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.

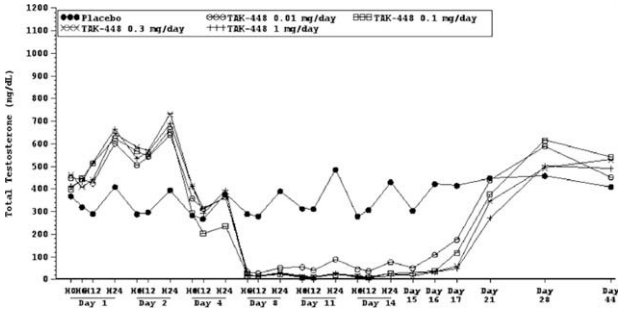

Figure. Mean testosterone concentrations in male volunteers (n = 30)

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

CRC with full clinico-pathological data. Furthermore, gene expression levels were assessed on CRC tissues by quantitative PCR.
Finally, in order to characterize the phenotype of IL-17-positive cells, expression of IL-17, in combination with that of specific surface molecules, was analyzed on freshly excised CRC specimens by flow cytometry.
Results: Frequencies of IL-17-producing cells, as well as IL-17 gene expression levels were significantly increased in tumour tissues as compared to autologous normal mucosa. IL-17-producing cells isolated from clinical specimens were exclusively comprised within the lymphocyte population and expressed CD4, but not CD8, and surprisingly,Foxp3 molecules. Accordingly, mRNA levels of genes encoding for cytokines favouring IL-17 acquisition by Foxp3+ T cells, including IL-6, IL-1beta, TGF-beta and IL-23, were found more elevated in CRC tissues as comparing to corresponding healthy mucosa.
High infiltration by IL-17 producing cells significantly correlated with low T and N stages, and, most importantly, with prolonged survival time in mismatch repair (MMR)- proficient, but not-deficient CRC. Moreover, the simultaneous CRC-infiltration by IL-17+ and Foxp3+ cells was significantly associated with improved survival in both MMR-proficient and -deficient tumours.
Conclusions: Our data suggest that IL-17 produced by tumour-infiltrating either CD4+ or Foxp3+ cells may promote a benign clinical outcome in CRC.

659 POSTER

A novel mechanism of action of platinum-drugs: breaking STAT6-mediated suppression of immune responses against cancer

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Tumor micro-environments feature inhibitory mechanisms that prevent T cells from generating effective immune responses. Therapeutic interventions aimed at disrupting these inhibitory mechanisms have been shown to result in enhanced anti-tumor immunity, but lack direct cytotoxic effects. We investigated the effect of cytotoxic chemotherapeutics on dendritic cell function and on tumor cell immunogenicity.
Using allogeneic and antigen-specific in vitro models, we found that when dendritic cells (major regulators of cellular immune responses) were activated in the presence of platinum-based chemotherapy, their T cell stimulatory capacity was strongly enhanced. Expression of the immune-inhibitory molecule *Programmed death receptor-ligand* 2 (PD-L2) by dendritic cells was markedly reduced upon platinum exposure. The enhanced T cell stimulatory capacity by dendritic cells upon platinum exposure was abrogated in the presence of PD-L2 blocking antibodies. This was also observed when the regulator of PD-L2 expression, *signal transducer and activator of transcription* 6 (STAT6), was knocked down using siRNA.
In addition, we also found in tumor cells that STAT6 is dephosphorylated by platinum compounds, leading to marked downregulation of PD-L2 and resulting in enhanced recognition by tumor-specific T cells.
In line with these in vitro findings, we observed in a retrospective study that patients with STAT6-expressing head and neck cancer displayed significantly enhanced recurrence-free survival upon treatment with cisplatin-based chemoradiation compared to patients with STAT6-negative tumors, demonstrating the clinical relevance of platinum-induced STAT6 modulation.
The PD-L2/STAT6 pathway is known as a major immunosuppressive network that paralyzes the immune system and builds an immune-evasive tumor microenvironment. Our findings demonstrate that platinum compounds not only directly kill tumor cells but also enhance T cell stimulation by dendritic cells. At the same time tumor cells are also sensitized to lysis by cytotoxic T cells through inactivation of this pathway. This novel action of platinum compounds, which are part of the standard treatment of many cancer types, may extend their therapeutic application and provides a rationale for their use in combination with other immunostimulatory compounds to increase the clinical efficacy of cancer treatment.

660 POSTER

Discovery of a novel series of indoleamine 2,3-dioxygenase 2 (IDO2) selective inhibitors for probing IDO2 function in cancer

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Indoleamine 2,3-dioxygenase (IDO or IDO1) is a tryptophan (trp)-catabolizing enzyme implicated in immune suppression during pregnancy, transplantation and in diseases such as infection and cancer. The role of IDO in cancer has been supported by studies with the IDO inhibitor, 1-methyl-tryptophan (1MT), which has been shown to improve the antitumor effects of chemo- or immunotherapeutic agents in tumor models by reversing IDO-mediated T cell suppression. A related protein, IDO2, was recently identified, but its role in cancer is unclear. Studies suggest that the L stereoisomer of 1MT inhibits trp to kynurenine (kyn) conversion by IDO1 *in vitro*, whereas the D isomer of 1MT is more selective for inhibiting IDO2 and also exhibits better activity than L-1MT in murine tumor models. D-1MT has since been advanced into clinical trials for cancer. Although both IDO1 and 2 can be detected in human tumors, emerging data indicate that only murine IDO2 can efficiently convert trp to kyn and that human IDO2 may not do so effectively. Given the conflicting data, a potent IDO2-selective inhibitor would provide a valuable tool to study IDO2 function and explore the potential utility of IDO2 inhibition in cancer therapy. Here we describe a novel series of IDO2 inhibitors. Due to the ineffectiveness of human IDO2 in catabolizing trp, we screened compounds in assays measuring trp to kyn conversion using mouse IDO1 or IDO2-transfected HEK293 cells. Representative lead compounds potently inhibited IDO2-mediated trp conversion and exhibited selectivity (up to 100-fold) over mouse IDO1. These inhibitors exhibited significantly weaker activity against human IDO1 compared to their activity against mouse IDO2. L- and D-1MT were >1000-fold less active against mouse IDO2 in these assays. Using IDO1 and IDO2 selective inhibitors, we find that IDO2 activity is not responsible for trp $\rightarrow$ kyn conversion in either human dendritic cells (DCs) or tumor cells that were induced to express IDO2. Further, in co-cultures of human allogeneic lymphocytes with IDO1/2-positive DCs, IDO2 selective inhibitors did not reverse T cell suppression at doses that significantly inhibit murine IDO2 activity, supporting that IDO2 is not involved in T cell suppression via this particular mechanism. In summary, we have identified a novel series of IDO2 selective inhibitors and our preliminary data suggest that, unlike IDO1, in man IDO2 may lack activity in catabolizing trp and consequently in regulating immune responses.

661 POSTER

A pharmacokinetic, pharmacodynamic and electrocardiographic study of L-MTP-PE in healthy volunteers

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Background: L-MTP-PE (liposomal muramyl tripeptide phosphatidyl-ethanolamine; mifamurtide; MEPACT®) is an activator of monocytes and macrophages. In Europe, L-MTP-PE is indicated for treatment of high-grade resectable non-metastatic osteosarcoma in children, adolescents, and young adults after macroscopically complete surgical resection in combination with postoperative chemotherapy. The recommended mifamurtide dose is 2 mg/m^2. This study aimed to characterize the pharmacokinetics (PK) and pharmacodynamics (PD) of single-dose L-MTP-PE, and evaluate effects on QTc interval in healthy adults.
Materials and Methods: Adults with normal baseline cardiac function and no risk factors for cardiac arrhythmias received a single 4 mg intravenous (IV) infusion of L-MTP-PE over 30 mins. Blood samples were collected pre-dose and serially post-dose for PK (serum MTP-PE) and PD (serum IL-6, TNF-α and CRP) measurements for noncompartmental data analysis. Continuous Holter ECG monitoring was performed over 48 hr, starting 24 hr pre-dose, to analyze changes in QTc (ΔQTc) relative to time-matched baseline values.
Results: 21 adults were enrolled (median age 31 years [range 20−58], 57% male, 71% African American). Maximum serum MTP-PE concentration (mean $\pm$ SD, 15.7$\pm$3.72 nM) was reached at the end of the infusion. Mean $\pm$ SD MTP-PE PK parameters were: clearance 3,409$\pm$928 mL/min (1,747$\pm$390 mL/min/m^2), terminal phase volume of distribution 589$\pm$138 L (305$\pm$69.9 L/m^2), steady-state volume of distribution 406$\pm$120 L