Results: Dose response testing showed 2/4 neuroblastoma and 3/3 ALL models achieving an objective response at 10 mg/kg (50% of MTD), with the most sensitive neuroblastoma model (NB-1643) achieving an objective response at 5 mg/kg and with 2/3 ALL models showing good leukemia growth control during treatment at 5 mg/kg. Two additional neuroblastomas were not responsive to MLN8237 at a dose of 20 mg/kg. MLN8237 induced an increase in mitotic index and %pHistH3 positive cells following a single dose of agent that peaked at 12 hrs, returning to baseline levels within 24 hrs.
Conclusions: Dose response testing indicates MLN8237 efficacy at 50% of its MTD in a subset of responsive neuroblastoma and ALL models. PD studies are consistent with in vivo anti-neuroblastoma activity through inhibition of Aurora A kinase. Further preclinical studies of MLN8237 focusing on combinations with other agents are anticipated, and pediatric clinical development of MLN8237 is proceeding.
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Fragment-based discovery of AT9283; a multi-targeted kinase inhibitor with potent Aurora kinase activity

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Today there is widespread acceptance that chemical fragments can be progressed into nanomolar (nM) lead series and on into clinical candidates. In the field of oncology there are a number of compounds, currently undergoing clinical evaluation, which can be attributed to a fragment-based approach. Examples include AT7519 (CDK inhibitor), ABT-263 (Bcl-XL inhibitor), AT13387 and NVP-AUY922 (both Hsp90 inhibitors). Here we describe the fragment-based discovery of AT9283, a multi targeted kinase inhibitor with potent Aurora kinase activity, which is currently in clinical trials. In this work, a low molecular weight heterocyclic fragment provided the starting point for a structure-based medicinal chemistry programme. Typically, a detailed structural understanding of the binding interactions between the fragment and its target protein is required to pursue a fragment-based approach. In this case, X-ray crystallographic structures were generated using a novel soakable form of Aurora A and were used to drive the optimisation towards potent (<10 nM) dual Aurora A/Aurora B inhibitors. These compounds inhibited growth and survival in HCT116 cells and produced the polyploid cellular phenotype typically associated with Aurora kinase inhibition. Optimisation of cellular activity and physicochemical properties ultimately led to the identification of AT9283. In addition to Aurora A and Aurora B, AT9283 was also found to inhibit a number of other kinases including JAK2, Flt3 and Abl T315I (<10 nM). AT9283 demonstrated excellent in vivo efficacy in mouse xenograft models and was selected for pre-clinical development.
In conclusion, low molecular weight fragments provide good chemical starting points for the discovery of drug candidates. During this programme, structure-based optimisation of a heterocyclic fragment led to the identification of AT9283 which is currently in Phase I clinical trials for the treatment of cancer.
The structure of AT9283 will be fully disclosed at the meeting.

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The selective Aurora B kinase inhibitor AZD1152 inhibits in vitro growth in small cell lung cancer (SCLC) cell lines

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Background: Aurora B kinase (AURKB) regulates mitotic histone modification, chromatid separation and cytokinesis, and is overexpressed in a range of cancers. Analysis of Affymetrix gene expression profiles revealed AURKB overexpression in our panel of small cell lung cancer (SCLC) lines compared with normal lung. In this study we investigated the effects of the selective aurora B kinase inhibitor AZD1152 in a panel of 14 SCLC lines; nine with known p53 and Rb status, and five with unknown status.
Methods: In vitro analyses included growth inhibition by MTT assays. Changes in DNA ploidy and cell cycle arrest were evaluated by FACS analysis. Cell-line p53 status was determined by direct sequencing. Rb mutational status was determined from the literature.
Results: AZD1152 treatment resulted in cytotoxic effects in 6-day MTT assays in five lines with IC50s in the therapeutically relevant range of 5–300 nM. A further five lines exhibited primarily cytostatic effects at 6 days post drug with IC50 values of 30–100 nM although cell death could be induced by extending the incubation time to 12 days and increasing AZD1152 concentrations above the initial IC50 value. The remaining four lines showed no growth inhibition in a 6-day assay and only one line reverted to cytotoxicity in an extended assay (IC50 ~25 nm). p53 and Rb mutational status did not correlate with sensitivity to AZD1152 in the MTT assays. G2M cell cycle arrest with no changes in DNA ploidy was observed at 24H hours post AZD1152 in p53-Rb wild-type lines. At 48 hours, however, these wild-type cells had progressed to 4N ploidy and a G1 arrest. SCLC lines mutant for both p53 and Rb went from 2N (S arrest) to 4N (G1 arrest) in 24 hours. Cell cycle changes in a SCLC line with wild-type p53 and mutant Rb mirrored the double mutants at 24 hours. Finally a SCLC line mutant for p53 alone saw an increase to 4N ploidy and arrest in G2M.
Conclusions: AZD1152 inhibited the growth of SCLC lines, induced increases in DNA ploidy and complex patterns of cycle arrest. Future studies will evaluate cell death mechanisms and changes in histone3 phosphorylation by FACS and FISH analyses will evaluate the AURKB gene copy number and potential correlation with AZD1152 sensitivity.

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Aurora A kinase inhibition abrogates the mitotic delay induced by microtubule perturbing agents

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Background: The spindle assembly checkpoint functions during mitosis to ensure that chromosomes are properly aligned in mitotic cells prior to the onset of anaphase, thereby securing an even segregation of genetic material to each daughter cell. Defects in the function of this checkpoint lead to aneuploidy, and eventually to cell death or senescence. Aurora B kinase has been shown to play a role in regulating the spindle assembly checkpoint. In this study, we demonstrate that Aurora A activity is required for maintainance of the spindle assembly checkpoint-mediated mitotic delay induced by microtubule perturbing agents.
Methods and Results: Inhibition of Aurora A using MLN8054, a selective small-molecule inhibitor of Aurora A, in paclitaxel- or nocodazole-pretreated cells induces cells to become multinucleated. This phenotype is consistent with disrupted spindle assembly checkpoint activity. Using time-lapse video microscopy, we demonstrate that this phenotype arises via mitotic slippage, which is accelerated upon Aurora A inhibition. Moreover, we use flow cytometry and western blot analysis to demonstrate that Aurora B remains active in these mitotic cells, strongly suggesting that the mitotic slippage induced by MLN8054 is due to the inhibition of Aurora A. This finding was corroborated by demonstrating that Aurora A depletion using RNA interference in paclitaxel-treated cells also induces multinucleation. When Aurora A is inhibited in the presence of paclitaxel, the spindle assembly checkpoint protein BubR1 remains localized to kinetochores. This suggests that the mitotic slippage induced by Aurora A inhibtion does not directly involve depletion of the spindle assembly checkpoint signal, but occurs through another mechanism related to checkpoint adaptation.
Conclusions: Taken together, these results suggest that Aurora A participates in prolonging the mitotic arrest activated in response to microtubule-perturbing agents. This finding may have implications in how Aurora A inhibitors will respond in combination with other anti-mitotics in a clinical setting.

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Pyrrolo[2,1-f][1,2,4]triazine-based inhibitors of Aurora kinases

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Protein kinases have emerged as key regulators of cellular functions, including cell growth, differentiation, and apoptosis, leading to extensive efforts to develop small molecule kinase inhibitors for the treatment of a wide range of cancers. Members of the Aurora family of serine/threonine kinases have recently drawn intense attention because of increasing evidence that links these kinases to oncogenesis and their regulatory roles in mitotic progression. Aurora A localizes to centrosomes and has a crucial role in mitotic spindle formation, whereas Aurora B is a 'chromosomal passenger' protein that is required for chromosome segregation and cytokinesis. Aurora A and B are overexpressed in a wide range of human primary tumors. Using RNA interference (siRNA specific for each Aurora kinase), we have shown that depletion of Aurora A and B kinases causes polyploidy, apoptosis, and decreased tumor cell survival. Depletion of Aurora C also potently induced apoptosis but did cause polyploidy