive nephrectomy within the phase 2 CABOPRE trial, Oncotarget, 2020.

Second author.

Case Report

A case series of advanced renal cell carcinoma patients treated with neoadjuvant cabozantinib prior to cytoreductive nephrectomy within the phase 2 CABOPRE trial

Guillermo de Velasco¹, Lucia Carril-Ajuria¹, Felix Guerrero-Ramos², Teresa Alonso-Gordoa³, Juan F. Rodríguez-Moreno⁴, Alberto Carretero¹, Maricruz Martín-Soberón¹, Federico de la Rosa-Kehrmann² and Daniel Castellano¹

¹Medical Oncology Department, Hospital Universitario 12 de Octubre, Comunidad de Madrid, Madrid, Spain

²Urology Department, Hospital Universitario 12 de Octubre, Comunidad de Madrid, Madrid, Spain

³Medical Oncology Department, Hospital Universitario Ramón y Cajal, Comunidad de Madrid, Madrid, Spain

⁴Medical Oncology Department, Hospital Universitario HM Sanchinarro, Comunidad de Madrid, Madrid, Spain

Correspondence to: Guillermo de Velasco, email: gdvelasco.gdv@gmail.com

Keywords: kidney cancer; renal cell carcinoma; neoadjuvant treatment; cabozantinib; trial

Received: September 18, 2020

Accepted: October 27, 2020

Published: November 24, 2020

Copyright: © 2020 de Velasco et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/3.0/) (CC BY 3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

Cytoreductive nephrectomy has long been used to improve disease control in metastatic Renal Cell Carcinoma (mRCC). However, based on the results of the CARMENA and SURTIME trials, cytoreductive nephrectomy is no longer the standard of care in patients requiring upfront systemic treatment and it should be avoided in most poor-risk patients. Nevertheless, it should still be considered in patients responding to systemic therapy and good-risk patients not requiring systemic treatment.

This case series of the phase 2 CABOPRE trial suggests neoadjuvant cabozantinib may be able to induce rapid and significant responses in some intermediate-risk advanced renal cell carcinoma patients facilitating resectability.

INTRODUCTION

Systemic treatment (ST) has been the cornerstone of advanced renal cell carcinoma (aRCC) but CN has also long been used to improve disease control [1–5]. Currently, the role of CN for aRCC patients has yet to be defined. The SURTIME and the CARMENA trials provided new data but the definitive role of CN remains unclear [6, 7]. The SURTIME trial, suggested the non-inferiority of deferred CN compared to upfront CN [7]. The CARMENA trial demonstrated the non-inferiority of sunitinib alone compared to immediate CN followed by sunitinib [6]. These results support that CN should no longer be the standard of care in patients requiring ST upfront and suggest that surgery should be avoided in IMDC International Metastatic RCC database Consortium (IMDC)-poor risk patients. Nevertheless, CN could remain as an option for selected patients responding to ST and good-risk patients not requiring ST [8].

Within this context the CABOPRE trial (EudraCT Number: 2018-001201-93) was designed as an open-arm phase II trial, to assess the efficacy and safety of

cabozantinib 60 mg/daily, given as perioperative treatment for aRCC patients in potential candidates to CN. The primary endpoint was objective response rate (ORR) by RECIST 1.1 (Response Criteria Evaluation Criteria in Solid Tumors) at 12 weeks. The goal of this case series is to illustrate the preliminary experience of the CABOPRE trial with some illustrative cases of aRCC treated with neoadjuvant (NA) cabozantinib prior to CN.

Patient 1: Tumour thrombus downsize in a locally advanced (LA) RCC patient treated with NA cabozantinib

A 60 year-old man with no relevant medical history, presented in the Emergency Department (ED) with back pain and hematuria in March of 2019. A thoracoabdominal-pelvic (TAP) computed tomography (CT) scan revealed a left renal mass of 9 cm with renal vein and inferior vena cava (ICV) invasion, paraaortic lymph nodes and small bilateral lung metastases. A CT guided biopsy of the renal mass revealed a Fuhrman grade 3 clear cell RCC (ccRCC).

IV. ANNEXE IV. ORIGINAL ARTICLE 2

Response to systemic therapy in fumarate hydratase-deficient renal cell carcinoma, European Journal of Cancer, 2021.

First author.

European Journal of Cancer 151 (2021) 106–114

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

Response to systemic therapy in fumarate hydratase—deficient renal cell carcinoma



Lucia Carril-Ajuria ^{a,b}, Emeline Colomba ^a, Luigi Cerbone ^a,
Carmen Romero-Ferreiro ^c, Laurence Crouzet ^d, Brigitte Laguerre ^d,
Constance Thibault ^e, Cécile Vicier ^f, Guillermo de Velasco ^b,
Aude Fléchon ^g, Carolina Saldana ^h, Patrick R. Benusiglio ⁱ,
Brigitte Bressac-de Paillerets ^j, Marine Guillaud-Bataille ^{ij},
Pauline Gaignard ^k, Jean-Yves Scoazec ^l, Stéphane Richard ^{m,n,o},
Olivier Caron ^p, Bernard Escudier ^{a,m}, Laurence Albiges ^{a,m,*}

^a Department of Medical Oncology, Gustave Roussy, Villejuif, France^b Department of Medical Oncology, University Hospital 12 de Octubre, Madrid, Spain^c CIBERESP, Instituto i+12, University Hospital 12 de Octubre, Madrid, Spain^d Department of Medical Oncology, Centre Eugene Marquis, Rennes, France^e Department of Medical Oncology, Hôpital Européen Georges-Pompidou, Paris, France^f Department of Medical Oncology, Institute Paoli-Calmettes, Marseille, France^g Department of Medical Oncology, Centre Léon-Bérard, Lyon, France^h Department of Medical Oncology, Hôpital Henri Mondor Créteil AP-HP, Franceⁱ UF d'Oncogénétique, Département de Génétique et Institut Universitaire de Cancérologie, GH Pitié-Salpêtrière, AP-HP Sorbonne Université F-75013 Paris, France^j Service de Génétique, Molecular Genetic Department, GRCC, 94805 Villejuif, France^k Biochemistry Department, Hôpital Bicêtre, APHP Université Paris-Saclay, Le Kremlin Bicêtre F-94275, France^l Department of Pathology, University Paris-Saclay, Gustave Roussy Cancer Center, Villejuif, France^m Réseau National de Référence pour Cancers Rares de l'Adulte PREDIR labellisé par l'INCa, Hôpital Bicêtre, AP-HP, Le Kremlin Bicêtre, Franceⁿ Génétique Oncologique EPHE, PSL Research University, Paris, France^o UMR9019 CNRS Genome Integrity and Cancers, Institute Gustave Roussy, Villejuif, France^p Laboratory Integrative Tumor Immunology and Genetic Oncology, Institute Gustave Roussy, Villejuif, France

Received 10 February 2021; received in revised form 31 March 2021; accepted 9 April 2021

Available online 8 May 2021

* Corresponding author: Gustave Roussy, Université Paris-Saclay, Département de médecine Oncologique, Villejuif, F-94805, France. Fax: +01 42 11 53 05.

E-mail address: laurence.albiges@gustaveroussy.fr (L. Albiges).

<https://doi.org/10.1016/j.ejca.2021.04.009>

0959-8049/© 2021 Published by Elsevier Ltd.

V. ANNEXE V. REVIEW 2

Complete response and renal cell carcinoma in the immunotherapy era: The paradox of good news, Cancer Treatment Reviews, 2021.

Co-first author.



Contents lists available at ScienceDirect

Cancer Treatment Reviews

journal homepage: www.elsevier.com/locate/ctrv

Hot Topic

Complete response and renal cell carcinoma in the immunotherapy era: The paradox of good news



Francisco Zambrana^{a,1}, Lucia Carril-Ajuria^{b,c,1}, Alfonso Gómez de Liaño^d,
Nieves Martínez Chanza^e, Ray Manneh^{c,f}, Daniel Castellano^c, Guillermo de Velasco^{c,*}

^a Medical Oncology Department, University Hospital Infanta Sofía, Madrid, Spain^b Medical Oncology Department, Institute Gustave Roussy, Villejuif, France^c Medical Oncology Department, University Hospital 12 de Octubre, Madrid, Spain^d Medical Oncology Department, Hospital Complex Insular-Materno Infantil, Las Palmas, Spain^e Medical Oncology Department, Institut Jules Bordet, Brussels, Belgium^f Sociedad de Oncología y Hematología del Cesar, Valledupar, Colombia

ARTICLE INFO

Keywords:

Renal cell carcinoma
Immunotherapy
Complete response
Immune checkpoint inhibitors
Kidney cancer

ABSTRACT

Immune-checkpoint inhibitor-based therapy has revolutionized the natural history of metastatic renal cell carcinoma (mRCC) providing better survival outcomes, higher rates of complete responses (CR) and durable remissions. Along with these advances, new challenges have emerged. RECIST and new immune-response criteria may be equivocal identifying complete responses. How to define a durable response and what is the optimal treatment duration remains unclear. Furthermore, the real value of a complete and deep response, whether or not it can be considered curation and whether or not immunotherapy discontinuation should be considered after complete response, are questions that remain open. The present article reviews the current evidence regarding the impact and challenges of managing complete and durable responses in mRCC treated with immune-checkpoint inhibitors.

Introduction

Renal cell carcinoma (RCC) is one of the most common urological cancers and the most frequent kidney solid tumor, being the clear cell RCC (ccRCC) the most frequent histological subtype [1]. Survival outcomes of metastatic RCC (mRCC) have improved greatly during the last two decades with the advent of vascular endothelial growth factor-tyrosine kinase inhibitors (antiangiogenic agents, AA) and the incorporation of immune-checkpoint inhibitors (ICI) into the treatment landscape during the last 5 years [2,3]. Immunotherapy has long been used in mRCC. In the 90 s, cytokine-based therapy was a mainstay of the systemic treatment. First spontaneous regressions were described in a small percentage (5–8%) of patients treated with high-dose interleukin-2 (HD-IL2) [4–6]. Later on, targeted therapies (TT) were incorporated providing improved survival outcomes with a better toxicity profile, but no further increase in complete response (CR) rate (~1%) [7,8]. However, the recent incorporation of ICI-based therapies, either in combination with another ICI or with an AA, has brought hope with

encouraging durable responses and a reported rate of CR of up to 8–16% in selected patients Fig. 1. [9–12]. This new scenario has raised optimism but also new challenges. What is the real value of CR and deep partial responses? Whether, and when, a maintained complete response may be considered curation? When treatment discontinuation should be performed?

Complete response: current definition and Relevance.

The incorporation of immunotherapy to the systemic treatment of different solid tumors has led to new patterns of response that are not well evaluated by the RECIST criteria used as the standard radiological assessment in most of the clinical trials using IO agents [13–17]. In addition to the conventional radiological types of response (complete response (CR), partial response (PR), stable disease (SD) and progression of disease (PD)), three atypical types of response (pseudoprogression, hyperprogression and dissociated response) have been incorporated [13]. Pseudoprogression refers to those patients which respond to ICI

* Corresponding author at: University Hospital 12 de Octubre, Madrid, Spain.

E-mail addresses: gvelasco.gdv@gmail.com, lucia.carril@gustaveroussy.fr (G. de Velasco).¹ Equal contribution.<https://doi.org/10.1016/j.ctrv.2021.102239>

Received 23 February 2021; Received in revised form 25 May 2021; Accepted 28 May 2021

Available online 1 June 2021

0305-7372/© 2021 Elsevier Ltd. All rights reserved.

VI. ANNEXE VI. ORIGINAL ARTICLE 3

Baseline circulating unswitched memory B cells and B-cell related soluble factors are associated with overall survival in patients with clear cell renal cell carcinoma treated with nivolumab within the NIVOREN GETUG-AFU 26 study, Journal of Immunotherapy of Cancer, 2022

First author.

Open access

Original research



Baseline circulating unswitched memory B cells and B-cell related soluble factors are associated with overall survival in patients with clear cell renal cell carcinoma treated with nivolumab within the NIVOREN GETUG-AFU 26 study

Lucia Carril-Ajuria ^{1,2}, Aude Desnoyer,^{2,3} Maxime Meylan,⁴ Cécile Dalban,⁵ Marie Naigeon,^{3,6,7} Lydie Cassard,² Yann Vano,^{4,8} Nathalie Rioux-Leclercq,⁹ Salem Chouaib,¹⁰ Benoit Beuselinck,¹¹ Sylvie Chabaud,⁵ Janice Barros-Monteiro,¹² Antoine Bougoüin,⁴ Guillaume Lacroix,⁴ Irelka Colina-Moreno,⁴ Florence Tantot,¹³ Lisa Boselli,² Caroline De Oliveira,² Wolf Herve Fridman ⁴, Bernard Escudier,¹ Catherine Sautes-Fridman ⁴, Laurence Albiges,^{1,7} Nathalie Chaput-Gras^{2,3}

To cite: Carril-Ajuria L, Desnoyer A, Meylan M, et al. Baseline circulating unswitched memory B cells and B-cell related soluble factors are associated with overall survival in patients with clear cell renal cell carcinoma treated with nivolumab within the NIVOREN GETUG-AFU 26 study. *Journal for ImmunoTherapy of Cancer* 2022;10:e004885. doi:10.1136/jitc-2022-004885

► Additional supplemental material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/jitc-2022-004885>).

LC-A and AD contributed equally. LA and NC-G contributed equally.

Accepted 23 April 2022



© Author(s) (or their employer(s)) 2022. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

Correspondence to
Dr Nathalie Chaput-Gras;
CHAPUT-GRAS.Nathalie@
gustaveroussy.fr

ABSTRACT

Background The phase II NIVOREN GETUG-AFU 26 study reported safety and efficacy of nivolumab in patients with metastatic clear cell renal cell carcinoma (m-ccRCC) in a 'real-world setting'. We conducted a translational-research program to determine whether specific circulating immune-cell populations and/or soluble factors at baseline were predictive of clinical outcomes in patients with m-ccRCC treated with nivolumab within the NIVOREN study.

Methods Absolute numbers of 106 circulating immune-cell populations were prospectively analyzed in patients treated at a single institution within the NIVOREN trial with available fresh-whole-blood, using dry formulation panels for multicolor flow cytometry. In addition, a panel of 14 predefined soluble factors was quantified for each baseline plasma sample using the Meso-Scale-Discovery immunoassay. The remaining patients with available plasma sample were used as a validation cohort for the soluble factor quantification analysis. Tumor immune microenvironment characterization of all patients included in the translational program of the study was available. The association of blood and tissue-based biomarkers, with overall survival (OS), progression-free survival (PFS) and response was analyzed.

Results Among the 44 patients, baseline unswitched memory B cells (NSwM B cells) were enriched in responders ($p=0.006$) and associated with improved OS ($HR=0.08$, $p=0.002$) and PFS ($HR=0.54$, $p=0.048$). Responders were enriched in circulating T follicular helper (Tfh) ($p=0.027$) and tertiary lymphoid structures (TLS) ($p=0.043$). Circulating NSwM B cells positively correlated with Tfh ($r=0.70$, $p<0.001$). Circulating NSwM B cells correlated positively with TLS and CD20 +B cells at the tumor center ($r=0.59$, $p=0.044$, and $r=0.52$, $p=0.033$)

and inversely correlated with BCA-1/CXCL13 and BAFF ($r=-0.55$ and $r=-0.42$, $p<0.001$). Tfh cells also inversely correlated with BCA-1/CXCL13 ($r=-0.61$, $p<0.001$). IL-6, BCA-1/CXCL13 and BAFF significantly associated with worse OS in the discovery ($n=40$) and validation cohorts ($n=313$).

Conclusion We report the first fresh blood immune-monitoring of patients with m-ccRCC treated with nivolumab. Baseline blood concentration of NSwM B cells was associated to response, PFS and OS in patients with m-ccRCC treated with nivolumab. BCA-1/CXCL13 and BAFF, inversely correlated to NSwM B cells, were both associated with worse OS in discovery and validation cohorts. Our data confirms a role for B cell subsets in the response to immune checkpoint blockade therapy in patients with m-ccRCC. Further studies are needed to confirm these findings.

BACKGROUND

Immunotherapy, particularly immune checkpoint blockade (ICB) therapy, has revolutionized the landscape of systemic therapy across different solid tumors by boosting the effector T cell responses.¹ Immune checkpoint inhibitors have the potential to provide sustained responses and long-term survival in different solid tumors, including metastatic clear cell renal cell carcinoma (m-ccRCC).² However, only a subset of patients will benefit from these treatments.³⁻⁶ The understanding of the underlying mechanisms of the antitumor immune response and the identification of

VII. ANNEXE VII. ORIGINAL ARTICLE 4

Frontline immune checkpoint inhibitor-based combination therapy in metastatic renal cell carcinoma patients with poor performance status, European Journal of Cancer, 2023.

First author.

European Journal of Cancer 180 (2023) 21–29

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

Frontline immune checkpoint inhibitor-based combination therapy in metastatic renal cell carcinoma patients with poor performance status



Lucía Carril-Ajuria ^a, Emeline Colomba ^a, Carmen Romero-Ferreiro ^{b,c},
Luigi Cerbone ^a, Raffaele Ratta ^d, Philippe Barthelemy ^e, Clarisse Vindry ^f,
Aude Fléchon ^f, François Cherifi ^g, Elouen Boughalem ^h,
Claude Linassier ⁱ, Giuseppe Fornarini ^j, Sara E. Rebuzzi ^j,
Marine Gross-Goupil ^k, Carolina Saldana ^l, Maricruz Martin-Soberón ^m,
Guillermo de Velasco ^m, Ray Manneh ⁿ, Cristina Pernaut ^o,
Ana Sanchez de Torre ^p, Ronan Flippot ^a, Bernard Escudier ^a,
Laurence Albiges ^{a,*}

^a Medical Oncology Department, Institute Gustave Roussy, Villejuif, France^b Instituto de Investigación Sanitaria, Hospital 12 de Octubre (imas12), Madrid, Spain^c Faculty of Health Sciences, Universidad Francisco de Vitoria, 28223 Madrid, Spain^d Medical Oncology, Foch Hospital, Suresnes 92151, France^e Medical Oncology, Hôpitaux Universitaires de Strasbourg/ICANS, Strasbourg, France^f Medical Oncology, Centre Léon Bérard, 69008 Lyon, France^g Medical Oncology, Centre François Baclesse, Caen, France^h Medical Oncology, Institut de Cancerologie de l'Ouest, 49055 Angers, Franceⁱ Medical Oncology, Centre Hospitalier Universitaire de Tours, Tours, France^j Medical Oncology, U.O. Oncologia Medica 1 RCCS Ospedale Policlinico San Martino, Genova, Italy^k Medical Oncology, Centre Hospitalier Universitaire Saint-André, Bordeaux, France^l Medical Oncology, Hôpital Henri Mondor, APHP, Univ Paris Est Creteil, Créteil, France^m Medical Oncology, University Hospital 12 de Octubre, Madrid, Spainⁿ Sociedad de Oncología y Hematología del Cesar, Valledupar, Colombia^o Medical Oncology, University Hospital Severo Ochoa, Leganés, Madrid, Spain^p Medical Oncology, University Hospital Infanta Cristina, Parla, Madrid, Spain

Received 24 August 2022; received in revised form 9 November 2022; accepted 10 November 2022

Available online 26 November 2022

* Corresponding author: Gustave Roussy, Université Paris-Saclay, Département de médecine Oncologique, Villejuif F-94805, France. Fax: +33 01 42 11 53 05.

E-mail address: laurence.albiges@gustaveroussy.fr (L. Albiges).

<https://doi.org/10.1016/j.ejca.2022.11.013>

0959-8049/© 2022 Elsevier Ltd. All rights reserved.

