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**TRANSLATIONAL RESEARCH STUDIES IN RENAL CANCER: Pharmacodynamic and Predictive Biomarkers for VEGF and mTOR Directed Therapies**

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**Introduction**

VEGF and mTOR pathway directed inhibitors have become standards of care for treatment of patients with metastatic renal cancer. Despite the paradigm shift these agents have introduced, overall survival benefit is modest, toxicities can be significant, development of clinical resistance is common and the choice of initial or subsequent drug to use in any specific patient remains unclear. As with many modern oncologic therapies, there is hope that judicial use of biomarkers can guide therapy.

In general a biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[1] Pharmacodynamic biomarkers reflect a pharmacologic effect of the agent on the host or tumor and predictive biomarkers provide suggest the likelihood of benefit or toxicity from a specific agent.[2] An example of the former is neutropenia following paclitaxel therapy and of the latter is expression of the estrogen receptor to predict benefit from tamoxifen. In this section, we will review pharmacodynamic and predictive biomarkers of mTOR and VEGF pathway directed therapy with a focus on those showing the greatest promise for guiding therapeutic selection.

**Tumor Characteristics as Biomarkers**

The simplest and most common predictive biomarker in oncology is tumor site of origin and histology. For renal cancer, it has been suggested that non-clear cell subtypes may have preferential benefit to mTOR pathway inhibitors.[3] These studies have, however, been limited by the lack of central pathologic review and suggestions that poor prognosis patients, who tend to have more poorly differentiated and less well characterized tumors, may have a preferential benefit to these agents, as well.[4]

In regards to tumor molecular characteristics, VHL mutation status has been evaluated as a predictive biomarker of VEGF pathway targeted therapy. As might be expected from the fact that VHL pathway alteration is pathognomonic for clear cell renal cancer, most studies have not shown significant correlations. One study suggested that loss of function mutations were associated with response but not progression free or overall survival.[5] For mTOR directed therapy, preclinical studies would suggest that HIF upregulation, which is present in essentially all clear cell renal cancers, and alterations of the AKT/PTEN pathway would be associated with treatment benefit.[6,7] High expression of phospho-S6 kinase and p-AKT were modestly associated with objective tumor response in one small study, but no general association between mTOR directed therapy benefit with VHL pathway or PTEN status has yet been demonstrated.[8,9] More recently, it has been suggested that clear cell renal cancer can be divided into subtypes based on HIF-1α specific expression.[10] Whether this has any therapeutic relevance remains to be determined.

**Therapy Toxicities as Biomarkers**

By definition, the most common toxicities associated with a particular therapy are pharmcodynamic biomarkers. In the context of VEGF pathway directed therapy, the most common on-target toxicity is elevated blood pressure (BP). Although the incidence of hypertension per standard toxicity scales is modest in phase III trials, careful BP measurements suggest that elevation occurs in the majority of patients usually within 24 hours of beginning therapy.[11] Improved progression free and overall survival has been reported with development of systolic BP greater than 140 or diastolic BP greater 90 in retrospective analyses of trials with sunitinib[12] and axitinib,[13] and with grade 2 or 3 hypertension in a trial with bevacizumab.[14] A prospective randomized phase II trial is evaluating the impact of axitinib dose titration to achieve hypentension on therapeutic outcome. (ClinicalTrials.gov NCT00835978).

Fewer studies have evaluated mTOR directed toxicities as biomarkers, but lipid and glucose elevations are target specific effects. Whether these have any predictive value remains to be determined, but careful evaluation suggests that glucose and triglyceride changes occur in the majority of patients and are not associated with each other.[15]

**Serum and Plasma Based Biomarkers**

A number of studies have demonstrated increased levels of plasma VEGF with VEGF pathway directed therapy, most convincingly with sunitinib,[16] but these changes have not necessarily had any predictive value. Studies evaluating baseline VEGF levels have more generally demonstrated a modest prognostic, but not necessarily predictive value.[16,17] One study suggested that lower levels of soluble VEGFR-3 might have some predictive value for benefit from sunitinib, but this has not been replicated. Retrospective analysis of the phase III temsirolimus data suggested that baseline LDH was not only prognostic, but possibly predictive marker for benefit from temsirolimus.[18]

**Imaging Based Biomarkers**

The most common imaging based biomarker is change in tumor size or burden with therapy. However, VEGF and mTOR pathway directed agents also slow disease growth and standard RECIST based response rates do not fully capture their anti-tumor activity. VEGF pathway inhibitors, however, target tumor vasculature and thus parameters derived from contrast-enhanced imaging have been evaluated as biomarkers. The simplest incorporation of these observations are the Morphology, Attenuation, Size and Structure (MASS) criteria.[19] Post treatment lesions are evaluated for central necrosis or decreased attenuation as well as size. MASS criteria favorable response is better correlated with progression and disease specific survival than standard RECIST response.

More quantitative vascular parameters can be derived from dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI). DCE-MRI tracks the diffusion of an intravenously administered paramagnetic contrast agent (i.e., gadolinium) into the extravascular tissue over time. Although several parameters can be calculated, the most useful biomarkers for VEGF pathway targeted therapy are $K^{trans}$ and the mathematically related $K^{ep}$. Derivation of these is beyond the scope of this article, but in essence they reflect a combination of tumor blood flow and permeability. Studies to date have indicated that decrease in $K^{trans}$ is a pharmacodynamic marker for VEGF pathway targeted therapy, but that the changes have little or no predictive value.[20,21] Some data suggests that high baseline values of $K^{trans}$ may be correlated with benefit from such therapy.[21] Similar

vascular parameters can be derived from dynamic contrast-enhanced ultrasonography (DCE-US). Reduction in tumor vascularity can be detected after 1 or 2 weeks of therapy and in preliminary studies is correlated with progression free and overall survival.[22] For mTOR directed therapy, decreased tumor glucose uptake, as demonstrated by FDG-PET imaging is a clear pharmacodynamic marker,[23,24] but is unlikely to be predictive. The preclinical suggestion that baseline FDG-PET uptake is predictive of benefit from mTOR directed therapy is being evaluated in a prospective trial (NCT00529802).

**Pharmacologic and Pharmacogenomic Based Biomarkers**

The value of pharmacokinetic parameters as predictors of patient outcome has not been well studied in the context of VEGF or mTOR pathway directed therapy for patients with renal cancer. For sunitinib, there has been a suggestion that increased exposure correlates with increased tumor shrinkage, and prolonged progression free and overall survival.[25] Even fewer studies have evaluated potential pharmacogenomic predictors despite the known metabolism of many VEGF and mTOR directed agents by highly polymorphic enzymes. There is some interesting preliminary data suggesting that certain VEGF or VEGFR single nucleotide polymorphisms may correlate with the development of hypertension and patient outcome.[26]

**Conclusion**

Although the outcome of patients with metastatic RCC has been substantially altered with administration of VEGF and mTOR directed therapies, selection of specific treatments for any individual patient remains challenging. A number of putative pharmacodynamic and predictive biomarkers have been suggested to be helpful. Nevertheless, none have been fully qualified, and substantial work, especially in a prospective manner, remains to be done before they can be recommended for general clinical use.

**Discussion**

**Dr. Atkins:** Does hypertension at baseline correlate in any way with benefit for VEGF blocking agents?

**Dr. Stadler:** No. Whether one has hypertension at baseline does correlate with development of hypertension as a toxicity. However it does not necessarily correlate with whether there is an increase in blood pressure relative to baseline. If you are already hypertensive and you get a delta of 10 then it is a toxicity, whereas if you are normal and get a delta of 10, you do not have toxicity yet.

**Dr. Atkins:** Is there any data about VEGF polymorphisms and the frequency of hypertension? Is there data from kidney cancer or other cancers about the relationship between VEGF polymorphisms and the frequency of hypertension on VEGF blocking agents?

**Dr. Stadler:** Yes. There seem to be some SNPs both in the VEGF as well as the VEGFR gene that correlate with development of either hypertension as a toxicity or a change in blood pressure. There are studies that have been proposed in the context of, for example, some prospective CALGB trials to look at that.

**Dr. Atkins**: It would seem to me that polymorphisms in the receptor would be more relevant than polymorphisms in VEGF in terms of predicting for either hypertension or for response to some of these agents. Have there been studies looking at polymorphisms in the receptor?

**Dr. Stadler:** There is a study, but it is not the greatest quality.

**Dr. Hutson:** One of the big problems I have is patients come to me and I try and figure out how often should I do CT scans or chest x-rays. There is no established recommendation for how you should monitor for disease progression like has been created for patients with melanoma or other cancers. Can you design a blood test with a number of these markers that would be helpful in determining disease progression or directing imaging?

**Dr. Stadler:** Could one? Maybe. Are these going to do it? Probably not based on what we know about variability within the population. I tell folks who want a blood test for their renal cancer to take a look at the prostate cancer literature and to be careful of what you wish for. Sometimes a sensitive marker of disease can cause more headaches than it can solve problems.

**Dr. Hutson:** Understood. But when a patient comes in, I honestly do not know how to determine what scanning is appropriate. Should I just not do any scans at all, wait until you become symptomatic? Do a CT once a year? Or should I do it on the basis of risk? If we had an inexpensive blood test we could just send off it would really help.

**Dr. Stadler:** Well, the biggest problem with that is that we already do not know what to do with patients who have come in with5 mm tumors. Do you start treatment early or do you start later? If I am presented with a new blood test that lets me detect tumor even earlier, it could create additional problems.

**Dr. Atkins:** You really want things that you can act on. And we have trouble knowing what action to take with small incidental findings on CT scan that are too small to biopsy. This problem could be exacerbated by a sensitive blood test. On the other hand a blood test might validate a non-specific imaging finding or vice versa. Furthermore, having a blood biomarker might prompt research studies that might elucidate important principles. For example, is there any evidence that VEGF levels are associated with tumor vascularity? Do other cytokine levels correlate with the onset of resistance to VEGF pathway inhibitors? Can such profiles guide therapy selection> d Even though we have a lot of imaging studies, we do not know whether what you see in the images correlates with something we could possibly detect in the blood.

**Dr. Stadler:** The answer is probably no. Of all the VEGF studies that have been done, VEGF in the blood is probably most closely correlated with platelet level. And if you do it in platelet-free plasma so that you get rid of the platelet problem then the amount of VEGF probably correlates best with tumor burden, sort of an expensive LDH

**ReferencesPharmacodynamic and Predictive Biomarkers for VEGF and mTOR Directed Therapies**

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