Unfortunately the lack of identification and utilization of this resistance marker in previous clinical trials led to the erroneous treatment of thousands of CRC patients with EGFR-targeted agents at the cost of considerable toxicity and no benefit. Although initially this resistance factor for EGFR-targeted therapy was thought to be relatively straightforward, subsequent studies using more robust analyses have revealed potentially important insights that may further refine the patient population selected for this class of agents. Furthermore, for those patients with KRAS mutations, treatment is restricted to first and second-line combinations of 5-FU/oxaliplatin/irinotecan/bevacizumab. No other options exist for this patient population, and although drug development is ongoing, preliminary results indicate that merely targeting putative resistance pathways may not be sufficient. Thus, there are numerous lessons learned and pathways forward in this disease, all of which rely upon the earlier development and integration of genomic technologies to refine patient selection and identify resistance pathways that may yield rational combination strategies.

SP 122

Debate on access to tissue specimens from clinical trials: when is the preliminary data strong enough to invest in highly annotated biospecimens?

J. Hall. *EORTC Headquarters, Belgium*

Panelists: J. Gastier-Foster, M. Hegi, S. Hilsenbeck, and JY. Blay. Access to well-annotated quality biospecimens is critical for tumor characterization and biomarker development and validation. Tissue specimens from clinical trials are often seen as a unique tissue resource that should mainly be used for the late stages of biomarker validation where considerable preliminary data already exists. Given that clinical trial tissue specimens are limited, how do we decide on the best use of samples? Should the trial specimens be saved for biomarker validation or could they also be used for large coordinated multidimensional -omics profiling? How to prioritize specimen use? Who decides on the scientific merit of proposed research and when are highly annotated trial specimens needed? The objective of the session is to explore the decision making processes behind the access to and feasibility of use of clinical trial specimens in multiple platforms and large scale genomic studies. Two short case studies on use of trial specimens in large-scale genomic studies from COG pediatric acute lymphoblastic leukemia (US) and glioblstoma trials (EU) will be presented followed by an open discussion on the topic by a multidisciplinary panel (biobanker, translational scientist, statistician and clinical oncologist).

SP 132

IDH1/IDH2 mutations predict survival in glioma and AML

M. Hegi. *CHUV, Switzerland*

Mutations in the isocitrate dehydrogenase family genes 1 or 2 (IDH1/2) have been discovered by high through put sequencing approaches in glioma and acute myeloid leukemia (AML) and related myeloproliferative neoplasms. In both diseases, the discovery of IDH mutations has identified a prognostically new subtype with distinct pathogenetic evolution. In gliomas mutations are mostly found in IDH1 (>90%). They are infrequent in primary glioblastoma (GBM) (<10%), but common in secondary GBM that evolve from lower grade glioma (60−90%). Mutations in IDH1 precede p53 mutations or 1p/19q co-deletions in sporadic low grade glioma, hence are an early evant. Co-deletions of 1p/19q, characteristic for oligodenroglioma, are highly associated with IDH1/2 mutations, while they are mutually exclusive with EGFR amplifications, a hall mark of primary GBM. IDH1 or 2 mutations are associated with younger patient age, but absent in childhood gliomas, and have a better prognosis that seems to be consistent in grade II through IV gliomas. In myeloid malignancies mutations are more likely in IDH2 and are found in de novo and secondary AML (12−18%) and pre-leukemic clonal malignancies (5% chronic; 20% transformed). IDH1/2 mutations are strongly associated with NPM1 mutations that are found in 30% of novo cytogenetically normal AML. In CN-AML with mutated NPM1, without FLT3 internal tandem duplication (ITD) IDH mutations constitute an adverse prognostic factor. Mutations in the metabolic enzymes IDH1 or 2 result in a neomorphic reaction, generating high levels of the metabolite 2-hydroxyglutarate (2-HG). IDH mutations are mutually exclusive with TET2 mutations in myeloid malignancies that led to the discovery that high levels of 2-HG inhibit the a-KG dependent dioxygenase TET2. TET2 is involved in epigenetic regulation and mediates demethylation of DNA. This mechanism is in accordance with the association of a methylator phenotype with loss of function of TET2 by mutation or indirectly by mutation of IDH1/2 in myeloid malignancies and gliomas, respectively.

Metabolism meets Epigenetics. These discoveries will have important clinical implications: IDH1/2 mutants may serve as unique targets for therapy. Further, the high concentrations of the onco-metabolite 2-HG generated by IDH1/2 mutants, may serve as biomarker in the serum of patients with myeloid malignancies and may be amenable by magnetic resonance spectroscopy in glioma patients.

SP 134

Predictive and pharmacodynamic markers of susceptibility for targeting IGF-1R receptor

F.R. Hirsch. *University of Colorado, USA*

Numerous human tumors have been shown to overexpress IGF-1R or have increased IGF-1R kinase activity resulting in enhanced proliferation, protection from apoptosis, stimulation of migration and invasion and stimulation of angiogenesis. Targeted therapies, including insulin-like growth factor (IGF) binding proteins, human monoclonal antibodies and small-molecule tyrosine kinase inhibitors, against IGF-1R, have been developed and show promise for therapeutic use in both in vitro and in vivo experiments. Several clinical studies with IGF-1R inhibitors are performed or currently on-going. In non-small cell lung cancer (NSCLC) most advanced in clinical development was studies with the monoclonal antibody, Figitinumab (Pfizer), which in randomized phase II study showed encouraging effect in combination with chemotherapy. However, large randomized phase III study in 1st line therapy was prematurely stopped due to futility and toxicity. None of the IGF-1R inhibitors studies was based on biomarker selection related to the IGF-1R pathway. The "negative" experience in patients with NSCLC have put clinical development of IGF-1R inhibitors on hold and calls for a better understanding of mechanisms development of predictive biomarkers. Retrospective analysis of the specimens from the figitumumab studies demonstrated significant association between plasma IGF and response and outcome. These findings have not yet been validated prospectively. However, several tissue assays might also be potential predictive assays which need to be validated. We demonstrated that many NSCLC tumors express IGF-1R protein by IHC and increased IGF-1R gene copy number occurs in many tumors, which represents potential tools for predictive assays. IGF-1R protein expression by AQUA-technology (HistoRx, USA) was in a retrospective analysis from the figitumumab studies also demonstrated to be associated with response. We recently demonstrated that IGF-1R activation might play a role as intrinsic resistant mechanisms for EGFR TKI therapy in patients with NSCLC, even in patients with tumors harboring activating EGFR-mutations, which raises a potential for use of IGF-1R inhibitors in combination with EGFR TKIs in order to overcome resistance to EGFR TKIs. In conclusion, while IGF-1R seems to play a role in tumor genesis of many cancer and use of IGF-1R targeted therapies in some early studies have demonstrated encouraging results, much focus have yet to be put into the development of predictive biomarkers. This needs to be done in preclinical studies and through retrospective analysis of specimens from the existing clinical trials.

SP 113

Translating molecular imaging agents into phase 3 trials

P. Jacobs. *National Cancer Institute, United States*

Drugs that have not been approved by regulatory authorities for marketing, including imaging agents, must be studied clinically under an Investigational New Drug (IND) exemption in the US or a Clinical Trial Authorization (CTA) in Europe or Canada. Many molecular imaging probes are short-lived radiopharmaceuticals with no intellectual property protection and relatively small market potential. Most commercial entities correctly view development of such discoveries as high risk (high cost, low potential revenue) that cannot be justified. Pre-investigational new drug application (IND) and early feasibility studies that are essential to moving drugs to the clinical investigational stage cannot generally be funded through the typical grant mechanisms because they are considered neither original research nor novel nor will they be funded by industry because of the lack of intellectual property. Multicenter trials with such agents present unique logistical, quality, and regulatory issues.

A few years ago, the Cancer Imaging Program at the National Cancer Institute began an effort to open multicenter trials with a few non-proprietary PET molecular imaging probes and encountered a number of hurdles. One was assuring that the radiopharmaceuticals used at each site were chemically equivalent. A second was the logistical barrier to supplying sites without cyclotrons and synthesis resources. The third was dealing with regulatory issues.

The strategies, failures, and successes of this effort will be discussed. An attempt to establish identical preparations of 18F-fluorothymidine at four academic sites with identical synthesis boxes was a failure. Commercial suppliers were then engaged to establish manufacturing to the identical specifications and to file Drug Master Files with FDA. These companies have gradually increased the number of sites preparing the agent to around 20 and can now supply most of the US. Under IND, the NCI is performing

multicenter trials in breast and lung cancer. Furthermore, the regulatory documents have been made publicly available and the NCI has freely provided letters of reference to investigators to file their own INDs; many academic and commercial entities have done so.
New strategies are needed for clinical development of non-proprietary short-lived radiopharmaceuticals with low market potential. Even newer strategies may be needed for commercialization of such agents.

SP 108
Resistance to tyrosine kinase inhibitors in chronic myeloid leukemia

T. Lion. *CCRI, Austria*

In patients with chronic myeloid leukemia, targeted therapy with tyrosine kinase inhibitors (TKIs) has been the standard of treatment for a number of years. Despite the extraordinary success of this approach, the occurrence of resistant disease remains a clinically important problem. Point mutations within the BCR/ABL1 tyrosine kinase domain (TKD) are currently regarded as the most important mechanism of TKI resistance. The European LeukemiaNet (ELN) has therefore recently provided guidelines for mutation testing and the therapeutic implications (Soverini et al., Blood 2011).
In line with the ELN-recommendations, mutation screening is most commonly performed by direct sequencing of the entire BCR/ABL1 TKD following amplification by PCR. This approach does not reveal the presence of mutant subclones below the level of 10−20% of the entire leukemic cell pool.
Although more than 100 different mutations have been described in the BCR/ABL1 TKD, a subset of 15 common mutations are observed in the great majority (>85%) of instances. Patients harbouring BCR/ABL1 TKD point mutations were reported to have a progression-free survival inferior to patients without point mutations. By contrast, detection of mutant subclones, especially by highly sensitive technical approaches, did not necessarily imply impending onset of clinically resistant disease. A number of mutations may be biologically neutral with regard to TKI resistance ("bystander" or "passenger" mutations), while other mutations are frequently associated with the onset of resistant disease ("driver mutations"). However, even mutant subclones commonly associated with resistance to TKI treatment have been described to disappear spontaneously below the limit of detection and to remain undetectable. Hence, the detection of mutations may be difficult to interpret with regard to clinical relevance and therapeutic consequences. Our recent observations indicate that the surveillance of subclone evolution by quantitative monitoring of mutant cells during treatment with TKIs provides information on their actual responsiveness to therapy and the imminent onset of resistant disease.
Judicious implementation of quantitative diagnostic approaches in the surveillance of CML patients could therefore improve our current options for timely treatment decisions, and help optimizing disease management in patients displaying point mutations in the BCR-ABL1 TKD or other sites of potential relevance.

SP 131
MicroRNAs as prognostic and predictive markers in breast cancer

J. Martens. *Erasmus MC, The Netherlands*

MicroRNAs (miRNAs) are small ribonucleotides that use the endogenous RNA interference pathway to modulate mRNA expression and translation thereby contributing to cancer biology. Our aim was to determine in breast cancer which miRNAs are associated with time to disease metastasis (TDM) in estrogen receptor positive (ER+) and in triple negative (ER-, PgR- and Her-2-negative) breast cancer and with clinical benefit of (endocrine) therapy in ER+ disease. In silico and functional studies have been and are being performed to address in which biological pathways the significant miRNAs operate.
To discovery prognostic miRNAs genome-wide miRNAs panels were analyzed by qRT-PCR in ER+ and triple negative primary breast cancers with short and long TDM. All patients included were lymph node negative and none had been treated with adjuvant systemic therapy. Candidate prognostic miRNAs were confirmed in independent cohorts. To identify candidate predictive miRNAs, a panel of selected miRNAs was studied in a large cohort of ER-positive breast cancers treated first-line with tamoxifen for their metastatic disease. Cox and logistic regression was used to associate variables with TDM and clinical benefit, respectively. Co-expression analyses; database searches and functional studies were performed to identify biological pathways connected to the significant miRNAs.
Four microRNAs were in uni- and multivariate analysis associated with TDM in ER+ patients; these microRNAs were associated with VEGF signaling, cell cycle progression/chromosomal instability and cytokine signaling. Twenty candidate prognostic microRNAs were discovered in triple negative breast cancer; validation is currently on going in collaborationwith EORTC-PBG members. Several microRNAs predictive of clinical benefit of tamoxifen therapy were also identified. These were related growth factor/RAC signaling, apoptosis and polycomb remodeling.
Our work connects microRNAs and their associated biology to breast cancer disease progression and therapy resistance.

SP 115
Cancer stem cells

W. Matsui. *Johns Hopkins University School of Medicine, USA*

Emerging data suggest that cells within an individual tumor are both phenotypically and functionally heterogeneous despite their clonal origins. In many malignancies, most cells appear to lack significant replicative potential and instead arise from relatively rare populations of phenotypically distinct cancer stem cells. In both hematologic malignancies and solid tumors cancer stem cells have been prospectively identified based on their ability to give rise to differentiated progeny that recapitulates the original tumor in the ectopic setting. Additionally, these cells are capable of self-renewal and may be relatively resistant to various anti-cancer agents. These unique functional properties suggest that cancer stem cells play a central role in disease initiation, relapse, and progression and that the development of effective strategies inhibiting these cells may ultimately improve long-term clinical outcomes. We have studied multiple myeloma and found that the malignant plasma cells forming the tumor bulk and characterizing the disease arise from cancer stem cells phenotypically resembling normal memory B cells. Recently, we have translated these findings and initiated novel clinical trials explicitly designed to target myeloma stem cells. We will discuss the strategies we have used to identify novel cancer stem cell-targeting agents in the laboratory and develop biomarker strategies to monitor their efficacy in the clinical setting.

SP 117
Using genomic landscapes to map biomarkers of drug sensitivity

U. McDermott. *Wellcome Trust Sanger Institute, UK*

Clinical responses to anticancer therapies are often restricted to a subset of patients. In some cases, mutated cancer genes are potent biomarkers of response to targeted agents. To uncover new biomarkers of sensitivity and resistance to cancer therapeutics, we screened a panel of several hundred cancer cell lines, which represent much of the tissue-type and genetic diversity of human cancers, with 130 drugs under clinical and preclinical investigation. In aggregate, we found mutated cancer genes played an important role in determining cellular response to most currently available cancer drugs.
We assembled 639 human tumour cell lines, which were subjected to systematic genomic and transcriptional profiling, including sequencing of the full coding exons of 65 commonly mutated cancer genes, genome-wide analysis of copy number gain and loss using SNP6.0 arrays, and expression profiling of 14,500 genes using Affymetrix HT-U133A microarrays. Cells were treated with drugs for 72 hours and effects on cell viability were measured and a curve-fitting algorithm was applied to derive the half maximal inhibitory concentration (IC50) and the slope of the dose response curve. The drugs selected for analysis covered a wide range of molecular targets and processes implicated in cancer biology.
We first used a MANOVA to identify statistically significant associations between individual mutated cancer genes and drugs across the cell line panel, applying a Benjamini-Hochberg false discovery rate (FDR) cutoff of 0.2 ($P < 0.0099$) to correct for multiple hypothesis testing. This analysis revealed a large number of individual gene-drug associations, a subset of which (448/9039, 5%) were highly significant. Remarkably, most of the cancer genes analyzed (including gene fusions) were significantly associated with either sensitivity or resistance to at least one drug in our panel.
The scope of this work provides a unique perspective on the factors that modify drug response and the use of biomarkers for the clinical stratification of cancer patients. The emergent picture is of a complex network of biological factors that affect response to the majority of cancer drugs. The clinical utility of genome-based biomarkers is likely to increase in the coming years as the genomic characterization of cancers increasingly becomes routine practice and this is combined with clinical information about patient response to treatment.