

2nd Annual Prostate Cancer Forum An Educational Initiative

Current and Emerging Biomarkers for Disease Management

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I. Biomarkers - the holy grail of personalized medicine?

This is an exciting time in medicine. The human genome has been sequenced in its entirety, and we have the capability of finding changes throughout the genome. These advances hold the promise of being able to tailor treatments to patients, and the potential benefits of clinically-relevant biomarkers would be improving clinical efficacy and decreasing toxicity by better treatment and patient selection.

II. Phases of Biomarker Development

There is essentially one biomarker that is in clinical use, and the processes that have been put in place by the FDA to find biomarkers that reflect clinically important outcomes have been broken into four phases. Ideally, in phase I you are looking to identify a causal relationship. After that phase, whatever assay you are looking at has to go through a process of laboratory validation. Analytic validation is equivalent to a phase II. Ultimately, to get to clinical use you want to qualify a biomarker, and that is where the confirmation with clinical outcomes happens after which it is hopefully brought into use for patients.

III. Clinical Utility of Biomarkers in Prostate Cancer

PSA is the biomarker that is probably the most familiar in prostate cancer, and one of the places that it is used in detection is for screening. Another commonly used biomarker is the Gleason score, which is where we look at the tumor itself in its differentiation. An additional clinical scenario where a biomarker may be helpful in making a clinical decision would be prediction. That would be whether the patient is going to respond to the prescribed treatment. There are no such biomarkers in prostate cancer currently. Testosterone is a pharmacodynamic marker in prostate cancer, and it measures whether the hormone therapy is actually working or doing what it is supposed to do. Finally, clinical surrogates measure not only whether the treatment is working and doing what it is supposed to do but also whether it is going to result in a better outcome for the patient. A PSA decline is a tumor marker in prostate cancer.

IV. Prostate Cancer Clinical States and Treatment Decisions

Biomarkers in prostate cancer could potentially help us when we are deciding when to start treatment, which treatment to use, whether a treatment is working and/or whether a treatment is helping the patient.

V. Current Landscape of Treatments for Castrate-Resistant Metastatic Prostate Cancer

After the failure of hormone therapy in prostate cancer, the options for treatment include sipuleucel-T, chemotherapy, second-line therapy, and third-line therapy, and docetaxel has been first-line chemotherapy for prostate cancer for the last ten years. The problem with all of these therapies is that they ultimately will stop working. Moreover, some patients don't respond at all to the initiation of chemotherapy. If you could identify ahead of time the patients that would respond, it would help in providing better treatment to patients.

VI. Response Markers in Prostate Cancer

The markers that we currently use to measure a response to treatment for patients with prostate cancer include the PSA, pain, bone scans and CT scans/MRI. The problem with prostate cancer is that all of these markers have limitation. For example, not all patients have pain, and it is very hard to document a response to chemotherapy with a bone scan. The recommendation from the Prostate Cancer Working Group is that, "In the absence of clinically compelling indicators of disease progression, early changes (within 12 weeks) in indicators such as serum PSA, patient-reported pain, and radionuclide bone scan be ignored."

VII. PSA Responses

An issue with the current response markers in prostate cancer, including the PSA, is the heterogeneity of responses in patients. The PSA is still, however, a useful marker. In the phase III SWOG cooperative group study, they compared docetaxel/estramustine to mitoxantrone/prednisone, and the PSA response occurred in 76% of the docetaxel patients compared to 40% of the mitoxantrone patients. The study ultimately did lead to the approval of docetaxel in combination with another docetaxel study.

VIII. Circulating Tumor Cells (CTC)

The biomarker that is closest to being used clinically in prostate cancer is circulating tumor cells. For patients with solid cancers, on occasion we can detect the cancerous tumor cells in the circulation. It is thought that as tumor cells travel through the circulation that is ultimately part of the process to becoming metastatic. In addition to being used as a biomarker, circulating tumor cells are also being looked at as a way to analyze cancer cells whereby you can actually do profiling on even very small numbers of tumor cells. Various companies and institutions have developed methodologies for measuring CTC in the blood, and the CellSearch assay has gone through the process of analytic validation. Their cutoff for favorable versus unfavorable is 5 circulating tumor cells per 7.5 mL of blood, and it is FDA-approved. Investigators are looking at whether we can use CTC as a marker of response and also comparing it to what we currently have available, which is PSA. It looks like CTC

can be used to predict outcomes for patients with castrate-resistant prostate cancer patients who are starting chemotherapy. In a comparison of CTC versus PSA, a change in CTC does appear to have more prognostic information than the PSA decline.

IX. Clinical Utility of Biomarkers in Prostate Cancer, Revisited

We have biomarkers for detection even though they are imperfect. We have biomarkers for prognosis, and we have biomarkers to some degree showing whether therapies are working. That is not true for all therapies, but it is true for GnRH agonists. We are able to use PSA decline even with limitations as a tumor marker, but we currently don't have anything in prostate cancer showing us whether or not a patient is predicted to respond to a certain therapy.

X. Markers that Correlate with Docetaxel Sensitivity

In my lab, I was looking at RNA profiling different cell lines in a retrospective data-mining type of study, and I came up with a list of the various sets that were correlated with response. SKP2 was over-expressed in cell lines that were sensitive to docetaxel, and I was able to show that if you knock that particular gene down, you affect docetaxel sensitivity. Hopefully in the future I will be looking at this in tumor tissue itself to see if we can use the information to predict in advance those patients that would respond to chemotherapy.

XI. Conclusions

PSA decline can be used as a surrogate marker for response although it is at a later time point. Baseline levels of CTC are prognostic for overall survival. Conversion of CTC, going from favorable to unfavorable, correlates with overall survival, but there are some caveats in terms of taking it into clinical practice right away. There is room in the field for more biomarker studies to try to help us identify new biomarkers and to figure out how to use them to get the best outcomes for patients.

Steven Lucas, MD-Karmanos Cancer Institute

I. Why are biomarkers important?

Prostate cancer is diagnosed in 200,000 men annually in the United States, and there are 30,000 cancer-specific deaths per year. Yet, a substantial number of cancers diagnosed through PSA screening do not progress to clinically significant disease.

II. Problem with PSA Screening

One of the problems with PSA screening is that it is not specific resulting in a negative biopsy rate of up to 70% in some series. The importance of the PSA screening issues is highlighted in two series published in the *New England Journal of Medicine* this year. In a European randomized trial, they determined that it would take 1,410 men screened and 48 treated to prevent 1 death from prostate cancer. However if you look at the watchful waiting trial from Sweden, the relative risk of dying from

surgery was 0.62 suggesting that it was protective and that the number needed to treat was a lot lower, 15 overall and 7 for men younger than 65.

III. How do we improve screening and treatment decisions?

We have been improving screening and treatment decisions by utilizing clinical nomograms, which include things like Gleason scores, the number of positive cores, percent involvement of cores, and PSA. Other risk factors include family history, age and race. We may be able to further improve the information by using some of the new biomarkers.

IV. How can biomarkers improve management?

Biomarkers could help supplement or replace PSA in identifying patients who might be at risk for prostate cancer who could go on to a prostate biopsy. After prostate biopsy, there is the decision of what to do with the cancer, and biomarkers may be able to help determine which cancers are more aggressive and deserve aggressive therapy and which cancers can be watched. Furthermore, biomarkers could help determine after the primary treatment whether the patient might be at risk of recurrence and might need additional therapy. Finally, in the more advanced stage, biomarkers could help determine which patients are at risk of progressing to death from their prostate cancer.

V. Categories of Biomarkers

1. Urine-Based Biomarkers

Urine-based biomarkers can be categorized into proteins, DNA and RNA. Perhaps the most widely used urinary biomarkers now are the RNA-based markers. Focusing on PCA3, it was developed from differential expression of noncoding RNA's in prostate cancer versus other prostate conditions. It is commercially available and an approved diagnostic test. It is collected from a urine sample following a firm digital rectal exam, and it could function as a first-line screen or prognostic indicator.

There are several studies that have looked at PCA3 as a first-line screen, and they demonstrated overall superiority of PCA3 in terms of specificity of 80 to 90%, but they included only patients with an elevated PSA. As a comparison, if you look at the placebo arm of the REDUCE trial, the sensitivity of PSA in the placebo arm was 0.51 and the specificity was 0.63. The specificity of PCA3 in elevated PSA levels of 4 to 10 is pretty good, and the sensitivity is a little bit lower. However, if you put PCA3 and PSA together in their own head-to-head comparison where each independently acts as a trigger for biopsy, we found there was only a slight improvement on PSA with PCA3. Another way to try to improve the diagnostic accuracy of PCA3 is to use gene fusion in which a strong androgen promoter is fused with an oncogene. In one study, it augmented the predictive accuracy of PCA3 from PCA3 alone to using PCA3 with gene fusion and PSA altogether at 0.8.

There is some conflicting data on whether PCA3 is a prognostic indicator. Some studies show a positive relationship with the aggressiveness of cancer. However, in the REDUCE trial they looked at PCA3 and only demonstrated a weak association between PCA3 and Gleason 7 or higher cancer. The odds ratio was 1.02. However, the number of high-grade prostate cancers was low somewhat diminishing the power of the study.

2. Blood-Based Biomarkers

Blood-based biomarkers include all of the PSA isoforms as well as some of the newer prognostic indicators including human kallikrein 2, urokinase plasminogen activator, transforming growth factor beta-1, interleukin-6 and endoglin. There are some limitations of the total PSA, and one of the reasons is that neoplastic cells produce varying levels of PSA. An additional problem is that there's inherent biologic variation within the individual. Another complicating issue is there are different assays that measure PSA, and there is some question on what the cut-off should be for PSA.

Before throwing out PSA, however, it is an extremely strong predictor in terms of the long-term risk of developing cancer. The Malmo Preventative Medicine Study found that when they went back and looked at the PSA at age 44 to 50 in 462 prostate cancers matched to 1,200 controls, as the PSA went up the risk of developing prostate cancer went up. When it got to between two and three, it was almost 20-fold.

PSA velocity is another PSA manipulation, and basically it is the measurement of change in total PSA over time. Two large prospective trials, however, found no independent predictive value beyond total PSA and other standard variables. That being said, PSA velocity may be more valuable as a prognostic indicator in that several series have found an increased risk of mortality from prostate cancer with PSA velocities that depending on the series ranged from 0.35 to 2.0 ng/mL per year. Some believe this may not be predictive of early progression but an indication that aggressive disease has gone beyond the treatment window.

Percent free PSA is the isoform of PSA that remains unbound in plasma. It is FDA-approved as an adjunct to total PSA between PSA values of 4 to 10. If the free PSA is less than 25%, it can be used as a trigger for biopsy. This was evaluated in a multicenter, prospective trial, and the specificity was 95%. The sensitivity was improved 20% over PSA. The predictive accuracy was 0.72 versus PSA alone at 0.53. However, we are now doing 10-12 core biopsies, and that diminishes the predictive accuracy.

In all likelihood, the most helpful solution will be to combine the PSA isoforms into a panel and devise a nomogram that can be added to the clinical nomogram. In a side arm of the European Randomized Screening for Prostate Cancer Trial, by using these various factors applying them to a theoretical guess of who should be biopsied and who shouldn't, they determined that they could reduce the biopsy rate in 1,000 men by 573. With that, they would miss 31 out of 152 low-grade cancers and 3 out of 40 high-grade prostate cancers.

Endoglin is a marker for immature blood vessels so becomes a surrogate marker for angiogenesis. Studies have shown that before prostatectomy the levels may predict a higher Gleason score or PSA recurrence. It has also been used to attempt to detect the presence of lymph node metastases in prostate cancer although that hasn't been valued as well. Biomarkers will likely be used to supplement not replace clinical data to improve the accuracy of prognosis.

3. Tissue-Based Biomarkers

Many of the biomarkers in the diagnostic category of tissue-based biomarkers are related to staining, and some of the tissue-based biomarkers have had some prognostic value.

Focusing on the prostate specific membrane antigen, it is negatively regulated by androgens and over expressed in androgen-independent prostate cancer. It has been used to help improve diagnostic accuracy, but more interestingly it may be associated with higher grade and increased rate of biochemical recurrence after therapy.

VI. Translating Biomarkers into Therapeutic Targets

In PSMA, there is an antibody drug conjugate currently in a phase 1 trial for castration-resistant metastatic prostate cancer. In endoglin, there is a monoclonal antibody that binds to endoglin thus inhibiting angiogenesis, and that is in a phase 1/phase 2 clinical trial.

VII. Summary

Biomarkers serve as a powerful adjunct to the diagnosis and management of prostate cancer, and they are testable in the urine, blood, and prostate cancer tissue. Further validation of these biomarkers and research into potential therapeutic targets is needed.