sentinel node metastases (micrometastases). The aim of this study was to characterize the early metastatic cells molecularly and to find the best diagnostic markers for metastatic melanomas by genome-wide gene expression analyses of melanoma lymph node micrometastases and macrometastases, and of primary melanomas and benign nevi. Significance analysis of microarrays with a false discovery rate of 0.93% identified 22 over- and 5 under-expressed genes with >4-fold changes in the micrometastases. Of these genes, melan-A (MLANA), tyrosinase (TYR), melanoma inhibitory activity (MIA), v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (ERBB3), preferentially expressed antigen in melanoma (PRAME), and secreted phosphoprotein 1 (SPP1) were tested as potential markers in RT-PCR and immunohistochemistry. In a prospective study of 160 patients, graded MLANA- and TYR-RT-PCR analyses could disclose clinically significant metastases and stratify patients (in regard to tumour burden) into distinct risk groups for recurrence better than did histological and immunohistochemical examinations. In the light of these data, quantifiable RT-PCR assays should be implemented in clinical use to confirm and complement pathological examination of sentinel node metastases. In addition, SPP1 and PRAME proved valuable as melanoma-specific markers capable of differentiating melanoma cells from benign nevocytes occasionally present in the sentinel lymph nodes. Most molecular traits of the micrometastases were already present in the primary tumors, suggesting that micrometastasis to lymph nodes is a fairly non-selective process. Taken together, these findings offer clues to the development of melanoma micrometastases and provide biomarkers for more accurate and earlier detection of significant metastases as well as rational targets for therapy.

425 Poster

Loss of PTEN expression in colorectal cancer (CRC) metastases (mets) but not in primary tumors predicts lack of activity of cetuximab plus irinotecan treatment

I. Stasi[1], F. Loupakis[1], L. Pollina[2], G. Masi[1], N. Funel[2], M. Scartozzi[3], I. Petrini[4], D. Santini[5], S. Cascinu[3], A. Falcone[6]
[1]Azienda USL 6, U.O. Oncologia Medica, Livorno, Italy; [2] Università di Pisa, Dipartimento di Oncologia, Pisa, Italy; [3] Azienda Ospedaliera-Ospedali Riuniti-Università Politecnica delle Marche, U.O. Oncologia Medica, Ancona, Italy; [4] Azienda Ospedaliera ASL 5, U.O. Oncologia Medica, Pisa, Italy; [5] Università Campus Biomedico, U.O. Oncologia Medica, Roma, Italy; [6] Università di Pisa-Azienda USL 6, U.O. Oncologia Medica, Pisa, Italy

Introduction: PTEN is a key tumor suppressor that inactivates PI3K, a downstream effector of the EGFR cascade. Mutations resulting in PTEN loss lead to uncontrolled activation of PI3K/AKT signalling pathway that may result in resistance to EGFR-blockade. Methods: We retrospectively investigated the role of PTEN immunoreactivity loss (anti-PTEN antibody clone 17.A, Immunomarkers) both on primary CRC and related mets in predicting the activity of cetuximab plus irinotecan combination treatment in EGFR-positive irinotecan-refractory metastatic CRC patients (pts). Results: As of today 102 pts have been included. M/F=60/42, median age=62 (38-78), median number of previous lines of chemotherapy=2 (1-5). Among the 100 pts evaluable for response we observed a partial (PR) or a complete response (CR) in 13 and 1 cases respectively for an overall response rate of 14%. PTEN immunostaining resulted positive (+), negative (-) or unconclusive (NE) in respectively 48, 36, 11 out 98 primary tumors. On 57 mets PTEN analysis was +, - or NE in 31, 22, 4 cases respectively. PTEN positivity or negativity on primaries was confirmed on 45 related mts in 27 cases (60%) while 7 (16%) + and 11 (24%) - primaries resulted respectively - and + on mets. PTEN status tested on primary tumor was not significantly predictive of response nor PFS. Defining as responders those pts obtaining a PR or CR (RECIST) or SD lasting >6 mos and clearly progressed on previous irinotecan-based regimen with a TTP<3 mos (5 pts), analysis of PTEN on mets showed: 1- vs 12+ responders and 21- vs 19+ non responders (p=0.008). Median PFS in pts with PTEN+ mets was 4.8 vs 3.3 mos in PTEN- (p= 0.009, HR=0.50, 95% CI 0.23-0.81). Conclusions: Loss of PTEN immunoreactivity tested on mets may predict the activity of cetuximab plus irinotecan combination treatment. Further analysis on KRAS mutational status and p-AKT immunostaining are ongoing. Final data will be presented at the meeting. Supported by A.R.C.O. Foundation.

426 Poster

Expression of the HER4 isoform JM-a/CYT2 correlates to improved survival in bladder cancer patients lacking Estrogen receptor alpha

M. Munk[1], A.A. Memon[1], T. Thykjaer[2], E. Nexo[1], B.S. Sorensen[1]
[1]Aarhus University Hospital NBG, Clinical Biochemistry, Aarhus, Denmark; [2] Aarhus University Hospital Skejby, Clinical Biochemistry, Aarhus, Denmark

The epidermal growth factor receptor HER4 consists of several isoforms, generated by alternative mRNA splicing. Two isoforms differ in the juxta-membranous domain (JM-a and JM-b) and two in the cytoplasmic domain (CYT1 and CYT2). The HER4 isoforms JM-a/CYT1 and JM-a/CYT2 can undergo intracellular cleavage and the released peptide (4ICD) acts as a transcription factor when complexed with the Estrogen receptor α (ER-α). When 4ICD is not complexed with ER-α, 4ICD can induce apoptosis in cancer cells in vitro. Previous studies indicate an improved survival in bladder cancer patients expressing high HER4 levels, but the isoform composition was not examined. In the present study we examine the expression of the individual isoforms of HER4 and the expression of ER-α in biopsies from patients with bladder cancer.

Quantitative mRNA assays specific for HER4 isoforms JM-a, JM-b, CYT1, and CYT2 as well as ER-α were established and used to analyse tumour samples from 86 bladder cancer patients. Expression of the isoforms was compared to overall survival with a median follow up time of 39.2 months.

No HER4 expression was identified in 58% (n=50) of the bladder cancer samples. HER4 positive samples (n=36) all expressed JM-a/CYT2. In addition the CYT1 isoform was co-expressed in half of these samples. As previously described the expression of HER4 (n=36) was associated to improved survival (P=0.008) and the expression correlated inversely to tumour stage, grade, and type (all P<0.05). ER-α was expressed in 37% (n=32) of the samples while 63% (n=54) were ER-α negative. A survival benefit was observed only for patients expressing HER4 JM-a/CYT2 but no ER-α (n=17, P=0.007). When HER4 JM-a/CYT2 and ER-α was co-expressed no difference in survival was observed (n=19, P=0.347).

We show that the HER4 isoform JM-a/CYT2 or a combination of JM-a/CYT1 and JM-a/CYT2 are expressed in bladder cancer biopsies. Interestingly, expression of HER4 JM-a/CYT2 is related with a favourable prognosis only in patients with no expression of ER-α.

427 Poster

API-2/Triciribine functions as chemoradio-sensitizer in human cancer by specific inhibition of constitutively active AKT including AKT1-E17K signaling

J.Q. Cheng[1], S.M. Sebti[1], M. Sun[1], D. Kim[1], H. Yang[1]
[1]H. Lee Moffitt Cancer Center, Molecular Oncology and Drug Discovery, Tampa, USA

The serine/threonine kinase Akt/PKB is frequently hyperactivated in human cancer and functions as a cardinal nodal point for transducing extracellular and intracellular oncogenic signals. In addition, mounting studies showed that activation of AKT is closely associated with chemo-, radio- and TKI (tyrosine kinase inhibitor)-resistance. Thus, AKT presents an exciting target for molecular therapeutics. We previously identified an AKT inhibitor, API-2/triciribine. Recent phase I clinical trials showed promising results of API-2/triciribine as single agent in solid tumors and advanced hematological malignancies. Here, we reported that API-2/triciribine sensitizes cancer cells to apoptosis and growth arrest induced by radiation, mTOR inhibitor and conventional chemotherapeutic agents, which include cisplatin and taxol in human ovarian and lung cancer, velcade in multiple myeloma and temazolamide in glioblastoma. In addition, API-2/triciribine overcame cisplatin-, taxol- and gefitinib-resistance in ovarian and lung cancer. We further demonstrated that API-2//triciribine inhibits constitutively activated Akt kinase activity, including myr-Akt, Akt1-E40K and naturally occurring mutation AKT1-E17K which is insensitive to allosteric Akt kinase inhibitor. AKT plasma membrane translocation induced by growth factor was also blocked by API2//triciribine. Notably, API-2//triciribine inhibits mTOR inhibitor feed back activated AKT and synergizes with RAD001 to induce cell cycle arrest and apoptosis. We also revealed a novel molecular mechanism by which RAD001 and rapamycin activate AKT pathway. These findings indicate that API-2//triciribine is a chemoradio-sensitizer and overrides chemoresistance by directly targeting the AKT pathway, and thus lay the foundation for clinical trial using API-2//triciribine combined with conventional chemotherapeutic agents, TKI and radiation to treat human malignancy.

428 Poster

Incidence and the clinical outcomes of epidermal growth factor receptor (EGFR) mutations in male smokers with squamous cell carcinoma of lung

S. Park[1], E. Cho[2], D. Shin[2]
[1]Sungkyunkwan University Samsung Medical Center, Medical Oncology Internal Medicine, Seoul, Korea; [2] Gachon University Gil Medical Center, Medical Oncology Internal Medicine, Incheon, Korea

Background: EGFR mutations in non-small cell lung cancer (NSCLC) have been reported to be related to certain clinical characteristics (i.e., female, non-smokers with adenocarcinoma) and gefitinib responsiveness.

Materials and methods: This exploratory analysis was performed within a prospective phase II study to determine the clinical outcome and the incidence of EGFR mutations in male smokers with squamous cell carcinoma, who were treated with EGFR tyrosine kinase inhibitors. We analyzed the incidence of EGFR mutations in NSCLC specimens from 69 Korean patients who were treated with gefitinib or erlotinib in a prospective study. For a subset of 20 male patients with squamous cell carcinoma and a history of smoking, pretreatment tumor tissue samples were obtained and analyzed for EGFR mutations (exons 18 to 21).

Results: EGFR mutations were found in 3 (15%) out of 20 patients, including in-frame deletions within exon 19 (n=2) and L858R missence mutation in exon 21 (n=1). The 3 patients with EGFR mutations responded to EGFR inhibitor therapy, whereas only one of remaining 17 patients with wild-type EGFR achieved clinical response. Trends toward longer progression-free survival (5.8 vs. 2.4 months; P=0.07) and overall survival (9.6 vs. 7.2 months; P=0.76) were noted in patients with EGFR mutations compared to those with wild-type EGFR, respectively.

Conclusions: Although male smokers with squamous cell carcinoma have not been considered ideal candidates for treatment with EGFR tyrosine kinase inhibitors, significant incidence of EGFR mutations was observed.

429 Poster

A decline in circulating HER2 DNA predicts treatment response and survival in breast cancer patients treated with trastuzumab

B. Sorensen[1], L.S. Mortensen[2], J. Andersen[2], E. Nexo[1]
[1]Aarhus University Hospital, Dept. of Clinical-Biochemistry AS, Aarhus, Denmark; [2] Aarhus University Hospital, Dept. of Oncology AS, Aarhus, Denmark

Some, but not all breast cancer patients, who over express HER2 (human epidermal growth factor receptor 2), respond to the HER2 inhibitor trastuzumab. It is the purpose of this study to identify patients responding to trastuzumab treatment by a novel technique measuring the amplification of the HER2 gene in DNA circulating in the plasma of patients with metastatic breast cancer. An already established method determines the release of the extracellular domain (ECD) of HER2 into the circulation, and it is the aim to compare the two methods.

DNA was isolated from plasma collected just prior to the start of trastuzumab treatment from 28 patients with metastatic breast cancer who all contained an amplified HER2 gene in their primary tumour. From 22 patients an additional blood sample was also collected three weeks after the start of trastuzumab treatment. HER2 gene amplification was measured with real time PCR and expressed relative to the un-amplified gastrin gene. The cut off value (ratio of 1.14) was calculated based on analysis of plasma from 20 control subjects without breast cancer (mean + 2 SD). HER2 ECD was measured with the HER2/neu kit (Bayer), and the cut of value given by the manufacturer (15 ng/ml) was used.

Prior to treatment, HER2 DNA was increased in 52% and HER2 ECD was increased in 57% of the patients, but in neither case did this correlate to treatment response or overall survival. In 9 of 22 patients a reduction in the amount of the amplified HER2 gene in the circulating DNA was observed following trastuzumab treatment (more than 14% (2 SD) was considered a reduction). This reduction correlated to treatment response (P=0.02, log rank test), as well as to an improved overall survival (P=0.05). No correlation between clinical data and the kinetics of HER2 ECD was observed.

In conclusion we demonstrate that the dynamics of circulating HER2 DNA following trastuzumab treatment predict treatment response in patients with metastatic breast cancer. In contrast to previous studies we did not observe a correlation between HER2 ECD and response to trastuzumab.

430 Poster

Resistance to cetuximab - implication of PTEN expression in the cellular sensitivity to cetuximab (Erbitux®) of Head and Neck Squamous Cell Carcinoma (HNSCC)

J. Mriouah[1], C. Chartier[1], C. Boura[1], A. Fifre[1], I. Fries[1], C. Ramacci[2], J.L. Merlin[2], B.M.A. Faivre[1]
[1]Université Henri Poincaré, EA 3452 Faculté de Pharmacie, Nancy, France; [2] Centre Alexis Vautrin, Unité de Biologie des Tumeurs, Vandoeuvre-lès-Nancy, France

Targeted therapies using monoclonal antibodies and tyrosine kinase inhibitors are very efficient for treatment of different cancers and theoretically less toxic than usual therapies. Nevertheless, a significant number of resistance cases to targeted therapies has been reported. In case of cetuximab (Erbitux®), a chimeric human/murine monoclonal antibody targeting Epidermal growth factor receptor (EGFR or Human Epidermal Receptor 1, HER1), such acquired resistance has been reported in colorectal treatment and explained by an overproduction of vascular endothelium growth factor (VEGF). Because of its control on the VEGF expression in tumoral cells, the Pi3K/AKT signalling pathway would be the true resistance mechanism and more specially, the protein PTEN (a tumor suppressor gene) and AKT phosphorylation.

The aim of our study is to precise the roles of PTEN and phospho-AKT proteins in a quite new indication of cetuximab: Head and Neck Squamous Cell Carcinoma (HNSCC) by using small interfering RNA. For this purpose, Cal 27 (human HNSCC cell line) choosen as a model of sensitive cell line to cetuximab, was transfected by PTEN-siRNA and then treated by cetuximab during 48 hours in the period of inhibition of PTEN expression. Cell cycle analysis was performed by flow cytometry after 24 hours of exposition to cetuximab. Proteins extraction and MTT assays were done after 48 hours of exposition. Western blot and Bioplex proteins array were used to check PTEN and p-AKT expression and to evaluate activation level of signalling pathways. Our results showed a significant increase of cell proliferation and metabolic activity, correlated with a significant increase of AKT phosphorylation in PTEN-SiRNA transfected cells versus non transfected cells after cetuximab treatment.

In conclusion, we demonstrated that the loss of expression of tumor suppressor gene PTEN in human HNSCC cell line, creates a resistance to cetuximab. This loss of PTEN expression would have consequences on VEGF expression.

431 Poster

Preclinical evaluation of dasatinib in melanoma cell lines

A.J. Eustace[1], J. Crown[2], M. Clynes[1], N. O'Donovan[1]
[1]National Insititute for Cellular Biotechnology, Dublin City University, Dublin, Ireland; [2] St. Vincents Hospital Dublin 4, Department of Oncology, Dublin, Ireland

Introduction: Newer targeted therapies, alone and/or in combination with chemotherapy, offer new hope of improving prognosis for malignant melanoma. In this study we evaluated the potential benefits of dasatinib as a targeted therapy for melanoma.

Methods: Dasatinib was tested alone and in combination with temozolomide in a panel of melanoma cell lines, using the acid phosphatase proliferation assay. The effects of dasatinib on invasion, migration, apoptosis and cell cycle arrest were assessed in the melanoma cells. Expression of Src kinase and EphA2, and the effect of dasatinib treatment on expression and activation of Src and EphA2 were measured by immunoblotting.

Results: Four of the six melanoma cell lines were responsive to dasatinib. Lox-IMVI had an IC50 of 35.4 nM (± 8.8 nM). HT144, Malme-3M and M14 also display sensitivity with a maximum growth inhibition of 40 %, 30 % and 15 %, respectively, achieved in these cell lines with 1 μM dasatinib. When combined with temozolomide in both HT144 and Malme-3M, dasatinib enhanced response to temozolomide. In Lox-IMVI, CI values (CI value at ED50 = 0.88) revealed the combination of dasatinib and temozolomide was slightly synergistic. Dasatinib significantly decreased invasion of both HT144 and M14 cells (in M14 dasatinib (25 nM) reduced invasion by 65 %; p = 0.005). Dasatinib also significantly decreased the level of migration in HT144 and M14 in a dose dependant manner (in M14 dasatinib (25 nM) reduced migration by 83 %; p = 0.004). Dasatinib (200nM) induced apoptosis after 72 hours in LOX-IMVI (12 %) and Malme-3M (20 %). Cell cycle analysis of dasatinib treatment in the melanoma cells indicated that dasatinib increased G1 arrest in HT144 (17 %) and Lox-IMVI (23 %). Src kinase and low levels of phosphorylated Src kinase were detected in all cell lines tested. The level of Src kinase phosphorylation decreased in HT144, Lox-IMVI and Malme-3M when treated with dasatinib, but the level of phosphorylation was increased in Sk-Mel-28 cells treated with dasatinib. Finally EPHA2 expression was higher in the dasatinib sensitive cell lines, so EPHA2 expression can predict response to dasatinib in our panel of cell lines.

Conclusions: Our results show that dasatinib has anti-proliferative, pro-apoptotic and anti-invasive effects in dasatinib-sensitive melanoma cells. Therefore, combining dasatinib with temozolomide, may improve response to treatment in these tumours.