sought to extend our previous results on gene expression grade genes capturing mainly proliferation by adding into the model other relevant gene expression modules representing several biological processes in breast cancer such as estrogen receptor signaling and ERBB2. We sought to depict the connection between these modules and the previously reported molecular classification, several prognostic classifiers and the most established clinico-pathological variables. A number of interesting conclusions were drawn from this collaborative effort.
First, the disparity of the gene lists produced by several investigators can be attributed to heterogeneity in patient characteristics, expression profiling methodologies and sampling variation due to small sample size relative to the number of genes examined. Second, breast tumors were grouped into three main subtypes corresponding roughly to ER−/ERBB2−, ERBB2+ and ER+ tumors. Third, with respect to proliferation, both, ER−/ERBB2− and ERBB2+ subtypes were characterized by high proliferation, whereas the ER+ subtype appeared to be more heterogeneous. The latter was divided into two distinct subpopulations, the ER+/low and the ER+/high proliferation tumors resembling to luminal A and B subtypes respectively. Fourth, all previously reported prognostics signatures despite the disparity in their gene lists carry similar information with regards to prognostication. Fifth, proliferation genes appear to be the common driving force. Sixth, all these prognostic signatures are very useful for determining the risk of recurrence in the ER+ subgroup and less informative for ER− and ERBB2+ disease. Finally, nodal status and tumor size still retain important prognostic information.
Conclusions: This meta-analysis reveals for the first time connections between clinico-pathological traditional prognostic factors, expression-based sub-typing and prognostic signatures, highlighting the important role of proliferation in breast cancer prognosis.

S14

From gene expression signature to diagnostic test: Challenges in applying genomic technology to molecular diagnostics

J. Warrington. *Affymetrix Inc., USA*

Many exciting discoveries in cancer research have been reported using whole genome gene expression assays, yet few actual diagnostic tests have been developed and cleared for use in clinical practice. Successful adaptation of microarray technology into routine clinical practice requires establishing analytic reproducibility, consensus on quality, standard controls and best practice guidelines. Multiple international standards development efforts are underway that will accelerate acceptance and adoption of microarray technology in clinical studies, clinical trials and diagnostics. In this talk I will describe two recent initiatives aimed at addressing the first of many of the needs that must be resolved to fully realize the benefits of genome technology as well as provide an overview of the challenges and issues facing the development community.

S15

Interpretation of microarray data in cancer: a statistical viewpoint

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Introduction: Gene expression profiling is increasingly used in cancer research. The main objectives of microarray studies are (1) to identify homogeneous subtypes of a disease on the basis of gene expression, or (2) to find genes that are differentially expressed in tumours with different characteristics, or (3) to develop a rule on the basis of gene expression allowing the prediction of patient prognosis or of the response to a particular treatment.
Main message: Using pioneering work on breast cancer as an example, I shall review some of the problems in interpreting the results of these types of study, and discuss the statistical power, the validity and the clinical usefulness of the findings.
Conclusion: The example of breast cancer illustrates a problem that is central to the interpretation of microarray data. The hypothesis underlying each study should be stated clearly and the primary objective of a study should aim at its rejection. Studies with a solid experimental design and larger sample sizes are required before gene expression profiling can be used in the clinic to predict outcome.

S16

Tumor biomarkers, the need for a new way to conduct business. Perspective from the US FDA

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Introduction: Despite of major advances in biotechnology and life sciences, new drugs applications to US FDA are not increasing and clinical research and the process of development is getting longer and more expensive. Furthermore, the predictability of drugs entering clinical trials to reach the market is shrinking.
Main Message: We are currently using tools of the 1960's and 1970's for the science of the 21st century. We are conducting clinical trials with designs intended to avoid bias of variability in an age where variability is at the heart of personalized medicine.
Conclusions: A paradigm shift in the way we do business in drug development from early discovery to clinical trial design has to be implemented and a concerted effort of all stake-holders is needed for a new way we do business.

S17

Epigenetic biomarkers in human cancer

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Introduction: Recent years have seen the mapping of increasing numbers of genes in which promoter CpG islands are hypermethylated in cancer.
Main Message: Such DNA-methylation mapping has revealed unique profiles of hypermethylated CpG islands

that define each neoplasia. The specificity of the assay is increased only if those DNA methylation markers that are always unmethylated in normal 'healthy' cells are included in this panel. In some cases, such as prostate cancer, a single hypermethylated marker, glutathione S-transferase-d (GSTP1), is informative in 80–90% of cases. For the various cancers for which DNA-methylation profiles are available, CpG-island hypermethylation has been used as a tool to detect cancer cells in all types of biological fluid and biopsy. One of its main advantages over other classical markers is the extreme sensitivity of some of the methods used for the detection of aberrant methylation. Another important finding has been that the CpG island hypermethylation of tumour-suppressor genes occurs early in tumorigenesis. This finding might be useful in early-detection screenings, especially in individuals with a high familial risk of developing cancer who have similar patterns of CpG-island hypermethylation as sporadic cases. Another interesting aspect are the use of DNA-methylation profiles as predictorsof outcome. There are instances in which a tumour suppressor that undergoes methylation-associated silencing is a potential candidate for testing as a predictor of tumour prognosis. For example, death-associated protein kinase (DAPK), p16INK4a and epithelial membrane protein 3 (EMP3) hypermethylation have been linked to tumour prognosis in lung, colorectal and brain cancer patients. The final issue correponds to pharmacoepigenetics: DNA methylation as a predictor of response to chemotherapy. The most compelling evidence that epigenomic profiles can predict responses of cancer to therapy is provided by the methylation-associated silencing of the DNA-repair protein MGMT in human brain tumours. MGMT is directly responsible for reversing the addition of alkyl groups to the guanine base of DNA and this base is the preferred point of attack in the DNA of several alkylating chemotherapeutic drugs, including BCNU (carmustine), ACNU (nimustine), procarbazine, streptozotocin and temozolamide. MGMT hypermethylation is the best independent predictor of response to BCNU and temozolomide in gliomas. The potential of MGMT to predict the chemoresponse of human tumours to alkylating agents can also be extended to other drugs with similar modes of action, such as cyclophosphamide.

Conclusions: DNA methylation biomarkers in human cancer are here to stay.

S18

Mining the proteome for clinically useful lung cancer signatures: Technology and trade-offs

D. Carbone. *Vanderbilt University, Nashville, TN, USA*

Introduction: Unlike some tumor types, the majority of the common solid tumors appear not to be driven by single dominant targetable pathways. Instead, diseases such as lung cancer are likely to be much more complex and heterogeneous, with many distinct and overlapping subsets of tumors within the class, each of which will demand an in depth analysis to define the optimal therapeutic approach. These groups are starting to be defined by multiple technologies, and the simplest example is the small subset of patients with tumors expressing mutant EGFR, who achieve substantial clinical benefit from minimally toxic targeted therapy. Even for this small subset of patients with mutant epidermal growth factor receptors (EGFR), multiple resistance mechanisms have emerged requiring different salvage strategies. DNA sequence analysis will likely yield other small subgroups with direct therapeutic implications, and expression arrays are beginning to identify others, but analysis of the proteome has many theoretical advantages, for a complete knowledge of the proteome would encompass all known mechanisms of functional dysregulation associated with the development of cancer, including DNA mutations, rearrangements, transcriptional alterations and promoter methylation, but also post-translational modifications. This depth of information is still far from reality, however, and the true information content of today's technologies leaves a lot to be desired, but indications of utility are now being seen.

Main Message: Using the simple, inexpensive, and rapid technology of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI MS) we studied unfractionated, pretreatment sera to identify NSCLC patients with improved survival after treatment with the EGFR TKIs gefitinib and erlotinib. Mass spectra, independently acquired at two institutions, gave highly concordant results, and were used to generate an algorithm predictive of time to progression and survival. This prediction algorithm was then validated in a blinded manner in two independent cohorts of NSCLC patients treated with EGFR TKIs. This classification algorithm did not predict outcome in three independent cohorts of patients who did not receive treatment with EGFR TKIs. Thus, if upheld in prospective clinical trials, this analysis of pre-treatment peripheral blood might be useful in selecting therapy for advanced non-small cell lung cancer patients.

Conclusion: New technologies, such as shotgun proteomics, that give far more detailed information have also been far more cumbersome and less reproducible. However, we are now able to achieve a depth of information comparable to expression microarray analysis using shotgun proteomics of tumor and normal samples, with improving reproducibility. This is allowing for the more practical analysis of single samples, and definition of activated pathways in tumor cells in real-time. Direct quantitation of specific peptides of interest can also be achieved. It is likely that as the technology improves, proteomic signatures of cancer will be a significant source of information enabling the development of clinically useful individualized of risk assessments and therapeutic decision-making.

S19

Diagnostic classification of pediatric cancers using microRNA profiles

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Introduction: While most conventional genes encode proteins to carry out their biological functions, the