For MRS analyses, cells were extracted using a dual phase method and ^{31}P and ^{13}C spectra of the aqueous fractions recorded on a 500 MHz Bruker spectrometer. Metabolite concentrations were normalised relative to cell number and internal standards.
Results: Inhibition with CI-1040 for 24h caused a marked decline in levels of P-ERK1/2 that was visible at 0.2 μM, 0.5 μM and 1 μM as detected by Western blotting. Cyclin D1 levels also decreased at 0.2 μM CI-1040 and were reduced further as the drug concentration increased. ^{31}P MRS analysis showed that this treatment was associated with a dose-dependent reduction in PC levels to 85±6% ($p = 0.07$), 57±8% ($p = 0.02$) and 65±7% ($p = 0.03$) of controls at 0.2 μM, 0.5 μM and 1 μM CI-1040 respectively. Time course analysis with 1 μM CI-1040 showed that the reduction in P-ERK1/2 levels seen at 24h was also present at 3h, 6h and 16h. ^{31}P MRS showed that PC levels remained unchanged at 3h (108±10%, $n = 2$) and 6h (99±10%; $n = 3$, $p = 0.9$) but decreased later at 16h reaching 64±7% of control ($n = 2$). ^{13}C MRS analysis of extracts from cells incubated in ^{13}C-choline showed that the levels of ^{13}C-labelled PC formed from choline decreased to 64±7% following exposure to 1 μM CI-1040 ($n = 3$, $p = 0.02$) suggesting reduction of de novo PC synthesis via inhibition of choline transport and/or phosphorylation.
Conclusions: Our results show that inhibition with CI-1040 in human melanoma cells is associated with a time- and concentration-dependent reduction in PC levels that results from decreased choline transport and/or phosphorylation. Thus, PC could have potential as a biomarker for monitoring the action of MEK inhibitors in melanomas during clinical trials.
Funding: Cancer Research UK [CUK] Grant # C1060/A808.

References

[1] M Beloueche-Babari et al. Cancer Res 2005; 65(8): 3356.

563 POSTER

In vitro activity of the multi-targeted kinase inhibitor sorafenib (BAY43–9006) against gastrointestinal stromal tumor (GIST) mutants refractory to imatinib mesylate

H. Prenen[1], B. Dewaele[2], J. Cools[2], N. Mentens[2], C. Stefan[1], R. Sciot[3], P. Schöffski[1], P. Marynen[2], A.T. van Oosterom[1], M. Debiec-Rychter[2].
[1]Catholic University Leuven, Department of General Medical Oncology, Leuven, Belgium; [2]Catholic University Leuven, Center for Human Genetics, Leuven, Belgium; [3]Catholic University Leuven, Department of Pathology, Leuven, Belgium

Although most GIST patients show good response to imatinib, many of them develop resistance during further treatment. The development of resistance to small molecule kinase inhibitors has emerged an important problem for targeted therapy in cancer. Acquired resistance commonly occurs via secondary gene mutation in the KIT kinase domain. Sorafenib was initially identified as a potent RAF and VEGFR inhibitor and was subsequently shown to also inhibit the related receptor tyrosine kinases FLT3, KIT and PDGFR. Sorafenib was recently approved for the treatment of advanced renal cell carcinoma.
We tested the ability of sorafenib to inhibit imatinib-resistant mutants. Primary imatinib resistant tumors cells and/or murine Ba/F3 cells, expressing imatinib-resistant KIT-V654A, KIT-T670I or PDGFRA-D842 mutations were evaluated for sensitivity to sorafenib by Western blotting and proliferation assays. Sorafenib inhibited the KIT kinase activity of V654A and T670I mutants as measured by Western blots at concentrations ranging from 1 to 5 μM. Sorafenib also suppressed proliferation of the cells expressing these mutations. In contrast, sorafenib did not inhibit the PDGFRA-D842V mutant. Clinical studies with sorafenib have shown that serum concentrations up to 13.3 μM could be safely achieved in patients receiving the standard dose of 400 mg twice daily. Therefore, our findings suggest that sorafenib can be an efficient therapy for patients with GIST that carry the acquired KIT-V654A or KIT-T670I mutations.
In conclusion, our in *vitro and ex vivo* findings indicate that sorafenib has good inhibitory activity against the V654A and T670I mutations in KIT that confer resistance to imatinib, in contrast to PDGFRA-D842V that confers resistance to both agents.

564 POSTER

Small molecule inhibitor BMS-536924 completely reverses IGF-IR-mediated transformation of immortalized mammary epithelial cells

B. Litzenburger[1], H.J. Kim[1], J. Carboni[2], M. Gottardis[2], T.W. Wong[2], R. Attar[2], X. Cui[3], A.V. Lee[3]. *[1]Baylor College of Medicine, Breast Center, Houston, Texas, USA; [2]Bristol-Myers Squibb Research Institute, Oncology Drug Discovery, Princeton, New Jersey, USA; [3]Baylor College of Medicine, Breast Center, Houston, Texas, USA*

Type I insulin-like growth factor receptor (IGF-IR) is overexpressed in a number of cancers and contributes to tumor invasion and metastasis. We have previously reported that a dominant active IGF-IR (CD8-IGF-IR) causes rapid mammary tumorigenesis when overexpressed in the mouse mammary gland. To elucidate the molecular mechanisms of tumor formation, we stably overexpressed CD8-IGF-IR in immortalized, but non-transformed, mammary epithelial cells (MCF-10A). MCF-10A-CD8-IGF-IR cells showed constitutive IGF-IR phosphorylation in the absence of any IGF stimulation. MCF-10A-CD8-IGF-IR showed numerous features of transformation including growth in the absence of serum, lack of contact inhibition in monolayer and foci formation, anchorage-independent growth in soft-agar, and invasion through matrigel. Interestingly, MCF-10A-CD8-IGF-IR cells were also able to grow as xenografts in immunocomprimised mice (when injected with or without matrigel) an uncommon feature following transformation of MCF-10A cells with a single oncogene. BMS-536924 was effective at blocking both IGF-I stimulated wild-type IGF-IR and also CD8-IGF-IR activity. Inhibition was observed at 10–100 nM and was maximal at 1 microM, a concentration which didn't affect epidermal growth factor (EGF)-mediated activation of EGFR signaling. Monolayer growth assays showed that BMS 536924 induced a dose dependent inhibition of proliferation with an IC_{50} of 0.4–0.8 μM, whereas the IC_{50} in anchorage independent growth was nearly a log-fold lower. Flow cytometry indicated that BMS-536924 caused a G0/G1 block in the cell cycle. BMS-536924 was also able to completely reverse the CD8-IGF-IR induced invasion. Finally BMS-536924 at 100 mg/kg/day caused a 70% reduction in MCF-10A-CD8-IGF-IR xenograft volume. These results demonstrate that the new small molecule, BMS 536924 is an effective inhibitor of IGF-IR, causing complete reversion of an IGF-IR-mediated transformed phenotype *in vitro* and blocking growth *in vivo*.

565 POSTER

Growth-inhibitory and anti-angiogenic effects of the novel MEK inhibitor PD0325901 in preclinical models of human malignant melanoma

L. Ciuffreda[1], M. Desideri[2], D. Trisciuoglio[2], L. Steelman[3], A. Anichini[4], F. Cognetti[1], J. McCubrey[3], G. Zupi[2], D. Del Bufalo[2], M. Milella[1].
[1]Regina Elena National Cancer Institute, Division of Medical Oncology A, Rome, Italy; [2]Regina Elena National Cancer Institute, Laboratory of Experimental Preclinical Chemotherapy, Rome, Italy; [3]Brody School of Medicine at East Carolina University, Department of Microbiology and Immunology, Greenville (NC), USA; [4]Istituto Nazionale Tumori, Human Tumors Immunobiology Unit, Milan, Italy

The Raf/MEK/ERK signaling module is a central mediator of tumor cell proliferation, survival, and angiogenesis. *BRAF* mutations may sensitize cancer cells to the growth-inhibitory effects of small-molecule MEK inhibitors; we therefore tested the effects of PD0325901, a novel MEK inhibitor, in a panel of human melanoma cell lines, with or without *BRAF* mutations. Cells were exposed to increasing concentrations of PD0325901 and analyzed for ERK phosphorylation, cell growth/proliferation, and apoptosis. VEGF and IL-8 production were also assessed, under normoxic and hypoxic conditions. PD0325901 strongly inhibited ERK phosphorylation in a dose- and time-dependent manner; inhibition was already evident at 1 nM and almost complete at ⩾10 nM, was detectable after 15 min, and persisted for at least 48h. PD0325901 potently inhibited cell growth (IC_{50}: 10−40 nM) in human melanoma cells harboring either mutant (M14, A375P, ME10538, ME4686) or wild-type (ME4405, ME13923) *BRAF*; the wild-type *BRAF* cell lines ME1007 and ME8959, conversely, proved relatively resistant ($IC_{50} > 100$ nM). Cell growth inhibition was due to inhibition of cell cycle progression, with depletion of S-phase cells and accumulation in G_0/G_1, and subsequent induction of apoptosis, both of which were further enhanced by decreasing the concentration of serum in the culture medium. We also investigated the anti-angiogenic potential of PD0325901 in the mutant *BRAF* cell line M14; in this model, PD0325901 significantly decreased VEGF protein secretion under both normoxic and hypoxic conditions. Inhibition of VEGF production took place at the transcriptional level, as demonstrated by the PD0325901-induced, dose-dependent decrease of HIF-1a protein expression and transcriptional activity. In addition, PD0325901 also strikingly decreased the production

of IL-8, an inflammatory chemokine that has been shown to play an important role in tumor growth and angiogenesis. Moreover, the mutant *BRAF* V600E gene was introduced into WT *BRAF* melanoma cell lines to directly determine the effects of inheritance of a mutant *BRAF* gene on sensitivity to PD0325901, VEGF/IL-8 secretion, and angiogenesis. In summary, the novel MEK inhibitor PD0325901 is endowed with potent growth-inhibitory, pro-apoptotic, and anti-angiogenic activity in preclinical models of human melanoma. Molecular mechanisms of action are currently under investigation, but preliminary results warrant further preclinical/clinical development of this compound.

566 POSTER

Anti-leukemic activity of the novel MEK inhibitor PD0325901

M. Milella[1], M.R. Ricciardi[2], C. Gregorj[2], S. Abrams[3], L. Steelman[3], S. Chiaretti[2], F. Cognetti[1], R. Foà[2], J. McCubrey[3], A. Tafuri[2]. [1]*Regina Elena National Cancer Institute, Division of Medical Oncology A, Rome, Italy;* [2]*University of Rome "La Sapienza", Department of Cellular Biotechnologies and Hematology, Rome, Italy;* [3]*Brody School of Medicine at East Carolina University, Department of Microbiology and Immunology, Greenville (NC), USA*

The Raf/MEK/ERK signaling module is frequently dysregulated in hematological malignancies. We investigated the effects of PD0325901, a novel MEK inhibitor, on phospho-protein expression, gene expression profiles, cell proliferation, and apoptosis in cell line models of AML, ALL, multiple myeloma (MM), *ex vivo*-cultured primary AML blasts, and oncogene-transformed hematopoietic cells. AML cell lines (OCI-AML2, OCI-AML3, HL-60) were strikingly sensitive to PD0325901 (IC_{50}: 5–19 nM), NB4 (APL) and U266 (MM) showed intermediate sensitivity (IC_{50}: 822 and 724 nM), while all the lymphoid cell lines tested and the myeloid cell lines U937 and KG1 were resistant (IC_{50} > 1000 nM). Cell growth inhibition was due to inhibition of cell cycle progression and induction of apoptosis. A statistically significant reduction in the proportion of S-phase cells ($p = 0.01$) and increase in the percentage of apoptotic cells ($p = 0.019$) was also observed in 18 primary AML samples in response to 100 nM PD0325901. PD0325901 effects were also examined in a panel of IL-3-dependent murine myeloid FDC-P1 cell lines transformed to grow in response to 11 different oncogenes in the absence of IL-3. Fms-, Ras-, Raf-1-, B-Raf-, MEK1-, IGF-1R-, and STAT5a-transformed FDC-P1 cells were very sensitive to PD0325901 (IC_{50}: ~1 nM), while A-Raf-, BCR-ABL-, EGFR- or Src-transformed cells were 10 to 100 fold less sensitive (IC_{50}: 10 to 100 nM); the parental, IL-3 dependent FDC-P1 cell line had an IC_{50} > 1000 nM. Analysis of the phosphorylation levels of 18 different target proteins after treatment with 10 nM PD0325901 showed a 5- to 8-fold reduction in ERK-1/2 and a 2-fold reduction in JNK and STAT3 phosphorylation. Conversely, increased phosphorylation in response to PD0325901 was observed for Raf-1 (2.5-fold), MEK1/2 (2.4-fold), AKT (2-fold), and $p70^{S6K}$ (2-fold). PD0325901 (10 nM) treatment also profoundly altered the gene expression profile of the sensitive cell line OCI-AML3: 96 genes were modulated after 24 h (37 up- and 59 down-regulated), most of which involved in cell cycle regulation. Changes in cyclin D1 and D3, cyclin E, and cdc 25A were also validated at the protein level. Overall, PD0325901 shows potent growth-inhibitory and pro-apoptotic activity, indicating that MEK may be an appropriate therapeutic target in an array of different hematological malignancies. Further preclinical/clinical development of this compound is warranted, particularly in myeloid leukemias.

567 POSTER

Efficacy of BIBW 2992, an irreversible dual EGFR/HER2 receptor tyrosine kinase inhibitor, in combination with cytotoxic agents

F. Solca[1], A. Baum[1], F. Himmelsbach[2], A. Amelsberg[3], G. Adolf[1]. [1]*Boehringer Ingelheim Austria, R&D Pharmacology, Vienna, Austria;* [2]*Boehringer Ingelheim Pharma GmbH&Co KG, Chemistry, Biberach/Riss, Germany;* [3]*Boehringer Ingelheim Pharmaceuticals Inc., Medicine, Ridgefield, CT, USA*

BIBW 2992 is an orally active irreversible dual EGFR/HER2 receptor tyrosine kinase inhibitor which is currently in clinical development. In preclinical models, BIBW 2992 has demonstrated single-agent activity in a variety of xenograft models of human cancer (e.g., A431 squamous cell carcinoma, MDA-MB-453 breast, NCI-N87 gastric and SKOV-3 ovarian carcinomas). Combinations of EGFR and/or HER2 targeted agents with standard chemotherapeutic agents have demonstrated efficacy in clinical trials. The present study aimed to assess the effect of BIBW 2992 in combination with established drugs *in vitro* and *in vivo*. Colony forming assays in soft agar revealed that concomitant treatment with BIBW 2992 and either docetaxel, doxorubicin, or 5-fluorouracil induces supra-additive inhibitory effects in SKOV-3 ovarian carcinoma cells. For cisplatin and carboplatin, combination experiments with BIBW 2992 were not performed as the EC^{50} values for the platinum-derived compounds were above 5000 nM in this assay system. Ensuing *in vivo* experiments in nude mice bearing subcutaneous SKOV-3 xenografts using docetaxel and doxorubicin in concomitant treatment combinations with daily doses of BIBW 2992 confirmed the *in vitro* observations. A refined assessment of docetaxel/BIBW 2992 combination schedules was performed using the SKOV-3 xenograft model. Pulsatile weekly treatment (qdx2) of tumor bearing mice for 5 weeks with BIBW 2992 at a dose of 35 mg/kg/d as single agent resulted in good anti-tumor activity (T/C=8 %). Weekly treatment with 10 mg/kg docetaxel also showed efficacy in this model (T/C=22%). The combination treatments irrespective of schedule resulted in better efficacy with T/C values between 1–3 %. The treatment schedule using docetaxel followed by BIBW 2992 resulted in tumor regressions (defined as $V^{rel} < 50\%$) in all treated animals. For comparison the inverse treatment schedule resulted in regressions in 30% of the cases (p-value: 0.0072). Taken together, these observations suggest that clinical trials of BIBW 2992 in combination with established chemotherapeutic drugs are warranted.

568 POSTER

In vitro and in vivo pharmacological properties of the potent phosphatidylinositol 3-kinase (PI3K) family inhibitor PI103

F. Raynaud, S. Eccles, P. Clarke, A. Hayes, F. Di Stefano, Z. Ahmad, S. Guillard, S. Patel, P. Workman. *Institute of Cancer Research, Cancer Research UK Centre for Cancer Therapeutics, Sutton, United Kingdom*

PI103 (PIramed) is a potent and competitive pyridofuropyrimidine inhibitor of class 1 PI3K with IC50 of 2–15 nM, as well as inhibiting mTOR. PI103 exhibited growth inhibitory (GI50 = 0.1−1 μM) activity against a panel of human cancer cell lines, including prostate, lung, ovarian, colon and breast, that have different genetic abnormalities in the PI3K signalling pathway, eg PTEN deletion, PIK3CA mutation or over-expressed receptor tyrosine kinases. Consistent with inhibition of PI3K, treatment with 1× and 5× GI50 concentrations of PI103 resulted in decreased phosphorylation of AKT on Ser473 in all cell lines. The effects of PI103 on the downstream components of the PI3 kinase pathway were rapid, with inhibition of phosphorylation of AKT on Ser473 and Thr308 and on Ser21 of GSK-3β as early as 10 min post treatment. In addition, PI103 induced redistribution of GFP-tagged FOXO4 from the cytoplasm to the nucleus (IC50 = 30 nM) in U2OS cells. PI103 also inhibited the re-localisation of GFP-AKT1, 2 and 3 and GFP-PDK1 to the plasma membrane in CHO cells with IC50s of 17, 13, 11 and 66 nM respectively. PI103 decreased cyclin D1 expression as early as 4h post-treatment, consistent with the G1 cell cycle arrest that was detected in all cancer cell lines tested, and this was both time- and concentration-dependent. Apoptosis was not seen, as measured by sub-G1 peak or by caspase 3/7 cleavage. PI103 inhibited the chemomigration and invasion properties *in vitro* of a variety of tumour cells (eg HCT116 colon carcinoma, MDA MB 468 breast carcinoma and PC3 prostate carcinoma (over 80% inhibition at 450 nM) and U87MG glioblastoma (44% inhibition at 50 nM). Despite extensive glucuronidation *in vitro* in microsomal incubations and *in vivo* in mice, PI103 distributed to liver, kidney, spleen and tumour (U87MG xenografts) resulting in tumour levels above GI50 for 2–4h following 40 or 70 mg/kg PI103 ip. This resulted in target inhibition in vivo with decreased AKT phosphorylation on Ser473. Significant antitumour activity was observed in a number of human xenograft models including U87MG glioma, HCT116 colon, PC3 prostate, MDA MB-468 breast and MDA MB 435 breast cancers. In the latter case we also observed an inhibition of vascular and muscular invasion. In the late-stage orthotopic ovarian carcinoma model OVCAR-3, PI103 reduced tumour burden (T/C 60%) and tumour cells showed decreased levels of AKT phosphorylation. We also noted a substantial decrease in intraperitoneal invasion of all major sites, with complete control of liver, diaphragm, kidney and ovary invasion and lower incidences of mesenteric and lymph node spread. The results demonstrate the therapeutic potential of potent PI3 kinase inhibitors for the treatment of a range of cancers in which deregulation of the PI3 kinase pathway contributes to oncogenesis.

569 POSTER

Antitumor activity of PLX4032, a novel B-Raf V600E Inhibitor

J.T. Lee[1], J. Kong[1], K. Smalley[1], J. Tsai[2], H. Cho[2], G. Bollag[2], M. Herlyn[1]. [1]*The Wistar Institute, Molecular & Cellular Oncogenesis, Philadelphia, PA, USA;* [2]*Plexxikon, Berkeley, CA, USA*

The concept of targeted therapy in cancer treatment has become clouded with therapeutic compounds that inhibit entire pathways rather than mutated gene products exclusive to the oncogenic tumor itself. While nearly 70% of melanoma patients harbor an activating mutation in B-Raf (V600E) that renders constitutive activity to the MAPK signaling pathway, no compound to date has successfully inhibited this mutation without off-target effects.