tumor, followed by lymphatic extension and specific mortality. Percutaneous injection and adjunction of mouse sarcoma proteins are associated with decreased perioperative mortality and increased hematogenous dissemination. This model will be of interest to study oncogenesis and to assess new treatments.

283 POSTER

New preclinical models of metastatic colon cancer: towards bridging the gap between bench and bedside therapeutic outcomes

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Background: In 2007, the small molecule receptor tyrosine kinase inhibitor sorafenib was FDA-approved for the treatment of advanced hepatocellular carcinoma (HCC). In contrast, little is known about the efficacy of adjuvant sorafenib for early stage HCC. As HCC is an intrinsically chemotherapy-resistant malignancy and as most patients suffering from HCC have reduced liver function thus not tolerating conventional chemotherapy, the impact of sorafenib-based regimens for this malignancy in earlier stages of disease progression may be important as a means to improve the clinical management of this highly lethal malignancy.

Methods: The human HCC cell line Hep3B was transfected with a hCG.pIRES vector and β-hCG expressing variants were obtained by puromycin selection. Analysis of β-hCG expression enables in vivo monitoring of relative tumor burden. Cells were orthotopically injected into the right lower lobe of the liver in a total of 50 CB17 SCID mice. Control vehicle or Sorafenib (15 or 30 mg/kg) was administered by daily gavage starting either immediately after wound healing (day 7) before circulating β-hCG was detected or after evidence of established tumors as determined by β-hCG analysis (days 14−21). Monitoring was carried out by analysis of β-hCG secretion, survival analysis and endpoint necropsy. Tissue was preserved for immunohistochemistry.

Results: All control animals needed to be sacrificed within 65 days due to primary tumor burden and ascites. No animal of this group showed local or distant metastasis. In contrast, all four dosing regimens of sorafenib significantly inhibited primary tumor growth, inhibited the formation of ascites and prolonged overall survival. However, possibly as a result of the prolonged survival, 56% (19/34) of the animals treated with sorafenib developed local, mesenteric and omental lymph node metastasis and 21% (7/34) developed secondary liver metastases. Metastatic cell lines were re-adapted to cell culture for future analysis.

Conclusions: Sorafenib prolongs survival and successfully controls primary tumor growth in an orthotopic model approximating early-stage HCC. However, it does not inhibit the development of secondary liver metastases or local and distant lymph node metastasis. The nature of these secondary growths will be addressed in follow-up experiments. Future analyses will also include adjuvant therapy of microscopic metastases following resection of the primary. Furthermore, experiments will be repeated using MHCC97-H as a second HCC cell line. Results of this ongoing study will be presented at the conference.

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Establishment and characterization of individualized patient-derived low passage human tumor models: Development, validation and evaluation for clinical correlation analysis

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The majority of patients with advanced solid tumors die from an absence of effective therapy. This is despite three decades of drug development using the current human tumor model screening platform derived from ex-vivo passaged cancer cells. While cell-derived models are useful for high throughput lead candidate identification and early stage preclinical single agent and combination optimization, these lines represent a cross section of different tumors but physically represent only a fraction of genetic and biological abnormalities which are now known to play a role in the pathogenesis and progression of human cancers. Patient-derived tumor models passaged only a few times in vivo retain physical and molecular characteristics of human cancer and may prove essential in identifying disease biomarkers and drug targets in later stage development.

To address this unmet need, we have implanted tissue from donor patients into immunocompromised mice to develop low passage models more representative of human cancer. To date one hundred seventy-four samples have been implanted over thirteen tumor types with a model development success rate of approximately sixty percent:

Tumor Type	No. of Models	%Total
Brain	8	5%
Breast	16	9%
Gastrointestinal (esophagus, colon)	(3, 18)	12%
Genitourinary (Renal, Bladder)	(5, 1)	4%
Head & Neck	16	9%
Hematopoietic	10	6%
Lung	16	9%
Neuroendocrine	3	2%
Ovary	32	18%
Prostate	2	1%
Pancreas	6	3%
Sarcoma	15	9%
Skin (melanoma, vulva)	(22, 1)	13%

Molecular and clinical outcome data was collected from donor patients and compared with data obtained from model characterization with excellent correlation. Several low passage models including colorectal, lung and ovary cancers and melanoma were screened for sensitivity to relevant standards of care with results correlating to clinical response 70% of the time. Taken together, these results demonstrate the ability to generate improved models of human cancer which retain molecular and clinical characteristics which may be used for patient drug sensitivity screening and improved oncology drug development.

285 POSTER

Constitutive overexpression of Id-1 in mammary glands of transgenic mice results in precocious and increased formation of terminal end buds, enhanced alveologenesis, delayed involution

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Inhibitor of differentiation-1 (Id-1) has been shown to play an essential role in cell proliferation, invasion, migration and anti-apoptosis. However, the effect of Id-1 in mammary gland development in in vivo remains unknown. Here, we analyzed the effect of Id-1 overexpression in mammary gland development of MMTV-Id-1 transgenic mice during virgin, pregnancy and involution. In virgin mice, overexpression of Id-1 led to precocious development and delayed regression of terminal end buds (TEBs) compared with wild type mice. The number of BrdUpositive cells, an indicator of cell proliferation, and the expression of Wnt signaling molecules, β-catenin and cyclin D1, which regulate ductal extension and TEB formation in virgin, were statistically higher in Id-1 transgenic mice than in wild type mice. Id-1 also had an effect on the formation and proliferation of lobuloalveolar structures during early and mid-pregnancy. The Id-1 transgenic mice had more lobulated and prominent alveolar budding than wild type mice and had significantly greater counts of lobuloalveolar structures in early pregnancy. The expression of BrdU, β-catenin and cyclin D1 was also predominantly increased in Id-1 transgenic mice. Moreover, Id-1 transgenic mice showed delayed involution in mammary gland development. Id-1 regulated the expression levels of anti-apoptotic Bcl-2 and pro-apoptotic Bax, and resulted in delay of apoptotic peak during postlactational involution. Taken together, our results suggest that Id-1 plays a pivotal role in mammary gland development through Wnt signaling-mediated acceleration of precocity and alveologenesis and Bcl-2 family members-mediated delay of involution.

Clinical methodology

286 POSTER

Tailored dosing of tasisulam-sodium (LY573636-sodium) to reduce hematologic toxicity and improve therapeutic index

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Background: Tasisulam (LY573636) is an acylsulfonamide with novel anti-cancer activity across a broad range of cell lines that induces apoptosis by a mitochondrial-mediated mechanism.

Material and Methods: In a phase I study and four subsequent phase 2 studies, tasisulam was administered by a 2-hour infusion using a lean body weight-based formula (calculated from a patient's gender, height and weight) that significantly reduced the inter-individual variability in C_{max} of tasisulam compared to flat dosing. Key observations during the first human dose study JZAA were that the dose limiting toxicity of tasisulam was bone marrow suppression, particularly thrombocytopenia, and that tasisulam had a long terminal half life (approximately 11 days), consistent with high albumin binding (99.7% to 99.9%). Based on the pharmacokinetic exposures of cancer patients in Phase 2 studies who achieved RECIST-defined response, an area under the curve (AUC) above a certain threshold was calculated for all patients and used to define a therapeutic range that minimized the risk of Grade 4 (G4) hematological toxicity (defined as developing either G4 thrombocytopenia or neutropenia in either Cycle 1 or Cycle 2).

Results: The risk in patients above this therapeutic range was ~50% whereas the incidence in patients within the hypothesized therapeutic range was ~15% or less, which was considered acceptable in the metastatic cancer setting. These findings have led to refinement of the current dosing algorithm to include patient predose albumin and lean body weight.

Conclusions: The goal of this tailored dosing paradigm for tasisulam is reducing the individual risk of Grade 4 hematological toxicity, while preserving the hypothesized therapeutic range. This dosing regimen is novel in cancer medicine to our knowledge and may be more widely applicable to other highly protein-bound cancer drugs. A dose and schedule using this approach have been defined and will be implemented in ongoing studies, including a Phase 3 study in second-line metastatic melanoma.

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Development and validation of a real-time multiplex PCR assay for the simultaneous quantification of *CK-19, MAGE-A3, HER-2* and *PBGD* in circulating tumor cells of breast cancer patients

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Introduction: Circulating tumor cells (CTCs) represent an important biological link in the spread of breast cancer from primary to metastatic disease. CTCs have already been established as strong predictors of prognosis in patients with metastatic breast cancer. The aim of our work was to develop a quantitative real-time multiplex PCR assay for *CK-19, MAGE-A3, HER-2* and *PBGD* and validate its performance in CTCs of early and metastatic breast cancer patients.

Materials and Methods: A tetraplex quantitative real time assay for *CK-19, MAGE-A3, HER-2* and *PBGD* was developed in the LightCycler 2.0 platform (Roche, Diagnostics). Specificity and sensitivity experiments were performed using the SKBR-3 cancer cell line. The method was applied in 66 patients with early breast cancer before the administration of adjuvant chemotherapy, 26 patients with verified metastasis and 16 female healthy volunteers. Peripheral blood (20 mL in EDTA) was obtained and after density gradient centrifugation, immunomagnetic Ber-EP4 coated capture beads were used to enrich for epithelial cells, keeping for each sample two fractions: the CTC and corresponding PBMC fraction. Messenger RNA was isolated from enriched epithelial cells using oligo $(dT)_{25}$ coated magnetic beads. After cDNA synthesis the expression of *CK-19, MAGE-A3, HER-2* and *PBGD* was tested, in both fractions.

Results: The analytical performance of the method was evaluated in SKBR-3 tumor cell line in respect to analytical sensitivity and specificity. Cross reaction studies, performed for each gene target in the presence of all other targets have shown a very high specificity for each analyte. RNA quality in all samples was evaluated by *PBGD* gene expression. We found 28/66 (42.4%) patients with early breast cancer positive for *CK-19*, 14/66 (21.2%) for *MAGE-A3* and 9/66 positive for *HER-2* (13.6%). In patients with verified metastasis we found 14/26 patients positive for *CK-19* (53.8%), 4/26 for *MAGE-A3* (15.4%), 5/26 patients positive for *HER-2* (19.2%). All healthy volunteers were found negative in their CTCs fractions for *CK-19* (0%), for *MAGE A3* (0%) and *HER-2* (0%).

Conclusions: We report for the first time a highly specific, reproducible and sensitive quantitative multiplex real-time PCR assay for the simultaneous detection of *CK-19, MAGE-A3, HER-2* and *PBGD*. The expression of these genes in CTCs will be further examined in a larger number of patients and results will be correlated with their clinical outcome.

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Pharmacokinetics and pharmacodynamics of the novel proteasome inhibitor CEP-18770 during a phase I trial in patients with solid tumor, non-Hodgkin lymphoma or multiple myeloma

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Background: CEP-18770 is a new proteasome inhibitor with boronic acid based structure under evaluation in phase I study in patients with solid tumors, non- Hodgkin lymphoma (NHL), or multiple myeloma (MM). It is an active inhibitor of the chymotrypsin-like activity of mammalian proteasome with IC_{50} of 3.0 nM. Its antitumor activity was demonstrated in mouse models of several tumors, particularly against the human multiple myeloma RPMI 8226 after repeated i.v. or oral administrations. It has also shown potent induction of apoptosis in human MM cell lines and in patient-derived cells.

Aims: to assess the pharmacokinetics (PK) and pharmacodynamics (PD) of CEP-18770 in patients with solid tumors or NHL participating to a phase I, dose-escalating study designed to determine the Maximum Tolerated Dose (MTD) of CEP-18770.

Patients and Methods: CEP-18770, supplied by Cephalon, was administered as i.v. bolus on days 1, 4, 8 and 11 of a 21-day cycle. The dose escalation followed a modified Fibonacci sequence starting from 0.1 mg/m^2. Blood samples were collected at pre-dose, after the administration on day 1 (from 5 min to 48 h), at pre-dose on days 4, 8, 11 and pre-dose on day 1 of the 2nd cycle. The plasma concentration of CEP-18770 was measured by a validated method based on liquid-chromatography coupled with tandem mass spectrometry; the method is highly sensitive (LOQ 0.2 ng/mL) with a precision CV% $\leqslant$8.3% and an accuracy range 93.8–107.7%. The % inhibition of the chymotryptic activity of 20S proteasome was assessed in blood by a fluorogenic kinetic assay.

Results: Dose was escalated up to 1.8 mg/m^2 through 8 dose levels in a total of 40 patients, with MTD defined at 1.5 mg/m^2. PK evaluation performed on day 1, showed linear PK of CEP-18770 over the dose range evaluated and acceptable inter-patient variability. In 16 patients at the MTD, mean±sd values of Cmax, AUCexp and terminal half-life were 366.0±117.4 ng/mL, 1048.8±536.5 ng/mL*h and 60.3±28.0 h, respectively. CEP-18770 was detectable up to 72 h at levels twice the LOQ. CEP-18770 inhibited 20S proteasome activity with effect increasing with dose and achieving maximal inhibition of 55±9% in patients at 1.8 mg/m^2 and of 45±12% in patients at MTD.

Conclusions: A PK and PD evaluation performed during the phase I clinical trial of the novel proteasome inhibitor CEP-18770 indicates that the drug achieves plasma levels that are able to inhibit proteasome activity.

Combinatorial chemistry, drug screening and synthesis

289 POSTER

3D culture systems for cancer drug evaluation

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Background: 2-dimensional (2D) monolayer cultures are generally used for testing anti cancer agents in vitro. However, 3D cultures (e.g. multicellular tumour spheroids; MTS) are of increasing interest in such applications. These systems mimic more faithfully the in vivo microenvironment in terms of cell–cell interactions, matrix deposition, nutrient and oxygen gradients and thus better recapitulate features of micrometastases or intervascular tumour regions. The aim of this study was to establish MTS-based growth and functional assays for target validation and drug screening.

Methods: A variety of techniques were explored to generate MTS and a standardised method established. MTS were characterised in terms of: (i) growth kinetics, (ii) cell viability, (iii) protein expression, (iv) migration on extracellular matrix proteins and endothelial monolayers, (v) invasion into MatrigelTM and (vi) co-culture with embryoid bodies to model invasion/angiogenesis. All assays were in microplate format (96 well) except migration (48 well). Highly malignant human glioblastoma (U87MG) and breast carcinoma (MDA MB 231) cells were selected and treated with the HSP90 inhibitor 17-AAG to exemplify assays utility.

Results: Our standardised MTS microplate method generates a single spheroid per well. MTS are highly reproducible in size and easy to handle. Acquisition of sequential images of MTS cultured for up to 14 days for