of the cohort. The first two pts who received 7.0 mg/kg experienced DLTs of grade 3 diarrhea, requiring dose reduction to 4.68 mg/kg. Expansion of the 4.68 mg/kg cohort to 6 pts occurred without further DLTs. Therefore, 4.68 mg/kg is considered the maximum tolerated dose (MTD). All ongoing patients had their XL647 dose converted to a fixed dose of 350 mg. Five additional pts have been enrolled into the 350 mg cohort. PK analysis indicates that XL647 shows approximately dose-proportional exposure, a mean time to maximal concentration (t_{max}) of 6–9 hours, and an elimination half-life of about 70 hours. To date, 1 pt (NSCLC) from Cohort 1 had a partial response and 12 others (NSCLC 3, chordoma 2, adenoid cystic carcinoma 2, adrenocortical carcinoma, CRC, mesothelioma, ovarian carcinoma, head and neck cancer) had prolonged stable disease (>3 months).

Conclusions: XL647 was well tolerated. A MTD of 4.68 mg/kg oral dosing for 5 consecutive days every 14 days was established. This dose was converted to a fixed dose of 350 mg, which is well tolerated. Exploration of additional dosing schedules is ongoing, including extended daily dosing.

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Phase I dose escalation study of the aurora kinase inhibitor PHA-739358 administered as a 24 hours infusion in a 14-day cycle in patients with advanced/metastatic solid tumor

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Background: PHA-739358 is a novel small molecule that selectively inhibits Aurora kinases (AKs) A and B. AK A is implicated in regulating mitotic entry, centrosome maturation, and spindle assembly. AK B is required for proper chromosome segregation and cytokinesis. Many tumor types overexpress AKs. In this Phase I trial, PHA-739358 is administered to patients (pts) with advanced/metastatic solid tumors by an IV 24-h infusion, every two weeks, to determine the maximum tolerated dose (MTD), safety, PK profiles, and evidence for antitumor activity.

Materials and Methods: This is a dose escalation study. Cohorts of 3–6 pts were sequentially allotted to progressively higher dose levels (DL) of PHA-739358 based on the number of dose limiting toxicities (DLTs) observed. As per protocol, definition of DLTs were grade (G) 4 neutropenia lasting >7 days, febrile neutropenia, neutropenic infection, any G3 or 4 non-hematological drug related toxicities during the first cycle of treatment.

Results: Thirty pts have been enrolled. Six DL were explored (45, 90, 180, 360, 500 and 650 mg/m^2). Most of the pts at the 500 and 650 mg/m^2 DLs had G3 and 4 leucopenia/neutropenia. No other G3 or G4 hematological toxicity was reported. Other than one G3 diarrhea all other drug related non-hematological adverse events were G1 or 2 (fatigue, anorexia, nausea, vomiting, and ejection fraction decreased). One DLT (neutropenic infection) in the 360 mg/m^2 and 2 DLTs (neutropenic infection and febrile neutropenia) at DL 650 mg/m^2 DL were reported. The MTD has been exceeded at 650 mg/m^2. An intermediate DL is being explored (580 mg/m^2). Nine pts showed stable diseases as best response and in 3 of them the response duration was $\geqslant$ 6 months. Inhibition of histone H3 phosphorylation induced by PHA-739358 is evident in skin biopsies of pts treated at $\geqslant$ 500 mg/m^2. PHA-739358 clearance was 0.3–0.6 L/h/kg, with a volume of distribution 2–4 times total body water and a terminal half-life of 18−30 hr. PHA-739358 showed dose-proportional and time-independent behavior.

Conclusions: Neutropenia was the dose-limiting toxicity for PHA-739358 and easily managed. Non-hematological drug related adverse events were mild or moderate. Disease stabilization has been observed. Biomarker modulation occurred at $\geqslant$500 mg/m^2. PK was linear with low interindividual variability. Refinement of the recommended dose for Phase II studies is ongoing.

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Dissecting the roles of Chk1 and Chk2 in mitotic catastrophe using chemical genetics

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Cell cycle checkpoint inhibition represents a novel strategy for enhancing the activity of genotoxic agents. EXEL-9844 (XL844) is a novel and specific inhibitor of both Chk1 and Chk2, with K_I values in the low nanomolar and sub-nanomolar range, respectively. In vitro, EXEL-9844 abrogates the DNA damage checkpoint induced by daunorubicin (Dnr), as measured by cdc2 activation and cell cycle analysis, in a CML cell line. In a CML survival model in nude mice, treatment with EXEL-9844 in combination with Dnr causes a significant increase in median survival time relative to Dnr alone. In addition, some of the animals in the combination treatment groups exhibit asymptomatic long-term survival, in contrast to animals treated with either agent alone.

The mechanism of Dnr potentiation was further evaluated using solid tumors in vivo. Administration of Dnr or EXEL-9844 as single agents caused substantial apoptosis, while dosing the agents in combination lead to widespread apoptotic destruction of tumor cells. In addition, cells treated with Dnr and EXEL-9844 exhibited many large histone-H3-positive cells, suggesting that premature mitotic entry and subsequent mitotic failure might contribute to tumor cell death. In vitro FACS analysis indicated that cells treated with a combination of Dnr and EXEL-9844 (but not with either agent alone) exhibited a large N>4 DNA content, with a prominent N = 8 peak. Parallel immunofluorescence studies showed that these cells formed large multinuclear aggregates, indicative of endoreduplication and mitotic catastrophe. We performed additional FACS analysis using a related compound (EXEL-3611) that has similar potency for Chk1, but >20,000-fold selectivity for Chk1 vs. Chk2. These studies showed little evidence for endopolyploid cell formation, indicating that Chk2 inhibition may play an important role in this process.

Overall, these data provide a rational basis for the development of EXEL-9844 for the sensitization of tumors to DNA damaging therapy. A phase I clinical trial for EXEL-9844 is in progress.

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Identification and characterization of small molecule inhibitors of ubiquitin-specific protease 8, a newly validated cancer target

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Deregulations of the ubiquitin/proteasome system have been implicated in the pathogenesis of many human diseases, including cancer. The approval of the proteasome inhibitor Velcade establishes this system as a valid target in cancer therapy. A promising alternative to targeting the proteasome itself would be to interact at the level of the upstream, ubiquitin conjugation/deconjugation system to generate more specific, less toxic anticancer agents.

Here, we silenced each of the 50 catalytically active ubiquitin-specific proteases (USPs) of the human genome by RNA interference and tested for cancer-relevant phenotypes. We identified a few USP among which USP8 (or UBPY) as regulators of cell cycle, proliferation and/or survival. Silencing of USP8 was found to induce growth arrest in several tumor cell lines. Phenotypic analysis of a colon cancer cell line stably transfected with an inducible USP8 shRNA construct further showed that USP8 knock-down leads to G1 arrest and apoptosis. Of interest, USP8 silencing resulted in less efficient growth arrest in a non tumoral epithelial cell line. To get insights into the molecular mechanisms of USP8 function, a yeast two-hybrid analysis was performed using various USP8 bait fragments against several cDNA libraries. Oncogenic kinases, centrosome-associated factors and proteins involved in receptor endocytosis and trafficking were identified as potential USP8 partners or substrates. Consistent with this, USP8 was recently suggested to regulate epidermal growth factor receptor ubiquitination and down-regulation.

To identify small molecule inhibitors of USPs, we developed and run high-throughput screens on 65,000 compounds from a chemically diverse library. Several hits demonstrated inhibitory activity towards USP8 with sub-micromolar IC50. A lead optimization program identified compounds that selectively inhibit USP8 over various cysteine proteases including other deubiquitinating enzymes (Table). Consistent with the RNAi data, these compounds also exhibited antiproliferative activity with sub-micromolar GI50 in different cancer cell lines.

Compound	IC50(μM)							
HBX	USP8	USP7	USP5	Uch-L1	Uch-L3	SENP1	Caspase 3	Cathepsin B
92,540	0.238	>100	32	>100	0.538	>100	>100	>100
96,819	0.278	>100	>100	>100	1.3	–	>100	>100
90,397	0.559	>100	>100	>100	10	>100	>100	>100
90,659	0.850	>100	>100	>100	>100	>100	>100	>100

Our data suggest that modulating the activity of USP8 could provide a novel way for controlling cancer cell proliferation. We also report the identification of a family of compounds that will help further validate this potential target and may provide a structural basis for the generation of new drugs.