was 63 days (33–336). The main side-effects of gefitinib were grade 1–2 skin rash, diarrhea and transaminitis. Data analyses on QoL are ongoing. Four patients underwent prostate biopsy. EGFR was overexpressed in 2 patients, cerbB2 was absent in all tissue samples. Serum HER2 ECD was assessed in 12 patients. Mean basal value was 10.1 ng/ml (6.5–14.4). After 2 months mean value was 11.7 (9.0–15.7). Serum EGFR was assessed in 14 patients. Mean basal value was 55.5 ng/ml (41.4–64.8). Mean value at 2 months was 52.8 ng/ml (47.5–58.3). Gefitinib has been associated with infrequent PSA responses and no objective response in patients with metastatic HRPC. Further evaluation of data from this study will clarify the effect on QoL and the correlation between serum EGFR and HER2 and clinical outcome.

386 POSTER

Effect of angio-sonography to monitor response during imatinib treatment in patients with metastatic gastrointestinal stromal tumor (GIST): a preliminary report

U. De Giorgi[1], C. Aliberti[2], G. Benea[2], M. Conti[1], M. Marangolo[1]. [1]*Santa Maria delle Croci Hospital, Istituto Oncologico Romagnolo-Department of Oncology, Ravenna, Italy;* [2]*Delta Hospital, Department of Imaging, Lagosanto (Ferrara), Italy*

GIST metastases are typically intra-abdominal and hypervascular. BR1 is a new blood pool ultrasound second-generation contrast agent, which consists of stabilized microbubbles, which allows angio-sonography through continuous real time examination during different vascular phases of contrast enhancement using low transmission power. We assessed angiosonography to monitor response during imatinib treatment (400 mg orally once daily) in patients with metastatic cKIT+ GIST. Ten consecutive patients with known advanced cKIT+ GIST were investigated with angio-sonography and CT. We also monitored the serum levels of vascular endothelial growth factor (VEGF). Angio-sonography showed an early reduction in tumor vascularization in all 10 cases. The tumor perfusion appeared reduced in the central part of the GIST metastases. With a median follow-up of 15 months (range 3–21), a reduction in tumor vascularization was continuously observed in all 10 patients, but in 2 progressive disease (PD) was documented after 12 and 21 months of imatinib treatment. CT documented tumor response according to standardized criteria in 6 patients (median time to response 4 months, range 1–9), stable disease (SD) in 2 lasting 18+ and 21+ months, and PD in 2 according to angio-sonography. Serum VEGF levels behaved in an heterogeneous manner, but an early reduction in serum VEGF levels was observed as early as 1 week in the 2 cases with higher pretreatment serum VEGF levels. In a single case receiving a strict angio-sonographic evaluation with angio-sonography at 1, 2, 4, 6, 8 weeks, a reduction in tumor vascularization was observed as early as 2 weeks but standardized tumor response based on CT was reported only after 9 months. A reduction in tumor vascularization observed before a reduction in tumor size coupled with the observation that the perfusion is mainly reduced in the central part of the treated tumors is in line with recently performed studies of monitoring antiangiogenic therapy with vascular functional imaging. Imatinib-mediated antiangiogenic properties have been demonstrated in experimental models and in vivo in CML and neuroblastoma. Imatinib could induce antiangiogenic effects in GIST. This effect could be easily monitored with angio-sonography. Large studies are warranted.

387 POSTER

A phase I study of AEE788, a novel multi-targeted inhibitor of ErbB and VEGF receptor family tyrosine kinases

H. Dumez[1], P. Clement[1], C.H. Takimoto[2], A.T. van Oosterom[1], A. Mita[2], K. Parker[3], E.K. Rowinsky[2], R. Salazar[4], E. Martinelli[4], J. Baselga[4]. [1]*University Hospital Gasthuisberg, Oncology, KU-Leuven, Belgium;* [2]*IDD/Cancer Therapy and Research Center, San Antonio, USA;* [3]*Novartis Pharmaceuticals Corp, East Hanover, USA;* [4]*Hospital Vall d'Hebron, Barcelona, Spain*

Background: Combined blockade of multiple signal transduction pathways may result in improved antitumor effects. AEE788 is an orally active, reversible, small molecule multi-targeted kinase inhibitor with potent inhibitory activity against ErbB and VEGF receptor family of tyrosine kinases. In preclinical *in vitro* and *in vivo* studies, inhibition of both the ErbB and VEGF receptor pathways has been shown. AEE788 has an IC_{50} of less than 100 nM against EGFR, ErbB2, VEGFR2. This phase I study was to assess the safety, pharmacokinetics (PK), MTD/DLT dose levels, and optimal biological dose of AEE788.
Methods: Patients (pts) with advanced solid tumors were enrolled. Dose escalation approximated a modified Fibonacci series with 3–6 pts/cohort. Safety monitoring included additional cardiac assessment. No prior EGFR/ErbB2 or VEGF/VEGFR directed therapies were permitted. Pharmacodynamic markers were analyzed in pre- and post-treatment skin and tumor biopsies. A 24 hr PK profile was obtained on days 1, 15 and 28, with trough sampling on days 8 and 22.
Results: To date, 27 advanced cancer pts (15 male, 12 female), median age 55 (range 25–78), have been treated with AEE788 at doses of 25 (5), 50 (6), 100 (5), 150 (5) or 225 mg (6) per day. AEE788 was given on a continuous daily schedule. Tumor types treated were breast (5), colon (5), bladder (2), melanoma (2), liver (2), soft tissue sarcoma (2), and 9 other tumor types (1 each). No dose limiting toxicities have been reported. The most common adverse events (>10%) included diarrhea (41%), nausea (33%), fatigue (30%), skin rash (18%) anorexia (15%), cough (15%), vomiting (15%), anemia (11%), asthenia (11%), cancer pain (11%), constipation (11%), pruritus (11%) and pyrexia (11%). 6 pts had diarrhea suspected to be related to AEE788; (50 mg-1 pt grade (gr) 1; 150 mg-1 pt gr 1, 1 pt gr 2; and 225 mg-3 pts gr 1). 5 pts had drug-related skin rash (100 mg-1pt gr 1, 150 mg-1pt gr 1, 225 mg-2 pts gr 1, 1 pt gr 2). There were no study drug-related grade 3 or 4 adverse events or lab abnormalities. There was no QTc > 500 ms in over 1000 ECGs. Exposure to AEE788 increased overproportionately with dose, with estimated half-life of 24–30 hrs. The ratio of an active metabolite (AQM674) to parent (AEE788) was on average ~ 0.7 (range 0.2 to 2). Exposure to AEE788 and AQM674 was similar after 15 and 28 days of dosing (with the exception of the 25 mg dose), suggesting that PK equilibrium was reached on or before day 15. The best response was stable disease (SD). To date, 7 patients have received AEE788 for > 2 cycles. The median number of cycles of AEE788 was 1.6 (range 0.5–8.8).
Conclusion: AEE788 was well tolerated at daily doses up to 225 mg/day. The study is continuing, the MTD/DLT dose level has not yet been reached.

388 POSTER

The abrogation of rapamycin-induced AKT activity by the small molecule IGF-IR inhibitor, AEW541, and the enhanced antitumor activity of combined mTOR and IGF-IR inhibition

K. O'Reilly[1], Q. She[1], H. Lane[2], F. Hofmann[2], D. Solit[1], N. Rosen[1]. [1]*Memorial Sloan-Kettering Cancer Center, Molecular Pharmacology and Chemistry, New York, USA;* [2]*Novartis Pharma AG, Institute for Biomedical Research, Basel, Switzerland*

mTOR (the mammalian target of rapamycin) is a serine/threonine kinase that senses nutrient availability and acts as a central regulator of cell growth. mTOR activates p70 S6 kinase (RSK) which increases translation of mRNA with 5′ polypyrimidine tracts and inhibits the translational repressor 4E-BP1. Small molecule inhibitors of mTOR (rapamycin, CCI-779, RAD001) have antitumor activity in pre-clinical cancer models and modest single agent activity in cancer patients. We hypothesized that resistance to mTOR inhibitors may be the result of adaptive induction of parallel survival pathways following mTOR inhibition. We observed that exposure of MDA-468 (breast) and DU-145 (prostate) cells to rapamycin (1nM) resulted in the induction of IRS-1, a key adapter protein in the IGF-1 signal transduction pathway. IRS-1 (insulin receptor substrates-1) mediates insulin and IGF-1 signaling by linking the IGF-1 and insulin receptor tyrosine kinases to multiple downstream signaling proteins, including p85, the regulatory subunit of PI3K, via interaction with the p85 SH2 domain. We found that rapamycin-stimulated IRS-1 induction was accompanied by increased AKT activity and phosphorylation of its downstream substrate GSK3β. Furthermore, inhibition of IGF-1R with AEW541, an inhibitor of IGF-1 receptor tyrosine kinase, abrogated the upregulation of p-Akt seen following mTOR inhibition. This combination of rapamycin and AEW541 also synergistically inhibited cell growth. The data suggest that IGF-1R inhibition sensitizes cancer cells to mTOR inhibition by counteracting the rapamycin-induced positive feedback upregulation of IGF-1 signalling molecules. This evidence provides a rationale for testing combined inhibition of IGF-1R and mTOR in cancer patients.

389 POSTER

CF101, an agonist to the A3 adenosine receptor enhances the chemotherapeutic effect of 5-fluorouracil in a colon carcinoma murine model

P. Fishman, S. Bar Yehuda, L. Madi, D. Silberman, G. Slosman, M. Shkapenuk. *Can-Fite BioPharma, Petach Tikva, Israel*

Background: The molecular mechanism underlying chemo-resistance of tumor cells to cytotoxic drugs entails high levels of NF-kB and the upstream kinase PKB/Akt, acting as inhibitors of apoptosis. A3 adenosine receptor (A3AR) activation with the specific agonist CF101 has been shown to inhibit the development of colon carcinoma growth in vitro and in vivo. In addition CF101 protected mice against myelotoxic effects of chemotherapy via its capability to induce G-CSF production. In this study we examined the

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,