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

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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

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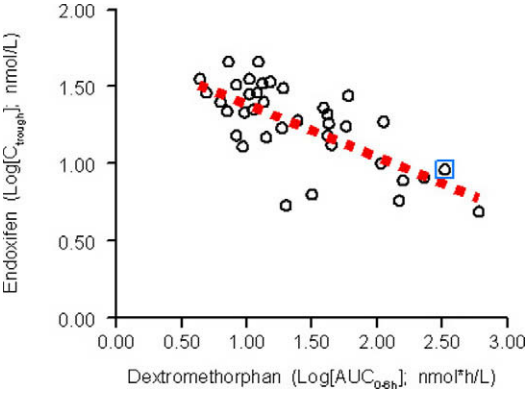
Background: Tamoxifen, a widely used selective estrogen receptor modulator for the prevention and treatment of breast cancer, is mainly metabolized by CYP2D6 and CYP3A4, to form the active metabolite endoxifen. Unfortunately, variability in toxicity and efficacy of this drug is substantial. Recently, 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

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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

that irreversibly inhibits generation of adrenal steroids, would have anti-tumour activity in ERα+ or ERα−/AR+ patients.
Patients and Methods: Post-menopausal women with ERα+ or ERα-/AR+ advanced or metastatic breast cancer who had failed at least 2 lines of hormone therapies were enrolled on a phase I/II study of once daily abiraterone at increasing doses (250 to 2000 mg) in 6-patient cohorts. Abiraterone was initially administered as a single agent to allow endocrine evaluation, with low dose hydrocortisone being commenced for hypermineralocorticoid toxicity.
Results: To date, 18 patients have been treated in this ongoing phase I trial (250, 500 and 1000 mg dose levels). Abiraterone has been well-tolerated in this patient population with the majority of related adverse events (AEs) such as fatigue, nausea, anorexia, dyspnoea, dizziness and hot flushes, classified as Common Toxicity Criteria (CTCAE) grades 1 or 2. Hypokalemia was commonly seen as might be expected from a secondary mineralocorticoid syndrome; in 3 patients, CTCAE grade 3/4 hypokalemia occurred. This was easily and effectively managed with a combination of potassium supplementation, hydrocortisone (20 mg bd) and eplerenone (50–200 mg). CTCAE grade 3 neutropenia and grade 3 reduction in left ventricular ejection fraction were observed in one patient. To date, two patients on 1000 mg daily abiraterone have continued on study beyond 4 months. One of these patients has shown an unconfirmed partial response by RECIST criteria, with a 69% reduction in Ca15.3 tumour marker from baseline, following 4 cycles of treatment.
Conclusions: Abiraterone is well tolerated in advanced breast cancer patients with preliminary evidence of antitumour activity. Mechanism based side-effects eg. hypokalemia are the predominant AEs and are managed expectantly and effectively.

656 POSTER

Suppression of testosterone release by chronic administration of investigational novel metastin analogues in male dogs and monkeys, and in healthy male volunteers

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Background: Metastin/kisspeptin is the cognate endogenous ligand for GPR54 and a key regulator of the gonadotropin-releasing hormone (GnRH) system. We previously reported that chronic administration of novel investigational metastin analogues TAK-448 or TAK-683, strongly suppresses testosterone release in normal male rats with superior activity compared to leuprolide acetate (LA). Here, we describe the effects of chronic administration of TAK-448 or TAK-683 vs LA on testosterone (T) release in intact male dogs and monkeys, as well as a phase I evaluation of TAK-448 in healthy male volunteers.
Materials and Methods: Adult male beagle dogs and cynomolgus monkeys received continuous infusion of TAK-448, TAK-683 or LA subcutaneously (sc) using osmotic ALZET® mini pumps (n = 3 animals/group). Plasma T levels were determined by radioimmunoassay (RIA) in dogs and monkeys; plasma TAK-448 and/or TAK-683 levels in monkeys were measured by liquid chromatography-tandem mass spectrometry (LC/MS/MS). Healthy male volunteers (n = 30) aged ⩾50 yrs received an sc bolus of TAK-448 0.1 mg (Day 1), followed by 13 days' continuous sc infusion (TAK-448: 0.01, 0.1, 0.3, or 1 mg/day or placebo). Blood samples were collected at 6,12,24 hrs post-dose on days 2,4,8,11,14 to determine plasma T levels via RIA; tolerability of TAK-448 was also assessed.

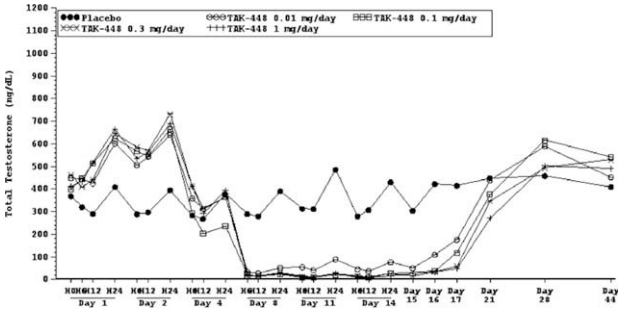

Figure. Mean testosterone concentrations in male volunteers (n = 30)

Results: Chronic administration of TAK-448 or TAK-683 exerted rapid and continuous suppressive effects on T release in a dose-dependent manner in dogs/monkeys. Suppression of T appeared greater with TAK-448 or TAK-683 vs LA, in terms of required dose and time to onset-of-effect. TAK-448 required 3-fold smaller dose than TAK-683 to achieve equivalent testosterone reduction both in dogs and monkeys. TAK-448 plasma concentrations at a given dose were approximately 3-fold higher than those of TAK-683. In healthy volunteers, continuous sc infusion of TAK-448 rapidly decreased T at all doses (Figure). 14/23 volunteers experienced an AE considered to be related to TAK-448.
Conclusions: TAK-448 and TAK-683 showed greater and more rapid reduction in plasma T vs LA in dogs and monkeys, and TAK-448 achieved superior *in vivo* T reducing activity compared with TAK-683. In healthy volunteers, continuous infusion resulted in rapid decreases in T levels. These findings suggest metastin analogues could be novel effective hormonal agents in prostate cancer therapy.

657 POSTER

Anti-tumor growth effect of TAK-683, a metastin analogue, in preclinical androgen-dependent prostate cancer models

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Background: The G-protein-coupled receptor GPR54 and its ligand metastin/kisspeptin are considered to play a pivotal role in the secretion of gonadotropin-releasing hormone (GnRH). We previously showed that chronic administration of metastin analogues, TAK-683 and TAK-448, reduced plasma testosterone levels in male SD rats. In this study, we compared the effects of chronic administration of TAK-683 with a GnRH analogue leuprolide acetate (LA) or orchiectomy (ORX) on testosterone levels and tumor growth in prostate cancer model *in vivo*.
Materials and Methods: (1) Tumor volume and plasma testosterone levels were assessed in male Copenhagen rats bearing subcutaneous R3327-G tumors (n = 7−8). Rats were treated with either ORX, chronic administration of TAK-683 (5.2, 16, or 52 nmol/kg/W) or LA (16, 52, or 700 nmol/kg/W), starting 12 days post-inoculation. (2) Serum prostate-specific antigen (PSA) levels as a biomarker of tumor growth were assessed in male F344/N nude rats bearing JDCaP human prostate cancer tumors, transplanted under the renal capsule (n = 7). Treatment involved either ORX, chronic administration of TAK-683 (10 or 50 nmol/kg/W) or LA (10 or 50 nmol/kg/W), starting 48 days post-tumor transplantation.
Results: In Copenhagen rats bearing R3327-G tumors, TAK-683 rapidly reduced testosterone vs LA. At 10 weeks after initiation of dosing, both TAK-683 (16 nmol/kg/W, p = 0.018) and LA (52 nmol/kg/W, p = 0.023) significantly reduced tumor volume compared with vehicle control. ORX showed a trend (p = 0.072) to reduce tumor volume in this setting. In nude rats bearing JDCaP xenografts, serum PSA levels were reduced below the detectable limit in all rats by Day 7 (ORX), Day 14 (TAK-683), or Day ⩾42 (LA) after treatment initiation; suggesting a more rapid PSA reduction by TAK-683 vs LA in this model. The observed PSA reducing effects associated with TAK-683 may reflect an earlier (vs LA) onset of testosterone reduction by metastin analogue.
Conclusions: TAK-683 exhibited anti-tumor activity in both the R3327-G and JDCaP prostate cancer models. Metastin analogues may have promise as potential new therapeutic agents for prostate cancer.

Immune system

658 POSTER

Prevalence, phenotype and prognostic significance of IL-17-producing cells infiltrating human colorectal cancers

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Background: Lymphocytic infiltration is known to be associated with a favourable prognosis in human colorectal cancer (CRC). In particular, the presence of CD8+ T cells and, unexpectedly, of Foxp3+ regulatory T cells, has been found to be associated with improved patient survival. Recent evidence suggests that IL-17 and T helper (Th) 17 cells might also have an impact on anti-tumour immune responses. We have investigated prevalence, phenotype and prognostic significance of IL-17-producing cells in human CRC.
Material and Methods: IL-17 expression was evaluated by immunohisto-chemistry on a tissue micro-array (TMA) including 1420 cases of primary