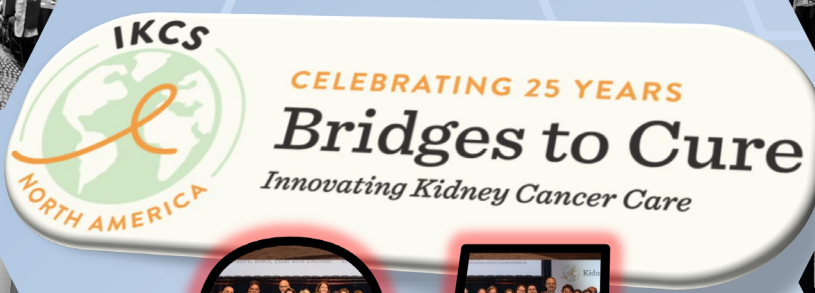


# Kidney Cancer JOURNAL



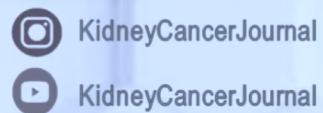
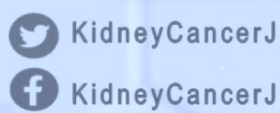
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SCAN ME



## 2024 IKCS NA PROCEEDINGS



KCJ Publishes the Finest & Most Comprehensive Peer-reviewed Research in Kidney Cancer

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The purpose of *Kidney Cancer Journal* is to serve as a comprehensive resource of information for physicians regarding advances in the diagnosis and treatment of renal cell carcinoma. Content of the journal focuses on the impact of translational research in oncology and urology and also provides a forum for cancer patient advocacy. *Kidney Cancer Journal* is circulated to medical oncologists, hematologist-oncologists, and urologists.

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- Clinical case studies.

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# Celebrating 25 Years of Progress: The 2024 IKCSNA

**Thomas E Hutson, DO, PharmD**

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<https://doi.org/10.52733/IKCS24-Proc-e>



Dear fellow clinicians and researchers,

**I**t is with immense pleasure and a profound sense of shared purpose that we present to you the proceedings of the International Kidney Cancer Symposium (IKCS) 2024. This year's symposium holds particular significance, resonating deeply within our community, as it coincides with the momentous 25th anniversary of our esteemed partner, the Kidney Cancer Association (KCA). This milestone is not merely a marker of time but a testament to a quarter-century of unwavering dedication to the fight against kidney cancer. The convergence of IKCS and this anniversary creates a unique opportunity to reflect on the remarkable journey we've undertaken together, celebrating the triumphs achieved and acknowledging the challenges that remain."

For a quarter of a century, the KCA has been a beacon of hope and a driving force in the fight against kidney cancer. Their unwavering commitment to patient advocacy, research funding, and education has transformed the landscape of kidney cancer care, shaping clinical practice and research directions. As Editor-in-Chief of Kidney Cancer Jour-

nal, I am deeply honored to collaborate with them on this special publication, which captures the essence of this transformative period. The IKCS24 proceedings serve as a testament to the vibrant and dynamic landscape of kidney cancer research and care, a landscape that has been profoundly shaped by the Kidney Cancer Association's unwavering dedication over the past 25 years. This collection is a mirror to the progress made, and a map to the future directions we must take."

Within these pages, you will find a rich tapestry of cutting-edge research, mirroring the advancements witnessed during this remarkable period. We have curated insights from leading experts who are pushing the boundaries of our understanding, from groundbreaking discoveries in the molecular underpinnings of clear cell and non-clear cell renal cell carcinoma to the latest clinical applications of immunotherapy and targeted therapies. You will encounter thought-provoking discussions on the evolving role of biomarkers in personalized medicine, the challenges and opportunities presented by novel imaging techniques, and the critical importance of patient-centered care. Each article, each presentation, represents a





step forward in our collective pursuit of improved outcomes for patients. We have endeavored to capture not only the data, but the spirit of innovation and dedication that characterized the symposium."

As we reflect on the significant strides made in survival rates and improved patient quality of life, it's evident that the collaborative spirit fostered by the KCA and events like IKCS are essential drivers of progress. This collection represents the forefront of our collective efforts to translate scientific breakthroughs into tangible benefits for patients, while acknowledging the critical need for continued research and innovation to address the remaining challenges in conquering kidney cancer. These proceedings serve as a practical resource for clinicians, offering insights that can be directly applied to patient care, and a source of inspiration for researchers, encouraging them to pursue new avenues of investigation."

The IKCS24 proceedings serve not only as a record of our current knowledge but also as a catalyst for future innovation. It is through the dissemination of these findings and the fostering of collaboration that we will continue to advance the field and bring hope to patients and their families. This publication is intended to spark dialogue, to encourage cross-disciplinary collaboration, and to inspire the next generation of researchers and clinicians to join us in this critical work. We believe that by

sharing these insights, we can accelerate the pace of progress and bring us closer to a future where kidney cancer is no longer a life-threatening disease."

On behalf of the journal and in partnership with the Kidney Cancer Association, I extend my sincere gratitude to all the authors, presenters, reviewers, and attendees who contributed to the success of IKCS24. Your dedication, expertise, and commitment to advancing kidney cancer care are truly inspiring. We are immensely proud to be part of this collaborative effort and look forward to continuing our shared mission of conquering kidney cancer. It is the collective energy and unwavering focus of this community that gives us the strength to persist."

As we close this chapter of the IKCS24 proceedings, we look forward with anticipation to the future advancements that will undoubtedly emerge from the seeds planted here. We are confident that the knowledge shared within these pages will fuel new discoveries and innovations, ultimately leading to improved outcomes for patients worldwide. With deepest respect and anticipation, we reaffirm our commitment to supporting the kidney cancer community and to working alongside you in this vital endeavor. Together, we will continue to push the boundaries of what is possible, bringing hope and healing to those affected by kidney cancer.

# Bridges Built, Pathways Opened: Celebrating 25 Years of Innovating Kidney Cancer Care

**Salvatore La Rosa, PhD**  
Chief Scientific Officer  
Kidney Cancer Association

<https://doi.org/10.52733/IKCS24-Proc-cso>



Reflecting on the 25th anniversary of the Kidney Cancer Association's (KCA's) International Kidney Cancer Symposium (IKCS), held recently in Louisville, KY with the theme "Bridges to Cure: Innovating Kidney Cancer Care," fills me with pride and optimism. For a quarter-century, IKCS has served as a cornerstone event, uniting leading researchers, clinicians, and advocates in a shared mission to revolutionize kidney cancer care.

Since its inception, IKCS has evolved beyond a traditional scientific meeting to become one of the most comprehensive and influential forums dedicated to kidney cancer. Today, IKCS includes not only premier scientific presentations but also expert think tanks, focused workshops, sessions

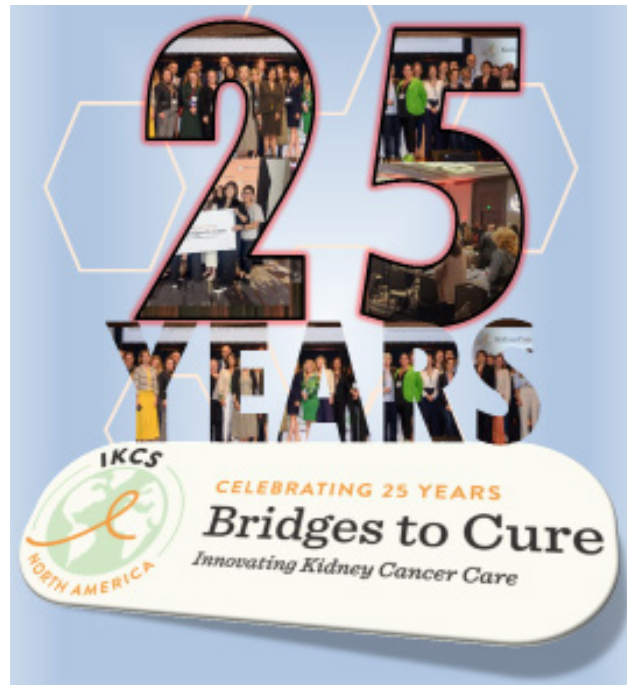
highlighting cutting-edge preclinical research, and mentorship groups. These initiatives enrich our community, stimulate innovation, and foster the growth of future kidney cancer leaders.

Over the years, we've witnessed transformative advancements in kidney cancer — moving from limited treatment options to a robust portfolio of over a dozen FDA-approved therapies. Groundbreaking research, including Nobel Prize-winning discoveries, has continuously expanded our understanding and opened new avenues for targeted treatments, significantly enhancing survival rates and patient quality of life.

Perhaps most inspiring is how IKCS meetings have nurtured talent within our scientific community. Many renowned experts and key opinion leaders presenting today at

IKCS first grew their careers through early engagement with KCA programs and activities, significantly shaped by their participation in these very symposiums.

unmet needs continue to challenge us. Many kidney cancers still present limited response to existing therapies, rare variants continue to have dismal prognoses, and the absence of definitive biomarkers highlights the urgent



Our ongoing commitment to innovation is further exemplified by one of our recent initiatives — the 2024 IKCS: North America conference proceedings. This publication captures the central messages of the conference, creating an enduring piece and reference for the future. These proceedings exemplify our dedication to exploring and adopting novel tools and methods designed to amplify our collective knowledge and support the kidney cancer community more effectively.

Yet, even as we celebrate these incredible milestones, we must acknowledge the challenges ahead. Kidney cancer remains a complex disease, and significant gaps persist in our understanding and management. Critical questions remain unanswered, and

need for ongoing innovation and research.

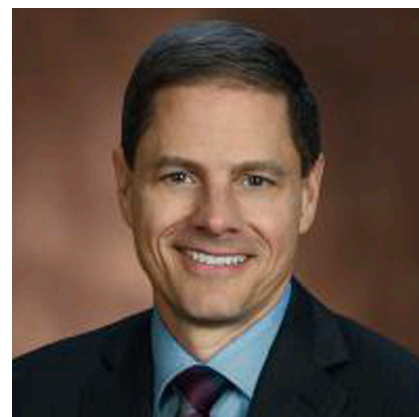
The very experts and changemakers we see today, nurtured through the KCA and IKCS, remind us that innovation thrives where collaboration and curiosity are actively encouraged. The next 25 years demand even greater vision, determination, and groundbreaking ideas.

Let us continue building bridges, pioneering pathways, and pushing boundaries toward a future where kidney cancer becomes fully manageable and curable. Together, with relentless determination, we remain unstoppable.

# An Oncologist's Perspective on Kidney Cancer Research Over the Last 25 Years

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<https://doi.org/10.52733/IKCS24-Proc-br>

I appreciate the opportunity to reflect on the last 25 years of kidney cancer research. I saw my first kidney cancer patients as a second-year fellow at the University of Chicago exactly 25 years ago under the mentorship of Drs. Nicholas Vogelzang and Walter Stadler. From day one, I found the biology of the disease interesting, even though at the time there were almost no therapeutics and most of our patients received clinical trial therapy. I remember one early patient encounter of a young man with advanced disease. I had been in the GU clinic for several months, and entered his room with my rehearsed speech about the therapeutic options, including interferon and interleukin-2, and then launched into clinical trial options. Feeling proud that I had mastered both of those topics and delivered them perfectly, I was quickly brought back to earth when the wife of the patient looked at me and said, "Do you mean that's all you've

got?". I slithered out of the room and have used that experience and perspective to drive my own personal journey of kidney cancer drug development. I learned from Dr. Vogelzang to always appreciate and advocate for the patient perspective and to do everything possible to optimize patient outcomes.

The early days of kidney cancer saw rare, but spectacular, responses to immunotherapeutics. For many years afterward, until the advent of checkpoint inhibitors, immunotherapy clinical trials often showed anecdotal positive responses and intriguing immune activity. However, they generally lacked broad clinical effect that could significantly alter the course of the disease. I was an Assistant Professor at UCSF working with Eric Small when a small company called Sugan invited me to give a talk and participate in development of a drug called SU11248 that later became known as sunitinib. I remember during



the first investigator call for the Phase 2 trial, Dror Michaelson said "you've got an active drug on your hands" based on a dramatic response he had seen in clinic. It was pretty clear from the early days of VEGF Tyrosine Kinase Inhibitors (VEGF TKIs) that there was a tumor-shrinking effect that had not been evident in the cytokine era. This was clearly a milestone in kidney cancer drug development as several phase 2 and then ultimately phase 3 trials established that these drugs were very good at controlling disease albeit without curative potential. My early days at Cleveland Clinic with Ron Bukowski and Jim Finke were priceless in terms of understanding RCC drug development and important translational immune work that would lay the foundation for years to come. Having been fortunate to be involved from the early days of TKIs, we spent time thinking about comparing these drugs and studying them in sequence and even re-challenging with drugs previously used, in large part out of desperation. In those days, biomarker development was limited,

largely due to the high cost of assays like whole exome and RNA sequencing, which are now routine. While other antiangiogenic agents were tested, they never meaningfully improved VEGF TKI monotherapy. Consequently, sequential treatment became the standard of care, though it was often limited by chronic toxicity.

The next pivot point in kidney cancer drug development came with the emergence of checkpoint inhibitors, building upon the success of ipilimumab in melanoma. Initially nivolumab monotherapy demonstrated activity, and then checkpoint inhibitors moved rapidly into the front-line setting where immune-based doublets are currently the standard of care. I remember the early days of dose-finding studies of Ipi/Nivo in a refractory setting with impressive and durable responses. Checkmate 214 was a landmark study establishing dual immunotherapy as a standard of care in this disease, and for the first time we started to use the word 'cure' in a subset of patients.



Given the success of combining drug mechanisms in curing other solid tumors, Immuno-Oncology (IO) and TKI combinations quickly followed. These regimens have incredible tumor shrinking/controlling effects with durability of response in a subset of patients. The biomarker efforts in the Immuno-Oncology (IO) doublet era have been more impressive with better data collection, more sophisticated and large-scale assays and some key insights into kidney cancer biology. PD-L1 expression was an intriguing early biomarker that has not proven clinically useful, but other RNA sequencing-based assays have shown value in retrospective analysis and are awaiting prospective testing. Kidney cancer remains challenged by matching the best therapy to individual patient biology both in front line and refractory settings. IO agents more recently were tested in the adjuvant setting to prevent RCC recurrence after resection of high-risk disease. Only pembrolizumab demonstrated benefit and is now a standard of care. However, challenges in application remain given that a large percentage of high-risk patients are not destined to recur, and application of any drug in this setting only has the potential for harm and not benefit.

Building on this important advance through better patient selection criteria including use of emerging circulating markers such as ctDNA and kidney injury molecule-1 (KIM-1) is paramount. The most recent innovation in kidney cancer drug development has been hypoxia inducible factor (HIF) inhibitors with activity in a refractory setting and an array of studies in several different settings designed to determine whether combination therapy and/or earlier use can provide greater benefit. Biomarker development will be especially key in the development of HIF inhibitors. Kidney cancer is the most biologically diverse solid tumor in my opinion, and taking advantage of indolent biology has also emerged as an option in the form of active surveillance and

metastasis-directed therapy. Surgical resection of metastases has always been part of kidney cancer patient management, and more recently stereotactic body radiotherapy (SBRT) has come into common use. Rigorous prospective testing is required for proper application of the many different tools at our disposal to care for RCC patients.

Despite the 25 years of advances that I have described, there is a long way to go to cure every patient that walks in the door. We must double down our efforts to uncover novel mechanisms that can complement existing therapies, perform difficult and expensive prospective work to validate clinically useful biomarkers and understand better how to incorporate the patient voice in drug development. A dedicated and selfless effort towards mentoring the next generation of kidney cancer researchers should be our defining legacy.

The most satisfying part of my job is feeling the true appreciation of patients and their families. This appreciation is not based on outcomes, but rather on participation in their journey. A few months after the passing of a young patient that I had grown especially close to, his wife wrote me a note with the following message "You gave us more time- more memories, more stories and more love. Because of your research our kids knew their dad. They have stories to share, and lessons learned. It is all priceless." This is the reason I show up to work every day - to keep fighting for better outcomes, to keep doing difficult investigator-initiated trials, to reflect on poor outcomes to drive further research, and to hope that one day that there is indeed a world without kidney cancer.

# Body composition, ClearCode34, and survival in localized clear cell renal cell cancer

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## ABSTRACT

**BACKGROUND:** Poor muscle health is an adverse prognostic factor in clear cell renal cell cancer (ccRCC), but mechanisms are unknown. We examined associations among six body composition features, ClearCode34 tumor molecular subtype, and survival.

**METHODS:** In a cohort of 837 patients with localized ccRCC, we segmented pre-surgical CT scans for cross-sectional areas and radiodensities of skeletal muscle, visceral adiposity, and subcutaneous adiposity. RNA was extracted from archived tumor specimens and run on a custom Nanostring array. A centroid-based classification categorized tumors as ccA (less aggressive) or ccB (more aggressive) ClearCode34 subtype. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for associations between body composition and ClearCode34 subtype. Hazard ratios (HRs) and 95% CIs were estimated for individual and joint effects of body composition features and ClearCode34 subtype on 5-year disease-free survival (DFS).

**RESULTS:** The cohort was predominantly male, white, and had Stage I disease; 26% of tumors were ccB subtype. 95 DFS events occurred over 3532.2 person-years of follow-up. Patients with lower skeletal muscle radiodensity (SMD) were more likely to harbor ccB tumors (OR per 10-unit decrease in SMD: 1.30, 95% CI: 1.00-1.69). Exploratory joint effects analyses suggested patients with lower SMD and ccB subtype experienced the lowest DFS compared to patients without those characteristics (HR 2.68, 95% CI: 1.43-5.00).

**CONCLUSIONS:** ccRCC patients with lower SMD (more fat-infiltrated skeletal muscle) at diagnosis may experience worse survival because they harbor more aggressive tumors. Future studies should determine how post-nephrectomy changes in body composition features are associated with survival.

## INTRODUCTION

In both localized and metastatic clear cell renal cell cancer (ccRCC), lower skeletal muscle quantity (i.e., mass or index) and quality (i.e., radiodensity) have emerged as adverse prognostic factors<sup>6,19,17,23,30</sup>, but mechanisms underlying these associations are poorly understood. Given that ccRCC is a molecularly heterogeneous disease<sup>7,8,13</sup>, it is possible that differences in tumor biology contribute to observed associations between body composition features and survival. To our knowledge, Kays et al. is the only group to have explored the inter-relationships among body composition, molecular tumor features, and survival in ccRCC<sup>17</sup>. Among 217 high-risk ccRCC patients in The Cancer Genome Atlas (TCGA) whose computed tomography (CT) scans at diagnosis were segmented for body composition features, the authors examined how quantities of skeletal muscle, visceral adipose tissue, and sub-

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cutaneous adipose tissue as well as cachexia-related tumor gene expression patterns were associated with survival. They found a strong association between lower skeletal muscle mass (i.e., quantity) and increased tumor interleukin-6 expression, and that ccRCC patients who presented with both factors experienced the worst survival. These findings establish the first link between body composition and one feature of ccRCC tumor biology but should be further explored. In particular, considering a broader range of body composition features (e.g. radiodensities) and molecular features may aid in understanding how body composition impacts cancer outcomes in the localized ccRCC setting.

ClearCode34 is a validated 34-gene signature patients with localized ccRCC and prognostic of risk of recurrence and death<sup>7,8,14</sup>. The signature uses prediction analysis of microarrays (PAM) to classify samples into two subtypes (ccA and ccB) first identified by Brannon et al<sup>7</sup>. ccA tumors, which are more indolent, exhibit increased angiogenesis, fatty acid metabolism, and hypoxia-related signaling, while ccB tumors are characterized by more aggressive characteristics including increased transforming growth factor beta (TGF-β) and epithelial-to-mesenchymal transition<sup>7</sup>. Patients with the more aggressive ccB subtype tumors experience a higher rate of metastasis, and worse recurrence-free, cancer-specific,

and overall survival than those with the less aggressive ccA subtype<sup>7,8,14</sup>. However, associations between molecular subtype and other prognostic indicators remain unclear. In our large cohort of localized ccRCC patients treated by nephrectomy at Memorial Sloan Kettering (MSK), we recently reported that patients with lower skeletal muscle quality (i.e., radiodensity, SMD) were more likely to present with advanced stage and high-grade tumors<sup>12</sup>, and to experience

worse survival<sup>6</sup>. Notably, skeletal muscle quantity was initially associated with advanced pathological features and worse outcomes, but associations did not achieve statistical significance in multivariable models that accounted for all body composition variables simultaneously. These patterns emphasize the importance of evaluating both the quantities and qualities of body composition features in one model. In this report we extend this line of

CHARACTERISTIC	OVERALL <sup>1</sup> , N=837	ClearCode34 Subtype		P-VALUE <sup>2</sup>
		CCA <sup>1</sup> , N=219	CCB <sup>1</sup> , N=618	
Age	58 (49, 66)	58 (50, 66)	57 (48, 66)	0.5
Sex				0.4
Female	388 (31%)	119 (30%)	269 (32%)	
Male	851 (69%)	283 (70%)	568 (68%)	
Race				0.7
Asian	55 (4.4%)	15 (3.7%)	40 (4.8%)	
Black	36 (2.9%)	11 (2.7%)	25 (3.0%)	
Other	17 (1.4%)	7 (1.7%)	10 (1.2%)	
Unknown	28 (2.3%)	7 (1.7%)	21 (2.5%)	
White	1,103 (89%)	362 (90%)	741 (89%)	
Tumor grade				0.11
1	29 (2.3%)	15 (3.7%)	14 (1.7%)	
2	582 (47%)	180 (45%)	402 (48%)	
3	533 (43%)	173 (43%)	360 (43%)	
4	95 (7.7%)	34 (8.5%)	61 (7.3%)	
Tumor stage				0.028
1	795 (64%)	248 (62%)	547 (65%)	
2	63 (5.1%)	30 (7.5%)	33 (3.9%)	
3	381 (31%)	124 (31%)	257 (31%)	
Tumor size	4.00 (2.50, 6.00)	4.00 (2.50, 6.50)	4.00 (2.50, 6.00)	0.5
Ever smoked				>0.9
Ever	604 (49%)	197 (49%)	407 (49%)	
Never	635 (51%)	205 (51%)	430 (51%)	
History of diabetes	193 (16%)	53 (13%)	140 (17%)	0.11
History of hypertension	674 (54%)	217 (54%)	457 (55%)	0.8
History of hyperlipidemia	469 (38%)	147 (37%)	322 (38%)	0.5
<sup>1</sup> Median (IQR) or n (%)				
<sup>2</sup> Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test				

**Table 1.** Demographic and clinical characteristics of the cohort



research by investigating the inter-relationships between the quantities and qualities of skeletal muscle, subcutaneous adipose, and visceral adipose tissues, a validated molecular subtype (ClearCode34), and survival in a large cohort of localized ccRCC patients.

METHODS  
Study Population

The Resolve study is a retrospective study of 1,239 patients with stage I-III ccRCC treated at MSK between January 2000 and March 2020<sup>6,12</sup>. Eligible patients were aged 18 to 85, underwent either radical or partial nephrectomy without systemic therapy, and had no prior history of cancer. All patients had a CT scan taken at most three months before surgery and consented to their tumor

specimens being used for research. The study was approved by the MSK Cancer Center Institutional Review Board and performed in accordance with the ethical standards of the 1964 Declaration of Helsinki, its later amendments, and all relevant state laws.

Body Composition

A single slice CT scan at the level of the third lumbar vertebrae was retrieved for each patient and segmented for body composition features using Automatica software<sup>22</sup>. We derived the cross-sectional areas (cm<sup>2</sup>) and radiodensities [Hounsfield units (HU)] of skeletal muscle, visceral adipose, and subcutaneous adipose tissues as previously described<sup>12</sup>. For indexed measurements, we

standardized the cross-sectional areas by dividing by squared height in meters (cm<sup>2</sup>/m<sup>2</sup>). The six body composition features evaluated included skeletal muscle index (SMI) and radiodensity (SMD), visceral adipose tissue index (VATI) and radiodensity (VATD), and subcutaneous adipose tissue index (SATI) and radiodensity (SATD). We regarded lower SMD and higher VATD and SATD as pathological, as they represent lipid-enriched or lipid-depleted tissues, respectively<sup>5,15,26</sup>.

Gene Expression Assay

Formalin-fixed, paraffin-embedded tumor tissue samples were available for 929 (75%) of the 1,239 Resolve patients, which were assayed for gene expression. To address potential molecular tumor heterogeneity

Body Composition Feature	Model 1 <sup>1</sup>		Model 2 <sup>2</sup>		Model 3 <sup>3</sup>	
	OR	95% CI	OR	95% CI	OR	95% CI
SMI per -10 cm <sup>2</sup> /m <sup>2</sup>	0.90	0.78, 1.03	0.97	0.82, 1.16	0.97	0.79, 1.20
SMD per -10 HU	1.06	0.88, 1.27	1.21	0.97, 1.49	1.30	1.00, 1.69
VATI per +40 cm <sup>2</sup> /m <sup>2</sup>	1.14	0.96, 1.35	1.12	0.93, 1.34	1.27	0.93, 1.73
VATD per +10 HU	1.04	0.85, 1.27	1.07	0.87, 1.31	1.25	0.85, 1.84
SATI per +40 cm <sup>2</sup> /m <sup>2</sup>	0.96	0.83, 1.09	0.99	0.85, 1.15	0.90	0.74, 1.08
SATD per +10 HU	1.18	0.96, 1.45	1.15	0.93, 1.42	1.09	0.78, 1.53
<sup>1</sup> Model 1: Estimates from individual models that included each body composition feature, unadjusted						
<sup>2</sup> Model 2: Estimates from individual models that included each body composition feature, adjusted for age, biological sex, and smoking						
<sup>3</sup> Model 3: Estimates from model that included all body composition features simultaneous, adjusted for age, biological sex, and smoking						

**Table 2.** Odds Ratios and 95% Confidence intervals (OR, 95% CI) for associations between continuous body composition variables and presenting with ccB tumor (vs ccA tumor)

related to large tumor size<sup>29</sup>, the study pathologist selected two specimens for transcriptomic profiling if tumors were >7 cm. RNA was extracted and analyzed using the Nanostring nCounter system on a custom panel of 356 endogenous genes, including the 34 genes used in the ClearCode34 assay. Only samples that passed Nanostring quality control were included in the analytic cohort. Data were visualized using PCA plots, normalized using Remove Unwanted Variation using the RUVSeq package<sup>24</sup>, as has previously been detailed<sup>4</sup>, and log2-transformed. Of the 929 patients with tumor tissue available, samples from 915 patients had sufficient RNA concentration to be assayed on the nCounter system (98.5%). Of these, gene expression data of tumors from 837 patients (91.5%) passed Nanostring quality control and comprised the analytic cohort (Supplemental Figure 1). Most patient characteristics were similar between those with molecular data and those without, suggesting that our analytic sample is representative of the population of eligible patients; however, mortality was non-significantly lower among patients who were molecularly profiled relative to those who were not (5-year disease-free survival HR, 95% CI: 0.77, 0.55-1.06; 5-year overall survival HR, 95% CI: 0.82, 0.50-1.35).

Samples were classified into either ccA or ccB

ClearCode34 classes using a reference dataset and a previously validated prediction analysis of microarrays (PAM) centroid-based classification<sup>8,9</sup>. Briefly, a reference sample set was used to compute centroids for each ClearCode34 class, which were applied to the Resolve samples to predict class membership. If the subtype calls for large tumors (>7 cm) were discordant (n=16, 13%), the more aggressive subtype (ccB) was chosen.

### Survival Outcomes

For these analyses, we used five-year disease-free survival (DFS) as the primary survival outcome. DFS was determined by medical records (disease progression, local or contralateral recurrence, and distant metastasis) and the National Death Index (death from any cause). Follow-up time was calculated between the date of nephrectomy and death, disease-related events, or last follow-up (for those without events). Patients without events at the time of their last follow-up

	BODY COMP LEVEL	CLEARCODE34 SUBTYPE	SINGLE REFERENCE HR (95% CI) <sup>1</sup>	RERI (95% CI)
SMI	53.8 cm <sup>2</sup> /m <sup>2</sup>	ccA	1	-0.29 (-0.99, 0.41)
		ccB	1.37 (0.81, 2.31)	
	43.8 cm <sup>2</sup> /m <sup>2</sup>	ccA	1.34 (0.94, 1.92)	
		ccB	1.42 (0.70, 2.89)	
SMD	37.4 HU	ccA	1	0.86 (-0.59, 2.31)
		ccB	1.37 (0.81, 2.31)	
	27.4 HU	ccA	1.45 (0.98, 2.16)	
		ccB	2.68 (1.43, 5.00)	
VATI	65.6 cm2/m2	ccA	1	0.43 (-0.71, 1.56)
		ccB	1.37 (0.81, 2.31)	
	105.6 cm <sup>2</sup> /m <sup>2</sup>	ccA	1.01 (0.60, 1.72)	
		ccB	1.81 (0.90, 3.64)	
VATD	-96.5 HU	ccA	1	0.88 (-0.97, 2.73)
		ccB	1.37 (0.81, 2.31)	
	-86.5 HU	ccA	1.02 (0.53, 1.97)	
		ccB	2.27 (1.01, 5.13)	
SATI	73.7 cm <sup>2</sup> /m <sup>2</sup>	ccA	1	-0.20 (-0.78, 0.38)
		ccB	1.37 (0.81, 2.31)	
	113.7 cm <sup>2</sup> /m <sup>2</sup>	ccA	1.07 (0.80, 1.44)	
		ccB	1.24 (0.65, 2.40)	
SATD	-101.6 HU	ccA	1	-0.35 (-1.45, 0.75)
		ccB	1.37 (0.81, 2.31)	
	-91.6 HU	ccA	1.31 (0.77, 2.23)	
		ccB	1.33 (0.58, 3.02)	
1. Model contains all body composition variables, ClearCode34 subtype, and their interactions				

**Table 3.** Joint effects analysis for the interaction between body composition variables and ClearCode34 subtype

were administratively censored at that time point. In total, 3532.2 person-years were accrued across the cohort for the first five years of follow-up.

While the cohort does contain data for longer follow-up, we hypothesized that tumor molecular features assessed at the time of nephrectomy would have the greatest impact on early DFS, and analysis of ClearCode34 in TCGA exhibited that the strongest relationships between the signature and recurrence-free survival were evident within the first five to ten years of follow-up.

### Statistical Analysis

We report medians and interquartile ranges (IQRs) for continuous variables and counts and percentages for categorical variables. Body composition variables were scaled so that effect estimates represent approximately a one standard deviation change in each variable, which translated to 10 units for SMI ( $\text{cm}^2/\text{m}^2$ ), SMD (HU), VATD (HU), and SATD (HU) and 40 units for VATI ( $\text{cm}^2/\text{m}^2$ ) and SATI ( $\text{cm}^2/\text{m}^2$ ). We first estimated hazard ratios (HRs) and 95% confidence intervals (CIs) for 5-year DFS using Cox proportional hazards models for body composition variables as well as ClearCode34 subtype. The proportional hazards assumption was tested at an alpha of 0.05. To determine the association between each body composition variable and ClearCode34 tumor subtype, we estimated odds ratios (ORs) and 95% CI in multivariable logistic regression models that

included all body composition features simultaneously and were adjusted for age, sex, and smoking history.

In exploratory analyses, we evaluated the joint associations of body composition and ClearCode34 subtype on survival by assessing interaction on the additive scale using the relative excess risk due to interaction (RERI), which measures the extent to which simultaneous exposure is related to a greater than expected absolute difference in risk given the independent effects of each factor<sup>18</sup>. An RERI of 0 indicates no interaction,  $\text{RERI} < 0$  indicates that simultaneous exposure to both factors corresponds to a lower increase in risk than their individual effects suggest, and  $\text{RERI} > 0$  suggests that the two factors act synergistically.

Sensitivity results excluding patients with either contrast-enhanced CTs or those with subcutaneous adipose tissue that extended beyond the limit of detection on the CT scan were performed to assess potential bias. All analysis was performed in R version 4.3.3 (<https://www.r-project.org>). Cox proportional hazards models were fit using the survival package<sup>28</sup>, joint effects analyses were conducted using the multcomp package<sup>16</sup>, and RERI estimates were generated using the interactionR package<sup>2</sup>.

### RESULTS

As shown in Table 1, the overall cohort ( $n=837$ ) was predominantly male (68%),

White (89%), and had a median age at diagnosis of 58 years (IQR: 49, 66). Most patients presented with stage I (65%) and high grade II or III disease (48% and 43%, respectively). There were 95 DFS events over 3532.2 person-years of follow-up in the cohort. The more aggressive ccB ClearCode34 tumor subtype was detected in 26% of patients, and was associated with higher stage at diagnosis, larger tumor size, and higher grade. Demographic characteristics were similar across ClearCode34 subtypes. Distributions of body composition variables are detailed in Supplemental Table 1.

Supplemental Tables 2 and 3 presents HRs and 95% CIs for each body composition feature as well as ClearCode34 tumor subtype with 5-year DFS. Consistent with our previous analysis with longer follow-up, lower SMD was the only body composition variable significantly associated with worse 5-year survival after multivariable adjustment of age, sex, smoking, and all body composition variables (Supplemental Table 2; compare with Bradshaw et al, 2024). We also confirmed that ccB subtype was associated with worse 5-year survival (Supplemental Table 3; HR, 95% CI: 1.66, 1.09-2.52). Associations between body composition features and ClearCode34 tumor subtype are shown in Table 2. In multivariable models that accounted for age, sex, and all body composition variables simultaneously, lower SMD was significantly associated with ccB



subtype. The odds of presenting with the aggressive ccB tumor subtype increased by 30% per 10 HU decrease in SMD (OR 1.30, 95% CI: 1.00-1.69). Similar patterns of associations were detected for decreasing VATD and increasing VATI but neither of these variables were related to survival. While none of the RERIs for the joint effect analyses of body composition features and ClearCode34 subtype on DFS achieved statistical significance, the strongest RERIs were observed for SMD [RERI 0.86 (-0.59, 2.31)] and for VATD [0.88 (-0.97, 2.73)], which suggests a potentially synergistic effect of presenting these body composition features and harboring a more aggressive ccB subtype on 5-year DFS survival (Table 3). The HR of the doubly exposed group of lower SMD and ccB subtype was 2.68 (95% CI: 1.43-5.00), which exceeded the expected combination of the individual effects of SMD (HR 1.45, 95% CI: 0.98-2.16) or ClearCode34 subtype (HR 1.37, 95% CI: 0.81-2.31). Sensitivity analyses where we excluded either those with contrast CTs or those with subcutaneous adipose tissue cut-off did not substantially change the results.

## DISCUSSION

To our knowledge this is the first study to evaluate the individual and joint effects of body composition features and ClearCode34 tumor subtype in relation to survival among localized ccRCC patients. After confirming that lower SMD and the more aggressive ccB subtype were both associated

with worse 5-year DFS in our cohort, we found that patients with lower SMD were more likely to present with more aggressive ccB tumors. Qualitatively, our joint effects analysis suggests that patients who present with fat infiltrated muscle and harbor a more aggressive ccB tumor are at highest risk for disease recurrence or death, although these findings were not statistically significant. Our findings support the hypothesis that differences in tumor biology may contribute to the observed associations between SMD and clinical outcomes in ccRCC.

Our results are generally consistent with Kays *et al.* who also reported that a marker of poor muscle health was associated with an adverse molecular tumor characteristic and that patients with both features experienced the worst survival<sup>17</sup>. Our two studies differed with respect to sample size, gene expression, and body composition features assessed. Kays *et al.* analyzed a smaller cohort of 217 patients, some of whom had metastatic disease and therefore higher mortality rates. Notably, they only evaluated the quantities of skeletal muscle and adipose tissues while we also considered the qualities (i.e., radiodensities) of these tissues. Our prior research demonstrates the importance of adjusting for both the quantity and quality of all body composition features simultaneously in multivariable models<sup>6,12</sup>. However, because Kays did not include radiodensity measures in their analysis, the influence of these measures on our disparate results could not be

explored.

Radiodensities of skeletal muscle and adipose tissues are regarded as markers of lipid content, with lower radiodensities reflecting more fat infiltrated tissue<sup>15</sup>. In both the general population and in patients with various cancers, fat-infiltrated skeletal muscle or myosteatosis is a marker of metabolic dysregulation and has been associated with poor survival, increased incidence of complications, and drug toxicity<sup>1,3,6,10,11,19,20,21,25</sup>.

However, biological mechanisms linking myosteatosis to poorer cancer outcomes have not been fully elucidated. In this report we found that patients with low SMD were more likely to present with aggressive ccB tumors, and that patients with both features experienced the worst survival. It is possible that we detected an association between SMD and ccB because ccB is characterized by an upregulation of genes involved in epithelial-to-mesenchymal transition, which has been linked with systemic inflammation<sup>27</sup>. Because our analysis was cross-sectional in nature and body composition features were measured around the same time as ClearCode34 subtype (i.e., the time of diagnosis), it is unknown whether low SMD is a cause or a consequence of tumor aggressiveness. Either is possible as prior research suggests that ectopic fat accumulation in skeletal muscle is multifactorial and can result from lifestyle factors such as poor nutrition and



physical inactivity but also chronic diseases including cancer<sup>3</sup>. Insights into whether tumor-induced wasting contribute to adverse body composition variables could be gained by focusing on changes in body composition post-nephrectomy. Such investigations could also identify which body composition features should be targeted to improve clinical outcomes.

Strengths of our study include a large cohort of localized ccRCC patients who had both pre-diagnosis CT scans and gene expression data as well as well-annotated survival data. Multivariable models accounted for all body composition features simultaneously and sensitivity analyses were performed to determine the impact of contrast enhanced CTs (which can artificially inflate radiodensity estimates) and subcutaneous adipose cut-off (which may lead to underestimates of this tissue component). We acknowledge our study limitations which included potential selection bias from unavailable or depleted tumor blocks and a lack of representation from racially diverse ccRCC patients. Outcomes may be underestimated as patients missing tumor blocks had worse survival. While ClearCode34 is a validated ccRCC signature for localized patients, it does not represent all genetic pathways dysregulated in ccRCC. Finally, we acknowledge residual confounding may affect our findings. If an upstream factor that is associated with both body composition at the time of

diagnosis as well as ClearCode34 subtype is not accounted for in the multivariable analysis, this could result in the appearance of an association between body composition and ClearCode34 subtype without there being a direct relationship between the two variables. While possible, we consider this unlikely as, to date, no such relationships have been established. Future studies could evaluate additional markers of tumor biology, such as immune-related gene expression patterns, to extend these findings. Changes in weight or body composition after surgical resection of the tumor may also elucidate how tumor biology impacts body composition and weight management among ccRCC survivors.

## DECLARATION OF INTEREST

The authors have no conflicts to declare.

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# Challenges of Utilizing Artificial Intelligence in Identifying Prognostic Patterns in Localized Renal Cell Carcinoma

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## ABSTRACT

Artificial intelligence (AI) tools are increasingly being adopted across a wide range of scientific disciplines, offering transformative potential in data analysis and interpretation and can enhance the understanding and optimization of medical databases. Specifically, in the context of renal cell carcinoma (RCC), prognostic models such as UISS, SSIGN, and ASSURE scores are commonly utilized to estimate recurrence risk and overall survival. However, these models rely heavily on conventional statistical methods and predefined clinical parameters. Our aim was to investigate the feasibility of leveraging readily available AI tools to identify novel prognostic factors for overall survival in RCC patients undergoing surgery and to aid in developing an enhanced, data-driven prognostic scoring system.

## MATERIAL AND METHODS

Data from 1,273 consecutive patients who underwent surgery for renal cell carcinoma between 2010 and 2020 at a German tertiary care center were analyzed. The de-identified dataset included demographics, clinical and pathological variables, surgical details, clinical scores such as R.E.N.A.L. score, and time to event endpoints such as recurrence and overall survival. Five publicly accessible AI tools (chatGPT, Tableau, Julius AI, Microsoft Power BI, Polymer) were utilized to identify key prognostic parameters and develop a prognostic scoring system for overall survival in RCC patients. These bots were asked to first identify prognostic parameters and then develop a multi-variate prognostic model in response to the question: "Build a prognostic score for overall survival based on a multivariate analysis of the most predictive parameters". The dataset was divided into a training (n=746) and test (n=527) subsets for score development and validation. Kaplan-Meier survival analysis and log rank testing were performed to evaluate the prognostic accuracy of the generated scoring systems.

## RESULTS

Julius AI proved to be the simplest assistant to utilize, with workflows and programming derived from natural speech that are automatically converted into code. Key factors in predicting outcomes with this AI included duration until recurrence (r=0.75), surgery duration (r=0.35), initial metastasis status (r=0.34), tumor size (r=0.28), female gender (r=0.27), presence of necrosis (r=0.25), T-stage (r=0.24), grading (r=0.24), surgeon (r=0.24), and histology type (clear cell versus non-clear cell) at r=0.21. The AI automatically selected fields to construct a score, incorporating age, gender, ECOG performance status, surgical duration, surgeon, histology, pT stage, tumor size, necrosis presence, resection status, grading, type of nephrectomy (partial vs radical), and R.E.N.A.L. score. Patients were stratified into high-, intermediate-, and low-risk groups, and Kaplan-Meier analysis demonstrating significant survival differences among the groups (log-rank test p < 0.0001). However, one major limitation of the tools was that repeated analyses often yielded varying results, indicating that human judgment remains necessary.

## CONCLUSIONS

AI displays potential in identifying parameters that predict survival outcomes in renal cell carcinoma (RCC) patients based on clinical factors. Nonetheless, human oversight remains crucial. In addition to established scoring systems like SSIGN, UISS, and ASSURE, key factors such as surgeon expertise, surgical duration, and the RENAL score, which have been historically underutilized, should be integrated into future prognostic models. These factors, combined with tumor characteristics such as histology, size, grading, and necrosis, offer an opportunity to enhance the accuracy and utility of prognostic tools. Refining AI systems for greater reliability and reproducibility is critical for advancing their application in clinical decision-making and personalized patient care.

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## INTRODUCTION

Artificial intelligence (AI) tools are increasingly employed across various scientific disciplines. Numerous accessible AI tools have the ability to enhance the understanding and optimization of medical databases.

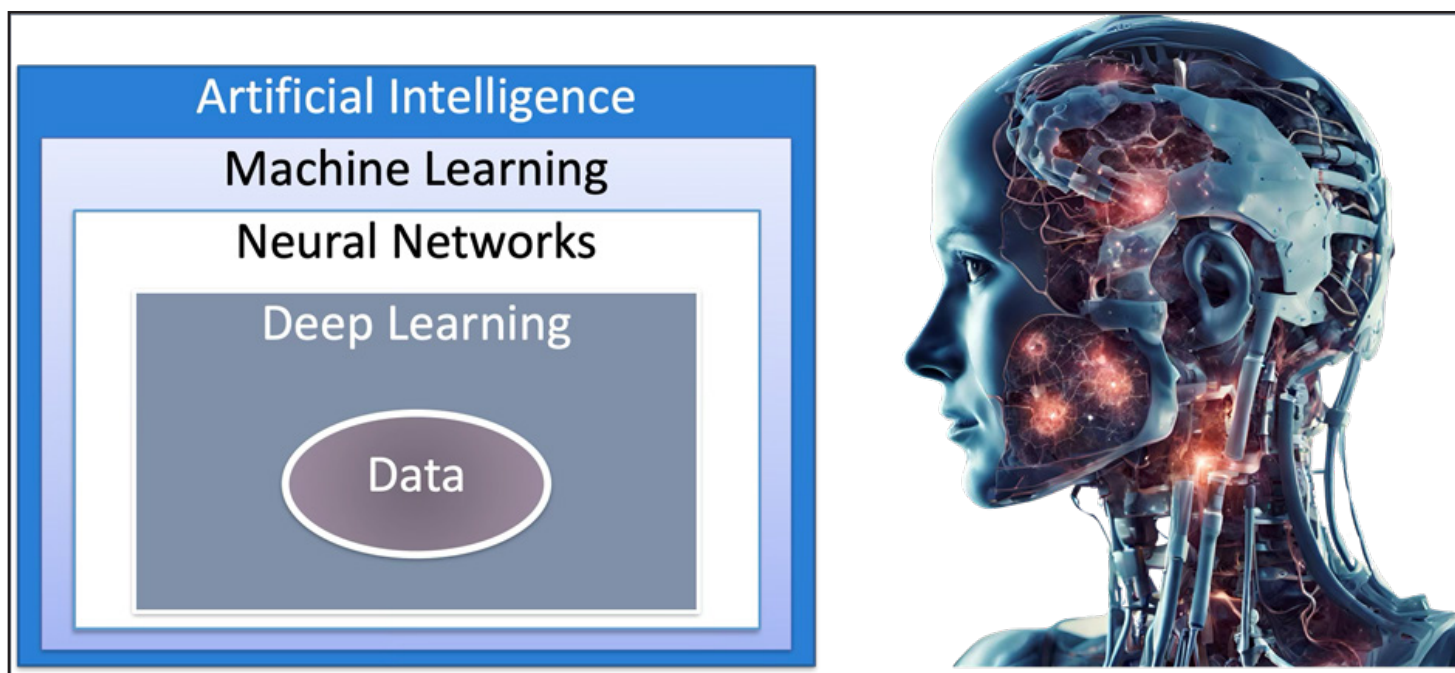
The availability of high-dimensional datasets, advances in high-performance computing, and innovative deep learning architectures have significantly increased AI use in various aspects of oncology research. These applications include the detection and classification of cancer, molecular characterization of tumors and their microenvironment, drug discovery and repurposing, and predicting treatment

outcomes for patients.<sup>1</sup>

With the introduction of adjuvant therapy into perioperative treatment of patients, it was shown that the majority of patients belonging to high-risk categories as defined by trial inclusion criteria, do not tend to progress.<sup>2</sup> Therefore, it is increasingly important to enhance predictive tools for local and distant progression.

In the context of renal cell carcinoma (RCC), prognostic models such as UISS, SSIGN, and ASSURE scores are commonly utilized to predict recurrence risk and overall survival.<sup>3-5</sup> They are mainly based on size, grading, and adverse/histopathological features, such as vascular invasion, sarcomatoid dedifferentiation, and the presence of necro-

sis.. These features have been analyzed and validated by numerous groups but were not revealed by AI but by conventional statistical analysis based on the judgement calls of physicians and statisticians. The principles of AI extend beyond merely analyzing data from large sources, such as clinical databases, to contextualizing the findings within the framework of existing data (Figure 1).<sup>6</sup> The advantage of this approach lies in the potential to identify new predictive factors within existing datasets, independent of prior assumptions, while simultaneously validating their significance. Thus, this study aimed to assess the feasibility of leveraging readily available AI tools to identify prognostic factors for overall survival in patients undergoing RCC surgery



**Figure 1.** AI and its subcategories; of note AI seems to be predominantly female if asked to draw a picture of itself.



and assist in developing a prognostic scoring system.

## MATERIALS AND METHODS

Data of 1,273 consecutive patients who underwent surgery for RCC between 2010 and 2020 were included in the analysis. De-identified data included age, gender, Eastern Cooperative Oncology Group performance status (ECOG PS), baseline creatinine and platelet levels, histology, laterality, pathological and clinical T-stage, grading, TNM stage, necrosis, lymphovascular invasion, vascular invasion, resection margin, Leibovich score, RENAL score, tumor size, duration of

surgery, type of surgery, surgeon, surgical approach, blood loss, complications (graded by Clavien-Dindo), time to recurrence, time to death, and cause of death. duration until recurrence and duration until death and reason of death.

Five publicly accessible AI tools (chatGPT, Tableau, Julius AI, Microsoft Power BI, Polymer) were asked to first identify prognostic parameters and develop a prognostic score for RCC outcome. These tools were asked to first identify prognostic parameters and then develop a prognostic score for RCC outcome by responding to the question: *"Build a prognostic score for overall survival based on a multivariate*

*analysis of the most predictive parameters"*.

The dataset was split into a training (n=746) and test set (n=527) for score development, with Kaplan-Meier survival analysis and log-rank testing conducted to assess the score's accuracy.

## RESULTS

Julius AI proved to be the simplest assistant to utilize, with workflows and programming derived from natural speech that are automatically converted into code. Key factors in predicting outcomes included duration until recurrence ( $r=0.75$ ), surgery duration ( $r=0.35$ ), initial metastasis status ( $r=0.34$ ), tumor

The dataframe contains various fields.

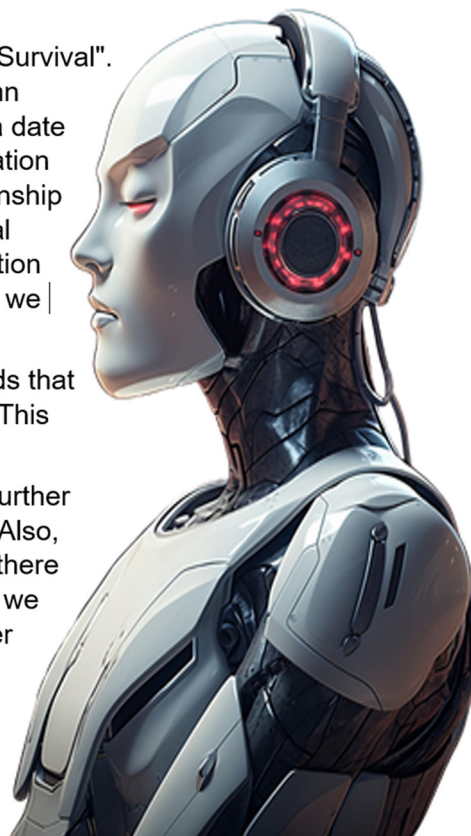
Next, I will identify the column that represents "Duration of Survival". If it's not immediately clear, I may need to create this column based on other columns (such as a date of diagnosis and a date of death or last follow-up). After that, I will perform a correlation analysis to identify the fields that have the strongest relationship with "Duration of survival". This will help us identify potential predictors. The dataframe contains a column named "Duration of Survival", which likely represents the duration of survival we are interested in.

Next, I will perform a correlation analysis to identify the fields that have the strongest relationship with "Duration of Survival". This will help us identify potential predictors.

Please note that correlation does not imply causation and further statistical tests may be needed to confirm these findings. Also, correlation analysis only works with numerical variables. If there are categorical variables that you think might be important, we might need to convert them into numerical form or use other statistical methods to analyze their relationship with the "Duration of Survival".

Let's proceed with the correlation analysis.

Here are the fields that have the strongest correlation with "Duration of Survival": see Figure 3.



**Figure 2:** Text based AI workflow

size ( $r=0.28$ ), female gender ( $r=0.27$ ), presence of necrosis ( $r=0.25$ ), T-stage ( $r=0.24$ ), grading ( $r=0.24$ ), surgeon ( $r=0.24$ ), and histology type (clear cell versus non-clear cell;  $r=0.21$ ).

The AI automatically selected fields to construct a score, incorporating age, gender, ECOG performance status, surgical duration, surgeon, histology, pT stage, tumor size, necrosis presence, resection status, grading, type of nephrectomy (partial vs radical), and RENAL score. Patients were categorized into high, intermediate, and low-risk groups, and Kaplan-Meier analysis revealed significant differences in survival, with a log-rank test  $p<0.001$ .

However, a major limitation of

the tools was that repeated analyses often yielded varying results, indicating the continued necessity of human judgment.

## DISCUSSION

AI can assist in identifying parameters to predict overall survival based on clinical factors. Factors such as the surgeon, duration of surgery, and RENAL score have been underestimated and should be considered alongside tumor characteristics like histology, size grading, and necrosis. Increasing evidence suggests that surgical volume, which can be interpreted as a criterion of surgical experience, is associated with RCC surgical outcomes.<sup>7-10</sup> Surgical volume is typically assessed at the hospital level rather

than the individual level in the literature, and volume alone does not necessarily indicate individual surgical quality. Performing a procedure frequently does not guarantee it is performed correctly, as repeated mistakes can occur if not identified and addressed in a constructive, evidence-based environment. A more accurate assessment of an individual surgeon's performance may be better reflected by adherence to clinical guidelines and demonstrated surgical expertise.<sup>9, 11</sup> In our dataset, it was inherent that some surgeons had an inferior survival of their patients in multivariate analysis compared to others who had an up to 30% better survival. To date, no standard operating procedures (SOPs) for quality

control have been implemented to address these differences and ensure optimized training and outcomes.

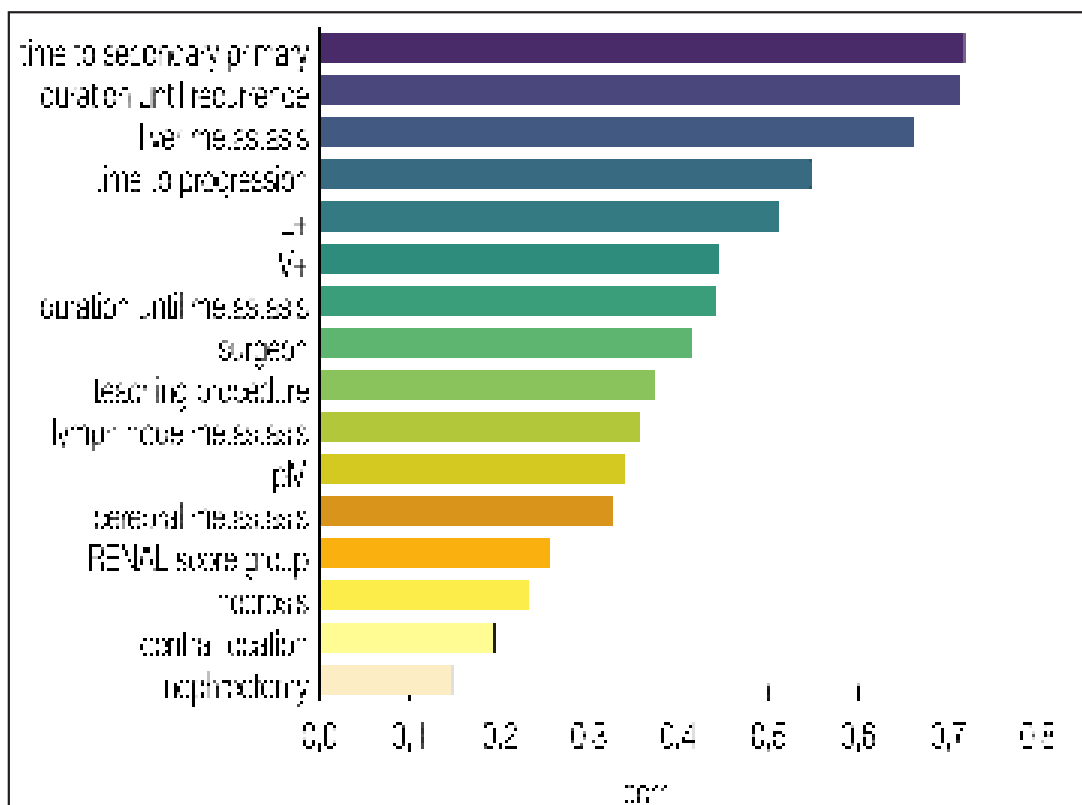


Figure 3. Identified fields of the database correlating to overall survival. Some of the fields can't be used as predictors as they described endpoints around survival

Duration of surgery might not only reflect the intraoperative situation depending on the oncological stage but could also be connected to surgical expertise. Because resection status was identified as a predictor, factors such as the use of intraoperative frozen sections and the decision to utilize them largely depend on the surgeon's judgment, as well as the availability and capacity of pathology services. These factors have not been incorporated into prognostic scores to date, primarily due to the lack of independent, objective measures of surgical expertise and the associated ethical complexities. Additionally, surgical performance is a sensitive topic, as no physician would typically self-identify as lacking proficiency, nor would healthcare providers wish to label individuals in this way. However, given the impact of surgical expertise on patient outcomes, it is essential to initiate discussions around this issue. In some cases, this may lead to the restriction of certain procedures to specific institutions or surgeons—a concept already partially implemented within the UK's NHS framework. Surgical factors are currently dependent on individual humans and cannot be standardized. AI has identified some previously recognized factors as prognostically relevant, such as pathological T-stage, tumor size, and presence of necrosis.<sup>4, 5</sup> Alongside grading and histology, these predictors of survival are not modifiable but can only be

reported. Histology has not been considered prognostic in most models to date; however, further efforts should focus on incorporating histological variants, such as chromophobe, papillary, or translocation tumors, into prognostic models. Larger cohorts of these subtypes would be required; however it is a valuable pursuit as objective prospective data are currently limited and patients may receive inaccurate counseling if prognostic insights are solely based on clear cell RCC data. The prognostic importance of histological factors also warrants for standardized pathological reporting. As the pathology reports incorporated in this analysis are not standardized, features such as the presence or absence of necrosis, certain IHC staining results, specimen color, texture, cellular size, stromal involvement and other potential histological characteristics can only lead to further speculation rather than accurate, reproducible data.

The AI models also revealed the RENAL score as an independent predictor (data submitted elsewhere). This has not been investigated enough in the past, but it might reveal anatomy as a clinically relevant factor of prognosis. This might fuel the debate around performing partial nephrectomies for highly complex tumors, a topic that has been discussed at several major urology meetings in the past

An obvious limitation was the

inability of AI to differentiate between endpoints and variables, making human adjustment and critical interpretation essential.

Some existing tools fail to produce consistent results, with repeated analyses often yielding different outcomes. In fact, some of the tools could not identify any new prognostic parameters besides the existing ones as their own results became general knowledge and there would not allow for a new scoring system as no newer signals were identified. Rerunning the test after resetting the AI three months later made it possible to reproduce some results, as data were previously deleted and seemingly forgotten.

Another limitation identified was the variability in AI tool outputs and the lack of transparency regarding the origins of these inconsistencies therefore raising questions about the objectivity of the results. Additionally, the dataset is limited by the included variables, the involved surgeons, and the characteristics of the patient population at a tertiary referral center. Future AI training may benefit from using comprehensive patient data rather than being limited to specific cancer databases for indications. Achieving this will require standardized reporting on both pathological findings and surgical procedures and outcomes.

## CONCLUSION

AI can help predict survival outcomes in RCC patients using clin-



ical factors, but human oversight is essential. Important factors like the surgeon, surgery length, and RENAL score should be included alongside tumor characteristics such as histology, size grading, and necrosis in future prognostic models. Additionally, some existing tools fail to produce consistent reproducible data.

Furthermore, integrating AI into clinical decision-making processes can potentially enhance the accuracy and reliability of predicting patient outcomes. By refining algorithms to account for a more comprehensive range of variables, we can improve personalized treatment plans. The inclusion of detailed patient history, lifestyle factors, and genetic information could provide deeper insights into disease progression.

Moreover, continuous validation and updating of AI models with new data are crucial to maintaining their relevance and accuracy over time. Collaborations between data scientists, oncologists, and surgeons will ensure that these models are both scientifically robust and practically applicable in clinical settings. Ethical considerations and transparency in AI-driven decisions must also be prioritized to maintain trust and accountability in medical practice.

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# Serum Protein Markers in Clear Cell Renal Cell Carcinoma

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## ABSTRACT

### BACKGROUND

Renal Cell Carcinoma (RCC) is one of the most frequently diagnosed malignancies, yet non-invasive biomarkers for early detection and prognosis in RCC remain scarce. This study aims to identify serum protein markers predictive of clear cell RCC (ccRCC) development and prognostic of survival outcomes.

### METHODS

Using the UK Biobank, a prospective cohort of >500,000 individuals, we analyzed serum proteomic data from participants who developed ccRCC after serum collection (Group 2), those diagnosed prior to serum collection (Group 1), and controls (Group 3). Proteomic measurements were performed using the Olink Proximity Extension Assay (PEA). Cox proportional hazards regression models estimated hazard ratios (HRs) for cancer risk and survival, adjusting for age, sex, BMI, smoking status, and renal function. Kaplan-Meier survival analysis evaluated prognostic markers.

**RESULTS:** Five serum proteins—HAVCR1, REN, INHBB, NCR3LG1, and PGF—were significantly associated with future ccRCC development. HAVCR1 exhibited the strongest predictive performance (HR 5.1, 95% CI: 3.6–7.3,  $p < 0.001$ ; AUC 0.8756). Among all patients with ccRCC, NCR3LG1 (HR 2.6, 95% CI: 1.4–5.1,  $p < 0.002$ ), PGF (HR 2.2, 95% CI: 1.3–3.8,  $p < 0.001$ ), and GDF15 (HR 1.8, 95% CI: 1.1–2.8,  $p < 0.02$ ) were associated with reduced survival.

### CONCLUSION

This study identifies HAVCR1 as a promising diagnostic biomarker for early ccRCC detection, with NCR3LG1, PGF, and GDF15 serving as potential prognostic markers. Further validation in independent cohorts is needed to facilitate clinical translation into diagnostic and prognostic tools for ccRCC.

**KEYWORDS:** Renal Cell Carcinoma, Serum Biomarkers, Proteomics, HAVCR1/KIM-1

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INTRODUCTION

Renal Cell Carcinoma (RCC) is among the most frequently diagnosed malignancies in both genders with over 81,000 estimated cases in 2024<sup>1</sup>. Incidence of renal mass detection has steadily increased over the past decades, largely due to increased cross sectional imaging revealing incidental renal masses at a lower stage<sup>2</sup>. Despite improved early detection of RCC, imaging remains cost-prohibitive and is not without risk to the patient, therefore image based screening is not recommended for average risk patients. While non-invasive diagnostic and prognostic biomarkers for other urologic cancers including PSA for prostate cancer and AFP and  $\beta$ HCG in testes cancer have been previously recognized and validated, accurate and reliable markers for RCC have proven much more elusive with no clinically actionable markers identified at present.<sup>3</sup> Though many potential biomarkers have been investigated in RCC in the past, most have been targeted towards predicting response to the armamentarium of RCC treatment options rather than focusing on use for cancer screening or detection of recurrence. While cell-free and circulating tumor DNA appear promising in early diagnosis or recurrence detection in RCC, they have yet to be adopted into

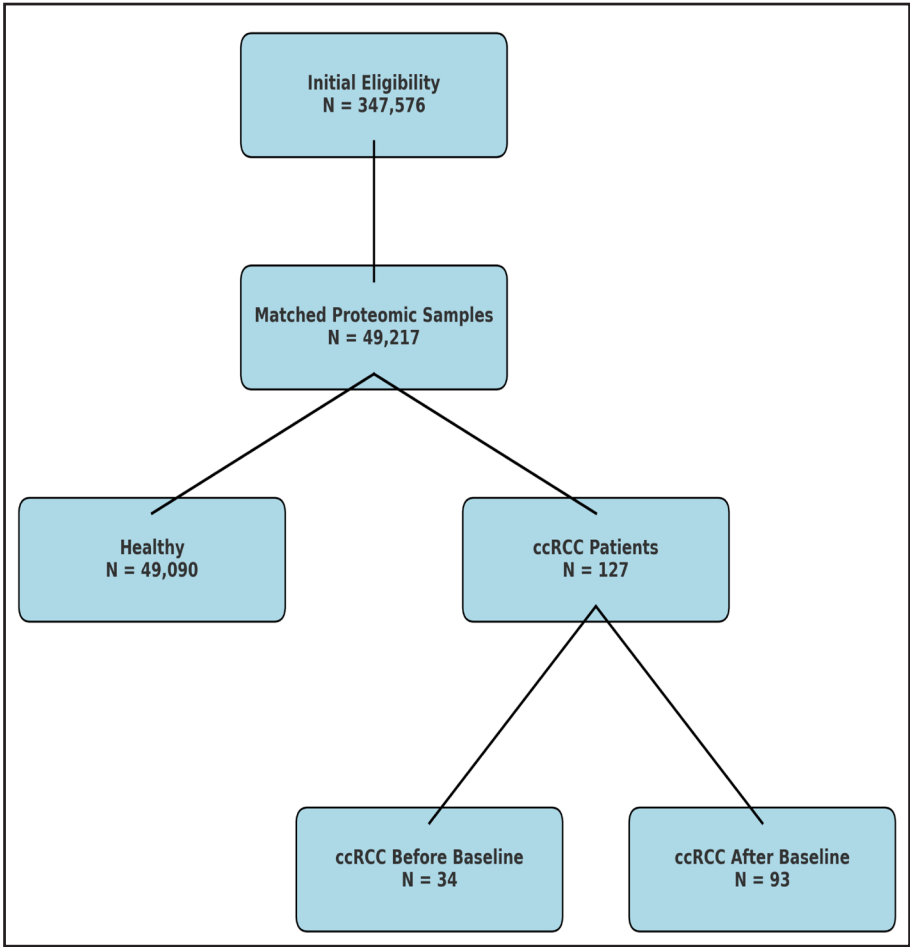


Figure 1. Flowchart of patient cohorts.

Characteristic	Group 1 (Had RCC at Baseline)	Group 2 (Developed RCC Later)
Total Patients	34	93
Mean Age at Recruitment (±SD)	61.2 (6.4)	59.3 (7.0)
Sex (Male/Female)	26 / 8	54 / 39
Mean BMI (±SD)	30.0 (5.5)	29.4 (4.8)
Smoking: Never (%)	9 (26.5%)	28 (30.1%)
Smoking: Current (%)	25 (73.5%)	65 (69.9%)
GFR (Creatinine, Mean ± SD)	60.1 (20.0)	84.2 (14.9)
Time to Diagnosis (Mean ± SD)	3.6 (3.7)	-6.5 (3.6)

Table 1: Patient characteristics by cohort. BMI = body mass index (measured in  $\text{kg}/\text{m}^2$ ); GFR = glomerular filtration rate (measured in  $\text{mL}/\text{min}/1.73\text{m}^3$ ); SD= standard deviation.

practice due to the low levels of DNA shedding that are the hallmark of the disease. Additionally, the costliness of these tests may pose a barrier to widespread clinical use.

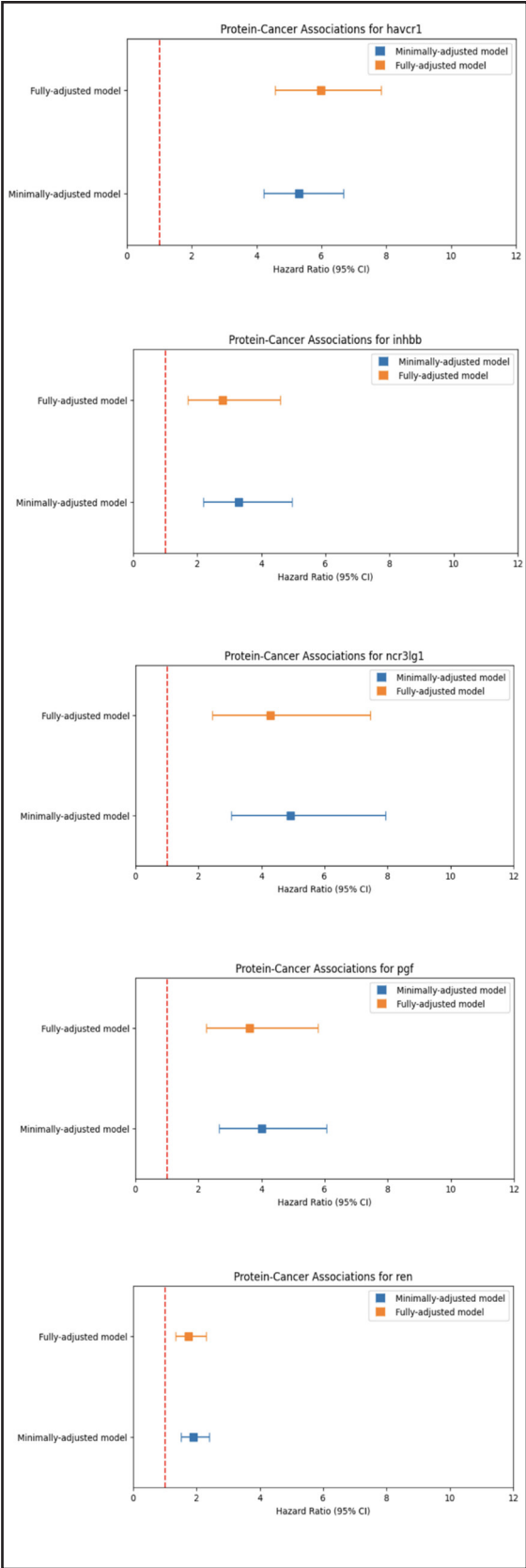
A recent publication by Papier *et al.* examined proteomic risk factors for 19 different cancers including kidney cancer utilizing data from the UK Biobank (UKBB). While the authors identified 51 proteins associated with kidney cancer risk, these proteins were not stratified by histologic subtype. As kidney cancer is known to be a disease with significant heterogeneity, biomarkers that are powerful predictors for one subtype of kidney cancer will not necessarily be useful in others<sup>4</sup>. With this in mind, we hypothesized that specific serum protein biomarkers can differentiate patients who later develop clear cell RCC (ccRCC) from those who do not, and help predict outcomes in patients who have been diagnosed with ccRCC. To test this hypothesis, our study utilized data from the UKBB to identify diagnostic serum protein biomarkers in patients who did not have ccRCC at the time of serum sampling but later developed it. We also examined serum samples of all patients diagnosed with ccRCC to identify potential prognostic markers using proteomic analysis.

### MATERIALS AND METHODS

To support our aims, we conducted a population-level examination of serum proteomic markers associated with ccRCC using data from the UKBB, a large population based

prospective cohort of over 500,000 individuals ages 40-69 recruited between 2006-2010 in the United Kingdom. All participants completed baseline assessments, including demographic and lifestyle data (e.g., BMI, smoking status) and physical measurements (Table 1). Non-fasting blood samples were also collected at baseline, which were processed into plasma and stored at -80 °C. As part of the UK Biobank Pharma Proteomics Project (UKB-PPP), protein measurements were performed using the Olink Proximity Extension Assay (PEA).

The relative abundance of proteins was quantified across four 384-plex panels designed to profile inflammatory, oncologic, cardiometabolic, and neurologic markers. These measurements, expressed as log2-transformed normalized protein expression (NPX) values, provided a comprehensive proteomic landscape for subsequent analyses. Cancer incidence and mortality data were obtained via linkage to national registries (NHS Digital for England and Wales, and the NHS Central Register for Scotland), and participants were followed until December 31, 2020, in England and Wales or November 30, 2021, in Scotland, or until death, withdrawal of consent, or emigration. For the observational analyses, kidney cancer (ICD codes C64–C65) was the endpoint, further stratified to ccRCC when histologic data permitted. (Figure 1) Our patient cohorts included individuals with established RCC that had been diagnosed before serum collection (Group 1) vs. patients without ccRCC at the time of recruitment who devel-



**Figure 2:** Protein-Cancer Associations for proteins of interest in previously undiagnosed ccRCC patients.



oped the disease later (Group 2) vs a group of healthy controls (Group 3). All were matched by age, sex, and BMI. We estimated hazard ratios (HRs), 95% confidence intervals (CI), and survival while adjusting for the aforementioned covariates using Cox proportional regression models. We investigated protein and ccRCC cancer-risk associations to examine the effects of reverse causality and conducted analyses to generate area under the curve (AUC) values to evaluate diagnostic performance of biomarkers. Finally, we utilized Kaplan-Meier curves to estimate survival rates based on high or low serum concentrations of proteins of interest.

### Study Design & Data Selection

This study was conducted using the UKBB, a population-based cohort of approximately 500,000 individuals aged 40-69 recruited between 2006-2010. Participants completed baseline assessments, including demographic and lifestyle data (e.g., BMI, smoking status), physical measurements, and blood sampling. Our analysis further focused on individuals with available multiplex proteomic data performed using Olink Proximity Extension Assay (PEA) as part of the UK Biobank Pharma Proteomics Project (UKB-PPP),

To be included in the analysis, participants needed complete data on age, sex, BMI, smoking status, and serum creatinine for calculating estimated glomerular filtration rate (eGFR) using the CKD-EPI formula. From the full cohort, of 347,567 patients, 346,432 individ-

uals were identified who had complete phenotypic data and serum creatinine. Of these, 49,217 also had proteomic data available. Only individuals with valid proteomic data (non-missing NPX values) were retained. Individuals with missing or unusable data on demographics or proteomics were excluded from the study.

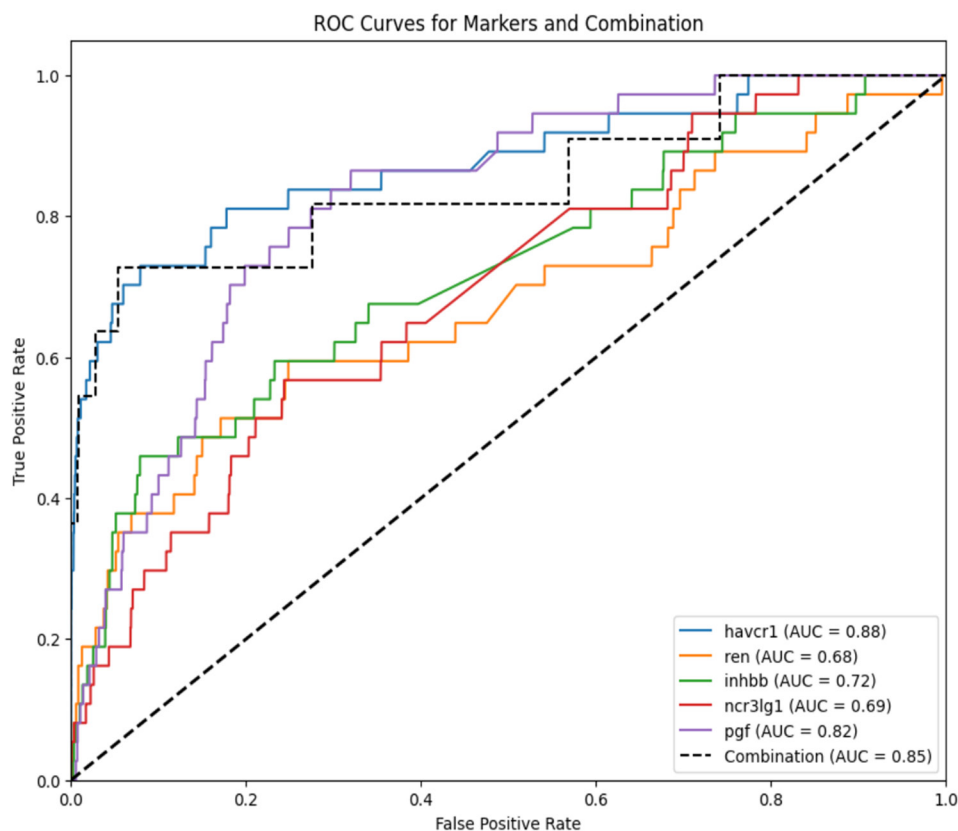
Renal cell carcinoma (RCC) cases were identified using ICD-10 codes C64 and C65. Among the 127 patients diagnosed with clear cell RCC, 34 were diagnosed at or before serum collection, while 93 were initially cancer-free and developed the disease later. The remaining participants formed the healthy control

group (n= 49,090).

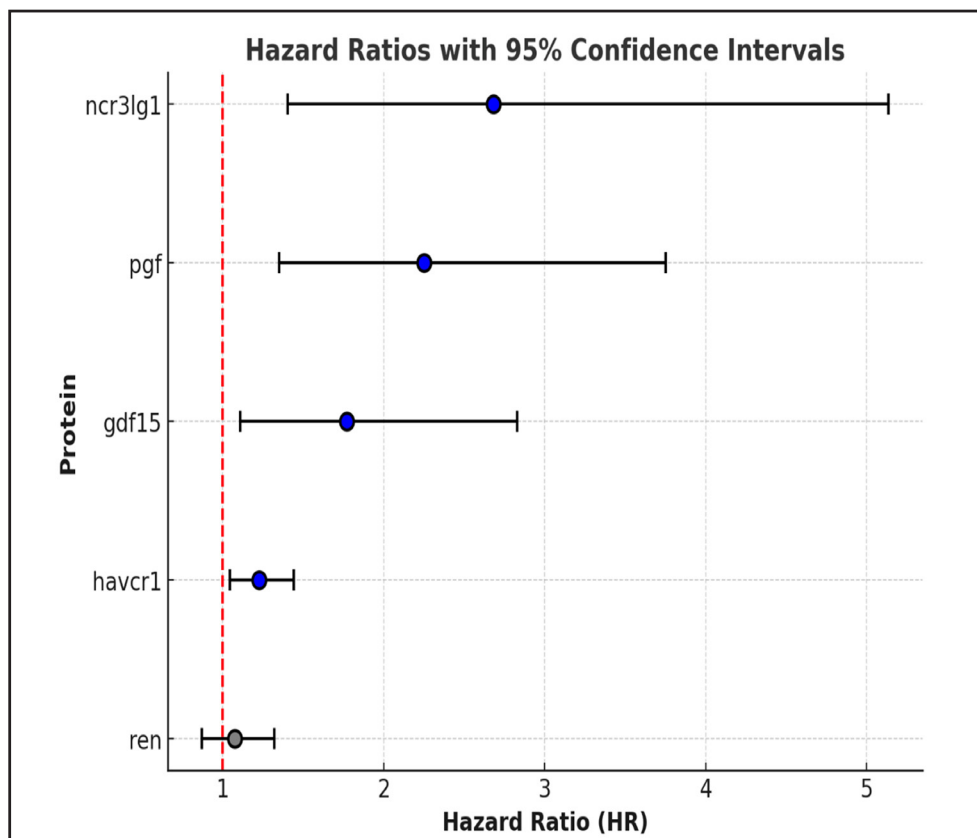
### Preprocessing & Quality Control:

We performed extensive preprocessing to ensure that the data were of high quality for analysis. Proteomic data were generated using Olink's multiplex PEA, which produce Normalized Protein eXpression (NPX) values on a relative log2-like scale. These proteomic values were integrated with the UKBB phenotype data based on participant identifiers. Any discrepancies, such as duplicate records or inconsistent IDs, were reviewed and resolved or discarded.

Proteins with more than



**Figure 3.** ROC curves for proteins of interest in previously undiagnosed ccRCC patients.



**Figure 4.** Prognostic Hazard ratios for proteins of interest in all patients diagnosed with ccRCC.

10% missing data were flagged for review, and if the missing data were considered random, single imputation methods (such as median imputation) were applied for proteins deemed essential to the study. We also conducted outlier detection on proteomic data by generating boxplots and calculating z-scores (greater than  $\pm 4$  standard deviations). Outliers were either excluded or adjusted (Winsorized) when technical errors or assay noise were suspected. Inconsistent or implausible BMI values were reviewed, and any unusual date entries were reexamined and standardized.

To ensure data consistency, Python (version 3.9) was utilized for

data merging, outlier detection, and normalization. We employed Pandas, Numpy, Scipy, and Scikit-Learn libraries for data processing and statistical checks. The Tidyverse package in R (version 4.1.2) was used for additional quality control, including verifying missing data patterns and examining distributions of variables.

#### Statistical Analysis:

We employed time-to-event analyses using Cox proportional hazards regression to investigate the relationship between baseline proteomic profiles and ccRCC risk, as well as overall survival. Of the 127 ccRCC cases identified, 34 were classified as

prevalent and 93 as incident, allowing us to study both disease onset and outcomes. Proteins were treated as key explanatory variables, with raw NPX values and standardized z-scores used to model hazard ratios (HRs). These analyses were adjusted for age, sex, BMI, smoking status, and eGFR.

To address multiple comparisons, we applied false discovery rate (FDR) corrections or effective number of tests to adjust for the correlation between proteins. Proteins with FDR-adjusted p-values less than 0.05 were considered significant. To visualize survival data and cumulative incidence of ccRCC diagnoses, Kaplan-Meier curves were generated. We also categorized participants by high or low protein levels (based on median NPX concentration) and performed log-rank tests to compare risk across these groups. In cases where we suspected a dose-response relationship, we further divided the groups into quartiles or tertiles to assess whether risk increased with higher protein levels.

To minimize reverse causation bias, we conducted sensitivity analyses by excluding individuals who developed ccRCC or died within two to three years of baseline. We also stratified analyses by eGFR ( $<60$  vs.  $\geq 60$  mL/min/1.73 m<sup>2</sup>) and sex to investigate whether associations varied by kidney function or demographic factors.

#### Reproducibility & Ethical Approvals

The computing environment was documented, including versions of

R (v4.1.2) and Python (3.9), to ensure that the analysis could be replicated. Internal consistency checks were performed to ensure data integrity before final analysis. UKBB participants provided written informed consent, and data were fully de-identified to maintain confidentiality. Ethical approval was granted by the North West Multi-centre Research Ethics Committee, and all procedures adhered to the principles outlined in the Declaration of Helsinki. Although UKBB data cannot be shared publicly due to confidentiality agreements, annotated R scripts, Jupyter notebooks, and data dictionaries are available upon request. Other researchers with approved access to the UKBB can replicate or extend the analysis by using the same methods and scripts. The analysis pipeline can be adapted for other proteomic or omics datasets with similar data structures, such as participant-level CSV files and log-transformed continuous markers.

## RESULTS

503,317 adults aged 39–73 years were analyzed utilizing the UK Biobank. Of these 347,576 met eligibility criteria, Olink PEA was performed on 49,217 of these participants. We then identified our cohorts as defined above with 34, 93 and 49,090 patients in Group 1, 2 and 3 respectively.

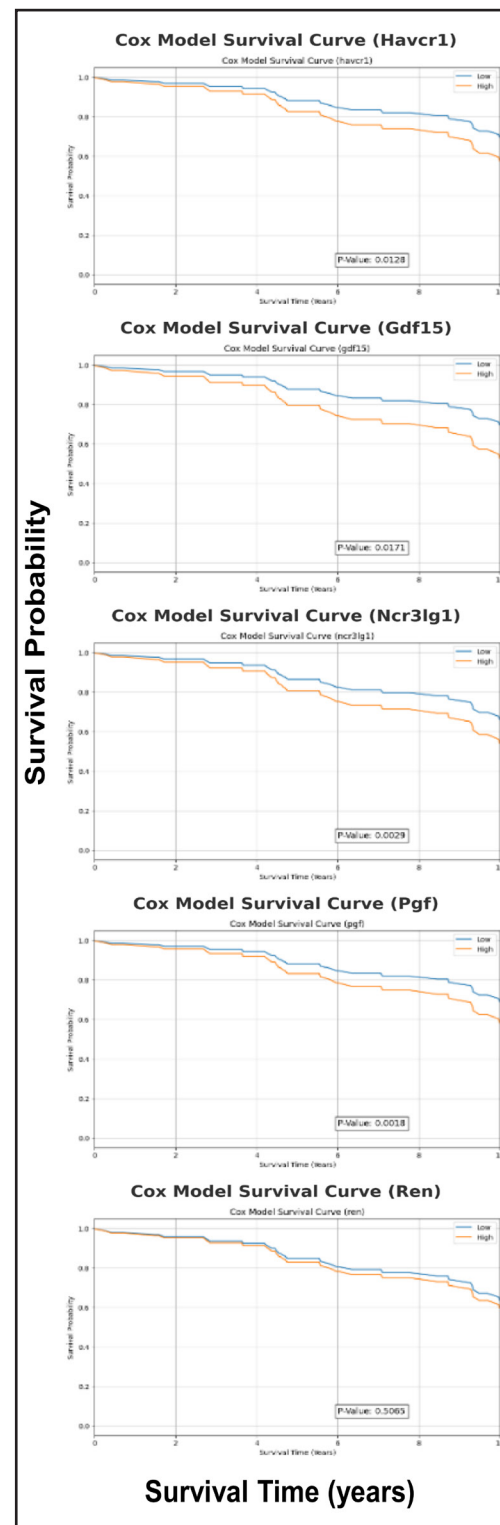
### Predictive serum biomarkers for ccRCC

Our findings revealed the strongest predictive associations between

elevated levels of HAVCR1, REN, INHBB, NCR3LG1, and previously undiagnosed ccRCC (Group 2), with these proteins showing increased HR for cancer association (95% CI) (Figure 2). Among these, HAVCR1 was most pronounced (HR 5.1, 95% CI: 3.6–7.3,  $p < 0.001$ ) underscoring its potential as a powerful diagnostic indicator in ccRCC screening. REN, INHBB, NCR3LG1, and PGF all exhibited strong performance as well, showing HR 1.5 95% CI: 1.1–2.2,  $p < 0.02$ ; HR 1.8 95% CI: 0.9–3.5,  $p < 0.09$ ; HR 3.7 95% CI: 1.7–8.1,  $p < 0.001$ ; HR 3.9 95% CI: 1.2–13.1,  $p < 0.03$ ; respectively. Operating characteristic curve analysis demonstrated strong predictive capabilities for each protein of interest as follows: HAVCR1 (AUC 0.8756; SN 0.8108; SP 0.7798), REN (AUC 0.6777; SN 0.5135; SP 0.7720), INHBB (AUC 0.7189; SN 0.4865; SP 0.8530), NCR3LG1 (AUC 0.6935; SN 0.1892; SP 0.9325), and PGF (AUC 0.8216; SN 0.2703; SP 0.9475). These biomarkers, individually and collectively, exhibited high sensitivity and specificity for ccRCC detection (Figure 3).

### Prognostic serum biomarkers for ccRCC

In all patients previously diagnosed with ccRCC (Groups 1 and 2 combined), we examined protein-to-cancer associations as potential indicators of prognosis, or overall survival. Note that the hazard ratios in the previous analysis for Group 2 specifically reflect the likelihood of developing ccRCC, whereas in this analysis, the hazard ratios pertain to overall survival in diagnosed pa-



**Figure 5.** Cox Survival Curves for proteins of interest in all patients diagnosed with ccRCC.



tients. Among the identified biomarkers, NCR3LG1 demonstrated the strongest prognostic association with survival in ccRCC, exhibiting the highest hazard ratio (HR 2.6, 95% CI: 1.4–5.1,  $p < 0.002$ ). This was followed by PGF (HR 2.2, 95% CI: 1.3–3.8,  $p < 0.001$ ) and GDF15 (HR 1.8, 95% CI: 1.1–2.8,  $p < 0.02$ ). HAVCR (HR 1.2, 95% CI: 1.0–1.4,  $p < 0.01$ ) and REN (HR 1.1, 95% CI: 0.8–1.3,  $p = 0.5$ ) also emerged as notable candidates, though with comparatively lower effect sizes. (Figure 4).

Cox regression curves were generated for the proteins identified above separated by high vs. low serum protein concentration. Higher concentrations of all proteins, except REN, were significantly associated with decreased survival probability. (Figure 5).

Kaplan-Meier analysis was performed for proteins of interest in this group, stratified by high versus low protein concentration, while adjusting for GFR. Over the 10-year follow-up, all proteins exhibited a trend toward decreased survival. However, only PGF demonstrated a statistically significant association with reduced survival ( $p < 0.006$ ). HAVCR1 ( $p = 0.07$ ), GDF15 ( $p = 0.1$ ), and NCR3LG1 ( $p = 0.2$ ) followed a similar trend but did not reach statistical significance. REN remained the only protein showing no survival difference based on concentration ( $p = 0.6$ ). (Supplementary Figure 1).

## DISCUSSION

Our study found significant association with elevations of HAVCR1, REN, INHBB, NCR3LG1, and PGF and previously undiagnosed ccRCC, indicating their potential as diagnostic markers. In particular, HAVCR1 was identified as a potential predictive biomarker for ccRCC, with a hazard ratio of 5.1. NCR3LG1, PGF, HAVCR1, and GDF15 were also associated with decreased survival in patients with diagnosed ccRCC.

In addition to examining protein-cancer risk associations, we also assessed the predictive capability of high performing protein markers in Group 2, or patients who later developed ccRCC, using AUC metrics. This analysis confirmed HAVCR1 as a top performer, demonstrating the highest predictive power (AUC 0.8756; SN 0.8108; SP 0.7798). By demonstrating the highest sensitivity among all other protein biomarkers, HAVCR1 has the potential as a marker for early detection of ccRCC. By contrast, NCR3LG1 (SP 0.9325) and PG (SP 0.9475) exhibited the strongest specificities in our cohort, identifying them as ideal candidates for confirmation of ccRCC in patients suspected to have the disease.

In our group of all ccRCC patients, NCR3LG1 showed the greatest promise as a prognostic marker with a hazard ratio of 2.6, and decreasing survival probability with increasing protein concentration. Kaplan-Meier analysis showed that PGF exhibited a statistically significant decrease

in survival ( $p < 0.006$ ) with higher protein concentrations, highlighting its potential as a prognostic marker in disease surveillance for ccRCC patients. Other proteins, including HAVCR1, GDF15, and NCR3LG1, showed trends toward decreased survival, but none reached statistical significance. This suggests that while these proteins might indicate a negative prognosis, further investigation is required to confirm their role in treatment outcomes.

The biological functions of these proteins solidify their potential use in diagnosis and prognosis of ccRCC, as several of these proteins have already been implicated in RCC carcinogenesis. HAVCR1 (Hepatitis A Virus Cellular Receptor 1), alternatively known as KIM-1 (Kidney Injury Molecule 1), is a transmembrane receptor that is induced in response to kidney injury. It activates the IL-6/STAT-3 pathway, known to drive tumorigenesis by stimulating proliferation, immune evasion, and metastasis. Overexpression of HAVCR1 has been linked to greater tumor aggressiveness in RCC, supporting its potential as a prognostic marker<sup>5,6</sup>. Previous studies show that increased HAVCR1 expression correlates with higher IL-6 levels, a known marker for renal malignancy.<sup>7,8</sup> Additionally, studies have suggested that elevated HAVCR1/KIM-1 pre-nephrectomy is associated with worse outcomes, such as metastasis-free survival (MFS) and overall survival (OS) and that higher HAVCR1/KIM-1 levels after treatment with nivolumab and

ipilimumab correlated with lower OS<sup>9,10</sup>. These findings are consistent with our own, which show strong associations with elevated HAVCR1 with development of ccRCC, and the risk of decreased survival. However, contradictory findings by Lee *et al.* suggest a possible positive prognostic role for HAVCR1 in some cases<sup>11</sup>.

NCR3LG1 (Natural Cytotoxicity Triggering Receptor 3 Ligand 1), a ligand for Nkp30 on natural killer cells, has been linked to immune evasion in RCC. Altered NCR3 splice variants contribute to deficient NK cell function, enabling tumor cells to evade immune surveillance.<sup>12</sup> PGF (Placental Growth Factor), a member of the VEGF family, plays a key role in angiogenesis. Elevated PGF levels are associated with higher RCC tumor grades and increased vascularity, making it a promising prognostic marker and therapeutic target<sup>13</sup>.

Similarly, GDF15 (Growth Differentiation Factor 15), a member of the TGF $\beta$  family, is implicated in carcinogenesis, promoting tumor progression and treatment resistance in various cancers<sup>14–16</sup>. However, a recent study by Yang *et al.* found decreased GDF15 in ccRCC, challenging its role as a prognostic factor.<sup>17</sup> In contrast, GDF15 elevation in our analysis was linked to poor prognosis, suggesting a complex role in RCC progression.

REN (Renin), typically involved in blood pressure regulation, may influence RCC indirectly through the renin-angiotensin system (RAS). In fact, a study by Nuzzo *et al.* found that combining RAS inhibitors with immune checkpoint inhibitors improved OS in metastat-

ic RCC patients, suggesting a potential therapeutic role for REN in RCC management<sup>18</sup>.

Our findings highlight the potential of HAVCR1, PGF, NCR3LG1, and REN as emerging biomarkers for both the early detection and prognosis of ccRCC in line with many previous studies mentioned above. HAVCR1, in particular, demonstrated the strongest predictive power, with high sensitivity for early detection and strong associations with ccRCC. PGF and NCR3LG1 exhibited strong prognostic potential, making them ideal candidates for confirming ccRCC diagnoses or progression in suspected patients or guiding risk based treatment strategies. These proteins, especially when considered together in a multi-biomarker panel, may offer more robust diagnostic and prognostic capabilities than relying on individual markers alone. While further research is needed to validate these findings in larger cohorts, these biomarkers offer promise for improving ccRCC diagnosis, patient stratification, and monitoring of disease outcomes.

## LIMITATIONS

Our study's findings may not be generalizable to non-European populations due to the UKBB's demographic composition. Additionally, treatment information following diagnosis of ccRCC was also limited which in turn limited the granularity of the study. Finally, while we adjusted for common covariates, residual confounding factors, such as other medical conditions could still affect the results. Future studies with larger sample sizes and longer follow-up are needed to confirm the clinical utility of these biomarkers.

## FUTURE DIRECTIONS

Future research could expand the proteomic panel and integrate complementary modalities, such as genomics and radiomics, to refine RCC risk prediction models. These biomarkers could eventually be incorporated into multi-institutional studies and risk prediction tools for targeted screening. However, validation in independent cohorts will be crucial before clinical implementation.

## CONCLUSION

Our study identifies several promising serum biomarkers—HAVCR1, PGF, and NCR3LG1—associated with both early detection and prognosis of ccRCC. Notably, HAVCR1 exhibits strong potential for early detection, while PGF and NCR3LG1 demonstrate significant prognostic value in diagnosed patients. Further validation in larger cohorts is necessary to confirm these findings and evaluate their clinical utility across diverse populations.

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# Hypoxia-Induced Immunosuppression in Kidney Carcinoma Cells

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## ABSTRACT

### BACKGROUND:

Clear cell renal cell carcinoma (ccRCC) is the most prevalent subtype of renal cell carcinoma and accounts for 70-80% of all kidney tumors. It originates in the proximal convoluted tubules in the nephron and is characterized by cells with clear cytoplasm due to large intracellular lipid droplets and free cholesterol. The current study examined the impact of hypoxia on kidney carcinoma cells 786-0.

**MATERIAL AND METHODS:** A publicly available dataset GSE225253 was analyzed, which contains RNA-seq transcriptomic data of kidney carcinoma cells 786-0 subject to normoxic and hypoxic conditions. Differential gene expression (DGE) analysis and gene ontology (GO) enrichment analysis were performed by R packages.

**RESULTS:** Following hypoxia, 2366 genes were upregulated and 1799 were downregulated in 786-0 cells (FDR < 0.05). GO enrichment analysis revealed key upregulated pathways including upregulation of cell adhesion, gland development, and monosaccharide metabolic process, and downregulated pathways including ribonucleoprotein complex biogenesis, RNA splicing, and ribosome biogenesis. Hypoxia induced higher expression levels of growth factors (IGF2, CSF1, CSF2, TGFB1, TGFB2, VEGFA), inflammatory chemokines (CCL2, CXCL16) and cytokines (IL12A, IL32), and immune checkpoint ligands and receptors (CD274, PDCD1LG2, CD276, TNFRSF9, ADORA2A, TNFRSF14) in 786-0 cells.

**CONCLUSION:** Hypoxia induced significant gene expression changes in kidney carcinoma cells, enhancing growth factor signaling, inflammation, immune checkpoint expression, and angiogenesis. These changes likely contribute to a tumor-supportive microenvironment, promoting cancer cell survival, proliferation, and immune evasion. Further in vitro and ex vivo studies are warranted to validate these findings and explore potential therapeutic strategies targeting hypoxia-induced pathways in ccRCC. in body composition features are associated with survival.

**KEYWORDS:** RNA-seq, growth factor, cytokine, chemokine, immune checkpoint, immune evasion

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## INTRODUCTION

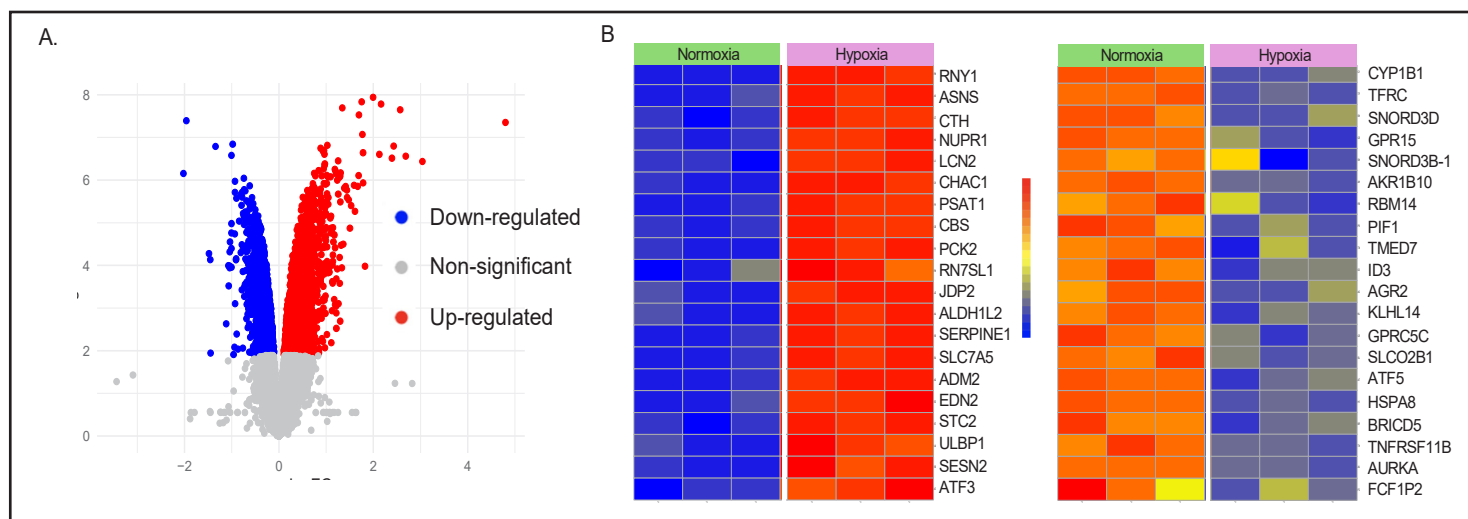
**C**lear cell renal cell carcinoma (ccRCC) is the most prevalent subtype of renal cell carcinoma (RCC) and accounts for 70-80% of all kidney tumors<sup>1</sup>. This tumor contains cells with a clear, bubble-like cytoplasm filled with large intracellular lipid droplets and free cholesterol<sup>1</sup>. The cut surface, which contains areas of hemorrhage and necrosis, can appear yellow or orange from lipid accumulation<sup>1</sup>. Despite its aggressive nature, ccRCC remains asymptomatic during early stages, and 60% of tumors are detected incidentally while conducting routine imaging tests<sup>2</sup>. The traditional symptoms of haematuria, flank pain, and abdominal mass only present in 10-15% of cases<sup>1</sup>, and

25-30% of patients diagnosed with ccRCC present with metastatic disease when diagnosed<sup>2</sup>.

Understanding the mechanisms driving ccRCC progression is essential for developing therapeutic interventions. Genetic studies have shown that the majority of sporadic ccRCC cases (~90%) have the loss of function in chromosome 3p, which houses a few tumor suppressor genes including VHL (von Hippel-Lindau), PBRM1 (PPARGC1 And ESRR Induced Regulator, Muscle 1), SETD2 (SET domain containing 2) and BAP1 (BRCA-associated protein-1)<sup>3</sup>. Unlike the other three genes PBRM1, SETD2, and BAP1, which promote tumor growth by promoting tumor cell growth metabolism, pVHL is a tumor suppressor gene that promotes angiogenesis metabolism<sup>4</sup>. Loss of pVHL function promotes tumorigenesis through the

dysregulation of the  $\alpha$ -subunits of hypoxia-inducible factors (HIFs), particularly HIF-1 $\alpha$  and HIF-2 $\alpha$ <sup>5</sup>. In normoxic conditions, hydroxylation of HIFs occurs by an oxygen-dependent enzyme known as prolyl hydroxylases (PHDs)<sup>6</sup>. This process triggers binding of pVHL to the HIF, leading to ubiquitination and proteasomal degradation of HIFs<sup>6</sup>. However, in hypoxic conditions, hydroxylation is suppressed, and HIFs stabilize and translocate into the nucleus<sup>6</sup>. HIFs bind to hypoxia-response elements inside the nucleus, activating several genes involved in angiogenesis, glycolysis, and erythropoiesis<sup>7</sup>. These studies indicate hypoxia may synergize with VHL mutation to accelerate the ccRCC tumor growth.

This study investigated the impact of hypoxia on gene expressions of kidney carcinoma cell 786-o compared with normoxia condition.



**Figure 1. A volcano plot and heatmaps showed the DEGs of 786-o cells in hypoxia compared with normoxia.** (A) A volcano plot of the DEGs. The x-axis represents the log<sub>2</sub> fold change (logFC) in gene expression, with positive values indicating upregulation and negative values indicating downregulation in the hypoxia-treated group compared with the normoxia group. The y-axis represents the -log<sub>10</sub> of the p-value, with higher values indicating greater statistical significance. Upregulated genes (logFC > 0 and FDR < 0.05) are shown in red, downregulated genes (logFC < 0 and FDR < 0.05) are shown in blue, and insignificant genes are shown in gray (FDR ≥ 0.01). (B.) The heat maps displayed the expression levels of top 20 up- (left) and downregulated (right) genes in hypoxia compared to normoxia. The color bar represents expression levels, with red indicating higher and blue indicating lower levels of expression. Each row represents a gene.

Dysregulated genes and pathways identified herein suggest their important involvements in the hypoxic tumor microenvironment.

## METHODS AND MATERIALS

### Dataset

Dataset GSE225253 was downloaded from NCBI (<https://www.ncbi.nlm.nih.gov/geo/>).

786-o kidney cells were cultured in normoxic and hypoxic conditions to investigate the influence of hypoxia on the gene expression of 786-o cells. Total RNA samples were collected, and the purities of RNA samples were assessed with a NanoPhotometer spectrophotometer. RNA libraries were made and sequenced with Illumina<sup>8</sup>.

## Bulk RNA-seq data analysis

Filtering and a log<sub>2</sub> transformation were applied to the Fragments Per Kilobase of transcript per Million mapped reads (FPKM) data. A linear fit model was implemented using limma. A data frame of differentially expressed genes was extracted from the model adjusted with the Benjamini-Hochberg method. All genes with  $|\log_2FC|$  (Log<sub>2</sub>Fold change) > 0 and FDR < 0.05 were considered significantly differentially expressed genes (DFGs), and all genes with FDR ≥ 0.05 were considered insignificant<sup>9</sup>. Gene Ontology (GO) enrichment analysis was conducted using clusterProfiler (q-value < 0.05). Three ontology aspects of GO were run: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF)<sup>10</sup>.

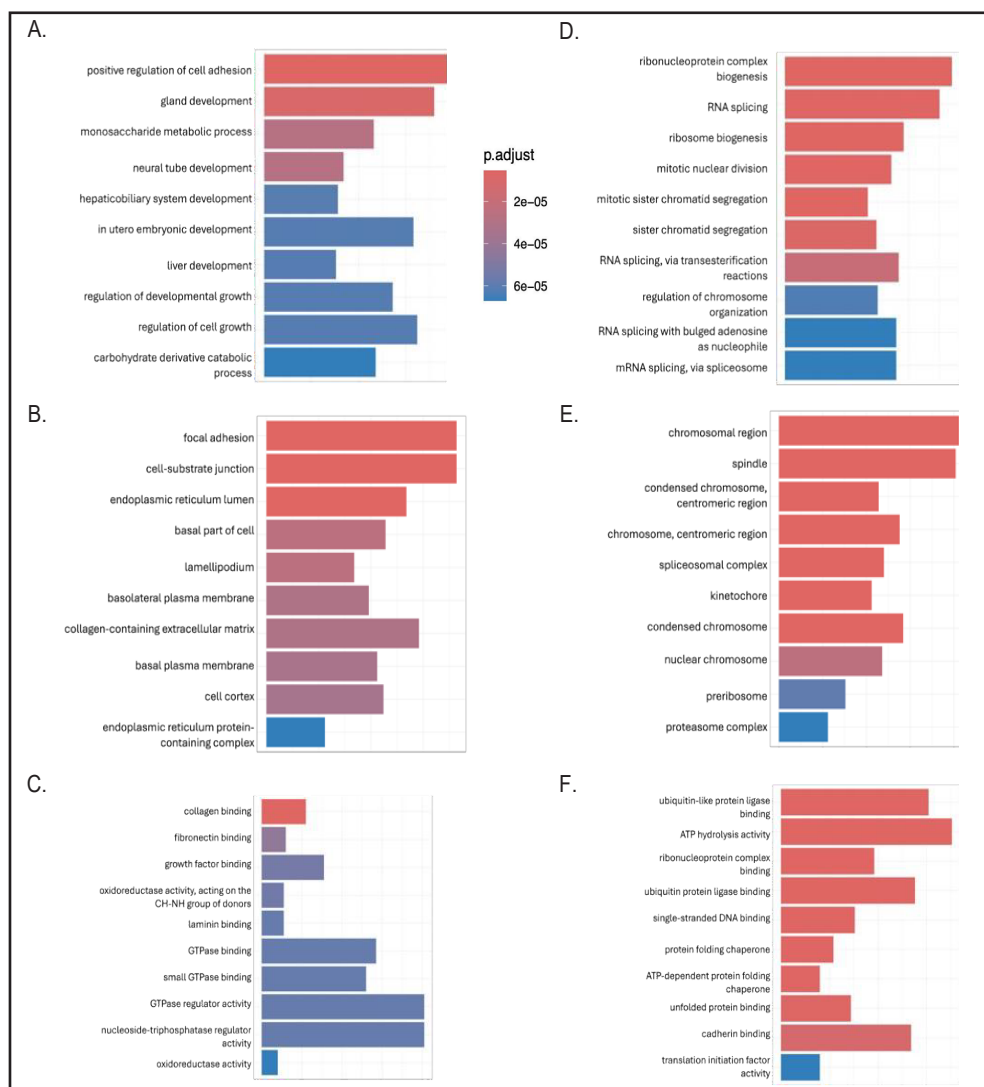
## STATISTICAL ANALYSIS

For single comparisons of RNA-seq genes presented in bar graphs, a student t test was used to assess statistical significance. N.S, P ≥ 0.05, \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.

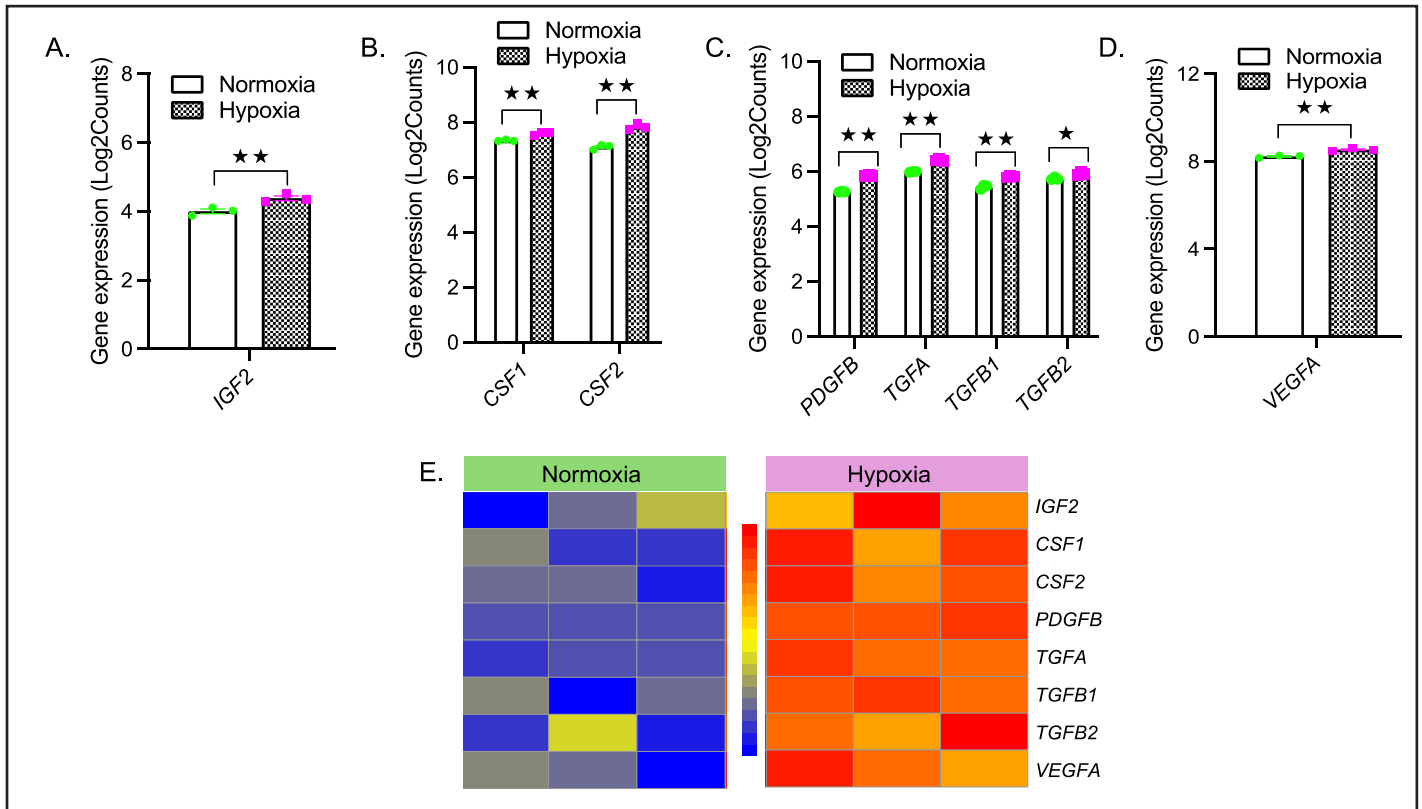
## RESULTS

### 1. Transcriptional reprogramming in hypoxia

Analysis of the dataset GSE225253, which contains RNA-seq data of hypoxia-treated and normoxia-treated kidney carcinoma 786-o cells, revealed significant transcriptional reprogramming in 786-o cells under hypoxia conditions. A total of 2366 genes were upregulated, and 1799 were downregulated (FDR < 0.05). A volcano plot visualized the significantly upregulated (red) and downregulated genes (blue) (Figure 1A). The heatmaps analysis showed the top 20 up- (left) and



**Figure 2. Gene ontology analysis revealed up- and downregulated pathways in hypoxia.** (A, D) Bar plots of top 10 up- (A) and downregulated (D) BP pathways in hypoxia. (B, E) Bar plots of top 10 up- (B) and downregulated (E) CC pathways in hypoxia. (C, F) Bar plots of top 10 up- (C) and downregulated (F) MF pathways in hypoxia.



**Figure 3. Hypoxia induced significantly higher expression levels of growth factors.** For all panels, N.S,  $P \geq 0.05$ , \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . (A) Hypoxia induced significantly higher expression levels of growth factor IGF2. (B) Hypoxia induced significantly higher expression levels of myeloid lineage growth factors CSF1 and CSF2. (C) Hypoxia induced significantly higher expression levels of fibroblast growth factors PDGFB, TGFA, TGFB1, and TGFB2. (D) Hypoxia induced significantly higher expression levels of vascular growth factor VEGFA. (E) The heatmaps of the significant upregulated growth factors in hypoxia (right) and normoxia (left).

downregulated (right) genes in hypoxia-treated 786-o cells compared with normoxia-treated 786-o cells (Figure 1B).

## 2. Dysregulated pathways in hypoxia

GO Biological Process (BP) analysis identified key upregulated pathways, including cell adhesion, gland development, monosaccharide metabolic process, neural tube development, hepaticobiliary system development, in utero embryonic development, liver development, regulation of developmental growth, regulation

of cell growth, and carbohydrate derivative catabolic process (Figure 2A). Cellular Component (CC) analysis revealed key upregulated pathways, including focal adhesion, cell-substrate junction, endoplasmic reticulum lumen, basal part of cell, lamellipodium, basolateral plasma membrane, collagen-containing extracellular matrix, basal plasma membrane, cell cortex, and endoplasmic reticulum protein-containing complex (Figure 2B). Molecular Function (MF) analysis identified key upregulated pathways, including collagen binding, fibronectin binding, growth factor binding, oxidoreductase

activity acting on the CH-NH group of donors, laminin binding, GTPase binding, small GTPase binding, GTPase regulator activity, nucleoside-triphosphatase regulator activity, and oxidoreductase activity acting on the CH-NH group of donors with NAD or NADP as acceptor (Figure 2C).

GO BP analysis also identified downregulated key pathways, including ribonucleoprotein complex biogenesis, RNA splicing, ribosome biogenesis, mitotic nuclear division, mitotic sister chromatid segregation, sister chromatid segregation, RNA



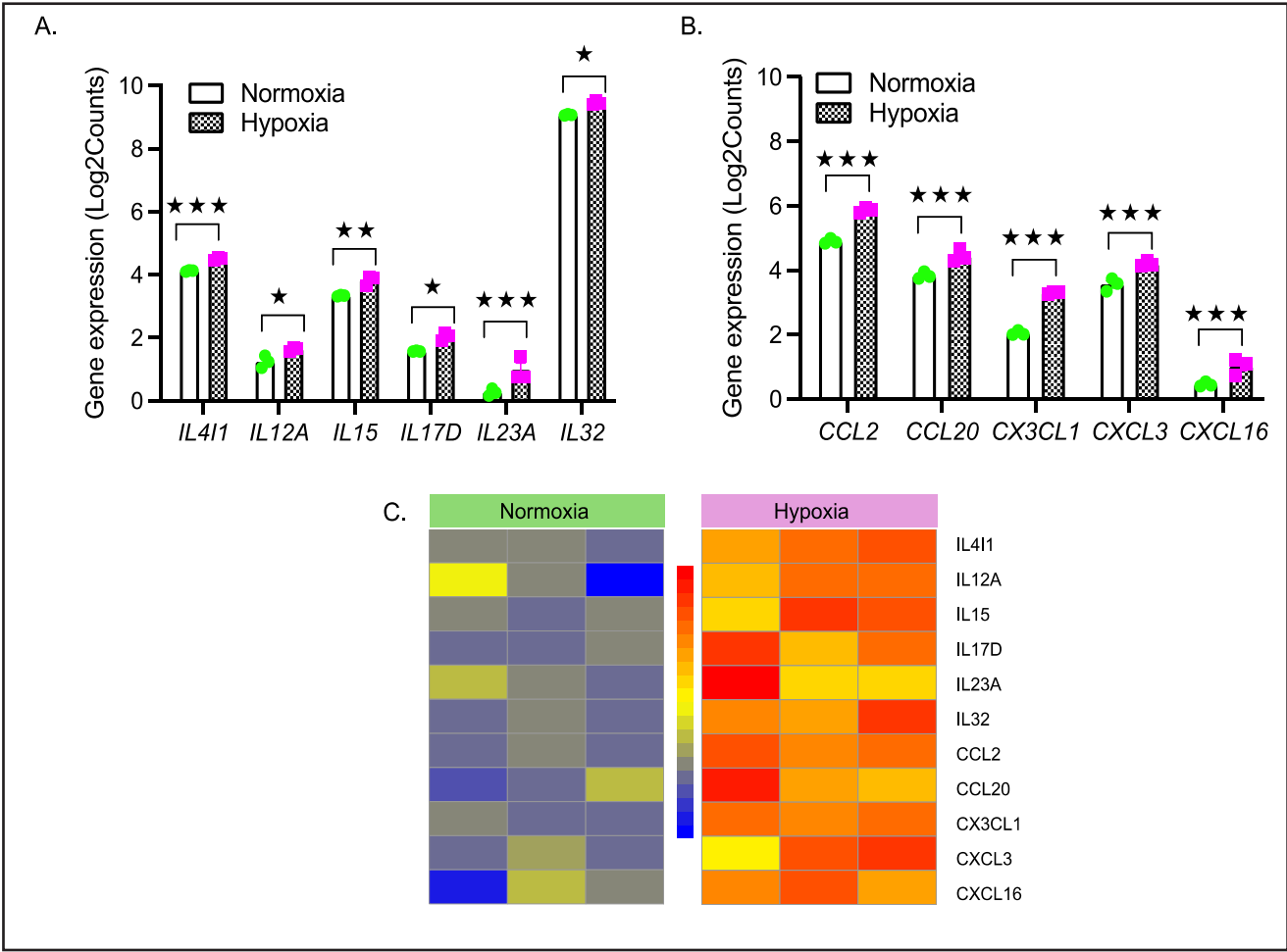
splicing via transesterification reactions, regulation of chromosome organization, RNA splicing via transesterification reactions with bulged adenosine as nucleophile, and mRNA splicing via spliceosome (Figure 2D). CC analysis revealed key downregulation of several pathways including chromosomal region, spindle, condensed chromosome centromeric region, chromosome centromeric region, spliceosomal complex, kinetochore, nuclear chromosome, preribosome, and proteasome complex (Figure 2E). MF analysis identified key downregulated pathways

including ubiquitin-like protein ligase binding, ATP hydrolysis activity, ribonucleoprotein complex binding, ubiquitin protein ligase binding, single-stranded DNA binding, protein folding chaperone, ATP-dependent protein folding chaperone, unfolded protein binding, cadherin binding, and translation initiation factor activity (Figure 2F).

### 3. Upregulation of growth factors induced by hypoxia

Under hypoxic conditions, kidney carcinoma cell line 786-o exhibited significant upregulation of several

growth factors, which play critical roles in tumor progression and the modulation of the tumor microenvironment (TME). Among these, insulin-like growth factor 2 (IGF2) was notably increased compared to normoxia (Figure 3A). IGF2 is primarily secreted by stromal cells in normal physiology, where it regulates metabolism, proliferation, survival, and differentiation across various cell types<sup>11</sup>. In the context of cancer, however, IGF2 plays a pivotal role in driving cancer stem-like cell transformation, which enhances tumorigenicity and resistance to



**Figure 4. Hypoxia induced significantly higher expression levels of proinflammatory cytokines (A) and chemokines (B).** For all panels, N.S,  $P \geq 0.05$ , \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . (A) Cytokines IL4I1, IL12A, IL15, IL17D, IL23A, IL32. (B) Chemokines CCL2, CCL20, CX3CL1, CXCL16, CXCL3. (C) The heatmaps of up-regulated cytokines and chemokines under hypoxic conditions

therapies<sup>12</sup>. The elevated expression of IGF2 in hypoxic 786-o cells suggested its potential contribution to promoting stem-like cell formation, thereby conferring a survival and proliferative advantage within the hypoxic TME.

In addition to IGF2, hypoxia significantly increased the expression of myeloid lineage growth factors CSF1 and CSF2 (Figure 3B, E). These factors are instrumental in recruiting monocytes to the TME, a hallmark of tumor-driven inflammation. Once recruited, CSF1 and CSF2 promote the proliferation of undifferentiated Mo macrophages and their subsequent polarization to the M1 phenotype, which enhances local inflammation. This inflammatory milieu can paradoxically facilitate

tumor progression by modifying the TME to support cancer cell survival and immune evasion.

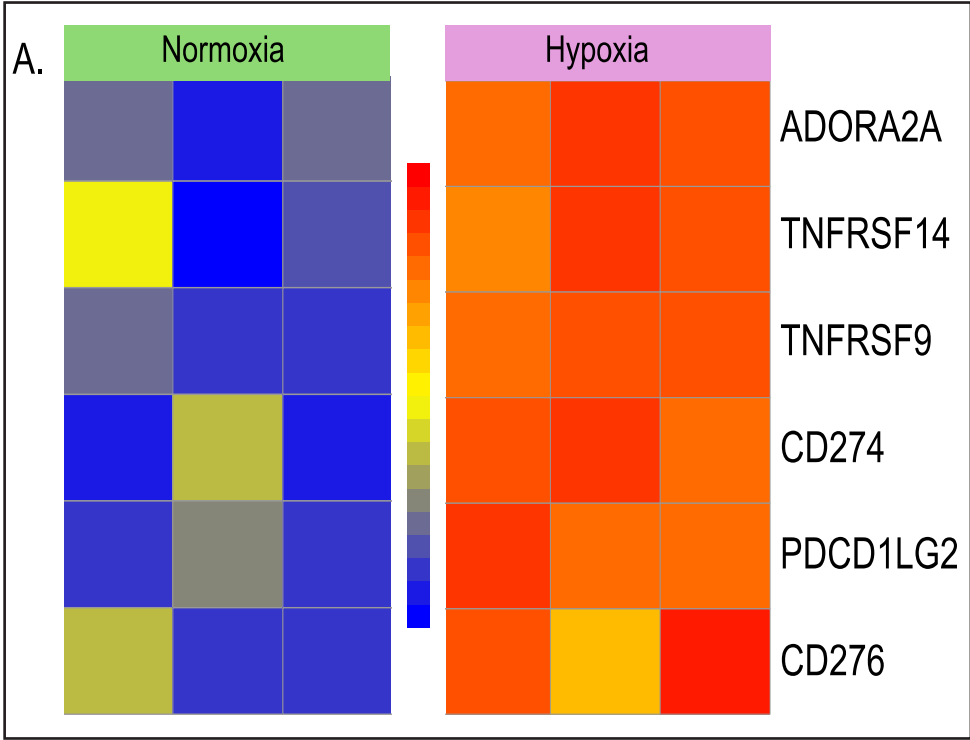
Hypoxia led to the upregulation of fibrotic-promoting growth factors, including PDGFB, TGFA, TGFB1, and TGFB2 in kidney carcinoma cell line 786-o (Figure 3C, E). PDGFB, for instance, is critical for the recruitment of cancer-associated fibroblasts (CAFs) and the deposition of extracellular matrix (ECM) components in the TME<sup>13</sup>. This ECM remodeling creates a supportive niche that fosters tumor growth and progression. TGFA, an epidermal growth factor receptor (EGFR) ligand, further contributes to tumor proliferation by acting as a potent mitogenic signal<sup>14</sup>. TGFB1 and TGFB2 play a dual role in

cancer: during the early stages of tumorigenesis, they act as tumor suppressors by inducing apoptosis and inhibiting cell cycle progression in normal and premalignant cells, whereas in later stages, they transition to tumor promoters, driving epithelial-mesenchymal transition (EMT), which enhances metastasis and promotes resistance to chemotherapy<sup>15</sup>.

A notable aspect of hypoxia-driven tumor progression observed in this study is the upregulation of vascular endothelial growth factor A (VEGFA) (Figure 3D, E). VEGFA is a well-known angiogenic factor that plays an indispensable role in cancer development by promoting the formation of new blood vessels. This ensures an adequate supply of oxygen and nutrients to proliferating tumor cells, while also contributing to the creation of an abnormal vasculature that perpetuates hypoxia in the TME<sup>16</sup>. By enhancing angiogenesis, VEGFA facilitates tumor growth and metastasis, establishing a cycle of hypoxia and tumor progression.

#### 4. Hypoxia-induced regulation of proinflammatory cytokines and chemokines

Hypoxia led to a significant upregulation of proinflammatory cytokines, including IL4I1, IL12A, IL15, IL17D, IL23A, and IL32, in kidney carcinoma cells (Figure 4A). These cytokines play pivotal roles in modulating the TME. Specifically, cytokines such as IL12A, IL15, IL17D, IL23A, and IL32 have been shown to influence regulatory T-cell (Treg) differentiation, promoting an immunosuppressive TME that favors tumor progression. By enhancing the recruitment and differentiation of Tregs, hypoxia promotes an inhibitory TME that shields tumor cells from immune



**Figure 5. Hypoxia induced higher expression levels of immune checkpoint receptors and ligands.** (A) Hypoxia induced higher expression levels of stimulated immune checkpoint receptors ADORA2A, TNFRSF14. (B) Hypoxia induced higher expression levels of stimulating and inhibitory immune checkpoint ligands TNFSF9, CD274, PDCD1LG2, CD276.

attack. This environment not only supports immune evasion but also facilitates tumor growth and survival in adverse conditions.

In addition to cytokines, hypoxia significantly increased the expression of chemokines such as CCL2, CCL20, CX3CL1, CXCL3, and CXCL16 (Figure 4B). Each of these chemokines plays distinct roles in promoting tumor progression and shaping the TME. For example, CCL2 is known to directly enhance cancer cell proliferation, migration, and invasion. Furthermore, it recruits monocytes and tumor-associated macrophages (TAMs) to the tumor site, where TAMs contribute to an immunosuppressive and tumor-supportive niche<sup>17</sup>. Similarly, CCL20 facilitates angiogenesis by recruiting endothelial cells to the tumor site, thereby promoting new blood vessel formation and enhancing the nutrient supply to the tumor<sup>18</sup>.

The upregulation of CXCL16 and CXCL3 implicates their role in linking tumor stromal cells with cancer cells, facilitating communication and promoting tumor growth. These chemokines contribute to the remodeling of the TME, fostering conditions that support cancer cell survival and proliferation<sup>19,20</sup>. By bridging interactions between stromal and tumor cells, they amplify signals that sustain tumor expansion and metastatic potential.

## 5. Hypoxia-induced upregulation of immune checkpoint receptors and ligands

Under hypoxic conditions, kidney carcinoma cell line 786-o exhibited a significant upregulation of immune checkpoint molecules, including both stimulatory receptors (ADORA2A and TNFRSF14), and stimulation and inhibitory ligands (TNFSF9, CD274, PDCD1LG2, and CD276) (Figure 5). These

immune checkpoints are critical regulators of the tumor immune microenvironment and play a dual role in modulating immune responses, often tipping the balance toward immune evasion and tumor progression.

The upregulations of stimulatory immune checkpoint receptors such as ADORA2A and TNFRSF14 were observed in hypoxia-treated 786-o cells. ADORA2A plays a key role in suppressing adaptive immune responses by modulating T cell activity. Through this mechanism, increased ADORA2A expression allows cancer cells to evade immune targeting, thereby promoting tumor growth and survival under hypoxic conditions<sup>21</sup>. Similarly, TNFRSF14 (also known as HVEM) functions as a receptor that can mediate both stimulatory and inhibitory signals within the immune system, depending on its interaction partners. Its upregulation under hypoxia suggests its involvement in shaping the immunosuppressive microenvironment.

TNFSF9, also known as CD137L, is another notable molecule whose expression was increased under hypoxia. This ligand interacts with its receptor, CD137, providing stimulatory signals that promote the proliferation, activation, and survival of CD137-positive immune cells. Although TNFSF9 has a stimulatory function, its precise role in the TME can vary depending on the cellular context and immune landscape, potentially contributing to tumor-associated immune regulation.

Among the inhibitory ligands, PD-L1 (CD274), PD-L2 (PDCD1LG2), and CD276 are well-documented immune checkpoint molecules expressed on tumor cells. These proteins inhibit immune responses, particularly the adaptive immune response targeting tumors, by interacting with corresponding

receptors on T cells, thereby suppressing their activation and cytotoxicity<sup>22</sup>. The overexpression of these inhibitory ligands in hypoxic 786-o cells suggests that they play a role in dampening anti-tumor immunity and facilitating immune escape.

## DISCUSSION

Through RNA-seq data analysis, this study revealed the changes in gene expression patterns of kidney carcinoma cell 786-o under hypoxia condition. Hypoxia-treated kidney carcinoma cell 786-o expressed higher levels of growth factors such as IGF2, CSF1, CSF2, PDGFB, TGFA, TGFB1, TGFB2, and particularly angiogenic growth factors VEGFA, as well as proinflammatory chemokines such as CCL2, CCL20, CX3CL1, CXCL3, CXCL16 and cytokines IL4I1, IL12A, IL15, IL17D, IL23A, IL32. Notably, hypoxia induced higher expression levels of immune checkpoint receptors and ligands including CD274, PDCD1LG2, CD276, TNFRSF9, ADORA2A and TNFRSF14.

The coordinated upregulation of these factors under hypoxia demonstrates the complex interplay of cellular processes that enable tumor cells to adapt and survive in challenging conditions. Growth factors such as VEGFA play a pivotal role in tumor progression by promoting angiogenesis, which in turn ensures a steady supply of oxygen and nutrients to the tumor, even in hypoxic regions. Proinflammatory cytokines and chemokines contribute to the formation of an inflammatory TME, which supports cancer cell proliferation, migration, and invasion. This inflammatory milieu, combined with angiogenesis, creates a favorable environment for tumor growth and metastasis.

Hypoxia also drives the upregulation of genes associated with immune evasion. The



increased expression of immune checkpoint ligands, including CD274, PDCD1LG2, and CD276, suppresses T cell activation, allowing tumor cells to evade immune detection. The upregulation of stimulatory molecules such as TNFSF9 and receptors such as ADORA2A further modulates the TME by promoting immune cell proliferation and regulating T cell responses, often tipping the balance toward immune suppression. These changes collectively establish an immunosuppressive microenvironment that facilitates tumor survival and progression.

Hypoxia, a common hallmark of cancer, is particularly prominent in ccRCC due to mutations in the VHL gene, which destabilize the HIF pathway. The dysregulated rapid growth of tumor cells creates imbalances in blood vessel formation, leading to localized oxygen deficiency. This hypoxic state drives critical processes such as angiogenesis, EMT, and immune evasion, further exacerbating tumor aggressiveness.

The hypoxic TME generated by 786-o cells is characterized by increased tumor cell proliferation, chronic inflammation driven by cytokine and chemokine upregulation, and immune suppression through enhanced checkpoint gene expression. Understanding these mechanisms should provide important insights into potential therapeutic targets. For example, targeting hypoxia-responsive pathways, such as angiogenesis inhibitors or immune checkpoint blockade therapies, could disrupt the tumor-supportive TME and enhance treatment efficacy in ccRCC.

## CONCLUSION

In summary, hypoxia induces extensive transcriptional reprogramming in kidney carcinoma cells, including upregulation of growth factors,

angiogenic factors, proinflammatory cytokines, chemokines, and immune checkpoint molecules. These changes enable tumor cells to survive, evade immune detection, and adapt to hypoxic stress, contributing to tumor progression, metastasis, and recurrence. Further studies on hypoxia-induced mechanisms in the TME could lead to novel therapeutic strategies that improve outcomes for patients with ccRCC.

## DECLARATION OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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# Quality improvement in treatment of patients with Renal Cell Cancer in Denmark. Overview and results from The DARENCA register

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## ABSTRACT

### BACKGROUND

The incidence of Renal Cell Cancer (RCC) is on the rise globally. In the period 1994-2003, the relative five-year survival after RCC in Denmark was 39 percent for men and 44 percent for women. We aimed to describe whether the establishment of a nationwide clinical quality database for RCC was followed by improved care and survival.

### PURPOSE

The purpose of the nationwide quality register in Denmark is to standardize treatment and improve prognosis by documenting the quality of healthcare treatment and contribute to improving prevention, diagnostics, treatment and rehabilitation

### MATERIAL AND METHOD

In Denmark it is mandatory for all departments treating RCC to report relevant quality data on all patients. Every Danish resident has a unique Civil Registration (CPR) number which allow linkage to the national patient register and the pathology register. These registers contain information about all examinations and treatments in Danish hospitals. In a national steering group of the clinical register for RCC, different quality indicators have been selected for monitoring the quality of the treatment. The group chose nine indicators to be measured each year. The group meet several times a year to prepare an annual quality report, which is published and sent to hospitals, healthcare regions and the Danish Health Authority.

### RESULTS

The register was established 1 August 2010 and contains 12.193 new cases of RCC, including 1 091 from the current year (the period 1 August 2022 to 31 July 2023). Over the years, a clear improvement has been observed both nationally and differences between regions and hospital departments decreased and thereby the equality of treatment and outcomes in the country has improved.

### CONCLUSION

Through systematic work with quality results, interdisciplinary collaboration and the introduction of new treatments, we have observed a uniform treatment and increased survival in patients with renal cancer, which is at an international level.

**KEYWORDS:** Renal Cancer, survival, register, clinical quality

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INTRODUCTION

In Denmark, approximately 80 approved nationwide clinical quality registers support the Danish healthcare system with data analysis and reports to secure development and improvement of the clinical quality. These registers typically cover all patients with a specific disease or those undergoing a specific procedure and includes 27 clinical quality registries in cancer care and cancer screening in collaboration with the Danish Multidisciplinary Cancer Group.<sup>1,2</sup> The registers are operated by the Danish Healthcare Quality Institute (DHQI) (formerly the *Regional Clinical Quality Development Program*)<sup>1</sup>, which contributes to the daily operation, analysis and reporting. To obtain approval and public funding, the

program requires that a quality register covers at least 90% of the Danish patients with a disease or within a clinical area.<sup>3</sup>

For each register, a professional steering group has been established to determine what the content should be – including the individual quality indicators and quality targets goals). DMCG.dk was founded in 2004 and is the association of Danish Multidisciplinary Cancer Groups, whose main task is to promote cancer treatment in Denmark.<sup>4</sup> There are 25 DMCGs that focus on, among other things, supporting clinical quality registers and preparing clinical guidelines for diagnostics and treatment (Figure 1).

Clinical quality development and indicator sets in the clinical quality registers

The primary purpose of the clinical quality register is to monitor the quality of the healthcare system's efforts and results for a defined group

of patients in order to contribute to healthcare quality improvements. Registrations in these registers are mandatory for all the relevant clinical departments and service providers. Reporting to the quality register can be done without patient consent.<sup>5</sup> The first Danish clinical registers were established by enthusiastic clinicians who felt a need to get insights into the clinical profile of the patient population including the treatments they were offered and the clinical results.<sup>6</sup> The current focus is on supporting the development and improvement of high and uniform quality of care in the treatment process across the country based on international understandings of the concept of quality.<sup>7,8</sup> Quality can be viewed from a clinical, patient-experienced and organizational perspective. In the registers, the clinical perspective has been predominant, but work is done to ensure that the patient perspective will increase by i.e. including patients' representatives in the steering group. The overall

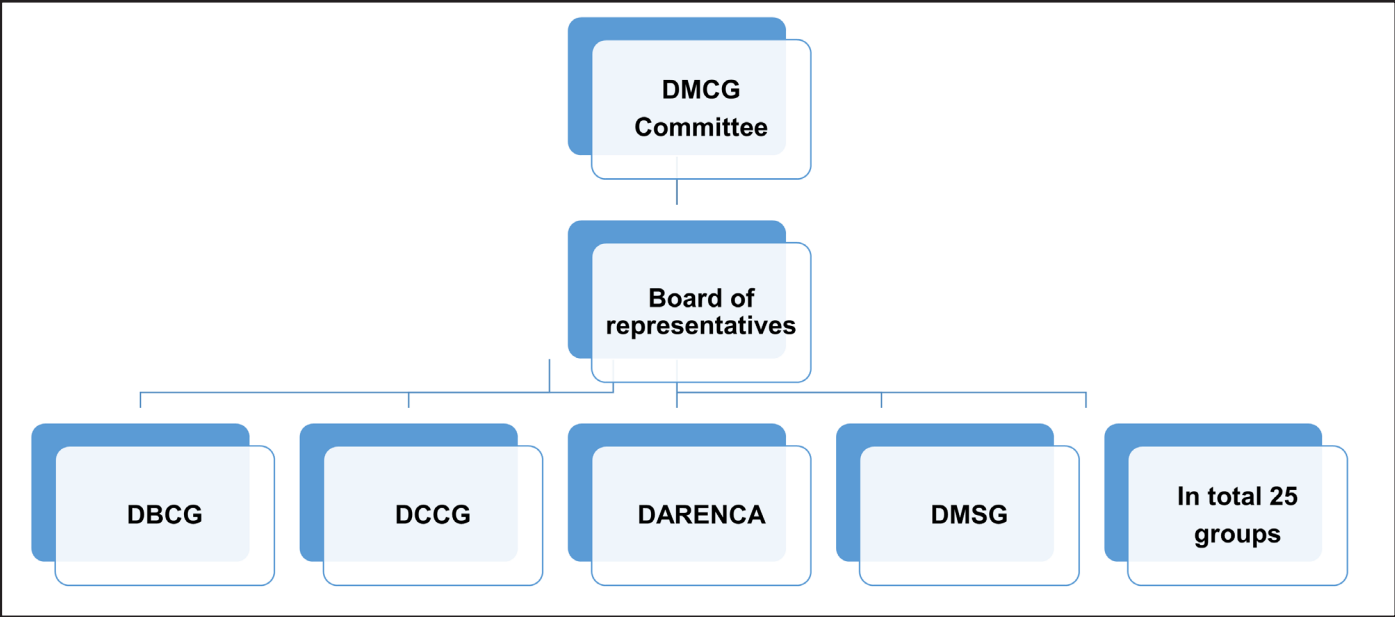


Figure 1. Danish Multidisciplinary Cancer Groups.

aim is to secure development and improvement of quality throughout the entire patient journey across the healthcare sectors (specialist practice, general practice, hospital, and municipality).

The clinical quality register allow for uniform national monitoring across the country. The target group and purpose of use are crucial for how frequently and how data should be analyzed and knowledge communicated. The overall thinking in the work is an improvement model with the following elements:

a) Improvement goals b) Establish measurement (indicator) c) Select change (intervention) d) Test change e) Assessment (audit). The efforts are focused on creating national consensus on quality and challenges in the current disease area, and on measuring, assessing and reporting results.

Darenca is the DMCG for kidney cancer. The group consist of 5 urologists, 3 oncologists, 1 clinical epidemiologist, 2 pathologists, 2 diagnostic imaging specialists, 1 clinical geneticist and 1 patient representative. The Darenca group

meet several times during the year to prepare an annual quality report, which is sent to all hospitals, hospital management, politicians and the Danish Health Authority. The annual report is finalized by DaRenCa members in collaboration with methodological experts from DHQI. The register DaRenCaData includes all persons with a first-time diagnosis of renal cancer in Denmark since August 2010.<sup>9</sup>

**The annual DaRenCa quality report**

The annual report includes two

INDICATOR AREA	INDICATOR	TYPE	STANDARD
Laparoscopic procedure	Indicator 1: Proportion of nephrectomy patients regardless of stage, who have been operated on with laparoscopic, including robot-assisted, technique	Proces	≥ 65 %
Re-operation/complications	Indicator 2: Proportion of surgically (operatively or ablatively) treated patients who are reoperated on or have complications within 30 days	Result	≤ 5 %
Recurrence	Indicator 3: Proportion of patients, surgically treated with curative intent, with recurrence within 3 years	Result	≤ 25 %
30 day mortality (after surgical treatment)	Indicator 4: Proportion of patients who die within 30 days after surgery or ablative procedure	Result	≤ 0,5 %
Survival after the diagnose	Indicator 5a: Proportion of patients who are alive 1 year after the diagnosis of kidney cancer is made	Result	≥ 80 %
	Indicator 5b: Proportion of patients who are alive 5 years after the diagnosis of kidney cancer is made	Result	≥ 60 %
Length of hospital stay	Indicator 6: Proportion of endoscopically or ablatively treated patients discharged within 3 days of the procedure	Result	≥ 85 %
Nefrectomy (T1a tumors)	Indicator 7: Proportion of patients with T1a tumors who undergo nephrectomy	Proces	≤10 %
SNOMED-coding	Indicator 8a: Proportion of patients who undergo nephrectomy or partial nephrectomy with coding of tumor size	Proces	≥ 95%
	Indicator 8b: Proportion of patients who undergo nephrectomy or partial nephrectomy with coding of pT stage	Proces	≥ 95%
Oncology	Indicator 9a: Proportion of patients with metastatic disease at diagnosis who initiate oncological treatment	Proces	Not determined
	Indicator 9b: Proportion of patients, surgically treated with curative intent, who develop metastatic disease who initiate oncological treatment	Proces	Not determinedat

Table 1. Characteristics of Indicator.

patient populations: A. Patients with newly diagnosed histologically or cytologically verified kidney cancer (Patient population 1) and B. All surgically treated patients with histologically verified kidney cancer (Patient population 2). Population 1 is based on pathological diagnoses recorded in the National Registry of Pathology (LRP), while Population 2 is based on data from both the Danish National Patient Register (DNRP) and the LRP.

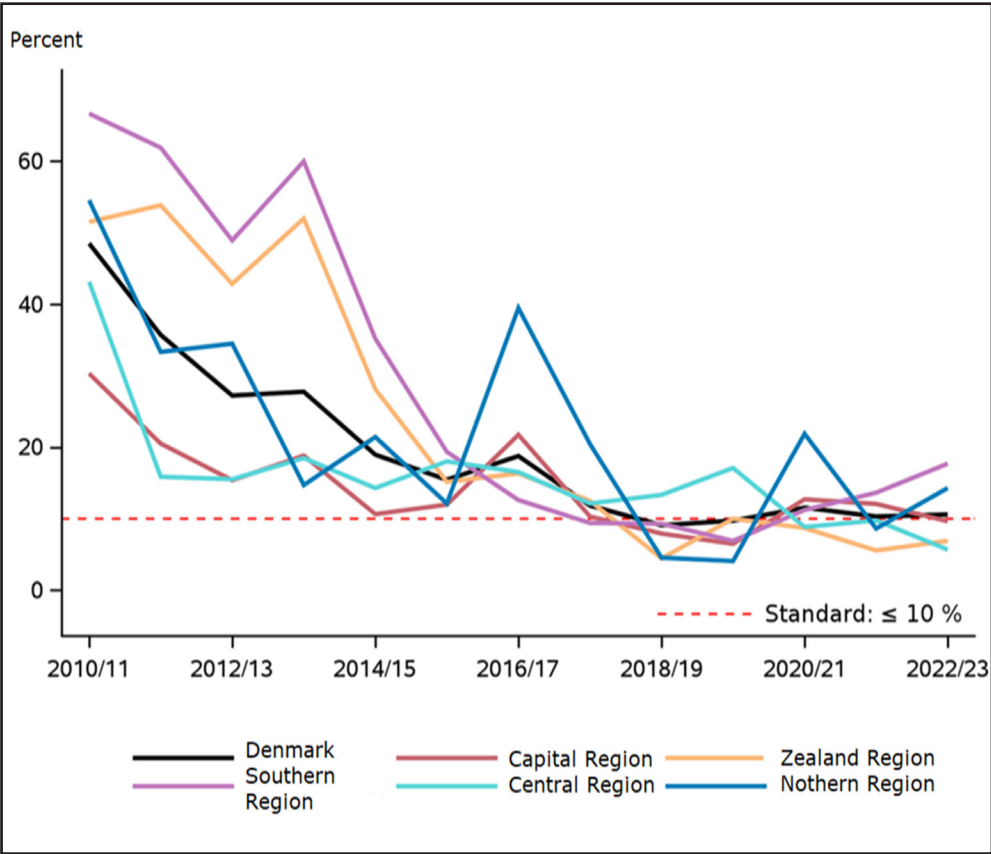
An overview of indicator set as published in 2024 is shown in table 1 and some results in table 2. At the end of 2023, the register included 12,193 new cases of kidney cancer in Denmark from its inception on 1 August 2010 to 31 July 2023, of which 1091 in the current year (the period 1 August 2022 to 31 July 2023).

Only patients with histologically or cytologically verified kidney cancer are included in the register. This is unique to Denmark, as patients in other countries registers are also included if the diagnosis is based solely on imaging.

Incidence rates of kidney cancer have shown an increasing trend in most countries over the past 10 years<sup>5</sup>, which is also the case in Denmark. In the 2023 report, the incidence rate was 17.3 per 100,000 inhabitants, and in 2022-23 it was 25.5 for men, increasing, and 10.3 per 100,000 for women, stable over time. The disease is most frequently diagnosed at the age of 60-70, with a median age of 69 years in the 2023 report and the distribution between men and women was constant, 69% and 31% respectively.

Survival after kidney cancer is increasing in Denmark. The observed 1-year survival was in 2023 90%, which indicated a slightly increasing trend compared to 89% and 88% in the 2 previous periods (Table 2). The observed 5-year survival was 68% compared to 70% and 65% in the 2 previous periods. The relative 5-year survival, which is the most often stated in international publications, was 75% for both men and women. In the period 1994-2003, the relative 5-year survival for kidney cancer in Denmark was 39% for men and 44% for women. Over the years, there has been a significant improvement in the survival after kidney cancer, as an outcome of different activities including publications from the clinical register and national audits of the results, systematic work with national treatment guidelines, as well as introduction of new treatments. A contributing factor to the improved survival also be the increase in the proportion of patients diagnosed at an early stage of the disease. However, at the same time, improved treatment options for patients with locally advanced and metastatic disease have meant that survival is also increasing for patients with stage III or IV disease.

The treatment of kidney cancer is primarily surgical, and surgical treatment continues to move towards an increased proportion of kidney-preserving procedures. The standards mentioned in table 1 and 2 are based in the current literature. During the current period, 931 patients were treated surgically (operation or ablation), and of these, 55% underwent kidney-preserving interventions. The vast majority of kidney cancer patients are now operated on using laparoscopic surgery. The standard was > 65%, but with some variation between



**Figure 2.** Proportion of nephrectomies over time for T1A tumors.



the five Danish regions (68-96%). The proportion of patients operated on using laparoscopic techniques has increased from 65 to 85 percent in the last five years alone. Nine out of ten patients operated on laparoscopically can be discharged within three days, and very few patients experience complications or require reoperation.

During the years, the chairperson in Darenca has an audit with the chairs of the individual departments if the report shows that there was an opportunity for improvement. This has shown to be essential because it clearly demonstrated that performing minimal invasive surgery was for the benefit of the patients.

Some departments performed most cases by open surgery and performed nephrectomy for T1A tumors. The chairs could see the advantage of this development because it gave the opportunity to get teams that were more robust, better equipment and facilities at the theater. From 2024 and onwards the indicator 1 has been changed so that the standard is now that > 85% of the surgeries should be performed robotic assisted or laparoscopically. This demonstrated how collaboration in the register has been a lever for the development of professional work - both locally and nationally.

There length of hospital stay after the procedures was short (< 3 days),

while the postoperative morbidity of 3% and the postoperative mortality of just over 0.5% within 30 days were stably low (Table 2). Morbidity and mortality were at an international level. 10 Among patients with small tumors (stage T1a), 11% underwent nephrectomy rather than kidney-conserving treatment demonstrating a decreasing proportion of nephrectomies over time (Figure 3). The result has become very close to the defined standard for this indicator and was uniform between the regions. This evolution was created in the same fashion as mentioned above.

Nephrectomies were performed laparoscopically incl. robot-assisted

INDICATOR	UNDISCLOSED		INDICATOR IFULFILLMENT		
	STANDARD	%	PROPORTION (95% CI) 01.08.2022 - 31.07.2023	PROPORTION 2021/22	PROPORTION 2020/21
Indicator 1a: Proportion of nephrectomy patients regardless of stage, who have been operated on with laparoscopic, including robot-assisted, technique	≥ 65	0	81 (77-84)	82	78
Indicator 2: Proportion of surgically (operatively or ablatively) treated patients who are reoperated on or have complications within 30 days	≤ 5	0	2 (1-4)	4	4
Indicator 3: Proportion of patients, surgically treated with curative intent, with recurrence within 3 years*	≤ 25	0	01.08.2019 - 31.07.2020 9 (7-11)	2018/19 11	2017/18 11
Indicator 4: Proportion of patients who die within 30 days after surgery or ablative procedure.	≤ 0,5	0	01.08.2022 - 31.07.2023 1 (0-1)	2021/22 1	2020/21 0
Indicator 5a: Proportion of patients who are alive 1 year after the diagnosis of kidney cancer is made *	≥ 80	0	01.08.2021 - 31.07.2022 90 (88-92)	2020/21 89	2019/20 88
Indicator 5b: Proportion of patients who are alive 5 years after the diagnosis of kidney cancer is made*	≥ 60	0	01.08.2017 - 31.07.2018 68 (65-71)	2016/17 70	2015/16 65
Indicator 6: Proportion of endoscopically or ablatively treated patients discharged within 3 days of the procedure.	≥ 85	0	01.08.2022 - 31.07.2023 90 (88-92)	2021/22 85	2020/21 87
Indicator 7: Proportion of patients with T1a tumors who undergo nephrectomy	≤ 10	0	11 (8-14)	10	12
Indicator 8a: : Proportion of patients who undergo nephrectomy or partial nephrectomy with coding of tumor size	≥ 95	0	98 (96-99)	98	98
Indicator 8b: Proportion of patients who undergo nephrectomy or partial nephrectomy with coding of pT stage	≥ 95	0	99 (98-99)	98	97
Indicator 9a: : Proportion of patients with metastatic disease at diagnosis who initiate oncological treatment*		0	01.08.2021 - 31.07.2022 89 (81-94)	2020/21 78	2019/20 78
Indicator 9b: Proportion of patients, surgically treated with curative intent, who develop metastatic disease who initiate oncological treatment*		0	68 (58-76)	77	80

**Table 2..** An overview of the indicator results. \* Time-lagged indicator, current year is moved back in time to allow for necessary follow-up time.

81% (last year 82%) with some variation between departments. The proportion of endoscopically or ablatively treated patients who were discharged within 3 days after the procedure is 90%, also with some variation between departments. A positive development has been seen nationally and in 4 of the 8 treating departments. Part of the report's data is drawn directly from the LRP, to which data from the country's pathology departments is automatically transferred. As in previous annual reports, there was a high overall data completeness for coding pT stage and tumor size. In 2023, 97% of all nephrectomies and partial nephrectomies/renal resections were registered with a pT stage with very low variation among pathology departments. Tumor size (tumor diameter) was reported for 98% of all nephrectomies/partial nephrectomies/renal resections, also with a small variation. In general, a great effort is being made to classify the histological subtype, which has prognostic and therapeutic significance, as only 5% were recorded as 'unclassifiable renal cell carcinoma', which is in line with international calculations.<sup>10</sup>

Recently, this annual report has compiled indicators describing the oncological treatment of kidney cancer, which show that 89% of newly diagnosed patients with metastatic disease who were seen at an oncological treatment facility receive oncological treatment. Work on improving the data quality on oncological treatment decisions is still being optimized.

The DaRenCa group has decided to have two new indicators starting from year 2024 report: Multidisciplinary team (MDT) meeting, and the use of pembrolizumab.

## CONCLUSION

Through systematic work with quality results, interdisciplinary collaboration and the introduction of new treatments, we have observed a uniform treatment and increased survival in patients with kidney cancer.

## Availability of data and Materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

## Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

## Conflicts of interest

All authors declare no conflicts of interest, financial or otherwise, related to this manuscript.

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## Contributions

All authors played a substantial role in conception, design, acquisition, analysis, interpretation, writing, and critical review of the manuscript. All authors approved the final content and accepted

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