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Publication of pre-clinical belinostat abstracts at AACR

In total six belinostat abstracts (pre-clinical) are now available for viewing at American Association for Cancer Research 2011 meeting. (www.AACR.org)

Abstract number: 5277

Presentation Title: **Non-invasive metabolic biomarkers of histone deacetylase inhibition in human colon cancer cells and tumors**

Presentation Time: Wednesday, Apr 06, 2011, 8:00 AM -12:00 PM

Location: Exhibit Hall A4-C, Poster Section 23

Poster Section: 23, Poster Board Number: 7

Abstract Number: 5320

Presentation Title: **Changes in [18]F-FLT and [18]F-FDG positron emission tomography following treatment with belinostat alone and in combination with paclitaxel/carboplatin in human ovary cancer xenografts in mice**

Presentation Time: Wednesday, Apr 06, 2011, 8:00 AM -12:00 PM

Location: Exhibit Hall A4-C, Poster Section 24

Poster Section: 24, Poster Board Number: 20

Abstract Number: 178

Presentation Title: **Pre-treatment with histone deacetylase inhibitor (HDACI) synergizes the anti-cancer effect of sorafenib in renal cell carcinoma cells**

Presentation Time: Sunday, Apr 03, 2011, 1:00 PM - 5:00 PM

Location: Exhibit Hall A4-C, Poster Section 8

Poster Section: 8, Poster Board Number: 2

Abstract Number: 178

Abstract Number: 4547

Presentation Title: **Treatment of human medullary thyroid carcinoma (MTC) with either proteasome (Pr) or histone deacetylase (HDAC) inhibitors leads to a fall in RET mRNA levels and, in turn, a decrease in RET protein expression providing alternate strategies to reduce RET expression in a tyrosine-kinase driven disease**

Presentation Time: Tuesday, Apr 05, 2011, 1:00 PM - 5:00 PM

Location: Exhibit Hall A4-C, Poster Section 29

Poster Section: 29, Poster Board Number: 16

Abstract Number: 597

Presentation Title: **The cellular and genomic response to histone deacetylase inhibitors in diffuse large b-cell lymphoma**

Presentation Time: Sunday, Apr 03, 2011, 1:00 PM - 5:00 PM

Location: Exhibit Hall A4-C, Poster Section 25

Poster Section: 25, Poster Board Number: 21

Abstract Number: 2624

Presentation Title: **Elevated expression of phosphorylated mitogen activated protein kinase kinase (MEK) as a mechanism of resistance to the histone deacetylase inhibitor romidepsin in HUT 78 cutaneous T-cell lymphoma cells**

Presentation Time: Monday, Apr 04, 2011, 1:00 PM - 5:00 PM

Location: Exhibit Hall A4-C, Poster Section 29

Poster Section: 29, Poster Board Number: 20

Abstract 5277

Non-invasive metabolic biomarkers of histone deacetylase inhibition in human colon cancer cells and tumors

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Abstract: Introduction: Histone deacetylase (HDAC) inhibitors are targeted anti-cancer agents currently approved for cutaneous T-cell lymphoma and in mid-late stage trials for other cancers (Ma et al, Drugs 2009). Developing non-invasive biomarkers of drug activity could provide a useful tool to aid clinical development. We have previously shown that the HDAC inhibitor LAQ824 increases phosphocholine (PC) levels in human colon cancer cells and tumors as detected by magnetic resonance spectroscopy (MRS), a non-invasive method for monitoring tissue metabolism (Chung et al, Neoplasia 2008).

Here we sought to confirm this effect using a different chemotype probe (PXD101, belinostat) and investigate the mechanism(s) underlying it.

Methods: Human HT29 colon cancer cells were treated for 24h with DMSO or 2 M PXD101 in DMEM ± 28 µM [1,2-¹³C]-choline. For in vivo evaluation, female nude mice bearing HT29 xenografts were treated i.p. with vehicle (n=6) or 60mg/kg PXD101 (n=6) for 3 days. In vitro metabolism was assessed using ³¹P or ¹³C MRS on cell extracts, while in vivo metabolism was evaluated using ¹H and ³¹P MRS pre- (d0) and 3 days post-treatment, followed by analysis of excised tumor extracts. Western blotting for acetyl histone-3 was used to verify inhibitor action.

Results: PXD101 treatment in HT29 cells induced histone-3 acetylation and decreased cell counts to 55±6% of controls (p=0.005). In vivo, PXD101 treatment resulted in tumor stasis (grew by 2% from d0) while the control tumors grew by 20% from d0. ³¹P MRS showed increased cellular PC content to 156±13% (p=0.04) post-PXD101 treatment. In vivo ¹H and ³¹P MRS of tumors revealed rises in total choline/water (169±25%; p=0.02) and phosphomonoester (i.e. PC+phosphoethanolamine)/total phosphate ratios (p=0.01). Ex-vivo

analysis attributed this effect to a rise in PC up to 1.4-fold ($p=0.03$) in the PXD101-treated tumors relative to controls.

PC is formed mainly through phosphorylation of choline via choline kinase (de novo route) or hydrolysis of phosphatidylcholine via phospholipases. ^{13}C MRS analysis of cells grown in the presence of the tracer $[1,2-^{13}\text{C}]$ -choline showed increased ^{13}C - PC formation post-PXD101 treatment ($180\pm 19\%$ of controls, $p=0.02$) consistent with increased de novo PC synthesis.

Conclusions: Our data show that HDAC inhibition with PXD101 increases PC levels in human colon cancer cells and tumors thus confirming our previous findings with the different chemotype agent LAQ824 (Chung et al, Neoplasia 2008). Importantly, we show that this effect is driven by a rise in de novo PC synthesis. These data further support the role of PC as a potential non-invasive biomarker for monitoring the action of HDAC inhibitors.

Abstract 5320

Changes in $[^{18}\text{F}]$ -FLT and $[^{18}\text{F}]$ -FDG positron emission tomography following treatment with belinostat alone and in combination with paclitaxel/carboplatin in human ovary cancer xenografts in mice

Author: Mette Munk Jensen¹, Kamille Dumong Erichsen², Camilla Bardram Johnbeck¹, Fredrik Björkling², Jacob Madsen³, Peter Buhl Jensen², Maxwell Sehested², Liselotte Højgaard¹, Andreas Kjær¹. ¹Cluster for Molecular Imaging, Univ. of Copenhagen and Dept. of Clinical Phys., Nuclear Med. & PET, Copenhagen, Denmark; ²Topotarget A/S, Copenhagen, Denmark; ³Dept. of Clinical Phys., Nuclear Med. & PET, Rigshospitalet, Copenhagen, Denmark

Abstract: Introduction: Belinostat is a histone deacetylase inhibitor with anti-tumor effect in several pre-clinical tumor models and clinical trials. The aim of the study was to evaluate early changes in cell proliferation and glucose uptake by use of 3'-deoxy-3'- $[^{18}\text{F}]$ fluorothymidine (FLT) and 2-deoxy-2- $[^{18}\text{F}]$ fluoro-D-glucose (FDG) positron emission tomography (PET) following treatment with belinostat alone or in combination with carboplatin and paclitaxel (CaP).

Methods: In vivo uptake of FLT and FDG in human ovary cancer xenografts in mice (A2780) was studied after treatment initiation. When tumor volumes were approximately 100 mm³ mice were divided in 4 groups receiving belinostat, CaP, combination of these (BelCaP) or vehicle. Doses were 40 mg/kg ip twice daily for 10 days for belinostat, 10 mg/kg iv day 2+9 for paclitaxel and 40 mg/kg ip day 2+9 for carboplatin. Baseline FLT or FDG scans were made before treatment day 0 and repeated at day 3, 6 and 10. FLT uptake was quantified using small animal PET/CT. One hour after iv injection of 10 MBq FLT or FDG, PET scans were performed for 10 minutes and region of interests (ROIs) covering whole tumor were defined on PET/CT images for calculation of standard uptake values (SUV).

Results: Tumors in the control group had volumes that were $769\pm 74\%$ (926 mm³) at day 10 relative to baseline day 0. In the belinostat group tumors were $462\pm 62\%$ (640 mm³) ($P=0.004$ vs. control), in the CaP group $359\pm 41\%$ (490 mm³) ($P<0.001$ vs. control), and in the BelCaP group $332\pm 35\%$ (341 mm³) ($P<0.001$ vs. control) all at day 10 relative to baseline.

On day 10 FLT SUV_{max} was significantly lower in the CaP group (-21.5%; $P<0.05$) and BelCaP group (-35.6%; $P<0.001$) compared to control. On day 3 FLT SUV_{max} was significantly lower for the CaP (-23.4%; $P<0.05$) and the BelCaP group (-22.1%; $P<0.05$) compared to the

Belinostat group. On day 10 FDG SUVmax was significantly decreased in the belinostat group (-22.4%; $P < 0.05$), the CaP group (-27.4%; $P < 0.01$) and the BelCaP combination group (-29.1%; $P < 0.001$) compared to control.

Within treatment groups FLT SUVmean day 3 was significantly correlated with relative tumor volume day10/day0 in the belinostat group ($R^2 = 0.67$; $P = 0.025$) but not in the CaP and BelCaP groups. FDG SUVmean day 3 was not correlated with relative tumor volume day10/day0 in any of the treatment groups, however FDG SUVmean day 6 correlated significantly with tumor volume day10/day0 in the belinostat group ($R^2 = 0.68$; $P = 0.023$).

Conclusions: In the belinostat group FLT uptake day 3 and FDG uptake day 6 predicted relative tumor volume changes at day 10. FDG uptake was significantly decreased day 10 for all treatment groups compared to control. Our data indicate that FLT and FDG PET may be used for non-invasive assessment of anti-tumor effects of belinostat both alone and in combination with carboplatin and paclitaxel.

Abstract Number: 178

Pre-treatment with histone deacetylase inhibitor HDACI) synergizes the anti-cancer effect of sorafenib in renal cell carcinoma cells Author: Dong Eun Kim¹, Mi Joung Kim¹, Sejin Jang¹, Je-Hwan Lee¹, Jene Choi¹, Seonggu Ro², Choung-Soo Kim¹, Jung Jin Hwang¹. ¹Univ. of Ulsan Asan Medical Ctr., Seoul, Korea, Republic of; ²CrystalGenomics, Inc., Seoul, Korea, Republic of

Abstract: Background: Renal cell carcinoma (RCC), account for 3% of all adult malignances and 75% of RCCs are clear cell renal cell carcinoma (ccRCC), which are highly vascularised tumors. Although a multi-kinase inhibitor, sorafenib has been used for treatment of advanced renal cell carcinoma, it has limitation such as toxicity and long term resistance. Recently, it was reported that histone deacetylase (HDAC) activity is increased in cancer, which leads to suppression of the genes that are related to tumor suppressor and differentiation. Therefore, histone deacetylase inhibitors (HDACIs) are considered as a new anti-cancer drug. In this study, we evaluated the effect of HDACI on anti-tumor effect of sorafenib in ccRCC cells.

Materials and Methods: We used various types of RCC cells including Caki-1(VHL+/+), ACHN (VHL+/+), 786-O (VHL-/-), A498 (VHL-/-), SN12C, TK-10, RFX393, and MDR-over-expressing UO31 cells. We calculated the viability of RCC cells treated with HDACIs (belinostat or vorinostat) alone or in combination with sorafenib using MTS Assay. Combination indexes were determined by CalcuSyn software. We analyzed activation of caspases, and the levels of Mcl-1, Bcl-xl, p-Erk by Western Bolt. Levels of secreted vascular endothelial growth factor (VEGF) were measured by ELISA kit.

Results: Mono-treatment with HDACI (belinostat or vorinostat) induced apoptosis via activation of intrinsic and extrinsic pathways in all RCC cells. Moreover, pre-treatment with belinostat or vorinostat synergistically potentiated cell death induced by sorafenib in Caki-1 and 786-O ccRCC cells. The combination treatment activated caspase-8, -3 and -9 and down-regulated Mcl-1, Bcl-xl, phosphorylation of Erk (p-Erk) and secretion of VEGF in Caki-1 and 786-O cells.

Conclusions: HDACI alone induced apoptotic cell death in various RCC cells. In addition, combination treatment with HDACIs and sorafenib represented synergistic anti-cancer effect in ccRCC cells, indicating that this combination may be a new therapeutic modality for RCC.

Abstract Number: 4547

Treatment of human medullary thyroid carcinoma (MTC) with either proteasome (Pr) or histone deacetylase (HDAC) inhibitors leads to a fall in RET mRNA levels and, in turn, a decrease in RET protein expression providing alternate strategies to reduce RET expression in a tyrosine-kinase driven disease

Author: Marianne S. Poruchynsky, Ann W. Gramza, Samuel A. Wells, Tito Fojo. NIH-NCI, Bethesda, MD

Abstract: Medullary thyroid carcinoma (MTC), a neuro-endocrine cancer of parafollicular C-cells accounts for 4-10% of all thyroid cancers and has low response rates to “cytotoxic chemotherapy”. The RET proto-oncogene encodes the RET (Rearranged during Transfection) transmembrane tyrosine kinase (TK) receptor, a pivotal player and a logical target for treatment of MTC. While trials with vandetanib (V) a RET TK inhibitor (TKI) in patients with MTC have been promising, we recognized a TKI is unlikely to have maximum benefit administered alone. We sought to combine V + a drug with a broad activity profile and no overlapping toxicities. Recognizing the failure of traditional “cytotoxic” agents we investigated the proteasome (Pr) inhibitor, bortezomib (B) not yet explored clinically in MTC. Initial experiments using TT MTC cells with a mutant C634W RET, found V + B non-antagonistic in cytotoxicity assays, and V showed decreased phospho-RET expression in TT cells with 0.5 or 1 μ M in 1.5h. But while V did not change RET protein levels, B decreased RET protein 7 fold and secondarily phospho-RET levels. That this was secondary to Pr inhibition and not unique to B was confirmed by treating TT cells 6h or 24h with three other Pr inhibitors - epoxomicin, lactacystin or MG132. We observed 5-9 fold decreases in RET protein, which followed a time course and a dose response, with greater reduction at 24h than at 6h. To assess if the decrease in RET protein was due to decreased RET mRNA levels, we assessed RET mRNA expression and were surprised to find a 4-9 fold decrease with Pr inhibitors. Since Pr inhibitors reduced RET by reducing mRNA levels, and reasoning this would provide an alternate strategy to impact RET function, we studied the effect of three HDAC inhibitors on RET mRNA and protein expression. Treatment of TT cells with vorinostat, belinostat, or romidepsin (R) decreased RET mRNA 3-4 fold at 24h and protein levels 4 and 7-14 fold, at 24 and 48h respectively. Furthermore B + R had greater impact than either drug separately, depressing RET protein in TT cells an additional 2-3 fold to levels 4-12 fold less than that in untreated cells, suggesting mechanisms whereby Pr and HDAC inhibitors reduce mRNA levels might differ. We have pursued the latter by examining mRNA and protein levels of 3 candidate transcription factors identified in a literature search. To date we have shown TTF1 and E2F1 mRNA levels are decreased 4-58 fold by both Pr and HDAC inhibitors whereas EGR1 levels only fall with Pr inhibitors. siRNA studies that modulate transcription factor levels will clarify their possible roles in the Pr and HDAC inhibitor drug effects and help elucidate whether these inhibitors, either alone or in combination, can prove useful in MTC therapy by reducing the crucial RET TK.

Abstract Number: 597

The cellular and genomic response to histone deacetylase inhibitors in diffuse large b-cell lymphoma

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Abstract: Diffuse Large B-cell lymphoma (DLBCL) is the most frequent subtype of Non-Hodgkin Lymphoma (NHL) in all countries around the world and in all age groups. Several drug regimens have been used in treatment of DLBCL; however, this disease remains eventually fatal in 30 - 40% of the patients. Chemotherapy resistance can be partly

explained by the fact that DLBCL is a heterogeneous group of NHLs, with the two most prevalent subtypes being “Activated B-cell Like” (ABC) and “Germinal Center B-cell like” (GCB). Patients with the ABC subtype have the poorest prognosis under the current treatment regimen. Therefore, there is a pressing need for new therapeutics that can increase survival rates in DLBCL patients. Histone deacetylase inhibitors (HDIs) have proven to be promising drugs in the treatment of blood malignancies. Even though their mechanism of action has not been fully characterized, two HDIs, (Vorinostat and Romidepsin) have been approved for the treatment of cutaneous T-cell lymphoma (CTCL). Therefore the purpose of the current study is to investigate the response of DLBCL subtypes to HDIs, with a particular focus on subtype-specific mechanisms of action. Our current working hypothesis is that a comprehensive analysis of the genomic and proteomic response to histone deacetylase inhibitors (HDIs) including gene expression and transcription factor acetylation will reveal both mechanisms and potential biomarkers of HDI action in lymphomas DLBCL.

In the current study we have focused on the cellular and genomic effects of the hydroxamate HDI, Belinostat (PXD101), on cell lines representing the GCB subtype of DLBCL. We show that PXD101 inhibits growth of four GCB-type cell lines with 24 h IC50s in the low micromolar range (SUDHL6 = 0.15uM, OCI Ly19 = 0.3uM, SUDHL4 = 0.45uM, DB = 0.77uM). Flow cytometry analysis has shown that three of these cell lines (SUDHL6, OCI Ly19 and DB) arrest in the G2/M phase of the cell cycle by 24 hours of treatment at the IC50 dose and then die by apoptosis. In contrast, the SUDHL4 cell line reversibly arrests in the G1 phase without undergoing cell death. Western blot analysis of PARP and caspase-3 cleavage has further confirmed the presence/absence of apoptosis. We suggest that the SUDHL4 cell line represents DLBCL tumors that are refractory to the apoptosis-inducing effects of HDIs. Thus, we are using this cell line to identify other therapeutics which could be used in combination with PXD101 to induce cell death. The mechanistic basis for the differential cellular response between the GCB type cell lines is currently under investigation using expression profiling data obtained from OCI Ly19 and SUDHL4 cells treated with PXD101. Preliminary data indicates divergent responses in expression of GADD45 and p21, the Myc/Max family of proteins, and the clock genes, Per1 and Cry2.

Abstract Number: 2624

Elevated expression of phosphorylated mitogen activated protein kinase kinase (MEK) as a mechanism of resistance to the histone deacetylase inhibitor romidepsin in HUT 78 cutaneous T-cell lymphoma cells

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Abstract Number: 2624

Elevated expression of phosphorylated mitogen activated protein kinase kinase (MEK) as a mechanism of resistance to the histone deacetylase inhibitor romidepsin in HUT 78 cutaneous T-cell lymphoma cells

Abstract: Elevated expression of phosphorylated mitogen activated protein kinase kinase (MEK) as a mechanism of resistance to the histone deacetylase inhibitor romidepsin in HUT 78 cutaneous T-cell lymphoma cells.

Histone deacetylase inhibitors (HDIs) have shown promise in the treatment of T-cell lymphomas including cutaneous and peripheral T-cell lymphomas. However, resistance to romidepsin limits its activity in some patients. A detailed understanding of the mechanisms of resistance to HDIs may lead to strategies designed to increase clinical efficacy. To study mechanisms of resistance to the HDI romidepsin, the HUT78 cutaneous T-cell lymphoma cell

line was exposed to increasing concentrations of romidepsin in the presence of the P-glycoprotein (P-gp) inhibitors verapamil or valspodar (PSC-833) to prevent the emergence of P-gp, a known resistance mechanism. The DpVp35 and DpVp50 sublines are maintained in 35 ng/ml and 50 ng/ml romidepsin, respectively, in the presence of 5 µg/ml verapamil while DpP75 is maintained in 75 ng/ml romidepsin and 3 µg/ml valspodar. In 4-day cytotoxicity assays, the sublines are approximately 55-fold resistant to romidepsin and are not cross resistant to the HDIs belinostat, panobinostat or vorinostat. Low but detectable levels of P-gp do not explain the resistance. We used a custom drug resistance gene expression array and found increased expression of insulin receptor (IR) in the resistant cells that was confirmed by immunoblot analysis. Elevated expression of phosphorylated mitogen activated protein kinase kinase (MEK), a downstream effector of the IR pathway, was also observed in the resistant cells compared to the parental cells. Interestingly, resistant cells were found to be exquisitely sensitive to MEK inhibition, as significant apoptosis was observed after 48 h in the presence of 5 nM of the MEK inhibitor PD0325901 and 10 nM of the MEK inhibitor AZD 6244 as measured by flow cytometry with annexin V and by immunoblot examining poly (ADP-ribose) polymerase (PARP) cleavage. No significant apoptosis was observed in parental cells at concentrations up to 500 nM. Resistant cells were not, however, sensitive to extracellular related kinase (ERK) inhibition or phosphatidylinositol 3-kinase (PI3K) inhibition in as determined by annexin V assay. In summary, we hypothesize that activated MEK can mediate resistance to romidepsin, but may also lead to collateral sensitivity to MEK inhibitors. The emerging role of activated MEK as a mechanism of resistance to romidepsin suggests combination of romidepsin with MEK inhibitors in future clinical trials.