Multiplexed IHC
Predicting Response To Anti-Angiogenic and mTOR inhibitor in Kidney Cancer

20/20 GeneSystems is developing a test to predict whether patients with advanced renal cell carcinoma will respond to anti-angiogenic drugs such as sunitinib and mTOR inhibitors such as everolimus or temsirolimus.

The test is based on 20/20’s Layered Immunohistochemistry (L-IHC) technology. It uses a single tissue section from a standard FFPE tissue block to measure the expression levels of key proteins in the VEGF and PI3K/AKT/mTOR pathways.

Renal Cell Carcinoma (RCC) is the 6th leading cause of cancer death but represents only 3% of US cancers. Sixty percent of RCC are detected early (localized cancer) and detected on scans for other indications. Up to 50% of these patients will develop distant metastasis in the year following surgery.

Targeted cancer therapies, in particular anti-angiogenic therapy and mTOR inhibitors, have improved the outlook for RCC.

However, it is impossible to predict if a patient will respond to a targeted therapy. Objective response rates to anti-angiogenic therapy (sunitinib) is 30-40% for sunitinib, and 10% for mTOR inhibitors (everolimus and temsirolimus). As both adverse reactions and treatment costs are significant, there is an unmet medical need for a method to identify responders and non-responders.

Sunitinib (as well as other anti-angiogenic therapies) often causes serious adverse reactions, such as bleeding, shortness of breath, and nausea (see www.sutent.com) and costs of up to $50,000 for a standard 8 month course of treatment.

20-20’s assay has the potential to help select the best treatment options for kidney cancer patients. Of 59 patients treated with sunitinib 32 had scores of 24 or higher, and were considered potential responders. 27 had scores below 24. (Figure 1)

The median time on treatment for patients in the high score group is 12 months with 95% CI (8, 24).

The median time on treatment in the low score group is 3 months with 95% CI (3, 12).

Like sunitinib, mTOR inhibitors are also associated with serious side effects, significant expense, and low objective response rate (10%).

It appears that the kidney cancer assay can predict who is likely to respond to treatment with an anti-angiogenic drug or an mTOR inhibitor. The study is currently being validated in a larger sample cohort.

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![Figure 1: The kidney cancer test uses a predictive score based on 3 biomarkers in the multikinase pathway to differentiate responders from non-responders to anti-angiogenic therapy. Of 59 patients treated with sunitinib 32 had scores of 24 or higher. 27 had scores below 24.](image1)

![Figure 2: The kidney cancer test measures 3 biomarkers to differentiate responders and non-responders to treatment with mTOR inhibitor. Using 6 or higher as the cutoff, the sum of the markers for each of the cases correctly identifies 8/12 (67%) responders, and 17/21 (93%) non-responders.](image2)
**Discussion**

Anti-angiogenic therapy is an important tool to treat RCC. Pfizer’s drug sunitinib (SUTENT) is one of several targeted therapies that have been approved by U.S. regulators in the last 10 years to treat advanced kidney cancer. It is currently used as the first-line therapy for this indication.

The mTOR pathway also plays an important role in cancer growth and metastasis. Several drugs, temsirolimus (TORISEL) and everolimus (AFINITOR), that target this pathway have been approved by the FDA for the treatment of metastatic kidney cancer.

This assay is likely to improve the standard of care. Early identification of potential responders to the correct targeted therapy could change the order of treatment and positively impact patient outcome.

Although the optimal treatment strategy for metastatic RCC continues to evolve, 3 agents that target angiogenesis (sunitinib, bevacizumab, and pazopanib) and a mammalian target of rapamycin (mTOR)–targeted therapy (temsirolimus and everolimus) have been approved as frontline agents.

![Diagram](image1)

**Figure 3**: Several treatment options are available for renal cell carcinoma. The two most important therapeutic approaches are antiangiogenesis drugs (e.g. sunitinib) with a 30-40% objective response rate, and mTOR inhibitors (temsirioimus or everolimus) with an objective response rate of 10%.

Anti-angiogenic therapy is typically the first choice of targeted therapy following cancer relapse after nephrectomy and in patients who present with stage IV cancer. mTOR inhibitors are normally used after failure of treatment with sunitinib or sorafenib. With this new kidney cancer test patients that test negative to response to anti-angiogenic therapy and positive for mTOR inhibitor therapy would be treated with an mTOR inhibitor as a first line therapy, followed by a VEGFR inhibitor as a second line therapy.

**Figure 3: Example of Kidney Cancer Test results.** The upper case shows a partial responder (left to right): Standard H&E section followed by three membranes stained for different protein biomarkers and one blank reference with no primary antibody. Upregulated biomarkers are clearly visible. In the non-responder case, below, one biomarker is upregulated. However, in 20/20’s statistical analysis of the biomarkers across many cases it was clear that only a combination of the three biomarkers could help separate responders from non-responders.
20/20 GeneSystems develops companion diagnostics and offers L-IHC tissue analysis services for multiplex biomarker analysis. We support all stages of drug development, from target validation through clinical trials for patient stratification and monitoring of therapeutic response.

Our laboratory routinely works with assays for pathway profiling, target validation, and clinical diagnosis including prognosis, prediction of therapeutic efficacy and monitoring of treatment outcome.

The technology conserves precious clinical specimens. Up to 10 biomarkers can be analyzed using a single section from any kind of FFPE tissue sample; needle biopsies, surgical resections, or even tissue microarrays.

L-IHC gives results comparable to IHC but with a broad dynamic range and a continuous measurement scale. Total vs. activated (e.g. phosphorylated) biomarkers can be measured simultaneously.

A robust, specific and reproducible approach to tissue proteomics, L-IHC introduces a high degree of quantitation to IHC. It allows for intra and inter experimental normalization, and ratio-metric measurements.

The L-IHC platform is a versatile and enabling approach to tissue proteomic analysis. It has a broad range of applications; from analysis of preclinical and animal models and translational research, to clinical trial assays and companion diagnostics.

The L-IHC platform was developed by 20/20 GeneSystems in collaboration with the National Cancer Institute’s Laboratory of Pathology. It has been validated with a large number of different antibodies and tissue types.

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