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ANTI-ANGIOGENIC AND TARGETED THERAPY IN RENAL CANCER:
Targeting the mTOR Pathway in Renal Cell Carcinoma
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Introduction
The mammalian Target of Rapamycin (mTOR) is the second molecular target (after VEGF signaling) for which small molecule inhibitors have been developed and shown to have significant clinical activity in patients with advanced renal cell carcinoma (RCC). The mTOR inhibitor temsirolimus is now FDA-approved for the first-line treatment of patients with RCC. It's structural analogue everolimus is similarly approved as second line therapy for RCC patients who have failed treatment with sunitinib and/or sorafenib.[1,2] Responses to these agents, however, remain limited to a subset of patients and all patients treated with these drugs eventually develop progressive disease. In this article, we will review the clinical experience with mTOR inhibitors in RCC, relevant class-specific toxicities, and future clinical directions. We will also discuss possible novel strategies to target this signaling pathway.

Clinical experience with mTOR inhibitors in patients with RCC
Temsirolimus and everolimus have both demonstrated clinical efficacy in large randomized phase III trials in patients with advanced RCC. After showing promising activity in a phase II trial randomizing patients with metastatic RCC to three different doses,[3] temsirolimus was assessed in a randomized three-arm Phase III trial comparing temsirolimus alone versus interferon-α (IFN-α) alone versus the combination of both.[1] As the phase II study suggested potentially unique efficacy in patients with poor prognostic features, the phase III study chose to focus on patients with metastatic RCC and ≥3 of 6 risk factors; (5 MSKCC risk factors + >1 metastatic site). Overall, 626 previously untreated patients were enrolled and randomized in a 1:1:1 fashion to receive IFN-α alone, temsirolimus alone, or the combination. The overall survival of patients treated with temsirolimus alone was statistically longer than those treated with IFN-α alone (7.3 versus 10.9 months; 0.73 hazard ratio, p=0.0069). There was no statistical difference between patients treated with IFN-α alone and the combination of IFN-α and temsirolimus. Temsirolimus was thus the first molecularly targeted agent to demonstrate a statistically significant survival benefit in first-line therapy of patients with metastatic RCC. Accordingly, temsirolimus was approved by the FDA for therapy in advanced RCC on May 30, 2007 and is now considered a standard first-line therapeutic option for patients with poor prognostic features.

While temsirolimus was assessed in untreated patients, everolimus was assessed in a randomized, double-blind, placebo-controlled phase III in patients with advanced RCC who had failed prior treatment with either sorafenib, sunitinib, or both within the preceding 6 months (REnal Cell cancer treatment with Oral RAD001 given Daily-1 [RECORD-1]).[2] Overall, 416 patients were enrolled and randomized in a 2:1 fashion to receive either everolimus (n=277) or placebo (n=139) each together with best supportive care. The primary endpoint was PFS as randomization was unblinded at time of progression and patients on placebo were allowed to crossover to open-label everolimus, confounding any potential differences in overall survival. The trial was halted at the second interim analysis after 191 progression events had been observed. At the final central radiology assessment the median PFS for patients treated with everolimus was 4.88 months as compared with 1.87 months in the placebo group (hazard ratio 0.33, [95% CI 0.25-0.43] $p < 0.0001$).[4] Five patients (2%) in the everolimus group experienced partial responses vs. none in the placebo group. Based on these results, everolimus was approved the FDA in March, 2009 for the treatment of patients with advanced RCC who failed either sorafenib, sunitinib or both.

Important toxicities of mTOR inhibitors
Although in general well tolerated, treatment with either everolimus or temsirolimus can be associated with many of the same side-effects observed with the VEGF-targeted TKIs. These most commonly include rash, nausea, diarrhea, stomatitis/mucositis, cytopenias, and fever. However the rapalogues can also induce toxicities which are distinct from those seen with VEGF pathway targeted therapies in RCC and are worthy of specific discussion. These toxicities include pneumonitis, endocrine abnormalities and the possibility of immunosuppression.

Pneumonitis.
Pneumonitis has been observed with all the rapalogues and appears to be a class effect of the allosteric inhibitors of mTOR.[5] The exact incidence of this toxicity seems to vary widely from study to study. In the phase III trials of temsirolimus and everolimus discussed above, the incidence of pneumonitis was fairly low, with incidences of only 2% and 8%, respectively, of any grade event.[2,6] However, in a retrospective study in patients with non-small cell lung cancer, White et al reported that 16 (25%) of 64 patients examined showed radiographic evidence of pneumonitis which was felt to be attributable to everolimus.[7] Other studies have suggested that pneumonitis from TORC1 inhibitors may be more common in patients with pre-existing pulmonary conditions.[8] Pneumonitis may be more commonly appreciated radiographically, where it most frequently presents as ground glass-opacity and occasionally as parenchymal consolidations and pleural effusion, than clinically. When symptoms are present, most patients experience dypsnea on exertion and cough, occasionally accompanied by fever, malaise, and hypoxia. While many mechanisms have been proposed, including cell-mediated auto-immunity and T-cell-mediated delayed-type hypersensitivity,[4,9] the exact molecular basis for this toxicity remains unknown. Although there are currently no specific guidelines to the management of rapalogue-associated pneumonitis, other etiologies, particularly infectious, should be first excluded. Most investigators appear to agree that treatment should be held in patients with overt symptoms attributable to pneumonitis and a brief course of steroids may be considered. Treatment resumption, usually at a lower dose, may be considered following resolution of symptoms. There does not appear to be consensus for patients with only radiographic findings of pneumonitis, but continuing therapy with careful observation or lowering the dose appear to be common interventions.

Endocrine side effects.
Treatment with rapalogues has also been associated with several endocrine abnormalities, namely hyperlipidemia and hyperglycemia. These toxicities appear quite common in patients with RCC treated with either temsirolimus or everolimus. In the phase III trial of temsirolimus, the incidence of hypercholesterolemia, hyperlipidemia and hyperglycemia in patients treated with temsirolimus alone was 24%, 27%, and 26% respectively.[1] In the RECORD-1 study, the incidence of hypercholesterolemia, hypertriglyceridemia, and hyperglycemia in patients treated with everolimus was 76%, 71%, and 50%, respectively.[2] Studies with rapamycin suggest that the

hyperlipidemia (observed as elevations in HDL, LDL, cholesterol, and triglycerides) induced by rapalogues is due to reduced catabolism of lipoprotein particles.[10] While this toxicity is quite common and therefore requires continuous monitoring, rapalogue-induced hyperlipidemia is usually manageable with statins or gemfibrozil (for hypertriglyceridemia) and typically does not require treatment cessation. Similarly, animal studies with rapamcyin have shown that hyperglycemia is a direct side effect of treatment with rapalogues due to enhancement of insulin resistance and reduction of β-islet cell mass and function.[11] Therefore, monitoring of fasting glucose levels is recommended for all patients treated with mTOR inhibitors, particularly those with pre-existing diabetes. Therefore initiation of oral anti-glycemic agents or escalation of current diabetic regimen may be indicated.

Immunosuppression.
As the rapalogues were developed first as immunosuppressive agents in the transplant setting, treatment with these agents has always raised concerns regarding the potential for immunosuppression in cancer patients. Recent studies have suggested that rapamycin may actually enhance the immune response to infections by both enhancing the CD8+ T-cell response and by increasing the differentiation of effector cells into potent memory T-cells.[12,13] Nonetheless, the reported incidence of infection in patients treated with either temsirolimus or everolimus in phase III trials was higher than that for their respective control arms (27% in patients treated with temsirolimus alone versus 14% in those treated with IFN; 10% in patients treated with everolimus versus 2% in those treated with placebo).[1,2] Therefore, the issue of whether rapalogues may be immunosuppressive cannot be considered to be completely resolved. Although current data does not support the use of antibiotic prophylaxis, clinical vigilance is recommended to the possibility of increased frequency of infections, particularly in those patients with pre-existing chronic viral infections or immunosuppressive conditions. In particular, recent reports filed through Medwatch, have indicated that treatment with everolimus may trigger the activation of hepatitis B in patients with a history of resolved or inactive hepatitis B.[14] In these patients, initiation of anti-hepatitis medication such as lamuvidine is recommended prior to the initiation of everolimus.

Future clinical directions
Although both temsirolimus and everolimus are approved by the FDA for the treatment of patients with advanced RCC, the role of these TORC1 inhibitors will likely continue to evolve as many questions regarding their efficacy in specific therapeutic situations are addressed. Both agents are being studied or considered in multiple other clinical scenarios and therapeutic strategies including sequential therapy with VEGF pathway inhibitors, combinational regimens the adjuvant setting, and in patients with non-clear cell histology.

Sequential therapy.
Multiple retrospective analyses have suggested that there is no true cross-resistance for VEGF pathway and mTOR inhibitors given in sequence.[15,16] Investigators have therefore proposed to examine specific sequences of novel agents given as single agents in an effort to identify a particular sequence of agents that may result in maximal duration of disease control while perhaps also minimizing toxicity. With respect to everolimus, this is specifically being examined in the RECORD-3 trial, a large phase II trial in which previously untreated patients with metastatic clear cell RCC will be randomized to receive either first-line everolimus followed by second-line sunitinib or first-line sunitinib followed by second-line everolimus. Similarly, temsirolimus is currently being investigated in a phase III trial versus sorafenib in patients who have failed initial therapy with sunitinib.

Combinational therapy.
Given the distinct targets of recently approved treatments for patients with RCC (i.e. inhibition of VEGF signaling vs. inhibition of mTOR), there has been considerable interest in whether combinations of these two classes of agents may lead to additional therapeutic efficacy. Perhaps the most studied approach thus far involves the combination of TORC1 inhibitors with bevacizumab. The combination of temsirolimus and bevacizumab showed encouraging efficacy in a phase II trial in patients with advanced RCC who have failed VEGF-targeted TKI therapy with an overall response rate of 16%.[17] However, in a separate randomized phase II study in which untreated RCC patients were randomized to receive either the combination of temsirolimus and bevacizumab, sunitinib, or the combination of bevacizumab and IFN-α, the response rates in the individual arms were 25%, 24%, and 34%, respectively.[18] These results, combined with the observation of significant premature treatment stoppage in the temsirolimus-bevacizumab arm (43%), have raised questions about both the additive efficacy and the toxicity of this combination. Nonetheless, the combination is also actively being assessed in multiple larger trials including in an arm the Eastern Cooperative Oncology Group (ECOG) Trial 2804 and in a large phase III trial in which patients are randomized to the combination of either temsirolimus and bevacizumab or bevacizumab and IFN-α (INTORACT Trial), so more information should be forthcoming.

Similar combinational studies with everolimus are also underway. A phase II trial of the combination of everolimus and bevacizumab produced five partial responses (17%) and a median progression-free survival of 11 months in 29 patients who had received prior VEGF receptor TKI therapy.[19] This data, plus the desire to examine the role of maintained VEGF pathway blockade following sunitinib or sorafenib resistance, has led the CALGB to propose an intergroup phase III trial randomizing patients whose disease has progressed following sorafenib and/or sunitinib to either everolimus alone or the combination of everolimus and bevacizumab.

Adjuvant therapy.
Although there are no therapies approved for the adjuvant treatment of patients with high-risk RCC, the recent approval of multiple therapies in the metastatic setting has prompted the assessment of these agents in the adjuvant setting. Studies involving sorafenib and/or sunitinib are currently underway and anticipated to reach accrual goals in the near future, but mature results are not envisioned for several years. In particular, the efficacy of everolimus in patients with metastatic RCC, together with its novel mechanism of action, favorable toxicity profile and oral administration make it an attractive agent to also test in the adjuvant setting. Accordingly, a large randomized placebo controlled phase III trial is being planned within the U.S. Intergroup mechanism to formally assess the role of adjuvant everolimus in patients with resected high-risk RCC.

Non-clear cell RCC.
Although the efficacy of TORC1 inhibitors has primarily been established in clear cell RCC, further analysis of the pivotal phase III trial leading to the FDA approval of temsirolimus suggested this TORC1 inhibitor may be even more effective compared with interferon in patients with non-clear cell RCC than clear cell RCC.[20] The median overall survival of temsirolimus versus interferon was 11.6 vs. 4.3 months in patients with non-clear cell histology (75% of which were of papillary sub-type) compared with 10.7 vs. 8.2 months

in patients with clear cell RCC. The possibility that TORC1 inhibitors in general may have unique efficacy in non-clear cell RCC has prompted the initiation of a randomized phase II trial of temsirolimus versus sunitinib in European patients with metastatic non-clear cell RCC. Likewise, everolimus will also be studied in a phase II trial in 60 European patients with metastatic papillary RCC (RAPTOR Trial [RAD001 in Advanced Papillary Tumor Program in Europe]). These two phase II trials should provide critical information regarding the efficacy of TORC1 inhibitors in patients with non-clear cell histology RCC.

Beyond first generation mTOR inhibitors

Despite the established efficacy of temsirolimus and everolimus in RCC, only a subset of patients with advanced RCC experience substantial clinical responses from treatment with these agents. Furthermore, these clinical responses are neither complete nor durable off therapy and all patients will eventually experience disease progression. The efficacy of these allosteric inhibitors of mTOR may be limited in part because they primarily inhibit the function of TORC1, the complex including mTOR and raptor, and have less activity against TORC2, the complex including mTOR and rictor (rapamycin insensitive companion of TOR). Recent studies have suggested that the expression of Hypoxia Inducible Factor (HIF)-2α, argued by many to be the more relevant HIF in RCC, is dependent almost completely upon TORC2 and largely independent of TORC1 function.[21] Furthermore, some pre-clinical studies have suggested that inhibition of TORC1 can lead to activation of signaling pathways upstream of mTOR including those mediated by phophatidy-linositol 3-kinasee (PI3-K) and Akt (Protein Kinase B).[22,23] As PI3-K and Akt activate numerous kinases, transcription factors and other proteins associated with cell growth and survival in addition to mTOR, persistent activation of these pathways might undermine the efficacy of TORC1 inhibition.

Not surprisingly, a new generation of agents targeting the PI3-K/Akt/mTOR pathway is in active clinical development. Inhibitors which directly inhibit the kinase function of mTOR, and thereby suppress the activity of both TORC1 and TORC2, are now entering clinical assessment. Given the aforementioned dependence of HIF-2α expression on TORC2, these direct mTOR kinase inhibitors would have the advantage of inhibiting the expression of both HIF-1α and HIF-2α. The possibility of rapalogue-induced feedback activation of PI3-K/Akt has made the dev-elopment of inhibitors of these upstream kinases an attractive strategy and many such agents are now in clinical development. Whether the many theoretical advantages of PI3-K/Akt or direct mTOR kinase inhibitors translates into superior clinical efficacy in patients with advanced RCC, however, remains to be seen.

Conclusion

With the recent FDA approvals of both temsirolimus and everolimus for the treatment of patients with advanced RCC, TORC1 inhibitors have now joined the antagonists of VEGF signaling and non-specific immune-therapies in a crowded therapeutic field in RCC. Despite these dev-elopments, however, only a subset of patients with RCC experience substantial clinical responses following treatment with TORC1 inhibitors. Therefore, efforts must continue to explore mechanisms of resistance to these agents to aid in the development of more effective agents directed against this critical pathway. Efforts must also focus on identifying predictive biomarkers of response to the rapalogues in order to develop more effective patient selection strategies. Through these efforts, the role of mTOR inhibitors in RCC therapy will almost certainly continue to evolve as it enters clinical assessment in a multitude of clinical settings including sequential, combinational, and adjuvant therapy as well as in patients with non-clear cell RCC

Discussion

Dr. Atkins: Are the dual TOR inhibitors or PI3-kinase inhibitors that you discussed working by a different mechanism than the VEGF inhibitors in treating the kidney tumors?

Dr. Cho: Our preclinical work suggests they are working by a different mechanism; we do not see any evidence of an anti-angiogenic effect and yet we see diminished proliferation and diminished tumor growth.

Dr. Kaelin: Well, frankly I will be surprised if they are not working in part by blocking angiogenesis. Certainly many models, many of which are based on preclinical experiments including using genetically defined mice and zebra fish, would suggest that the PI3Kinase, mTOR pathway should be important for angiogenesis.

Dr. Stadler: We have focused on the tumor. I mean on the cancer cell itself. We cannot forget that the mTOR pathway, especial-ly the TOR-1 pathway, is critically important in the immune system as well and that these–at least the rapalogues–are potent immune suppressive agents. So what do you know about the TOR-1 /TOR-2 inhibitors and their affect on various components of the immune system?

Dr. Cho: From Phase 1 trials we have not observed significant opportunistic infections, but those are things that we will keep an eye out for.

Dr. Stadler: But it is clearly known that these drugs produce fairly profound immune suppression.

Dr. Atkins: Right. By the way, it is interesting that we are seeing benefits with mTOR inhibitors in some patients with aggressive tumors. That fact that an approach associated with immunosuppression works against these tumors, perhaps changes the discussion about where you would want to test immunotherapies such as the PD1 antibody.

Dr. Choueiri: It looked like your interpretation of MTOR inhibition was that it was really primarily through S-6 kinase down-regulation that you are suggesting this mechanism.

Dr. Cho: I think clinically you could argue that those who benefit are have sufficiently high blood levels to also inhibit TORC-2 to some extent.

Dr. Atkins: Once again, the concept that different tumors respond better to different treatments puts a premium on biomarker studies.

Dr. Kaelin: I think one other thing we could think about going forward is whether autophagy plays a role here. Just to spice things up, another gene on 5Q is a gene involved in autophagy

References

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