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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.

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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.

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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.

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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.