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

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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

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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

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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

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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.

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

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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

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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

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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

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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.