PP87

Etoposide/platinum therapy, UGT1A1 and GSTP1 polymorphisms, and toxicity in children with solid tumors

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Background: Etoposide is a commonly used chemotherapy agent with a broad range of antitutmor activity. It stabilize the double-stranded DNA cleavage normally catalyzed by topoisomerase II and inhibit faithful relegation of DNA breaks. Glutathione and glucuronide conjugation seem to inactivate parent drug and metabolite, and are mediated by GSTT1/GSTP1 and UGT1A1. Recently, 64 genetic variants that contribute to etoposide-induced cytotoxicity were identified through a whole-genome association study. UGT1A1 gene promoter polymorphism can affect the expression level of UDP glucuronosyltransferase. The polymorphism consists of an insertion of a TA nucleotide sequence into a (TA)6 TAA sequence in the gene promoter resulting in (TA)7 TAA (UGT1A1*28). The longer TA repeats lower the enzyme expression level and glucuronidation. Decreased glucuronidation leads to severe hematotoxicity and mucositis. The SNP polymorphism GSTP1 313A>G (I105V in exon 5) has been associated with reduced enzyme activity and anticancer drug resistance and toxicity. The aim of our research was the correlation between UGT1A1 and GSTP1 polymorphisms and etoposide/platinum therapy in children with solid tumors in Croatian population.
Materials and Methods: Our study was performed on 44 childrens with solid tumors treated in Children's Hospital Zagreb. All patients received standardized adjuvant chemotherapy, etoposide (in range 100–150 mg/m^2) in combination with platinum derivates. Toxicity was assessed according to the NCI Common toxicity criteria (version 2.0). The study was approved by the Ethics Committee of the Children's Hospital Zagreb.
UGT1A1 polymorphisms was analyzed by heteroduplex analysis on Elchrom Spreadex EL-300 gels stained with SYBRGreen. GSTP1 polymorphism was analysed using predeveloped real-time PCR Taqman® SNP genotyping assay.
Results: Correlation between UGT1A1 and GSTP1 genotypes and grade of hematotoxicity were found. Hematotoxicity grade 3 was more frequent in children with GSTP1 G allele and UGT1A1*28 allele. There was no correlation between GSTP1 and UGT1A1 genotypes and mucositis.
Conclusion: These results are a part of prospective pharmacogenetic study to examine the correlation between SNPs in genes regulating chemotherapeutic metabolism and solid tumor chemotherapy in children in Croatian population. We hope that the results of this project will be used in the future for improvement of children's solid tumors therapy.

PP128

Expression profiling of BRCA1 and BRCA2 deficient human tumours and cell-lines using a breast specific platform to identify a biomarker of DNA repair deficiency

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Background: Recent evidence suggests that PARP inhibitors can be used to selectively kill cancers defective in DNA repair. Cancers with mutations in the breast cancer associated BRCA1 and BRCA2 genes fall into this category. However, there is no method to accurately identify other tumours defective in DNA repair that may also benefit from this type of therapy.
Materials and Methods: BRCA1 and BRCA2 were silenced in a panel of ERa-positive and ERa-negative cell-line models using siRNA transfection technology. Levels of mRNA knockdown were determined by qPCR. Functional loss of BRCA1 and BRCA2 was demonstrated using a PARP-1 inhibitor sensitivity assay. A cohort of BRCA1 and BRCA2 mutant FFPE tumours and matched sporadic breast cancer controls were collected at Mayo Clinic. Gene expression profiling of cell line and tumour samples was carried out using the Almac Breast Cancer DSATM microarray. Feature selection and unsupervised analyses were carried out on the gene expression data and functional analysis was performed using the GeneGo MetacoreTM knowledgebase.
Results: Differentially expressed genes derived from the cell-lines were used for comparison with, and functional enrichment of, the gene expression profiles of the tumor samples. We found that the differentially expressed genes common to both the cell-line and tumor data were primarily concerned with cell-cycle control and DNA-damage response. Unsupervised analysis using this list of common genes identified a subset of sporadic tumour samples that were classified amongst the BRCA1 and BRCA2 mutant tumours. Further analysis of the genes characteristic of these sporadic tumours and the BRCA1 mutant tumours show a strong correlation in abrogation of DNA response pathways in both cohorts.
Conclusion: This study demonstrates the power of using gene expression profiling of cell-line models to refine the analysis of expression data from FFPE archived human tumor samples. In this case this approach suggests that a subset of sporadic breast tumours are defective in DNA-repair response, and are therefore candidates for targeted therapy such as PARP-1 inhibitors.

PP117

Increased EGFR gene copy number is variably associated with both EGFR and KRAS Mutation (Mt) status and influences Progression Free Survival (PFS) to first-line chemotherapy in EGFR/KRAS wildtype (WT) patients

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Background: EGFR IHC, FISH and KRAS/EGFR Mt status have been explored as independent factors for EGFR TKI benefit in NSCLC. Associations between test results in different clinical/demographic groups and the additional contribution of overlapping biomarkers to outcomes remains under-explored.
Materials and Methods: 200 patients with stage IIIB/IV NSCLC with molecular analysis performed after June 2008 were eligible. Patients with trimodality testing for EGFR plus KRAS Mt data (quadruple testing) had retrospective PFS data collected for EGFR TKI regimens (any line) and first line chemo (without EGFR TKI). IHC H-score >100 was considered positive (+). FISH score 5 (high polysomy) and 6 (true amplification) were considered +. Never-smokers (NS) smoked <100 cigarettes/lifetime. PFS was defined from therapy initiation to radiographic/clinical progression. If PFS was not met, data was censored at the time of analysis provided treatment time was $\geqslant$6 mo. Chi-square or Student's T tests were used in categorical and PFS comparisons, using a p-value of 0.05 for significance.
Results: 71% (142/200) of patients had quadruple testing. Adenocarcinoma was dominant among males (M), females (F), NS and smokers (S). S had a higher % of squamous than NS [20% (19) vs 8% (4), p = 0.069]. 85% (121/142) were + by IHC. EGFR IHC was more likely to be negative in S than NS [20% (19) vs. 4% (2), p = 0.011]. A higher proportion of S were FISH + than NS (55% vs 48%, p = 0.714). EGFR Mt was more common in NS [15% (7) vs. 7% (7), p = 0.177]. No patients had both EGFR and KRAS Mts. S had a higher proportion of KRAS Mt than NS [22% (21) vs. 4% (2), p = 0.005]. Patients with IHC+, FISH $\geqslant$5 were more likely to have both EGFR Mt (21.5% vs 11%, p = 0.042) and KRAS Mt (17% vs 5%, p = 0.087) than those with IHC+, FISH < 5. PFS with chemo if IHC+, EGFR/KRAS WT, FISH < 5, compared to IHC+, EGFR/KRAS WT, FISH $\geqslant$5 was 2.94 vs 6.35 mo, respectively (p = 0.0022).
Conclusion: Clinical and demographic factors are associated with different proportions of EGFR/KRAS tests being positive. EGFR FISH + was associated with EGFR Mt and a non-statistically significant trend towards association with KRAS Mt. In EGFR/KRAS WT patients EGFR FISH influences PFS to first line chemo. Overlapping biomarker data sets defining different molecular syndromes in NSCLC may reveal previously unsuspected contributions from EGFR gene copy number to outcome. Updated PFS data with chemo and with EGFR TKIs will be presented.

PP126

Quantitative protein analysis in FFPE tissue: Application to the tissue microenvironment

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Background: Measuring drug target proteins directly in patient-derived formalin fixed tissue can provide for personalized medicine. The best example of this is measuring Her2 by IHC. While IHC provides visual cellular detection of Her2 expression within the context of the tissue microenvironment, the assay suffers from a lack of reproducibility, sensitivity, and quantitative objectivity. We have developed a quantitative Her2 assay designed to achieve objective quantitation of Her2 directly in formalin fixed patient tissue and have applied it to the analysis of protein expression within the tissue microenvironment to understand differential Her2 expression with respect to various regions of the tumor tissue.
Materials and Methods: Utilizing Director® tissue microdissection, Liquid Tissue® preparation reagents and protocol, combined with quantitative MRM mass spectrometry we developed a quantitative Her2 assay that measures Her2 within FFPE tissue down to amole sensitivity. This assay was applied to a cohort of breast cancer tissues with varying levels of Her2 expression and gene amplification. This assay was applied to a single breast cancer tissue showing variable Her2 expression across the tumor microenvironment. Different histological regions were microdissected and multiple lysates from this single tissue block were prepared for analysis by the Her2 assay.

Results: Results across the cohort indicate Her2 MRM results correlate precisely with IHC and FISH demonstrating Her2 quantitation in Liquid Tissue lysates obtained by microdissecting multiple cancerous regions across the same tissue section and processing them together to produce a single lysate. Results of this assay across different regions within the same tumor tissue indicate different levels of Her2 are detected in different cancerous and stromal regions within the same tissue section that correlate precisely with IHC analysis in these various regions.
Conclusion: These results indicate the need for a sampling strategy for measuring quantitative levels of specific proteins directly in patient tissue that requires tissue microdissection with implications for the molecular analysis of tumor tissue for advanced diagnostic applications.

PP116

Lycopene and prostate cancer

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Background: The purpose of this Phase II randomized-controlled trial was to evaluate the safety and effect of administering several doses of lycopene to men with clinically localized prostate cancer, on intermediate endpoint biomarkers implicated in prostate carcinogenesis.
Materials and Methods: Forty-five eligible men with clinically localized prostate cancer were supplemented with 15, 30 or 45 mg of lycopene or no supplement from biopsy to prostatectomy. Compliance to study agent, toxicity, changes in plasma lycopene, serum steroid hormones, PSA and tissue Ki-67 were analyzed from baseline to completion of intervention
Results: Forty-two of forty-five five subjects completed the intervention for approximately 30 days from the time of biopsy until prostatectomy. Plasma lycopene increased from baseline to post treatment in all treatment groups with greatest increase observed in the 45 mg lycopene-supplemented arm compared to the control arm without producing any toxicity. Overall, subjects with prostate cancer had lower baseline levels of plasma lycopene similar to those observed in previous studies in men with prostate cancer. Serum free testosterone decreased with 30 mg lycopene supplementation and total estradiol increased significantly with 30 mg and 45 mg supplementation from baseline to end of treatment, with no significant increases in serum PSA or tissue Ki-67. These changes were not significant compared to the control arm for this sample size and duration of intervention.
Conclusion: Although antioxidant properties of lycopene have been hypothesized to be primarily responsible for its beneficial effects, our study suggests that other mechanisms mediated by steroid hormones may also be involved

PP60

Correlation of absolute lymphocyte count with clinical benefit and overall survival: results of compassionate-use trial of ipilimumab in advanced melanoma at Memorial Sloan-Kettering Cancer Center

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Background: Ipilimumab (ipi) is a monoclonal antibody which antagonizes cytotoxic T lymphocyte antigen (CTLA)-4, a negative regulator of the immune system. We report on advanced refractory melanoma Pts treated on a trial of compassionate-use ipi at the Memorial Sloan-Kettering Cancer Center.
Materials and Methods: Eligibility criteria included stage III (unresectable) or stage IV melanoma. Pts had experienced progressive disease to at least one prior systemic therapy (except for those with ocular primary tumors, who were required to have local control of their disease). Pts with primary ocular or mucosal melanomas were eligible, as were those with brain metastases. Pts received ipi 10 mg/kg every three weeks for four induction doses. Those Pts with evidence of clinical benefit (CB) at Week 24 − complete or partial response (CR or PR) or stable disease (SD) as defined by modified WHO criteria − then received maintenance ipi every 12 weeks.
Results: 53 Pts were enrolled, with 51 evaluable (one was lost to follow-up after one ipi treatment while the other received chemotherapy between ipi treatments). The median age of Pts was 62 years (range, 38−86 years). 64% of Pts were male and most had an excellent performance status (85% with ECOG status 0−1). 25% of Pts had an abnormally elevated lactate dehydrogenase (LDH) level $\leqslant 2\times$ the upper limit of normal (ULN) and 32% had a baseline LDH $> 2 \times$ ULN. Grade 3/4 immune-related adverse events (irAEs) were noted in 29% of Pts, with the most common irAEs being pruritus (43%), rash (37%) and diarrhea (33%). The response rate (CR+PR) was 12% (95% CI: 5%, 25%) while 29% had SD (95% CI: 18%, 44%). Median progression-free survival was 2.5 months while median overall survival (OS) was 7.2 months (95% CI: 4.0, 13.3). Pts with grade 3/4 irAEs appeared to have improved Week 24 CB rate. Pts with an absolute lymphocyte count (ALC) $\geqslant$1,000/μL (33/41 Pts) after two ipi treatments (week 7) had significantly improved CB rate (45% versus 0%, $p = 0.02$) and median OS (11.9 versus 1.4 months, $p < 0.001$) compared to those with an ALC < 1,000/μL (8/41 Pts). Six and 12 month OS were 75% vs. 0% and 47% vs. 0% when stratified by Week 7 ALC. This association remained significant when controlled for baseline LDH level.
Conclusion: Our results confirm that ipi is clinically active in Pts with advanced refractory melanoma. The ALC after two ipi treatments appears to strongly correlate with CB and OS and should be prospectively validated.

PP52

Amplification of the chromosome 17 q22 amplicon containing TOP2A gene is correlated with better survival in HER2 amplified/ hormone receptors negative breast cancers

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Background: HER2 amplified breast cancers are considered as a homogeneous sub-group of breast cancers. It has been described that amplifications in the chromosome 17 (ch17) could affect different genes implicated in cancer development. The aim of the study was to determine, by quantitative PCR, the levels of amplification of different genes located on ch17q (on the centromeric side and distal side of the chromosome in regards of HER2 localization), and their relation with patient's survival.
Materials and Methods: We determined MED1 (centromeric side) and TOP2A IGFBP4, CCR7, KRT20, KRT19 and GAS (distal side) gene copy numbers by quantitative PCR in 87 HER2 amplified breast tumors. Patients were included between 2002 and 2006 (median follow-up = 40.3 month). They received radiotherapy (100%), anthracyclin based regimen (78%), taxanes (2%), herceptin (24%) and hormonotherapy (40%) as adjuvant therapy according to therapeutic recommandations used at the time of surgery.
Results: Gene amplification occurs in 65.6% for MED1 (57/87), 23% for TOP2A (22/65), 19.5% for IGFBP4 (17/87), 18.4% for CCR7 (16/87), 13.8% for KRT20 (12/87), 11.5% for KRT19 (10/87), and 6.9% for GAS (6/87). The level of amplification of HER2 is correlated with the level of amplification of MED1 ($p < 0.0001$) but not with others gene amplification's levels. HER2-MED1 amplicon is associated mostly with hormone receptors positive breast tumor ($p < 0.01$). Hormone receptors negative patients have the worst overall survival (OS) (HR 0.356; 95% CI 0.16−0.783; $p < 0.01$), median progression-free survival (PFS) after recurrence was 36.5 compare to 43 months for positive hormone receptors patients. In hormone negative patients, amplifications of HER 2 +/− MED1 genes have a worse OS (HR 0.277; 95% CI 0.131−0.588; $p < 0.0008$) than patient with amplification of genes located on the distal side; PFS after recurrence was 35 compared to 43 months, respectively.
Conclusion: HER2 and MED1 are located on the same amplicons. The Amplification of genes located on the distal side of ch17 determines a profile with a better survival in hormone receptors negative breast cancer. This could be explained by a better response to anthracyclin based regimen for hormone-independent -TOP2A amplified breast cancer.

PP114

Non-coding mitochondrial RNA differential expression: a new biomarker for noninvasive cancer diagnosis

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Background: Recently, we described the existence in human cells of a family of non-coding mitochondrial RNAs (ncmtRNAs) formed by the sense or antisense 16S mitochondrial rRNA, and an inverted repeat (IR) of variable size covalently linked to its 5′ end. These transcripts were named sense ncmtRNA (SncmtRNA) and antisense ncmtRNA (ASncmtRNA) respectively. The expression of these transcripts varies depending of the proliferative status of the cell. In resting cells, the sense and antisense transcripts are down-regulated. In normal proliferating cells, both transcripts are highly represented. In tumor cells, however, only the antisense transcript is selectively repressed. Based on the universal representation and the differential expression of these ncmtRNAs, the goal of this research is to demonstrate the enormous potential in cancer diagnostic of these molecules and their use as a new biomarker in human neoplasias. By double in situ fluorescent hybridization (FISH), we are developing a non invasive approach for the diagnosis of prostate and bladder cancer