The Last Word

Predictive Biomarkers for Molecularly Targeted Therapies in Renal Cell Carcinoma

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A significant challenge in oncology is the identification of predictive biomarkers of therapeutic benefit. The article in this issue by Fay et al (page 820) illustrates this issue in renal cell carcinoma (RCC). The challenge encompasses all RCC therapies, including immunotherapies (interleukin-2 and nivolumab) and molecularly targeted therapies, which comprise angiogenesis inhibitors (bevacizumab, sunitinib, sorafenib, pazopanib, axitinib, and, with a characteristic target spectrum, the recently approved drugs, lenvatinib and cabozantinib) and inhibitors of mTOR complex 1 (mTORC1), specifically the sirolimus analogues temsirolimus and everolimus. Response rates (almost always partial) to targeted therapies in the first or second line range between 10% and 40%, and primary resistance may be seen in a somewhat lower but still significant percentage of patients. Whether a patient will or will not benefit from a particular therapy cannot be predicted.

An understanding of the molecular mechanism of drug action, the target, and corresponding pathway has assisted in pinpointing potential predictive biomarkers for mTORC1 inhibitors. Sirolimus analogues are highly specific—they form a complex with FK506-binding proteins (ie, FKBP12, also called FKBP1A) and bind to mTOR in a gatekeeper domain (FKBP12-rapamycin–binding [FRB] domain).[1] When bound to mTOR, they restrict substrate access and inhibit mTORC1 function. mTORC1 is activated by Ras homolog enriched in brain (RHEB), a small GTPase, which in turn is regulated by a GTPase-activating protein complex made up of tuberous sclerosis (TSC) 1 and 2 proteins and TBC1D7. Function-altering mutations acquired during tumor development in the corresponding genes, *MTOR*,[2] *RHEB*,[3] *TSC1*,[4] and *TSC2*,[5–7] likely reveal a heightened dependency on mTORC1 and may identify patients more likely to benefit from mTORC1 inhibitors.[4,8,9] Mutations in genes encoding signaling nodes further upstream, such as phosphatase and tensin homolog (*PTEN*) and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*), may also confer dependency on mTORC1.[9]

For patients with mutations in mTORC1 pathway genes, the clinical benefit of mTORC1 inhibitors may be significant. Time to progression typically decreases with each subsequent line of therapy, and in these patients, mTORC1 inhibitors may increase progression-free survival (PFS) by more than 2-fold compared with previous lines of therapy.[8] However, the frequency of mTORC1 pathway mutations is low (5%–20% in aggregate), and 20% of the patients with mutant tumors may not benefit significantly from mTORC1 inhibitor therapy (possibly due to nonfunction-altering and subclonal mutations). Further, mutations are only detected in approximately 50% of patients with substantial benefit from mTORC1 inhibitors.[9] Whether immunohistochemistry (IHC) for markers of mTORC1 activation[10] would improve the sensitivity and specificity afforded by mutation detection is unclear. Nevertheless, I speculate that the prospective identification of patients with function-altering somatic mutations in proximal mTORC1 regulators would significantly enrich for patients likely to benefit from mTORC1 inhibitors.

Estimates from the International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) suggest that fewer than 40% of patients receive an mTORC1 inhibitor in first- or second-line therapy.[11] Moreover, this number is likely to decrease further given results of recent phase III trials showing superiority of nivolumab and cabozantinib compared with everolimus.[12,13] Thus, the identification of patients likely to benefit from mTORC1 inhibitor therapy may become even more critical.

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In contrast to mTORC1 inhibitors, less is understood about the mechanism of action of angiogenesis inhibitors (except for bevacizumab). Specifically, the relative contribution of vascular endothelial growth factor (VEGF) receptor 2 versus other kinases is poorly understood, and as catalytic inhibitors, these small molecules have pleiotropic effects. To identify determinants of sensitivity to angiogenesis inhibitors, Fay et al evaluated 27 patients with extreme response to first-line sunitinib or pazopanib (including 7 from The Cancer Genome Atlas [TCGA]). The series included 13 patients with an outstanding response (partial or complete response for ≥3 years) and 14 patients with rapid progression (within the first 3 months). Notwithstanding patients from TCGA, formalin-fixed paraffin-embedded (FFPE) samples (largely from archival nephrectomy specimens) were subjected to whole-exome sequencing. The authors found polybromo 1 (PBRM1) mutations enriched in tumors from the responding group (7 of 13 vs 1 of 14; unadjusted P=.01).

These results are consistent with a retrospective analysis of patients enrolled in RECORD-3 (a large randomized phase II trial comparing sunitinib and everolimus sequences), which found that PBRM1 mutations were associated with longer PFS to first-line sunitinib and also everolimus.[14] However, one caveat is that PBRM1 mutations could be prognostic. Indeed, overall survival was also improved in patients with PBRM1-mutant tumors in the study by Fay et al. In addition, at least in the localized setting, patients with tumors deficient for PBRM1 exhibit improved RCC-specific and overall survival compared with those with other genotypes.[15,16]

In addition, Fay et al report an association between TP53 mutations and the resistant group (4 of 14 vs 0 of 13; unadjusted P=.09). However, patients with TP53-mutant tumors also had a significantly worse overall survival compared with those with tumors wild-type for TP53. Thus, whether TP53 and PBRM1 are indeed predictive or simply prognostic remains to be determined.

The identification of predictive biomarkers of responsiveness to angiogenesis inhibitors has been particularly challenging, because these drugs do not target the cancer cell directly but rather target the vasculature.[17] Tumor angiogenesis likely involves paracrine effects from tumor cells on endothelial cells, and experimental systems suitable to dissect the crosstalk have been lacking. Recently, we showed that primary cultures from RCC activate mitogenic signaling in endothelial cells in coculture experiments.[18] Endothelial cell activation could be blocked by sunitinib. However, primary cultures derived from patients with sunitinib-resistant tumors retained the ability to induce mitogenic signaling in endothelial cells despite sunitinib. This in vitro system appears therefore to reproduce the situation in patients.

Interestingly, dovitinib, an inhibitor of angiogenesis that also targets fibroblast growth factor receptors, blocked endothelial cell activation by sunitinib-resistant RCCs.[18] Dovitinib was not better than sorafenib in a phase III trial, however.[19] One potential explanation for these results is the differential effect of dovitinib on endothelial cells and fibroblasts. Although dovitinib effectively inhibits endothelial cell activation by RCC cells, it fails to consistently inhibit fibroblasts, a cell type that may contribute to tumorigenesis.[18] Thus, failure to inhibit other cell types may explain the modest activity of dovitinib against RCC. Like dovitinib, lenvatinib (which was recently approved in combination with everolimus[20]) also targets fibroblast growth factor receptors, but how it would affect signaling by RCC is unknown.

Given the paracrine effects of the tumor on the vasculature, a potential avenue to identify biomarkers involves gene expression analyses. A study using gene expression arrays involving 98 clear cell RCCs identified several characteristic gene expression patterns in unsupervised analyses, and the groups identified differed in their responsiveness to sunitinib.[21] However, these patterns failed to clearly separate responsive from resistant tumors, and the patterns were associated with different overall survival in patients. This raises the concern that they may be prognostic rather than predictive.

Overall, these data suggest that sensitivity and resistance may not be associated with distinct biologic entities identifiable by unsupervised gene expression analyses. Rather, supervised analyses focusing on cytokines and growth factors may be required.

VEGF has been extensively evaluated as a candidate predictive biomarker of angiogenesis inhibitors. Most studies have focused, however, on circulating VEGF isoforms (or soluble forms of the receptors),[22,23] which may be impacted by other factors besides the tumor. However, VEGF expression in tumors may more accurately reflect tumor angiogenesis, and in co-culture experiments, sunitinib sensitivity correlates with VEGF production.[18] Notably, VEGF production may differ not only across tumors from different patients but also among metastases within the same patient. This is evidenced by an elegant, albeit small, study using [89]Zr-bevacizumab PET.[24] Different mechanisms of angiogenesis across metastases further complicates the identification of predictive biomarkers.

In summary, despite more than a decade of molecularly targeted therapies in RCC, clinically actionable predictive biomarkers remain unavailable. Progress has been hampered by biological complexity (and tumor heterogeneity) as well as drugs targeting the stroma with poorly understood mechanisms of action. The development of experimental approaches suitable to dissect the crosstalk between tumor and stroma, and a deeper understanding of the biology, may pave the way to identification of candidate predictive biomarkers.

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