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

J.F. Eary[1], J.M. Link[2], E.U. Conrad[3], M. Muzi[2], F. O'Sullivan[4], K.A. Krohn[2]. *[1]University of Washington, Radiology and Orthopedics, Seattle WA, USA; [2]University of Washington, Radiology, Seattle WA, USA; [3]University of Washington, Orthopedics, Seattle WA, USA; [4]University College Cork, Statistics, Cork, Ireland*

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

J.D. Cheng[1], R. Tibes[2], J.H. Schellens[3], G.I. Shapiro[4], A.C. Pavlick[5], A.M. Oza[6], Y. Xu[7], Y. Song[8], R.L.H. Lam[8], T. Demuth[1]. *[1]Merck & Co., Clinical Oncology, North Wales PA, USA; [2]Translational Genomics Research Institute, Medical Oncology, Scottsdale AZ, USA; [3]Netherlands Cancer Institute, Medical Oncology, Amsterdam, The Netherlands; [4]Dana-Farber Cancer Institute, Medical Oncology, Boston MA, USA; [5]New York University, Medical Oncology, New York NY, USA; [6]Princess Margaret Hospital, Medical Oncology, Toronto Ontario, Canada; [7]Merck & Co., Clinical Pharmacology, West Point PA, USA; [8]Merck & Co., Biostatistics, North Wales PA, USA*

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

652 POSTER

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

F. Salvador Moreno[1], M.A. Horst[1], R. Pierri[1], A. de Conti[1], R. Heidor[1], J.F. Ortega[1], S.A.R. Paiva[2], T.P. Ong[1]. *[1]Faculty of Pharmaceutical Sciences of the University of Sao Paulo, Food and Experimental Nutrition, SAO PAULO, Brazil; [2]Faculdade de Medicina de Botucatu Universidade Estadual Paulista Júlio de Mesquita Filho − UNESP, Departamento de Clínica Médica, Botucatu, Brazil*

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

evolution to hepatocarcinomas) and increased ($p < 0.05$) number and % of remodeling PNL (rPNL; lesions that tend to disappear). Mechanisms associated to modulation of p53 subcellular compartimentalization could be involved with PNL aggressivity. Imunohistochemistry analysis showed that compared to CO group, TB group presented a smaller ($p < 0.05$) frequency of pPNL with aberrant p53 cytoplasmic localization. Furthermore, both pPNL and rPNL of TB group showed smaller ($p < 0.05$) and greater ($p < 0.05$) frequency of cytoplasmic and nuclear immunostaining of CRM1 (an exportin involved with p53 nuclear-cytoplasmic traffic), respectively. TB group also presented increased ($p < 0.05$) hepatic histone H3K9 acetylation specifically in PNL, as well as higher ($p < 0.05$) p21 expression, suggesting that TB acted as an HDACi. Also compared to CO group, TB group displayed increased ($p < 0.05$) hepatic expression of $p33^{ING1A}$, a tumor suppressor gene that plays an inhibitory role in p53 cytoplasmic degradation and that was shown to be downregulated ($p < 0.05$) in this phase of hepatocarcinogenesis. The present data suggest that TB presents suppressive chemopreventive activities of hepatocarcinogenesis by acting as an HDACi. In addition, $p33^{ING1A}$ and CRM1 seem to represent relevant targets for TB modulation of p53 compartimentalization. Financial assistance: FAPESP(2009/53407−5)/CNPq/CAPES.

653 POSTER

Evaluation of strychnine, a plant alkaloid for in vitro anti-angiogenesis, apoptosis and antioxidant potential in MCF-7 cancer cells

S. Saraswati[1], R. Mathur[1], S.S. Agrawal[1]. [1]*Delhi Institute of Pharmaceutical Sciences and Research, Genome Research Laboratory, Delhi, India*

Background: It is widely accepted that the growth of a solid tumor such as breast cancer is dependent on angiogenesis. Mechanism of action of strychnine on VEGF and other proangiogenic factors (TNF-α, IL 12) and about the possible role of VEGF regulation of breast cancer growth has not been elucidated yet. Thus, the study was designed to evaluate *in vitro* anticancer and anti-angiogenic effect of strychnine, an alkaloid isolated from *Strychnos nux-vomica* on human mammary tumor cell line (MCF-7).
Material and Methods: The effect of strychnine on cell death and intracellular targets that affect angiogenesis (VEGF), inflammation (IL-12, TNF-α), apoptosis (caspase-3, -8 & -9) and antioxidant (superoxide dismutase & catalase) were determined by MTT assay, ELISA and enzymatic activity assay. In addition, Anti-VEGF neutralization effect was evaluated alone and in combination with strychnine, to assess whether it could result in augmented anticancer efficacy than the single agent.
Result: Strychnine inhibited growth of cancer cells in a dose and time-dependent manner. Experiments aiming to investigate the anti-angiogenic activity of strychnine against MCF-7, revealed that following the treatment, a dose-dependent decrease ($p < 0.001$) in the levels of VEGF secreted by the cells was recorded. In another set of experiments, strychnine potentiated ($p < 0.001$) the cell death induced by anti-VEGF antibody. VEGF and its receptors are established as major mediators of tumor cell growth and invasiveness; taken together; the results of these experiments suggest that strychnine possesses antiangiogenic activity. Although strychnine appeared to decrease the levels of tumorigenesis factor, TNF-α ($p < 0.05$), it did not alter IL-12 level significantly. The pro-apoptotic effect of strychnine was confirmed by significant ($p < 0.001$) increase in caspases-3 and -9 but not 8 activity. Significant increase in antioxidant enzymes (SOD, catalase) activity was also recorded.
Conclusion: Strychnine acts via multiple albeit specific molecular targets to elicit anti-carcinogenic activity thus might be a candidate for developing multifunctional anti-cancer agent through its inhibitory activity on several aspects of tumor growth and angiogenesis.

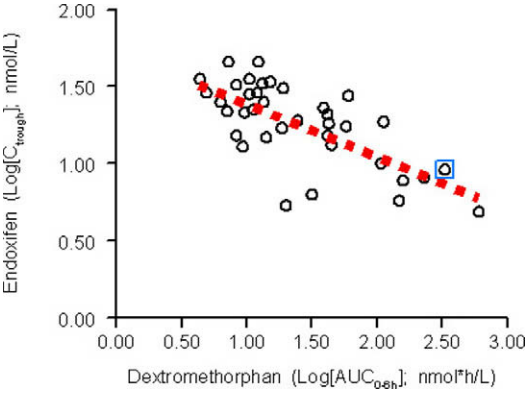
Hormonal agents

654 POSTER

Predictive value of a dextromethorphan phenotyping test for endoxifen exposure

A.J. de Graan[1], S.F. Teunissen[2], F.Y. de Vos[1], W.J. Loos[1], R.H. van Schaik[3], F.E. de Jongh[4], C. Seynaeve[1], J. Verweij[1], J.H. Beijnen[2], R.H. Mathijssen[1]. [1]*Erasmus MC, Medical Oncology, Rotterdam, The Netherlands;* [2]*Slotervaart Hospital, Pharmacy & Pharmacology, Amsterdam, The Netherlands;* [3]*Erasmus MC, Clinical Chemistry, Rotterdam, The Netherlands;* [4]*Ikazia Hospital, Internal Medicine, Rotterdam, The Netherlands*

Background: Tamoxifen, a widely used selective estrogen receptor modulator for the prevention and treatment of breast cancer, is mainly metabolized by CYP2D6 and CYP3A4, to form the active metabolite endoxifen. Unfortunately, variability in toxicity and efficacy of this drug is substantial. Recently, genotyping for CYP2D6 polymorphisms was suggested to individualize tamoxifen therapy, which also translated into a shorter relapse free survival of CYP2D6 poor metabolizers (Schroth *et al*, JAMA 2009). However, other studies fail to confirm this observation. The inter-individual variability in the pharmacokinetics of tamoxifen is not only influenced by genetic profile, but is also affected by lifestyle factors and co-medication, interacting with cytochrome P450 activity. Therefore, we studied the usage of dextromethorphan, a known probe drug for both CYP2D6 and CYP3A4, as a potential phenotyping probe for tamoxifen metabolism by exploring correlations between the pharmacokinetics of dextromethorphan and tamoxifen.
Material and Methods: In this prospective study, 40 women with breast cancer using tamoxifen on steady state received a single dose of 30 mg dextromethorphan orally, 2 hours after oral tamoxifen intake (daily dose 20 mg in adjuvant setting or 40 mg for metastatic disease). Dextromethorphan and metabolites (dextrorphan, 3-methoxymorphinan, and 3-hydroxymorphinan) and tamoxifen and metabolites (4-hydroxy tamoxifen, N-desmethyl tamoxifen and endoxifen) were quantitated by LC-MS/MS. Next, C_{trough} levels, exposures and clearances of all compounds were estimated (WinNonLin), log transformed and subsequently correlated with a two-sided Pearson's correlation test (SPSS).
Results: A highly significant correlation ($r = -0.72$, $p = 0.0001$) was found between the clearances (CL/F) of dextromethorphan (0−6 h) and endoxifen (0−24 h). Also, between the AUC of dextromethorphan (0−6 h) and the daily trough endoxifen concentrations a highly significant correlation was observed ($r = -0.70$, $p = 0.0001$); see figure. In one patient (indicated in the figure by a box) using the strong CYP2D6 inhibitor paroxetine, the expected low endoxifen concentration caused by inhibition of CYP2D6 by paroxetine of this patient was accurately predicted by the dextromethorphan probe.
Conclusions: The dextromethorphan phenotyping probe showed to be an excellent tool to predict endoxifen exposure. This test could aid in future studies on the association of tamoxifen and CYP2D6 genotypes/inhibitors in relation to outcome, and in the further personalization of tamoxifen treatment by optimizing therapeutic benefit and reducing side-effects in individual patients.



655 POSTER

Preliminary report of efficacy of abiraterone acetate in patients with estrogen (ER) or androgen receptor (AR) positive, advanced breast carcinoma resistant to standard endocrine therapies

B. Basu[1], J. Ang[1], M. Blanco[1], M. Dowsett[2], J. Spicer[3], A. Tutt[4], S. Johnston[5], S. Wan[6], J. De Bono[1], C. Swanton[5]. [1]*Royal Marsden Hospital, Drug Development Unit, Sutton, United Kingdom;* [2]*Institute Cancer Research, Academic Department of Biochemistry, Sutton, United Kingdom;* [3]*Guy's and St. Thomas' Foundation Hospital NHS Trust, Department of Medical Oncology, London, United Kingdom;* [4]*Guy's Hospital, Breakthrough Breast Cancer Research Unit, London, United Kingdom;* [5]*Royal Marsden Hospital, Breast Unit, Sutton, United Kingdom;* [6]*Cancer Research UK, Drug Development Office, London, United Kingdom*

Background: Approximately 50% of patients with estrogen receptor positive (ER+) breast cancer display intrinsic resistance to endocrine treatment with the remainder acquiring resistance. Epidemiological, preclinical and clinical data suggest that androgenic steroids upstream of aromatase drive steroid receptor signalling, that is critical to tumour growth. There is also preclinical evidence for the existence of an AR driven, ERα negative, subset of breast cancers transcriptionally similar to ER+ disease. We hypothesized that abiraterone acetate, a cytochrome (CYP) 17 inhibitor