353 POSTER

MEK blockade converts AML differentiating response to retinoic acid (RA) into extensive apoptosis: involvement of Bcl-2 modulation and ROS accumulation

M. Milella[1], M. Konopleva[2], Y. Tabe[2], C. Precupanu[1], C. Gregorj[3], M.R. Ricciardi[2], M.T. Petrucci[3], F. Cognetti[1], A. Tafuri[3], M. Andreeff[2]. *[1]Regina Elena National Cancer Institute, Division of Medical Oncology A, Rome, Italy; [2]University of Texas M.D. Anderson Cancer Center, Section of Molecular Hematology and Therapy, Houston, USA; [3]University of Rome "La Sapienza", Dipartimento di Biotecnologie Cellulari ed Ematologia, Rome, Italy*

In this study we investigated whether constitutive MAPK activation, could explain the clinical resistance of non-M3 AML to RA action. AML/APL cell lines were pretreated with selective MEK inhibitors (MI, 25 microM PD98059 or 0.5 microM CI-1040) and then exposed to ATRA or 9-cis RA (0.01–1 microM). MI blocked ATRA-induced differentiation, reduced histone H3 phosphorylation and acetylation, and blocked ATRA-mediated RARbeta mRNA induction. However, in the absence of constitutive MAPK activation, MI, alone or combined with RA, had no discernible effect on cell growth and survival. Conversely, combined treatment resulted in > 95% cell growth inhibition and massive induction of apoptosis (>70% net apoptosis induction) in AML/APL cells with constitutively active MAPK. Isobologram analysis demonstrated that this interaction was indeed highly synergistic (CI < 0.1 and < 0.2, for cell growth inhibition and apoptosis induction, respectively). Both RAR-(TTNPB) and RXR-(LGD1069 and methoprene acid) selective ligands resulted in the synergistic induction of apoptosis when combined with MI (CI < 0.4 and < 0.3 for TTNPB and LGD1069, respectively). Moreover, RA-induced growth inhibition and pro-apoptotic synergism with MI were both abrogated in the RA-resistant HL-60R cell line, which carries a dominant negative mutation in the RARalpha gene: RXRalpha overexpression restored pro-apoptotic synergism, while wild-type RARalpha overexpression resulted in hypersensitivity to the apoptotic effects of CI-1040 (ED50 for apoptosis induction 0.33 and 0.03 microM in HL-60R and HL-60R/RARalpha, respectively). While death-inducing ligand/receptor pairs do not appear to play a major role in MI/RA combination-induced apoptosis, preliminary data suggest the involvement of RA-induced Bcl-2 downregulation and ROS accumulation. Both ATRA and 9-cis RA, indeed, efficiently decrease Bcl-2 expression levels, resulting in the massive accumulation of ROS in cells simultaneously exposed to MI and RA; moreover, forced Bcl-2 overexpression partly inhibits and significantly delays the apoptotic response to combined MI and RA. Altogether, our findings indicate that MEK blockade and RA receptor engagement synergistically induce apoptosis in AML/APL cells with constitutive MAPK activation; given the high prevalence of constitutive MAPK activation in primary AML (75%), MI could be used to revert the clinical resistance to RA ubiquitously observed in non-M3 AML.

354 POSTER

Farnesyl transferase inhibition in circulating peripheral blood mononuclear cells with the novel oral prenyl transferase inhibitor AZD3409 following single and multiple doses in volunteer studies

D. Wilson, T. Stephens, M. Bate, F. Le Maulf, P. McCormack, A. Hughes. *AstraZeneca, Macclesfield, UK*

Background: AZD3409 is a novel, oral, antitumour agent that acts as a prenyl transferase inhibitor. In preclinical studies, AZD3409 has achieved up to 90% inhibition of farnesyl transferase (FTase) in tumours at well-tolerated doses.
Methods: Two clinical studies have been conducted. In the single ascending dose study, a maximum of eight healthy male volunteers were dosed at each dose level (6 AZD3409, 2 placebo) in a randomised, double-blind, alternating panel design, with doses escalated from 20 mg to 2500 mg. FTase activity in circulating peripheral blood mononuclear cells (PBMCs) using a K-ras substrate was measured at pre-dose, and at 2, 4, 12, and 24 hours post-dose. In the multiple dose study, a maximum of 16 healthy male volunteers (12 AZD3409, 4 placebo) were administered the same once-daily dose for 7 consecutive days at the following ascending doses for three consecutive cohorts: 500 mg, 1000 mg, and 1750 mg. Pharmacodynamic data to determine the degree of FTase inhibition using two substrates, K-ras and lamin-B, in circulating PBMCs were collected at pre-dose on Day 1, and then at 2, 12, and 24 hours post-dose on Days 1 and 7.
Results: In the single dose study, evidence of FTase inhibition on the K-ras substrate assay was seen at early time points at high dose levels (1350 mg and above). In the multiple dose study, inhibition against K-ras and lamin-B was seen across all dose groups at 2 hours post-dose at steady state (Day 7), and these differences were statistically significant when compared with placebo (p<0.001). Mean levels of approximately 60% inhibition on lamin-B and 50% on K-ras were reached at the 1750 mg dose. Evidence of some inhibition was still apparent at 12 hours on the lamin-B assay at 1000 mg and 1750 mg on Day 7, and these differences were statistically significant when compared with placebo (p=0.02). Mean levels of inhibition were approximately 25% when compared with placebo. At 24 hours post-dose on Day 7, there was some evidence of inhibition at the 1750 mg dose on the lamin-B assay when compared with placebo, although this was not as consistent as the 12-hour data.
Conclusions: Evidence of FTase inhibition has been observed on once-daily dosing at steady state at AZD3409 doses of 500 mg and above on the K-ras and lamin-B substrate assays.

355 POSTER

Inhibition of human small cell lung cancer growth by simvastatin reveals selective functions of Ras isoforms in growth factor signalling

A. Arcaro[1], U.K. Khanzada[2], M.J. Seckl[2]. *[1]University of Zurich, Department of Pediatrics, Zurich, Switzerland; [2]Imperial College, Department of Cancer Medicine, London, UK*

Lung cancer is the most frequent cause of neoplastic death and small cell lung cancer (SCLC) represents a very aggressive type of lung cancer associated with poor survival rates. Since current treatments such as chemotherapy are ineffective, there is an urgent need to develop novel therapies for SCLC. 3-Hydroxy-3-methylglutaryl CoA reductase inhibitors (statins), such as simvastatin have recently been shown to inhibit the growth of a variety of human tumours, but have not been investigated in SCLC. The mechanism of statin action in tumours is thought to be through inhibition of prenylation of proteins of the Ras superfamily. However, other mechanisms may be important and include depletion of cholesterol in lipid rafts resulting in reduced growth factor receptor activation and/or signalling. Here we show that simvastatin profoundly impaired both basal and stem cell factor (SCF)-stimulated SCLC cell growth *in vitro*. This correlated with induction of apoptosis by simvastatin and inhibition of SCF-activated extracellular signal-regulated kinase (Erk), protein kinase B (PKB) and ribosomal S6 kinase (S6K). These results suggested that simvastatin might act proximally in SCF-induced signalling between the receptor (c-Kit) and Ras activation. Simvastatin did not directly affect activation of c-Kit or its localisation to lipid rafts. However, the drug did block the localisation of Ras family proteins to the membrane. Strikingly, H-Ras protein expression was down-regulated whilst N-Ras, K-Ras, RhoA and Rac-1 were unaffected by simvastatin. This selective down-regulation occurred post-transcriptionally since H-Ras mRNA levels were unaffected by the drug. The inhibition of the Ras/Erk pathway by simvastatin appeared to be of crucial importance for growth inhibition, as SCLC cells expressing an activated mutant of mitogen-activated Erk kinase (MEK) were no longer sensitive to the drug. Whilst the functional relevance of the selective down-regulation of H-Ras by simvastatin urgently requires further investigation, crucially, administration of this drug orally induced profound inhibition of SCLC xenograft growth in nude mice. Thus simvastatin may represent a novel candidate drug for inhibition of SCLC growth *in vivo*.

356 POSTER

Rationale for therapeutically inhibiting NFkB activity in hormone-dependent breast cancers

Y. Zhou[1], S. Eppenberger-Castori[2], G. Scott[1], C. Marx[1], U. Eppenberger[2], C. Benz[1]. *[1]Buck Institute for Age Research, Developmental Therapeutics Program, Novato, USA; [2]Stiftung Tumorbank Basel, Riehen, Switzerland*

NFκB activity is required for mammary gland development, and its upregulation as measured by nuclear translocation and DNA-binding has previously been linked to the progression of hormone-independent estrogen receptor (ER)-negative breast cancers. To investigate the clinical importance of NFκB activation in hormone-dependent ER-positive breast cancers, we evaluated NFκB DNA-binding (measured by EMSA or ELISA-based TransAM/ActiveMotif assay detecting p65 vs. p50 DNA-bound components) in two groups of hormone-dependent primary human breast tumors, one with higher (>100 fmol/mg; group A, n=22) and another with lower (20–99 fmol/mg; group B, n=59) ER content. Group A tumors were found to possess 2–4 fold lower NFκB activity (p50 and p65) than group B tumors, which had been selected *a priori* for their uniform stage (T1/2, N0), known adjuvant treatment and clinical outcome (median 52 month follow-up for DFS), and previous biomarker analyses. Group B tumors destined to relapse (n=13) had significantly higher NFκB p50 (but not p65) DNA-binding than those not destined to relapse (n=46; p=0.04). NFκB p50 DNA-binding showed significant outcome association by univariate Cox model analysis and by Kaplan-Meier DFS curves. Cell culture studies were performed to explore the therapeutic potential of inhibiting NFκB as a treatment for some ER-positive breast cancers. Drugs shown to

prevent oxidant (K3)-induced upregulation of NFκB DNA-binding in ER-positive breast cancer cells (MCF-7) included the IKK inhibitor parthenolide (PA), the antioxidant dithiocarbamate (PDTC), and proteasome inhibitors (MG-132, PS-341/bortezomib). PA given in combination with tamoxifen (Tam) to ER-positive breast cancer cells with reduced Tam sensitivity (BT474, MCF-7/HER2) produced greater than additive reduction in cell survival ($p<0.001$) as compared to treatment with Tam or PA alone. In conclusion, clinical studies indicate that ER-positive breast cancers may be prognostically subdivided according to NFκB activity. Breast cancer cell culture studies indicate that inhibition of NFκB activity by several different drug strategies is feasible, and that inhibition of NFκB activity may synergistically enhance the antitumor activity of ER-targeted endocrine agents like tamoxifen.

357 POSTER

IGF-I receptor kinase inhibitor NVP-AEW541-NX-7 abolishes MCF-7 breast cancer cell responsivity to estradiol

Y. Zhao[1], F. Hofmann[2], M. Pollak[1]. [1]*S.M.B.D. Jewish General Hospital and McGill University, Oncology, Montreal, Canada;* [2]*Novartis Pharma AG, Basel, Switzerland*

There is prior evidence for interaction of estrogen receptor and IGF-I receptor signalling in breast cancer. A recent report (Mitsiades, C. et al, *Cancer Cell* 5: 221–230, 2004) showed progressive reduction in survival of estrogen receptor positive MCF-7 human breast cancer cells induced by the IGF-I receptor kinase inhibitor NVP-ADW-742 (Novartis) in concentrations ranging from 0.1 to 10 micromolar, in 10% charcoal-stripped fetal calf serum culture conditions. We observed 40% growth stimulation of MCF-7 cells by 0.1 nM estradiol under 5% charcoal-stripped fetal calf serum culture conditions, and carried out experiments to determine if estradiol influences the activity of NVP-AEW541-NX-7 (a IGF-1 receptor inhibitor related to NVP-ADW-742). We observed that NVP-AEW541-NX-7 at 0.1 micromolar completely blocks estradiol induced cell proliferation. Conversely, estradiol alters the dose-response characteristics of NVP-AEW541-NX-7 as a suppressor of MCF-7 cell proliferation as assessed by MTT assay.
Under our conditions, NVP-AEW541-NX-7 had detectable anti-proliferation effects in the absence of estradiol over a wide concentration range (25% growth inhibition at 0.01 uM to 87% growth inhibition at 1uM). estradiol protected cells, particularly at low NVP-AEW-NX-7 concentrations. Equal growth inhibition was observed at 0.01 micromolar NVP-AEW-NX-7 in the absence of estradiol and 0.15 micromolar in its presence. These results are consistent with recently reported IGF-I receptor – estradiol receptor interactions (Song, R. et al, *PNAS* 101:2078–81,2004), and raise the possibility of therapeutically useful co-blockade of estrogen and IGF-I receptors. Studies of the influence of estradiol on phosphorylation changes induced by NVP-AEW541-NX-7 at the IGF-1R and downstream are in progress.

358 POSTER

Inhibitors of the Ras/Raf/MAPK signaling pathway sensitize pancreatic cancer cells resistant to EGFR inhibitors

V. Grünwald, M. Morgan, A. Ganser, C. Reuter. *Medical School Hannover, Hematology, Hemostaseology and Oncology, Hannover, Germany*

The epidermal growth factor receptor (EGFR) is frequently over-expressed in pancreatic cancers and is currently exploited as a novel therapeutic target in early clinical trials. Ligand binding to the EGFR mediates biological responses through activation of downstream signaling pathways, including the Ras/Raf/MAPK signaling cascade. Over 90% of pancreatic cancers have also activating K-Ras mutations, which can result in constitutive activity of the Ras/Raf/MAPK signaling cascade and represent a possible mechanism of resistance to EGFR inhibition. The aim of this study was to determine the role of constitutively active Ras/Raf/MAPK signaling in pancreatic cancer cells which demonstrate resistance to EGFR inhibitors. Constitutive activity of MAPK was determined by Western blot analyses of pancreatic cancer cell lines, which contain activating K-Ras mutations. MTT survival assays were used to analyzes a panel of pancreatic cancer cells for response to treatment with AG1478, which is an irreversible inhibitor of the EGFR tyrosine kinase domain. These assays demonstrated that pancreatic cancer cells were relatively resistant to EGFR inhibition. By Western blot analyses, treatment with AG1478 failed to inhibit MAPK phosphorylation, expression of SKP2, a mediator of p27Kip1 degradation in the proteasome, was also unaffected. Co-treatment with the MEK1 inhibitor PD98059 or lovastatin, a prenylation inhibitor, sensitized pancreatic cancer cells to treatment with AG1478. Combination therapies were found to block MAPK phosphorylation, SKP2 expression and rendered cells sensitive on a molecular level. In conclusion, these data provide the rationale to explore combined targeted therapies in clinical trials in K-Ras mutated pancreatic cancer.

359 POSTER

Phase Ib and pharmacokinetic studies of Everolimus (RAD001), a novel oral mTOR-inhibitor, with paclitaxel in patients with advanced solid tumors

M. Campone[1], V. Levy[2], V. Delecroix[1], E. Bourbouloux[1], D. Bootle[3], C. Tanaka[3], N. Shand[3], F. Calvo[2], E. Raymond[2]. [1]*Centre René-Gauduchea, Department of Medicine, Nantes, France;* [2]*Saint-Louis Hospital, CIC & Department of Oncology, Paris, France;* [3]*Novartis Pharma, Oncology, Basel, Switzerland*

Background: RAD001 (everolimus) is a novel mTOR inhibitor that displays in-vitro and in-vivo additive or synergistic effects of with paclitaxel when the two drugs were administered simultaneously.
Methods: This study investigates the safety and pharmacokinetic interactions of everolimus given orally at escalated doses simultaneously with a fixed dose of 80mg/m^2 paclitaxel on days 1, 8 and 15 every 4 weeks. In this study, doses of everolimus were selected on the basis of previous phase I single agent safety data with everolimus, pharmacokinetic/pharmacodynamic analysis demonstrating that everolimus inhibits the phosphorylation of $P_{70}S_6$kinase downstream to mTOR at doses ranging 20–30mg, and early evidence of antitumor activity. So far, two dose levels were successively investigated: 15mg (cohort 1) and then 30mg (cohort 2).
Results: Twelve patients have been enrolled onto the study (male/female: 2/10; median age 59y, range 38–69y). Tumor types consisted of breast (5pts), ovarian (2pts), thyroid (1pt) carcinomas; melanoma (1pt); and sarcomas (3pts). All patients were previously treated with chemotherapy, including 9 patients who were previously exposed to paclitaxel. Three pts were entered in cohort 1 and received a range of 3–4 cycles with no dose-limiting toxicity. Therefore 9 pts were included in cohort 2 with so far no dose-limiting toxicity. As expected for paclitaxel-based chemotherapy, the most frequent toxicity was grade 1–2 neutropenia in 50% of patients. Adverse events related to everolimus administration were mild to moderate grade 1–2 skin reactions, mucositis and diarrhea. None of the patients required treatment withdrawal or discontinuation due to toxicity. No clinically relevant pharmacokinetic interaction between the drugs was found in cohort 1. Antitumor activity was observed in a patient with breast cancer previously exposed to a taxane and in patients with paclitaxel-pretreated ovarian cancer (2 stable diseases with decrasing CA125 at 3 and 4 months).
Conclusions: The combination of everolimus to weekly paclitaxel has a safe toxicity profile comparable to that observed with paclitaxel and everolimus when used single agents. So far, no pharmacokinetic interaction between everolimus and paclitaxel has been detectable. Based on early evidence of activity, our results encourage subsequent explorations of this combination in phase 2 studies.

360 POSTER

NFkB expression and disease outcome in prostate cancer

J. Ross[1], B. Kallakury[2], C. Sheehan[1], T. Jennings[1]. [1]*Albany Medical College, Pathology and Laboratory Medicine, Albany, USA;* [2]*Georgetown University, Pathology, Georgetown, USA*

Background: The activation of nuclear transcription factor NFkB is regulated by the binding to its specific inhibitor IkB. NFkB binds to multiple DNA sequences controlling the downstream expression of cell cycle regulatory proteins, cytokines, angiogenesis factors, cell adhesion molecules, enzymes and pro- and anti-apoptotic proteins. IkB is in turn regulated by degradation through the ubiquitin proteasome pathway.
Methods: Using prostatectomy specimens, immunohistochemical staining (IHC) for NFkB and IkB (Santa Cruz Biotechnology) was performed on formalin-fixed paraffin-embedded sections of 136 cases of PCA. Cytoplasmic immunoreactivity was scored for intensity and distribution and results were correlated with tumor grade, stage, DNA ploidy status (Feulgen spectroscopy) and biochemical disease recurrence.
Results: 49% of PCAs over-expressed NFkB and 63% showed decreased expression of IkB. NFkB over-expression correlated with advanced tumor stage ($p=0.048$), aneuploidy ($p=0.022$), and biochemical disease recurrence ($p=0.001$). Decreased expression of IkB correlated with high tumor grade ($p=0.015$). On multivariate analysis, tumor stage ($p=0.043$) and NFkB expression ($p=0.006$) were independent predictors of disease recurrence.
Conclusions: Over-expression of NFkB in primary PCA predicts advanced tumor stage and independently predicts disease recurrence. Drugs such as proteasome inhibitors that target NFkB should be considered for the treatment of prostate cancer.