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.

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

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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.

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

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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.

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

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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.

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

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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