its expression levels increased in diverse tumors, especially in carcinomas. Based on that, this study has aimed at evaluating the gene alterations associated with high protein expression levels.
Material and Methods: Immunohistochemistry (IHC) against EGFR was performed in 195 penile carcinoma samples selected from the files of AC Camargo Hospital, Brazil. Cases showing strong and complete membrane staining in more than 10% of the tumor cells were considered positive and were submitted to dual-color fluorescence *in situ* hybridization (FISH). Reactions were carried out using fluorescenet-labeled probes for EGFR locus and chromosome 7 centromere (Zytovision™) in samples over-expressing EGFR, previously selected by immunohistochemistry. Cases showing two signals of each probe were considered non-altered, those showing more than two signals of each probe were considered polissomyc and those showing more EGFR signals compared to centromere signals were considered amplified.
Results: In this series, 67 (49,7%) penile carcinoma samples overexpressed EGFR by IHC and were selected for FISH. Protein overexpression was associated with greater risk of recurrence in univariate analysis (p = 0,031). Regarding FISH, 31 cases (46%) were uninterpretable and, out of 36 valid cases, 22 (61,1%) were non-altered cases, 12 (33,3%) were polissomyc of cromossome 7 and 2 (5,6%) cases presented EGFR amplification.
Conclusions: The high number of uninterpretable cases in FISH seems to be related to technical artifacts due to the high quantity of cytokeratin which may block probe penetration in cytoplasm and nuclei of these tumor cells. Although EGFR overexpression seems to be associated with worse prognosis, neither gene copy number nor polysomy of chromosome 7 is the main cause of this abnormality in penile tumors. Further studies concerning mutational analysis and clinical data are needed and might be useful for identifying patients who may benefit from EGFR-target therapy.

650 POSTER

Multiparameter PET imaging for assessing risk/outcome in sarcoma

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Background: PET molecular imaging with biologically specific agents has the potential to assess phenotypic expression for multiple tumor pathways non-invasively and serially during treatment. To demonstrate feasibility and expression ranges in a clinical population, patients with sarcoma were examined with a multi-agent imaging sequence of [^{11}C]-thymidine (Tdr) to quantify tumor proliferation, [^{18}F]-misonidazole (FMISO) to determine tumor hypoxic volume, [^{11}C]-verapamil (Verap) to assess P-glycoprotein activity, and [^{15}O]-water to quantify tumor blood flow (BF). Images were compared to FDG scans.
Materials and Methods: Ten patients with soft tissue sarcoma were imaged in this pilot study involving neo-adjuvant adriamycin chemotherapy. Studies with all imaging agents were done at baseline and prior to surgery. At mid-chemotherapy, FMISO and Tdr were repeated. Image analysis was by a five-compartment model for Tdr, a one-compartment model with BF normalization for Verap, and determination of tumor hypoxic volume in mL for FMISO. Patient outcome was measured as months from baseline scan to tumor recurrence, metastasis or death.
Results: Image analysis in this pilot study showed heterogeneity in tumor baseline levels for each agent and patient. These did not correlate with FDG uptake or show other associations, suggesting that expression for each of pathway is measuring an independent aspect of tumor biology. Repeat imaging during therapy showed that most patients had reductions in Tdr flux. Some patients showed return of FMISO hypoxia images to normoxic levels. For Verap there was a range of tumor uptake rates normalized to BF at baseline, but all patients showed a decreased Verap uptake in tumor after neo-adjuvant therapy but unchanged background muscle uptake. In this pilot dataset, high FMISO levels at baseline and decreased tumor Verap uptake after adriamycin were associated with the worst patient outcomes. This latter finding may indicate induction of P-gp multi-drug resistance and other genomic changes as a consequence of chronic hypoxia.
Conclusions: Multi-parameter PET imaging for assessment of tumor phenotype that may be predictive of poor outcome in sarcoma is feasible. The pathways imaged for each agent provided unique tumor measurements, which may predict risk for poor response. This pilot study in sarcoma patients supports further evaluation in a multi-center trial and similar protocols in multiple tumor histologies.
Supported by NCI P01 CA42045−21.

651 POSTER DISCUSSION

Pharmacodynamic evaluation of pCDC2 and Wee1 signature as biomarkers of target engagement for the Wee1 tyrosine kinase inhibitor MK-1775

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Background: MK-1775 is a first-in-class inhibitor of Wee1, a kinase that phosphorylates CDC2 to inactivate the CDC2/cyclin B complex thereby regulating the G2/M checkpoint. Biomarkers that inform this class of therapeutics remain to be fully developed, including assays that demonstrate target engagement. Such biomarkers were evaluated in the context of a phase I first-in-man clinical trial of MK-1775.
Material and Methods: MK-1775 was administered p.o. in dose escalating cohorts both as monotherapy and in combination with either gemcitabine, cisplatin, or carboplatin. Pharmacokinetics (PK) and pharmacodynamics (PD) of MK-1775 were evaluated and benchmarked against targets identified in preclinical models. CDC2 phosphorylation (pCDC2) was assessed by immunohistochemistry (IHC) in serial skin biopsies obtained at baseline, 8 hrs or 48 hrs after MK-1775. Wee1 gene expression signature was analyzed by quantitative polymerase chain reaction (qPCR) from plucked hair samples.
Results: To date, 118 pts have been treated with MK-1775 monotherapy or in combination with chemotherapy at doses ranging from 25 mg to 1300 mg to define the maximum tolerated doses (MTD). Statistically significant, dose dependant decreases in pCDC2 were observed in skin biopsies from patients across multiple dose levels of MK-1775. Pooled analysis of pCDC2 by dose across chemotherapy arms in this study suggests that doses $\geqslant$100 mg MK-1775 appear to approach 50% inhibition of pCDC2. In contrast chemotherapy alone resulted in significant upregulation of pCDC2. Supporting evidence of target engagement was also observed with single agent MK-1775 modulation of a Wee1 gene expression signature.
PK increases were approximately dose proportional at all the tested dose levels of MK-1775 both as monotherapy and in combination with chemotherapy. A strong positive correlation between plasma MK-1775 concentrations and MK-1775 dose, and a negative correlation between plasma concentrations of MK-1775 and skin pCDC2 levels was seen.
Conclusions: MK-1775 is a first-in-class Wee1 inhibitor that demonstrates significant target engagement at tolerable doses both as a single agent and in combination with chemotherapy. Clinical activity was observed in combination with gemcitabine, cisplatin or carboplatin.

Chemoprevention

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The p53 pathway as a molecular target for the suppressive chemopreventive action of the histone deacetylase inhibitor tributyrin in rat hepatocarcinogenesis

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Epigenetic mechanisms and pathways involved with regulation of p53 nuclear-cytoplasm translocation have been proposed as molecular targets for carcinogenesis control. Histone deacetylase inhibitors (HDACi) such as tributyrin (TB), a butyric acid prodrug, represent promissing anti-cancer agents. In this study we evaluated the chemopreventive activity of TB when administered to rats during promotion phase of hepatocarcinogenesis. Moreover, epigenetic mechanisms and p53 pathway as molecular targets of TB were also investigated. After being submitted to the resistant hepatocyte model rats received TB (200 mg/100 g b.w.; TB group) or maltodextrin (300 mg/100 g b.w., isocaloric control; CO group) during 5 consecutive weeks. The macroscopic analysis of the livers revealed that compared to CO group, TB group presented smaller ($p < 0.05$) number of nodules. Hepatic GSTP-positive preneoplastic lesions (PNL) morphometry showed that compared to CO group, TB group presented smaller ($p < 0.05$) number, area and % of liver section occupied by persistent PNL (pPNL; sites of