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Vorinostat sensitizes colorectal cancer cell (CRC) lines to AZD6244 and results in synergistic inhibitory effects on proliferation

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Background: AZD6244 (ARRY-142886) is a potent and selective small molecule inhibitor of MEK1/2 with nM potency against CRC cancer cell lines in vitro and is active in several in vivo models of CRC. In phase I trials of AZD6244, inhibition of p-ERK was noted in PBMCs and tumor biopsies at tolerable dose levels, with the best outcome being stable disease. Despite the initial enthusiasm for this class of agents in RAS or BRAF mutant tumors, it is clear that new approaches, including combination therapies, are needed. Vorinostat (Vo) is an inhibitor of histone deacetylases (HDACs) and has been shown to cause growth arrest of cell lines derived from several human cancers including, colon, pancreatic and hepatocellular carcinoma. The goal of this study was to assess the effectiveness of combining AZD6244 with Vo in CRC cell lines.
Methods and Results: Initially, we assessed sensitivity to varying doses of AZD6244 or Vo as single agents against a panel of CRC cell lines. Inhibition of proliferation was determined and cell lines were designated sensitive (S, IC50 <1.0 μM) or resistant (R, IC50 >1 μM) for each drug. We then selected cell lines according to the following conditions: (1) S to both drugs, (2) R to both drugs, (3) S to AZD6244 but R to Vo, and (4) R to AZD6244 but S to Vo, and treated them with varying doses of the combination of the two drugs. The combination of AZD6244 and Vo resulted in synergistic inhibition of proliferation in cell lines that were previously R to AZD6244 but S or R to Vo, as determined by the method of Chou and Talalay. Cell lines that were S to single agent AZD6244 also demonstrated a synergistic effect when combined with Vo with combination indices <1.0. Strikingly, the SW480 cell line, which was R for both drugs as single agents, was converted to S in response to the combination treatment. To determine the effects on known target proteins of these drugs we performed western blot analysis for phospho-ERK (pERK) and actetylated histone H3 in SW480 cells treated with varying doses of AZD6244 or Vo as single agents and in combination. Single agent AZD6244 treatment resulted in an expected dose-dependent decrease in pERK, and histone H3 acetylation was similarly increased with single agent Vo. Interestingly, we also observed a significant increase in pERK levels in cells treated with Vo alone, which may indicate an increased dependence on the MEK pathway as a result of the inhibition of alternative pathways through modulation of HDACs.
Conclusions: We determined the combination of AZD6244 with Vo resulted in synergistic inhibition of proliferation of CRC cell lines. Lines that were R to either drug alone were converted to the S phenotype when treated in combination. Vo increased pERK levels, which we hypothesize may be involved in sensitizing the cells to AZD6244. Our pre-clinical results suggest that combination treatment with AZD6244 and Vo may be a more effective therapy for treatment of CRC versus either agent alone.

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Measurement of mechanistic markers of histone deacetylase (HDAC) inhibition in samples from clinical trials

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Introduction: Assays used to analyse clinical samples should be robust, sensitive and quantitative. Western blots are the 'gold standard' but are slow, have low throughput and are not easily quantifiable. Other assay technologies e.g. ELISAs offer improved characteristics for analysis of clinical samples. HDACs are chromatin modifying enzymes which modulate the transcriptional activity of genes important for tumour development, growth and survival. Increased acetylation of histones is an important marker of HDAC inhibition.
Methods: We have optimised a quantitative Meso Scale Discovery sandwich ELISA for acetylated histone H3 (AcH3), the results of which are quantitated by the inclusion of a calibration curve. Immunofluorescence (IF) has been used to measure AcH3 levels in plucked eyebrow hair follicles. AcH3 was detected using primary antibody (Upstate 06–599) and secondary antirabbit IgG conjugated to Alexa 488 (Invitrogen). TO-PRO-3 (Invitrogen) was used as a nuclear marker. AcH3 was visualised using confocal microscopy (Leica SP2). Fluorescence intensity in hair follicles was quantified using INCell Translator (GE Healthcare).
Results: Using ELISA, AcH3 increased 3 and 1.5-fold following exposure of HCT116 human colorectal tumour cells to the HDAC inhibitors SAHA and MS-275 (5XGI50 for 24 h) respectively. This assay was suitable for measuring changes in AcH3 in peripheral mononuclear cells (PMC) treated ex vivo with the same HDAC inhibitors for 4 h and was validated for Good Clinical Laboratory Practice (GCLP). Within and between plate variation was 9.6%, and 12.5% respectively with a recovery for a 60 ng/ml AcH3 peptide spike of 113±32.7%. The assay was used to measure changes in AcH3 in PMC samples from patients taking part in a Phase 1 clinical trial of an HDAC inhibitor (R306465). A minimal rise in AcH3 was detected in patients receiving the lowest drug doses (100, 200 and 300 mg). A 5−10 fold increase in AcH3 was measured in 2/6 patients receiving the highest dose (400 mg). The assay has been re-optimised for increased sensitivity and will be used to measure samples from future HDAC inhibitor clinical trials. Hair follicles offer an alternative surrogate tissue for measurement of mechanistic markers. Nuclear AcH3 increased 5-fold in plucked hair follicles treated ex vivo with SAHA (5xGI50 for 4 h).
Conclusion: This IF technique will be used in future clinical trials of HDAC inhibitors and the results compared to those obtained with the ELISA.

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Identification of kinases that are potential molecular determinants of cellular response to radiation using antibody arrays

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Background: Deregulated expression and activity of receptor tyrosine kinases (RTKs) and associated proteins are implicated in carcinogenesis, cancer progression and metastasis of several human malignancies, including head and neck squamous cell carcinomas (HNSCCs). Resistance of HNSCCs to therapy is a major challenge and recent reports show that redundant and overlapping signal transduction pathways shared by various kinases are responsible for poor treatment outcome. The goal of this study is to identify kinases that may play a role in cellular response to radiation therapy. This will enable us to develop novel multimodality treatment strategies to overcome cancer cell resistance to therapy.
Materials and Methods: Four HNSCC lines (HN-5, Fadu, HN-30 and UMSCC-10) were used for the study. Clonogenic cell survival assays were performed to characterize these HNSCC lines for their response to radiation (2−6 Gy). Lysates of untreated or irradiated cells (collected 2 or 30 min after 4 Gy) were analyzed by antibody arrays (Proteome ProfilerTM, R&D Systems, Inc., Minneapolis, MN, USA) generated for quantifying expression levels of phosphorylated RTKs (human phospho-RTK array kit) and phosphorylated forms of other kinases (human phospho-kinase array kit). Western blot analysis was performed to validate the expression levels.
Results: Clonogenic cell survival data showed that HN-5 and Fadu cells were relatively radioresistant with surviving fractions after 2 Gy (SF2) of 0.82 and 0.65 respectively. HN-30 and UMSCC-10, were more sensitive with SF2 of 0.5 and 0.52 respectively. Among the RTKs investigated, basal levels of EGFR, ErbB3, VEGFR3, EphA7 and Dtk were significantly over-expressed in HN-5 and Fadu cells than in HN-30 and UMSCC-10 cells. Among other associated kinases, cSrc was over-expressed in HN-5 and Fadu cells and Erk1/2 was over-expressed in HN-30 and UMSCC-10 cells. Exposure to radiation resulted in up-regulated phosphorylation of EGFR, ErbB3, VEGFR3, cSrc, and Chk-2. Among these proteins, EGFR, ErbB3, VEGFR3 and cSrc were validated by western blot analysis.
Conclusions: Our data showed that (1) antibody arrays serve as a powerful tool to determine the expression levels of phospho kinases (2) our data suggest that EGFR, ErbB3, VEGFR3, and cSrc may contribute to governing cellular responses to radiation. Further investigation on specific role for these proteins in cell radiosensitivity is underway.
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Small molecule antagonists of very late antigen (VLA)-4 inhibit metastasis formation and tumor growth of melanoma

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Very late antigen-4 (VLA-4) constitutes an interesting molecular target for innovative therapy against melanoma progression. In previous studies, we have reported that treatment of melanoma cells with a novel family of VLA-4 antagonists abrogate their adhesion to cytokine-activated endothelial cells and efficiently inhibit their metastatic development in vivo. Other studies evidenced that VLA-4 is an endothelial cell receptor that modulates angiogenesis. Herein, we have evaluated the in vivo antimetastatic and

anti-tumor activity of these VLA-4 antagonists through experimental models of hepatic metastasis and subcutaneous xenografts by using both murine B16 melanoma (B16M) cells and primary cultures of human malignant melanoma cells. An in vitro assay based on B16M cell adhesion to immobilized vascular cell adhesion molecule-1 (VCAM-1) substrates was performed in order to calculate the IC50 of the compound. Tumor cell proliferation and hepatic sinusoidal endothelial (HSE) cell migration assays were determined in order to examine the mechanism of action of the VLA-4 antagonists.
Results showed that when mice were treated 3 days/week intraperitoneally with 2.5 mg/kg, metastatic development decreased by 50% and 80% in mice bearing B16M cells and human melanoma cells respectively. In addition, the tumor volume was reduced by 80% and 50% in those mice inoculated subcutaneously with B16M and human melanoma cells, as compared to untreated mice. Histological analysis demonstrated that proliferating KI67 expressing cancer cells, CD31 expressing endothelial cells and smooth-alpha actin expressing myofibroblasts, significantly decreased in metastases and subcutaneous xenografts from mice receiving VLA-4 antagonist. Furthermore, in vitro assays showed that VLA-4 antagonist inhibited B16M cell adhesion to immobilized VCAM-1 in a dose-dependent manner (IC50= 4 μM). Moreover, VLA-4 antagonist inhibited tumor-induced HSE cell migration through collagen type I as well as the tumor cell proliferation in response to vascular endothelial growth factor (VEGF).
In summary, these results demonstrated that small molecule antagonists of VLA-4 possess anti-metastatic, anti-angiogenic and anti-proliferative activity and constitute novel promising agents in the chemoprevention of cancer progression.

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Therapeutic potential of YM155 alone and in combination with chemotherapeutics against human non-small cell lung cancer in carcinoma xenograft models

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Background: YM155 is a novel survivin suppressant currently in phase II trials. Consistent with the evidence that survivin is preferentialy expressed in most human neoplasms and regulates cancer cell proliferation, YM155 shows potent antiproliferative activity against various cancer cell lines and induces cell death preferentially in tumor cells rather than in normal cells. In an established human hormone refractory prostate cancer model, YM155 induces substantial tumor regression without overt toxicities. In this study, we showed the antitumor activity of YM155 alone and in combination with various chemotherapeutics in several established human non-small cell lung carcinoma (NSCLC) xenograft models.
Material and Methods: For each model, male BALB/c nu/nu mice were implanted subcutaneously with 3×10^6 cancer cells and treatment was initiated after the tumors were established (100–200 mm3).
Results: In human NCI-H358 NSCLC xenograft model, continuous infusion of YM155 (3 and 10 mg/kg/day) completely inhibited tumor growth and induced substantial tumor regression with no decrease in body weight and blood cell counts. The antitumor activity of YM155 was more potent than that of cisplatin (3 mg/kg/day, i.v., 5 times weekly), and comparable to paclitaxel (20 mg/kg/day, i.v., 5 times weekly). On the other hand, the MTD of paclitaxel-induced severe systemic toxicities as evidenced by significantly decreased body weight and blood cell counts. In a combination study using an established Calu-6 human NSCLC xenograft model, YM155 in concomitant treatment with paclitaxel, cisplatin, doxorubicin or irinotecan showed substantial tumor regression for longer periods than with each treatment administered singly. Similar findings were observed in the case of sequential treatment of YM155 with carboplatin, vinorelbine or gemcitabine regardless of the dosing sequences. When the combinational efficacy of YM155 plus docetaxel was examined with 18F-FDG-PET imaging, concomitant administration of YM155 and docetaxel significantly inhibited tumor growth and the corresponding intratumoral accumulation of 18F-FDG more extensively when compared with the each treatment administered singly. Consistent with the regression of the tumor volume, the complete inhibition of intratumoral uptake of 18F-FDG was observed only in the combination group.
Conclusions: These results suggest that YM155 is a promising candidate for NSCLC treatment as a novel apoptosis inducer with potent antiproliferative activity and no hematologic toxicity. In addition, YM155 potentiates the antitumor activity of various cytotoxics without an increase in systemic toxicity, which provides a rational approach to combination regimens of YM155 with other chemotherapeutics in clinical tumor treatment.

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Membrane androgen receptor activation triggers down-regulation of PI-3K/AKT/NF-κB activity and induces apoptotic responses via FasL, caspase 3 and Bad in DU145 prostate cancer cells

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Background: Membrane Androgen Receptor (mAR) is a novel, yet unknown G-protein coupled receptor functionally distinct from the classical intracellular Androgen Receptor (iAR). mAR is frequently over-expressed in aggressive prostate cancer and initiates rapid non-genomic signaling resulting in tumor cell death by apoptosis in both iAR-positive and iAR-negative cell lines.
Materials and Methods: Using membrane impermeable conjugates of serum albumin covalently linked to testosterone, we have evaluated the effects of mAR activation on gene products implicated in cell survival and apoptosis in iAR-negative DU145 prostate cancer cells. We have assessed the activity of PI-3K and AKT or NF-κB using either antibodies selective for the activated form of the proteins (p85/PI-3K, Ser473 and Thr308/AKT) or a kit measuring activity of NF-κB's p50 and p65 subunits. Furthermore, we have measured the expression levels of pro-apoptotic FasL, the activity of caspase 3 and the phosphorylation status of Bad upon mAR activation. Actin cytoskeleton disruptors (cytochalasin B) or ROCK inhibitors (Y-27632) previously shown to block mAR-dependent apoptosis were also included as controls.
Results: mAR activation inhibited phosphorylation of the p85 subunit of PI-3K already at 2 hours post stimulation. Similarly, Ser473 andThr308 AKT phosphorylation and NF-κB activity were significantly reduced. Testosterone-albumin conjugates induced apoptotic cell death by activating FasL expression and a FasL blocking peptide was capable of blocking mAR-dependent apoptosis. Caspase 3 activity was strongly increased and Bad was dephosphorylated. Finally, cytochalasin B and Y-27632 were capable of blocking FasL induction and caspase 3 activation in mAR-treated DU145 cells.
Conclusions: Our results provide mechanistic insights on the mAR induced apoptotic regression of prostate cancer cells and corroborate previously published observations on the potential use of mAR agonists as novel anti-tumor agents targeting key survival and apoptotic pathways in prostate cancer cells.

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Updated safety and efficacy data from a first-in-human, first-in-class, phase I study of Hedgehog pathway antagonist, GDC-0449

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Background: Aberrant Hedgehog (Hh) signaling pathway activation via both Hh ligand-independent and ligand-dependent mechanisms has been implicated in a variety of cancers including basal cell carcinomas (BCC) and medulloblastoma. GDC-0449 is a potent oral systemic inhibitor of Hh signal transduction. Efficacy in advanced BCC patients and an unusually long half-life and plasma accumulation of GDC-0449 have been previously reported.
Methods: Pts with advanced solid tumors were enrolled in a phase 1 study to evaluate safety, tolerability PK, and pharmacodynamics at one of 3 dose levels: 150, 270, or 540 mg GDC-0449 orally. Surrogate tissue was assessed for expression levels of Hh target gene, GLI1.
Results: To date, 40 pts have been enrolled (01MAY08 data cutoff date): 150 mg (n = 25), 270 mg (n = 11), 540 mg (n = 4); days on study (range 10+ – 465+ d, median 60 d). There have been no dose-limiting toxicities. Possibly drug-related Gr 1−2 AEs include alopecia, anorexia, arthralgia, dermatitis acneiform, dysgeusia, dyspepsia, fatigue, hypoesthesia, hypomagnesaemia, hyponatremia, madrosis, nausea, skin exfoliation, vomiting, and weight loss. Drug-related reversible AEs of Gr 3 hyponatremia (n = 2) and fatigue (n = 1) were reported. In 12 pts with BCC and medulloblastoma (tumors likely to harbor Hedgehog pathway mutations), 6 PRs (2 RECIST, duration 164+ and 293+ days; 4 clinical exam, duration, 113+ – 214+ d), 2 SDs (duration, 147+, 158+ d), and 1 PD were reported; 3 pts are too early to evaluate. Updated safety and response data for all patients will be reported. GDC-0449 showed a prolonged terminal half-life (>7 days) and drug accumulation, resulting in