combined effect of CF101 and 5-FU on the growth of colon carcinoma and the molecular mechanism involved.
Materials and Methods: HCT-116 human colon carcinoma cells were cultured in vitro in the presence of 5-FU in combination with CF101. MTT and colony formation assays were used to monitor proliferation and western blott analysis to evaluate protein expression level of cell growth regulatory proteins. *In vivo* studies included xenografts of the colon carcinoma cells in nude mice treated with the combined drugs.
Results: In HCT-116 human colon carcinoma cells, a combined treatment of 5-FU and CF101 enhanced the cytotoxic effect of 5-FU in the MTT and colony formation assay and in the xenograft model. Western blot analysis of protein extracts derived from HCT-116 cells treated with 5-FU + CF101 revealed down-regulation of PKB/AKT, NF-kB and cyclin D1 and up-regulation of caspase-3 expression level in comparison to cells treated with 5-FU alone. Similar profile was observed in protein extracts derived from tumor lesions excised from mice treated with the combined therapy or 5-FU alone. In the group of mice treated with 5-FU + CF101, myelotoxicity was prevented and was evidenced by normal levels of white blood cells (WBC) and neutrophils.
Conclusions: These results support the notion that CF101 acts *in vitro* and *in vivo* via a similar molecular mechanism to potentiate the cytotoxic effect of 5-FU thus preventing drug resistance. The myeloprotective effect of CF101 grants the molecule an added value and suggests its development as a supportive treatment to 5-FU.

390 POSTER

A phase I trial of weekly AP23573, a novel mTOR inhibitor, in patients with advanced or refractory malignancies: a pharmacokinetic (PK) and pharmacodynamic (PD) analysis

A.A. Desai[1], L. Janisch[1], L. Berk[2], H.L. Knowles[2], V.M. Rivera[2], H.L. Kindler[1], W.M. Stadler[1], C.L. Bedrosian[2], M.J. Ratain[1].
[1]The University of Chicago, Medicine, Chicago, USA; [2]ARIAD Pharmaceuticals, Inc., Cambridge, USA

Background: AP23573 is a non-prodrug rapamycin analog that potently inhibits mTOR, a downstream effector of the PI3K/Akt and nutrient pathways. AP23573 demonstrated powerful antiproliferative activity in vitro and antitumor activity in mouse xenograft studies.
Materials and Methods: This trial utilizes an accelerated dose escalation scheme to determine safety and tolerability, establish a maximum tolerated dose (MTD), and characterize the PK and PD of AP23573. AP23573 is administered as 30-minute IV infusion wkly on 4-week cycles, and tumor responses are evaluated every 2 cycles. Potential PD markers are assessed using western blot analysis of peripheral blood mononuclear cell lysates.
Results: To date, 17 pts (11M/6F), median age 62 years (range 27–79 years), have received a total of 34 cycles in 6 dose level cohorts ranging from 6.25 to 100 mg. Cycle 1 side effects were considered for determining dose limiting toxicity (DLT). Two pts experienced DLT of reversible grade (gr) 3 oral mucositis at the 100 mg dose level, which, by definition, exceeds the MTD. Additional reversible non-hematologic side effects for first cycle included gr 1–2 anorexia, diarrhea, fatigue, rash, and mucositis. Two pts had reversible gr 1 thrombocytopenia, and one pt had gr 2 anemia. PK analyses (doses 6.25 to 25 mg) suggest a median estimated AP23573 half-life of 49 hours [hrs] (range 31 to 55 hrs). The mean $\pm$ standard deviation of AP23573 clearance is 2.8$\pm$1.2 liters/hr, which is independent of both dose and pt body surface area. Also, there is minimal intra-individual variability between Days 1 and 8 post-dose AP23573 blood levels. PD analyses (doses 6.25 to 100 mg) show significant inhibition of mTOR activity until the next wkly dose as measured by decrease in phosphorylated 4EBP1 levels. Two of 12 evaluable pts exhibited stable disease for $\geqslant$ 4 months; one pt each with metastatic cholangiocarcinoma and medullary thyroid cancer.
Conclusions: AP23573 can be administered safely using this schedule. There is evidence of straightforward pharmacokinetics, substantial PD effects, and early evidence of antitumor activity. Given the promising findings, pt enrollment and dosing continue at the 50 and 75 mg dose levels to identify MTD and maximum effective AP23573 dose based on PK/PD relationships. If substantial inter-individual PK variability is observed, the trial is prospectively designed to evaluate the relevance of genetic variants in candidate drug metabolism genes.

391 POSTER

Proof of principle trial uncovers cyclin D1 as a marker of response to erlotinib

W.J. Petty[1], K.H. Dragnev[2], V.A. Memoli[2], T.H. Davis[2], L.D. Lewis[2], W.C. Nugent[3], J.R. Rigas[2], E. Dmitrovsky[1]. *[1]Dartmouth Medical School, Department of Pharmacology and Toxicology, Hanover, NH, USA; [2]Dartmouth-Hitchcock Medical Center, Department of Medicine, Lebanon, NH, USA; [3]Dartmouth-Hitchcock Medical Center, Department of Surgery, Lebanon, NH, USA*

Background: Active targeted agents for lung cancer therapy exist. Mechanisms engaged during clinical responses to these agents need to be determined. We reported that cyclin D1 is frequently overexpressed during lung carcinogenesis. We have highlighted the proteasomal degradation of cyclin D1 as important for therapeutic or chemopreventive response to certain targeted agents. To uncover mechanisms for responses to an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI), erlotinib, or the rexinoid, bexarotene, we performed in vitro studies and conducted two proof of principle clinical trials.
Materials and Methods: Human bronchial epithelial (HBE) cells were treated with erlotinib or bexarotene at varying dosages and effects on growth, cell cycle distribution, and cell cycle regulatory proteins were determined using established techniques. We then sought to validate candidate biomarkers through conduct of proof of principle trials for each of these single agents. Patients enrolled onto these trials had a pre-treatment biopsy followed by a short course of treatment with either bexarotene or erlotinib. On the final day of drug administration, patients underwent post-treatment tumor biopsy or resection. Detailed plasma pharmacokinetics were performed and tumor tissue drug concentrations were also measured. Biomarker responses were assessed by comparing immunohistochemical expression of pre- and post-treatment biopsies.
Results: Erlotinib induced dose-dependent growth suppression of HBE cells through induction of G1 arrest. Immunoblot analyses confirmed that cyclin D1 was preferentially repressed before onset of G1 arrest. These responses were also observed in erlotinib-sensitive lung cancer cell lines. Bexarotene induced repression of cyclin D1 more than cyclin D3 without appreciable changes in other examined cell cycle regulatory proteins. During these clinical trials, both agents were well tolerated with no treatment-related deaths or serious adverse events. Two patients had evidence of pathologic response with the appearance of necrosis in post-treatment versus pre-treatment biopsies. These responding cases achieved appreciably greater tumor tissue erlotinib levels than did non-responding cases. Cyclin D1 was substantially repressed in tumors of responding cases. Notably, no change in cyclin D1 immunostaining was observed in non-responding cases. Accrual to the bexarotene trial has been completed. Bexarotene tumor tissue concentrations showed appreciable tumor penetration. Pathologic and biomarker responses are under study.
Conclusions: Cyclin D1 is repressed in tumors during pre-clinical and clinical responses to erlotinib. Tissue erlotinib concentrations are substantially higher in these responding as compared to non-responding cases. The proof of principle clinical trial design is useful to validate molecular targets. This study has highlighted cyclin D1 as a marker of response to an EGFR-TKI.

392 POSTER

Targeting janus kinase 3 with JANEX-1 to attenuate the severity of acute graft-versus-host disease across the major histocompatibility barrier in mice

M. Cetkovic-Cvrlje[2], B. Waurzyniak[3], X.P. Liu[4], F. Uckun[1]. *[1]Parker Hughes Cancer Center, Department of Immunology, St. Paul, USA; [2]Paradigm Pharmaceuticals, LLC, Department of Immunology, St. Paul, USA; [3]Paradigm Pharmaceuticals, LLC, Department of Pathology, St. Paul, USA; [4]Paradigm Pharmaceuticals, LLC, Department of Chemistry, St. Paul, USA*

GVHD significantly limits the success of allogeneic bone marrow transplantation (BMT) for patients with leukemia. In an attempt aimed at preventing the development of acute graft-versus-host disease (GVHD) in lethally irradiated C57BL/6 (H-2b) recipient mice transplanted with bone marrow/splenocyte grafts from MHC disparate BALB/c mice (H-2d), recipient mice were treated with the rationally designed JAK3 inhibitor JANEX-1 [4-(4′-hydroxyphenyl)-amino-6,7-dimethoxyquinazoline] every day from the day of BMT until the end of the 85-day observation period. TBI-conditioned, vehicle-treated control C57BL/6 mice (N=38) receiving bone marrow/splenocyte grafts from BALB/c mice survived the acute TBI toxicity, but they all developed histologically confirmed severe multi-organ GVHD and died with a median survival of 37 days. JANEX-1 treatment prolonged the median survival of the BMT recipients to 56 days. The probability of survival at 2 months post-BMT was 11$\pm$5% for vehicle-treated control mice (N=38) and 41$\pm$9% for mice treated with JANEX-1 (N=32) ($P<0.0001$). Notably,

the combination regimen JANEX-1 plus the standard anti-GVHD drug Methotrexate (MTX) was more effective than JANEX-1 alone or MTX alone. More than half of the C57BL/6 recipients receiving this most effective GVHD prophylaxis remained alive and healthy throughout the 85-day observation period with a cumulative survival probability of 70±10%. Taken together, these results indicate that targeting JAK3 in alloreactive donor lymphocytes with a chemical inhibitor such as JANEX-1 may attenuate the severity of GVHD after BMT.

393 POSTER

A phase II study of OSI-774 given in combination with carboplatin in patients with recurrent ovarian cancer (NCIC IND.149)

H. Hirte[1], K. Swenerton[2], A. Oza[3], S. Ellard[4], M. Plante[5], R. Grimshaw[6], M. Carey[7], L. Seymour[8], B. Fisher[8], W. Walsh[8]. [1]*Juravinski Cancer Centre, Medical Oncology, Hamilton, Canada;* [2]*BC Cancer Agency, Medical Oncology, Vancouver, Canada;* [3]*Princess Margaret Hospital, Medical Oncology, Toronto, Canada;* [4]*BC Cancer Agency, Medical Oncology, Kelowna, Canada;* [5]*CHUM – Pavillon Notre Dame, Gynecologic Oncology, Montreal, Canada;* [6]*Nova Scotia Cancer Centre, Gynecologic Oncology, Halifax, Canada;* [7]*London Regional Cancer Centre, Gynecologic Oncology, London, Canada;* [8]*NCIC Clinical Trials Group, Kingston, Canada*

Approximately 50% of ovarian cancers have elevated levels of epidermal growth factor receptor (EGFR) and this overexpression is correlated with a poor prognosis. Preclinical evidence suggests that EGFR tyrosine kinase inhibitors (TKI's), such as OSI-774, may potentiate the antitumour effects of cytotoxic agents, including carboplatin. EGFR TKI's may also beneficially modulate drug resistance, and EGFR may be causal in the development of resistance to platinum. Blocking the EGFR could thus potentially reverse drug resistance.

This study was designed to determine the efficacy of the addition of OSI-774 to carboplatin in patients with recurrent ovarian cancer. Patients enrolled on this study had recurrent ovarian cancer with measurable disease. They may have had up to 2 prior chemotherapy regimens, one of which must have contained platinum, and they must have responded to prior platinum therapy. The patients were treated with OSI-774 150 mg daily on a continuous dosing schedule, and carboplatin at an AUC of 5 every 21 days. Patients were stratified by platinum sensitivity (>6 months from last dose of platinum agent to relapse). The primary objective of the study was to assess the response rate of OSI-774 in patients with recurrent ovarian cancer who were receiving carboplatin.

Fifty patients with recurrent ovarian cancer entered the study, 33 in the platinum sensitive arm and 17 in the platinum-resistant arm. Of patients evaluable for response, there were 16 partial responses (PR) of 24 evaluable for response (67% response rate (RR)) in the platinum-sensitive arm, and 1 PR of 14 evaluable for response (7% RR) (Table 1). The hematologic and biochemical toxicities were those expected with carboplatin and OSI-774. Of 21 patients who had tumour samples tested for EGFR status, 17 (81%) tested positive. A review of the responses is ongoing and the results will be presented.

Table 1. Response rate

Response	Platinum Sensitive (n=24)	Platinum resistant (n=14)
CR	1* (4%)	0
PR	15 (63%)	1
SD	8	10
PD	0	3
Overall RR	67% (95% CI 45–84%)	7% (95% CI 0.2–33.8%)

*CA-125 remains elevated.

The combination of OSI-774 and carboplatin was active in patients with platinum sensitive disease, but not in platinum resistant disease. The toxicities seen were those expected with carboplatin and OSI-774. The majority of patients had EGFR+ tumours. To determine the effect of OSI-774 in enhancing the efficacy of carboplatin in patients with platinum-sensitive disease, a randomized controlled combination study is under development.

394 POSTER

Inhibition of Kit-dependent tumor growth by OSI-930, a novel selective tyrosine kinase inhibitor

A.J. Garton[1], L. Castaldo[1], J. Kahler[1], M. O'Connor[1], S. Nitzan[1], S. Winski[2], M. Srebernak[2], J. Keily[3], A.-H. Li[1], G. Wynne[3]. [1]*OSI Pharmaceuticals, Inc., Farmingdale, NY, USA;* [2]*OSI Pharmaceuticals, Inc., Boulder, CO, USA;* [3]*OSI Pharmaceuticals, Inc., Oxford, UK*

The receptor tyrosine kinase Kit has been implicated in multiple human tumor types. These include GIST and mast cell leukemia in which mutant, constitutively active forms of Kit are thought to play a major role in tumor progression. In addition, in small-cell lung cancer (SCLC) co-expression of wild-type Kit and its ligand (SCF) is thought to provide continuous growth and survival signals that may contribute to tumor growth. We have recently identified a series of 2,3-substituted thiophenes with potent inhibitory activity against Kit, as well as the related tyrosine kinases KDR and PDGFRa/b, and OSI-930 has emerged from this series of compounds as an IND-track clinical candidate. In cell-based assays OSI-930 has been found to inhibit with low nanoMolar potency both the wild-type enzyme found in SCLC cells (H526) and the exon 11 juxtamembrane mutant form of Kit (V560G) found in HMC-1 cells, which is similar to the most common type of mutation identified in tumors from GIST patients. Furthermore, OSI-930 inhibits the growth of both HMC-1 and H526 cell lines in vitro and potently induces apoptosis in HMC-1 cells, where the mutant Kit enzyme appears to provide an essential cell survival signal. These in vitro effects correlated with potent effects of OSI-930 on the phosphorylation state of the downstream signaling effectors Erk1/2, Akt and S6, which are established mediators of cell growth and survival pathways. Following oral dosing of OSI-930 in mice, a reduction in the level of tyrosine phosphorylation of Kit has been observed in extracts prepared from HMC-1 and H526 tumor xenografts, and this effect could be maintained for up to 24h. The ability of OSI-930 to inhibit Kit in vivo correlated with potent anti-tumor activity in both the mutant Kit-expressing HMC-1 model and wild-type Kit-expressing SCLC models (H526, H209 and WBA). These results suggest that OSI-930 may have clinical utility in tumor types that are dependent on Kit tyrosine kinase activity.

395 POSTER

Blocking the interaction between HIF-1alpha and p300 by a 32 amino acid fragment of p35srj inhibits the hypoxia induced transcriptional activity of HIF-1alpha in human U87MG glioma cells

S. Latini, K.S. Jensen, L.S. Olsen, J. Finneman, M.W. Madsen. *LEO Pharma, Biochemistry, Ballerup, Denmark*

The Hypoxia Inducible Factor-1α (HIF-1α) is the oxygen- and growth factor-regulated subunit of HIF, an αβ-heterodimer and a transcriptional activator of several genes involved in the regulation of angiogenesis, glycolysis, and tissue invasion. The binding of the carboxyl terminal activation domain of HIF-1α (TAD-C) to the transcriptional co-activator proteins p300 and CBP (CREB-binding protein) is essential for the transcription of HIF target genes and occurs at the cysteine-histidine-rich CH1 domain of p300 and CBP. A 35 kD nuclear protein containing a serine rich junction (p35srj) is constitutively bound via its amino acid residues 224–255 to the CH1 domain of p300, inhibiting the binding of HIF-1α to p300. As p35srj is encoded by a HIF-1 regulated gene, and the binding of p35srj to p300 restricts the access of HIF-1α to p300, it is believed that p35srj is a natural feedback inhibitor of the activation of HIF-1α (Bhattacharya, S., et al. Genes and Development. 13:64–75, 1999). We have confirmed that p35srj (224–255) inhibited the interaction of the TAD-C domain of HIF-1α and the CH1 domain of p300, using an Amplified Luminescence Proximity Homogeneous Assay (AlphaScreen™). Coupling p35srj (224–255) to a cell permeable peptide resulted in a product that inhibited the hypoxia-induced transcriptional activity of HIF-1α, as determined by a reporter gene assay in the human U87MG glioma cell line. We believe that the interaction of HIF-1α with the p300 co-activator protein can be a target for the development of new therapeutic agents that inhibit the hypoxia-induced transcriptional activity of HIF-1α.