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