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The Next Challenge in the Treatment of Renal Cell Carcinoma: Overcoming the Resistance Mechanisms to Antiangiogenic Agents

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1. Introduction

In recent years, important advances have been made in the medical therapy of metastatic renal cell carcinoma (mRCC). These advances are due on the one hand to the availability of many new molecules directed at specific biomolecular targets, and on the other hand to the understanding of both the pathogenetic mechanisms which have led to the identification of the key role of some gene mutations and angiogenesis, fundamental mechanisms in the process of tumour proliferation (1,2). In particular, there have been great developments in molecules capable of inhibiting the activity of the pro-angiogenesis receptors of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) such as tyrosine-kinase inhibitors (TKI) sunitinib, sorafenib, pazopanib, and monoclonal antibodies bevacizumab. Also inhibitors of specific pathways correlated with tumour growth such as the mTOR inhibitors temsirolimus and everolimus have become crucial drugs in the management of mRCC (3).

In the last few years, these drugs have radically changed the course of medical therapy of mRCC and other molecules currently in an advanced stage of clinical development will soon further enrich the therapeutic options of mRCC: axitinib (new, powerful anti-tyrosine kinases inhibitor), dovitinib (multi-target inhibitor particularly active against Beta Fibroblast Growth Factor Receptor (FGFR)), volociximab (new chimeric antibody with powerful anti-angiogenic activity directed towards the $\alpha 5 \beta 1$ integrin), regorafenib, cediranib etc.

As is known, RCC is a highly vascularized neoplasm which is dependent on VEGF-mediated angiogenesis. In fact, mRCC is among neoplasms showing the highest level of circulating VEGF. The importance of VEGF signaling for tumoral growth is also supported by the high frequency of von Hippel-Lindau (VHL) gene mutations found in about 70% of clear cell RCC. The VHL gene product regulates VEGF expression through suppression of the HIF transcription factor. Loss of function mutations in VHL lead to unregulated activation of HIF and overexpression of VEGF and other proangiogenic factors. For these reasons, anti-angiogenic drugs are particularly active in clear cell RCC and these drugs are currently considered the standard of care for first-line treatment. They include the monoclonal antibody bevacizumab which binds to the soluble ligand of VEGF, and the inhibitors of multiple receptor TK for vascular endothelial growth factor receptors (VEGFR-

1, VEGFR-2, and VEGFR-3), PDGFR- α and PDGFR- β , FLT3, the stem cell growth factor receptor KIT, and RET (4).

Despite the efficacy of TKI and bevacizumab therapy, the development of resistance is of major clinical concern; in fact, almost all patients with mRCC develop resistance and the disease inexorably progresses.

Conventionally, patients are categorised as “early progressors” when they develop resistance within approximately 6 months of the beginning of first-line therapy, and “late progressors” when they develop resistance later. About 30% of patients present a primary resistance to these drugs with a rapid spreading of disease and a very poor survival (primary refractory). Another 40% of patients, after an initial positive response, exhibit disease progression after about 1 year of treatment (5).

Consequently, the number of patients who receive a second line therapy after anti-angiogenic agents is only about half of the total. In the registrative phase III trial which compared sunitinib to interferon alpha, of 375 patients treated in the sunitinib arm, only 182 patients, corresponding to 56% of the total, received a second line therapy with an anti-mTOR or with a second anti-angiogenic drug (6). Similarly, in the AVOREN study with bevacizumab plus interferon *vs* interferon alone, of 325 patients in the bevacizumab plus interferon arm only 180 patients corresponding to 55% received a second line therapy (7). These data have been confirmed in the similar CALGB 90206 study (8). Notably, outside large controlled studies the percentage of patients receiving a second line treatment after anti-angiogenic agents is much lower. In a recent retrospective analysis of 645 patients from 7 centers and recruited in various studies, only 216 (30%) underwent second line therapy with anti-VEGF/anti-mammalian target of rapamycin (mTOR) drugs (9). Of interest, basal performance status resulted the only significant independent predictor of receiving second-line targeted therapy. Moreover, patients who received a second-line anti-VEGF drug appeared to have a similar overall survival to those who receive a second-line anti-mTOR drug (9).

The adoption of alternative angiogenic signaling pathways to compensate for inhibition of VEGF/VEGFR-mediated signaling seems to be the main, but not the only, common mechanism for the development of cancer resistance to VEGF pathway inhibitors. Nevertheless, to date very few data are available in literature about which alternative pathways are involved in resistant disease. Therefore, understanding the escape mechanisms of resistance to anti-angiogenic agents could improve clinical outcomes and the number of responsive patients.

2. Mechanisms of resistance in MRC

Resistance is generally defined as the capability of tumors to evade the antineoplastic effects of various treatments. About 30% of mRCC have an innate resistance to all available treatments independently from the type of anti-angiogenic agent used. Furthermore, treatment with mTORi as second line therapy results in primary resistance in about 20% of patients.

In this chapter we will attempt to give some partial responses to the numerous questions regarding the significance of resistance in mRCC: what is the definition of resistance? Which mechanisms sustain it? How can we overcome the resistance mechanisms?

2.1 Definition of resistance and its clinical implications

Resistance is divided into primary (also “refractoriness” or “intrinsic responsiveness”), which is characterized by a lack of efficacy to anti-angiogenic agents from the start of therapy, and secondary (also “acquired” or “adaptive” or “evasive” or “angiogenesis escape”), which begins after an initial response to TKI lasting for a period of time of variable length. Notably, early treatment failure involves all anti-angiogenic agents and all type of patients with mRCC.

Nevertheless, primary resistance to TKI in mRCC is heavily influenced by the patient risk score (low-intermediate *vs* poor) and by the type of first line therapy used. Primary refractory patients are about 20% in good-intermediate risk patients treated with different TKI, and it arises over 30% in poor risk patients (6, 8, 10, 11). In addition, the mTORi everolimus generally utilized as second line therapy is characterized by a resistance involving about 20% of patients (12). It is not clear if the patients who present primary resistance are the same as those who also present secondary resistance as data on this topic are not available.

The influence of prior therapies on the risk of primary resistance in patients with mRCC treated with sunitinib as first line has recently been reported in a systemic review and meta-analysis of 10 clinical studies including a total of 4,320 (13). The overall incidence of primary resistance to sunitinib was 22.4%. Moreover, the risk of developing primary resistance was significantly lower in patients with clear-cell cancer compared with non-clear-cell cancer. Notably, patients with prior cytokine therapy exhibited a significantly higher risk of primary progressive disease with sunitinib compared with those who had no prior treatment (RR, 1.18, 95% CI, 1.05-1.34, $p=0.007$). Although not statistically significant, there was a trend supporting that prior treatment with another mTKI sorafenib increased the risk of resistance to sunitinib in comparison with no prior treatment (RR 1.33, 95% CI: 0.98-1.80, $p=0.069$).

The conclusions of the Authors are that the risk of primary resistance to sunitinib may vary with tumor histology and prior therapies. In particular, previous exposure to cytokines significantly increased the risk of primary resistance suggesting that an immune mechanism may underlie the resistance to this drug.

A similar meta-analysis was done in patients treated with sorafenib as first line therapy (14). A total of 3,269 patients from 20 studies were included for the analysis. The overall incidence of primary resistance was 22.6% without significant difference between clear cell and non-clear cell nor between prior cytokine therapies and no prior treatment. Notably, patients with prior exposure to sunitinib had a significantly higher incidence of resistance when treated with sorafenib (52.2%). The conclusions of the Authors are that prior exposure to sunitinib but not cytokines significantly increased the risk of resistance with sorafenib in mRCC patients, suggesting that initial therapy with angiogenesis inhibitors may promote the development of resistance to sorafenib.

The conclusive considerations regarding the primary resistance to anti-angiogenic agents in mRCC are that about 30% of mRCC have an innate resistance to all available treatments and the resistance to angiogenic drugs seems to be independent from the type of TKI used.

In second line treatment, resistance to mTORi everolimus occurs in about 20% of patients (12), but when a second TKI was used, the risk of resistance increased to about 50%.

Therefore, considering that only 30%-50% of patients receive second line therapy, the re-challenge with a second TKI is an option available for very few and selected patients.

2.2 The resistance mechanisms

Resistance has yet to be thoroughly understood in kidney cancer. The “angiogenic escape” to anti-VEGF treatment may be dependent both on cancer cell phenomena or endothelial cell phenomena. It is believed that multiple factors affect resistance including factors that decrease angiogenesis and factors that increase angiogenesis. Often these mechanisms are present contemporarily in a single patient. Several of these factors need to be accounted for when developing a comprehensive treatment approach and in understanding why a patient may be resistant to any one approach.

Hypoxia is a known inducer of angiogenic response in a wide variety of tumors. Nevertheless, it is strongly believed that hypoxia is also the key mechanism of angiogenic escape. It involves induction of gene expression via HIF transcription factor of various pro-angiogenic factors including VEGF, FGFs and ephrins. When angiogenesis is inhibited, tumors are in a hypoxic state and develop new alternative pathways to guarantee their further growth (15).

2.3 Primary resistance mechanisms

It is thought that patients with primary resistance to TKI have already activated one or more alternative mechanisms of resistance in response to the selective pressure of their microenvironment. Probably these cases are not, or not only, sustained by angiogenesis mechanisms. Moreover, in patients with primary resistance there is frequently an upregulation of alternative pro-angiogenic pathways mediated by FGFR, interleukin-8 (IL-8), insulin-like GFR, ephrins, and angiopoietins. In particular, FGF/FGFR system has been reported as one of the most important escape pathways of anti-VEGFR therapies.

Other possible mechanisms include the pre-existing inflammatory cell-mediated vascular protection (myeloid cell); an hypovascularity status with consequent indifference toward angiogenesis inhibitors (desmoplastic stroma); the co-option of normal vessels without requisite angiogenesis (4, 16-18).

2.4 Secondary resistance mechanisms

Regarding secondary resistance, many Authors believe that it is precisely the state of **hypoxia** determined by anti-angiogenic drugs which is at the root of the onset of the *escape* mechanisms sustained by new HIF, FGF, IL-8, ephrine etc transcript factors, which lead to the activation of alternative pathways which support a “*new angiogenic wave*” (15). It is notable that during therapy with anti-VEGF the expression of new and ever-increasing pro-angiogenic factors is observed. It is known that the early phase of angiogenesis is generally characterized by a response to anti-VEGF treatment. On the contrary, the late phase of angiogenesis is characterized by the escape to anti-VEGF treatment. This late phase is sustained by FGF, IL-8 and other factors. It has been reported that in the presence of sunitinib the tumor is able to produce until 19 pro-angiogenic factors to rescue endothelia cell proliferation (19,20).

Function-blocking antibodies to VEGF receptors R1 and R2 were used to probe their roles in controlling angiogenesis in a mouse model of pancreatic islet carcinogenesis. Inhibition of VEGFR2 but not VEGFR1 markedly disrupted angiogenic switching, persistent angiogenesis, and initial tumor growth. In late-stage tumors, phenotypic resistance to VEGFR2 blockade emerged, as tumors regrew during treatment after an initial period of growth suppression. This resistance to VEGF blockade involves reactivation of tumor angiogenesis, independent of VEGF and associated with hypoxia-mediated induction of other proangiogenic factors, including members of the FGF family. These other proangiogenic signals are functionally implicated in the revascularization and regrowth of tumors in the evasion phase, as FGF blockade impairs progression in the face of VEGF inhibition (15).

Recently, it has been demonstrated that the FGF pathway is important in patients who develop resistance to sunitinib. Welti and colleagues (21) reported that FGF2 supports endothelial proliferation and de novo tubule formation in the presence of sunitinib and that FGF2 can suppress sunitinib-induced retraction of tubules. Importantly, these effects of FGF2 were ablated by PD173074, a small molecule inhibitor of FGF receptor signalling. They also showed that FGF2 can stimulate pro-angiogenic signalling pathways in endothelial cells despite the presence of sunitinib. Finally, analysis of clinical renal-cancer samples demonstrated that a large proportion of renal cancers strongly express FGF2. In conclusion, they suggest that therapeutic strategies designed to simultaneously target both VEGF and FGF2 signalling may prove more efficacious than sunitinib in renal cancer patients whose tumours express FGF2.

Interestingly, it has been demonstrated that FGFR is highly expressed in RCC. Tsimafeyeu and colleagues analyzed the expression of FGFR1 in 140 patients with mRCC. Expression of FGFR1 was observed in 98% of primary tumors and in 82.5% of lymph node metastases. Moreover, a significant rise in plasma bFGF levels was reported in patients with disease progression but a non-significant fall in patients with response or stable disease. Plasma VEGF-A level increased in patients with response whereas no detectable changes in plasma VEGF-A level was found in patients with progressive disease. The conclusions of the Authors are that plasma levels of bFGF and VEGF-A are altered in MRCC patients receiving sunitinib, and the increases in bFGF levels may represent biomarker of resistance to targeted therapy (22). Recently it has confirmed that the subset of clear cell RCC tumors with increased expression of FGFR1 is associated with a shorter progression free survival (23).

Also the role of IL-8 in resistance mechanisms seems to be determinant. In xenograft models, sunitinib resistance/refractoriness has been reported associated to higher levels of IL-8 (16). Moreover, the resistance to sunitinib was associated with a higher microvessel density, indicating an escape from anti-angiogenesis mechanisms. Finally, the addition of monoclonal antibody anti-IL-8 resensitized the tumor to sunitinib activity. The conclusions of the Authors are that IL-8 mediates resistance to sunitinib and could represent a candidate target to reverse acquired or intrinsic resistance to sunitinib.

Higher levels of IL-8 were associated with shorter progression free survival in mRCC patients treated in phase III trials of pazopanib (24).

Some Authors also demonstrated in pre-clinical models that antiangiogenic drugs could elicit malignant progression of tumors with an increase of local invasion and distant

metastasis. In particular, it has been reported that short-term treatment with a potent inhibitor of tumor angiogenesis is able to induce an acceleration of metastasis formation (25). Moreover, other Authors reported that angiogenesis inhibitors targeting the VEGF pathway had antitumor effects in mouse models of pancreatic neuroendocrine carcinoma and glioblastoma, but concomitantly these drugs elicit tumor adaptation and progression to stages of greater malignancy, with heightened invasiveness and in some cases increased lymphatic and distant metastasis (26). Increased invasiveness is also seen by genetic ablation of the VEGF-A gene in both models, substantiating the results of the pharmacological inhibitors. The realization that potent angiogenesis inhibition can alter the natural history of tumors by increasing invasion and metastasis warrants clinical investigation, as the prospect has important implications for the development of enduring antiangiogenic therapies (26).

Other two main mechanisms that could partially explain the ability of the tumor to become resistant to treatment are their capability to epithelial-mesenchymal transformation and the intra-tumoral heterogeneity.

The **epithelial to mesenchymal transition** (EMT) process has been described in different neoplasms and associated with metastatic disease, drug resistance, and development of angiogenesis (27-30). Treatment-associated tumor hypoxia has been reported to induce an EMT in several tumor models (31). How EMT as a mechanism of acquired resistance occurs in human tumors is unknown and deserves further investigation. In RCC, sarcomatoid phenotype is observed across all histological subtypes, and associated with a poorer prognosis and an increased resistance to VEGF inhibitors. A growing number of interdependent pathways have been linked to the induction of EMT, which, by definition, is a potentially transient/reversible phenotype of epithelial cancers. The reverted histologic phenotype observed in the xenografts also suggests that this escape mechanisms against anti-VEGF therapies may be transient (30, 32, 33).

According to this hypothesis, patients who have initially received clinical benefit from treatment with TKIs and then developed resistant disease may respond again to TKIs following a break from anti-VEGF therapies. The “holiday” period from anti-VEGF therapies may lead to “reset” the tumor microenvironment and reestablish a primarily EGF driven tumor growth. This hypothesis is supported by anecdotic reports of patients who were treated with sunitinib with initial response and subsequent progression who responded again to sunitinib following different targeted therapies such as mTOR inhibitors. The apparent transient/reversible mechanism of resistance to anti-VEGF therapies may also explain why clinical benefit has been reported by sequencing different anti-VEGF therapies despite the fact that these agents target the same VEGF pathway.

Regarding **intratumoral heterogeneity**, it has been demonstrated that mRCC, like other cancer, is characterized by a significant chromosomal instability that creates a selection of multiple clonal tumor subpopulations with an intrinsic multidrug resistance. Multiple intermixed cell subpopulations within one tumour differ by large genomic events as focal amplifications and deletions. For this reason, it is thought that single biopsy is often not representative of mutational landscape of the tumor (34). Recently have been developed methods able to study multiple subpopulations from different anatomic locations of neoplastic tissue (35).

	First line	Second line		Predictive factors
Drug/Author	N. Pts	Type of therapy	N. of Pts (%)	
Sunitinib <i>Motzer et al, JCO 2009</i>	375 (sunitinib arm)	Anti-VEGF/ anti-mTOR	182 (56)	-
Beva + IFN <i>Escudier et al, JCO 2010</i>	325 (bevacizumab-IFN arm)	TKI	180 (55)	-
TKI* <i>Vickers et al, Urology 2010</i>	645	Anti-VEGF/ anti-mTOR	216 (30)	Basal PS

* Multi-institutional studies
Abbreviations: PS: Performance status

Table 1. Percentage of patients who access to a second line treatment after TKi in mRCC

Setting	Author	Drug	% of incidence resistance
1 ^a line therapy Good-intermediate prognosis	Motzer, 2007	Sunitinib	22.4
	Ranpura, 2010*	Sorafenib	22.6
	Su, 2010*		
1 ^a line Poor prognosis	Hudes 2007	Temsirolimus	33
2 ^a line therapy	Motzer, 2008	Everolimus after TKI	20
	Ranpura, 2010*	Sorafenib after Sunitinib	33
	Su, 2010*	Sunitinib after Sorafenib	52.2

*Meta-analysis

Table 2. Percentage of patients with resistance according to the risk score and treatments in mRCC

Due to this genomic instability, it is strongly believed that resistance is a dynamic mechanism changing in different conditions (treatment pressure, hypoxia pressure, etc) and during the tumor growth. This aspect could explain the response obtained in some patients re-challenged with sunitinib. It thought that during treatment interruption, the selective pressure from drugs is removed and drug-sensitive clones re-growth. Recently, Zama and colleagues reported the results of a retrospective study describing 5 partial response (22%) of 23 mRCC patients re-treated with sunitinib (36, 37).

Also a “holiday” period from anti-VEGF therapies it is thought able to determine a reacquired drug-sensitivity by clones become resistant to TKI drugs.

Various genes associated with resistance have been identified which could become a target for future treatments. Recently, Sanjmyatas and colleagues also reported a specific gene expression signature able to characterize the different metastatic potential in ccRCC (38).

It has been demonstrated that some genes are hyperexpressed when there is resistance, for example the gene which encodes sphingosine kinase, calvasculin, chemokine receptor 4 (CXCR4), NNP1, arginase II, hypoxia-inducible protein-2 (HIG2) and VEGF. Other anti-angiogenic genes, however, show reduced expression in resistant tumors, such as the genes which encode cytokines associated with interferon-gamma, in particular IP10 (CXCL10) and Mig (CXCL9) (39). Sphingosine-1-phosphate (S1P), a pleiotropic bioactive lipid derived from sphingosine through sphingosine kinase (SphK) action, is dysregulated in a variety of disease conditions including cancer. S1P is a tumorigenic and angiogenic growth factor produced normally by blood platelets, mast cells and possibly fibroblasts in the tumour microenvironment. It is capable of determining proliferation and migration of endothelial cells, favouring angiogenesis and tumour proliferation. Notably, several tumors up regulate the expression of SPHK1, which may greatly contribute to the putative increased levels of S1P. In experimental models it has been demonstrated that SphK and S1P expression was increased during sunitinib resistance (39).

In xenografts models Bhatt and colleagues provided evidence that resistance to VEGF receptor therapy is due at least in part to resumption of angiogenesis in association with reduction of IFN γ -related angiostatic chemokines, and that this resistance can be delayed by restoration of angiostatic signalling with the concomitant administration of CXCL9 (40).

An emerging area of drug discovery called lipidomic-based therapeutics is in rapid develop. It directly targets pleiotropic bioactive lipids involved in cancer as well as other disorders. It has been postulated that S1P antibodies could represented a potential therapeutic strategies in the treatment of renal cancer (41).

Other mechanisms, not completely known, sustaining secondary resistance in mRCC include: **secondary mutations in tyrosine kinase receptors** (analogous to EGFR TKI); **recruitment of bone marrow-derived pro-angiogenic cells** which can obviate the necessity of VEGF signalling, thereby affecting re-initiaton and continuance of tumour angiogenesis; **increasing of pericyte** coverage of the tumour vasculature, serving to support its integrity and attenuate the necessity for VEGF-mediated survival signalling has been described; activation and enhancement of invasion and metastasis to provide **access to normal tissue vasculature** without obligate neovascularisation (4).

In table 3 are reported the main mechanisms of primary and secondary resistance in mRCC.

3. How can we overcome resistance to anti-angiogenic agents?

Many attempts have been made in the effort to overcome resistance to anti-VEGF treatments, but so far the results are disappointing. They include the use of non cross-resistant drugs, integrating or combining current treatment, optimization of sequential therapies and TKI re-challenge. Finally, several ongoing studies are trying to clarify the optimal sequence of the different drugs and the significance of the rechallenge with TKI in the treatment strategies.

As regards primary resistance, other than new experimental molecules the main route taken up until now has been to the combination of drugs for different biomolecular targets.

Primary resistance	<ul style="list-style-type: none">- Alternative pro-angiogenic pathways mediated by FGFR, interleukin-8 (IL-8), insulin-like GFR, ephrins, and angiopoietins;- Non angiogenic mechanisms<ul style="list-style-type: none">-Pre-existing inflammatory cell-mediated vascular protection (myeloid cell);-Hypovascularity status with consequent indifference toward angiogenesis inhibitors (desmoplastic stroma);-Co-option of normal vessels without requisite angiogenesis-Non clear cell histology
Secondary resistance	<ul style="list-style-type: none">- New angiogenic wave induced by hypoxia determined by anti-angiogenic drugs- Epithelial to mesenchymal transition- Intra-tumoral heterogeneity- Gene instability and gene iperexpression- Secondary mutations in tyrosine kinase receptors- Bone marrow-derived pro-angiogenic cells which can obviate the necessity of VEGF signalling;- Increasing of pericyte coverage of the tumour vasculature, serving to support its integrity and attenuate the necessity for VEGF-mediated survival;- Access to normal tissue vasculature without obligeate neovascularisation

Note: to bibliographic references see the text

Table 3. Main mechanisms of primary and secondary resistance in mRCC

To overcome secondary resistance, various strategies are being explored: increasing the dose of the current drug, the use of non cross-resistant drugs (for example changing to a mTOR inhibitor such as everolimus after a anti-angiogenic drug), changing to another VEGF inhibitor (for example sunitinib after bevacizumab, or sorafenib after sunitinib, or axitinib after sorafenib), the use of a “drug holiday” (12, 42, 43). However, results obtained so far have been modest, above all because in general the choice of strategy has been empirical rather than determined by a strong biological rationale. It is therefore desirable that new studies are founded on convincing preclinical data.

3.1 Drug combinations

As previously mentioned, several studies using combinations of drugs targeted to different biomolecular targets have been started with the aim of increasing clinical activity. Many attempts have been made to verify if the combination of drugs with different mechanisms of action was able to improve the results of single agent therapy. Unfortunately, so far this strategy has given disappointing or negative results with a heavier profile of toxicity.

Figure 1 shows the possible drug combination strategies in mCRC therapy.

Some combinations have proved to be very toxic and relatively inactive and therefore they were quickly abandoned, as was the case of the combination of TKI and bevacizumab (44, 45).

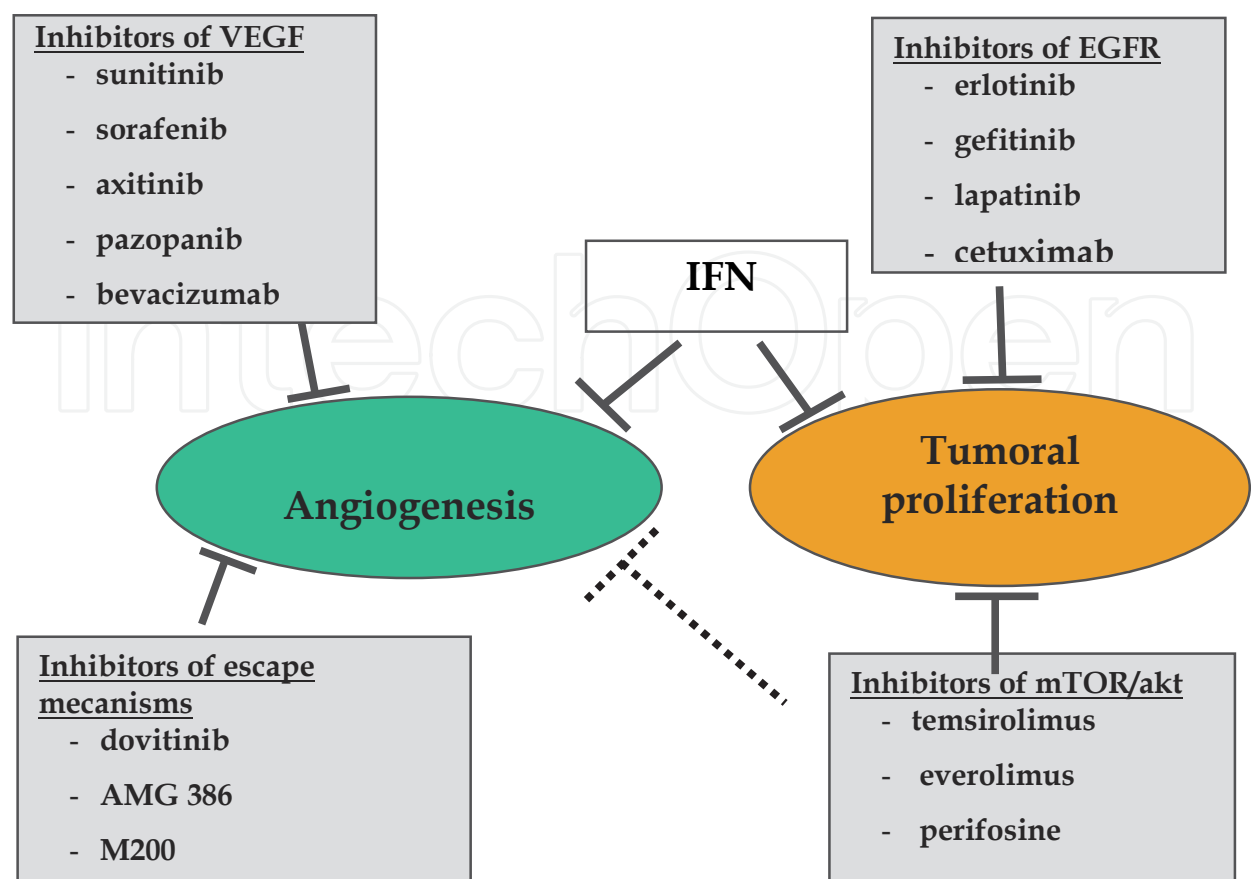


Fig. 1. Possible strategies of drugs association in mRCC

The high expression of EGFR in renal tumours from 50 to 90% (46), has also encouraged the use of anti-EGFR drugs in combination with anti-angiogenic agents. A study has recently been published by Motzer and colleagues at the Memorial Sloan-Kettering Cancer Center in New York in which gefitinib was combined with sunitinib in order to realise a double target. However, the reported results were similar to those obtained with sunitinib alone, but with an increase in toxicity. The Authors therefore discourage further studies on this combination (47).

Another route which seems more promising is the combination of bevacizumab and m-TOR inhibitors. However, after some encouraging early experiences (48-50), more recent studies are re-dimensioning the preliminary results. Of particular note are the results of a randomized phase II trial which compared temsirolimus and bevacizumab *vs* sunitinib *vs* interferon alfa and bevacizumab (TORAVA study). Unfortunately, in view of clearly higher toxicity in the temsirolimus plus bevacizumab arm, superiority of this combination compared to other arms was not reported (51). The conclusions of Authors are that the toxicity of the temsirolimus and bevacizumab combination was much higher than anticipated and limited treatment continuation over time, whereas clinical activity was low compared with the benefit expected from sequential use of each targeted therapy. Thus, this combination cannot be recommended for first-line treatment in patients with mRCC.

The combination of targeted drugs with immunological molecules such as interferon is proving to be more interesting. In particular, encouraging results have been reported on the combination of sorafenib and interferon alpha (52).

Generally speaking, even if it is necessary to wait for definitive results of ongoing phase III trials, the results reported so far do not encourage this therapeutic strategy.

Table 4 shows the most significant experiences of the different drug combinations used.

Drugs combinations/ Authors	Setting	N. of pts	PFS (Mo)	OR/SD	Toxicity	Notes
Sorafenib + Interferone <i>Gollob et al, JCO 2007</i>	phase II (1 ^a e 2 ^a line)	40	10	33% PR 29% SD	moderate	50% pts in 2 ^a line Good activity
Beva + Sunitinib* <i>Garcia et al, ASCO 2008</i>	phase I	31 (varius histology)	-	3/7 mRCC 1 /3 melanoma 1 surrene	moderate	Good activity
Beva + Sorafenib <i>Sosman et al, ASCO 2008</i>	phase I-II	48	14	25% PR 18% SD	high (hypertensi on, stomatite, hand-foot syndrome)	A negative experience
Sunitinib + Gefitinib <i>Motzer et al, AJCO 2010</i>	phase II	42	11	37% OR 34% SD	acceptable (diarrea G3-4 in 14% of pts)	Activity similar to that of sunitinib alone
Beva + Everolimus <i>Whorf et al, ASCO '08</i> <i>Hainsworth, Whorf, JCO 2010</i>	phase II 1 ^a e 2 ^a lines	80	9,1 7,1	30/23% OR 50/64% SD (1 ^a /2 ^a linea)	moderate	Good activity
Beva + Temsirolimus <i>Merchan et al, JCO 2009</i>	phae I-II	45	18/5.3	14,5%	acceptable	Long PFS
Beva + Temsirolimus vs Sunitinib vs IFN + Beva <i>Escudier et al, ASCO 2010</i> <i>(TORAVA Trial)</i>	phase II R Beva + Tem		8,2	27,3% OR 47,7% SD	High (41% of pts stop therapy)	Higher toxicity for experimental arm
	Sunitinib		8,2	23,8% OR 50% SD	As aspected	No confirmed results of phase II studies
	Beva + alpha IFN		16,8	39% OR 34% SD	As aspected	

Abbreviations: PFS: progression free survival.

Table 4. Most significant experiences with drug combinations in mRCC

3.2 New drugs and sequences

Clearly, another approach to overcoming resistance mechanisms is the use of new molecules which have a more powerful anti-angiogenic activity or which are more directly aimed at the targets involved in resistance mechanisms. Axitinib and dovitinib are of particular

interest here. In preclinical trials, axitinib has shown much more powerful antiangiogenic activity than other TKIs (53, 54). Furthermore, interesting results have been reported in phase II studies as a second line therapy after sorafenib with an overall response of 23% and a stable disease of 55%; interestingly, the progression free survival was 7.4 months, one of the longest ever reported (42).

Recently, beta FGFR has been identified as a new target for anti-angiogenic therapy. The system FGF/FGF receptor (FGFR) has been frequently reported as one of the most important escape pathways of anti-VEGFR therapies. It is involved in primary and secondary resistance mechanisms. The activation of FGFR3 is associated with cell proliferation and survival in certain cancer cell types. Thus, beta FGFR is proving to be a new interesting target for anti-angiogenic therapy.

Dovitinib, a new small multi-target molecule, is able to strongly binds to FGFR3 and inhibits its phosphorylation, which may result in the inhibition of tumor cell proliferation and the induction of tumor cell death. In addition, this agent may inhibit other members of the TK receptors superfamily, including the VEGFR; FGFR1; PDGFR3; FMS-like tyrosine kinase 3; stem cell factor receptor (c-KIT); and colony-stimulating factor receptor 1; this may result in an additional reduction in cellular proliferation and angiogenesis, and the induction of tumor cell apoptosis. A phase I/II has been recently concluded (55) and a large phase III clinical trial is ongoing to evaluate the efficacy of this drug as third line therapy in mRCC.

Other drugs of great interest are the monoclonal antibody anti-S1P, a molecule directly involved in resistance mechanisms already being developed clinically, and the anti- IL-8 and anti-IL-12 antibodies, which are still being studied in preclinical trials.

Regarding **sequences**, factors that could drive the choice of a more appropriate second line therapy are the response to primary treatment with TKI, the side effects reported in first line therapy, the patient risk score, and the histology of the tumor.

At present, the use of non cross-resistant mTOR inhibitor everolimus is the only registered agent available as second line therapy for mRCC resistant to anti-angiogenic drugs. In fact, the registrative trial showed a significant benefit in terms of PFS of 4.9 months for everolimus *vs* 1.9 months for placebo (12).

A second TKI as second line therapy is another option to consider for patients resistant to antiangiogenetic agents. Nevertheless, it is thought that this treatment must be propose only in carefully selected patients who did not show a rapid progression at the first line TKI. At present, this choice has a weaker recommendation because no definitive data from phase III studies are available yet.

Notably, in the AVOREN study it has been reported a median overall survival of 23.3 months for the sequence alfa interferone/bevacizumab followed by a second antiangiogenetic agent (TKI), with respect to only 21.3 months for the sequence alfa interferone/placebo followed by a TKI (7).

Some Authors believe that better clinical outcomes are correlated with a higher number of lines of treatments used rather than with the sequences utilized. Consequently, they have hypothesize specific sequences with the aim to utilize the maximum of therapeutic options available. Others suppose that the sequence TKI-TKI is to prefer to that with TKI-mTORi on

the basis of some preliminary experiences. Also the rechallenge with the same drug has been proposed, especially when a “holiday” period from anti-VEGF therapies is given to the patient. This break could be able to determine a reacquired drug-sensitivity by clones become resistant to TKI. Nevertheless, at present the majority of data are from small and retrospective studies regarding selected patients (36,37,56-61).

Recently, the results of the phase III study with Axitinib as second line therapy have been published (62). Axitinib resulted in significantly longer PFS compared with sorafenib. Nevertheless, in the subgroup of the patients treated previously with the TKI inhibitor the PFS was similar to what has been reported for the mTOR inhibitor everolimus (4.8 vs. 4.9 months).

Of course, further controlled studies are needed to determine the real effect of prior anti-angiogenesis therapy on the development of resistance to further therapies. A series of planned trials are evaluating what are the best sequences and timing.

4. Conclusions

The adoption of alternative angiogenic signaling pathways to compensate for inhibition of VEGF/VEGFR-mediated signaling seems to be the common mechanism for the development of cancer resistance to VEGF pathway inhibitors. Nevertheless, until now very few data are known about which alternative pathways are involved in resistant disease.

Many attempts have been proposed to overcome resistance. These include the use of non cross-resistant drugs, the optimization of sequential therapies, and the use of combined therapies. Unfortunately, all these approaches have given only modest results. Therefore, the overcome resistance mechanisms to antiangiogenic agents remains the next challenge in the treatment of renal cell carcinoma.

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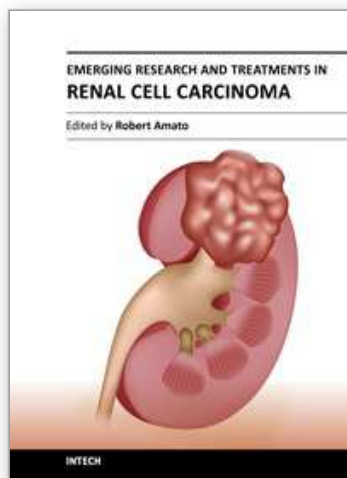
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The field of renal cell cancer has undergone a significant resurgence. This book summarizes up-to-date research and innovative ideas for the future in this rapidly changing field, which encompasses medicine, surgery, radiation oncology, basic science, pathology, radiology, and supportive care. This book is aimed at the clinician or scientist who has an interest in renal cell cancer, whether they are academic or nonacademic. The book covers tumor biology, molecular biology, surgery techniques, radiation therapy, personal testimonies, and present and future treatments of the disease that are on the horizon. The goal was to produce a textbook that would act as an authoritative source for scientists and clinicians and interpret the field for trainees in surgery, medicine, radiation oncology, and pathology.

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