photo-affinity linker and the cell lysates that overexressed DsRed or DsRed-fused Pirin were used.
Results: We identified a small-molecule that binds to Pirin by using the chemical array screening method. The K_d value of the small-molecule to Pirin was 614 nM by isothermal titration calorimetry experiments. The pull-down assay showed that the small-molecule inhibited the interaction of Pirin with Bcl3 *in vivo* and *in vitro*. To get a insights into the effect of the small-moleclue against cells, we examined the cytotoxic activity against a number of different human cancer cell lines. As a result, the small-molecule did not show potent cytotoxic activity. In contrast, the inhibition of migration in melanoma cells was observed in the dose-dependent treatments of the small-molecule, but not the treatment of the negative control that does not bind to Pirin. We found that the treatment with the cultured tumor cells with the small-molecule or Pirin-targeted siRNA resulted in suppression of migration, but not cell proliferation.
Conclusions: We have discovered the first inhibitor of Pirin, functions in cellular system is unclear. The inhibitor disrupted the interaction of Pirin with Bcl3 *in vivo* and *in vitro*. By use the inhibitor and siRNA, it is suggested that Pirin regulates migration of the tumor cells, and the inhibitor of Pirin might be a new chemotherapeutic potential for invasive tumors.

356 POSTER

An innovative platform technology accelerates drug screening

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Fluorescence Correlation Spectroscopy (FCS) and Fluorescence Cross-Correlation Spectroscopy (FCCS) are biophysical techniques that enable precise and sensitive analysis of molecular interactions in solution. FCCS allows analysis of reactions in realtime at nanomolar concentrations and can be applied to the monitoring of binding events in cellular lysates and living cells.
FCS and FCCS are single molecule sensitive fluctuation analysis methods; in contrast to other fluorescence techniques, the parameter of primary interest is not the emission intensity itself, but rather spontaneous intensity fluctuations caused by minute deviations from thermal equilibrium. Consequently, FCS/FCCS can only function properly if concentrations and observation volumes are reduced such that only few molecules are simultaneously detected. For this, the observation volume is defined by strongly focused, overlapping excitation laser beams and a confocally arranged optical setup. Fluorescent molecules entering and leaving this open, illuminated spot of below 10^{-15} liters volume by diffusion emit photons, as long as they dwell in the observation focus. In combination with single photon sensitive detectors the photon count trace is recorded and subjected to a mathematical operation, the auto- and cross correlation analysis, to extract information on particle number, brightness, size and interactions.
The authors will demonstrate the application of FCCS addressing several relevant cancer targets and reference inhibitors. Esp. the quantitative determination of binding constants within cellular lysates enables the efficient and sensible characterization of kinase inhibitors with long residence time.

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Antitumor triazoloacridinone C-1305 as a potent FLT3 tyrosine kinase inhibitor in human acute myeloid leukemia (AML) cells

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Background: An internal tandem duplication (ITD) in the juxtamembrane domain of Fms-like tyrosine kinase 3 (FLT3) is the most common molecular defect associated with acute myeloid leukemia (AML) and predicts for poor prognosis. This mutation causes constitutive activation of the FLT3 receptor in the absence of natural FLT3 ligand (FL). Here, we investigated whether antitumor triazoloacridinone C-1305, selected for extended preclinical trials, exhibits inhibitory activity toward FLT3 kinase.
Materials and Methods: C-1305 activity was evaluated in human leukemia cell lines, MV4;11 (FLT3 ITD) and RS4;11 (FLT3 WT). The inhibitory effect of C-1305 on FLT3 receptor activation was determined by analysis of protein phosphorylation by ELISA. Flow cytometry for annexin V/PI assay, caspase-3 activation and sub-G1 DNA fraction was applied to identify apoptosis. DAPI staining was used to analyse cellular morphology.
Results: Ligand-independent phosphorylation of FLT3 in MV4;11 cells was inhibited by C-1305 with an IC_{50} of approximately 7 μM. Treatment of MV4;11 cells with the drug for 72 h suppressed cell proliferation with EC_{50} concentration equal to 0.2 μM. To determine whether blocking of FLT3 activity modulates cellular response upon C-1305 treatment, MV-4;11 cells were exposed to various drug concentrations. C-1305 was found to exerts its cytotoxic effect by the induction of apoptosis as evidenced by chromatin condensation, phosphatydilserine externalization, caspase-3 activation and presence of sub-G1 fraction. In all tests, the number of apoptotic cells increased after treatment with C-1305 in a time- and dose-dependent manner and reached about 80% after 72 h of drug exposure. Importantly, apoptotic cell death was observed at concentration of drug required to block FLT3 phosphorylation, suggesting that inhibition of FLT3 kinase by C-1305 may account for its cytotoxic activity in MV4;11 cells. Since majority of AML harbor WT-FLT3, we further tested the effect of C-1305 on RS4;11 cells. Significantly higher concentration of the drug (EC_{50} 1,8 μM) was required to inhibit the growth of RS4;11 cells, compared to MV4;11, suggesting potent selectivity of C-1305 towards FLT3 ITD cells.
Conclusions: Our results indicate that C-1305 shows high potency and selectivity toward acute leukemia cells harboring FLT3 ITD activating mutations as evidenced by inhibition of cellular proliferation, induction of apoptosis and inhibition of FLT3 autophosphorylation in these cells.

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Study of cetuximab and panitumumab activity in an 80 cell line panel to evaluate specificity of small molecule EGFR inhibitors

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Developed and broadly applied in the Developmental Therapeutics Program of NCI/NIH, multiple cell line panels became essential platforms for anticancer drug discovery and development efforts. An activity profile of an anticancer agent in a large cell line panel can lead to hypotheses about its mode of action when compared with reference agents. Antibodies with naturally inherited specificity of action could be better reference molecules, but they are hardly ever used for these purposes. This can be explained by a number of factors. First, antibody responses cannot be described by a common sigmoid dose response curve. Therefore, statistical correlation methods cannot be applied for comparison as performed with small molecules. Secondly, antibody activity *in vivo* is linked to secondary responses, e.g. Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC), which partially explain only minor effects of antibodies on the growth of tumor cell lines *in vitro*.
We will present an approach to implementing activity profiles of antibodies for studying the mode of action of small molecules. We tested cetuximab and panitumumab in a panel of 80 cell lines *in vitro*. Both agents were applied to cells in a range of 0.01 μg/ml to 100 μg/ml for 72 hours, and their activity was measured by the sulforhodamine B assay. Based on the dose response curve we selected three different groups of cell lines. The first group included cell lines, e.g.NCIH292, most probably dependent on EGFR activation, and very sensitive (already at 0.1 μg/ml) to the antibody action. The second group comprised cell lines, e.g. A431, that were less sensitive to the antibodies. Resistant cell lines were combined into the third group. Activity profiles of small molecules were used to match activity patterns of these three groups. Among more than 300 reference anticancer agents, the strongest similarity was observed for EGFR inhibitors, e.g. lapatinib or erlotinib. Less, but still significant similarity was observed for agents known to be involved in the EGFR pathway: some Akt, MEK and PI3K inhibitors. These results indicate that antibodies can be applied to study small molecule's mode of action. In addition, we observed that the molecular profile of cell lines sensitive to the antibodies correlated well with the known clinical outcome for these antibodies. This fact suggests that larger sets of cell line models should be considered for pre-clinical evaluation of antibodies independently from an ADCC study.

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Effect of the TGF-beta 2 specific antisense oligodeoxynucleotide trabedersen on TGF-beta 2 and -beta 1 expression in human glioma cells: Cross-regulatory loops regulate TGF-beta isoform expression

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Background: Transforming growth factor-beta (TGF-beta) plays a key role in cancer progression, by regulating crucial cancer mechanisms. Three mammalian isoforms, i.e. TGF-beta 1, 2, and 3, have been isolated. TGF-beta 2 plays a particular role in several cancers, and our newly developed therapeutic approach based on the specific inhibition of TGF-beta 2 synthesis by the antisense oligodeoxynucleotide trabedersen (AP 12009) has been successfully tested in clinical trials in patients with advanced stage glioma, pancreatic cancer or malignant melanoma.
Apart from TGF-beta 2, most tumors also express considerable amounts of TGF-beta 1. Since auto-induction and cross-regulation of different TGF-beta isoforms has been reported in some tissues, we were investigating, if this phenomenon is also present in human tumor cells and