from the known hUPP1 structure. This high resolution structure revealed unequivocally the presence of an intramolecular disulfide bridge that repositions a critical, active-site, phosphate-coordinating arginine residue (Arg100) to a location that does not support catalysis of the enzyme's phosphorolytic activity. Consistent with this structural finding, in vitro comparison of mammalian UPP1 and UPP2 activity reveals a substantial sensitivity to oxidative inactivation in the latter isoform. Together these results demonstrate that UPP2 is intracellularly controlled by a redox mechanism that could be exploited to inactivate the enzyme and therefore limit the activation of Capecitabine in the liver and other organs expressing this UPP isoform.

Toxicology-side effects

449 POSTER

Increased levels of serum creatine kinase caused by skin toxicity of molecularly targeted anticancer agents in phase 1 clinical trials

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Background: New anticancer agents are often associated with skin toxicity, especially those molecularly targeted agents (MTA) targeting EGFR-RAS-RAF-MEK pathway. Creatine kinase (CK) is an enzyme that catalyses the generation of phosphocreatine from ATP and creatine, and is located in tissues such as brain and muscle, human epidermis and hair follicles. We investigated whether rash caused by MTA could increase serum CK levels.
Material and Methods: Retrospectively reviewed 25 Phase 1 Clinical Trials that included CK measurement in the protocol, conducted in the Drug Development Unit (Royal Marsden Hospital) from June 2002 to May 2010. Trials included MTA directed against EGFR/HER2 (41 pt), mTOR (48 pt), VEGFR (31 pt), Src/Abl (26 pt), Aurora kinases (19 pt), vascular disrupting agents (16 pt), BRAF/MEK (12 pt), PARP (6 pt), CDK (6 pt), A5B1 integrin (3 pt) and other targets (34) or viruses (53 pt). Rash was considered as maculo/papular or papulo/pustular drug induced skin toxicity. Fishers exact test was used to calculate differences in incidence or raised CK between groups who developed rash and those who did not. A Kruskal-Wallis one way analysis of variance test was used to determine differences between CK levels and the grade of rash.
Results: 295 patients were included for analysis. Male/female ratio was 55/45 and median age was 59 years. In 49 pt (17%) an elevated serum CK was found after starting treatment. Overall 20% (58/295) patients developed rash. Patients who developed a rash had a higher incidence of raised CK than those who did not 24/58(41%) Vs 25/237 (10%) ($p < 0.001$). There was an association between the grade of rash and CK levels. The rash was grouped into three cohorts; Group A (No Rash: mean CK = 90 IU/L), Group B (G1 rash: Mean CK IU/ = 138), Group C (G2/3 rash: mean CK = 406 IU/L)($p < 0.001$). When the analysis was limited to patients treated with inhibitors of the EGFR/BRAF or MEK ($n = 53$), the incidence of elevated CK was significantly higher in those who had a rash 16/44 than those who did not 0/9, $p = 0.02$. Rash appeared a median of 11 days before the first increased level of CK (range −18–165). No electrolyte disorder or acute renal failure was associated with the increase of CK.
Conclusions: For the first time we have shown that elevated CK is associated with skin rash caused by MTA. It should be studied further as a surrogate for skin toxicity.

450 POSTER

Glucocorticoids frequently induce survival and growth in tumor cells by activation of classical survival and proliferation pathways which should be avoided during anti-cancer therapy

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Background: Glucocorticoids (GCs) such as Dexamethasone (Dex) are widely used in cancer patients. In the treatment of hematopoietic tumors, GCs are given as cytotoxic drugs to treat the tumor. In solid tumors, GCs are given as adjuvants to alleviate adverse effects such as nausea and headache. Nevertheless, in vitro data, animal trials and clinical showed that the use of GCs was accompanied by inverse prognosis, e.g., in patients with lung cancer. This study aimed to systematically characterize non-apoptotic effects of GCs on tumor cell lines and primary tumor cells together with underlying signaling mechanisms.
Materials and Methods: 16 tumor cell lines from different origins were studied together with 139 primary, patient-derived tumor cells obtained from children with acute leukemia before onset of treatment; one cell line was tested in a preclinical subcutaneous nude-mouse model. To study intracellular signaling mechanisms, cells were transfected using siRNA and subjected to functional assays and Western Blots, including phospho-specific antibodies.
Results: GCs enhanced cell growth in 9 out of 16 solid tumor cell lines in vitro. In one cell line, GCs doubled the growth rate of the tumor cells. When cytotoxic drugs were added, only those drugs inducing significant apoptosis were able to inhibit GC-induced tumor cells growth. In contrast, GCs induced significant proliferation even in the presence of cytotoxic drugs with low or absent potential for induction of apoptosis.
When the lung cancer cell line CALU-6 was transplanted subcutaneously into nude mice, Dexamethasone significantly induced the growth rate leading to increased tumor burden.
On 139 fresh primary, patient-derived tumor cells GCs increased survival in 15% of these cells. 20 samples were tested for GC-induced proliferation. In 1/20 samples, both GCs induced cell growth and the formation of new tumor cells as shown by BrdU incorporation. Thus, GCs induced survival and growth in both tumor cell lines and primary tumor cells freshly from patients.
To characterize signalling mechanisms, we found early and sustained phosphorylation of the glucocorticoid receptor followed by its degradation. Transfection of tumor cells with siRNA directed against the glucocorticoid receptor completely inhibited proliferation by Dexamethasone. Dexamethasone activated the pro-survival and proliferation signaling pathways of protein kinase B/Akt and p38 mitogen-activated protein kinase; inhibitors of these pathways abrogated Dex-induced tumor cell growth.
Conclusion: Translated into clinical praxis, our data argue towards a restricted use of GCs during anti-cancer therapy. Whenever possible, GCs might be replaced by other adjuvant drugs. More effort is required to define biomarkers and/or clinical criteria, how GCs can be used safely in cancer patients. Clinical studies are needed to evaluate, whether inhibitors of Akt and/or p38 MAPK can be used to inhibit GC-induced proliferation in cancer patients.

451 POSTER

Cumulative drug toxicity experience of ARQ 197, a selective c-Met inhibitor, and its correlation with pharmacokinetic (PK) and pharmacogenomic (PG) parameters

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Background: ARQ 197 is an oral, selective, non-ATP competitive inhibitor of c-Met, a receptor tyrosine kinase implicated in cancer cell migration, invasion, and proliferation. Since its first clinical trial in 2006, ARQ 197 has been administered to more than 400 cancer patients (pts) either as monotherapy or in combination. A maximum tolerated dose (MTD)/recommended Phase 2 dose (RP2D) of 360 mg administered orally twice daily (BID) as monotherapy was established previously. Here we summarize cumulative safety data from single-agent clinical trials of ARQ 197 and the correlation among toxicity, PK, and PG parameters.
Methods: Data from four single-agent trials were assessed. Adverse events (AEs) were graded using NCI CTCAE v. 3.0 guidelines and coded using MedDRA terminology. Causalities were assessed by study investigators. For PK analyses, plasma concentrations were determined using LC/MS/MS methodology, and PK parameter estimation was performed using noncompartmental analyses. CYP450 2C19 (2C19) genotyping was tested using a FDA-approved Amplichip CYP450 reagent kit manufactured by Roche Diagnostics.
Results: The most common drug-related AEs (≥5%) in the first 175 pts treated with ARQ 197 monotherapy were fatigue (20.0%), nausea (18.3%), vomiting (9.1%), and diarrhoea (6.3%). Dose-limiting toxicities (DLTs) observed in two Phase 1 monotherapy studies ($n = 120$) mainly consisted of myelosuppression events including 1 Grade (G) 4 and 2 G3 febrile neutropenia, 1 G4 neutropenia, 1 G4 leukopenia, and 1 G4 thrombocytopenia. The remaining DLTs included 1 G3 fatigue, 1 G3 palmar-plantar erythrodysaesthesia (hand-foot) syndrome, 2 G3 stomatitis/oral mucosal inflammation, 1 G3 hypokalaemia, 1 G3 vomiting and 1 G3 dehydration. Preliminary PK data suggested a dose-related increase in exposure, although this increase appeared to be less than dose proportional. PK data in pts with DLTs suggested that in general DLTs were associated with drug accumulation after repeated dosing, a phenomenon likely related to reduced clearance. A high degree of inter-pt variability in C_{max} and $AUC_{(0-12)}$ was observed with coefficients of variation of 75% and 90% respectively. Inter-pt variability appeared to be due in part to 2C19 polymorphism status. One pt was identified as a 2C19 poor metabolizer (PM). Drug exposure in the PM was high in comparison to extensive metabolizers, and this pt experienced G4 febrile neutropenia, G3 stomatitis and other G1/2 drug-related AEs.
Conclusions: ARQ 197 demonstrated a manageable safety profile at treatment doses up to and including the MTD/RP2D and were well tolerated

by most pts. DLTs consisted mainly of myelosuppression events and appeared to be associated with drug accumulation following repeated dosing. High inter-pt PK variability appeared partially due to 2C19 polymorphism status. Updated data and PK/PG correlative analyses will be presented during the meeting.

452 POSTER

Novel in vivo imaging of acute chemotherapy-induced vascular toxicity

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Background: Chemotherapy may induce deleterious effects in normal tissues, leading to organ damage. Direct vascular injury is the least characterized side effect. Studies performed using organ cultures have shown that doxorubicin induces apoptosis in endothelial cells, leading to impaired vasodilatory response of arteries. Our aim was to establish a real-time, *in vivo* molecular imaging platform for evaluating the potential vascular toxicity of doxorubicin in mice.
Methods: Ovaries served as a prototype for evaluating toxicity in normal tissues. Femoral vasculature was imaged as a control vessel. Mice ovarian perfusion and femoral blood flow were measured in real time during and after doxorubicin administration (8 mg/kg intravenously). Ovarian blood flow was imaged by ultrasound biomicroscopy (Vevo2100) with microbubbles as a contrast agent, and by fluorescence optical imaging system, equipped with a confocal fiber microscope (Cell-viZio). Femoral blood flow was imaged by pulse wave Doppler ultrasound and by Cell-viZio.
Results: Using microbubbles as a contrast agent revealed a 33% ($P < 0.01$) decrease in ovarian perfusion already 3 minutes after doxorubicin injection. The same was true for the femoral arterial blood flow. Cell-viZio imaging depicted a pattern of vessel injury at around the same time after doxorubicin injection: the wall of the large blood vessels was disintegrated whereas the small blood vessels were damaged with malformed walls. The fluorescence signal displayed by the small vessels was nearly diminished.
Conclusions: We have established a platform of innovative high resolution molecular imaging, suitable for the imaging ovaries and blood vessels, that enables prolonged real-time detection of chemotherapy-induced effects in the same individuals. The acute reduction in ovarian and femoral blood flow and the impairment of the blood vessels wall may represent an acute universal doxorubicin-related vascular toxicity, an initial event in organ injury.

453 POSTER

Population pharmacokinetics (PK) and exposure/response relationships of the receptor tyrosine kinase inhibitor E7080 in phase I studies

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Background: E7080 is a once-daily, orally administered, receptor tyrosine kinase inhibitor with anti-angiogenic and anti-proliferative activity. Anti-tumor activity has been reported in phase I studies. Separate dose-finding Phase 1 studies were conducted with continuous once-daily (qd) and twice daily (bid) administration, with empirical maximum tolerated doses (MTDs) at 25 and 10 mg/day, respectively. To aid selection of the dose schedule for further investigation, exposure–efficacy and −safety relationships were evaluated for E7080.
Methods: A population PK model was developed using data from two E7080 dose-finding studies, investigating continuous qd and bid dosing in 28-day cycles (tumor assessments every 2 cycles). Steady-state individual exposure parameters ($C_{max,ss}$, $AUC_{0-24,ss}$, and $C_{min,ss}$) were derived to correspond with starting dose. Logistic regression was used to model the probability of developing the main adverse events, hypertension and proteinuria, in relation to E7080 exposure and ECOG performance status, and the relationship of response (PR or durable stable disease of $\geqslant$23 weeks [dSD]) with E7080 exposure, ECOG performance status and development of hypertension or proteinuria. Progression-free survival (PFS) was modeled as a function of E7080 exposure and other covariates using Cox regression. Disposition for subjects with PK information is presented in the table.
Results: E7080 PK profile was best described by a two-compartment model with sequential zero- and first-order absorption and first-order elimination. All (log-transformed) E7080 steady-state exposure parameters ($C_{max,ss}$, $C_{min,ss}$ and $AUC_{0-24,ss}$) were significant predictors of the probability of developing $\geqslant$grade-2 hypertension ($p < 0.0001$ for all) and proteinuria ($p < 0.05$ for all), whereas dosing frequency and ECOG performance status were not. PFS correlated with increasing E7080 exposure. The probability of tumor responses increased with increasing $C_{max,ss}$ and $AUC_{0-24,ss}$ ($p < 0.01$ for all), while $C_{min,ss}$ and other covariates were not significantly correlated.

	Schedule	
	QD	BID
Response (evaluable subjects, *n*)		
Total	62	35
PR	7	10
dSD	22	9
Safety (subjects experiencing AE $\geqslant$grade 2, *n*)		
Total	81	41
Hypertension	27	21
Proteinuria	21	15

Conclusions: The probability of response and developing hypertension and proteinuria are correlated with E7080 exposure ($C_{max,ss}$ and $AUC_{0-24,ss}$). Based on the higher exposure achieved at the MTD of the once-daily dosing, 25 mg qd was selected for further clinical development.

454 POSTER

A clinical study to characterize the occurrence of mild-to-moderate diarrhea after administration of neratinib either once daily or twice daily for 14 days

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Background: Neratinib (HKI-272 [NER]) is a potent, low molecular weight, orally administered, irreversible pan-ErbB receptor tyrosine kinase inhibitor in development for the treatment of ErbB2-positive breast cancer. We examined whether NER 120 mg twice daily (BID) could reduce the occurrence of diarrhea relative to NER 240 mg once daily (QD), and characterized the pharmacokinetics after single and multiple doses.
Methods: In a randomized, double-blind, parallel-group study, healthy adults aged 18−50 years were eligible to receive NER 240 QD or NER 120 mg BID with a standard meal for 14 days. Severity of diarrhea was graded. Drug was withdrawn in subjects with diarrhea graded at least moderate. Blood samples were obtained through 24 hours postdose on days 1 and 7 for PK analyses of NER and metabolites. ABCG2 genotyping was done on blood. Subjects who had diarrhea graded at least moderate or finished 14 days of dosing were considered evaluable for the primary endpoint.
Results: 50 subjects (48 M, 2 F) aged 19−49 years (median 29.5) were enrolled. 5 subjects discontinued for moderate adverse events (AEs) other than diarrhea. All 50 subjects developed at least mild diarrhea ~4 days into drug administration. The frequency of moderate diarrhea was comparable between regimens: 11/22 (50% [90% CI: 28−72%]) in the QD group and 17/23 (74% [52−90%]) in the BID group. No severe AEs occurred. ABCG2 genotype did not appear to affect the severity or onset of diarrhea that was graded at least moderate. NER 120 mg BID resulted in lower exposures than did NER 240 mg QD: mean (%coefficient of variation [%CV]) for peak plasma concentration (C_{max}) was 37.4 (29%) ng/mL vs 71.8 (34%) ng/mL on day 1 and 49.6 (32%) ng/mL vs 73.1 (35%) ng/mL on day 7, and mean (%CV) area under the concentration-time curve from 0 to 24 hours (AUC_{0-24h}) was 569 (26%) ng·h/mL vs 891 (28%) ng·h/mL on day 1 and 873 (36%) ng·h/mL vs 1060 (25%) ng·h/mL on day 7. Steady-state exposures (AUC_{0-24h}) of NER following 120 mg BID and 240 mg QD on day 7 in this study appeared similar to that previously reported in patients with cancer following the therapeutic dose of NER 240 mg QD with food (AUC_{0-24h}: 939 ng·h/mL; Wong et al. Clin Cancer Res 2009;15(7):2552−8).
Conclusions: NER 240 mg QD and NER 120 mg BID regimens were comparable for severity, frequency, and onset of diarrhea. The observed steady-state exposures of NER in this study appeared consistent with that reported in patients with cancer following the therapeutic dose of NER 240 mg QD with food.