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The tumor microenvironment in renal cell cancer

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Abstract

Purpose of review: In addition to the provision of nutrients and growth factors that facilitate tumor cell proliferation and metastasis, the tumor microenvironment (MEV) restricts immune surveillance of tumor-associated antigens and limits the efficacy of immune checkpoint inhibitors (ICIs), tumor vaccines, and other immune therapies. This review will focus on the immunosuppressive mechanisms operative within the tumor MVE of renal cell carcinoma (RCC).

Recent findings: Several of the immunosuppressive mechanisms within the tumor MEV have been identified and are potentially druggable. Clinical trials with agents that target several of these inhibitory pathways are currently underway.

Summary: Although RCC is one of several tumor types responsive to ICIs, the effectiveness of these agents is likely to be limited by the various tumor-infiltrating bone marrow-derived myeloid cells that comprise the MEV. Several strategies to combat the recruitment of these cells into tumor tissue or to neutralize their immunosuppressive function have shown encouraging results in animal tumor models and clinical trials.

Keywords

microenvironment (MEV); renal cell carcinoma (RCC); myeloid-derived suppressor cells (MDSC); tumor-associated macrophages (TAM); microvasculature; tumor-infiltrating lymphocytes (TIL); cytotoxic T lymphocytes (CTL); regulatory T cells (Tregs); immune checkpoint inhibitor (ICI); vascular endothelial growth factor (VEGF); hypoxia-inducible factor (HIF); arginase; indoleamine dioxygenase (IDO)

INTRODUCTION

The RCC MEV consists of various lymphoid and myeloid cells, myofibroblasts, and endothelial cells recruited from nearby tissue or derived from precursors that originate in the bone marrow. To a large extent, the MEV of a typical clear cell RCC (ccRCC) resembles that of other tumor types, but there are a few distinguishing features peculiar to RCC – notably the extent of the leukocytic infiltrate and the luxurious, VEGF-driven vasculature. The cells that constitute the RCC MEV not only promote the growth and metastasis of the tumor cells but, through a host of elaborate mechanisms, undermine the ability of the immune system to recognize and eradicate neoplastic cells.

Conflicts of interest

There are no conflicts of interest.

CELLULAR COMPOSITION OF THE RCC MICROENVIRONMENT

Tumor-Infiltrating Lymphocytes:

Perhaps more than any other tumor type, ccRCC are infiltrated with T cells of various phenotype and function [1,2]. This is particularly the case with granzyme A- and perforin-expressing CD8⁺ cytotoxic T cells (CTL), which, according to an analysis carried out by Rooney *et al*, are more abundant in ccRCC than in any of 17 other tumor types examined [3]. Tregs and Th2 cells are correspondingly underrepresented in ccRCC [4]. Although these histologic findings might be predicted from the documented instances of “spontaneous” regression seen with RCC [5] and the well-known sensitivity of the disease to treatment with IL-2 [6] and immune checkpoint inhibitors [7–9], there are several aspects of ccRCC biology that predict that these tumors should not be especially immunogenic. The response to immune checkpoint inhibitors has been shown to correlate with the mutational burden of the tumor cell [10,11] and the number of mutations in a typical ccRCC is several orders of magnitude less than that observed in melanoma or NSCLC [12], suggesting that ccRCC should have limited expression of novel tumor-specific antigens and at best, a modest immune cell infiltrate. The molecular basis for the robust immune cell infiltration and response to immunotherapy observed with RCC is not entirely clear but may be due to the upregulation of genes associated with antigen processing and peptide loading onto HLA Class I molecules such as TAP1 and TAP2 [13] or antigen presentation such as HLA-A, B, and C, and β_2 -microglobulin. Senbabaoglu *et al* recently compared the expression of these genes in various tumors with that of adjoining normal tissue and observed the greatest variance between the paired tissues in ccRCC tumors [4]. The mechanism by which the genetic aberration of ccRCC (functional loss of VHL) results in the up regulation of the antigen-presenting machinery and the role played by these genes in the regulation of the immune infiltrate remain to be defined.

The nature and extent of the tumor infiltrate has prognostic significance in RCC. In the aforementioned study by Senbabaoglu *et al*, the ccRCC subset with the greatest accumulation of T cells was found to have the poorest survival [4]. This negative correlation was attributed to the high levels of CTLA-4 and PD-1 expressed on these invading T cells and the possibility that their cytolytic function might be impaired by interaction with other cells within the tumor microenvironment. Infiltration by Th2 cells and Tregs was associated with negative outcomes. On the other hand, the number of infiltrating Th17 cells correlated with improved survival, as did the CD8⁺ T cell/Treg ratio.

Myeloid-derived suppressor cells (MDSC):

In addition to Tregs, tumors are infiltrated to a varying extent with a population of bone marrow- derived myeloid cells (myeloid-derived suppressor cells, MDSC) that serve to promote tumor angiogenesis, metastasis, and to undermine the antitumor effects of infiltrating CTLs. These cells are usually characterized as a subpopulation of activated neutrophils which express high levels of CD66b, CD11b, and VEGFR1 and low levels of the Fc receptor CD16 [14]. They are, however, actually quite heterogeneous and are often classified as neutrophilic, monocytic, or immature based on their morphology and surface phenotype [15,16]. The differentiation of these cells and their accumulation within tumor

infiltrates are driven by a number of factors, including cytokines or chemokines produced by the tumor cells [17]. Conditioned media from RCC cell lines, for example, induces the differentiation of monocytes into MDSC [18]. These treated cells have the phenotype and several of the immunosuppressive functions of patient-derived MDSC. They produce nitric oxide and suppress the proliferation of autologous CD3⁺ T cells. All of these acquired features can be partially blocked by inhibitors of GM-CSF or STAT3, suggesting that the production of GM-CSF by the tumor cells is one of the means by which MDSC are induced and recruited by RCC. In a study focused specifically on RCC, the influx of MDSC was found to be correlated with levels of CCL2 (MCP-1), IL-17, and IL-18, all of which were abundantly expressed in tumor tissue and present in the blood of patients [19]. Najjar *et al* observed a correlation between the infiltration of the neutrophilic type of MDSC in RCC with the expression of IL-1 β , IL-8, CXCL5, and Mip-1 α [20]. RCC infiltration by the immature type of MDSC was found to correlate with tumor levels of IL-8 and CXCL5. In the murine RCC model Renca, the administration of antibodies against IL-1 β or CXCR2 (the receptor for IL-8 and other pro-angiogenic chemokines) was shown to reduce MDSC accumulation, augment tumor infiltration by CD4⁺ and CD8⁺ T cells, and to retard tumor growth, suggesting that tumor cell production of various inflammatory cytokines is an important mechanism by which RCC orchestrate the tumor microenvironment. Finally, the release of the nuclear protein HMGB1 by damaged RCC tumor cells was found to contribute to the differentiation and accumulation of MDSC [21]. Administration of an anti-HMGB1 antibody inhibited tumor growth and prolonged survival in the Renca model. This beneficial effect was absent in mice independently depleted of MDSC by treatment with an anti-Gr-1 antibody.

Tumor-Associated Macrophages (TAMs):

RCC are extensively infiltrated with macrophages of various type [22,23]. These cells support tumor growth and metastatic predisposition through the production of proteases capable of remodeling the extracellular matrix. They also contribute to the general state of immunosuppression of the tumor microenvironment. Although the total number of (CD68⁺) macrophages infiltrating RCC appears to be only weakly predictive of tumor behavior, the abundance of tumor-promoting M2 (CD163⁺, CD204⁺, or CD206⁺) macrophages or the ratio of M2/M1 (CD11c⁺) cells present within the tumor infiltrate has consistently been shown to correlate with poor survival [22–26]. Chevrier *et al* have observed that the abundance of CD204⁺ (M2) macrophages within RCC correlates with the number of T cells expressing an exhausted or regulatory phenotype [23]. These M2 macrophages were found to express the PD1 ligands CD273 and CD274 and to produce the immunosuppressive cytokine IL-10. Fu *et al* have shown that these cells undermine the immune response to tumor antigens in part by producing IL-23, which induces Treg proliferation and activates their production of IL-10 and TGF- β [27]. Thus, the accumulation of M2 macrophages within the tumor infiltrate would be expected to contribute, along with Tregs and MDSC, to the immunosuppressive state of the RCC microenvironment.

RCC Microvasculature:

RCC is one of the most highly vascularized of all tumor types. The robust microcirculation of RCC is the result of the massive overproduction of vascular endothelial growth factor

(VEGF) and other pro-angiogenic cytokines by the tumor cells and to a lesser extent, the supporting stroma. The generation of VEGF by RCC is in turn due to high levels of the transcription factors hypoxia-inducible factor-1 and -2 (HIF-1 and -2), which mediate the cellular response to hypoxia [28]. In RCC, the high levels of HIF-1 and -2 are the result of the loss of a functional VHL gene, the protein product of which is part of a multi-protein complex that ubiquitinates and destabilizes HIF-1 and -2 when they have undergone oxygen-dependent proline hydroxylation [29, 30]. The loss of VHL through mutation, deletion, or epigenetic silencing ensures that the two HIFs are allowed to accumulate in tumor cells regardless of the availability of oxygen.

Stabilization of HIF-1 and -2 through the loss of VHL is not the only factor contributing to the high levels of these transcription factors in RCC. Mathew *et al* have shown that RCC have low levels of the microRNAs miR-30-2-3p and miR-30a-3p compared to the normal renal parenchyma [31,32]. These microRNAs interfere with the translation of the HIF mRNAs and their absence from RCC cells allows for the HIF transcripts to be efficiently translated. This effect, coupled with the inherent stability of the HIF proteins due to the VHL loss, guarantees the abundance of these transcription factors in RCC cells. Another genetic quirk of RCC is the consistent loss of the glycolytic enzyme fructose-1,6-bisphosphatase (FBP1)[33]. This enzyme has recently been shown to bind to and inhibit the nuclear function of HIF-1 and -2 through a mechanism that is completely independent of its catalytic function. The absence of FBP1 therefore not only promotes glycolysis (the Warburg effect) but ensures the function of HIF-1 and -2 in RCC cells. The downstream consequence of the loss or down modulation of VHL, the miR-30 micro-RNAs, and FBP1 is to maintain high levels of HIF transcription factors and the expression of proangiogenic cytokines such as VEGF. This ensures a robust microcirculation within the tumor but also creates a unique vulnerability to the effects of VEGF receptor inhibition. Indeed, RCC was the first tumor type for which drugs that block VEGFR signaling were approved by the FDA as single agents [34–36].

The microvasculature within RCC consists of several types of microvessels that differ with respect to their degree of differentiation. Qian *et al* have analyzed the morphology and phenotype of the RCC endothelium and have identified differentiated vessels, which express both CD31 and CD34, and a less differentiated type of vessel in which the endothelial cells express only CD31 [37]. The latter tend to have small lumens and no pericyte coverage and are located primarily within the centers of tumor deposits. The more differentiated vessels are covered with pericytes and are more abundant in the tumor periphery. The undifferentiated vessels are particularly abundant in high grade RCC and their presence is associated with short patient survival [38].

Similar studies have been carried out by investigators in the Dvorak laboratory using an adenovirus engineered to express VEGF-A [39–42]. Implantation of this virus triggers an influx of vessels that resemble those found in RCC. Sitohy *et al* and Nagy *et al* have identified six distinct vessel types that develop at the site of virus-induced VEGF production, including “mother vessels”(MV) that emerge early from preexisting venules [39–41]. These mother vessels evolve into glomeruloid microvascular proliferations (GMP) and later into capillaries and microvascular malformations (VM). Subsequent remodeling gives rise to

feeding arteries (FA) and draining veins (DV) similar to those found in RCC. The early MV and GMP express high levels of VEGFR-2 and are particularly sensitive to the inhibition of VEGF signaling from the administration of the VEGF trap aflibercept, for example. Late-appearing capillaries, VM, FA, and DV on the other hand ultimately lose their initial dependency on VEGF and are unresponsive to VEGF inhibition. This loss of dependency on VEGF, and the persistence of all but two of the microvessel types despite the inhibition of the growth factor that triggered the development of vessels in the first place, are thought to limit the efficacy of agents that block VEGFR signaling in the treatment of patients with RCC.

Wragg *et al* have compared the gene expression profiles of tumor-associated with nonmalignant endothelium from a variety of tumor types and have identified melanoma cell adhesion molecule (MCAM) and its extracellular matrix ligand laminin- α 4 (LAMA4) as consistently overexpressed in the microvasculature of RCC [43]. These microarray data were confirmed by immunohistochemistry. MCAM expression is induced by exposure to VEGF and its expression in RCC was linked to poor patient survival. Given its specificity for the microvasculature of RCC, the authors suggest that MCAM might be therapeutically targeted by monoclonal antibodies as part of a treatment strategy for this disease.

MECHANISMS OF IMMUNOSUPPRESSION: STRATEGIES TO OVERCOME RESISTANCE TO IMMUNOTHERAPY

The tumor MEV limits the antitumor activity of invading T cells through a variety of mechanisms. Certainly one constraining factor is the microenvironmental depletion of key nutrients essential for optimal immune function such as glucose and glutamine, both of which are avidly taken up by proliferating tumor cells. Glutamine is essential for the expression of the T cell transcription factor Tbet and the differentiation of IFN- γ -producing TH1 cells and in its absence, CD4⁺ T cells differentiate into Foxp3⁺ Tregs [44]. Although inhibitors of the enzyme glutaminase such as CB-839 have multiple effects on tumor cell metabolism, oxidative stress, and DNA methylation that might contribute to their antitumor activity, their ability to increase glutamine levels in the MEV and reverse some aspects of immune dysfunction may be an important component of their overall antitumor effect [45].

The amino acid arginine is often lacking in the MEV extracellular fluid due to its uptake by MDSC and the secretion of arginase by these cells [14]. MDSC express one of the isoforms of the enzyme nitric oxide synthase (iNOS) that generates NO from arginine. This free radical directly nitrates proteins such as STAT1, rendering the T cells incapable of responding to antigen-presenting dendritic cells [46]. Several investigators have conjectured that NO production by tumor-infiltrating MDSC is one of the dominant mechanisms by which the tumor MEV undermines immune function.

RCC cells are generally unable to generate their own arginine due to a lack of the enzyme argininosuccinate synthetase-1 [47]. One might therefore expect that microenvironmental arginine depletion would limit RCC growth. The net effect of the arginase secretion, however, turns out to be tumor-promoting due to its inhibitory effect on immune function. In the absence of arginine, T cells accumulate uncharged tRNA, which activates the eIF-2 α

kinase GCN2, which in turn arrests protein synthesis. Certain elements of the T cell signaling apparatus – notably the TCR- ζ chain, the kinase p56^{lck}, and components of the NF- κ B pathway – are particularly affected by eIF-2 α phosphorylation, and the down modulation of these molecules profoundly inhibits T cell function [48]. Recently, inhibitors of arginase (e.g. INCB001158) have entered clinical trials as adjuncts to immune checkpoint inhibitors [49], and it is hoped that they might improve the efficacy of these agents.

Metabolism of the amino acid tryptophan by the enzyme indoleamine dioxygenase (IDO) not only depletes the MEV of this critical amino acid but results in the accumulation of metabolites (kynurenines) that are particularly toxic for T cells [50]. Although IDO has been shown to mediate immunosuppression in numerous animal tumor models, the extent to which the tryptophan-kynurenine metabolic pathway disrupts immune function in the MEV of human cancer remains unclear [51]. Another immunosuppressive compound, adenosine, is present in the MEV extracellular fluid as a result of the action of the ectonucleotidase CD73, which is expressed by many tumor cells. This nucleotide metabolite suppresses immune function through the engagement of its purinergic receptor [52], and studies are currently underway to determine if inhibition of this pathway is able to restore immune function and augment the efficacy of immune checkpoint inhibitors.

Several of the strategies to improve the efficacy of immune checkpoint inhibitors currently under investigation involve the combination of an anti-PD1 antibody with a drug designed to block the differentiation of MDSC or their trafficking to tumor sites [53–55]. These potentially important agents include the histone deacetylase inhibitor entinostat, which reduces arginase and NO production by MDSC [56], as well as STAT3 inhibitors, which block the differentiation and function of MDSC [57]. Drugs that selectively inhibit PI3K- γ , the PI3K isoform needed for MDSC to suppress immune function [58], are also being paired with anti-PD1 antibodies in early clinical trials. In a similar vein, agents that block the chemokine CSF-1 (e.g. cabiralizumab) have been combined with nivolumab in an effort to exclude MDSC from the tumor MEV in RCC and other malignancies [59].

Finally, it is worth mentioning the various VEGF- or VEGFR-targeted drugs that have recently been shown to augment the efficacy of anti-PD1 or –PDL1 antibodies in the treatment of RCC [60–62]. VEGF is known to interfere with dendritic cell function and to induce the expression of FasL on tumor endothelium [63], which should promote apoptosis in T cells in the act of extravasation. VEGFR blockade would be expected to reverse these effects. In addition, VEGF has been shown to reduce STAT3 signaling in MDSC and to limit the trafficking of these cells into tumor tissue [53,64,65]. It is therefore conceivable that the ability of these agents to enhance the efficacy of immune checkpoint inhibitors is at least partly mediated through an immunological mechanism involving MDSC within the RCC MEV independent of their anti-angiogenic effects.

CONCLUSION

ccRCC are extensively infiltrated with regulatory T cells, myeloid, and other cell types that are capable of suppressing immune function through mechanisms completely independent of PD1. These cells constitute a tumor MEV that limits the effectiveness of immune

surveillance and the activity of immune checkpoint inhibitors. Several of the mechanisms by which these cells inhibit tumor eradication by CTL and NK cells have been characterized, and drugs designed to block their function are currently being tested as adjuncts to immune checkpoint inhibitors. It appears likely that the future management of ccRCC will entail the use of a combination of drugs with complementary effects on the immune system.

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REFERENCES

1. Yoshihara K, Shahmoradgoli M, Martinez E, et al. Inferring tumour purity and stromal and immune cell admixture from expression data. *Nat Commun* 2013; 4: 2612. [PubMed: 24113773]
2. Thompson RH, Dong H, Lohse CM, et al. PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. *Clin Cancer Res* 2007; 13: 1757–61. [PubMed: 17363529]
3. Rooney MS, Shukla SA, Wu CJ, et al. Molecular and genetic properties associated with local immune cytolytic activity. *Cell* 2015; 160: 48–61. [PubMed: 25594174]
4. Senbabaoglu Y, Gejman RS, Winer AG, et al. Tumor immune microenvironment characterization in clear cell renal cell carcinoma identifies prognostic and immunotherapeutically relevant messenger RNA signatures. *Genome Biol* 2016; 17: 231. [PubMed: 27855702] This extensive transcriptome analysis defines the heterogeneity of the immune infiltrate in ccRCC and identifies correlations between the abundance of various cell types with clinical outcome.
5. Janiszewska AD, Poletajew S, Wasiutynski A. Spontaneous regression of renal cell carcinoma. *Contemp Oncol* 2013; 17: 123–7.
6. Rosenberg SA. IL-2: the first effective immunotherapy for human cancer. *J Immunol* 2014; 192: 5451–8. [PubMed: 24907378]
7. Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus everolimus in advanced renal cell carcinoma. *N Eng J Med* 2015; 373: 1803–13.
8. Ball MW, Allaf ME, Drake CG. Recent advances in the immunotherapy for kidney cancer. *Discov Med* 2016; 116: 305–13.
9. Motzer RJ, Tannir NM, McDermott DF, et al. Nivolumab plus ipilimumab versus sunitinib in advanced renal cell carcinoma. *N Eng J Med* 2018; 378: 1277–90.
10. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Eng J Med* 2014; 37: 2189–99.
11. Rizvi NA, Hellmann MD, Snyder A, et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small lung cancer. *Science* 2015; 348: 124–8. [PubMed: 25765070]
12. Lawrence MS, Stojanov P, Polak P, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature* 2013; 499: 214–8. [PubMed: 23770567]
13. Abele R, Tampé R. Moving the cellular peptidome by transporters. *Front Cell Dev Biol* 2018; 6: 43. [PubMed: 29761100]
14. Rodriguez PC, Ernstoff MS, Hernandez C, et al. Arginase 1-producing myeloid-derived suppressor cells in renal cell carcinoma are a subpopulation of activated granulocytes. *Cancer Res* 2009; 69: 1553–60. [PubMed: 19201693]
15. Groth C, Hu X, Weber R, et al. Immunosuppression mediated by myeloid-derived suppressor cells (MDSCs) during tumour progression. *Br J Cancer* 2018;
16. Budhwar S, Verma P, Verma R, Rai S, Singh K. The yin and yang of myeloid-derived suppressor cells. *Front Immunol* 2018; 9:2776. [PubMed: 30555467]
17. Susek KH, Karvouni M, Alici E, Lundqvist A. The role of CXC chemokine receptors 1–4 on immune cells in the tumor microenvironment. *Front Immunol* 2018; 9: 2159. [PubMed: 30319622]

18. Okada SL, Simmons RM, Franke-Welch S et al. Conditioned media from the renal cell carcinoma cell line 786-O drives human blood monocytes to a monocytic myeloid-derived suppressor cell phenotype. *Cell Immunol* 2018; 323: 49–58. [PubMed: 29103587]
19. Guan X, Liu Z, Zhang J, Jin X. Myeloid-derived suppressor cell accumulation in renal cell carcinoma is correlated with CCL2, IL-17, and IL-18 expression in blood and tumors. *Adv Clin Exp Med* 2018; 27: 947–53. [PubMed: 29905412]
20. Najjar YG, Rayman P, Jia X, et al. Myeloid-derived suppressor cell subset accumulation in renal cell carcinoma parenchyma is associated with intratumoral expression of IL-1 β , IL8, CXCL5, and Mip-1 α . *Clin Cancer Res* 2017; 23: 2346–55. [PubMed: 27799249]
21. Li J, Sun J, Li L, et al. HMGB1 promotes myeloid-derived suppressor cells and renal carcinoma immune escape. *Oncotarget* 2017; 8: 63290–8. [PubMed: 28968989]
22. Xu L, Zhu Y, Chen L, et al. Prognostic value of diametrically polarized tumor-associated macrophages in renal cell carcinoma. *Ann Surg Oncol* 2014; 21: 3142–50. [PubMed: 24615178]
23. Chevrier S, Levine JH, Zanotelli VRT, et al. An immune atlas of clear cell renal cell carcinoma. *Cell* 2017; 169: 736–49. [PubMed: 28475899] This is an exhaustive analysis of the heterogeneous immune landscape of RCC.
24. Kovaleva OV, Samoilova DV, Shitova MS, Gratchev A. Tumor associated macrophages in kidney cancer. *Anal Cell Pathol* 2016; 2016:930549.
25. Santoni M, Massari F, Amantini C, et al. Emerging role of tumor-associated macrophages as therapeutic targets in patients with metastatic renal cell carcinoma. *Cancer Immunol Immunother* 2013; 62: 1757–68. [PubMed: 24132754] Excellent discussion of the immunosuppressive function of TAM in RCC.
26. Motoshima T, Miura Y, Wakigami N, et al. Phenotypical change of tumor-associated macrophages in metastatic lesions of clear cell renal cell carcinoma. *Med Mol Morphol* 2018; 51: 57–63. [PubMed: 29218457]
27. Fu Q, Xu L, Wang Y, et al. Tumor-associated macrophage-derived Interleukin-23 interlinks kidney cancer glutamine addiction with immune evasion. *Eur Urol* 2018;
28. Shen C, Kaelin WG. The VHL/HIF axis in clear cell renal carcinoma. *Semin Cancer Biol* 2013; 23: 18–25. [PubMed: 22705278]
29. Iliopoulos O Molecular biology of renal cell cancer and the identification of therapeutic targets. *J Clin Oncol* 2006; 24: 5593–600. [PubMed: 17158545]
30. Kaelin WG. The von Hippel-Landau tumour suppressor protein: O₂ sensing and cancer. *Nat Rev Cancer* 2008; 8: 856–73. Comprehensive discussion of the VHL gene and the consequences of its loss in the development of ccRCC.
31. Mathew LK, Lee SS, Skuli N, et al. Restricted expression of miR-30c-2–3p and miR-30a-3p in clear cell renal cell carcinomas enhances HIF-2 α activity. *Cancer Discovery* 2014; 4:53–60. [PubMed: 24189146]
32. Moch H, Lukamowicz-Rajska M. miR-30c-2–3p and miR-30a-3p: New pieces of the jigsaw puzzle in HIF-2 α regulation. *Cancer Discovery* 2014; 4:22–4. [PubMed: 24402943]
33. Li B, Qui B, Walton ZE et al. Fructose-1,6-bisphosphatase opposes renal carcinoma progression. *Nature* 2014; 513: 251–5. [PubMed: 25043030]
34. Choueiri TK, Escudier B, Powles T, et al. Cabozantinib versus everolimus in advanced renal cell carcinoma (METEOR): final results from a randomized, open-label, phase 3 trial. *Lancet Oncol* 2016; 17: 917–27. [PubMed: 27279544]
35. Bellesoeur A, Carton E, Alexandre J, Goldwasser F, Huillard O. Axitinib in the treatment of renal cell carcinoma: design, development and place in therapy. *Drug Des Devel Ther* 2017; 11: 2801–11.
36. Motzer RJ, Hutson TE, Cella D, et al. Pazopanib versus sunitinib in metastatic renal-cell carcinoma. *N Engl J Med* 2013; 369: 722–31. [PubMed: 23964934]
37. Qian C-N, Huang D, Wondergem B, The BT. Complexity of tumor vasculature in clear cell renal cell carcinoma. *Cancer* 2009; 115: 2282–9. [PubMed: 19402071]
38. Cao Y, Zhang ZL, Zhou M, et al. Pericyte coverage of differentiated vessels inside tumor vasculature is an independent unfavorable prognostic factor for patients with clear cell renal cell carcinoma. *Cancer* 2013; 119: 313–24. [PubMed: 22811049]

39. Sitohy B, Nagy JA, Jaminet SC, Dvorak HF. Tumor-surrogate blood vessel subtypes exhibit differential susceptibility to anti-VEGF therapy. *Cancer Res* 2011; 71: 7021–8. [PubMed: 21937680]
40. Sitohy B, Nagy JA, Dvorak HF. Anti-VEGF/VEGFR therapy for cancer: reassessing the target. *Cancer Res* 2012; 72: 1909–14. [PubMed: 22508695]
41. Nagy JA, Dvorak HF. Heterogeneity of the tumor vasculature: the need for new tumor blood vessel type-specific targets. *Clin Exp Metastasis* 2012; 29: 657–62. [PubMed: 22692562]
42. Nagy JA, Chang SH, Dvorak AM, Dvorak HF. Why are tumour blood vessels abnormal and why is it important to know? *Br J Cancer* 2009; 100: 865–9. [PubMed: 19240721] This article describes the use of a novel VEGF-producing virus to generate blood vessels that simulate those found in ccRCC. It describes the heterogeneity of blood vessels arising in response to VEGF and the inconsistent effects of VEGF inhibition on the different vessel types.
43. Wragg JW, Finnity JP, Anderson JA, et al. MCAM and LAM4A are highly enriched in tumor blood vessels of renal cell carcinoma and predict patient outcome. *Cancer Res* 2016; 76: 2314–26. [PubMed: 26921326]
44. Klysz D, Tai X, Robert PA, et al. Glutamine-dependent α -ketoglutarate production regulates the balance between T helper 1 cell and regulatory T cell generation. *Sci Signal* 2015; 8(396):ra97. [PubMed: 26420908]
45. Gross MI, Demo SD, Dennison JB, et al. Antitumor activity of the glutaminase inhibitor CB-839 in triple negative breast cancer. *Mol Cancer Ther* 2014; 13: 890–901. [PubMed: 24523301]
46. Markowitz J, Wang J, Vangundy Z, et al. Nitric oxide mediated inhibition of antigen presentation from DCs to CD4+ T cells in cancer and measurement of STAT1 nitration. *Sci Rep* 2017;7: 15424. [PubMed: 29133913]
47. Delage B, Fennell DA, Nicholson L, et al. Arginine deprivation and arginosuccinate synthetase expression in the treatment of cancer. *Int J Cancer* 2010; 126: 2762–72. [PubMed: 20104527]
48. Rodriguez PC, Quiceno DG, Ochoa AC. L-arginine availability regulates T-lymphocyte cell cycle progression. *Blood* 2007; 109: 1568–73. [PubMed: 17023580] This article provides insights into how microenvironmental arginine depletion resulting from the secretion of arginase by MDSC in the tumor microenvironment (MEV) inactivates T cells.
49. See [ClinicalTrial.gov](https://clinicaltrials.gov).
50. Hornyak L, Dobos N, Koncz G, et al. The role of indoleamine-2,3-dioxygenase in cancer development, diagnostics, and therapy. *Front Immunol* 2018; 9:151. [PubMed: 29445380] This discusses the effects of tryptophan metabolism on the function of TIL and the value of IDO inhibition on immune surveillance.
51. Mitchell TC, Hamid O, Smith DC, et al. Epcadostat plus pembrolizumab in patients with advanced solid tumors: Phase I results from a multicenter, open-label phase I/II trial (ECHO-202/KEYNOTE-307). *J Clin Oncol* 2018;
52. Vijayan D, Young A, Teng MWL, Smyth MJ. Targeting immunosuppressive adenosine in cancer. *Nat Rev Cancer* 2017; 17: 7090–24. Review of adenosine-mediated immunosuppression and its potential reversibility.
53. Weber R, Fleming V, Hu X, et al. Myeloid-derived suppressor cells hinder the anti-cancer activity of immune checkpoint inhibitors. *Front Immunol* 2018; 11: 1310. Article implicating MDSC as one of the key microenvironmental factors limiting the clinical efficacy of immune checkpoint inhibitors.
54. Finke JH, Rayman PA, Ko JS, et al. Modification of the tumor microenvironment as a novel target in renal cell carcinoma therapeutics. *Cancer J* 2013; 19: 353–64. [PubMed: 23867518] This article discusses the use of VEGFR-targeted drugs such as sunitinib as a means of inhibiting the immunosuppressive effects of a particular subset of MDSC (i.e. those that are STAT3-dependent). The article supports the use of these agents as adjuncts to immune checkpoint inhibitors in the treatment of RCC – an idea recently validated in several clinical trials (see references 60–62).
55. Draghiciu O, Lubbers J, Nijman HW, Daemen T. Myeloid-derived suppressor cells – an overview of combat strategies to increase immunotherapy efficacy. *OncoImmunol* 2015; 4:e954829. A comprehensive review of different strategies to limit the immunosuppressive effects of MDSC in the tumor MEV.

56. Orillion A, Hashimoto A, Damayanti N, et al. Entinostat neutralizes myeloid-derived suppressor cells and enhances the antitumor effect of PD-1 inhibition in murine models of lung and renal carcinoma. *Clin Cancer Res* 2017; 23: 5187–5201. [PubMed: 28698201]
57. Liu JF, Deng WW, Chen L, et al. Inhibition of JAK2/STAT3 reduces tumor-induced angiogenesis and myeloid-derived suppressor cells in head and neck cancer. *Mol Carcinog* 2018; 57: 429–39. [PubMed: 29215754]
58. De Henau O, Rausch M, Winkler D, et al. Overcoming resistance to checkpoint blockade therapy by targeting PI3K γ in myeloid cells. *Nature* 2016; 539: 443–7. [PubMed: 27828943]
59. See [ClinicalTrial.gov](https://clinicaltrials.gov).
60. Atkins MB, Plimack ER, Puzanov I, et al. Axitinib in combination with pembrolizumab in patients with advanced renal cell cancer: a non-randomized, open-label, dose-finding and dose-expansion phase 1b trial. *Lancet Oncol* 2018; 19: 405–15. [PubMed: 29439857]
61. McDermott DF, Huseni MA, Atkins MB, et al. Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma. *Nat Med* 2018; 24: 749–57. [PubMed: 29867230]
62. Choueiri TK, Larkin J, Oya M, et al. Preliminary results for avelumab plus axitinib as first-line therapy in patients with advanced clear-cell renal cell carcinoma (JAVELIN Renal 100): an open label, dose-finding and dose-expansion phase 1b trial. *Lancet Oncol* 2018; 19: 451–60. [PubMed: 29530667]
63. Motz GT, Santoro SP, Wang LP, et al. Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors. *Nat Med* 2014; 20: 607–15. [PubMed: 24793239]
64. Yuan H, Cai P, Li Q, et al. Axitinib augments antitumor activity in renal cell carcinoma via STAT3-dependent reversal of myeloid-derived suppressor cell accumulation. *Biomed Pharmacother* 2014; 68: 751–6. [PubMed: 25081318]
65. Bex A, Etto T, Vyth-Dreese F, Blank C, Griffioen AW. Immunologic heterogeneity of the RCC microenvironment: Do targeted therapies influence immune response? *Curr Oncol Rep* 2012; 14: 230–9. [PubMed: 22362358]

- Clear cell RCC is one of several cancer types shown to respond to immune checkpoint inhibitors.
- The ccRCC microenvironment is extensively infiltrated by Tregs and various bone-marrow-derived cells (MDSC, TAMs) that suppress the immune system independent of the interaction between PD1 and its ligands.
- Several mechanisms by which these cells interfere with immune surveillance - e.g. amino acid depletion, NO and kynurenine production – have been identified.
- Pharmacologic agents that reduce the trafficking of these cells into tumor infiltrates or block their immunosuppressive function have been developed and are in clinical trials as adjuncts to immune checkpoint inhibitors.