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Angiogenic and immunological pathways in metastatic renal cell carcinoma: A counteracting paradigm or two faces of the same medal? The GIANUS Review

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ABSTRACT

In the so-called "antiangiogenic era" of recent years, a number of targeted therapies have been approved for the treatment of metastatic renal cell carcinoma (mRCC). Emerging information about the immunological features of mRCC and the immunomodulating properties of antiangiogenic agents, one of the standard treatments for mRCC, indicates that a more rational design of potentially synergistic combinations should be pursued. Indeed, immunotherapy has undergone a resurgence in clinical practice. In this narrative review, we discuss the immunological features of mRCC and the potential interactions that antiangiogenic agents may also exert on host immunity and tumor immunogenicity, possibly working on both sides of this complex cross-talk. Hence, the recall to Gianus, the ancient two-faced Roman God who was looking both at the future and the past. Treatment strategies will be also critically discussed.

1. Introduction

Over the last decade, in the so-called "*antiangiogenic era*", a number of targeted therapies have been approved for the treatment of metastatic renal cell carcinoma (mRCC) (Choueiri and Motzer, 2017; Vitale and Carteni, 2016). More recently, immunotherapy has undergone a resurgence in clinical practice (Gill and Agarwal, 2017). In 2016 the European Society for Medical Oncology (ESMO) guideline still recommended bevacizumab plus interferon (IFN)-α, sunitinib or pazopanib as first-line treatment in patients with good or intermediate risk and clear-cell histology and temsirolimus for the poor risk cases (Escudier et al., 2016). These indications have been completely changed with the recent publication of the Checkmate-214 results, comparing nivolumab + ipilimumab versus sunitinib in previously untreated advanced or metastatic RCC and suggesting the use of the two drug combination in cases with intermediate-poor prognosis but not in patients with good risk where sunitinib remains, at the moment, the standard treatment option (Powles et al., 2017; Motzer et al., 2018a,b). For patients treated beyond the first line, at the moment the most promising data derive from the nivolumab or cabozantinib studies, while other second-line treatments include axitinib, sorafenib or everolimus; enrolment into clinical trials is recommended where possible (Escudier et al., 2016).

The introduction of innovative immunotherapeutic agents to the treatment scenario for mRCC has undoubtedly provided clinicians with new therapeutic options, to be used either alone or in combination with existing drugs. Of note, modern treatment selection should rely upon a detailed knowledge of the molecular effects exerted by each molecule and not simply upon an empirical basis (Porta et al., 2017), although it must be admitted that gaining a precise definition of the pleiotropic mechanisms of action of immune checkpoint inhibitors (ICI) might be challenging. Furthermore, the emerging amount of information about the immunological and other features of RCC and the not-always acknowledged immunomodulating properties of most of the recent RCC standard treatments, such as tyrosine kinase inhibitors (TKI) or monoclonal antibodies, indicate that a more rational and careful design of potentially synergistic combinations can be achieved.

This narrative review discusses recent data on the immunological features of mRCC and the potential interactions that antiangiogenic agents may exert on host immunity and tumor immunogenicity,

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possibly functioning on both sides of this complex cross-talk. Hence the recall to Gianus, the ancient two-faced Roman God of beginning and transitions, who was looking at the same time to the future and to the past. On these bases, some speculations on how old and new drugs among the RCC treatment strategies could be rationally combined will also be discussed.

2. Metastatic renal cell carcinoma and the immune system

2.1. The paradox of high T-cell infiltrate correlating with poor prognosis, an exception confirming the rule?

Despite the overwhelming amount of new information that has enlightened the field of tumor immunology in recent years, the decades-old paradox about the correlation of the T-cell infiltrates with poor prognosis for RCC remains totally unexplained. In the difficult days when nobody believed in cancer immunotherapy, and we were struggling for evidence to strengthen our faith in the role of the immune system, the presence of cytotoxic T cell (CD)8+ T-cell infiltrate correlating with good prognosis in several solid cancers, including colorectal cancer, and the pioneering Immunoscore developed by Jérôme Galon (Galon et al., 2006, 2014), were some of the few positive things to hold onto. But in this scenario, RCC was already standing alone as an awkward exception: an immunogenic cancer responding to immunotherapy but showing a curious inverse association of the T-cell infiltrates with the worst patient outcome. Various hypotheses were formulated depicting a possible immunosuppressive nature of the infiltrating T cells, a prevalent role of natural killer (NK)-mediated immune surveillance, lack of human leukocyte antigen (HLA)-I expression, and HLA-G positivity. But that was not a random observation as, even now in this era of ICIs, the inverse correlation has been confirmed with multiple and sophisticated technical approaches, from immunophenotyping by conjugating mass spectrometry with CyTOF (Cytometry by Time-Of-Flight) to gene expression profiling (GEP), and it still remains totally unexplained. Indeed, are the RCC patients with the strongest CD8+, perforin+, IFN-γ+ signature T-cell infiltrates who respond to ICI in perfect alignment with the other cancer histologies, except for the not negligible difference that these are patients with the worse conditions and the most aggressive disease in which ICI may even mediate the so-called Lazarus effect and totally tilt the fate of patients? Hence, in line with the newest theories, we should be facing "hot tumors", highly programmed cell death (PD)-1 and programmed cell death-ligand (PD-L)1 expressing tumors in which immunity is inexplicably a negative prognostic factor. Although reasonable explanations are still lacking, it is tempting to speculate that what is driving immunogenicity of RCC is also driving disease aggressiveness. In other words, according to the most up-to-date trends, neoantigens (likely to stem from deletion/insertion events rather than mutations in RCC) possibly involve genes fostering tumor cell proliferation and progression. Alternatively, RCC is a quite immune-dependent tumor, hence the T-cell response, while there, is totally paralyzed by PD-1, thus the greater the immune response, the more immune escape there is and the more aggressive the disease gets: does this make sense?

It should be remembered that tumor-infiltrating lymphocytes are associated with bad prognosis, but this is rapidly reverted in the presence of PD-1 blockade, suggesting a connection. A final hypothesis, which is rather counter-intuitive but still likely, is based on the possibility that the T cell immune response is actually causing a detrimental effect on the tumor microenvironment, favoring tumor growth instead of hindering it. On the same basis that might underlie hyperprogression in patients receiving ICI, a strong type 1 IFN-related immunity might, for still unconceivable reasons, favor tumor proliferation and spreading. May tumor cells feed on IFN or tumor necrosis factor (TNF)? May they express specific receptors and receive a protumor instead of antitumor effect upon their triggering? PD-L1 expression is associated with resistance to IFN, but there is also limited evidence that, for instance, IFN-γ may have protumorigenic effects in particular circumstances (Zaidi and Merlino, 2011).

One key emerging issue is why tumor regression can occur in PD-L1 negative RCC tumors. Although this is still an open question in clinical setting, preclinical studies allow to speculate that tumor immunity is a systemic effect and, as such, can be modulated in sites other than tumor microenvironment. An interesting view on this topic comes from two recent studies investigating in murine models the role of PDL-1 as predictive factor of sensitivity to PD-1 blockade. By knocking-out PD-L1 expression in tumor cells or in host immune cells, both the studies conclude that host PD-L1+ antigen presenting cells (i.e. dendritic cells, monocytes, macrophages) play an essential part in the antitumor response mediated by anti-PD-1/PD-L1 mAbs. These data indicate that the expression of PD-L1 by these cells, that are present throughout the body (in lymph nodes, blood, bone marrow and many organs), could provide more predictive information about patient response to ICB therapy with respect to tumor microenvironment (Lin et al., 2018; Tang et al., 2018). Additional data sustaining the systemic nature of tumor immunity come from complex murine cancer models in which tumor rejection by different types of immunotherapies has been recently discovered to require a systemic response coordinated across tissues (Spitzer et al., 2017). This evidence again confirms that effective tumor immunity is only marginally influenced by tumor microenvironment while is critically regulated by systemic immune responses.

The question remains, "why should ICI work?" They probably work in those tumors where the T-cell infiltrate is so dense and "ready to be unleashed" that the tumor is rapidly eliminated upon PD-1 blockade.

2.2. Immunogenic patterns of mRCC as a potential predictor of response

The extraordinarily rich and heterogeneous immune scenario of clear cell RCC has made this cancer one of the most studied, and a training ground for novel and challenging techniques of molecular profiling. Among 19 human cancer histologies, high through-put analysis of immune infiltrate scored clear cell RCC as the histology showing the highest immune response both at a proteomic and transcriptional level. Of note, RCC tumors could be divided into T-cell enriched, heterogeneous and poorly infiltrated, with the former group including strong expression of pathways involved in T-cell activation, from antigen presenting cell machinery (APM) and HLA-class I expression, to CD8+T helper (Th)1-like responses, IFN-γ secretion, cytolytic granule release – in sum, all features of an antitumor acute full-fledged immune reaction (Şenbabaoğlu et al., 2016). These patients also express the highest level of immune checkpoints (Chen et al., 2016) and, as such, the highest chance of responding to ICI (Şenbabaoğlu et al., 2016), albeit at the same time, the most aggressive disease course and the worst prognosis.

Such an impressive immune reactivity does not seem to stem from a particularly high mutational load, i.e., from non-synonymous mutations that give rise to the so-called neoantigens, which makes RCC one of the "outliers" (together with triple-negative breast cancer) of the by now famous correlation between somatic mutation frequency and overall response rate to PD-1 blockade (Yarchoan et al., 2017). The puzzle has been solved only recently by the elegant work of Turajlic and collaborators, unraveling RCC immunogenicity to show that it relies on neoantigens arising from DNA deletions and insertions (rather than single nucleotide mutations) that are the highest in number in this cancer with respect to other histotypes (Turajlic et al., 2017). Thanks to its "exceptions", RCC is again pushing the field forwards by introducing the concept that T-cell neoantigens might originate from multiple DNA alterations if the resulting protein is sufficiently different from the wild-type counterpart to be detected as non-self by the immune system.

RCC was the first cancer where an" immune atlas" of the tumor microenvironment was developed and published, thanks to the introduction of a novel approach using conjugating mass-spectrometry with CyTOF, which allows the mapping at single cell level of the

cellular composition of the immune infiltrate. Astonishing data depict an unpredictable complexity of both the effector T cells and myeloid components, likely reflecting the multiplicity of "real-life" immune responses (Chevrier et al., 2017). Besides their graphical beauty, these analyses depict a dynamic, dense and tightly interconnected scenario where multiple T cell phenotypes interact with equivalent numbers of myeloid cell subsets, with functional outcomes that eventually influence the disease course and response to therapy. Although this study is a goldmine of invaluable information to be exploited in years to come, some key insights into the mechanisms of RCC-immune interaction have already emerged: PD-1 + T cells encompass different levels of exhaustion, from "fully exhausted", i.e., expressing a broad panel of immune checkpoints (TIM3, LAG3, Vista, cytotoxic T lymphocyte-associated protein 4 [CTLA-4], Gal9, GITR in addition, of course, to PD-1) to partially exhausted cells, showing for instance only PD-1 and/or CTLA-4. This evidence has key clinical implications, as the latter cells will be responding to PD-1 and /or CTLA-4 blockade while the first ones will be resistant and will need the blocking of additional pathways to be unleashed. The rich palette of myeloid cells and macrophage infiltrate also have important outcomes in terms of clinical transferability. Thanks indeed to the concomitant analysis of T cell profiles, it is possible to distinguish protumor and immunosuppressive myeloid cells (which would need to be eliminated) from antitumor and protective cells, which likely represent patrolling macrophages (Hanna et al., 2015) instead requiring to be potentiated.

Furthermore, macrophages associated with PD-1 + T cells and correlating with poor prognosis selectively express CD38 (Chevrier et al., 2017), which is intriguing evidence as an antagonist antibody directed against this molecule (daratumumab) is available for clinical use (Raedler, 2016).

Therefore, the RCC immune scenario is intriguing and a paradigm in the face of the complexity but also the remarkable therapeutic potentiality of antitumor immunity. Not to be forgotten, aside from T cells and macrophages, NK cells are also abundantly detected in RCC infiltrate (Geissler et al., 2015) and represent a potent ally in oncology (Bruno et al., 2014), thanks also to their powerful cytolytic activity against cancer cells, particularly those lacking HLA and antigenic determinants required for T-cell recognition (Vivier et al., 2012).

2.3. Immune escape and angiogenesis

It has long been known that angiogenesis and immunosuppression are companion processes in cancer (Motz and Coukos, 2011) and it is easy to understand why: when new vessels are required and tissues are healing, immunity has to be tolerant and allow these processes to occur. This takes place through a series of tightly regulated mechanisms, with the best example again to be found in RCC, through three main potential pathways: 1) direct immunosuppressive role of angiogenic factors; 2) accrual by angiogenesis and related mechanisms of immune regulatory effectors; and 3) detrimental effects of aberrant vasculature on immune physiology. Specifically:

In most cancers, hypoxia is the major driver of angiogenesis: low O_2 tension triggers the release of the transcription factor hypoxia-factor (HIF)1a, which in turn induces the expression of the proangiogenic vascular endothelial growth factor (VEGF), along with platelet-derived growth factor (PDGF)b and erythropoietin (EPO) to promote neoangiogenesis and compensate oxygen supply (Majeed et al., 2012). In RCC, HIF1a is constitutively induced by the hot-spot mutations of the Von Hippel Landau (VLH) gene, conferring to this tumor the characteristic angiogenesis-dependency (Cowey and Rathmell, 2009). Concomitantly, potent immunosuppressive factors are also boosted by HIF1a activation, including transforming growth factor (TGF)b and PDL-1, both responsible for restraining antitumor T-cell responses (Ruf et al., 2016). Elegant studies performed in preclinical models have recently enlightened the direct immunosuppressive role of VEGF. For instance, VEGF knockout (VEGF-KO) tumors display spontaneously increased T-cell infiltrate and activation, with a boost in chemokines such as CXCL10 and 9, both known to attract specific T-cell effectors at the tumor site (Courau et al., 2016). Most importantly, when tumors are silenced for the expression of VEGF, they grow much slower in immunocompetent mice but similarly to the wild-type tumor in immunocompromised animals. These are clear proofs that VEGF exerts immunosuppression and that the effects of antiangiogenic drugs may be passing through the recovery of immune-mediated tumor control. Furthermore, VEGFA induces the upregulation of immune checkpoints, fueling the exhaustion state in tumor-associated CD8 + T cells, while ICIs potentiate the therapeutic efficacy of antiangiogenic agents in murine tumor models (Voron et al., 2015).

HIF1a and VEGF are potent activators of inflammatory cells, particularly of a key subset of immunosuppressive effectors represented by myeloid-derived suppressor cells (MDSC) (Corzo et al., 2010; Noman et al., 2014). Accrual of MDSC at the tumor site is further potentiated by the ability of RCC cells to secrete multiple chemokines such as CCL2, CCL3, CCL4 5 and CXCL8, favoring myeloid cell migration to the tumor microenvironment, also enhanced by the activated state of microvascular endothelial cells (Parihar and Tunuguntla, 2014). MDSC, one of the deadliest culprits favoring cancer progression, contribute by establishing in the tumor milieu a vicious circle by promoting extra angiogenesis via VEGF secretion, supplemented by stroma remodeling, mesenchymal transition, and immunosuppression, altogether creating an unbreakable barrier to antitumor immunity (Kumar et al., 2016). Originating from the bone marrow, MDSC come from myeloid precursors as granulocytic and monocytic subsets and accumulate in the blood of patients with RCC in clear association with a bad prognosis (Najjar et al., 2017) and poor response to cancer vaccines (Walter et al., 2012). Upon reaching tumor lesions, these cells transdifferentiate into TAM (tumor-associated macrophages), contributing, together with resident macrophages, to creating the rich myeloid repertoire that is a feature of RCC (Chevrier et al., 2017). In line with this picture, neutrophil infiltration, likely reflecting granulocytic MDSC, is an independent prognostic factor for short recurrence-free and overall survival in localized clear cell RCC (Jensen et al., 2009). In addition, pretreatment neutrophil-to-lymphocytes ratio (NLR) significantly influences overall and progression-free survival of patients with RCC treated with first-line therapy (Keizman et al., 2012; Pichler et al., 2013). The increase of blood neutrophils and their relative ratio with blood lymphocytes are negative prognostic factors in most cancers, RCC included. Elevated NLR is associated with poor prognosis in a recent meta-analysis involving > 3000 RCC patients (Hu et al., 2015) as well as in the preoperative setting of non metastatic disease (Byun et al., 2016). As indicator of systemic inflammatory response, NLR at baseline or early decline are independent immunologic predictor of non-response to immunotherapy in RCC patients (Lalani et al., 2018). The reasons for the negative impact of neutrophils on cancer patients' prognosis stems for the recently emerged plasticity of granulocytic cells and their ability to polarize in diverse subpopulations with different functional properties including protumor and immunosuppressive effects (Zilio and Serafini, 2016).

Altered vasculature due to the neoangiogenesis process makes RCC vessels "resistant" to lymphocyte rolling, adhesion, and extravasation, contributing to limit the homing of antigen-primed T cells to the tumor site (Farsaci et al., 2014).

It is thus easy to understand that the RCC microenvironment might represent a type of "*immune trap*" where immune cells are attracted by tumor antigenicity, chemokine secretion, angiogenic and hypoxic mediators, and then rapidly converted into protumor forces, with T cells paralyzed by immune checkpoint regulation and myeloid cells conditioned towards immunosuppressive drivers. In such a scenario, we can speculate that ICIs might have limited room for action, as long as the immunosuppressive pressure of the angiogeneic-myeloid axis is in place (Fig. 1).

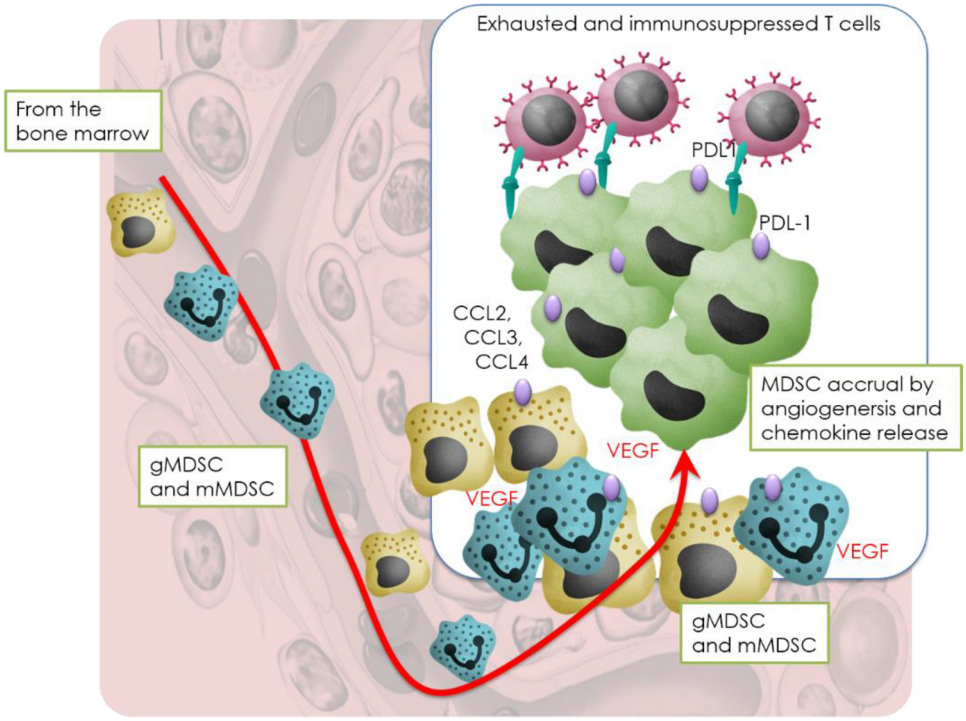

Fig. 1. Schematic representation of accrual of myeloid-derived suppressor cells (MDSC) by angiogenesis and chemokine release in the renal cell cancer tumor microenvironment. PDL, programmed cell death-ligand; VEGF, vascular endothelial growth factor.

3. VEGFRs-TKIs immunomodulatory properties

In keeping with the uniqueness of RCC, this cancer offers the invaluable option of testing antiangiogenics for their potential ability to interrupt the immunosuppressive vicious circle provoked by angiogenesis. Indeed, mounting evidence indicates that drugs interfering by diverse pathways with VEGF activity can also modulate hematopoiesis and immune functions (Powell et al., 2012; Santoni et al., 2012). While antagonists of VEGF and VEGFR work through the direct interference with immunosuppressive properties of the former or acting on immunosuppressive cells expressing the latter, the effects of TKIs on MDSC and myelopoiesis depend on their different selectivity for c-kit and fms-related tyrosine kinase 3 (FLT3) receptors expressed on hematopoietic stem cells and precursor cells (Santoni et al., 2014). Moreover, TKIs may modulate the tumor's ability to process and present antigens, promoting their immunogenicity through the expression of costimulatory molecules and changing the cytokine/chemokine profile in favor of a more immunogenic milieu.

However, in contrast with a strong rationale and convincing evidence in the preclinical setting, data available about the immunomodulating properties of antiangiogenics in RCC patients in terms of potential boosting of antitumor T-cell immunity and/or reduction of the immunosuppressive pressure of regulatory immune cells are often limited and contrasting. Furthermore, depending on the experimental conditions and the clinical settings, the same drug appears to exert positive or negative effects on tumor immunity and homeostasis, making the evaluation of the immunomodulating properties of antiangiogenics challenging to perform based on the available data. Nevertheless, some interesting data that could provide promising insights into the potentiality of antiangiogenics as immunomodulators have been reported over the last few years.

For instance, the TKI sunitinib ienhances RCC immunogenicity through the upregulation of innate immune receptors (the so-called *pattern recognition receptors*), mediating type I IFN induction pathways (Cao et al., 2016). Moreover, sunitinib regulates innate immune responses by influencing the processing and presentation of intracellular antigens by major histocompatibility complexes (MHC) and enabling the immune surveillance for intracellular antigens. Treatment with sunitinib reduces MDSC but acquired drug resistance is associated with the renewed expansion of these cells, often in combination with increased regulatory T cells (Finke et al., 2011). Importantly, MDSC inhibition correlates with reversal of suppression of type 1 T cell-mediated IFN-α production (Ko et al., 2010). Of note, neoadjuvant sunitinib improves tumor immunity in the RCC microenvironment by increasing CD8+ T-cell infiltrate and PD-1 expression by T cells, decreasing mMDSC and gMDSC frequencies, and improved *ex vivo* expansion of tumor-infiltrating cells (Guislain et al., 2015). Nevertheless, it is worth mentioning the potential detrimental effects that sunitinib may also exert on immune cells through its kinase inhibitor activity, such as the *in vitro* inhibition of PBMC proliferation and impairment of cytokine release (Gu et al., 2010). This effect is also evident with concomitant administration of an anti-PD monoclonal antibody (mAb), which reduced CD8+ cytolytic T-cell infiltrate in a preclinical model (Rayman et al., 2015). Finally, potential activity on glycogen accumulation and oxidative stress in myeloid cells may indicate a paradoxical worsening of immunosuppression in RCC patients (Amemiya et al., 2015).

Sorafenib can also suppress activation of human macrophages (Lin et al., 2013), thus enhancing antitumor T cell response (Edwards and Emens, 2010; Zhang et al., 2010). This drug can also mediate positive effects of cancer immunity by reducing the frequency of tumor-infiltrating regulatory T cells when administered in a neoadjuvant setting (Desar et al., 2011). In addition, sorafenib and bevacizumab, but not sunitinib, have been demonstrated to reverse the inhibitory effects of VEGF on monocyte-derived dendritic cell (DC) maturation and cell stimulation (Alfaro et al., 2009). Moreover, both sorafenib and sunitinib enhance tumor sensitivity to NK cell killing (Huang et al., 2011). On the other hand, several detrimental effects have also been reported for sorafenib. Hipp et al. reported that this drug, but not sunitinib, may inhibit DC antigen presentation and their capability to stimulate primary T cell responses, by reducing the secretion of cytokines and the expression of MHC and CD1a molecules (Hipp et al., 2008). *in vitro*, sorafenib, but not sunitinib, inhibited spontaneous and interleukin(IL)-

2-induced NK cell effector functions (Krusch et al., 2009).

More recently, pazopanib has also been investigated for its properties of regulating the tumor immune microenvironment. Unnikrishnan et al. reported that macrophage *in vitro* expansion is significantly inhibited by several TKIs, in particular with pazopanib (Chanmee et al., 2014; Unnikrishnan et al., 2015). Similarly to sunitinib, pazopanib can also restore DC immunostimulating functions by reducing their expression of VEGFR1 and VEGFR2, thus counteracting the immunosuppressive activity of VEGF (Dikov et al., 2005; Mimura et al., 2007; Porta, 2011). As for the other TKIs, pazopanib may inhibit T cells in their proliferation to IL-2 but not in their effector function, with a final effect of boosting T-cell memory Th1 responses (IFN-γ and IL-2 release) (Porta, 2011). Notably, it enhances PD-1 and PD-L1 expression, thus increasing the potential responsiveness to PD-1 blockade. Unlike sunitinib, pazopanib mediates direct apoptosis in RCC cells, blocks carbonic anhydrase activity, buffering tumor acidity, and is a potent inhibitor of the tumor-associated, hypoxia-inducible enzymes CA IX and XII, preserving the function of CTLs (Nakagawa et al., 2015; Shablak et al., 2014; Winum et al., 2012). In a very recent analysis presented at the 2017 American Society of Clinical Oncology (ASCO) meeting, monocyte-derived DCs from 10 healthy donors or patients with RCC were differentiated *in vitro* in the presence of pazopanib and sunitinib (Nuti et al., 2017). Strongly improved DC performance as antigen-presenting cells, together with the upregulation of HLA-DR, CD40 and CCR7 was observed with pazopanib but not sunitinib. The results were confirmed in DCs differentiated from RCC patients during pazopanib treatment (before and at 30 and 60 days) and suggest a reversal of the tumor-induced immunosuppression (Nuti et al., 2017). Moreover, in these patients only pazopanib treatment induced a defined circulating CD4+ T-cell population highly expressing the CD137 molecule. In another recent study, presented at the 2017 ESMO meeting, 16 patients with mRCC receiving first-line pazopanib were prospectively analyzed (Verzoni et al., 2017). Overall, pazopanib administration was associated with a significant rebalance of tumor immunity, more evident in patients experiencing clinical benefit (Rivoltini L, unpublished data).

As a potential clinical confirmation of the above-mentioned findings, a subgroup analysis of the CHECKMATE 025 trial presented at the 2016 Genitourinary Cancers Symposium showed that patients receiving prior pazopanib (32% of the total) had an overall survival not estimable in the nivolumab arm and 17.6 in the everolimus arm; on the other hand, corresponding figures for prior sunitinib recipients were 23.6 and 19.8 months, respectively (Motzer et al., 2016). This finding needs further exploring in dedicated studies.

Although the evidence appears intriguing, there is still a great deal of information to be discovered regarding the role of the RCC tumor environment and the modifications induced by antiangiogenic treatments. Deeper investigation of the functions of immune cells in the RCC microenvironment and of the immune-modulatory effects of TKIs will be crucial to optimize combined or sequential immunotherapeutic approaches with targeted agents in RCC advanced patients.

4. Combination or sequential approaches: rationale and clinical evidence

According to the above-mentioned evidence, there is a bidirectional link between angiogenesis and the immune system (Kuusk et al., 2017; Porta et al., 2017). This represents the basis for combination or sequential approaches with immunotherapy and VEGF inhibitors, where different antiangiogenic agents may exert immunomodulation with different activity and patterns. Moreover, VEGFR inhibition drives the activation of immune checkpoints (Kuusk et al., 2017). Of note, the efficacy of combination anti-VEGF therapy with immunotherapy (although not by checkpoint inhibition) was demonstrated in mRCC in 2007, in trials of bevacizumab plus INF-α (Bracarda et al., 2011; Escudier et al., 2007).

Despite this strong mechanistic rationale, the recent release of the positive preliminary results of the randomized phase II study ImMotion 150, testing the combination of bevacizumab plus atezolizumab versus atezolizumab alone or sunitinib (Sznol et al., 2015; McDermott et al., 2018) are of interest. The complete response rate was the highest in the atezolizumab-only arm (11%) followed by the combination (7%) and sunitinib arm (5%), and the rate of adverse events was high (41% for atezolizumab only, 64% for the combination therapy, and 69% with sunitinib); Intent-to-treat PFS HR for atezolizumab + bevacizumab or atezolizumab monotherapy versus sunitinib were 1.0 (95% CI, 0.69–1.45) and 1.19 (0.82–1.71), respectively; PD-L1+ PFS hazard ratios were 0.64 (0.38–1.08) and 1.03 (0.63–1.67), respectively (McDermott et al., 2018). However, at present evidence on these combination approaches is not completely established, with several studies still ongoing (see Kuusk 2017 (Kuusk et al., 2017) for an updated review of those investigations). Of note, most of these trials are in the phase I stage, and only a few phase II studies are being performed to date. It is worth noticing that a phase I study (Checkmate-016, NCT01472081) in mRCC has compared the combination regimens of nivolumab with sunitinib, pazopanib or ipilimumab (Amin et al., 2014). However, the results of this study showed efficacy but also relevant toxicities for the two TKI-based combinations. Several other trials have investigated pembrolizumab, an anti-PD-1 inhibitor, in combinations with bevacizumab or a TKI. In particular, a phase Ib study on the combination of pembrolizumab and bevacizumab in 16 patients with mRCC showed somewhat promising results, with a 71% rate of partial responses and no grade 3–4 adverse events (Dudek et al., 2016).

A phase Ib/II study on the combination of pembrolizumab and lenvatinib in the first-line setting documented an overall response rate of 69% and no dose-limiting toxicities (Taylor et al., 2016); this combination is now being investigated in a phase III study versus lenvatinib plus everolimus versus sunitinib (NCT02811861). The results of another phase I/II study combining pembrolizumab with pazopanib 600 or 800 mg (Keynote-018, NCT02014636), recently communicated, confirmed a significant toxicity profile for this combination (Chowdhury et al., 2017). Preliminary efficacy data were also reported for the combination of pembrolizumab and axitinib (Atkins et al., 2016). Axitinib was further investigated in a phase Ib combination study with the anti-PD-L1 inhibitor avelumab in the first-line setting (Larkin et al., 2016). No discontinuation due to treatment-related toxicity occurred, and a partial response was observed in six of 55 patients.

Early data from ASCO 2017 showed that an improved clinical benefit can be achieved in RCC patients by combining PD-1 blockade with IDO inhibition (Mellemgaard et al., 2017). Indeed, IDO (indoleamine 2,3-dioxygenase) is an enzyme involved in immune tolerance, that can be expressed by multiple components of TME including tumor and stromal cells. In the context of antitumor immune responses, IDO works as an immune checkpoint by inhibiting hyperactivated T and NK cells in a sort of negative feedback loop. Of note, RCC immunohistochemical evaluation revealed that IDO is almost exclusively expressed in tumor neo-vessels, and this expression seem to predict response to immunotherapy (Seeber et al., 2018). In this scenario, IDO inhibitors could be exploited not only to rescue suppressed immune responses but also to target tumor vessels and favor trafficking of tumor-specific T cells to tumor site (Prendergast et al., 2017).

However, The further development of IDO-based innovative combinations in mRCC has been recently blocked after the communication of initial negative results of this approach in melanoma (Cancer Discovery 2018).

To our knowledge, only one study has investigated antiangiogenic therapy (sunitinib) in combination with a monoclonal antibody against CTLA-4, namely tremelimumab (Rini et al., 2011). However, a high toxicity rate was reported, and therefore the combination was not considered worthy of further investigation. In line with the above-described, very preliminary evidence, the combination of VEGF Inhibitors

Table 1
Immunomodulating activity of antiangiogenics.

Drug	Adaptive Immunity	Innate Immunity
Bevacizumab	Induction of DC maturation and activation promoting antitumor T cell activity Reduction of Treg in blood of CRC patients Increase of HLA-I expression and CD8+ T-cell infiltration in synergy with PD-L1 blockade	Myeloid cell conversion into antigen-presenting cells (dendritic cells) by proper chemokine mileu (fractalkine)
Sorafenib	enhances therapeutic efficacy of a cancer vaccine by inhibiting STAT3 activation	reduces the frequency of infiltrating Treg in RCC neoadjuvant therapy decreases Treg in correlation with survival in HCC patients
Pazopanib	activated CD8+ T cells in peripheral blood of RCC patients	reduces monocytic and granulocytic MDSC, increasing cytolytic NK cells in RCC patients Enhances dendritic cell function
Sunitinib	Significantly decline in total leucocyte, CD3 total T-cell and CD4 counts in RCC patients Decreases of Tregs in association with better OS in RCC patients Raise of activated T cells in both peripheral blood and tumor site in sarcoma patients. Increased CD8+ PD-1+ T-cell infiltrate upon neo-adjuvant administration in RCC patients	Reduction of circulating neutrophils Monocytic MDSC decrease Reduced mMDSC and gMDSC frequency
Axitinib (mouse models)	Increase of CD3+T cell tumor infiltration Enhancement of antitumor CD8+T cell activity	Reduction of mMDSC immune suppressive activity STAT3-dependent reversal of MDSC accumulation

or TKIs and immunotherapy in patients with mRCC could be considered a rational strategy, although it appears burdened by substantial toxicity for some, but not all, of the TKIs tested to date. However, available results are derived only from phase I studies, and therefore investigation in phase II/III trials is necessary. Several phase III trials on this approach are ongoing and, in particular, initial results of the Immotion 151 trial (NCT02420821) on the combination of bevacizumab + atezolizumab have been preliminary presented and are expected to be updated for the end of 2018 (Motzer et al., 2018a,b)

On the other hand, sequential approaches appear more workable. For instance, nivolumab is active after one or more previous lines of TKIs, with some data from the CheckMate 025 trial suggesting, as previously stated, increased efficacy after a first-line therapy with pazopanib, compared with first-line treatment with sunitinib (Escudier et al., 2017a,b). We believe that the different immunogenic-modulating properties of TKIs should be considered in order to allow improved optimization of sequential regimens (Table 1).

In fact, a major challenge to precision medicine in cancer treatment is tumor heterogeneity, that can heavily hamper our ability to predict patient sensitivity to a defined treatment based, at least, on a single analysis of a single tumor biopsy. The complex technical approaches that are currently used to address this key issue are often applied to kidney cancer as a prototype of cancer displaying profound intra and inter-patient heterogeneity in tumor genetics, metabolomics and immunity (Okegawa et al., 2017; Thorsson et al., 2018).

One small retrospective study has investigated the optimal treatment choice after anti-PD-1 therapy (Nadal et al., 2015). Of 63 patients included, 84% had previously received at least one anti-VEGF agent prior to anti-PD-1 therapy. The percentage of objective responses observed with a VEGFR-TKI treatment (68% axitinib, 17% pazopanib and 1% by sorafenib) after anti-PD-1 antibodies was 27% (17/63; all partial responses), while 41% achieved disease stabilization, for an overall disease control rate of 68%. The incidence of grade 3–4 adverse events was overall modest (11% asthenia). It has been suggested that the prognosis of patients with mRCC may be further improved by the optimization and individualization of current sequential treatment with targeted agents and immunotherapy (Calvo et al., 2016).

5. Future perspectives

What can we expect, from a biological point of view, from these partially similar but potentially completely different approaches to the treatment of mRCC, namely different combination- and sequential-based regimens? In principle, we may expect similar overall results in terms of long-term responses but potentially different results in terms of response rates and progression-free survival data and cohorts of responders, such as in Checkmate 214 and IMmotion 151 studies. However, in the recent past sequential therapies proved to be more feasible than many combinations; certainly, what has happened to date is that the empirical sequential use of different agents was the therapeutic approach responsible for the overall improvement in the survival of patients with mRCC observed in the past decade or so. But will these potential differences in disease control translate into different probabilities of “chronicization”? Moreover, different cohorts of patients (i.e., cases with sarcomatoid features, different metabolic aspects also related to the volume of disease) would likely respond differently to different combination approaches.

Furthermore, it may be of interest to investigate the importance of the timing (time factor) of interaction between the two components of these possible combinations. And, in the case of confirmation of the importance of the “timing” factor, we should investigate which agent (or class of agents) should be started first and which should be initiated later and when, also considering a possible initial induction phase with both the agents (McDermott et al., 2018). Similarly, rules for stopping a therapy should also be investigated.

Nothing should be considered by itself in the world of biology. Only a few years ago, the discovery of angiogenesis and antiangiogenic agents, stimulated by the pivotal studies of Judah Folkman, represented a deep revolution which completely changed the treatment scenario of advanced mRCC. However, only limited advantage in overall survival, if any, was achieved, which was unexpected, especially in cases expressing high baseline levels of VEGF, the most awaited, but not confirmed, potential predictive factors of the “angiogenic era”.

We are now in the “new immunological era”. Modern immunotherapy approaches and data have for the third time completely changed the treatment scenario of advanced RCC with a rapid evolution toward a possibility of cure for an undefined, but possibly existing, percentage of patients, moreover characterized by an unexpected optimal candidate phenotype (sarcomatoid features, bulky disease, intermediate or poor prognosis). In this new and evolving scenario, antiangiogenic treatments may remain agents of interest with a major role not only within specific combination approaches, at the moment only speculative due to limited available data, but also within sequential regimens, e.g., in patients experiencing progression on a checkpoint inhibitor, a recent second-line condition that involves a majority of treated cases at the moment, or also as a remaining standard for good risk patients in the first line setting (McDermott et al., 2018).

The Immune atlas of surgically resected RCC indicates a highly

enriched immune microenvironment, where T cells and macrophages are key players of reciprocal cross-talks that influence disease-free survival and patient outcome.

Such a scenario encourages the application of immunotherapy approaches in a neoadjuvant setting for localized and locally advanced RCC as a strategy to render it surgically resectable. Despite the lack of large randomized clinical trials supporting this approach, and guidelines for presurgical therapies, immune checkpoints may be a promising approach in this setting, particularly in light of the positive experience in other cancer histologies. As a matter of fact, quite recent is the news that short-term treatment with nivolumab in a neoadjuvant schedule, beside being safe and not compromizing surgery, produces a quite high rate of pathological responses, implying a significant gain in terms of long-term benefit (Forde et al., 2018).

However, anything may change, and what we have at the moment is the possibility of a further consistent enlargement of the treatment scenario in mRCC: more weapons for a more tailored approach to the enemy, as recently suggested by the remarkable results of the first line ipilimumab plus nivolumab combination in intermediate and poor risk cases (Escudier et al., 2017a,b). This finding further corroborates the importance of T-cell infiltrate and tumor microenvironment in determining response to treatment - a new concept, but probably not the last in the crazy but continuously evolving scenario of advanced RCC

Conflict of interest statement

The Authors have no conflicts of interest directly relevant to this manuscript.

Author's contributions

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