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.