cells) polysomal RNA, total RNA and mRNA eluted from SKOV3 cells. We performed flow cytometry and Cr-release assay to check the peptide specificity and the cytotoxity against SKOV3-A2 cells. Western blot analysis was performed to check HER-2/neu and Heat shock protein70 (HSP70) expressions in these fractions.
Results: Polysomal RNA, as particulate Ag is phagocytosed by iDC. Polysomal RNA together with its bound proteins presented by iDC activate tumor cytolytic cells, which include CD8+ T cells. At equal amount of RNA, polysomal RNA fractions are more immunogenic than total RNA and mRNA. We found nascent HER-2 polypeptide from polysomal RNA of ovarian cancer cells expressing the indicator CTL epitope of HER2/neu (E75) to be immunogenic for functional CTL expansion and differentiation. It has been proposed that chemotherapy activates tumor antigen (TA)-specific immunity. The molecular composition of the immune activators from ovarian cancers, and their modifications by chemotherapy are unknown. Paclitaxel at sub-pharmacological doses induces formation of larger amounts of polysomal RNA. We show that paclitaxel induced polysomal RNA, chaperoned by heat shock proteins, activate immune cells such as CD8+ cells which respond by expressing perforin, IFN-gamma, and mediating lysis of tumor cells. The polysomal RNA is taken-up through scavenger receptors (CD91/Lox-1) and efficiently stimulate immature iDC to secrete IL-12.
Conclusion: These results demonstrate that polysomal RNA from ovarian cancers can induce tumor antigen-specific immunity. And Paclitaxel can intensify this CTL induction.

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Immunoglobulin G fragment C receptor polymorphisms and clinical outcome of EGFR-expressing metastatic colorectal cancer patients treated with cetuximab-based therapy

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Background: FcγR polymorphisms have been shown to predict clinical activity of monoclonal antibody rituximab in patients with follicular lymphoma and trastuzumab in metastatic HER-2 positive breast cancer patients. Cetuximab is a chimeric monoclonal IgG1 with demonstrated activity in metastatic colorectal cancer. In this study we tested wheter FcγR polymorphisms are associated with clinical outcome of colorectal cancer patients who received cetuximab.
Patients and Methods: Forty-five consecutive EGFR-positive advanced colorectal cancer patients receiving cetuximab-based therapy (irinotecan or oxaliplatin) were examined for the FcγR IIIa 158 valine (V)/phenylalanine (F), FcγR IIa 131 histidine (H)/arginine (R) and FcγR IIb 232 isoleucine (I)/threonine (T) polymorphisms. FcγR IIIa, FcγR IIa and FcγR IIb genotyping was performed by polymerase chain reaction followed by direct sequencing of the region of interest. Genomic DNA was used for FcγR IIa, FcγR IIIa genotyping, while FcγR IIb polymorphisms were detected on cDNA.
Results: Cetuximab and irinotecan was administered as second-line in 4 patients (9%) and after $\geqslant$3 lines of chemotherapy in 37 patients (82%). Four patients (9%) were treated with cetuximab and oxaliplatin. Our population was in Hardy-Weinberg equilibrium for the three polymorphisms. The FcγRIIIa 158 V/V genotype was associated with a higher clinical benefit rate (RR + SD; 60% v 40%) even though without statistical significance ($P = 0.46$). FcγR polymorphisms did not affect progression free survival of study cohort.
Conclusions: Despite previous data on other monoclonal antibodies, in our study the hypothetical role of FcγR polymorphisms on cetuximab activity was not demonstrated. Unknown clinical and/or molecular variables may have influenced our results. The analysis is ongoing and updated findings on an expanded number of cases will be presented.

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Understanding the role nitric oxide plays in tumour formation and progression

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Background: Previous work has shown that nitric oxide (NO) – a nitrogen-based free radical found within the human body – is overproduced in many human tumors. NO is produced by Nitric Oxide Synthase (NOS). Cells combat the exposure to NO by producing Glutathione-S-Transferase-pi (GST-pi), a protective mechanism. Herein, we assess the DNA damage caused by NO in cells exhibiting increased GST-pi expression.
Materials and Methods: In this study we utilized five head and neck squamous cell carcinoma cell lines that were previously adapted to high concentrations of NO by gradually introducing a nitric oxide donor into the cell culture media. The amounts of DNA damage in these adapted cell lines and their corresponding parent cell lines (cells grown in medium without nitric oxide) were determined using COMET assays. The lengths of the COMET tails were measured and compared between the NO-adapted cells and the parent cells, with the length of the COMET tail corresponding to the amount of DNA damage (i.e., increasing tail lengths correspond to greater amounts of DNA damage). Immunohistochemistry was also used to measure the expression levels of APE-1, a DNA repair enzyme.
Results: The high-NO adapted cells were found to have shorter COMET tails than their corresponding parent cells in four of the five cell lines studied. Higher levels of APE-1 expression were also found in four of the five NO-adapted cell lines.
Conclusions: The COMET assay results suggest that the elevated nitric oxide levels of the NO-adapted cells result in increased protective mechanisms that spare DNA from damage, and the immunohistochemistry findings suggest that the upregulation of APE-1 is one such mechanism that NO-adapted cells use to protect their DNA under these harsh conditions. Therefore clinically, tumors that express high levels of NOS, GST-pi, and APE-1 would be predicted to have a more aggressive behavior.

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UGT1A1*28 polymorphism is not associated to irinotecan-induced toxicity in pediatric patients

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Background: Hepatic uridine diphosphate glucuronosyltransferase isoform 1A1 (UGT1A1) catalyzes the glucuronidation of bilirubin and that of SN-38, the active metabolite of irinotecan. More than 50 genetic variations in the promoter and coding regions of UGT1A1 gene have been described. UGT1A1*28 polymorphism, characterized by an additional TA repeat $[A(TA)_7TAA]$ in the TATA sequence of UGT1A1 promoter, has been associated with reduced glucuronidation of SN-38 compared with the wild-type $[A(TA)_6TAA]$. Conflictive results have been reported on the association between this genotype and irinotecan toxicity, namely myelosupression and gastrointestinal toxicity. Moreover, little information exists on this association in pediatric patients. We analyzed the association between polymorphic variants in the TATA sequence of UGT1A1 promoter and the incidence of adverse events related to irinotecan in pediatric patients.
Patients and Methods: This is a retrospective analysis of 32 pediatric patients including 21 with central nervous system tumors (CNS), 4 Ewing's sarcomas, 6 rhabdomyosarcomas, and 1 neuroblastoma. Weekly irinotecan (50 mg/m^2) and cisplatin (30 mg/m^2) for CNS tumors or irinotecan (10 mg/m^2 [qdx5]x2) and oral temozolamide (100 mg/m^2 dailyx5) for systemic tumors was used. Patients were evaluated for gastrointestinal and hematological toxicity. UGT1A1*28 promoter polymorphism was genotyped by PCR and fragment size analysis. 74 children without oncological, neurological, metabolic or gastrointestinal diseases were analyzed as control population.
Results: Overall, genotype frequencies were 50% TA6/TA6, 37.7% TA6/TA7, 10.3% TA7/TA7, 0.9% TA6/TA8 and 0.9% TA5/TA7. Fifteen of the 32 patients (46.8%) displayed the TA6/TA6 genotype, 15 (46.8%) TA6/TA7, 1 (3%) TA7/TA7 and one patient had a TA8/TA6 variant (3%). The 2 patients with UGT1A1*28 polymorphism did not developed relevant irinotecan-related toxicity and did not had a greater baseline total bilirubin. The only one patient who developed irinotecan related life-threatening gastrointestinal toxicity displayed a TA6/TA7 genotype, had an underlying liver dysfunction and baseline bilirubin of 1.7. Other mutations and SNPs in the promoter and coding regions were analyzed in this particular case with negative results.
Conclusions: Severe toxicity was not increased in pediatric patients with UGT1A1*28 polymorphisms receiving low and repeated doses of irinotecan. Underlying liver disease is more important to predict irinotecan-toxicity in this particular set of patients.