Methylation-Specific-PCR (qMSP) to characterize potential "field effect" markers in DNA samples from tissue prints obtained from diagnostic prostate biopsies and confirmed the technical validity of the assay design. Biopsy tissue print techniques allowed us to design DNA-M marker panels that include up to 6 candidate field effect markers. Tissue prints also simplify the development of tests that include both DNA and RNA based assays.
Conclusion: By getting the most from the least tissue, a tissue print "field effect" biomarker test might be used with prostate biopsies to predict the presence of an adjacent cancer while reserving the FFPE specimens for histology.

PP121

High coexpression of both the epidermal growth factor receptor (EGFR) and insulin-like growth factor receptor-1 (IGF-1R) correlates with a poor patient prognosis in resected non-small cell lung cancer (NSCLC).

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Background: Following the success of the EGFR inhibitors a renewed interest in IGF-1R inhibitors has emerged. IGF-1R overexpression has been identified in several tumour types and protects cancer cells from apoptosis. Currently, several different approaches are being investigated for targeting the IGF-1R, including small-molecule kinase inhibitors, IGF1R monoclonal antibodies, antisense oligonucleotides and RNA interference. To date, it is not clear what factors influence sensitivity to IGF-1R blockade but it is likely that tumours that respond well to treatment will be those where IGF-1R overexpression results in a poor patient prognosis. Initial data show that tumour type may also determine response to therapy with squamous non-small cell lung cancers responding well to a combination of a IGF-1R monoclonal antibody and chemotherapy. The aim of this study was to elucidate the EGFR and IGF-1R expression profile in a cohort of NSCLC patients and correlate the results to patient clinico-pathological data and prognosis.
Materials and Methods: EGFR and IGF-1R expression were evaluated in 197 NSCLC patients (92 − squamous, 87 − adenocarcinoma, 18 − others) using immunohistochemistry analysis and the results were scored by a pathologist as follows: 0 (negative), 1+ (weak), 2+ (moderate) and 3+ (strong). Expression of EGFR and IGF-1R were also examined in a panel of cell lines (SKMES1, A549, HCC827, H1819, H1299) and patient samples (10 squamous and 10 adenocarcinomas) using Western Blot analysis.
Results: The panel of 6 NSCLC cell lines examined showed variability in IGF-1R expression. In the fresh frozen resected NSCLC tumours IGF-1R was overexpressed relative to matched normal tissues. Furthermore squamous cell carcinomas had higher levels of expression than adeno-carcinomas. Immunohistochemistry analysis demonstrated that squamous cell tumours have higher IGF-1R expression levels than adenocarcinomas (3+/2+ Squamous [70/197] versus 3+/2+ Adenocarcinoma 27/197)] $p < 0.0001$). Patients with squamous cell carcinoma also had higher EGFR expression than those with adenocarcinoma ($p = 0.002$). Patients with EGFR and IGF-1R overexpression had a poorer survival ($p = 0.043$).
Conclusion: Our findings indicate that while EGFR and IGF-1R expression alone are not independent prognostic markers of survival in NSCLC. Taken together overexpression of both proteins correlates to a poor survival. This subset of patients may benefit from a combination of TKIs/monoclonal antibodies and chemotherapy.

PP81

18F-FDG PET/CT for early detection of relapse in head and neck squamous cell carcinoma

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Background: A key prognostic factor in head and neck squamous cell carcinoma (HNSCC) is the loco-regional control of the disease. Early detection of relapse by selected imaging modalities is therefore of upmost importance. We evaluated the diagnostic accuracy of 18F-FDG PET/CT and MRI for the assessment of HNSCC relapse. Since early treatment might anticipate on inoperable relapse, we also evaluated if early 18F-FDG PET/CT might help in residual tumor detection despite treatment-related changes.
Materials and Methods: The study was prospectively performed on 32 patients with 36 primary HNSCC who underwent 18F-FDG PET/CT and MRI before treatment and at 4 and 12 mo after treatment completion. 18F-FDG PET/CT was also performed 2 weeks after the end of radiotherapy. All images were blindly and independently interpreted and graded on a 5-point scale. Histopathology or a minimum of 18 mo follow-up were used as gold standard.
Results: Before treatment 18F-FDG PET/CT and MRI detected all primary tumors except for 2 limited vocal fold lesions (sensitivity: 94%). MRI was more sensitive than 18F-FDG PET/CT for the detection of precise local extension sites (sensitivity: 75% versus 58%, $P < 0.05$) but at the cost of a higher rate of false positive results (positive predictive value: 74% versus 86%, $P < 0.05$). For relapse detection at 4 mo, sensitivity was significantly higher for 18F-FDG PET/CT (92%) than for MRI (73%) ($P < 0.05$), but the diagnostic performances were not significantly different at 12 mo post-treatment. For the detection of residual malignant tissue at 2 weeks post-radiotherapy, sensitivity and specificity of 18F-FDG PET/CT were respectively 86% and 85%, when using an SUV cut-off value of 5.8.
Conclusion: This study demonstrates that 18F-FDG PET/CT is effective in the differentiation between residual tumor and radiation-induced changes, as early as 2 weeks after treatment of a primary HNSCC. For follow-up, accuracy of 18F-FDG PET/CT and MRI are similar except for a higher sensitivity of 18F-FDG PET/CT at 4 mo.

PP14

RRM1 expression in muscle invasive, locally advanced urothelial cancer is associated with survival in younger patients

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Background: RRM1, the regulatory subunit of ribonucleotide reductase, plays an important role in DNA repair after chemotherapy damage and in regulation of tumor progression. Prior studies demonstrated a survival benefit to high expression in resected early stage lung cancer and a trend to longer time to progression in patients with low expression in unresectable advanced bladder cancer treated with gemcitabine-cisplatin therapy. We undertook this study to assess whether patients with resected locally advanced (T2−4NxM0) urothelial carcinoma (UC) whose tumors had higher RRM1 expression would have longer overall survival (OS).
Materials and Methods: 84 radical cystectomy specimens with muscle invasive UC were identified from existing tissue microarrays. The medical records of these patients were retrospectively reviewed to confirm pathology and stage. Specimens were analyzed for RRM1 expression using automated quantitative analysis (AQUA). The median value of RRM1 was established a priori as the cutoff for high and low expression. Older patients were defined as having an age $\geqslant$70 years.
Results: Median age was 69.3 years. 43 patients were <70 years; 41 were $\geqslant$70 years. There was near equal distribution of stages: 30%, 38%, and 32% for stage II, III, and IV respectively. The majority were high grade (99%) with no nodal involvement (69%). Median OS was 2.0 years (0−13.1). Tumoral RRM1 expression levels did not correlate with OS. However, when adjusted for age, high tumoral RRM1 expression in younger patients (<70 years) correlated with increased survival. Younger patients with high RRM1 had a median OS of 10.6 years compared to 1.6 years in older patients ($p = 0.0013$). No difference in survival was seen among low RRM1 expressors: 2.3 vs. 1.6 years in younger and older patients respectively, ($p = 0.215$). 40% of younger patients were high expressors. 32% of younger patients had nodal involvement compared to 29% of the older subset. In terms of T stage, 33% of younger patients had T3 disease compared to 54% of older patients and 33% of younger patients had T4 disease compared to only 15% of older patients.
Conclusion: Our results suggest that high RRM1 expression may be prognostic for improved survival in locally advanced UC patients less than 70 years old. This novel finding suggests that the biology of bladder cancer in "younger" patients is inherently different than their older cohort such that RRM1 gene expression should be the target of a larger investigation in this subset of patients.

PP16

Metronomic weekly use of zoledronic acid for breast cancer with bone metastases has more potent antitumor and bone-preserving effects than conventional zoledronic acid given every-four-weeks

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Background: Zoledronic acid (ZOL) has direct and indirect antitumor effects, however, the pharmacokinetics of the drug in breast cancer patients remain to be elucidated and optimized. The main study objectives were

to compare the effects of ZOL on bone resorption, angiogenesis, tumor markers and time to disease progression between a weekly low dose (the metronomic regimen) versus a conventional dosage.
Materials and Methods: Sixty breast cancer patients with bone metastases were recruited to a randomized phase II trial. They were randomized to either ZOL 1 mg IV weekly for 4 doses or a single dose of ZOL 4 mg IV. No other antitumor treatments were administered during the first month after randomization. Serial blood samples were collected on day 1, 15 and 29 to measure markers for bone resorption (N-telopeptide), angiogenesis (VEGF) and tumor burden (CEA and CA15-3).
Results: Compared to a single-dose administration, weekly low-dose of ZOL resulted within the first 4 weeks in significantly greater reductions in serum levels of VEGF and N-telopeptide, with more reduction towards the end of the first month of treatment. Compared with baseline serum VEGF levels, the percentages of more than 25% reduction with the metronomic regimen were 50% and 96.6% on day 15 and day 29, respectively, while the corresponding values with conventional dosing were 23.3% and 17.2%, respectively. Patients who received metronomic ZOL had a substantially longer median TTP (7.0 months, 95% CI, 6.1−7.9 months) than those who had a single dose of ZOL (2.8 months, 95% CI, 0−5.7 months; P = 0.076).
Conclusion: Metronomic use of low-dose ZOL appeared to be more effective than conventional regimen in sustained reduction of circulating VEGF and N-telopeptide levels, and in prolonging TTP. This dosing schedule should be further assessed in phase III trials.
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PP65

PIK3CA mutations in patients with advanced cancers treated in a phase I clinic

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Background: Phosphatidylinositol 3-kinase (PI3K) is thought to play an important role in tumorigenesis. Activating mutations of the p110á subunit of PI3K (PIK3CA) have been identified in a broad spectrum of tumors.
Materials and Methods: A mutational analysis (a PCR-based DNA sequencing) of exon 9 (helical domain) and exon 20 (kinase domain) of the PIK3CA was performed using DNA obtained from tumors of patients referred for clinical trials using targeted therapy. Patients with PIK3CA were preferably treated whenever possible with regimens containing inhibitors of PI3K-AKT-mTOR signaling pathway.
Results: To date 105 samples from patients with various advanced cancers have been collected. At the time of submission 80 results of mutational analysis were available (ovarian cancer, n = 17; colon cancer, n = 9; cervical cancer, n = 10; endometrial cancer, n = 7; breast cancer, n = 7; melanoma, n = 6; head and neck cancer, n = 4; soft tissue sarcoma, n = 4; renal cancer, n = 3; and other tumor types, n = 13). PIK3CA mutations were detected in 11 (14%) patients (2 in exon 9-helical domain, 9 in exon 20-kinase domain). In tumor types with more than 5 patients tested, PIK3CA mutations were most frequent in endometrial cancer (43%, 3 out of 7 patients), ovarian cancer (24%, 4 out of 17 patients), head and neck cancer (25%, 1 out of 4 patients), breast cancer 14% (1 out of 7 patients), and colon cancer (11%, 1 out of 9 patients). No mutations were identified in patients with melanoma or cervical cancer. The small number of patients at this point precludes statistical comparisons. Of the 11 patients with PIK3CA mutations, 9 were treated on a protocol that included a drug targeting the PI3K-AKT-mTOR pathway, and 4 (44%) responded (partial responses). Although numbers are small, in individual disease there were 2 (67%) responses in 3 endometrial cancers, 1 (33%) in 3 ovarian cancers, 1 (100%) in 1 breast cancer, and no response in 1 colorectal cancer patient.
Conclusion: PIK3CA mutations were detected in 14% of patients with various solid tumors. Patients with PIK3CA mutations had high response rates when treated with PI3K-AKT-mTOR inhibitors.

PP104

TYMS and DPYD polymorphisms and toxicity of 5-fluorouracil and capecitabine chemotherapy in colon cancer patients

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Background: Thymidylate synthase (TS) is key enzyme in the synthesis of thymidylate and is a target for 5-FU which is mainly catabolised by dihydropyrimidine dehydrogenase (DPD). VNTR and C>T SNP in the enhancer region of TYMS gene (TSER) as well as DPYD gene mutation (DPYD*2A) may influence toxicity of 5FU/Capecitabine-based chemotherapy. Aim of this study was to evaluate the correlation between TSER VNTR and the susceptibility to sporadic colon cancer and to correlate the genotype frequencies of TSER VNTR and DPYD*2A between Croatian and other european populations. We also aimed to correlate TSER polymorphisms and DPYD*2A and toxicity of 5FU/Capecitabine-based chemotherapy in colon cancer patients.
Materials and Methods: Genotyping was performed on 100 healthy unrelated Croatians and 100 colon cancer patients using PCR-RFLP method.
Results: Genotype frequencies of TSER VNTR did not differ statistically between controls and colon cancer patients. 49 patients were assesed for toxicity and two patients with worst toxicities were heterozygous for DPYD*2A. Among the remaining 47 patients, 33 were assigned into a 'low expression TSER genotype' group [13 (39.4%) with 2R/2R, 12 (36.4%) with 2R/3RC and 8 (24.2%) with 3RC/3RC TSER genotype] and 14 into a 'high expression TSER genotype' group [7 (50.0%) with 2R/3RG, 1 (7.14%) with 3RG/3RG and 6 (42.86%) with 3RG/3RC TSER genotype]. 25 patients (75.76%) from the 'low expression TSER genotype' group experienced a total of 65 toxicities. 6 patients (42.86%) from the 'high expression' group experienced total of 10 toxicities.
Conclusion: No correlation was found between TSER VNTR and the susceptibility to sporadic colon cancer. Genotype and allele frequencies were similar to other European populations. We assume that the worst toxicities experienced by two patients with DPYD*2A mutation were a consequence of that mutation but due to a small patient number, the impact of this mutation on risk of toxicity could not be proven to be statistically significant. The remaining forty-seven colon cancer patients were divided into two groups based on TSER genotype. 'Low expression TSER genotype' group of patients suffered from more and worse toxicities (grade III and IV) of 5FU/Capecitabine-based chemotherapy compared to a 'high expression TSER genotype' group ($p = 0.020606$, $p < 0.0001$, respectively). These results might have a prognostic role in the prediction of toxicity of 5FU/Capecitabine-based chemotherapy in colon cancer patients in Croatia.

PP51

Heat shock of tumor cells at sublethal temperatures causes an immediate phosphorylation of the C-terminal hydrophobic motive of Akt

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Background: Regional hyperthermia (RHT) combined with chemotherapy is proven to provide a new treatment option for high-risk soft-tissue sarcomas. The cellular signalling responses of heat exposed tumor cells at clinical relevant temperatures are of special interest. Here we investigate Akt signalling pathways of different human tumor cells in vitro. The protein kinase Akt is involved in major signalling events including important anti-apoptotic programs. Accordingly, mutations of the Akt pathway are frequently found in malignant transformed cells
Materials and Methods: For heat exposure, cells (FG-pancreatic carcinoma, MG63-osteosarcoma; A673-rhabdomyosarcoma cell lines) were incubated for 1−6 hrs at 41.8ºC (sublethal heat shock). Concentrations of phorphorylated and non-phosphorylated intracellular signalling molecules (PI3K, PIP3-DK, Akt, mTor, p70 S6K) and HSP70 induction were measured by SDS-PAGE and immunostaining.
Results: The inducible heat shock protein HSP70 is upregulated over this time-temperature exposure. In parallel, immediate activation of Akt signalling was detected after 1 hr heat exposure in all investigated tumor cell lines. Preliminary results indicate that sublethal heat exposure does not influence the activities of PI3K and PIP3-DK by changing their phosphorylation status. Accordingly − as shown by our results − the phosphorylation of Akt on its activation loop is not influenced. Usually this is the initial activation event after transmembrane RTK-activation and subsequent PIP3 synthesis by the PI3-kinase. In contrast, we found that Akt phosphorylation is selectively induced on its C-terminal hydrophobic motive as a consequence of sublethal heat shock. Furthermore, the phosphorylation of downstream targets (mTor, p70 S6K) indicates an increase of Akt activity.
Conclusion: The data suggest a specific and fast mechanism for heat stress related Akt activation. It is known that phosphorylation of the C-terminal hydrophobic motive − as observed under heat exposure − enhances Akt activity. Several protein kinases for example mTorC2, DNA-PK or PKCβII are discussed to be responsible for this phosphorylation event. In current experiments using different inhibitors and siRNAs we try to reveal further the mechanism of heat-induced Akt phosphorylation. Targeted modification of heat-induced Akt activation might enhance the efficacy of RHT without harming the tissues beyong the heated region.
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