Material and Methods: A bioanalytical assay based on mass spectrometry that allows the simultaneous monitoring of gem as well as its active (dFdCTP) and inactive (dFdU) metabolites was developed in the context of this study. The balance of gem and its active/inactive metabolites, a crucial indicator of efficacy, was evaluated in cell lines after treatment with gem or GSG. Furthermore, since GSG's structure contains a potent agonist peptide of the GnRH-R we investigated whether it could have a central effect through the pituitary by performing testosterone measurements in animals dosed with GSG. In addition, an in vitro colony forming hematotoxicity assay was developed in order to compare the hematoxic potential of GSG and gem.
Results and Discussion: GSG appears to offer a metabolic advantage in comparison to gem which is a beneficial attribute in conditions of gem acquired resistance. The ratios of dFdCTP/dFdU were significantly higher in DU145 cells incubated with GSG versus gem. When GSG was administered acutely in mice, it caused a rapid increase of testosterone concentrations in 2 h suggesting that GSG is a potent GnRH-R agonist. After continuous administration of GSG in mice (21 day protocol), testosterone levels dropped to baseline levels (59±11 vs 1±0.9 ng/mL in plasma and 2010±296 vs 400±197 ng/g in testes). Importantly, in an in vitro hematotoxicity colony forming assay we demonstrated that GSG was less toxic in comparison to gem (IC90 of 17.2 vs 6.8 nM).
Conclusion: The findings of our study along with the targeted delivery nature of the GSG analogue could offer a powerful and unique approach to prostate cancer treatment: a single nontoxic molecule can be used to reach the tumour site selectively, improve the distribution and metabolic properties of the cytotoxic agent (gem), and act as an agonist that ablates testosterone release following repeated dosing. The multiple modes of action of GSG are currently being evaluated by our team in appropriate prostate cancer animal models.
No conflict of interest.

862 Therapeutic efficacy of the paradox-breaking panRAF and SRC drug CCT3833/BAL3833 in KRAS-driven cancer models

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Introduction: KRAS is mutated in ~80% of pancreatic ductal adenocarcinoma (PDAC), ~35% of colorectal cancer (CRC) and ~20% of non-small-cell lung cancer (NSCLC). KRAS remains an intractable drug target and targeting the downstream pathway component is ineffective because feedback mechanisms or parallel pathways provide alternative routes to cell proliferation and/or survival.
Results and Discussion: Here we show that CCT3833, the clinical candidate from our in house panRAF/SRC inhibitor series, is active in KRASmutant PDAC, CRC and NSCLC. We demonstrate that CCT3833 inhibits tumor growth in several KRAS-driven cancers via inhibition of RAF and SRC, eliciting therapeutic efficacy at well-tolerated doses in mouse models of human cancer.
Conclusion: CCT3833 has entered clinical trials (NCT02437227) for BRAF mutant and BRAF inhibitor-resistant melanomas and our data here show that it is also effective in KRAS mutant cancers, potentially providing a new therapeutic option for these patients.
No conflict of interest.

863 Proteomic analysis and interactions network for penile cancer characterization according to HPV status

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Background: Penile Carcinoma (PeCa) corresponds to a mutilating men's malignant tumor. It is more frequent in underprivileged socioeconomic regions (e.g., N, NE Brazil). Five-year survival rates drops dramatically in patients with lymph node metastasis. The major risk factors include poor local hygiene, phimosis, and Human Papilloma Virus (HPV) infection. In the present study, it was our objective to provide molecular information regarding the differential protein pattern in PeCa and look for potential diagnostic, prognostic and/or predictive biomarkers.
Material and Methods: Twenty-four frozen samples were obtained from patients, fourteen cases of penile squamous cell carcinoma, usual type, and ten samples of normal control specimens, postectomy (n = 4) and adjacent normal tissue (n = 6), were submitted to 2D gel-based proteomic. Next, liquid chromatography coupled with a mass spectrometry was used to reveals the protein identity of the differential 2D spots from PeCa HPV positive versus PeCa HPV negative comparison. Furthermore, bioinformatics tools assessed the protein-protein interactions.
Results: Twenty-nine differentially expressed proteins were identified, 22 of them were downregulated and seven were upregulated in HPV-associated PeCa. The interactions network has demonstrated, among others, proteins, which were overexpressed in PeCa HPV negative samples, related to loss of cellular adhesion (e.g., moesin) and gain of oxidative stress defense (e.g., peroxiredoxin-2), that contribute to metastases and cellular survival, respectively. These results suggest that the absence of HPV infection might represents a facilitating factor of tumor progression, that could explains the frequent poorly prognosis associated with HPV negative PeCa.
Conclusions: Our data provide news insights on the characterization of PeCa and may be useful for the development of biomarkers with significant clinical value.
No conflict of interest.

864 RNA sequencing reveals a distinct transcriptomic profile predictive of clinical outcome in stage III colorectal cancer patients treated with adjuvant chemotherapy

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Introduction: 5-year overall survival of stage III colorectal cancer (CRC) patients (pts) treated with standard adjuvant chemotherapy (CHT) (a fluoropyrimidine − FP − with/without oxaliplatin − OHP) is still unsatisfactory and highly variable (42−88%). Although single molecular biomarkers and molecular signatures predictive of adjuvant CHT outcome have been identified in CRC, none of them has been validated. We hypothesized that differences in gene expression may be responsible for the variability of prognosis of stage III CRC pts. The goal of this study was to identify molecular biomarkers predictive of response to FP-based adjuvant CHT in stage III CRC pts. MATERIAL AND Methods: From a large case series of CRC pts who received standard adjuvant CHT (5-fluorouracil/capecitabine with/without OHP) we selected two groups with favorable (no evidence of disease recurrence within 5 yrs from CHT, n = 12) or unfavorable (evidence of disease recurrence within 3 yrs from CHT, n = 12) prognosis, according to stringent eligibility criteria. We analyzed fresh frozen primary CRC explants according to an IRB-approved protocol. Whole transcriptomic sequencing was performed by Ion Proton System (Ion Torrent Thermo Fisher Scientific). After quality control, produced reads were aligned to all transcripts to measure the gene expression levels in each sample. To identify differentially expressed genes between the two groups a statistical analysis was performed using DESeq2 package of R Bioconductor repository. Oncomine database was used to classify genes.
Results: Bioinformatic analysis identified 108 differentially expressed genes between the two groups (p value <0.01, False Discovery Rate <0.01): 104 genes were upregulated and 4 downregulated in the unfavorable prognosis group compared with the favorable prognosis group. Magnitude of fold changes was within −2.5 to +3.5. Among these, 42 genes belonging to the olfactory signaling pathways, were not further considered. Among 66 remaining genes, 19 were pseudogenes, 7 uncharacterized non-coding RNA, 4 were involved in the immune response (e.g. IFNs) and one was a miRNA (MIR548I1). All these genes were upregulated. Further 9 genes were cancer-related (6 upregulated (e.g. CETN1 involved in cell adhesion) and 3 downregulated (e.g. ROR2 involved in Wnt pathway). The remaining genes (n = 26), most of which involved in key cellular processes (e.g. RNA processing: UPF3A upregulated; apoptosis: TMEM150B downregulated) have not yet been associated with cancer and/or cancer prognosis. A validation of differentially expression genes is ongoing by RT-PCR.
Conclusion: Stage III CRC pts with favorable and unfavorable prognosis following adjuvant CHT differs at a transcriptomic level. These findings, after a proper validation in an independent cohort, may have important implications for FP-based adjuvant CHT.
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