in a murine renal tumor model (Renca). Despite its lack of effects on Renca cell growth in culture, SSG as a single agent in Balb/c mice induced 61% growth inhibition of Renca tumors coincident with increase (2 folds) of tumor-infiltrating macrophages (Mϕ). SSG/IL-2 combination induced 91% growth inhibition of Renca tumors in Balb/c mice accompanied by 4-fold increase in tumor infiltrating Mϕ. IL-2 by itself failed to inhibit Renca tumor growth as reported previously and did not induce tumor-infiltrating Mϕ. The safety of SSG and SSG/IL-2 combination was indicated by the tolerance of the treatments in these mice. Although SSG/IL-2 treatment did not increase tumor-infiltrating T cells in association with Renca tumor growth inhibition in the Balb/c mice, its lack of anti-Renca tumor activity in athymic nude mice indicated a T-cell-dependent immune action mechanism of the combination. Involvement of the tumor-infiltrating Mϕ in the action was supported by the capacity of SSG to increase cytotoxic activity (4 folds) of Raw 264.7 Mϕ against Renca cells in vitro. Evidence of more marked Mϕ increases in spleen in the mice treated with SSG (3 folds) or SSG/IL-2 combination (6 folds) indicated a systemic Mϕ expansion, which recapitulates a prominent feature of murine genetic SHP-1 deficiency and is consistent with in vivo inhibition of SHP-1 by SSG in these mice. These results together demonstrated an anti-Renca tumor activity of SSG that interacts with IL-2 via a T-cell-dependent immune action mechanism in connection with expansion/activation of Mϕ. They provided evidences suggesting a therapeutic potential of SSG in anti-RCC immune therapy and indicating SHP-1as a target molecule for augmenting anti-tumor immunity and improving the efficacy of immune therapies.

336 POSTER

De novo resistance to epidermal growth factor receptor blockade by gefitinib in colorectal cancer cells involves increased insulin receptor isoform A signalling

H.E. Jones[1], J.M.W. Gee[1], D. Barrow[1], A. Wakeling[2], S. Guy[2], R.I. Nicholson[1]. [1]*Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff, UK;* [2]*AstraZeneca, Macclesfield, UK*

Background: Gefitinib (Iressa®) is an epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) that has antitumour activity in a range of cancer types. It has become apparent, however, that high EGFR expression and activity do not always predict for response and clinical data have indicated the existence of both *de novo* and acquired resistance to gefitinib in tumours reported to be EGFR positive. In this study, the possible involvement of the insulin receptor isoform A (InsR-A) in *de novo* gefitinib resistance has been investigated in LoVo colorectal human cancer cells.
Materials and Methods: LoVo cells were exposed to 1 μM gefitinib for 7 days. Cell proliferation was determined by evaluating anchorage-dependent growth. Alterations in signalling pathways were assessed by immunocytochemical, Western blotting and/or reverse transcription polymerase chain reaction techniques. Cell sensitivity to the InsR/insulin-like growth factor (IGF)-1 receptor inhibitor ABDP (AstraZeneca) was also measured.
Results: The highly EGFR-positive LoVo cells demonstrated negligible growth inhibition (<15%) after exposure to 1 μM gefitinib. Compared with controls, the gefitinib-treated cells showed a reduction (45%) in phospho-EGFR (Tyr-845) activity, the tyrosine site located in the kinase domain of the receptor. However, levels of activity of the downstream receptor tyrosine sites Tyr-1068 and Tyr-1173 were unchanged in the presence of 1 μM gefitinib, as was the activity of phospho-ERK1/2. Compared with a panel of various cancer cell line types (A549, DU145 and MCF-7), it was noted that the LoVo cells produced markedly higher levels (9-fold) of IGF-II mRNA, unprocessed (~230 kD) inactive pro-IGF-1R protein, mature InsR protein (~130 kD α-subunit) and substantially elevated (10-fold) amounts of InsR-A mRNA, with minimal detection of InsR-B mRNA. Exposure of LoVo cells to insulin and IGF-II resulted in growth promotion (20% and 40%, respectively) and increased phospho-EGFR (Tyr-1068) and EGFR (Tyr-1173) activity over 60 min. Furthermore, challenge with the InsR/IGF-1 receptor inhibitor ABDP resulted in a dose-dependent decrease in basal growth (IC_{50}=0.25 μM). Interestingly, both phospho-InsR and phospho-Akt levels increased (2-fold and 5-fold, respectively) after treatment with gefitinib.
Conclusions: We propose that *de novo* resistance to gefitinib in LoVo colorectal cancer cells may, in part, be directed through the InsR-A, utilising IGF-II as a ligand. The interactions between InsR and EGFR are currently being further assessed. Evidence suggests that InsR-A overexpression is important in the promotion of cancer growth in IGF-II-producing tumours (Sciacca *et al*. Oncogene 2002;21:8240–50). Co-targeting cells with gefitinib and an InsR/IGF-1R inhibitor may prove more effective than gefitinib alone.
Iressa is a registered trademark of the AstraZeneca group of companies

337 POSTER

Velcade, an NF-kb inhibitor, enhances tamoxifen sensitivity in Akt-induced resistant breast cancer xenograft tumors

L. deGraffenried, W. Friedrichs, B. Chandrasekar, G. Weiss. *UT Health Science Center at San Antonio, Medical Oncology, San Antonio, USA*

Tamoxifen resistance is the underlying cause of treatment failure in a significant number of patients with breast cancer. Only approximately 50% of estrogen receptor α-positive tumors will initially respond to hormonal therapy, and of those tumors that do initially respond, a refractory phenotype will develop in a majority of them within 5 years. Activation of Akt, a downstream mediator in the PI3K signaling pathway has been implicated as one of the mechanisms involved in tamoxifen resistance. Breast cancers with heightened Akt activity are frequently associated with an aggressive disease and resistance to chemo- and hormone-therapy induced apoptosis. In previous studies we have demonstrated that MCF-7 breast cancer cell lines expressing a constitutively active Akt (myrAkt1) are resistant to tamoxifen, both *in vitro* as well as *in vivo* in xenograft models. One potential mechanism is through activation of NF-κB, a known regulator of pro-survival genes. In our model, Akt activity correlated with increased inhibition of IκB (the negative regulator of NF-κB), and increased NF-κB DNA binding. We found that co-treatment with an NF-κB inhibitor or transfection with a non-degradable IκB restored tamoxifen sensitivity to our refractory myrAkt1 MCF-7 cells. Velcade, a specific and reversible inhibitor of the proteasome that prevents NF-κB activation in cancer cells, is currently in clinical development for the treatment of several types of carcinomas, and recently received FDA approval for the treatment of multiple myeloma. We hypothesized that treatment with Velcade would increase the responsiveness of our Akt-induced resistant breast cancer cells to tamoxifen. Nude mice were injected contralaterally with either the myrAkt1 or Control MCF-7 cells, and tumors were allowed to develop to a size of 200 mm^3. Mice were then separated into four (4) treatment groups: Placebo, Velcade as a single agent, tamoxifen as a single agent, or the combination of Velcade with tamoxifen. We found that while Velcade as a single agent had no significant inhibitory effects on our myrAkt1 tumors, tumor growth inhibition was 73% greater with the combination of Velcade and tamoxifen compared to tamoxifen as a single agent. These findings suggest that Velcade as a co-treatment for breast cancer tumors with high levels of Akt activity could be an effective approach for overcoming growth-factor induced resistance and may have implications for future clinical studies of Velcade in breast cancer.

338 POSTER

Computer-aided immunohistochemical analysis of EGFR signaling in paired colorectal cancer and normal colon tissue samples

W. Messersmith[1], D. Oppenheimer[1], M. Amador[2], J. Paralba[1], E. Embuscado[3], M. Hidalgo[1], C. Iacobuzio-Donahue[3]. [1]*Johns Hopkins University, Medical Oncology, Baltimore, MD, USA;* [2]*Hospital Universitario Doce de Octubre, Medical Oncology, Madrid, Spain;* [3]*Johns Hopkins University, Pathology, Baltimore, MD, USA*

Background: The EGFR plays a role in multiple tumor cell processes including proliferation, survival, and susceptibility to chemotherapy. EGFR signaling is complex and multilayered, and appears to have several downstream effector pathways including PI3K-Akt and Ras-Raf-MAPK. The present study aimed to validate and apply a novel computer-aided immunohistochemical (IHC) technique to characterize the status of EGFR signaling in matched colorectal tumor and normal colon tissue samples. We previously presented results in which paired samples were poorly correlated, likely due to differing amounts of confounding stromal tissue in the sample. We present here a re-analysis using a "graphic microdissection" technique in which cell clusters of interest are analyzed separately in an effort to improve correlation between paired samples.
Materials and Methods: Tissue Microarrays (TMA) were made using samples from both cancerous and normal colorectal tissue in 18 patients and stained with antibodies against EGFR, phospho-EGFR (pEGFR), Akt, pAKT, MAPK, and pMAPK. TMA's were quantitatively scored using the Automated Cellular Imaging System (ACIS II, Chromavision, Inc), which couples a computer-controlled brightfield microscope to a camera with imaging analysis software. Prior to image analysis, cell clusters of interest were chosen under the supervision of a gastrointestinal pathologist and circled using the computer mouse. For validation, cell line pellets were analyzed using ACIS II and compared against Western blotting (A431 cells) and ELISA (8 head and neck cancers). Xenograft experiments were also performed in which A431 cells were implanted subcutaneously in nude mice treated with erlotinib versus control vehicle.
Results: ACIS analysis was highly reproducible and results were well correlated with Western blotting and ELISA. The "graphic microdessection" technique of analyzing heterogeneous human samples showed good corre-

lation between paired tumor samples [Pearson correlation of percentage × intensity score, 0.922 ($p<.001$)]. While total EGFR staining was similar between tumor and normal tissues, cancer samples had markedly higher staining of pEGFR, Akt, pAkt, MAPK, and pMAPK. ACIS analysis of xenografts was poorly reproducible. There did not appear to be preferential activation of a particular EGFR signaling pathway.
Conclusions: ACIS IHC is quantitative, reproducible, and correlates with Western blots and ELISA in cell line pellets. A graphic microdissection technique appears to overcome the issue of tissue heterogeneity. Colorectal tumors show higher staining of pEGFR and downstream effectors compared to matched normal colorectal tissues.

339 POSTER

Development of HDAC Class I and II specific assays in order to identify novel small molecule inhibitors

L.S. Collins[1], N. Khan[1], A. Finn[1], L.H. Jensen[2], F. McLaughlin[1], P.W. Finn[1], P. Buhl Jensen[2], M. Sehested[2]. [1]*TopoTarget UK Ltd, 87a Milton Park, Abingdon, UK;* [2]*TopoTarget A/S, Copenhagen, Denmark*

Histone deacetylase (HDAC) activity is associated with repression of gene expression. Aberrant gene expression is often observed in cancer, therefore, the enzymes involved in regulating gene expression are of particular interest as target proteins in oncology. HDACs are involved in deacetylating histone and non-histone proteins. e.g. HDAC6 functions as an α-tubulin deacetylase. Eleven members of the HDAC family have been identified in humans. Non-sirtuin HDACs can be divided into three distinct groups of Class **I** (HDAC1, 2, 3, 8), Class **II** (HDAC4, 5, 6, 7, 9, 10) and Class IV-proteins related to the human HDAC11 gene.
Topotarget has a novel HDAC inhibitor (HDACi), PXD101, currently undergoing Phase **I** clinical trials. Specific HDAC isotype *in vitro* biochemical assays have been developed and used to screen novel HDACi compounds. Details are given on the baculoviral expression and purification by affinity chromatography of a number of HDAC isotypes. Data on the optimization of the conditions for the Fleur de Lys™ HDAC assay is presented. A subset of small molecule HDACi compounds were screened, comprising 6 chemical classes – amides, sulphonamides, piperazine ketones, piperazine sulphones, heterocycles and ethers. The effect of these compounds on the activity of HDAC isotypes, representing both Class **I** and **II**, is described.
A cell-based assay was developed in order to study HDACi induced changes in α-tubulin and histone acetylation levels. These changes were detected using FACs and western blotting techniques. The kinetics of tubulin and histone acetylation was investigated following HDACi withdrawal. Data from these experiments are also presented.

340 POSTER

Effectiveness of a novel, selective inhibitor of the IGF-IR kinase against musculoskeletal tumors

K. Scotlandi[1], M.C. Manara[1], M. Serra[1], S. Croci[2], G. Nicoletti[2], C. Garcia-Echeverria[3], F. Hofmann[3], P. Picci[1]. [1]*Istituti Ortopedici Rizzoli, Laboratorio di Ricerca Oncologica, Bologna, Italy;* [2]*Sezione di Bologna, Dip. Patologia Sperimentale, Bologna, Italy;* [3]*Novartis Institutes for Biomedical Research, Novartis Pharma, Basel, Switzeland*

Background: The identification of new active agents against sarcoma is considered an important challenge in medical oncology. Several lines of evidence have indicated that Insulin-like Growth Factor-I (IGF-I) and its corresponding receptor (IGF-IR) were of major importance in deregulated sarcoma cell growth and pathogenesis, therefore representing a valuable therapeutic approach against these tumors. In this study, we analyzed the *in vitro* effects of the orally bioavailable, specific IGF-IR kinase inhibitor NVP-AEW541 in a panel of musculoskeletal tumor cell lines (5 human rhabdomyosarcoma; 10 human Ewing's sarcoma and 8 human osteosarcoma cell lines).
Methods and Results: A potent cell growth inhibitory activity of NVP-AEW541 was clearly observed either in monolayer and in anchorage-independent conditions. Ewing's sarcoma cells appeared to be particularly sensitive to the effects of this drug (IC_{50} ranging from 100 nM to 300 nM), whereas osteosarcoma cells were at least 10-fold more resistant to the drug, in agreement with previous observations obtained with the neutralizing anti-IGF-IR αIR3 antibody. The analysis of the effects of NVP-AEW541 on the cell cycle and apoptosis indicated a significant enhancement of the G1-phase rate and apoptotic rate in treated cells. In addition, NVP-AEW541 showed anti-angiogenetic activity since it significantly reduced the expression and secretion of VEGF-A by sarcoma cells, and supernatants of treated cells were unable to sustain the survival and proliferation of HUVEC endothelial cells. We also analyzed whether this agent is of value in being combined with conventional cytotoxic drugs for the design of more effective therapeutic regimens. Concurrent exposure of cells to NVP-AEW541 and other chemotherapeutic agents resulted in greater than additive interactions when vincristine and ifosfamide were used, whereas subadditive effects were observed with doxorubicin, cisplatin and actinomycin D.
Conclusions: All together, these results encourage future studies testing the *in vivo* therapeutic value and the general toxicity of this specific IGF-IR kinase inhibitor to be considered for innovative treatments of patients with sarcomas, particularly Ewing's sarcoma and rhabdomyosarcoma. A careful design of new regimens is required in order to identify the best therapeutic conditions and drug-drug interactions.

341 POSTER

MEK1inhibition enhances arsenic trioxide (ato) induced apoptosis in acute leukemia

P. Lunghi[1], F. Lo Coco[2], L. Salvatore[1], N. Noguera[2], A. Tabilio[3], P. Pelicci[4], A. Bonati[1]. [1]*University of Parma, Department of Clinical Sciences, Parma, Italy;* [2]*University of Rome "Tor Vergata", Department of Biopathology, Rome, Italy;* [3]*University of Perugia, Department of Clinical and Experimental Medicine, Perugia, Italy;* [4]*European Institute of Oncology, Department of Experimental Oncology, Milano, Italy*

According to recent laboratory studies, the blast cells of most acute myelogenous leukemias (AML) including acute promyelocytic leukemia (APL) show constitutive activation of extracellular signal-regulated kinases 1/2 (ERKs 1/2) as well as of the kinases immediately upstream of ERK, known as mitogen-activated protein (MAP)/ERK kinases (MEKs). Furthermore, we and others have demonstrated that down-modulation of MEK1 phosphorylation inhibits the proliferation and induces apoptosis of primary AML blasts. In this study, we firstly aimed at investigating whether the combination of Arsenic Trioxide (ATO) with agents that block the phosphorylation of MEK1 can potentiate the anti-leukemic action of ATO in APL. We then investigated whether this combination is capable to enhance apoptosis of non APL AML blasts. For our purposes we studied parental NB4 cell line, an arsenic-resistant NB4 subline (NB4-As^R) derived in our laboratory from the NB4 cell line, primary blast cells of typical hypergranular APL (M3) carrying PML/RARα fusion transcript, primary blast cells of AML (M1 or M2) carrying 47, XX, +8 or 46, XX inv (16), of acute monocytic leukemia (M5), of acute lymphocytic leukemia carrying 46, XX, del (11)(q23). Leukemic cells were pre-treated with PD98059 (Cell Signaling Technology, Beverly, MA) 10, 20 or 40 microM or PD184352 (kindly provided to us by Dr J. S. Sebolt-Leopold, Cancer Molecular Sciences, Pfizer Global Research & Development, Ann Arbor, MI) 1 or 2 microM, and then treated with ATO 0.5–2microM. We found that leukemia cells exploit the Ras-MAPK activation pathway to phosphorylate at Ser112 and to inactivate the pro-apoptotic protein Bad, delaying arsenic trioxide (ATO)-induced apoptosis. Both in APL cell line NB4 and in primary blasts, the inhibition of ERK1/2 activity and of Bad phosphorylation by MEK1 inhibitors enhanced and accelerated apoptosis in ATO-treated cells. NB4-As^R showed stronger ERK1/2 activity (2.7 fold increase) and Bad phosphorylation (2.4 fold increase) compared to parental NB4 cells in response to ATO treatment. Upon ATO exposure, both NB4 and NB4-As^R cell lines doubled protein levels of the death antagonist Bcl-xL but the amount of free Bcl-xL that did not heterodimerize with Bad was 1.8 fold greater in NB4-As^R than in the parental line. MEK1 inhibitors dephosphorylated Bad and inhibited the ATO-induced increase of Bcl-xL, overcoming ATO resistance in NB4-As^R. Synergism, additive effects, and antagonism were assessed using the Chou-Talalay method and Calcusyn software (Biosoft, Ferguson, MO). PD + ATO combination appears to synergize for the induction of apoptosis primarily in arsenic resistant but also in parental NB4 cells. Furthermore, the combination PD + ATO significantly increased the ATO-induced apoptosis in primary acute leukemia blasts ($P<0.001$) These results may provide a rationale to develop combined MEK1 inhibitors plus ATO therapy in APL and in other types of acute leukemia.

342 POSTER

Anti-tumor activity, pharmacokinetic and pharmacodynamic effects of the MEK inhibitor ARRY-142886 (AZD6244) in a BxPC3 pancreatic tumor xenograft model

J.D. Winkler[1], P.A. Lee[1], E. Wallace[1], G. Poch[1], K. Litwiler[1], T. Pheneger[1], J. Lyssikatos[1], M. Perrier[1], S.G. Eckhardt[2], T.C. Yeh[1]. [1]*Array BioPharma, Biology, Boulder, USA;* [2]*University of Colorado, Cancer Center, Aurora, USA*

Background: ARRY-142886(AZD6244), a potent, selective MEK1,2 inhibitor currently in Phase I trials, has demonstrated efficacy in numerous tumor models, including HT29, BxPC3, MIA PaCa2, A549, Colon26, PANC-1, LoVo, Calu6, HCT116, MDA-MB-231, ZR-75–1 and LOX. The