60 POSTER

Pre-clinical evaluation of combinations of PI3K & MEK inhibitors in colorectal carcinoma cell lines

E.J. Cox[1], S. Kyle[1], G.S. Beale[1], R.J. Maxwell[1], D.R. Newell[1]. [1]*Northern Institute for Cancer Research, Drug Discovery & Imaging, Newcastle upon Tyne, United Kingdom*

MAPK and PI3K signalling pathways are frequently activated in human cancers, and represent promising therapeutic targets. Previous studies suggest that combined targeting of these pathways may be necessary for optimal therapeutic activity, hence the aim of this study was to evaluate the MEK inhibitors, ARRY-142886 and PD 0325901, alone and in combination with the dual mTOR/PI3K inhibitor, NVP-BEZ235, or the pan class I PI3K inhibitor, GDC-0941, in colorectal cancer cell lines. Growth inhibition, survival and signal transduction were measured using the Sulforhodamine B assay, clonogenicity and western blotting, respectively. Median effect analysis revealed that all MEK/PI3K inhibitor combinations exhibited marked synergistic growth inhibition in both HCT116 and HT29 cell lines. GDC-0941 displayed the greatest synergy in combination with either MEK inhibitor. At concentrations up to 10 μM only NVP-BEZ235 was cytotoxic after 72 hours exposure in either colorectal cancer cell line with an LC_{50} of 0.51 μM in the HCT116 cell line and an LC_{50} of 0.49 μM in the HT29 cell line, and increased cytotoxicity was only observed with selected MEK/PI3K inhibitor combinations in the HT29 cell line. Western blotting revealed that NVP-BEZ235 exhibits stronger inhibition of 4EBP1 phosphorylation, and similar inhibition of S6 and AKT phosphorylation, compared to GDC-0941. Both PD0325901 and ARRY-142886 inhibited ERK phosphorylation. The additional effect on S6, ERK or AKT phosphorylation observed with MEK/PI3K inhibitor combinations was minimal. However, there is a significant difference in the magnitude of inhibition of p4EBP1, as with NVP-BEZ235 there is a high level of inhibition alone and in combination whereas there is minimal inhibition with GDC-0941. These studies confirm that single agent MEK and PI3K inhibitors are predominantly cytostatic, as opposed to cytotoxic; however, combination studies demonstrated marked synergism in the 2 cell lines investigated. The dual mTOR/PI3K inhibitory action of NVP-BEZ235 may increase its ability to inhibit 4EBP1 phosphorylation, and thereby protein translation. Furthermore, the lower level of synergy exhibited by NVP-BEZ235 in combination with MEK inhibitors, compared to GDC-0941, may be due to the inhibition of mTOR and thereby 4EBP1 phosphorylation. These studies confirm that dual targeting of PI3K and MEK can induce synergistic growth inhibition; however, the detailed effects of specific inhibitors should be investigated to identify optimal combinations.
The research was funded by grants from the Medical Research Council UK and UCB Celltech Ltd.

61 POSTER

Evaluation of the synthetic heat shock protein 90 inhibitors NVP-AUY922 and NVP-HSP990 in human prostate cancer tissue

M.M. Centenera[1], J.L. Treloar[1], A. Hanson[1], P. Sutherland[2], W.D. Tilley[1], L.M. Butler[1]. [1]*University of Adelaide and Hanson Institute, Dame Roma Mitchell Cancer Research Laboratories, Adelaide, Australia;* [2]*Royal Adelaide Hospital, Urology, Adelaide, Australia*

The molecular chaperone heat shock protein 90 (Hsp90) is an important target for cancer therapy as it is required for the correct maturation and function of its various client proteins, many of which are known oncogenes. In prostate cancer, targeting Hsp90 is particularly attractive as the androgen receptor (AR), the key mediator of prostate cancer cell growth and survival, is also an Hsp90 client protein. Despite promising results in pre-clinical studies, the first-in-class Hsp90 inhibitor 17-allylamino-demethoxygeldanamycin (17-AAG) has shown limited efficacy in phase I and II clinical trials for advanced prostate cancer, at least in part due to limitations in formulation, poor pharmacokinetics and hepatoxicity. In this study, we used cell based assays and a model of human prostate cancer to examine the efficacy of two new synthetic inhibitors of Hsp90, namely (i) NVP-AUY922 that has emerged as the most potent Hsp90 inhibitor developed to date, and (ii) the orally available NVP-HSP990. We demonstrate that both agents are significantly more potent than 17-AAG at killing prostate cancer cells. In the AR-positive LNCaP cell line, a 40 nM dose of NVP-AUY922 or NVP-HSP990 induced cell death in 70% and 30% of cells, respectively, compared to no cell death observed with 40 nM 17-AAG. The AR-negative cell line PC3 was more sensitive to both agents, with a 40 nM dose of NVP-AUY922 or NVP-HSP990 causing 80−90% cell death. Both NVP-AUY922 and NVP-HSP990 significantly reduce steady-state protein levels of the Hsp90 client proteins HER2, c-RAF-1 and AR, in addition to the AR-regulated protein PSA, and both inhibitors altered cell cycle distribution. In addition to our cell line studies, we have developed a unique model of human prostate cancer where specimens collected from men undergoing radical prostatectomy are cultured as explants. Using this model, we demonstrate for the first time how human prostate tumour tissue responds to NVP-AUY922 and NVP-HSP990, and demonstrate modulation of the established clinical biomarkers of Hsp90 inhibition, namely HSP70, c-RAF-1 and CDK4, in addition to AR. In summary, we provide the first extensive evaluation of the synthetic Hsp90 inhibitors NVP-AUY922 and NVP-HSP990 in prostate cancer cells and human prostate cancer tissue. Our studies support clinical development of these agents for the treatment of prostate cancer.

62 POSTER

Pre-clinical evaluation of LYS6KAKT1, a novel, highly selective, orally bioavailable dual inhibitor of p70 S6 Kinase and AKT currently in phase I clinical trials for cancer

S. Geeganage[1], L. Sams[1], S. Joseph[1], T. Shepherd[1], R. Dally[1], L. Brail[1], T. Wang[1], A. Capen[1], G. Rodgers[1], E. Perkins[1]. [1]*Eli Lilly & Co, Discovery Oncology, Indianapolis IN, USA*

PI3K/AKT/mTOR/S6K signaling pathway (AKT pathway) controls cell survival, cell-cycle progression, cell growth and metabolism through a cascade phosphorylation of a number of key substrates. This pathway is regulated by three well characterized tumor suppressors; *pten, tsc2,* and *lkb1*. Deletion of these genes results in activation of the AKT pathway and proliferative disorders. Similarly, activating mutations of the receptor tyrosine kinases or PI3 Kinase result in the activation of the pathway. Therefore, multiple nodes of the pathway have become drug targets. As part of a comprehensive drug discovery platform aimed at targeting the PI3K pathway, we have developed a potent small molecule dual inhibitor of p70 S6 kinase and AKT targeting two key nodes of the pathway.
LYS6KAKT1 is a potent, highly selective ATP competitive inhibitor against p70 S6 kinase and AKT with an IC50 of 6 nM and 14 nM respectively. *In vitro*, LYS6KAKT1 inhibits the phosphorylation of S6 ribosomal protein in U87MG glioblastoma cells with an IC50 of 120 nM and the phosphorylation of GSK3b with an IC50 of 1200 nM. It also inhibits the phosphorylation of other downstream AKT substrates such as PRAS40 and FOXO. Similar activity is seen in a broad range of other cell lines. *In vivo,* LYS6KAKT1 demonstrates potent phospho-S6 inhibition in nude mice bearing U87MG glioblastoma cells, with an ED50 value of 3 mg/kg and an ED90 value of 8 mg/kg 4 hours after a single oral dose. *In vivo* pharmacodynamic activity on phospho-S6 was demonstrated in other xenograft models such as A2780 (ovarian), H460 (lung), PC3 (prostate), and HCT116 (colon). In addition, pharmacodynamic activity was shown on other AKT markers such as phospho-PRAS, phospho-Forkhead, and phospho-S6K. In these studies, LYS6KAKT1 showed elevation of phospho-AKT in a dose dependent manner. This has been observed with other ATP competitive AKT inhibitors, whereas a previously disclosed p70 S6 kinase selective inhibitor LYS6K1, did not (Geeganage et al, Abstract 352, Pre-clinical evaluation of LYS6K1, a novel, highly selective, orally bioavailable inhibitor of p70 S6 Kinase currently in phase I clinical trials for cancer, AACR 2010). In vivo pharmacodynamic relationships on pS6 and other markers were dose, exposure, and time dependent.
In vitro, LYS6KAKT1 also showed cellular anti-proliferative activity in a broad range of cell lines in monolayer and colony formation assays. *In vivo,* LYS6KAKT1 effectively inhibits the growth of A2780 ovarian carcinoma xenografts in mice and the growth of U87MG glioblastoma tumor as a single agent at 8 mg/kg and 2.5 mg/kg given twice daily, respectively. It also inhibits the growth of 786-O renal xenograft growth when given orally once a day at 12.5 mg/kg.
Based on above pre-clinical observations, LYS6KAKT1 is currently being evaluated in phase I clinical studies for cancer.

63 POSTER

Efficacy evaluation of novel Pim kinase inhibitors with anticancer activity

K. Brzozka[1], A. Zarebski[1], R. Windak[1], E. Trebacz[1], W. Czardybon[1], M. Cholody[1], M. Millik[1], N. Beuzen[1]. [1]*Selvita, R&D, Krakow, Poland*

Pim-1, -2, and -3 serine-threonine kinases play an important role in intracellular signaling and contribute to pathways involved in cell survival, proliferation, stress response and cellular motility. Pim kinases emerged as a novel and interesting target of significant potential for therapeutic intervention in cancer. Overexpression of Pim kinases was reported for a variety of cancer types of both hematological and solid tumor type origin such as diffuse B cell lymphoma, chronic lymphocytic leukemia, Flt3-mediated acute myelogenous leukemia, prostate, pancreatic and hepatic cancers.
Selvita is presenting results of the currently performing lead optimization program of novel, small molecule Pim kinase inhibitors. Among the newly synthesized compounds we have identified molecules exerting substantial specificity and superior potency in inhibition of all three Pim kinase isoforms

with IC_{50} values in low nanomolar range. Anticancer effect of our new derivatives was investigated in several cancer cell lines of hematological and solid tumor origin where the compounds induced cell death with low micromolar ED_{50} values. Additionally, synergistic toxic effect was observed in combination with standard therapeutics. Biomarker analysis of Pim kinase downstream targets confirmed Pim-dependent mechanism of action of tested compounds. We have observed a potent inhibition of 4EBP1 and S6 phosphorylation, as well as downregulation of c-Myc levels already after 4h of the treatment with our Pim kinase inhibitors. Following the results obtained *in vitro* in cell culture, compounds were further profiled for their ADMET properties like permeability, metabolic stability and bioavailability. Oral administration of our small molecule Pim kinases inhibitors in a subcutaneous leukemia *in vivo* xenograft model revealed strong inhibition of tumor growth with almost 90% TGI. Moreover, histological analysis of the organs from the animals dosed sub-chronic for over 2 weeks with the compounds, did not reveal any significant organ damaging effects.

64 POSTER

PDL192, a humanized antibody to TweakR exhibits potent anti-tumor activity in non-small cell lung cancer models

B.L. Hylander[1], M. Sho[2], S. Tanlimco[3], D. Choi[2], H. Kim[3], D. Chao[2], E.A. Repasky[1], K. Wilson[3], G.C. Starling[2], P.A. Culp[2]. [1]*Roswell Park Cancer Institute, Immunology, Buffalo NY, USA;* [2]*Facet Biotech, Preclinical Program Research, Redwood City CA, USA;* [3]*Facet Biotech, Research Discovery, Redwood City CA, USA*

Background: Approximately 1.3 million new cases of lung cancer are diagnosed worldwide every year, with non-small cell lung cancer (NSCLC) comprising 80−85% of those cases. The 5-year survival rate for NSCLC is 10−15%, highlighting the need for novel effective therapies. We have generated PDL192, a humanized IgG1 antibody to TweakR (Fn14, TNFRSF12A, CD266), a cell surface protein and member of the TNF receptor superfamily. PDL192 has been shown to exhibit anti-tumor activity in xenograft models on a range of solid tumor types via both direct tumor cell growth inhibition and by antibody-dependent cellular cytotoxicity.
Materials and Methods: In this study, we examined the expression of TweakR in NSCLC patients and explored the activity of PDL192 on lung cancer cell lines *in vitro* and in lung cancer xenograft models derived from cell lines, as well as in primary tumor xenograft models.
Results: TweakR protein was found to be expressed at high levels in all four major subtypes of NSCLC: in 59% of lung adenocarcinomas, 42% of squamous cell carcinomas, 66% of large cell carcinomas, and 75% of bronchioalveolar carcinomas (BAC). In *in vitro* growth assays, PDL192 inhibited the growth of 10 of 19 NSCLC cell lines, which included cell lines derived from each of the four major subtypes. *In vivo*, PDL192 exhibited potent anti-tumor activity (68% inhibition) in the H358 BAC model and exhibited moderate tumor growth inhibition (25%) in the Calu6 adenocarcinoma model. In the H358 model, PDL192 treatment resulted in a decrease in the proliferation marker Ki67 and significantly enhanced the activity of both erlotinib and pemetrexed. PDL192 was also tested for its ability to inhibit the growth of primary tumor xenografts. PDL192 inhibited the growth of 1 of 4 adenocarcinomas tested and 2 of 4 large cell carcinomas. Studies exploring the activity of PDL192 on primary squamous cell carcinomas are currently ongoing.
Conclusions: The *in vitro* and *in vivo* activity of PDL192 demonstrate the potential of this antibody as a therapeutic in NSCLC, both as monotherapy and in combination with erlotinib or pemetrexed, agents currently used as standards-of-care in this disease. PDL192 is currently being evaluated in a Phase 1 safety study in patients with solid tumors.

65 POSTER

Targeting Ras-mutated tumors with novel multiplex PI3K inhibitors through inhibition of eIF-4E-mediated protein translation

M. Hamilton[1], A.K. Bernardino[1], Y. Liu[1], K. Provoncha[1], D. Paul[1], Y. Rotshteyn[1], A. Han[1], D. Qian[1]. [1]*Progenics Pharmaceuticals, Research & Development, Tarrytown NY, USA*

Background: The simultaneous dysregulation of both PI3K and Ras-MAPK pathways is characteristic of some of the most aggressive forms of human cancer (e.g. tumors with Ras mutations). It has been demonstrated in preclinical studies that effective treatment of Ras-mutated tumors requires blockade of both pathways. Therefore, the ability to achieve this outcome with a single agent holds great clinical promise.
One key function of PI3K and Ras-MAPK pathways is to converge at eIF-4E, a critical factor in cap-dependent translation of critical proteins involved in tumorigenesis and tumor cell survival (e.g., c-Myc and Mcl-1). The PI3K pathway activates eIF-4E via mTOR-mediated phosphorylation and suppression of 4E-BP1, a negative regulator of eIF-4E; the Ras-MAPK pathway modulates eIF4E function through phosphorylation by MNK, a downstream kinase of MAPK. A combination of rational drug design and conditional lethal screening led to the identification of a novel lead series of small molecule inhibitors with multiplex activities against PI3K, mTOR and MNK which have been characterized preclinically.
Methods and Results: The anti-tumor activity of novel multiplex PI3K inhibitors was assessed against a panel of 30 human tumor cell lines comprised of various genetic backgrounds and histotypes. Multiplex PI3K inhibitors demonstrate broad and potent anti-proliferative activity (EC_{50}: 10 nM − 500 nM) and induce cell death (i.e. >100% inhibition of cell proliferation) in all cell lines tested, including those harboring Ras mutations (e.g., PANC-1, HCT116 and A549). Mechanistically, multiplex PI3K inhibitors induce caspase activity, inhibit the phosphorylation of AKT, 4E-BP1, ribosomal S6 and eIF-4E, and inhibit the expression of c-Myc and Mcl-1 proteins. In contrast, the dual PI3K-mTOR inhibitor NVP-BEZ235, and the PI3K-selective inhibitor GDC-0941, were partially cytostatic in Ras-mutated tumor cell lines, inhibiting cell proliferation by 60−90%. NVP-BEZ235 and GDC-0941 failed to induce significant increases in caspase activity, inhibit eIF-4E phosphorylation, or inhibit c-Myc and Mcl-1 expression. Multiplex PI3K inhibitors display favorable drug-like properties. In human tumor xenografts *in vivo*, multiplex PI3K inhibitors demonstrate a robust anti-tumor effect and inhibition of pharmacodynamic endpoints (i.e. phosphorylation of AKT, 4E-BP1, ribosomal S6 and eIF-4E).
Conclusion: Newly-discovered novel multiplex PI3K inhibitors are capable of simultaneously targeting both PI3K and Ras-MAPK pathways and exhibit potent anti-tumor activity in Ras-mutated tumors through the inhibition of eIF-4E mediated protein translation. These inhibitors warrant further investigation for development as a potentially novel class of anti-cancer drugs.

66 POSTER

Tigatuzumab, a novel anti-human death receptor 5 antibody, shows synergistic efficacy against colon cancer in vitro and in vivo in combination with chemotherapy

A. Yada[1], Y. Ogitani[1], S. Ishida[1], K. Ichikawa[1], K. Fujiwara[1]. [1]*Daiichi Sankyo Co. Ltd., Oncology Research Laboratories, Tokyo, Japan*

Background: Tigatuzumab is a humanized anti-human death receptor 5 (DR5) antibody and induces apoptosis in several human tumor cell lines. The enhanced effect of anti-DR5 antibodies when combined with chemotherapy or radiotherapy has been reported in tests on several cancers. In this study, we investigated the anti-tumor effect of tigatuzumab combined with irinotecan or 5-fluorouracil (5-FU) in human colon carcinoma HCT 116.
Material and Methods: The effect of tigatuzumab alone or in combination with camptothecin or 5-FU on the proliferation of HCT 116 cells was evaluated. The apoptosis pathway, cell-cycle arrest and changes in DR5 expression induced by each single agent or in combination were analyzed *in vitro*. To study *in vivo* efficacy, HCT 116 cells were inoculated into the flanks of Balb/c nude mice. The mice were treated with tigatuzumab (6 mg/kg, weekly), irinotecan (65 mg/kg, single dose) and 5-FU (90 mg/kg, single dose).
Results: Both camptothecin and 5-FU arrested the proliferation of HCT 116 cells at G2/M phase and G1 phase, respectively. However, addition of tigatuzumab to each chemotherapeutic agent almost completely killed HCT 116 cells with apoptosis induction. These combination treatments were determined as synergism when evaluated by combination index, suggesting that both irinotecan and 5-FU in combination with tigatuzumab may augment the apoptosis induction. Both camptothecin and 5-FU upregulated p53 expression in the HCT 116 cells and induced phosphorylation of p53 at the site of Ser^{15} and Ser^{392}, with increased expression of p21/Cip1 and cell surface DR5. The addition of tigatuzumab resulted in the activation of the apoptosis pathway, along with inducing the upregulation of proapoptotic Bax and Bak, and enhanced cleavage of caspases and PARP when compared with those of each single agent treatment. In a xenograft model, tigatuzumab, irinotecan and 5-FU inhibited the growth of HCT 116 tumors by tumor growth inhibitions of 20%, 39% and 35%, respectively (on Day 31). Combined treatment of tigatuzumab with irinotecan and 5-FU significantly enhanced the anti-tumor activity of each single agent, resulting in tumor growth inhibitions of 54% and 56%, respectively. In addition, p53-dependent apoptosis induction was observed in the xenografted HCT 116 tumors treated with tigatuzumab combined with irinotecan and 5-FU.
Conclusions: These results demonstrate that irinotecan and 5-FU affect a synergistic enhancement on the anti-tumor activity of tigatuzumab by activating p53 and the mitochondrial apoptosis pathway. This combination efficacy was further confirmed in a xenograft model. This evidence supports the use of tigatuzumab in combination with FOLFIRI as an effective treatment for patients with CRC.