

ADVANCES IN ONCOLOGY

Current Developments in the Management of Solid Tumor Malignancies

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Molecular Assays to Predict Prognosis of Breast Cancer

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H&O What is the basis for the need to generate molecular prognostic data in breast cancer?

SP There are two primary reasons to use molecular assays in breast cancer. First, the clinical tools currently available do not predict patient outcome and response to treatment with high enough accuracy. Second, the type of data that is generated using RNA seems to be superior to the data generated with measuring proteins by immunohistochemistry. The data generated appear to have more linearity in relationship to outcome or treatment response. To me, this aspect of molecular prognostic data is important because if an assay is not linear, the data generated are not accurate and hence the prediction can be flawed. A typical example is the estrogen receptor in breast cancer. In the National Surgical Adjuvant Breast and Bowel Project (NSABP) trial B-14, which tested the blocking of the estrogen receptor with tamoxifen in patients with node-negative, estrogen receptor-positive breast cancer, the amount of estrogen receptor measured using a classic biochemical assay such as ligand binding or immunohistochemistry was found not to be a very reliable predictor of the degree of benefit to expect from tamoxifen due to the lack of linearity. When we examined the same cohort with the RNA assay, this proved to be an extremely linear predictor. From my personal viewpoint, this finding shows the reason why RNA-based measurement of any target is preferable to protein-based measurement.

A major concern with the use of molecular assays is that none of the molecules we assess can singlehandedly predict outcome or response. It is necessary to look at multiple markers. Many times looking at a single marker seems to be effective in one cohort but not in another because there is instability with the prediction. Only when multiple markers are combined is stability achieved.

If the same kind of patient cohort is repeatedly assessed, for example 400 estrogen receptor-positive patients treated the same way, after multiple experiments 1,000 genes predictive of prognosis may be found, but there is not a high degree of overlap with these genes from each experiment. Only a handful turn out to be reproducibly prognostic. The pitfall of starting with one target or just a few targets, rather than the whole genome, is that it is likely data that are not very reproducible will be generated, which has been proven over time in multiple studies. Many biomarkers that investigators have thought to be very exciting turn out not to be robust in other trials for this reason.

H&O Which subsets of patients are most interesting for research on predicting prognosis?

SP A good deal of interest has been in patients who were diagnosed with estrogen receptor-positive and axillary node-negative breast cancer, mainly because of the clinical data showing that when these patients are treated with endocrine therapy like tamoxifen, their prognosis is very good. Only 15% of these patients recur within 10 years. When these patients are given chemotherapy, there is some benefit, so it is important to identify the patients who recur and the patients who derive benefit from chemotherapy. Much of the focus of biomarker development in breast cancer has been in that particular subset. However, NSABP and other groups are conducting clinical trials in other subsets, trying to improve upon standard chemotherapy by changing the schedules or adding new drugs, including targeted therapies such as HER2 or angiogenesis inhibitors. These new drugs are effective only in particular subsets, so the molecular profiling in these trials is very timely and essential.

H&O Are there ongoing trials incorporating molecular profiling upfront?

SP There is only one trial worldwide using upfront stratification based on molecular profiling in breast cancer, which is the Trial Assigning Individualized Options for Treatment (TAILORx) sponsored by the National Cancer Institute. The stratification in this trial is based on the 21-gene assay developed with the NSABP called

Oncotype DX (Genomic Health). TAILORx is enrolling women with estrogen receptor and/or progesterone receptor–positive breast cancer who have undergone surgical resection; the patients receive either chemotherapy, hormonal therapy, or hormonal therapy in combination with chemotherapy based on the results of the assay. There is another trial in Europe using a microarray-based assay, called the MINDACT trial, with a slightly different aim. Otherwise, many trials are collecting tissue as part of the trial, with the goal of analyzing the tissue after the trial has ended in order to develop markers.

H&O How are molecular markers integrated with more traditional clinical prognostic markers?

SP It is of course possible to ignore the classic markers and decide to use molecular markers because they are more accurate and reproducible, but there are still useful clinical markers, such as tumor size. A tumor can have a poor prognosis despite a positive molecular profile if it is neglected to 5 cm at the time of diagnosis. Usual clinical parameters should not be ignored; rather, molecular markers should be integrated with traditional clinical markers of prognosis. There is an ongoing effort to combine molecular and conventional markers into a single algorithm. It is important for clinicians to understand that, for example, a prognostic signature from 70 genes must be transformed into a single mathematical algorithm that is linked with clinical outcome to form a combined prognostic index. It is not possible simply to use the raw data derived from the microarray.

H&O Are the assays that are commercially available useful in all settings of breast cancer?

SP Utilization of the 70-gene assay (MammaPrint, Agendia) can be problematic because this assay was developed for the setting of premenopausal patients who have not yet received therapy. The 21-gene assay was developed to be highly context-specific in its usage. As a clinical investigator, I prefer context-specific markers, with which the clinical utility can be defined from the outset. The 70-gene assay, though it appears useful on paper, may be difficult to use clinically.

H&O What are the next steps with molecular assays of prognosis?

SP From the conceptual viewpoint of management of breast cancer, what researchers have found so far from doing gene expression analysis is that there is an inherent link between poor prognosis and favorable response to chemotherapy, which is a positive finding. If a tumor has good prognostic features, not much benefit will be conferred by chemotherapy. On the other hand, those tumors with poor molecular prognostic features tend to gain significant benefit from standard chemotherapy.

Therefore, we now have a useful tool for triaging patients into risk categories that help define whether to administer therapy. Unfortunately, even when chemotherapy is given to patients whose tumors have poor prognostic features, their prognosis remains not as good as those with good-prognosis tumors who do not receive chemotherapy. What we discovered in the NSABP trial B-27 is that if chemotherapy is given prior to surgery in a neoadjuvant setting and response is measured and combined with information from gene expression, those patients who begin with a poor prognostic gene signature can have a very good prognosis if they respond to chemotherapy. Thus, by combining gene signature and response to neoadjuvant therapy with the tumor intact, we are essentially creating an *in vivo* chemosensitivity assay. The patients in whom the tumor disappears have a very good prognosis, even if the tumor originally had a bad prognostic signature. Furthermore, it is possible to identify those who start with a bad prognostic signature and who do not respond to chemotherapy. These patients become ideal candidates for trials of new therapies, such as antiangiogenic therapy. With gene expression in the neoadjuvant setting, we have been able to develop a new paradigm for trials, called a postneoadjuvant trial, in which new drugs can be tested in chemoresistant patients with poor prognosis based on molecular features. We are hopeful that this strategy will be beneficial to patients.

From a practical point of view, these assays need to be extremely reproducible. At this point, it is not clear whether we can apply microarray assays in everyday clinical settings as a routine assay. There is a great deal of variability in routine settings, though it can be controlled to some extent. The 70-gene MammaPrint assay was approved by the US Food and Drug Administration based on its reproducibility, so it is important to develop new molecular tests with reproducibility in routine settings in mind. Reasonable cost is another concern. The 21-gene Oncotype DX assay is highly reproducible, but it costs over \$3,600, which makes it inaccessible to patients in many countries around the world. One of the goals should also be to develop reasonably affordable assays.

Suggested Readings

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