role of pharmacogenetics in metabolizing enzymes (UGT1A1 and CYP2D6 respectively) seems important. However, if dosing based on these specific pharmacogenetic differences between patients will be truly successful remains controversial, as many non-inherited factors and other single-nucleotide polymorphisms may also influence the metabolism of these anti-cancer drugs. For instance, co-medication is capable to induce or to inhibit the activity of CYP3A4, a crucial enzyme involved in the metabolism of many drugs. Therefore, a 'phenotyping' dosing strategy might potentially be more promising than a 'genotyping' dosing strategy that is focussed on pharmacogenetic variability only. But also this phenotyping strategy has serious limitations. In this presentation differences between both dosing strategies will be discussed based on recent literature.

12 INVITED

Pharmacogenetics of drug transporters

R. Danesi[1]. [1]*University of Pisa, Department of Internal Medicine, Pisa, Italy*

Membrane transporters are major determinants of the absorption, distribution and elimination of many drugs. Functional genomic and clinical studies have provided new information regarding the contribution of coding variants in transporters to drug disposition and response. ATP-binding cassette (ABC) genes play a role in the resistance of malignant cells to anticancer agents. The ABC gene products, including ABCB1 (P-glycoprotein), ABCC1 (MRP1), ABCC2 (MRP2, cMOAT), and ABCG2 (BCRP, MXR, ABCP) are also known to influence oral absorption and disposition of a wide variety of drugs. As a result, the expression levels of these proteins in humans have important consequences for an individual's susceptibility to certain drug-induced side effects, interactions, and treatment efficacy.
The influence of polymorphisms in ABCB1 2677G>T/A, 3435C>T, and 1236C>T and progression-free and overall survival in patients treated with paclitaxel/carboplatin demonstrated that, compared to homozygote GG carriers at 2677, women with the minor T/A alleles were significantly less likely to relapse following treatment (Hamidovic et al., 2010). In a study on patients with androgen-independent prostate cancer given docetaxel and thalidomide, subjects receiving docetaxel alone and carrying the 1236C-2677G-3435C linked alleles had improved overall survival after treatment. Additionally, patients treated with docetaxel and thalidomide carrying the 2677T-3435T haplotype had a shorter median survival. Among both treatment arms together, individuals carrying the 2677GG genotype also had a significantly longer time to neuropathy. Finally, there was a strong trend toward patients carrying the 2677TT-3435TT diplotype having higher grades of neutropenia. Therefore, this study demonstrated that docetaxel-induced neuropathy, neutropenia grade, and overall survival could be linked to ABCB1 allelic variants with ensuing negative implications for docetaxel treatment in patients carrying ABCB1 variant genotypes (Sissung et al., 2008). A study investigated the relationships of polymorphisms in transporter genes ABCB1, ABCC2, and ABCG2 and CYP2D6 to clinical outcome of patients with hormone receptor-positive breast cancer receiving tamoxifen. CYP2D6 variants were significantly associated with shorter recurrence-free survival in patients with two variant alleles compared to subjects without variant alleles. Among 51 tag-SNPs in transporter genes, a significant association was found at rs3740065 in ABCC2 (in patients with AA vs. GG genotypes). The number of risk alleles of CYP2D6 and ABCC2 showed cumulative effects on recurrence-free survival. Thus, polymorphisms in CYP2D6 and ABCC2 are important predictors for the prognosis of patients with breast cancer treated with tamoxifen (Kiyotani et al., 2010). In conclusion, drug transporters play an important role in cancer chemotherapy and the full evaluation of their implication in stratified medicine should be assessed in prospective trials.

References

Hamidovic A, et al. Clinical significance of ABCB1 genotyping in oncology. J Oncol Pharm Pract 2010; 16: 39.

Sissung TM, et al. ABCB1 genetic variation influences the toxicity and clinical outcome of patients with androgen-independent prostate cancer treated with docetaxel. Clin Cancer Res 2008; 14: 4543.

Kiyotani K, et al. Significant effect of polymorphisms in CYP2D6 and ABCC2 on clinical outcomes of adjuvant tamoxifen therapy for breast cancer patients. J Clin Oncol 2010; 28: 1287.

13 INVITED

The role of gene expression profiling in early drug development

S. Loi[1], C. Sotiriou[1]. [1]*Jules Bordet Institute, Breast Cancer Translational Research Laboratory, Brussels, Belgium*

The advent of high through-put, whole genome gene expression profiling has increased our understanding of the molecular heterogeneity of many cancers. From a breast cancer viewpoint, the identification of distinct molecular subtypes has resulted in the majority of clinical trials no longer being conducted in the global population, but in subgroups such as ER+, HER2+ or triple negative disease (ER−/HER2−). Similarly, gene expression signatures have emerged as potential new prognostic tools and chemotherapy response predictors.
The potential role of gene expression profiling in early drug development is immense. Discovery of previously unidentified molecular subtypes can also provide information on the biology such as potential drug targets and biological pathway enrichment. Gene expression profiles induced by drugs may also provide an indication of the transcriptional phenotype most likely to respond. The importance of serial biopsies in early drug development has also begun to emerge as baseline biopsies may not provide the full picture with regards to prediction of response. Serial biopsies can also provide demonstration or confirmation of the molecular mechanisms of action of the new agent and highlight mechanisms of potential resistance. In breast cancer, several neoadjuvant clinical programs are running around the world to take advantage of the accessibility of serial biopsies in the early stage setting. Finally, gene expression profiles may be helpful as predictive biomarkers of response to drug therapies. These 'functional read-outs' may be more sensitive, objective and quantitative than a single marker by immuno-histochemistry, for example p-AKT or p-S6 but as yet, there has been no clinical validation of this concept.

14 INVITED

Genomic versus histological grading in soft tissue sarcomas

J. Coindre[1], F. Chibon[2]. [1]*Institut Bergonié, Anatomo-cyto-pathologie, Bordeaux, France;* [2]*Institut Bergonié, Pathology, Bordeaux, France*

For more than 20 years, histological grading has been the most important factor for predicting metastasis-free and overall survival in adult soft tissue sarcomas. The most common grading systems used are the National Cancer Institute grading and the French grading. The last system is based on a score obtained by evaluation of 3 parameters: tumor differentiation, mitotic rate and amount of tumor necrosis. The main value of grading has been for the most efficient use of chemotherapy. However, histological grading has several limitations: its reproducibility is questionable, it is not applicable to all types of sarcomas and it is not informative for grade 2 tumors which represent about 40% of sarcomas. Moreover, the value of grading is also limited by the universal use of core needle biopsies. Histological grading can be considered as a morphological translation of molecular events that determine tumor aggressiveness and we postulated that molecular parameters will be important for prognostication of sarcomas. In order to set up a molecular grading, we performed a genomic and expression profiling (array-CGH and Affymetrix) of 183 sarcomas with complex genetic profile and established an expression signature which is highly predictive of metastasis outcome in the whole group and the different subgroups such as limb sarcomas and leiomyosarcomas. This signature has been established by a bottom-up supervised strategy using genomic profile (array-CGH), histologic grade and a previously published chromosomal instability signature. The resulting signature is composed of 67 genes related to mitosis and chromosome management (CINSARC for Complexity Index in SARComas). This signature has been validated on an independent group of 127 sarcomas with complex genetic profile. It appears to be superior to the French grading system with no intermediate group. It has been tested in silico in GIST, breast carcinomas and lymphomas and it could be an important tool in GIST for selecting patients for adjuvant target therapy. Moreover, metastatic potential seems to be related to tumor complexity level and driver genes related to genome complexity could become therapeutic targets. In conclusion, the concept of grading should be maintained, histological grading is still useful in complement to tumor histotype/genotype but molecular grading should be developed and tested in sarcomas and GIST.

15 INVITED

Microarray and validation analyses performed on melanoma metastases identify TYRP1 as a prognostic marker for both DMFS and OS

G. Ghanem[1], L. van Kempen[2], H. Id Booufker[3], M.D. Galibert[4], M. Wiedig[1], A. Theunis[5], F. Salès[6], G. Laurent[7], A. Awada[8], F. Journé[1]. [1]*Institut J. Bordet, Lab of Oncology and Experimental Surgery, Brussels, Belgium;* [2]*Radbout university Nijmegen Medical Center, Pathology, Nijmegen, The Netherlands;* [3]*Institut J. Bordet, Lab of Oncology and Experimental Surgery, Brussels, Belgium;* [4]*University of Rennes 1, CNRS UMR 6061, Rennes, France;* [5]*Institut J. Bordet, Pathology, Brussels, Belgium;* [6]*Institut J. Bordet, Surgery, Brussels, Belgium;* [7]*University of Mons, Histology, Mons, Belgium;* [8]*Institut J. Bordet, Medical Oncology, Brussels, Belgium*

Using microarrays in 32 skin and lymph node metastases, we generated a 278 gene probe signature based on a survival cut-off of 30 months.

Among the first ranked genes, 6 were linked to pigmentation machinery [TYRP1(34×), SILV(16×), DCT(13×), OCA2(8x), TYR(6x) and MITF(3x)]. We then compared the whole gene set to both the 6 pigmentation genes and TYRP1 (tyrosinase related protein 1) and found that the latter alone could represent the information brought by the two other signatures. We also confirmed that TYRP1 expression was significantly higher in the group of patients with poorer survival ($p < 0.001$), especially in skin metastases. In a second step, we ran a first validation study in the same samples evaluating TYRP1 mRNA expression in the skin metastase subgroup by quantitative RTqPCR and confirmed the microarray data (rho = 0.780, $p = 0.002$). A second validation study addressed TYRP1 mRNA expression (RTqPCR) in an independent group of 101 skin metastases. We used the 25th percentile as a cut-off to divide the population into two groups with low and high TYRP1 mRNA levels, and found that high expression was significantly associated with a shorter DMFS ($p = 0.01$, HR = 0.49, 95% CI = 0.28–0.84, Kaplan-Meier analysis and Cox regression), a shorter OS ($p = 0.009$, HR = 0.47, 95% CI = 0.26–0.82) and, very interestingly, significantly correlated with Breslow thickness and Clark level. Furthermore, we observed that TYRP1 mRNA levels were maintained in successive skin metastases obtained over months or years. These data strongly suggest that TYRP1 mRNA expression in skin metastases, whatever was the lag time to their occurrence, was associated with the main prognosis parameters defined in the corresponding primary lesions and most probably maintained through melanoma progression.
Moreover, we found that high TYRP1 mRNA expression had a positive predictive value of 94% associated with DMFS at 7.5 years and of 96% with OS at 15 years. We also evaluated the expression of Tyrp1 protein in a panel of skin metastasis paraffin-embedded biopsies (n = 52) by IHC and observed that, in many cases (56%), the protein was not detected while mRNA was expressed at high levels, suggesting possible regulatory mechanisms. However, considering samples with positive-immunological staining for Tyrp1 only, we found a significant correlation between protein (IHC data) and mRNA (RTqPCR data) expressions (rho = 0.488, $p = 0.034$). Finally, we validated the TYRP1 mRNA detection by ISH (N = 40) and found significant correlation between ISH data and RTqPCR data (rho = 0.679, $p < 0.001$). TYRP1/Tyrp1 mRNA/protein expression (ISH/IHC) has also been assessed in primaries and found much more transcript than the protein expressed in the vertical invasion phase.
In conclusion, we found that TYRP1 mRNA can be regarded as a prognostic marker, at least in skin metastases, and may also be helpful where information on the primary are lacking. Its expression is most probably conserved during tumor progression and consequently, its suggested role as a target for therapy is here highly supported.

Wednesday, 17 November 2010 08:00–09:45

WORKSHOP 3

European and US initiatives for early drug development

16 INVITED

Molecular driven clinical trials: The EORTC Network Of Core Institutions (NOCI) Perspectives

D. Lacombe[1], M. Piccart[2]. [1]*European Organisation for Research and Treatment of Cancer, Headquarters, Brussels, Belgium;* [2]*Institut Jules Bordet, Medical Oncology, Brussels, Belgium*

As wealth of knowledge is constantly generated by researchers, clinical research methodology and network infrastructure have to evolve alongside the technical approaches to integrate various set of data for optimal drug development, improvement of therapeutic strategies and clinical decision making.
Clinical researchers are confronted with challenges to address the development of non cytotoxic/targeted agents and the path to optimized development is still a cumbersome process. New clinical trials design approaches are being developed such as biomarker based or adaptative designs. Enrichment strategies based on expression of molecular alterations or phase 0 designs have also shown benefit to the drug development agenda. Therefore, the next generation of clinical trial which will not only be based on clinical data but also on information generated from biological material or molecular imaging is at the door step. However, optimal choice of the best strategy may often depend on the target and selected tumor(s). In addition as the scope of our clinical trials is changing, the rapidly evolving ethical and regulatory environment adds another level of complexity.
The EORTC building its scientific strategy on strongly established tumor oriented groups is making a transition to clinical trials asking biological relevant questions. To support this strategy, several initiatives are in place including an imaging platform, revisited bio-banking policies and the network of core institution. NOCI was formed from a network of more than 20 key EORTC institutions selected for their recruitment capacities and high quality translational research infrastructure. NOCI supports the EORTC strategy to design and conduct sophisticated trials which are aimed at understanding the biology of the disease and the mechanism of action of new agents through the identification of molecular determinants predictive of activity or toxicity, related to host or tumor.
EORTC scientific strategy now also addresses molecular imaging through image centralization and on line quality assurance. NOCI institutions are fully mapped for their imaging infrastrcuture. Bio-banking policies address critical tissue storage and access. Examples of NOCI trials will be presented.

17 INVITED

NCI initiatives in developmental therapeutics

J.M. Collins[1]. [1]*National Cancer Institute, Development Therapeutics Program, Bethesda, USA*

NCI has many initiatives in developmental therapeutics, including re-vitalization of natural products, launch of the Chemical Biology Consortium, and re-invention of early clinical trials via the Phase Zero initiative. All have exciting potential to improve developmental therapeutics, but the time to direct patient benefit varies widely. This presentation highlights the NCI initiative for systematic exploration of combinations of approved anticancer drugs. The potentially rapid rate of translation from the bench to the bedside for this initiative is unmatched by other approaches.
“Rational” designs are highly preferred, especially in this conference. NCI supports hypothesis-driven, mechanism-based approaches to drug development, including combinations. In practice, it is more common is to combine a new drug with the standard-of-care. In the desire to be rational and relevant, our ability to craft all worthwhile combinations may be overestimated.
To discover combinations that would not be found by other strategies, we have begun the systematic exploration of all binary combinations of each approved anticancer drug with every other approved anticancer drug. There are about 100 anticancer drugs approved for human use world-wide. A systematic survey requires testing of 5,000 unique binary combinations. To ensure a broad evaluation, we chose to measure growth inhibition for each combination in the entire NCI-60 cell panel, for a total of 300,000 experiments. Bioinformatic tools such as the heat map facilitate the display of large sets of genomic data, and can also be used to summarize the data generated from this initiative. The results will be presented from our pilot phase, which covers 1% of the total set of combinations. Impressive variation in effect has been seen not only with different combinations, but also for the same combination across the full NCI-60 panel.
The demonstration that the activity in vitro of a drug combination exceeds the activity of either single agent is exciting, but only the first step. The most promising combinations discovered via this process are then tested in vivo, to assure that host tissues can tolerate the therapy. If only 2% of the full set of 5,000 unique binary combinations are sufficiently interesting to stimulate follow-up in vivo, then 100 combinations would require testing with human tumor xenografts. To minimize false negative results, multiple xenografts would be needed for each combination selected for testing.
Because the ultimate metric is improvement over single-agent antitumor activity, the design includes treatment groups for the dose-response of each agent alone, as well as various doses in combination. Among a series of equitoxic regimens, the preferred choice is the most active therapy, whether single or multi-agent. This second part of the initiative in at an early stage, but the results from a few key examples will be presented.
This initiative is unquestionably “big”, but why should it be attempted? Creation of a public database for combination data can stimulate improvements in the art and science of choosing combinations. Equally attractive, the truly unexpected discovery of a successful empiric combination can be the starting point that brings full attention to understanding the molecular basis. In some cases, immediate translation into a clinical trial of the combination provides the fastest route from an “interesting” lab finding to direct evaluation of patient benefit.