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.

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

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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.

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

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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.

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

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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.

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

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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.