Issues such as sensitivity, specificity, reproducibility, and standardization are not on the typical patient's radar screen, nor should they need to be so long as the research community (academic, government, and commercial) does their jobs correctly.
Instead, issues of protection of privacy, non-discrimination, and affordability dominate the radar screens of the vast majority of patients.
The spelling of the words "hope" and "hype" differs by only one letter − "o" instead of "y". However, their definitions are vastly different, and in biomarker research the difference between hope and hype can be as wide or as narrow as the people conducting and interpreting the research. Timing is everything and patients depend on researchers for hope, not hype.
Molecular advancements that connect a patient's biomarker with an event such as a drug response are occurring and will continue to occur with greater frequency and reliability. With these emerging molecular methods comes great responsibility in educating health professionals, policy makers, and the general public about the potential use and misuse of the information these methods produce.

SP162
Clinical application of biomarkers: discovery, validation, and application

E. Kohn. *National Cancer Institute, USA*

Introduction: Biomarkers can function as diagnostic, prognostic, or predictive tools, and can also lead to new therapeutics. Alternatively, they can be applied for proof of concept for the mechanism of action of novel agents. The findings of EGFR and K-ras mutations in tumor tissue have had important implications in the treatment of lung and colon cancers and have led to pre-treatment patient selection based upon retrospective analyses. Key to development of new agents is application of pathway and function-related markers to tissue in order to validate the target of the agent. This approach will also yield important insight into the "why" and "why not" of outcomes.
Purpose: To demonstrate the value of tissue-based analyses of pathway and function-related markers in order to characterize and validate molecular treatment targets.
Main message: Clinical trials of single and multi-agent targeted therapies were designed to include at least a pair of biopsies. Biopsies were immediately frozen in OCT and later cut, pathology evaluated, and then cells collected for analysis. Proteomic approaches were optimized to allow reverse-phase protein array analysis of serially diluted proteins. Both total and phospho-protein quantity was measured and then analyzed against patient outcome, toxicity, and demographics. A new trial schema was designed to allow proof-of-concept measurements in combination therapy. We demonstrated on-target activity of imatinib and gefitinib in ovarian cancer patients; this on-target activity correlated with toxicity. Triplet biopsies obtained from patients treated with bevacizumab and sorafenib were analyzed to evaluate single and combination agent signal inhibition activity. Confirmation of target activity was demonstrated for both agents and inhibition of pathway activation was associated with clinical benefit.
Recommendations: Clinical trial design and execution should include objectives to confirm target presence, activation, modulation by therapeutic intervention, and association with outcome. This will expedite selection of patients as well as lead agents and should lead to faster and improved results.
Conclusions: Optimizing application of the new molecularly targeted agents requires knowledge and application of pertinent biomarkers. Prospective validation of target and biomarker will allow future controlled patient selection and would be expected to yield improved patient outcomes.

SP150
Pharmacogenomics in colon cancer

H.-J. Lenz. *USC/Norris, USA*

Although the introduction of biologic agents and the development of associate molecular markers have shown promising results (K-ras, MSI, 18q del, TS, ERCC1), only a few of these biomarkers has been accepted into routine clinical practice. It is becoming increasingly apparent that complex pathways drive disease progression; analysis of one single marker is unlikely to predict efficacy and outcome. Tumors are being classified into specific tumor phenotypes based on molecular profiles. Two of these represent genetic instability classes. The majority of sporadic cases (85%) display chromosomal instability (CIN), defined as allelic imbalance (AI) at a number of chromosomal loci (including; chromosomes 5q, 8p, 17p, and 18q), chromosome amplification and translocation, which collectively contribute to tumor aneuploidy (Vogelstein, Fearon et al. 1988). In contrast, the remaining 15% of sporadic colon cancers demonstrate a high-frequency microsatellite instability phenotype (MSI-H), in which tumors display frameshift mutations and base pair substitutions commonly found in short tandemly repeated nucleotide sequences called microsatellites (Aaltonen, Peltomaki et al. 1993). The underlying genetic mechanism responsible for this phenotype is mutation and loss of function through gene silencing of DNA mismatch repair genes (MMR) (Kane, Loda et al. 1997). Recently the analysis of CpG island methylation as a mechanism of silencing genes in colon tumors has resulted in the identification of the CpG island methylator phenotype (CIMP). This phenotype appears to be complex and the overlap between this phenotype and MIN and CIN, and the subsequent prognostic significance in colon cancer patients has not been thoroughly investigated (Shen, Toyota et al. 2007). The design of new prospective trials must encompass a more comprehensive and disciplined approach with defined protocols, primary end points and increased statistical power. Follow-up studies are also required to identify the functional significance of the many mutations and polymorphic variants that exist in the patient population, such functional information will inevitably assist in unraveling the complex and multi-faceted mechanisms of drug metabolism and cytotoxicity. Markers of response to the novel therapeutic drugs including bevacizumab, cetuximab and panitumumab have been identified and need to validated so that the use of these agents can be targeted to those who will derive greatest benefit.

SP151
Current challenges in the design and conduct of pharmacogenetic and pharmacogenomic studies

G. Liu. *Princess Margaret Hospital, Canada*

Introduction and Purpose: For many cancers, multiple regimens are active. Yet these different regimens produce a variable response to therapy and sometimes unpredictable toxicity. Some of these variations may be explained by tumour genomics or the patient's genetics. Eludication of the mechanisms behind such variations is a critical component of "personalized" or "individualized" medicine, allowing an intelligent choice of available therapies. Clinically, the primary goals of such studies are to maximize drug efficacy, select responsive patients, and avoid adverse drug reactions. These clinical goals can be accomplished through research goals that link either variation in genotype of the patients or the tumour to a phenotype (an observable characteristic or trait such as patient response, survival or drug toxicity), that determine mechanisms responsible for that link, and that translate that link into enhanced understanding, treatment and prevention of disease or toxicity. Well conducted studies are necessary to advance this field.
Main Message and Recommendations: Challenges in the design and conduct of such trials include: (i) phenotyping accuracy (e.g. toxicity or response to therapy) across prospective and retrospective study designs; (ii) treatment issues, including controlled and observational designs; (iii) sample size determinations, whether planned or convenient; (iv) control of Type 1 error (exploratory versus corrected); (v) inclusion or exclusion of known molecular and clinicoepidemiologic prognostic factors in multivariate analyses; (vi) DNA/RNA sample source, including formalin-fixed versus fresh tissues, blood versus saliva/buccal; and (vii) whether interventional or non-interventional trials by pharmacogenetic or pharmacogenomic markers are required for clinical adoption. In addition for genetic studies, additional challenges include gene selection (candidate, pathway or genome-wide) and variant selection, through either functional or tagging approaches. For genomic studies, additional challenges include reproducibility within and across platforms and cancer heterogeneity. Illustrations and examples of such design challenges are presented.
Conclusion: Tackling these challenges are key to successful pharmacogenetic and pharmacogenomic trials.

SP145
PET imaging in upper GI Cancer − past experience and current EORTC initiatives

F. Lordick. *Clinic Brunswick, Hematology-Oncology, Hannover Medical School, Germany*

Introduction: Metabolic imaging and early response assessment by PET are gaining importance in guiding treatment of localised and metastatic cancer [Weber JNM 2009]. Consistent results have been obtained during neoadjuvant treatment of adenocarcinoma of the oesophagus and the oesophago-gastric junction.
Main messages: It was demonstrated that FDG-PET is highly accurate for identifying non-responding tumours within 2 weeks after the initiation of neoadjuvant chemotherapy when a quantitative threshold for metabolic response is used [Weber WA et al. JCO 2001; Ott K et al. JCO 2006]. In consecutive phase II studies we quantified the metabolic activity, defined by the standardised uptake (SUV) of 18-FDG before and during chemotherapy. We observed that after only two weeks of induction chemotherapy significant decreases of the SUV occurred. A drop of >35% measured

2 weeks after the start of chemotherapy revealed to be the most accurate cut-off value for prediction of clinical and histopathological response after a full-course of preoperative chemotherapy lasting for 12 weeks. We have further noticed that the metabolic response to induction chemotherapy is an independent and important prognostic factor in cases of locally advanced adenocarcinoma of the oesophago-gastric junction. This suggests that PET can be used to tailor treatment according to the chemosensitivity of tumours located at the oesophago-gastric junction. This concept has been realised in the MUNICON-1 trial [Lordick F et al. Lancet Oncol 2007]: In metabolic non-responders, chemotherapy could be discontinued at an early stage, thereby saving time, and reducing side-effects and costs. Compared to previous studies one can deduce that the outcome of metabolic non-responders was at least not compromised by the early discontinuation of chemotherapy.
Recommendations and Conclusions: Based on these results, integration of FDG-PET can be recommended for further clinical studies in oesophago-gastric cancer like the planned EORTC IMAGE trial.

SP169
Gap & priorities: Biomarker integration in drug development

G. Los. *PGRD, Pfizer, USA*

Substantial improvements in genomics, proteomics and the way that human tumors are characterized, are allowing clinical exploration of new targeted strategies. As a consequence, cancer treatment is shifting from a "one size fits all" therapeutic approach to a more personalized approach, in which specific cancer subpopulations are treated based on genetic defects. To be successful, such an approach requires the discovery and development of biomarkers to (a) select which patients to treat (Prognostic Biomarker); (b) determine whether the drug interacts with the target (Target Biomarker); (c) assess whether the drug elicits a biological effect (Mechanism Biomarker) and (d) determine whether the drug produces a positive clinical outcome following treatment (Outcome Biomarker). Although there have been clinical successes in targeting molecularly defined subsets of several tumor types using molecular targeted agents, the ability to apply such successes in a broader context is limited by the lack of a strategy to evaluate targeted agents in patients. The solution requires biomarkers integrated into the drug development process and the ability to reliably select patients with molecularly defined cancers. In this tutorial I will highlight key approaches for the use of such biomarkers, focusing on gaps and priorities.

SP172
Getting the most from the least tissue

P. Mack. *UC Davis Cancer Center, USA*

The field of clinical oncology is poised to undergo a paradigm shift where personalized therapies based on tumor and host molecular profiles supplant the current practice of empirical clinical decision-making based on tumor stage, age and performance status. Considering the heterogeneous and varied nature of most solid tumors, molecularly-targeted agents designed to inhibit abnormal signaling events will likely be of benefit only to a subset of patients whose tumors are uniquely dependent on the target(s) of such agents. Tumor profiling will also prove valuable for selection of optimal cytotoxic chemotherapies. However, individually tailored regimens administered in rational therapeutic combinations can only be accomplished if high quality tumor specimens are available for rigorous collection and analysis. Recently, tumor diagnosis and staging has become more reliant on fine needle aspirates or core biopsies, sufficient for pathological evaluation, but inadequate for much additional molecular characterization. Furthermore, DNA artifacts, produced by the formalin fixation process, interfere with PCR amplification and may lead to erroneous data if the starting material is too limited. Tumor heterogeneity and admixed normal cells (stroma, vascular, immune etc) also may contribute to ambiguous data. Multiple approaches are being explored to improve the predictive capacity of biomarkers from limited tissue sources. For instance, highly sensitive and precise methodologies are currently undergoing validation to improve quality of individual high-utility markers, including mutation detection, gene copy number and tumor RNA levels. Additionally, platforms designed to produce multiplex or even genome-wide molecular signatures are demonstrating notable promise for tumor prognostics and prediction of treatment sensitivity/resistance. Targets of measurement include DNA mutations, methylation and copy number; RNA levels and proteomics, among others. In the absence of sufficient archival tissue for these analyses, alternative sources of tumor material may be exploited for molecular diagnostics, including shed tumor DNA in peripheral circulation, circulating tumor cells and plasma "omic" profiles. For personalized therapy strategies to be introduced into mainstream practice, the infrastructure for specimen acquisition, processing, storage, pathological oversight and standardized analysis must be established.

SP163
BMP4 and the inhibitors of differentiation, Id-1 and Id-3, play an essential role in the maintenance of colon cancer-initiating cells

C. Obrien. *University of Toronto, Canada*

Cancer-Initiating cells have been identified in numerous solid tumors, including colon. Markers utilized to identify Colon C-IC (CC-IC) include CD133 and CD44. The aim of this study was to examine the role of bone morphogenic protein-4 (BMP4) and its major target genes, inhibitor of differentiation-1 and -3 (Id-1 and Id-3), in colon cancer and the CC-IC subset.
To study the effect of BMP4 on colon cancer 2×10^5 human colon cancer cells were injected subcutaneously (SQ) into NOD/SCID mice. A total of four groups were studied (n = 5 per group): (1) no treatment, (2) heparin-coated acrylic beads, (3) BMP4 (100 ng) and (4) Noggin (100 ng) (BMP4 inhibitor), both conjugated to heparin-coated acrylic beads. The experiment was repeated with 4 colon cancers (all smad 4+). Once tumor volume reached 0.5 cm^3 intra-tumoral injections were administered weekly until xenografts reached 1 cm^3, at which time the mice were sacrificed.
The administration of Noggin resulted in tumor regression in 11/20 mice, with a mean tumor weight of 92.4 ± 37.2 mg. In contrast, the mean tumor weights (mg) for the BMP4, acrylic bead, and untreated mice were: 673.8 ± 65, 734.6 ± 94, and 684.5 ± 100.8. The CD133+ fraction was significantly elevated in the BMP4 treated tumors, 27.15% vs. 0.36% in control tumors. To better understand the mechanism of action of BMP4 we looked at two of its major target genes, Id-1 and Id-3. Short hairpin RNA (shRNA) mediated knockdown of Id-1 and Id-3 was carried out in primary colon cancer cells. Five groups were included: (1) untransduced, (2) transduced control, (3) Id-1 shRNA, (4) Id-3 shRNA, (5) Id-1/3 shRNA. A total of 1×10^5 cells were injected SQ into NOD/SCID mice (n = 32/group) to assess tumor formation. The combined inhibition of Id1/3 resulted in decreased tumor formation, the mean tumor weight (mg) being 588.1 ± 89 in transduced controls (n = 32) vs. 56 ± 19.1 in Id1/3 knockdown (n = 32) ($p < 0.05$). The tumors in the Id1/3 knockdown group demonstrated decreased self-renewal and decreased chemoresistance to oxaliplatin.
These experiments indicate that BMP4 plays a central role in colon cancer and the maintenance of CC-ICs. Furthermore, the knockdown of two of the major target genes of BMP4, Id-1/3, also resulted in a decrease in xenograft formation. We have identified a multifunctional role for Id1/3 in CC-ICs that includes maintenance of self-renewal and chemoresistance. Current studies are underway to further investigate how Id-1 and Id-3 affect self-renewal in colon cancer-initiating cells.

SP154
The role of pharmacogenetics and pharmacogenomics in cancer therapy

G. Peters. *VU University Medical Center, The Netherlands*

Introduction and Purpose: Both pharmacogenetics and pharmacogenomics can affect the efficacy of cancer therapy with cytotoxic drugs targeted against DNA as well as drugs targeted against signalling. Pharmacogenetics is the impact of one or some genes on the effect of a drug, which includes both gene expression and genetic polymorphisms. Pharmacogenomics is the impact of a cluster of genes, e.g. by gains or losses. Next to non-genetic factors both can affect the pharmacokinetics and the pharmacodynamics of a drug, influencing either drug toxicity or the antitumor effect.
Main Message and Recommendations: The pharmacogenetics of a drug is usually the result of a genetic polymorphism, which is classified by a genetic variation in the DNA in more than 1% of the patients. Many candidate genetic polymorphisms have a rational preclinical basis and have subsequently been identified in retrospective studies; several of them have been validated in prospectively sampled studies, but few were sufficiently robust to be used for selection of patients and have been identified by the FDA as a potential risk factor. For most of these genetic polymorphisms data were not strong enough or too heterogeneous to predict an antitumor effect. Although there is a general concordance for most pharmacogenetic markers between germline and e.g. colorectal cancer, this does not seem to be sufficient to predict efficacy, also because in tumors gene regulation is often deregulated. Furthermore in combination therapy more genetic factors in the tumor play a role, so that risk of toxicity to one drug in a combination can be predicted more reliably then the chance to respond. Since combination therapy (cytotoxic drugs and/or targeted drugs) is common, it seems more appropriate to use a set of genes to test the tumor. A wide application also requires a robust source for RNA or DNA.