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OVERVIEW

Instrumentation for Flow and Static Cytomorphometry and Cytophotometry. Quality Control of Surrogate Endpoint Biomarkers

Session VII was technique-oriented, focusing on the quality and reproducibility of biomarker measurements from cytologic and histologic material (in contrast to tissue extracts). Dr. Robert Becker described key aspects of techniques comprising quantitative microscopy. Generation of numerical data, rather than a visual impression, is central to each technique. Standardization of methods is required in order to share and pool results for large studies conducted at diverse sites. Quality control is required to track technique performance specimen by specimen. Standardization and quality control efforts interact, since results from quality control of specific test phases can bring to light appropriate standards to be adopted for test performance. Using DNA densitometry as an example, the importance of proper technique and control was demonstrated for all test phases including specimen preparation, image acquisition, image segmentation, and image feature extraction. Most issues affecting DNA densitometry also apply to other quantitative microscopy techniques. There is not yet a consensus for standards and quality control measures in quantitative microscopy. Consensus development will likely require circulation of specimens, though distribution of archived images may be appropriate for some quantitative microscopy techniques.

Ms. Lynn Dressler discussed flow cytometry (FCM) for measurement of biomarkers associated with nuclear DNA content. S-phase fraction, in particular, is well established for prognosis of invasive breast carcinoma. Utility of flow cytometry results depends on the use of proper analytic methods, especially at the time of histogram analysis. Adherence to well-established criteria for presence of aneuploidy is critical to reproducibility across data sets and laboratories. Proper S-phase calculation is even more exacting than ploidy characterization, requiring use of sophisticated programs to compensate for debris, cell aggregates, and population overlap in histograms, and establishment of laboratory-specific reference values for low versus high S-phase results. Though FCM can be applied effectively to various types of specimens (*e.g.*, aspirates, fresh tissue, paraffin-embedded tissue), it is important to assure an adequate number of representative cells for analysis. This will be a special concern when trying to monitor progression or response of preinvasive disease.

Dr. Gary Clark indicated that establishment of surrogate endpoint biomarkers for development of invasive breast carcinoma is a goal yet to be accomplished. In studies working toward this goal, statistical considerations are critical in order to avoid false negative results due to inadequate study power, or false positive results due to inappropriate p-values derived from multivariate data. When sorting through numerous candidate biomarkers, it is especially important to focus on those with a plausible cause-effect relationship to the disease process. Determination of appropriate case source and number is crucial for conclusive results, especially in view of potential sampling bias. The ability to generalize study results to the larger population depends on avoiding introduction of bias during multivariate analysis. One approach is to refrain from unnecessary conversion of continuous biomarker values to categories via "cutpoints." Another is to discount significance of features tested in multiple comparisons against outcome. The best indicator of study validity remains effective biomarker performance in predicting outcome for test cases independent from those used in the original study.

Given our current knowledge, proposed Phase II trials may be most valuable in documenting biomarker expression in preinvasive disease. Long term follow-up for all patients enrolled in such studies will be critical in establishing the true value of those biomarkers for prognosis and as surrogate endpoints for disease progression.

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