

## Changing Landscapes in the Treatment of Advanced Renal Cell Carcinoma

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### INTRODUCTION

Renal Cell Carcinoma is a significant cause of worldwide mortality and morbidity. Globally, an estimated 338,000 new cases and 144,000 deaths occurred due to Renal Cell Carcinoma in 2012<sup>[1]</sup>. It is much more prevalent in regions such as North/Eastern Europe. In the US; incidence is approximately 62,000 cases per year with almost 14,000 deaths<sup>[2]</sup>. Caucasians and African Americans have a higher incidence in comparison to those of Asian or Pacific Islander descent<sup>[3]</sup>. Incidence of renal cell carcinoma continues to grow globally— perhaps driven by lifestyle risk factors such as smoking and increasing obesity<sup>[4]</sup>. The detection and diagnosis of incidentally found small renal masses is also showing a large increase, due to the availability of non-invasive imaging studies for other indications. Incidence of metastatic disease, which comprises almost a third of the total number of cases, also continues to increase.

While early stage Renal Cell Carcinoma usually have good outcomes, metastatic disease has been a challenging diagnosis, given its chemo-resistant nature. Outcomes have progressively improved over the past decade, and Renal Cell Carcinoma has become a poster child for a generation of targeted therapies. From 2005 onwards, 8 new agents have been approved by the FDA for the treatment of advanced RCC, with the 8<sup>th</sup> agent being approved in November 2015. Currently approved options include tyrosine kinase inhibitors targeted towards vascular endothelial growth factor (VEGF) such as Sorafenib, Pazopanib, Sunitinib and more recently Axitinib; as well as the mammalian target of Rapamycin (mTOR) inhibitors such as Everolimus and Temsirolimus and most recently, the anti-PD1 (Programmed Death) checkpoint inhibitor, Nivolumab. The encouraging results from these immune check point inhibitors such as Nivolumab, Ipilimumab, and Pembrolizumab hold promise for a considerable improvement in the overall prognosis of patients with advanced renal cell cancer<sup>[5]</sup>. We summarize here the development of the first generation of

### ABSTRACT

Historically, advanced renal cell cancer stood as a devastating diagnosis due to the paucity of systemic therapy options. Cytokine therapy including high dose IL2 and interferon alpha comprised the mainstay of advanced renal cell cancer treatment with modest benefit. Better understanding of the biology of the von Hippel-Lindau axis in the past 2 decades gave rise to antiangiogenic therapeutics starting late 2005 with currently 8 agents now approved by the FDA for the treatment of advanced renal cell cancer. Further, most recently approved is a check point inhibitor – a form of “targeted immunotherapy” – which has shown significant clinical efficacy in the management of this disease, marking a resurgence of immunotherapy in a more sophisticated fashion. In this review, the historical perspective, current treatment options and what is on the horizon in the treatment of renal cell cancer will be discussed. With advances in the understanding of this disease, our treatment armamentarium has remarkably expanded, thereby improving overall prognosis of patients affected with this disease.

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**Key words:** Renal cell cancer; Targeted therapy; Immunotherapy; VEGF inhibition; Check point inhibition

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targeted therapies as well as newer developments in the Renal Cell Carcinoma therapeutic space.

## VEGF TARGETING IN RENAL CELL CARCINOMA

### Biology of Kidney Cancer: Underlying mechanisms of VEGF activation

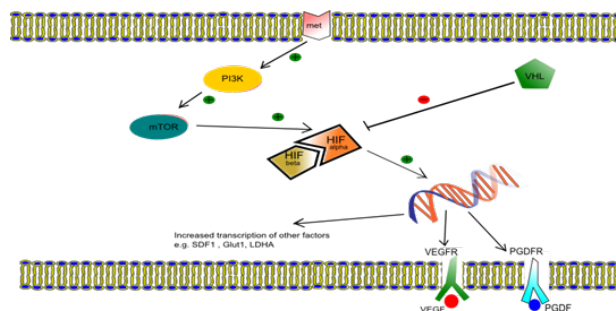
The activation of hypoxia pathways forms a critical underpinning for the pathogenesis of renal cancer. The inactivation of the VHL (von-Hippel-Lindau) gene through mutation or methylation is the most commonly described genetic anomaly associated with sporadic Renal Clear Cell Carcinoma, reportedly present in almost 75-90% of the cases of this subtype<sup>[6,7]</sup>. The VHL gene is responsible for degradation of the transcription factor HIF-1 $\alpha$  (Hypoxia-Inducible Factor 1-alpha) and HIF-2 $\alpha$ , which is a key driver of angiogenesis. The loss of VHL contributes to tumorigenesis through an unregulated buildup of the factor complex, in turn resulting in increased coding for VEGF receptors as described in the figure<sup>[6]</sup>. This downstream activation has made VEGF inhibition an attractive target for the development of targeted agents for Renal Cell Carcinoma. Activation of other receptors such as c-met (Figure 1) are also clinically significant and intricately involved in the VEGF activation pathway.

### First generation anti-VEGF therapy

Until the year 2002, the space for management of metastatic renal cell carcinoma was dominated by Interferon- $\alpha$  (IFN- $\alpha$ ) and Interleukin-2 (IL-2); systemic therapies that relied on generating a non-specific immune response<sup>[8]</sup>. Although these options provide a small number of durable responses in the order of 5-10% based on various reports, the vast majority of patients did not respond and progressed<sup>[8]</sup>. In 2002, Bevacizumab, a monoclonal antibody to VEGF ligand, was one of the first targeted molecules to enter clinical trials for RCC<sup>[9]</sup>. In a landmark phase II study, high dose Bevacizumab almost doubled time to progression in patients with metastatic RCC at 4.8 months vs. 2.5 months (HR 2.55,  $p < 0.001$ )<sup>[9]</sup>. This was a big proof of concept for the use of antiangiogenic therapy in patients with advanced renal cell cancer, and was followed by FDA approval for bevacizumab in combination with Interferon in the front line setting<sup>[10]</sup>.

### Oral Small molecule tyrosine kinase inhibitors of the VEGF receptor

With the relevance of VEGF pathway in RCC established, small molecule inhibitors, namely Tyrosine Kinase Inhibitors or TKIs were developed for more potent blockade of pro-angiogenic cellular signaling through the VEGF and PDGF pathways<sup>[11]</sup>. The first TKI to be approved for RCC was Sorafenib in 2005. Sorafenib is a multikinase inhibitor of VEGFR, Flt-3, PDGFR, and c-KIT<sup>[12]</sup>. Following early demonstration of tolerability and activity in phase I studies<sup>[13]</sup>, it was evaluated in a phase II trial in patients with advanced renal cell carcinoma. At 24 weeks, almost 50% of the Sorafenib-treated patients were progression free versus 18% of placebo-treated patients<sup>[14]</sup>. Sorafenib also demonstrated a similar progression free survival when compared with traditional Interferon therapy (5.7 months with Sorafenib arm vs. 5.6 months with Interferon) but with a much milder side effect profile. Also, as part of this trial, switching to Sorafenib after progression on Interferon therapy resulted in an improvement in PFS (5.3 months versus 3.6 months)<sup>[15]</sup>. Sorafenib was then finally evaluated in second line setting in a phase III trial involving 903 patients. This study showed



**Figure 1** Pathways of action of anti-angiogenic agents in Renal Cell Carcinoma: The shifting of the balance towards higher concentration of HIF- $\alpha$  leads to the increasing transcription of pro-angiogenic factors such as VEGFR, EGFR and PDGFR. The VHL gene increases the degradation of HIF- $\alpha$  thus preventing overactivation. mTOR inhibitors act upstream from HIF activation, while VEGF inhibitors act by blocking these receptors.

a significant improvement in the PFS (5.8 vs. 2.8 months) in favor of Sorafenib without any statistically significant overall survival (OS) benefit (17.8 vs. 15.2 months) on an intent to treat basis, likely due to a significant months cross-over effect in the final data analysis<sup>[16,17]</sup>. Sorafenib was approved by the FDA in December of 2005.

After the approval of Sorafenib, the next TKI to be approved was Sunitinib, a similar VEGFR, PDGFR, FLT-3 and c-KIT inhibitor. Sunitinib has since become the cornerstone of VEGF targeted therapy in the first line setting in renal cell carcinoma. Initial studies began in 2003, where Sunitinib showed an objective response to treatment in 3 out of 4 RCC patients treated in its first phase I trial<sup>[18]</sup>. In the follow-up phase II study, among 68 patients with cytokine refractory metastatic RCC, almost 40% of the patients achieved partial response and an additional 27% had stable disease for 3 months and greater<sup>[19]</sup>. This was a large step forward in the treatment of mRCC. Larger confirmatory trials were conducted such as a phase II trial of 106 patients, where 34% of the patients had a partial response with a median time to progression of 8.3 months. Fatigue and diarrhea were the most commonly reported side effects<sup>[20]</sup>. The magnitude of responses prompted the evaluation of Sunitinib in a first line setting, where it was compared with Interferon- $\alpha$  as the standard of care treatment at the time. Sunitinib achieved an objective response rate (ORR) of 31% compared to 6% for Interferon- $\alpha$ , and PFS doubled to 11 months compared with 5 months<sup>[21]</sup>. With these unprecedented results, Sunitinib was approved in the front line setting in Jan of 2006<sup>[22]</sup>. Sunitinib is now largely the preferred VEGF targeted first line treatment option for mRCC. There continue to be ongoing trials focusing on improving the efficacy and tolerability of Sunitinib by modifying dosing schedule or using it in combination therapy<sup>[23]</sup>.

Pazopanib was the next TKI to appear on the horizon around 2009. In a phase I trial, among 63 patients, of whom 12 patients had renal cell carcinoma, 2 patients had a partial response while 4 had stable disease<sup>[24]</sup>. Further studies in phase II showed that Pazopanib had a similar efficacy profile to Sunitinib, with overall response rates around 30%-35%, and a median PFS of approximately 11.1 months to 16 months depending on previous treatment<sup>[25,26]</sup>. While Pazopanib demonstrated a milder clinical side effect profile, it was associated with a higher incidence of liver injury<sup>[27]</sup>. This led to a head to head comparison between the de-facto standard Sunitinib and Pazopanib in a phase III setting<sup>[28]</sup>. The COMPARZ trial randomly assigned 1110 patients with metastatic clear cell carcinoma to receive Sunitinib or Pazopanib and was powered to show the non-inferiority of Pazopanib versus Sunitinib. In this study Pazopanib was showed to be non-

Table 1 FDA Approved Targeted Therapies for Renal Cell Carcinoma: Selected studies.						
Agent	Setting	Comparison(s)	Phase/Design	PFS	Year	Ref.
<b>VEGF Inhibitors</b>						
Bevacizumab	Front-Line or Prior IL-2 Therapy	Placebo	II	4.8 m (Bev) 2.5 m (Placebo)	2003	9
	Frontline, no prior therapy	Bev + IFN <i>vs</i> IFN	III, Randomized controlled trial	8.5 m (Bev+IFN) 5.2 m (IFN)	2008	10
	Frontline, no prior therapy	Bev + IFN <i>vs</i> IFN	III, Randomized controlled trial	10.2 m (Bev+IFN) 5.4 m (IFN)	2007	43
Sorafenib	Frontline, or prior therapy	Placebo	II, Randomized Discontinuation Trial	NR	2006	14
	Front Line only	IFN	II, open label	5.7m(Sorafenib) 5.6 m (IFN) PFS 5.3 m (Sorafenib) <i>vs</i> 3.6 m (IFN)**	2009	15
	Second Line	Placebo	III, Randomized controlled trial	5.5 m (Sorafenib) 2.8 m (Placebo)	2007	16
Sunitinib	Second Line, after cytokine failure	N/A	II	8.3 m	2003	20
	Front Line only	IFN	III, Randomized Controlled Trial	11 m (Sunitinib) 5 m (IFN)	2007	21
Pazopanib	Frontline, or prior therapy	N/A	II, open label	11.5 m	2010	26
	Second Line	Sunitinib	III, Randomized Controlled Trial	10.2 m (Pazopanib) 10.5 m (Sunitinib) ^ (NS)	2013	27
Axitinib	Prior IL-2 Therapy	N/A	II, Open label	15.7 m	2007	31
	Prior Sorafenib	N/A	II, Open Label	7.4 m	2009	44
	Second Line	Sorafenib	III, Randomized Controlled Trial	8.3 m (Axitinib) 5.7 m (Sorafenib)	2013	45
<b>mTOR inhibitors</b>						
Temozolimus	Frontline, or prior therapy	N/A	II, open label	7.4 m	2012	46
	Second Line, after prior Sunitinib	Sorafenib	III, Randomized Controlled Trial	4.3 m (Temozolimus) 3.9 m (Sorafenib)	2014	40
Everolimus (+ Bevacizumab)	Frontline, or prior therapy	N/A	II, Open label	9.1 m (frontline) 7.1 m (second-line)	2010	47
Everolimus	Second Line, after Sunitinib/Sorafenib	Placebo	III, Randomized Controlled Trial	4.9 m (Everolimus) 1.9 m (Placebo)	2008	48
<b>PD-1 inhibitors</b>						
Nivolumab	Second / third line	Everolimus	III, Randomized Controlled Trial	PFS 4.6 m (Nivolumab) 4.4 m (Everolimus) mOS 25 m (Nivolumab) 19.6 m (Everolimus)	2015	49

\*\*When switched to Sorafenib on progression. ^NS- Not significant. Legend: IFN: Interferon, m - months.

inferior to Sunitinib with respect to PFS, with a similar overall survival (hazard ratio for death with Pazopanib, 0.91; 95% CI, 0.76 to 1.08). Also, Pazopanib was favored with respect to quality of life (QoL) measures in 11 of 14 health-related quality-of-life domains. Although, interpretation of the QoL results was thought to be difficult due to timing of the assessments and different dosing schedule for these two agents<sup>[27]</sup>.

The newest multikinase TKI that was approved by FDA for RCC is Axitinib, a powerful inhibitor of VEGFR, PDGFR and c-KIT at very low concentrations (equivalent to 1% of other TKIs such as Sunitinib), without an effect on FLT-3 or RET<sup>[29,30]</sup>. In its first multicenter phase I trial in 36 patients, Axitinib was tolerated safely and demonstrated 2 partial responses among 6 patients with advanced RCC<sup>[30]</sup>. Investigated further in phase II trials, Axitinib delivered an overall response rate (ORR) of 44% with a Median Time to Progression of 15.7 months in 52 patients with cytokine refractory mRCC. These results showed superiority in efficacy compared to other TKIs in similar cohort of patients at the level of Phase II studies<sup>[31]</sup>. The side effect profile was similar to Sunitinib with diarrhea, fatigue, nausea and hypertension being the most commonly reported and what are now known as mostly VEGF inhibitor class effects<sup>[31]</sup>. Interestingly, several studies have reported a correlation between hypertension and response to treatment with Axitinib<sup>[32]</sup>. Axitinib was also effective at eliciting objective response in patients who had disease progression on treatment with Sorafenib, where

it had an ORR of approximately 23%, with a median PFS of 7.4m. 74% of patients on this study had been through two or more systemic months therapies prior to Axitinib<sup>[33]</sup>. Axitinib, however, did not meet the pre-determined statistical significance for the front line setting compared to Sorafenib, perhaps due to a highly rigorous statistical set point of the study (75% increase in ORR compared to Sorafenib). Therefore, Axitinib remains as the approved treatment option in a second line setting.

As with other anticancer treatments, emergence of resistance to antiangiogenics is observed in most patients and accordingly, the mechanisms of resistance to VEGF inhibition is under intense investigation<sup>[34]</sup>. Evidence points towards up-regulation of other pro-angiogenic pathways secondary to VEGF blockade. Induction of Fibroblast Growth Factor (FGF) was initially thought of as one such mechanism<sup>[35]</sup>. TKIs developed with FGF targeting in mind along with VEGF inhibition activity included Nintedanib, Dovitinib, Tivozanib and Regorafenib.

Tivozanib, one of these newer molecules, has been recently the subject of much debate. In 2013, FDA rejected the candidacy of Tivozanib for the indication of renal cell carcinoma, causing a large setback to the drug development industry. Many experts have commented that the results of the TIVO-1 study, which was to be the cornerstone behind the approval, was the victim of flawed trial design<sup>[36]</sup>. The drug was originally seen to be effective in phase I, inducing responses in almost a third of patients with

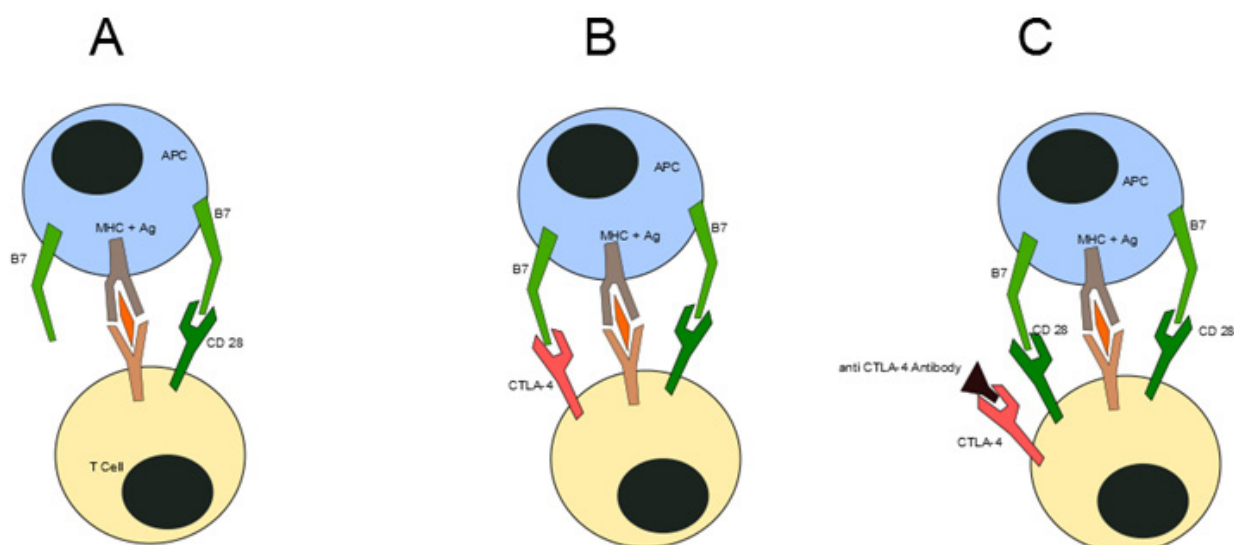


Figure2: Mechanism of action of anti-CTLA4 antibody: T cells require the costimulatory effect of the B7-CD28 interaction in order to proliferate and mount an immune response to the tumor (A). However, the proliferation as a result of this stimulation also causes T cells to express CTLA-4, an anti-proliferation signal. This is the mechanism to prevent excessive autoimmune responses, but leads to insufficient anti-tumor responses in cancers (B). The blocking of the negative effect of CTLA-4 then releases the brakes on the immune system, reactivating the T cells once again (C).

renal cell carcinoma along with others<sup>[37]</sup>. In a phase II randomized discontinuation trial it showed impressive efficacy in the overall trial population (n=272), almost half of which were treatment naïve (54%) with predominantly clear cell histology (83%). This trial reported an Objective Response Rate of 24% and PFS of 11.7 months<sup>[38]</sup>. Tivozanib reached the crucial phase III TIVO-1 trial, where it compared head to head with Sorafenib. Of this multicenter trial of 517 patients, the overwhelming majority of patients were from Eastern Europe<sup>[39]</sup>. This was also an open-label trial and patients who progressing on Sorafenib were given the opportunity to cross over to Tivozanib<sup>[39]</sup>. Although the trial met the primary endpoint of demonstrating superior PFS with Tivozanib (11. months vs. 9.1 months), Sorafenib showed superior OS; perhaps as a byproduct of a cross over effect, where almost 150 patients on Sorafenib had crossed over to Tivozanib. Unfortunately, the conduct of this trial in some resource limited settings meant that patients who were on the Tivozanib arm did not have access to other VEGF directed therapies to switch over to, likely creating an imbalance in VEGF antagonist exposure between the two arms.

## mTORT ARGETING IN RENAL CELL CARCINOMA

As described above, the PI3K /AKT/mTOR pathway has been a pro-growth signaling pathway activated in many cancers and in the majority of RCCs due to its regulation via HIFs as well. The FDA approved targeted agents developed towards this pathway include Temozolomide and Everolimus.

### Oral mTOR Inhibitors

Temozolomide was developed in parallel with the earlier VEGF TKIs, in the early 2000s, where it was found to be effective in RCC in its initial phase I studies. Temozolomide demonstrated confirmed partial response in a patient with metastatic treatment refractory RCC and a patient with breast cancer among 24 total patients in an early Phase I study<sup>[42]</sup>. However, Its FDA approval for mRCC came from a

Phase III randomized clinical trial of 626 patients with mRCC with poor and Intermediate MKSCC prognostic criteria. In this high to intermediate risk population the median survival was 7.3 months in the Interferon group, 10.9 months in the Temozolomide group, and 8.4 months with a combination-therapy group<sup>[50]</sup>. This trial forms the basis of the FDA approval of this agent and also for the NCCN's recommendation of Temozolomide as a first line agent in patients with poor prognostic criteria.

In addition to Temozolomide, Everolimus is the other mTOR inhibitor approved for treatment of mRCC. Everolimus was evaluated in the RECORD-1 trial, a double blind randomized controlled phase III trial conducted in 410 patients who had progressed on first line Sunitinib or Sorafenib. There was a significant improvement with Everolimus, with a doubling of median progression free survival to 4.0 months [95% CI 3.7-5.5] compared with 1.9 months [95% CI 1.8-1.9] on best supportive care. Stomatitis, fatigue and rash, a class effect of mTOR inhibitors occurred in a large minority of patients (40%, 25% and 20% respectively). Pneumonitis was the most common severe side effect; observed in 8 out of 272 patients on Everolimus reporting grade III events<sup>[51]</sup>. mTOR pathway inhibition is a promising strategy and continues to be evaluated in renal cell carcinoma. mTOR inhibition has also been studied in non-clear cell renal cell cancer with evidence for modest benefit<sup>[52]</sup>.

## FIRST GENERATION SYSTEMIC IMMUNOTHERAPIES

### Interleukin and Interferon Therapy

Although cancer immunotherapy has become the center of attention in the recent years, the early evidence of its effectiveness in cancer treatment and in RCC comes from the use of interferon-alpha (IFN- $\alpha$ ) and interleukin 2 (IL-2) in the earlier trials<sup>[53]</sup>. IL-2 stimulates a stress response to infection in the human body such as through the stimulation of T-cell proliferation. The first demonstration of efficacy was in a young woman with metastatic melanoma in 1984, where IL-2 caused diffuse shrinkage of her tumors and a complete response

**Table 2** Examples of ongoing trials evaluating new therapies in metastatic renal cell carcinoma.

Investigational Agent(s)	Mechanism of Action	Study Name/Description	ClinicalTrials.gov Identifier
HyperAcute®-Renal Immunotherapy	Cancer vaccine	A Phase I Study of HyperAcute-Renal (HAR) Immunotherapy In Patients With Metastatic Renal Cell Cancer	NCT02035358
Nivolumab, Ipilimumab	Anti PD1 ab + Anti CTLA4 ab	NivolumabvsNivolumab + BevacizumabvsNivolumab + Ipilimumab in Metastatic Renal Cell Carcinoma (mRCC)	NCT02210117
Nivolumab	Anti PD1 ab	A Study of Anti-PD1 (Nivolumab) Therapy as Pre- and Post-operative Therapy in Metastatic Renal Cell Cancer (ADAPTeR)	NCT02446860
Intuvax( vaccine)	Cancer Vaccine	Intratumoral Vaccination With Intuvax Pre-nephrectomy Followed by Sunitinib Post-nephrectomy vsSunitinib Post-nephrectomy in Newly Diagnosed Metastatic Renal Cell Carcinoma (mRCC) (MERECA)	NCT02432846
Varlilumab	anti-CD27 Ab	A Study of Varlilumab (Anti-CD27) and Sunitinib in Patients With Metastatic Clear Cell Renal Cell Carcinoma	NCT02386111
PT2385	HIF-2A Inhibitor	A Phase 1, Dose-Escalation Trial of PT2385 Tablets In Patients With Advanced Clear Cell Renal Cell Carcinoma	NCT02293980
Atezolimumab	Anti-PD-L1 Ab	A Study of MPDL3280A (Anti-Programmed Death Ligand 1 [PD-L1] Antibody) in Combination With Bevacizumab Versus Sunitinib in Patients With Untreated Advanced Renal Cell Carcinoma	NCT02420821
Avelumab	anti PD-L1 ab	Avelumab in Metastatic or Locally Advanced Solid Tumors (JAVELIN Solid Tumor)	NCT01772004
CAR-T cells for RCC	Anti-VEGFR2 Engineered CD8 + Cells	CAR-T Cell Receptor Immunotherapy Targeting VEGFR2 for Patients With Metastatic Cancer	NCT01218867

that persisting three decades<sup>[54]</sup>. IL-2 was also the first successful demonstration that extraneous activation of the immune system can have spectacular effects on cancer control. Renal Cell Carcinoma is an immunogenic tumor and has also benefited from IL-2 treatment. High dose IL-2 was developed for human use and approved by FDA in 1996 for metastatic Renal Cell Carcinoma on the basis of encouraging and durable responses in a small fraction of patients (5-10%)<sup>[55, 56, 57, 58]</sup>. Response rates as high as 28% have been reported in recent studies and expression of biomarkers such as PD-L1 or CA9 may serve as predictors of overall response rates<sup>[59, 60]</sup>. Significant acute toxicity and lack of benefit to a vast majority of treated patients continue to remain the main issue with this agent.

Interferon- alpha, another cytokine that has immunostimulatory effects was co-developed in the mid-1990s and used as an adjunct to IL-2. Among 425 patients in a trial, IL-2, Interferon-Alpha and the combination were associated with response rates of 6.5 %, 7.5 %, and 18.6 % respectively while event free survival rates at 1 year ranged from 12 to 20%<sup>[61]</sup>. Interferon-alpha has subsequently been tried in combination with some of the targeted therapies or as a comparison arm in trials of several targeted agents.

## SECOND GENERATION TARGETED IMMUNOTHERAPIES :CHECK POINT INHIBITION

First generation immune therapy with Interferon- $\alpha$  and IL-2 only provided limited benefit due to the ability of cancer cells to escape an extrinsic cellular anti-tumor response –a process now recognized as immunoeediting<sup>[62]</sup>. This process, driven by both the innate and adaptive immune systems comes into play when intrinsic cellular checkpoints such as tumor suppressors fail to contain abnormal growth, leading to nascent cancer cell formation Figure 2. Initial stages involve elimination of the tumor, but on rare occasions, the tumor cells that cannot be completely eliminated enter the ‘equilibrium’ phase, during this phase, the constant fight between the immune system and the tumor achieves an evolution of the final form of the tumor which is able to ‘escape’ immune surveillance. One of these mechanisms is through the expression of immune checkpoint modulators<sup>[63]</sup>.

### CTLA-4 inhibitors

The process of immune surveillance involves the identification of cancerous cells and then phagocytosis by antigen presenting cells (APCs). A protein that regulates this process is the CTLA-4; or Cytotoxic T lymphocyte antigen 4. CTLA-4 behaves like a brake for the immune system, preventing excessive activation. However, this brake is utilized by the cancer cells to override the immune response. CTLA-4 antibodies have been developed which release these brakes from the immune system. Figure 1 describes the mechanism of action of these drugs. Drugs in this category such as Ipilimumab have now obtained FDA approval in treatment of cancers such as melanoma.

Some of the earliest clinical evidence for Ipilimumab came from renal cell carcinoma patients, in 2007, Yang et al reported a phase II study where 5/40 patients on Ipilimumab at 3 mg/kg had a partial response to therapy with the drug<sup>[64]</sup>. These responses were in patients who had progressed on IL-2 therapy; however it appeared that responses were highly correlated with the frequency of immune related adverse events (irAEs) with 30% of those with adverse effects having responses while no patients without irAEs had any responses<sup>[65, 64]</sup>. However, Ipilimumab monotherapy for renal carcinoma remained on the backburner until the advent of the newer checkpoint inhibitors.

### PD-1/PD-L1 checkpoint inhibitors

One of the most promising agents to arrive on the horizon for cancer immunotherapy have been the PD-1 checkpoint inhibitors. PD-1/PD-L1 stand for programmed death receptor and programmed death ligand respectively. The PD-1 receptor is present on T cells and the PD-L1 ligand is present on the surface of antigen presenting cells (APC) or on Tumor cells. The interaction is immunosuppressive, and tumors expressing high concentrations are able to subdue the cellular immune response against tumors<sup>[66]</sup>. PD-1 inhibitors had showed significant activity in the recent years in tumors such as melanoma but data presented in June 2015 now demonstrates significant success in the treatment of a large number of tumor types including NSCLC, liver cancer, advanced head and neck cancers, and particular subsets of colorectal cancer patients.

Nivolumab, a well-tolerated anti-PD-1 antibody was investigated in mRCC settings and is first immune checkpoint inhibitor now

approved for metastatic renal carcinoma. Phase I trials showed good tolerability, with some responses in patients with mRCC in the cohort<sup>[66]</sup>. In a recent phase II study patients with mRCC refractory to treatment with VEGF inhibition were randomized 1:1:1 to Nivolumab in increasing doses of 0.3mg/kg, 2 mg/kg or 10 mg/kg. There was an ORR around 20%; but with a median OS of 25.5 months in patients being treated with Nivolumab 3 mg/kg group and 24.7 months with Nivolumab 2 mg/kg<sup>[67]</sup>. Side effects with PD-1 blockade is a stark contrast to those with agents such as IL-2 and Interferon, with 19/168 patients in the trial experiencing a grade III/IV adverse events, most commonly fatigue<sup>[67]</sup>. A smaller phase II trial with 34 patients and Nivolumab dosed at either 1 mg/kg or 10 mg/kg found an ORR of 29%, with an additional 27% with disease stabilization, and a median OS of 22.4 months<sup>[68]</sup>. This was finally followed by the CheckMate025 study, which compared Nivolumab to Everolimus in patients with advanced clear cell renal carcinoma that had progressed on first and second line anti-angiogenic therapies. 821 patients participated, and were randomized to receive either Nivolumab at 3mg/kg every 2 weeks or Everolimus 10 mg tablets daily. Median overall survival with Nivolumab was 25.0 months (95% CI 21.8 – Not estimable) and 19.6 months (95% CI 17.6 to 23.1) with Everolimus resulting in a Hazard Ratio [HR] of 0.73 (98.5% CI, 0.57 to 0.93;  $P=0.002$ ), indicating superiority of the Nivolumab regimen. As expected, this benefit also came with minimal risks, with Grade III/IV toxicity rates being 19% in the Nivolumab arm in comparison to 37% in patients on Everolimus<sup>[49]</sup>. This highly encouraging data has led to the FDA approval of Nivolumab for metastatic renal cancer patients.

Similar to PD1 inhibition Blockade of the PD-L1 ligand is also being investigated in kidney cancer. This includes agents such as MPDL3280A (now called atezolizumab). In a Phase I trial of 277 patients, including 69 patients with metastatic renal cell carcinoma administered MPDL3280A, there was an objective response rate of 14% with 8 out of 58 evaluable RCC patients with partial responses. The median response was 54 weeks, a very robust duration – and a characteristic of this therapy type, where those who benefit tends to have durable responses. PFS at 24 weeks was 53% in this cohort<sup>[69]</sup>.

## COMBINATIONS OF CHECKPOINT INHIBITORS

Given the relatively mild side effect profile of the newer targeted treatments and their clear efficacy, there is growing interest in evaluating combination therapies of immune agents, either with each another or with anti-angiogenic agents in mRCC.

Recent updated results from the Checkmate 016 study evaluated combination of the CTLA-4 and anti-PD1 checkpoint inhibitors Ipilimumab and Nivolumab respectively. Three groups were compared, with Nivolumab and Ipilimumab one low and high doses of 1mg/kg and 3 mg/kg each. The combined higher dose combination was not tolerated secondary to toxicity. Nivolumab (3mg/kg) + Ipilimumab (1mg/kg) had 34% patients with Grade 3-4 Adverse effects while it was 64% for the Nivolumab (1mg/kg) + Ipilimumab (3mg/kg) dosing. The Objective response rates in both arms were similar at around 40%, with a median PFS of 30-36 weeks<sup>[70]</sup>. Patients were intermediate-favorable per MSKCC criteria and approximately half had been treatment naïve.

The anti-PDL1 antibody MPDL3280A described earlier was also investigated in combination with Bevacizumab in a recent phase Ib study where it demonstrated good tolerability, with no grade 3-4 adverse events reported in the limited sample of 12 patients. Also,

there was an objective response rate of 40% in first –line patients. The data is still early, but encouraging for further follow up. A phase II was ongoing when last reported<sup>[71]</sup>.

## SEQUENCING OF AGENTS AND ROLE OF ADJUVANT THERAPY

### Adjuvant Therapy for Renal Cell Carcinoma

The concept of adjuvant chemotherapy for renal cell carcinoma was a subject of great interest due to the in-principle plausibility of benefit. A recent large trial (ASSURE) was conducted on 1943 patients with subjects randomized to 1:1:1 of Sunitinib, Sorafenib and placebo for locally advanced renal cancer in an adjuvant setting. Most recent updates from the trial unfortunately suggest no statistically significant benefit in either group<sup>[72]</sup>. The trial was also subject to significant rates of attrition due to side effects, with 26% in the experimental arms and thus had dose reductions (e.g. Sunitinib at 37.5 mg and Sorafenib at 400 mg ) reducing the attrition rate to 14%, however this is unlikely to have had an impact on the overall hazard ratio<sup>[72]</sup>. Although this has been a negative trial, it has not shut the door on the concept of adjuvant therapy for Renal Cell Carcinoma. It is still to be determined if new generation checkpoint inhibitors will be more tolerable and improve outcomes in this population.

### Sequencing of therapies

The relatively recent approval of the newer mTOR and VEGF inhibitors has resulted in some confusion on the most efficacious sequence of the use of these agents. There are now 7 FDA approved agents spanning both classes, all developed and approved within a few years. In first line settings, apart from Interleukin and Interferon-Sunitinib, Temsirolimus, Pazopanib, Axitinib and Sorafenib have all shown activity. The COMPARZ trial of Sunitinib and Pazopanib referred above did not show any significant benefit to either but sets up a case for patient-physician discussion based on side effect profiles<sup>[77]</sup>. A trial of Everolimus followed by Sunitinib or Vice versa has been explored and presented recently as the RECORD-3 trial. The trial analyzed individual PFS benefits as well as the OS benefit for each of the sequences in treatment. Median OS was 22.4 months for Everolimus followed by Sunitinib and 29.5 months for Sunitinib followed by Everolimus (HR, 1.09; 95% CI, 0.87-1.37), this suggests that the current practice of starting with VEGF targeted therapy maybe the most effective approach<sup>[73]</sup>.

In a second line setting the INTORSECT trial evaluated Temsirolimus and Sorafenib in patients who had previously progressed on Sunitinib therapy. Although the objective response rate for both arms were 8%, and Temsirolimus had a similar median PFS of 4.3 months versus 3.9 months for Sorafenib, there was an OS benefit for Sorafenib (16.6 months) compared with Temsirolimus (12.3 months) demonstrating that even disease progressive with VEGF blockade with one agent can respond to subsequent therapy<sup>[40]</sup>. Challenges to interpreting this data as a verdict on VEGF vs mTOR blockade in the second line setting include the proven efficacy of Axitinib in the AXIS trial and Everolimus in the RECORD-1 trial and there remains scope for further research. Another question for oncologists has been potential interchangeability between the FDA approved 2 mTOR inhibitors. A trial has now compared Everolimus and Temsirolimus as second line after therapy with an anti-VEGF TKI, which seems to suggest that Everolimus may lead to a superior OS (24.2 months vs 12.1months)<sup>[74]</sup>. A meta-analysis extrapolating data from available studies also supports the direction of this effect<sup>[75]</sup>.

## INVESTIGATIONAL THERAPIES FOR KIDNEY CANCER

Apart from the anti-angiogenic and immune checkpoint therapies discussed so far, there are several other promising targeted therapies that under development. Some of the strategies employed are either medications that can target resistance mechanisms to VEGF inhibitors, or act on novel molecular targets of renal cell carcinoma.

Resistance mechanisms to the commonly targeted processes such as VEGF inhibition are under intense investigation, a next generation of molecules are being developed which may target these resistance mechanisms<sup>[76]</sup>. Endoglin is an accessory receptor for TGF-β1 and has been seen to be expressed in higher amounts in tumors with advanced stage renal carcinoma, it is assumed to be an important piece in developing resistance to VEGF inhibitors<sup>[77]</sup>. In a recent phase I study of TRC105 in 18 mRCC patients refractory to multiple agents including prior Axitinib, an anti-Endoglin monoclonal antibody co-administered with Axitinib showed 18% RECIST response rate with almost half (47%) having a >10% decrease in tumor burden<sup>[78]</sup>.

Another agent that shares a similar mechanism, by decreasing resistance to VEGF therapies is Dalantercept, which is an activating receptor kinase-1 (ALK1) inhibitor: a recent phase I trial on 29 patients' refractory to previous mTOR, VEGF or immune therapies has shown very encouraging results. Dalantercept was combined with Axitinib at 5 mg PO twice a day dosing, there were no severe dose limiting toxicities with incremental dosing. While the ORR was 25% for the whole group, the median PFS was not reached at the maximum tolerated dose as of data reported until May 2015<sup>[79]</sup>. The study will now expand to a randomized phase II setting comparing Dalantercept versus placebo in combination with Axitinib<sup>[79]</sup>.

There has been an observation of increased c-MET expression in many renal cell carcinomas that are resistant to VEGF therapy, Cabozantinib is a small molecule tyrosine kinase inhibitor with action only on the VEGF receptor but also inhibitory action on Met, RET, AXL, KIT and FLT 3, however the c-MET inhibition alongwith VEGF inhibition are likely key elements of efficacy in renal cell carcinoma<sup>[80]</sup>. A Phase I study of Cabozantinib in heavily pretreated patients with RCC ( $n = 25$ ) demonstrated that it was tolerated well, with a partial response rate of 28%, and median progression free survival of 12.9 months, and mean overall survival of 15.0 months<sup>[81]</sup>. This was followed by a pivotal phase III randomized controlled trial of Cabozantinib versus Everolimus in 658 patients, in this trial median progression free survival with Cabozantinib was 7.4 months in comparison to 3.8 months with Everolimus- for a Hazard Ratio [HR] of 0.58 (95% CI 0.45-0.75) in favor of Cabozantinib. PFS also translated into an improvement in overall survival as well. Adverse events requiring discontinuation of therapy were similar across both arms (9% for Cabozantinib vs 10% Everolimus)<sup>[82]</sup>. Cabozantinib has been granted breakthrough therapy designation by the FDA and may likely be the 9th targeted therapy to be approved for kidney cancer.

While the checkpoint inhibitors have been recently discovered to augment the immune response against cancers, thus improving disease control, an alternative approach that preceded this discovery was the development of peptide vaccines for cancer. There are certain proteins expressed by malignant cells that can help the body identify the underlying process. These antigens, called Tumor Associated Antigens (TAA)s, have been thought of as antigenic stimulants for an immune response. IMA901 is a synthetic RCC-specific, multiantigen peptide vaccine that contains tumor associated peptides derived from the antigens overexpressed in RCC. In phase I, among 28 patients, 20

patients had a T cell response to atleast one tumor associated antigen and 8 had responses to more than one. Studies in RCC showed a correlation of multi-tumor associated antigen responses with OS, and there are phase III trials now ongoing to further evaluate its efficacy<sup>[83]</sup>. Similar such agents include Hyperacute- Renal Immunotherapy, and Intuvax. Unpublished communication from the maker suggests that of 11 patients on the phase I/II trial of this agent in 2012, 5 patients continue to survive until 2015 for a median overall survival of 29.8 months versus expected 15.2 months<sup>[84]</sup>.

Finally, as we discussed before, hypoxia inducible factors form the final common product of the hypoxia axis activation due to various mutations associated with renal cell cancer. Until recently there were no potent inhibitors of HIF. However, currently some trials are recruiting to evaluate efficacy of newly developed oral HIF inhibitors (refer table).

## DISCUSSION

Metastatic renal cell cancer has become a much more “treatable disease” in the past 10 years than it has ever been in the past. The last decade has seen an explosion of therapeutic options in patients with metastatic renal cell carcinoma. These developments have been led by our deeper understanding of the underlying biology of mRCC, ultimately leading to the development of targeted agents for the VEGF/angiogenesis pathway. More recently achieved is our improved understanding of immune mechanism in the setting of cancer, leading to approval of checkpoint inhibitors in not only kidney cancer but also in multiple other cancers including lung and melanoma. Other immunotherapy approaches including vaccines as well as combination approaches are currently undergoing investigation at this time with promising early results. Importantly, studies are also ongoing to identify biomarkers for response and toxicity to all therapies involving kidney cancer as well as cancer therapy as a whole in pursuit of “personalizing” cancer care. Going forward, as a result of the rapid development of these agents, several questions will be need to be answered which include optimal combination or sequencing of therapeutic agents, effectiveness of available agents in an adjuvant or neoadjuvant setting, and the development of biomarkers to identify those who may or may not respond and with what degree of tolerance. Overall, however, it is without a doubt that the landscape of renal cell carcinoma has changed significantly for the better and there are definitely more exciting times ahead.

## CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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