Improving Outcomes in Metastatic Clear Cell Renal Cell Carcinoma by Sequencing Therapy

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OVERVIEW

Targeted agents have substantially improved outcomes in metastatic clear cell renal cell carcinoma. However, due to multiple mechanisms of evasive resistance, almost all patients progress at some point and may require subsequent therapies. Various agents have been explored after failure of first-line treatment in randomized clinical trials. However, so far few questions about the optimal sequence have been answered. Both everolimus and axitinib have been considered standard of care after failure of first-line VEGF-TKI; sorafenib has been proposed as an additional option. In clinical practice, several factors may influence the choice of subsequent treatment: these include considerations on appropriate drug exposure in first-line, gained insights on prognostic and predictive factors as well as mechanisms of resistance. Once the decision in second-line has been made and treatment has been initiated, treating physicians may already be challenged by the question of what to offer in third- and later lines. Treatment beyond second-line treatment isn’t supported by strong evidence, and at this stage of disease, retrospective reports on rechallenge may help to guide decisions. In addition, local treatment approaches including metastasectomy and stereotactic radiosurgery may help to optimize outcomes in all treatment lines.

Within the last 7 years, we have witnessed a paradigm change in the treatment of metastatic clear cell renal cell carcinoma (mRCC). Seven new agents have been approved based on the ability to improve response rates, progression-free survival (PFS) and/or overall survival (OS) in first-line (sunitinib, bevacizumab plus interferon-alpha, pazopanib, temsirolimus) or second-line treatment (sorafenib, everolimus, pazopanib, axitinib). Targeted agents are now recommended as the standard of care in this disease.1-3

The effect of individual agents on survival was less clear in the past because most clinical trials focused on PFS as the primary endpoint. Moreover, crossover designs and/or varying access to subsequent therapies may have confounded the OS endpoint. However, with a wider availability of an increasing number of agents, the effect on survival has become more evident. Patients treated in more recent clinical studies were often offered effective treatment options upon progression on the study drug. This may have contributed to an extended OS when compared with patients from the first pivotal trials. For example, the median OS in the sunitinib arm in the pivotal phase III trial comparing sunitinib with interferon-alpha1-5 was 26.4 months. In the recently completed RECORD-3 trial, which compared first-line sunitinib followed by second-line everolimus with the opposite sequence, the median OS for patients assigned to the sunitinib—everolimus sequence was 32 months.6 It is widely acknowledged that targeted agents have indeed substantially improved the outcomes of patients with clear cell mRCC, specifically changing it from a rapidly progressing fatal disease to a more chronic condition.

The excitement about the effect of targeted agents in mRCC has, however, faded slightly because treating physicians have had to accept that the benefits are time limited and that almost all patients progress at some point, albeit less rapidly than in the cytokine era. This has led to an increase in research on resistance to vascular endothelial growth factor (VEGF)-targeted therapies, which is essential for the development of effective agents for patients who fail first-line treatment.

Meanwhile, several clinical trials have been conducted to investigate the benefits of novel agents in the second- and third-line settings. In addition, analyses have been undertaken to identify predictive and prognostic factors in first and later treatment lines. Simultaneously, results from basic research have offered various explanations of the mechanisms of resistance. Despite the enormous efforts, it appears that so far few questions about the optimal sequence of drugs have been answered. Moreover, study designs have been driven not only by a biologic rationale but also by the readiness of a
new pharmaceutical compound to be evaluated in phase III trials. In addition, marketing considerations may have influenced study designs, for example, by exploring an agent in a treatment-line where it would not compete with another agent in the company’s portfolio rather than in the most appropriate treatment line.

Thus, treatment decisions on optimal sequencing remain difficult and various different interpretations can be drawn from the current evidence, ranging from (1) “the sequence of agents does not matter at all as long as the patient has favorable prognostic features and access to targeted agents,” to (2) “one sequence is more promising than another,” to (3) “better therapy management and understanding of resistance rather than new agents are important,” to (4) “novel agents are urgently required, none of the available agents is promising enough,” or, (5) “mode of action should, or should not be changed in second-line” etc.

**CLINICAL EVIDENCE ON SEQUENCING, LIMITATIONS OF THE PIVOTAL TRIALS, GUIDELINES**

Several sequences have been studied in randomized clinical trials and/or prospective and retrospective studies.

**VEGFR Receptor-TKI followed by mTOR Inhibitor, RECORD-1: Change of Mode of Action Versus Placebo in Second-Line**

Everolimus was the first agent to receive approval for second-line treatment after tyrosine kinase inhibitor (TKI) failure. Approval was based on the observation in the RECORD-1 trial that patients assigned to everolimus had a significantly longer PFS than those on placebo (4.9 vs. 1.9 months; p < 0.001; HR 0.30; 95% CI 0.22–0.40).

Although it can be postulated that the PFS benefit might be overestimated in the context of an inactive comparator, additional questions arise from the details of this trial.

First, it may be argued that RECORD-1 is a third-line rather than second-line trial. Among 416 patients evaluable for efficacy, only 89 had received a single prior treatment whereas 327 had received more than one. In fact, the proportion of patients that were studied in the sequence of most interest (sunitinib as the first and only antineoplastic treatment followed by second-line everolimus) was quite low (16%).

Moreover, the trial also included patients who had discontinued TKI treatment because of intolerance rather than resistance. From a biologic point of view, this is a completely different population that may have had a better outcome with continuation of first-line VEGF receptor (VEGFR)-TKI and improved therapy management.

Thus, the question arises as to whether it is appropriate to claim that everolimus is the standard of care after sunitinib failure. Based on the patient profile in this trial, some guidelines recommend everolimus as a potential third-line option in mRCC. However, it has also been argued that the benefits observed with everolimus were more pronounced in patients who had failed only one rather than two TKIs (median PFS 5.4 months and 4.0 months, respectively).

**VEGFR-TKI Followed by VEGFR-TKI or mTOR Inhibitor: The INTORSECT Trial—Change of Mode of Action after Sunitinib or Maintaining Mode of Action with a Weaker VEGFR-TKI?**

In this randomized phase III INTORSECT trial, patients who had progressed on sunitinib were randomly assigned to sorafenib (VEGFR-TKI) or temsirolimus (mTOR inhibitor), with PFS as the primary study endpoint. No statistically significant difference was found between treatment arms with regard to PFS (temsirolimus: 4.28 months; 95% CI 4.01–5.43; sorafenib: 3.91 months; 95% CI 2.80–4.21), but OS was significantly longer for patients assigned to sorafenib when compared with temsirolimus (sorafenib: 16.64 months; 95% CI 13.55–18.72; temsirolimus: 12.27 months; 95% CI 10.13–14.80; p = 0.014). The beauty of this trial is that only sunitinib-resistant patients were included; sunitinib discontinuation because of intolerance and prior systemic therapy with agents other than sunitinib were both exclusion criteria.

It is tempting to interpret the results of this trial as supporting a preference for the sequence TKI followed by TKI rather than TKI followed by mTOR inhibitor. However, neither temsirolimus nor sorafenib are commonly used after sunitinib and, since there are extensive differences between agents of the same class (sorafenib/axitinib or temsirolimus/everolimus), it should not be assumed that the results would have been similar if axitinib and everolimus had been compared in sunitinib-refractory patients. Thus, a general translation of these results into a therapeutic concept might be inappropriate.

**KEY POINTS**

- Phase III evidence supports the sequential use of targeted agents in clear cell mRCC; axitinib, everolimus (and sorafenib) are considered standard options.
- Treatment decisions in second- or later lines remain difficult because few questions about the optimal sequence of drugs have been answered.
- Identified mechanisms of resistance may influence treatment decisions toward change or maintenance of mode of action.
- Local treatment approaches (e.g., metastasectomy and stereotactic radiosurgery) may offer additional benefits and improve outcomes.
- Patients may require a personalized treatment approach: there might be no standard paradigm for the optimal sequence.
sunitinib: 10.71 months; HR 1.43; 95% CI 1.15–1.77), combined PFS (everolimus→sunitinib: 21.13 months vs. sunitinib→everolimus: 25.79 months; HR 1.26; 95% CI 0.94–1.73) and OS (everolimus→sunitinib: 22.41 months vs. sunitinib→everolimus: 32.05 months; HR 1.24; 95% CI 0.94–1.64) were all longer for the sequence sunitinib followed by everolimus. The authors concluded that the sequence sunitinib followed by everolimus remains the standard treatment paradigm. Another important observation in this trial was the high proportion of patients who did not receive second-line therapy.

First-Line Treatment Followed By VEGFR-TKI, AXIS: Axitinib Versus Sorafenib in the Second Line, Weaker or Stronger VEGFR-TKI After Sunitinib?

The AXIS trial\textsuperscript{10} was the first randomized trial to compare a targeted agent with an active comparator in the second line. Patients who had progressed on sunitinib, cytokines, temsirolimus or bevacizumab were randomly assigned to receive axitinib or sorafenib. The median PFS was significantly longer with axitinib compared with sorafenib (6.7 vs. 4.7 months; HR 0.665; 95% CI 0.544–0.812; p < 0.0001). Based on these results, axitinib was approved for second-line treatment after failure of sunitinib or cytokines.

However, results from subgroup analyses as well as the final OS data have raised further questions.

First, the patient population studied in this trial was quite heterogeneous. Although the majority of patients (54% each arm) received sunitinib as a first-line antineoplastic agent, other first-line strategies included cytokines (35% each arm), bevacizumab (8% each arm) and temsirolimus (3% each arm). PFS for axitinib and sorafenib patients in the sunitinib-refractory population was 4.8 months and 3.4 months (p = 0.0107; HR 0.741; 95% CI 0.573–0.958), respectively; whereas, the longest PFS was observed in patients who had progressed on cytokines (12.1 vs. 6.5 months; p < 0.0001). Although the PFS achieved with axitinib was still significantly longer when compared with sorafenib in sunitinib-refractory patients, it is quite similar to that achieved with everolimus in sunitinib-refractory patients in RECORD-1 (4.8 vs. 4.6 months).

The final analysis\textsuperscript{11} revealed no significant survival benefit for either treatment; however, OS was numerically longer for sorafenib in sunitinib-refractory patients (axitinib: 15.2 months; 95% CI 12.8–18.3 vs. sorafenib: 16.5 months; 95% CI 13.7–19.2). In addition, a trend toward longer OS was observed in the 130 intermediate-risk profile patients treated with sorafenib (sorafenib plus intermediate risk OS: 23.9 months; 95% CI 19.4–34.5) when compared with the 123 intermediate-risk axitinib-patients (axitinib plus intermediate risk OS: 18.8 months; 95% CI 14.9–23.8). Based on these findings, the recommendation-level for sorafenib as a second-line strategy after failure of TKIs in the European Association of Urology (EAU) guidelines is similar to that for everolimus and axitinib (all recommendation 1b).

Thus, the question arises whether these results are convincingly enough to claim that axitinib is the new standard of care in the second line, particularly after sunitinib.

Other Studies on VEGFR-TKI Followed by VEGFR-TKI

Various other prospective and retrospective trials have sought to identify the best sequence with regard to VEGFR-TKIs, mostly sunitinib and sorafenib, but also sorafenib and axitinib.\textsuperscript{10,12–24} The authors of these trial reports have generally come to two conclusions. First, it appears that there is no absolute cross-resistance on occurrence of resistance to the first-line TKI. Second, combined PFS is mostly longer for the sequence sorafenib→sunitinib compared with sunitinib→sorafenib, supporting the concept of treating with a weaker TKI first followed by a stronger TKI. However, the recently presented data from the SWITCH-I trial,\textsuperscript{25} comparing the combined PFS of two sequences (sunitinib followed by sorafenib or vice versa) showed no difference between treatment arms. Moreover, such trials raise additional questions: (1) whether combined PFS is a valuable endpoint, and, (2) how trials with this unusual endpoint can be incorporated into the current evidence.

Other Sequences

Bevacizumab followed by a TKI (sunitinib). Retrospective subgroup analyses from the two pivotal studies of first-line bevacizumab plus interferon-alpha compared with interferon-alpha revealed that patients may benefit from subsequent TKI treatment (sunitinib). An unplanned exploratory analysis of the AVOREN trial\textsuperscript{26,27} showed a longer median OS in patients receiving a TKI after bevacizumab plus interferon-alpha compared with patients receiving a TKI after interferon-alpha plus placebo (OS 38.6 vs. 33.6 months, respectively; HR 0.80; 95% CI 0.56–1.13). Subsequent sunitinib appeared to have a particularly marked effect on median OS (43.6 months).\textsuperscript{28} A subgroup analysis from a similar trial\textsuperscript{29,30} showed that median OS was longer for patients who received any subsequent therapy, regardless of previous treatment arm (31.4 months and 26.8 months with subsequent treatment, respectively; 13.1 months and 9.1 months without subsequent treatment, respectively). Although the authors stressed the risk of bias associated with retrospective analyses, it appears that the sequence of bevacizumab plus interferon-alpha followed by a TKI merits further prospective investigation. The safety and efficacy of sunitinib in bevacizumab-refractory patients has also been explored in a phase II trial; in 61 patients, the objective response rate was 23% and the median PFS 30.4 weeks.\textsuperscript{29} Similarly, in patients refractory to bevacizumab, Choueiri et al reported a PFS of 9.3 and 4.2 months with sunitinib or sorafenib, respectively.\textsuperscript{12}

These findings suggest a potential benefit for the sequence of bevacizumab followed by a TKI. However, as these results were obtained from retrospective analyses or small phase II trials, the data should be interpreted cautiously and with awareness of all biases and limitations.
The Guidelines

Table 1 outlines the current National Comprehensive Cancer Network (NCCN), European Society for Medical Oncology (ESMO), and EAU guidelines for the treatment of mRCC.1–3 In the NCCN guidelines, everolimus and axitinib both have the highest level of recommendation (category 1) for use after failure of first-line targeted agents. According to the ESMO guidelines, axitinib has a higher level of recommendation (Ia) than everolimus (IIa). Finally, the EAU guidelines provide identical recommendation levels for axitinib, sorafenib and everolimus (all Ia) after failure of prior TKI-treatment. Hence, selecting the appropriate treatment to optimize the outcome for the individual patient may prove challenging.

### TABLE 1a. Guidelines for First-Line Treatment of Advanced/Metastatic Clear Cell Renal Cell Carcinoma

<table>
<thead>
<tr>
<th>NCCN Predominant Clear Cell Histology</th>
<th>Relapse or Stage IV and Medically or Surgically Unresectable</th>
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<tbody>
<tr>
<td>Clinical trial or</td>
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<tr>
<td>Sunitinib (category I) or</td>
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<tr>
<td>Temsirolimus (category I for poor prognostic patients), category 2b for selected patients of other risk groups or</td>
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<tr>
<td>Bevacizumab plus IFN (category I) or</td>
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<tr>
<td>Pazopanib (category I) or</td>
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<tr>
<td>High dose IL-2 for selected patients or</td>
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<tr>
<td>Sorafenib for selected patients and best supportive care</td>
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<tr>
<th>ESMO Clear-Cell Histology</th>
<th>Good or Intermediate Prognosis</th>
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<tbody>
<tr>
<td>Standard option</td>
<td>Sunitinib (Ia)</td>
</tr>
<tr>
<td>Bevacizumab plus IFN (Ila)</td>
<td></td>
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<tr>
<td>Pazopanib (Ila)</td>
<td></td>
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<tr>
<td>Alternative options</td>
<td>Sorafenib (IIb)</td>
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<td>IL-2 (IIlc)</td>
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<tr>
<th>Poor Prognosis</th>
<th>Standard option</th>
<th>Temsirolimus (IIa)</th>
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<tr>
<td>Alternative options</td>
<td>Sorafenib (IIb) best supportive care</td>
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<tr>
<th>EAU Clear Cell RCC</th>
<th>Favorable or Intermediate Risk Group</th>
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<tbody>
<tr>
<td>Standard</td>
<td>Sunitinib (Ib)</td>
</tr>
<tr>
<td>IFN plus bevacizum (Ib)</td>
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<tr>
<td>Pazopanib (Ib)</td>
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<tr>
<td>In selected patients</td>
<td>IFN-alpha (Ib)</td>
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<td>High-dose IL-2 (Ib)</td>
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<tr>
<th>Poor Risk</th>
<th>Temsirolimus (Ib)</th>
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### TABLE 1b. Guidelines for Second-Line Treatment

<table>
<thead>
<tr>
<th>NCCN Predominant Clear Cell</th>
<th>Clinical Trial or Targeted Therapy</th>
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<tbody>
<tr>
<td>After TKI</td>
<td>Everolimus (category I)</td>
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<tr>
<td></td>
<td>Axitinib (category I)</td>
</tr>
<tr>
<td></td>
<td>Sorafenib (category 2a)</td>
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<tr>
<td></td>
<td>Sunitinib (category 2a)</td>
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<tr>
<td></td>
<td>Temsirolimus (category 2b)</td>
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<tr>
<td></td>
<td>Bevacizumab (category 2b)</td>
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<td></td>
<td>Pazopanib (category 3)</td>
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<tr>
<th>After Cytokines</th>
<th>Axitinib (category I)</th>
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<tr>
<td>Sorafenib (category I)</td>
<td>Sunitinib (category I)</td>
</tr>
<tr>
<td>Pazopanib (category I)</td>
<td>Temsirolimus (category 2a)</td>
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<tr>
<td>High dose IL-2 (category 2b)</td>
<td>OR IL-2 (category 2b)</td>
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<td>AND best supportive care</td>
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<tr>
<th>After VEGF-Pathway-Inhibitor Standard Treatment Options</th>
</tr>
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<tbody>
<tr>
<td>Everolimus (Ila)</td>
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<tr>
<td>Axitinib (Ia)</td>
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<tr>
<th>After VEGF-Pathway Inhibitor Alternative Treatment Options</th>
</tr>
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<tbody>
<tr>
<td>Clinical trial</td>
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<tr>
<td>Shifting TKIs (IIIb)</td>
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<td>After Cytokines</td>
</tr>
<tr>
<td>Sorafenib (Ia)</td>
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<tr>
<td>Sunitinib (IIla)</td>
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<td>Pazopanib (IIa)</td>
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<td>Axitinib (Ia)</td>
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<tr>
<th>EAU Clear Cell RCC</th>
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<tr>
<td>After Prior TKI</td>
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<tr>
<td>Axitinib (Ib)</td>
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<tr>
<td>Sorafenib (Ib)</td>
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<tr>
<td>Everolimus (Ib)</td>
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<tr>
<th>After Cytokines</th>
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<tr>
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<td>Pazopanib (Ib)</td>
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Abbreviations: EAU, European Association of Urology; ESMO, European Society of Medical Oncology; IL, interleukin; IFN, interferon; NCCN, National Comprehensive Cancer Network; RCC, renal cell carcinoma; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor.

In clinical practice, treatment decisions may be based not only on guidelines, but also on clinical considerations, including a patient’s comorbidities, the expected toxicity, and prognostic factors identified. Moreover, with increasing research on mechanisms of resistance, physicians may be tempted to take molecular aspects into account when considering what the best second-line treatment for the patient is.
OPTIMIZING OUTCOMES: CLINICAL AND BIOLOGICAL CONSIDERATIONS

Apart from guidelines, several other considerations may influence decisions on how to best sequence the available agents in clinical practice.

Inappropriate Exposure, Increased Drug Metabolism, Inappropriate Treatment Duration in First Line

In clinical practice, it should be questioned whether resistance has occurred at all. Treatment-related toxicities may lead to dose reductions and an inappropriate individual dose may result in disease progression in the absence of acquired resistance. Figures 1a and 1b show CT-scans of a patient who initially achieved complete response (CR) of pancreatic metastases with sunitinib 50 mg, but who relapsed shortly after dose reduction to 37.5 mg because of stomatitis (1a). Dose escalation to 50 mg restored CR (1b), which has been maintained until now; the current first-line PFS of this patient (who was classified as intermediate risk) is longer than 42 months. Notably, this patient only experienced hypertension with a sunitinib dose of 50 mg. This observation is consistent with (1) a meta-analysis that found that increased sunitinib exposure was associated with clinical outcomes,31 and, (2) the observation that treatment-related hypertension is a reliable biomarker for treatment outcome.32

Inappropriate exposure leading to progression may also occur after initially successful treatment with the standard dose as a result of an increased drug metabolism and excretion.33 In this case, gradual dose intensification may represent the most appropriate strategy. For instance, Pili R et al34 observed that sunitinib-induced resistance may be overcome in part by dose escalations in both xenografts models and in patients with mRCC.

Thus, dose escalation may restore responsiveness to treatment and improve outcome in first and/or later lines of treatment. In this context, physicians should also be aware of the influence of other medications on the outcome. Concomitant administration of drugs such as cytochrome P450 3A4 inducers that interfere with the metabolism of the antitumor drug may also result in inappropriate drug exposure. In this situation, an increase in the dose of the antineoplastic drug might be required to achieve optimal drug exposure.

Prognostic Factors That May Influence Treatment Decisions in the Second Line

For both sequences, VEGFR-TKI followed by mTOR inhibitor and VEGFR-TKI followed by VEGFR-TKI, various factors that may have influenced PFS and/or OS have been studied.

In the RECORD-1 trial, independent prognostic factors for shorter PFS were prior sunitinib, presence of liver/bone/lymph node metastases, and elevated neutrophil counts (inflammation). Independent prognostic factors for OS were poor performance status, prior sunitinib, increased corrected serum calcium level, anemia, liver and bone metastases, and inflammation (increased neutrophils).35 Calvo et al showed that PFS with everolimus is longer if the patient had received only one rather than two prior TKIs.8

Other authors have found that the response to a first-line TKI and prolonged PFS on a first-line TKI are both associated with longer PFS and OS with everolimus in second-line.36

In the AXIS trial, the following factors were found to be associated with shorter survival: prior treatment with sunitinib, ECOG performance status of 1, less than 1 year from initial diagnosis to treatment in AXIS, more than one metastatic site, liver or bone metastases, anemia, increased corrected serum calcium or lactate dehydrogenase level, increased alkaline phosphatase level, and neutrophils. An

FIG 1. (A) Relapse of pancreatic metastases after sunitinib dose reduction from 50 mg to 37.5 mg. (B) Complete remission of pancreatic metastases after dose escalation to 50 mg.
increase of diastolic or systolic blood pressure was an independent predictor for improved OS.11

Many of these factors have been confirmed in other retrospective analyses and various treatment sequences.23,37,38

However, with the exception of hypertension, none of these prognostic factors seem to be useful to select treatment, because they reflect the individual biology/aggressiveness of the tumor rather than the sensitivity of patient’s tumor to a specific agent. Response to a first-line TKI was probably the strongest driver for selection of second-line treatment, since it reflects sensitivity to VEGF inhibition. However, a large analysis of 464 patients found no correlation between response and PFS with first-line treatment with sunitinib, sorafenib, or bevacizumab and subsequent second-line treatment (sunitinib, sorafenib, bevacizumab, pazopanib, axitinib).39

### Predictive Factors That May Influence Treatment Decisions in the Second Line

Little is known about predictive factors that may help guide therapy choices in second line. In a retrospective analysis, Nishikawa et al40 measured expression levels of multiple components in the mTOR-signaling pathway in nephrectomy specimens to identify predictive factors for susceptibility to mTOR inhibitors. Expression levels of five molecular markers PTEN, phosphorylated (p)-Akt, p-mTOR, p-p70 ribosomal S6 kinase, and p-4E-binding protein 1 (4E-BP1) were evaluated, as well as clinical parameters. On multivariate analysis, p4E-BP1-expression and presence of bone metastases were found to be significantly associated with PFS. The authors suggested that it would be useful to consider these markers when selecting patients for mTOR inhibitor treatment.

However, the expression profile of the primary tumor may not be reliable enough to guide treatment for metastases. Gerlinger et al41 used multiregion sequencing to show that there is a large amount of intratumor heterogeneity in multiple spatially separated samples from primary tumors and associated metastases.

### Mechanisms of Resistance That May Influence Treatment Decisions in the Second Line

Evasive resistance is observed in patients who initially derived benefits from first-line therapy and then progressed, despite adequate drug exposure. This phenomenon is not fully understood, but several experiments suggest that a hypoxic tumor microenvironment contributes strongly to acquired resistance.42 Several relevant observations have been made in this context including (1) devascularization of tumors with subsequent hypoxia-inducible factor 1(HIF1)-alpha upregulation followed by an increase in circulating VEGF; (2) upregulation of proangiogenic pathways that are VEGF independent or less VEGF dependent, involving, for example, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), interleukin-8 and others;43-45 (3) increased pericyte coverage to protect endothelial cells46; (4) increased mobilization of vascular progenitor cells from the bone marrow47; (5) increased motility of tumor cells to escape from the hypoxic microenvironment;46 and, (6) metabolic adaptation.48 Moreover, resistance has been linked to epithelial to mesenchymal transition (EMT), for example, from clear cell type to sarcomatoid type.42

Several of these findings may have an effect on treatment choices in second and later lines; however, it should be emphasized that this translation into clinical practice remains highly speculative.

### 1. Observations That May Influence a Decision toward a Change in Mode of Action or Drug Holidays from VEGF Inhibitors

1.1 The phenomenon of EMT has been described in the tumor of a patient who had progressed on sunitinib.42 The tumor was initially conventional clear cell RCC without sarcomatoid features, but, after progression on sunitinib, histologic examination of an excised skin metastasis showed pure sarcomatoid RCC without evidence of clear cell histology. The mesenchymal markers vimentin and HIF1-alpha were expressed, suggesting an epithelial-mesenchymal transition. Interestingly, after transplantation of the metastasis into mice, the sarcomatoid histology was lost; clear cell histology was restored and the xenografts regained sensitivity to sunitinib. Similar observations were made with sorafenib in a murine model, where a transcriptome profile of the tumors revealed that the gene expression profile of tumors after re-implantation resembled that of the untreated tumors and was distinct from the resistant tumors.49 These data suggest that the tumor microenvironment contributes to acquired resistance to VEGF-TKIs.

Changing the mode of action in second line by targeting the tumor microenvironment upon occurrence of resistance appears tempting in such a situation. However, it is quite challenging to identify the optimal target, since the tumor microenvironment consists of many different compartments (extracellular matrix, cellular, liquid) with multiple cells, growth factors, proteases, cytokines, and proteins involved.40

The reverted histologic phenotype observed in the xenograft model discussed above also suggests that the escape mechanisms from VEGF-therapy may only be temporary. Patients may respond to a TKI again after a break from anti-VEGF therapies. The “holiday” period may lead to resetting of the tumor microenvironment and re-establishment of a primarily VEGF-driven tumor. A change of mode of action after failure of sunitinib might therefore appear reasonable and it could be argued that the break from VEGFR-TKI might be the most important “mode of action” of everolimus in the second line.

1.2 Another observation that supports a change of mode of action was made by Paez-Ribes et al51 They demonstrated in two distinct genetically engineered mouse models of cancer that therapeutically effective antiangiogenic treatment can elicit an adaptive evasive response involving a more aggressive phenotype with increased invasion and metastatic spread. Thus, it might be worth considering that prolonged VEGF inhibition may be disadvantageous.

1.3 The concept of changing mode of action is also sup-
ported by the work by Rosa et al who investigated the association between key signaling proteins involved in angiogenesis and proliferation and response to TKI and mTOR inhibitors in first- and second-line murine models. They found that pretreatment with sunitinib reduced the response to subsequent VEGFR-TKIs (sunitinib and sorafenib) but not mTOR inhibitors. After first-line sunitinib, second-line everolimus was more effective than VEGFR-TKI rechallenge in interfering with signaling proteins, VEGF, and interleukin-8.

1.4 Another driver toward mTOR inhibition in the second line is the observation that RCC cells may adapt to a hypoxic antiangiogenic environment (caused by first-line VEGF inhibition) by activation of the mTOR-pathway. Activated mTOR was shown to be involved in metabolic adaptation by sensing the availability of amino acids, metabolic fuel, and energy, thus, supporting cells by increasing their access to nutrients. Hence, mTOR inhibitors may help to reduce the ability of RCC cells to cope with metabolic stress.

2. Observations That May Influence a Decision toward Maintenance of Mode of Action

2.1 Ebos et al demonstrated in a murine model that short-term sunitinib treatment may accelerate experimental multi-organ metastasis and decrease survival. This may be important not only in the context of adjuvant treatment or treatment discontinuation on complete remission, it may also suggest that VEGF inhibition beyond progression might be important.

2.2 The decision to maintain VEGFR inhibition may also be driven by the awareness of the limitations of currently available mTOR inhibitors in mRCC. The mTOR inhibitors currently approved for mRCC—everolimus and temsirolimus—primarily inhibit activation of mTOR complex 1 (mTORC1, RAPTOR), but not mTOR complex 2 (mTORC2, RICTOR). This may lead to a compensatory activation of PI3 kinase and AKT leading to resistance via upregulation of mTORC2 (RICTOR). Activated mTORC2 may drive tumor progression through involvement in AKT/PKB-regulation, angiogenesis, transcription of hypoxia-inducible factor 2 (HIF2)-alpha, expression of HIF1-alpha and HIF2-alpha, and activation of VEGF. Moreover, activated mTORC2 was shown to regulate stem cell behavior and to mediate tumor growth by activating tumor growth factor-alpha. It should be noted that these findings have been discussed and are considered controversial and dependent on both the tumor and agent investigated. Results of pharmacodynamic studies revealed continuous and almost complete inhibition of the mTOR pathway with 10 mg everolimus daily whereas temsirolimus only inhibited the mTOR pathway intermittently.

2.3 Pericytes are regarded as important supporters of endothelial cells in both normal and tumor vasculature. It has been suggested that as a result of VEGF-induced vascular regression, endothelial cells can in turn induce pericyte recruitment to protect themselves. PDGF signaling was shown to play an important role in pericyte-endothelial cell interactions. In this context, it may be speculated that a highly selective VEGF inhibitor (bevacizumab, axitinib) in the first line should be followed by a less-selective, multikinase inhibitor such as sunitinib, which is also a strong PDGFR receptor (PDGFR)-inhibitor in the second line.

2.4 Finally, it may be conjectured that the reverted histologic phenotype occurring on EMT may also facilitate a treatment decision toward a multikinase inhibitor, notably a strong inhibitor of c-KIT. C-KIT is commonly expressed in sarcomatoid RCC and multikinase inhibitors such as sunitinib and sorafenib have been shown to inhibit c-KIT. Zhang et al investigated whether expression levels of c-KIT in RCC tumors with sarcomatoid features may predict the efficacy of sorafenib treatment. They found that the benefits of treatment with sorafenib were greater in patients whose tumors strongly expressed c-KIT.

BEYOND SECOND-LINE TREATMENT

Treatment beyond the second line may be offered less commonly than one might expect. Levy et al reported that only 36% of the patients in a specialized center received third-line treatment, with the highest percentage (65%) for those who had been treated with bevacizumab in first line. Memorial Sloan-Kettering Cancer Center score and type of first-line treatment appeared to be the best factors to predict which patients would receive further treatment lines.

As a result of patient selection in the RECORD-1 trial, the ESMO and EAU guidelines recommend everolimus as a third-line treatment option after two VEGF-TKIs. So far, there is only one randomized trial (NCT01223027) that investigated the effect of third-line treatment in mRCC. Patients who had failed treatment with a VEGF-TKI and an mTOR-inhibitor were randomly assigned to receive either sorafenib or the oral TKI dovitinib (TKI258) that targets FGFR, VEGFR, PDGFR and other kinases. The rationale for selecting a FGFR-inhibitor is based on the hypothesis that FGFR pathway activation is an escape mechanism from VEGF-inhibiting agents. No differences were observed in median PFS or OS between treatment arms (PFS dovitinib: 3.9 months; 95% CI 3.7–5.1 vs. sorafenib: 3.9 months; 95% CI 3.7–5.0; OS dovitinib: 11.1 months; 95% CI 9.5–13.4 vs. sorafenib: 11.0 months; 95% CI 8.6–13.5). The authors concluded that this outcome highlights the need for more effective agents in this patient setting. However, it may be argued that the efficacy of dovitinib has not been investigated in the most appropriate target population. If FGF levels are believed to be elevated on occurrence of resistance to sunitinib, it would appear more reasonable to study the efficacy of an FGF inhibitor after failure of sunitinib rather than in third line after mTOR inhibitor failure. Marketing considerations may have influenced the trial design, since evaluating dovitinib in the second line might have been interpreted as developing a potential future competitor in the manufacturer’s own portfolio.

There are also data available from case reports or smaller patient series on successful outcomes with TKI rechallenges in later treatment lines. Zama et al performed a retrospec-
tive analysis on sunitinib-refractory patients who were offered rechallenge with sunitinib. Between first-line sunitinib and sunitinib-rechallenge, various treatment strategies were implemented including other VEGF inhibitors (26%), mTOR inhibitors (13%), both (26%), other systemic therapies including chemotherapy (17%), radiotherapy (17%), and surgery (9%). Among 23 patients who were rechallenged with sunitinib, 22% achieved objective partial response (PR); the median PFS was 13.7 months with initial treatment and 7.2 months with rechallenge. Patients with a longer than 6-month interval between initial sunitinib and rechallenge had a longer PFS with rechallenge than those who were rechallenged within 6 months (median PFS 16.5 vs. 6 months, p = 0.03). The authors concluded that selected patients may benefit from sunitinib rechallenge. Grünwald et al. came to similar conclusions; they observed a PR rate of 69% and a median PFS of 21 months with first-line sunitinib. After progression on second-line mTOR inhibitor treatment, rechallenge with sunitinib led to 15% of the patients achieving PR and a median PFS of 6.9 months. Although these data support the hypothesis that sunitinib resistance is reversible, the high proportion of responders to first-line sunitinib may reflect selection of patients who were highly sensitive to sunitinib. Rechallenges have also been reported with sorafenib. Nozawa et al. rechallenged 14 patients who had failed first-line sorafenib and second-line treatment with sunitinib, cytokines, mTOR inhibitors, and others. The median PFS on initial sorafenib and sorafenib rechallenge was 5.7 months and 5.4 months, respectively. In this series, the outcome of sorafenib rechallenge was not affected by response to initial sorafenib. Finally, Maj-Hes et al. reported a median treatment duration of 10.3 months (95% CI 8.8–19.2) and 5.8 months (95% CI 2.9–19.3) for mTOR rechallenges with either everolimus→TKI→temsirolimus or temsirolimus→TKI→everolimus, respectively.

**ADDITION OF LOCAL TREATMENT OPTIONS TO OPTIMIZE OUTCOMES**

Strategies to improve outcomes of treatment sequencing may include other nonpharmacologic approaches. Local treatment options such as metastasectomy and stereotactic radiosurgery should be considered whenever it appears clinically reasonable. Alt et al. reported survival data of 887 patients with mRCC and nephrectomy between 1976 and 2006. In these patients, resection of metastases was performed whenever complete resection appeared feasible. Cancer-specific survival was 4.8 years, 2.6 years, and 1.1 year for patients with complete, incomplete, and no resection, respectively. The authors concluded that patients who did not undergo surgery for metastases have a threefold increased risk for death from mRCC (HR 2.91; 95% CI 2.17–3.90; p < 0.001). The benefits of metastasectomy—even if incomplete—have also been demonstrated in other small patient series.

Local treatment approaches appear promising in light of mixed responses observed with targeted agents. It is possible for the majority of metastatic lesions to remain stable or in partial remission with ongoing treatment while other lesions progress despite treatment. This phenomenon of different responses of metastases to treatment may be explained by the work of Gerlinger et al. showing genetic differences between metastases. Moreover, differences in the tumor microenvironment of different metastases may further contribute to the occurrence of mixed responses. Thus, surgery (or stereotactic radiosurgery) for metastases that do not “behave” may be an interesting approach to extend treatment duration in each treatment line and to improve outcomes.

**DISCUSSION**

Phase III evidence supports the sequential use of targeted agents in clear cell mRCC and axitinib, everolimus, and sorafenib are considered standard options in the second line. However, the best sequence remains to be determined. In the last few years, findings from basic research on the subject of resistance may have influenced treatment choices. Translation of these observations into clinical practice remains both speculative and often contradictory. For example, it appears that both continued VEGF inhibition and brief VEGF inhibition could be drivers of the disease. Similarly, mTOR inhibition may either be helpful against metabolic adaptation of tumor cells or detrimental in light of compensatory mechanisms and AKT activation. Finally, it is possible that the choice of the sequence may not matter at all: patients without intrinsic resistance and good prognostic features may benefit from any treatment in any line.

The development of strategies that target alternative proangiogenic pathways and different components of the tumor microenvironment either alone or simultaneously with VEGF inhibition appears extremely important. So far, attempts to overcome resistance by combining agents have shown no advantages for this approach. However, several new and promising agents that target alternative pathways and novel immunomodulatory agents are currently under investigation in mRCC.

Novel sequences with existing agents may also merit further investigation. A good example is the sequence sunitinib followed by bevacizumab plus interferon-alpha: Pastorelli D et al. reported on a good response to bevacizumab plus interferon-alpha in a patient refractory to sunitinib. Such a strategy could indeed make sense, since in vitro studies have demonstrated that sunitinib decreases the level of regulatory T cells. As interferon-responders have been shown to have low numbers of regulatory T cells, offering interferon-alpha (plus bevacizumab) in second line would be logical.

Finally, it might be worth challenging some current treatment strategies and goals. So far, a widely accepted strategy has been to extract the maximum benefit from each treatment line. This approach is supported by findings from Levy et al showing that fewer patients than expected proceed to second- or third-line treatment. Moreover, Grünwald et al have demonstrated in a retrospective analysis that the magnitude of tumor shrinkage predicts survival. However, the
extent of tumor shrinkage may merely represent an epiphenomenon reflecting high sensitivity to VEGF-inhibition. In fact, high sensitivity to VEGF inhibition may be the prognostic factor rather than the actual achievement of objective response, since it represents a different disease with a different biology. Hence, strategies to avoid resistance and to maintain the current biology may be more important. It is questionable whether continuous treatment exposure until progression is really important. In a prospective phase II trial, Rini et al demonstrated that sunitinib dosing with periods of time off drug is associated with a reduction of side effects without compromising clinical efficacy.60 Time off drug may allow resetting of the tumor microenvironment and maintain sensitivity to VEGF inhibition. It has been suggested that resistance could be avoided by alternating agents with different modes of action before resistance occurs. The SUNRISE study (NCT01784978) is investigating alternating cycles of sunitinib and everolimus compared with sequential treatment of sunitinib followed by everolimus (on progression). However, results from a similar trial (EVERSUN) have been presented recently and did not support this therapeutic concept.81

Disclosures of Potential Conflicts of Interest

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked “L” indicate leadership positions. Relationships marked “I” are those held by an immediate family member; those marked “B” are held by the author and an immediate family member. Relationships marked “U” are uncompensated.

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