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.
(Supported by NCI NO1CM42216.)

287 POSTER

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

suggesting a distinct biologic role for this family member. Medicinal chemistry efforts have identified pyrrolo[2,1-f][1,2,4]triazine-based inhibitors of Aurora kinases. Structure–activity relationships were developed by introducing substituents at C4 and C6 positions of the pyrrolotriazine to optimize the potency of kinase inhibition. The lead Aurora kinase inhibitors caused dose dependent apoptosis and cell cycle effects that are consistent with the effects of Aurora A or B depletion. These results suggest that targeting the Aurora kinases present a novel opportunity for anti-mitotic cancer therapy.

Cyclins and CDK's

291 POSTER

Discovery of selective CDK9 small molecule inhibitors: CDK9 inhibition in tumor cells is associated with inhibition of proliferation and induction of apoptosis

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Background: CDK9 (in complex with Cyclin T or K) phosphorylates the C-terminal domain of RNA polymerase II (RNAPII CTD) as a required step for mRNA transcript elongation. CDK9 inhibition causes the rapid depletion of short-lived mRNA transcripts and their associated protein products. Since many genes encoding proteins involved in cell growth, proliferation, and survival are characterized by short-lived mRNAs and proteins, the consequences of CDK9 inhibition are expected to include anti-proliferative and pro-apoptotic effects through loss of function at many cellular pathways. Proteins with well-established roles in tumor development and growth that are depleted following CDK9 inhibition include Myc, Cyclin D1, and Mcl-1.
Methods: CDK9-selective small molecule inhibitors were discovered by high-throughput screens and chemical optimization using biochemical and in vitro cell-based assays, as well as x-ray crystallographic information from CDK9-inhibitor co-complexes.
Results: Identified CDK9 inhibitors demonstrate excellent activity in CDK9 biochemical and cell-based assays, with IC50 values <10 nM and 100 nM respectively. Representatives of this series show excellent selectivity for CDK9 vs other CDK family members. IC50 values in cellular proliferation and apoptosis assays were found to correlate well with inhibition of cellular RNAPII CTD Ser2 phosphorylation, indicating that CDK9 inhibition is associated with strong anti-growth and pro-apoptotic effects on tumor cells in vitro. Selected CDK9 inhibitors exhibit oral bioavailability and dose-dependent plasma exposure. In vivo pharmacodynamic analysis following oral administration of these inhibitors in mouse xenograft tumor models demonstrates potent and dose-dependent inhibition of RNAPII CTD SER2 phosphorylation, as well as reductions in Myc, Cyclin D1, and MCL-1 protein levels.
Conclusions: CDK9-selective small molecule inhibitors were discovered that demonstrate potent CDK9 inhibitory activity in both in vitro and in vivo studies, and show anti-proliferative and pro-apoptotic activity in multiple tumor cell lines. Further chemical optimization and biological characterization is proceeding, with the goal of identifying a CDK9-selective inhibitor candidate for clinical development as a cancer therapeutic.

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AZD5597, a novel CDK inhibitor causes inhibition of RNA polymerase II mediated signaling and stimulates apoptosis in vitro and in vivo

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Background: AZD5597 is a CDK inhibitor displaying potency against both the cell cycle CDKs 1 and 2 and the transcriptional CDKs 7 and 9. This profile could result in both cell cycle block and induction of apoptosis and therefore may be particularly efficacious as a novel anti-tumour therapy.
Materials and Methods: The *in vitro* effects of AZD5597 on CDK inhibition, cell cycle progression and apoptosis were investigated by incubating the compound with MCF-7 cells. Cells were fixed, then labeled immunocytochemically for intracellular phosphorylation of RNA polymerase II, DNA intensity, and Bak conformational changes respectively and quantified on a Cellomics ArrayScan. We subsequently investigated the effects of AZD5597 *in vivo* using a variety of tumour xenografts (BT474c; CoLo 205, A2780 and U87MG). Mice bearing established sub-cutaneous tumours (mean tumour volume 0.4–0.6 cm^3) were given a single dose of AZD5597 (25 mg/kg, ip). Tumours were taken 6 h (4 h for U87MG) post dose, halved and either frozen or fixed in formalin for subsequent analysis. The effects of AZD5597 on cell cycle were assessed by scoring mitosis; pRb IHC and phH3 IHC. Transcriptional effects of AZD5597 were assessed by scoring RNA polymerase II IHC. Assessments of apoptosis were based on examination of both cellular phenotype and cleaved caspase 3 IHC. AZD5597 was dosed (15 mg/kg, ip; Mon, Wed and Friday for 3 weeks) to mice bearing SW620 xenograft tumours to assess the effects on tumour growth.
Results: AZD5597 treatment of MCF-7 cells *in vitro* resulted in a cell cycle block (at G2/M), reduced the phosphorylation of RNA polymerase II, and increased initiation of cell apoptosis (as indicated by conformational changes in Bak). Consistent with these data, *in vivo* a single dose of AZD5597 resulted in cell cycle block (reduced mitosis, pRb and phH3 staining), a reduction in phospho RNA polymerase II staining, and induction of apoptosis. Similar results were seen in all four tumour types examined. In chronic dosing, AZD5597 resulted in a 55% inhibition of SW620 tumour growth *in vivo*.
Conclusions: AZD5597 results in cell cycle block and causes the inhibition of survival pathways that leads to induction of apoptosis *in vitro* and *in vivo*. These effects were observed in histologically distinct tumour models (breast, colorectal, ovarian and glioma).

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The complexity of cell cycle dynamic of anticancer drugs unraveled by the use of mathematical models suitable for a quantitative assessment of G1, S and G2M checkpoint activities

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Background: Current methods to assess cell cycle effects of drugs in cell populations are still limited to score the macroscopic variations on specific measurable quantities like percentages of cells in G1, S and G2M. These quantities result from the superimposition of the effects of cell cycle block and cell loss, their dynamics in the times before the measure, and the proliferation of surviving cells. They depend on but do not provide a direct measure of the activities of the molecular networks regulating G1, S and G2M checkpoints. A mathematical model was built based on the kinetics of cell cycle transit times of an ovarian cancer cell line and on parameters that directly describe the activity of each checkpoint, and giving as output cell cycle percentages and other macroscopic measurable quantities.
Material and Methods: Experiments were performed with a standardized protocol using the IGROV1 cell line with 5 clinically used anticancer drugs (cisplatin, taxol, doxorubicin, melphalan, topotecan) characterized by different molecular mechanisms of action. We measured cell cycle percentages and absolute cell number at four times after treatments (6, 24, 48, 72 h) with a wide range of drug concentrations. DNA-BrdUrd histograms and apoptosis were also collected at specific times.
Results: After obtaining a robust set of data of the concentration-dependent effects of all drugs by using different techniques we have analyzed the complexity of events (delayed cell cycle progression, short or long term blockades in different cell cycle phases, cell death) by the application of a mathematical model based on parameters that directly describe the activity of each checkpoint. By deciphering cellular data in terms of the global activities of G1, S and G2M checkpoints, each governed by specific molecular network, it became evident that there are differences in the complex cell response to each drug that have never been observed before by standard methodologies.
Conclusions: We found that the overall response of each drug is the result of the combination of a few types of checkpoint response, which operate with different strengths, and with specific drug concentration thresholds. The application of the simulation mathematical model to anticancer drug effects allows a quantitative knowledge of the dynamics of critical cellular events, that should be taken into account when using an anticancer drug alone or in combination in a less empirical and more rational way.

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Biological characterization of the dual CDK2/TRKA inhibitor PHA-848125

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Among the small molecule cyclin-dependent kinase modulators that are nowadays in the clinical arena the pyrazolo quinazoline PHA-848125 emerges as a potent inhibitor of the CDK2/cyclinA complex (IC50=45 nM) with the novelty of being particularly active also against TRKA (IC50=53 nM) proposing in this way to be a "selective dual inhibitor". It also maintains some activity against other cell cycle CDKs, even if