included abdominal or pelvic pain/discomfort [7 events; Gr1/2 (4), Gr3 (3)], infusion site reaction/thrombophlebitis [5 events; Gr1/2], malaise/fatigue [5 events; Gr1/2], and nausea [4 events; Gr1/2]. One Gr4 and eight Gr3 AEs, all attributed to underlying cancer, were observed and included pulmonary embolus (Gr4), abdominal/pelvic pain (3), ascites (2), pleuritic chest pain, vomiting, and constipation. The most common lab abnormalities observed during treatment included hyperbilirubinemia [4 pts; Gr1/2 (3), Gr3 (1)], hyperglycemia (4 pts; Gr1), and anemia [4 pts; Gr1/2 (4)]. Two Gr3 lab abnormalities were noted and included hypermagnesemia and hyperbilirubinemia that occurred in the presence of disease progression involving the liver. No DLTs were observed. To date, no consistent effects on hematologic parameters were observed. Preliminary PK data indicate that AUC and Cmax were proportional across doses; mean values were CL (~75 L/hr), Vss (~900 L), and t1/2 (~13hr). At the doses administered thus far, there is no evidence of anti-tumor activity.
Conclusions: No consistent mechanism-related toxicity has been observed and an MTD has yet to be defined. Dose escalation continues.

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A small molecule allosteric inhibitor of Polo-like kinase 3 induces apoptosis and disrupts the integrity of the mitotic spindle apparatus in cancer cells

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Proper functioning of the mitotic spindle apparatus is paramount for normal cellular division and maintenance of genomic integrity. Amplification and/or over-expression of proteins within the Aurora/centrosome signaling pathway, which include members of the Aurora and Polo like kinase families, lead to mitotic instability and malignant progression in many solid tumors. For these reasons, many pharmaceutical companies have created programs to identify inhibitors of the Aurora and Polo kinases. Utilizing a gene expression biomarker based platform (AvalonRx) we identified small molecule compounds that disrupt the centrosome signaling pathway. One compound series, LC-445, were predicted to be novel kinase inhibitors based upon gene expression biomarker profiling and comparison to a database of gene expression profiles from hundreds of small molecule drugs with known mechanisms. To identify the kinase targets of the compound series, we tested a panel of 220 kinases using a Fluorescent Resonance Energy Transfer-based assay. The compound was found to be highly selective with activity against only a few kinases, most notably of which was Polo-like Kinase 3. Polo-like Kinase 3 has been reported to mediate multiple mitotic process including bipolar spindle formation, activation of CDC25C, centrosome maturation and activation of the DNA damage response. LC-445 compounds induce cell cycle arrest and apoptosis and are broadly active against a large panel of malignant cell lines. We found that LC-445 causes a decrease in phosphorylation of Ser-191 of CDC25C (the activation site of CDC25C by PLK3) in a concentration dependent manner. Kinetic studies show that LC-445 is a non-ATP competitive, allosteric inhibitor of PLK3. Furthermore, LC-445 compounds affect not only PLK3 enzymatic activity but decrease levels of PLK3 protein both in cell culture and in xenograft animal models. Importantly, treatment of cancer cells with LC-445 induced formation of monopolar and tripolar spindles, abnormal chromosome alignment and a disruption of spindle structure reminiscent of the known spindle check point inhibitors. These data show that LC-445 compound series is a novel, specific allosteric inhibitor of PLK3 that inhibits kinase activity and PLK3 protein levels leading to mitotic catastrophe and subsequent cell death in cancer cells.

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Crystal structures of Plk1 kinase domain in complex with ATP-competitive inhibitors

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Background: Polo-like kinase 1 (Plk1) is a serine/threonine protein kinase involved in several processes during mitosis. It belongs to the Polo-like-kinase family, comprising the structurally related Plk1, 2, 3 and 4 proteins. All of these proteins are characterized by an N-terminal kinase domain and a C-terminal “polo-box” domain. Pkl1 is ubiquitously expressed in normal tissues and is over-expressed in a wide variety of human tumours, where it also correlates with poor prognosis. Inhibition of Plk-1 expression by siRNA or DNA antisense oligonucleotides further validates Plk1−1 as an attractive target for anticancer drug therapy.
Material and Methods: In order to help in the design of Plk1 small-molecule inhibitors, we initiated a program to determine the crystal structure of the Plk1 catalytic domain. A total of 33 protein constructs bearing different combinations of the N- and C-termini of the catalytic domain were cloned, expressed and purified for crystallization trials.
Results: In the end we succeeded in growing crystals by co-crystallization of the methylated construct Plk1(36–345) with the ATP analog AMP-PNP. The crystals belong to space group P3221 and have one molecule in the asymmetric unit. The resulting structure was solved at a resolution of 2.0 Å and comprises the residues 39–328. Interestingly, the crystal contains a dimer of symmetry-related molecules tethered by zinc ions, a feature that was also observed by other groups (1). Subsequently we solved the structure of Plk1(36–345) in complex with different classes of Plk1 inhibitors and the analysis of these complexes allowed us to identify key structural features of the investigated inhibitors. This information proved helpful in guiding the chemical expansion and obtaining potent and selective Plk1 inhibitors.
Conclusions: The crystal structure of Plk1(36–345) construct with different inhibitors will be shown and key features within the different chemical classes will be discussed in the poster.

References

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Antitumoral activity of pyrazoloquinazoline derivatives as potent oral Plk-1 specific inhibitors

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Background: Polo-like kinase 1 (Plk-1) is a serine / threonine protein kinase involved in different stages of mitosis with roles in centrosome maturation, bi-polar spindle formation, chromosome separation and cytokinesis. The expression, activity and localization of Plk-1 is dynamically regulated during the cell cycle and PLK-1 protein levels increase from the late S phase to mitosis. Plk-1 is over-expressed in a variety of human tumours including lung, colon, stomach, breast, ovary, head and neck, and melanomas where often correlates with poor prognosis. Inhibition of Plk-1 expression by siRNA or DNA antisense oligonucleotides further validates PLK-1 as an attractive target for anticancer drug therapy.
Material and Methods: From HTS screening several small molecules belonging to the pyrazoloquinazoline class emerged as interesting hits to target Plk-1 kinase. Chemical modifications at the R1, R2 and R3 residues of the pyrazoloquinazoline core scaffold reported in the figure resulted in new compounds with favourable drug-like properties. The Co-crystal structure of methylated construct Plk-1 (36–345) with some of the most interesting compounds was also determined.
Results: These compounds posses sub-nanomolar Ki in a Plk-1 biochemical assay, accompanied by a very high selectivity towards a panel of more than 250 kinases and no cross-reactivity with the other PLK family members. The compounds exhibit high potency in an antiproliferation assay having IC50 < 100 nM on a large number of cell lines, both from solid and haematological tumors, while demonstrating excellent PK properties and good oral bioavailability in rodent and non-rodent species. Oral administration of compounds causes tumor stabilization or regression in a variety of tumor xenograft models using flexible schedules. Notably also prolonged daily administration is well tolerated.
Conclusions: In summary, the data suggest that some members of this chemical class are very potent antiproliferative agents suitable for further development as oral and selective Plk-1 inhibitors for anticancer therapy.