Formalin fixed paraffin embedded tumors are a reliable source for DNA to perform Comparative Genomic Hybridisation arrays (CGH) using 44 kD oligonucleotides (or even more), enabling to identify even micro-gains and -losses. These can be used to identify patients who are likely to respond to currently used combinations.
Conclusions: Subsequent prospective testing of treatment algorithms is necessary to implement drug individualisation in the clinic.

SP143
Cancer stem cells and the microenvironment: The role of hypoxia

J. Rich. *Cleveland Clinic, USA*

Tumors are aberrant organ systems containing a neoplastic compartment and vascular, inflammatory, and stromal elements. Most cancers display a hierarchy of differentiation states within the tumor cells. Molecular signals that drive tumor formation and maintenance commonly overlap with those involved in normal development and wound responses − two processes in which normal stem cells function. Cancer stem cells need not be derived from normal stem cells but self renew and differentiate depending on conditions. The potential significance of cancer stem cells in cancer biology has been demonstrated by studies showing contributions to therapeutic resistance, angiogenesis, and tumor dispersal. Like somatic stem cells, cancer stem cells reside in specific niches (including the perivascular and hypoxic compartments).
Glioblastoma stem cell enriched and depleted populations were derived from human surgical biopsy specimens and xenografts and analyzed under normoxia and hypoxia.
We recently reported that physiologic oxygen levels differentially induce hypoxia inducible factor-2α (HIF2α) levels in cancer stem cells. HIF1α functioned in proliferation and survival of all cancer cells but also was activated in normal neural progenitors suggesting a potentially restricted therapeutic index while HIF2α was essential in only in cancer stem cells and was not expressed by normal neural progenitors demonstrating HIF2α is a cancer stem cell specific target. We now extend these studies to examine the role of hypoxia in regulating tumor cell plasticity. We find that hypoxia promotes the self-renewal capability of the stem and non-stem population as well as promoting a more stem-like phenotype in the non-stem population with increased neurosphere formation as well as upregulation of important stem cell factors, such as OCT4, NANOG, and c-MYC. The importance of HIF2α was further supported as forced expression of non-degradable HIF2α induced a cancer stem cell marker and augmented the tumorigenic potential of the non-stem population.
This novel finding may indicate a specific role of HIF2α in promoting glioma tumorigenesis. The unexpected plasticity of the non-stem glioma population and the stem-like phenotype emphasizes the importance of developing therapeutic strategies targeting the microenvironmental influence on the tumor in addition to cancer stem cells.

SP152
Beyond immunohistochemistry: Accurate, reproducible and quantitative measurement of protein analyte concentrations in fixed tissue

D. Rimm. *Yale University School of Medicine, USA*

Historically, the many analytical and pre-analytic variables associated with immunohistochemistry (IHC) have relegated it to a semi-quantitative or qualitative status. However, as companion diagnostics become more critical to patient management, new techniques have been invented that use the tools of ELISA assays and flow cytometry to move IHC to a fully quantitative assay. This presentation will describe use of the AQUA® method of quantitative immunofluorescence (QIF) to measure critical analytes in breast tissue showing data on accuracy and reproducibility. Then, using a series of cell lines which are standardized to recombinant protein, we have measured estrogen receptor in three separate cohorts of breast cancer cases. The quantitative approach reveals a cutpoint of 50 pg/ug total protein and suggests that conventional IHC may have a misclassification rate of around 15%. As accurate as QIF is, it does not address pre-analytical variables, most significantly including cold ischemic time, or the time between surgical removal of the tissue sample and fixation. We will show a series of QIF methods for antibody validation that allow neutralization of this variable in assessment of tumor tissue. In summary, measurement of protein on a pathology slide can now be achieved with accuracy and reproducibility of nucleic acid assays or ELISA assays. Combining QIF with rigorous methods of standardization and antibody validation allows companion diagnostic tests to be done on very small tissue fragments.

SP167
Methodology and design of phase 0 and phase 2 trials with biomarker variables and endpoints

L. Rubinstein. *National Cancer Institute, USA*

Current research is yielding an increasingly better understanding of molecular pathways and targets associated with the progression of cancer and, likewise, new target-oriented potential therapies. This presents the opportunity, in early clinical trials, to usefully incorporate biomarkers, associated with the molecular targets or pathways, as prognostic or predictive baseline variables and as correlative or primary endpoints.
We discuss various potential ways a biomarker may be used or explored in an early clinical trial, including:
1. As a prognostic baseline variable, defining patients with homogeneous expected outcome, independent of treatment, or as a predictive baseline variable, defining patients expected to benefit from a given treatment, a biomarker can be used to make a phase 2 trial more efficient and more informative in planning a subsequent phase 3 trial.
2. As a correlative endpoint in a phase 2 trial, a biomarker can be used to assess the effect of an agent or combination on a particular molecular target.
3. A phase 2 trial may be used to explore the use of biomarkers as potential baseline variables, prognostic or predictive, or as correlative endpoints, to assess molecular target effect, but the sample size will restrict discovery to baseline biomarkers associated with relatively large prognostic or predictive effects and correlative endpoints associated with relatively large treatment effects.
4. In relatively rare circumstances, a biomarker may be used as a surrogate primary endpoint in a phase 2 trial, to greatly increase its efficiency.
5. A biomarker may be used as a primary endpoint in a first-in-man phase 0 trial to establish the biologic effect of an agent and determine a biologically efficacious dose level with a very small number of patients.

We present specific statistical designs and guidelines for incorporating biomarkers as baseline or endpoint variables in phase 0 and phase 2 trials, including:
1. A biomarker may be used as a pharmacodynamic response endpoint in a phase 0 trial. For example, with 5−8 patients, a true and promising 40% response rate can be effectively distinguished from a false-positive 5% response rate.
2. With less than 100 PFS events, potentially predictive biomarkers can be successfully detected in phase 2 trials only if the treatment-biomarker interaction (measured as the ratio, for biomarker + vs. −, of treatment-related hazard ratios) is at least 3.

SP148
Emerging tissue-based cancer biomarkers in breast cancer

M. Schmitt. *Clinical Research Unit, Dept. Obstetrics & Gynecology, Technical University of Munich, Germany*

Introduction: Specific biomarkers indicating the course of the cancer disease and/or response to therapy are very much needed to help breast cancer treatment move from the current trial-and-error approach to more personalized treatment.
Purpose: Breast cancer is a heterogeneous disease that varies in morphology, biology, behavior and response to therapy. Since the first application of multiplex gene expression profiling to breast cancer, the molecular subtyping of breast cancer has advanced rapidly from a novel concept to a clinically valuable prognostic/predictive classification, including breast cancer cases based on histology combined with ER (estrogen receptor), PR (progesterone receptor), and HER2 (human epidermal growth factor receptor type-2) status. Now, systematic screening for novel cancer biomarkers at the gene/protein level in breast cancer subgroups has started for determining prognosis, predicting response to therapy and predicting severe toxicity related to treatment.
Main Message: In breast cancer, until end of 2007, only biomarkers ER, PR, and HER2 were recommended for treatment decision making, in addition to histomorphological factors (TNM-status, grading), by (inter)national guidelines. Then, the American Society of Clinical Oncology (ASCO) added biomarkers uPA and PAI-1 to the list. Similar to ER and PR, uPA and PAI-1 may also serve as prognostic factors and factors predicting response to systemic adjuvant therapy. High levels of the matrix metalloprotease (MMP) inhibitor TIMP-1 correlate with poor prognosis, too, absence of TIMP-1 is related to favorable outcome of breast cancer patients. Patients presenting with TIMP-1 overexpression would do equally well by receiving the far less toxic CMF instead of receiving anthracycline-containing chemotherapy. Besides ER, GPR30 (another estrogen binding receptor) is associated with tumor progression and metastatic disease. Moreover, associations with drug-resistance mechanisms in cancer patients have been identified for the Y-box-binding protein YB-1, a member of the cold-shock domain protein super-family.