bcl-b and Noxa. There was inhibition of cellular viability exhibited by trypan blue assay, while Ki67 and MIB1 markers exhibited inhibition of cell proliferation. BrdU, MTT and XTT assays exhibited inhibition of DNA synthesis and metabolic activity in CAV and endothelial cells. Finally, we observed induction of apoptosis or nuclear PCD type I, paraptosis (necrapoptosis) or cytoplasmic PCD type III and autophagic PCD type II leading to a bystander killing effect (BKE) of CAV and endothelial cells.
**Conclusion:** We achieved to eradicate chemoresistant CAV and endothelial cells by using a functional genomic tool such as siRNAs in a pegylated liposomal formulation which inhibited DNA methylation re-establishing normal function and expression of vital tumor suppressor genes combined with the cytostatic and apoptotic action of vinorelbine-tartrate leading to the synergistic inhibition of angiogenesis, lymphangiogenesis, metastasis and cellular proliferation after the induction of type I, II, III PCD.

**339** POSTER

**Implication of tumor suppressor maspin in the eradication of lung cancer**

C. Park, E. Nam, S. Yeom. *Sungkyunkwan University School of Medicine, Cancer Center, Samsung Medical Center, Seoul, Korea*

Our work focuses on identifying factors critical to the progression of lung cancer. Here, we investigated the possible role of maspin in this context. In breast and prostate cancers, maspin acts as a tumor suppressor capable of inhibiting motility, invasion and metastasis; in pancreatic cancer it functions as a malignant factor. Few studies to date have investigated the role of maspin in lung cancer, likely because it is not expressed in normal lung tissue. In the present study, we investigated whether engineered overexpression of maspin in NCI-H157 lung cancer cells cells, which do not express endogenous maspin, would suppress the tumorigenicity of this particular tumor cell type. NCI-H157 cells overexpressing maspin displayed a dramatically reduced growth rate compared to the parental cell line when subcutaneously implanted in athymic (nu/nu) mice. Furthermore, gene transfer of maspin suppressed the growth of established NCI-H157 tumors. The data suggest that maspin gene therapy and/or agents that increase maspin expression could have utility in the treatment of lung cancers.

**340** POSTER

**Gene therapy with plasmid IL-12 delivered by electroporation in patients with malignant melanoma: results of first human Phase I trial**

R. Heller[1,2,3], R. DeConti[3,4], J. Messina[3,5], S. Andrews[3], P. Urbas[3], K. Ugen[1,2], C. Puleo[3], V. Sondak[3,4], A. Riker[3,4], A. Daud[3,4]. *[1]University of South Florida, Molecular Medicine, Tampa, Florida, USA; [2]University of South Florida, Center for Molecular Delivery, Tampa, Florida, USA; [3]H. Lee Moffitt Cancer Center and Research Institute, Cutaneous Oncology, Tampa, Florida, USA; [4]University of South Florida, Department of Interdisciplinary Oncology, Tampa, Florida, USA; [5]University of South Florida, Pathology and Cell Biology, Tampa, Florida, USA*

Metastatic melanoma is a devastating disease lacking effective treatment. In the B16.F10 melanoma model, electroporation of plasmid encoding IL-12 (pIL-12) into established tumors resulted in an 80% cure rate as well as resistance of the cured mice to subsequent challenge with B16.F10 cells. Furthermore, minimal toxicity was observed when pIL-12 was delivered with electroporation as compared to untreated mice or mice that only received injection of pIL-12. These experiments provided the rationale for a Phase I safety, dose escalation and biological efficacy trial in patients with accessible subcutaneous metastases with melanoma.
**Methods and Study Design:** The primary objective was to determine the toxicity and maximum tolerated dose (MTD) of intra-tumorally electroporated pIL-12. The secondary objectives were to determine the efficacy of this treatment and to evaluate local and systemic cytokine response. Patients received intra-tumoral injection of pIL-12 followed immediately by 1300 V/cm 100 μs duration electric pulses at the tumor site. Electroporation treatments were performed on days 1, 5 and 8. Each patient had a minimum of 2–4 tumors treated. Dose escalation was performed by varying the plasmid amount, (0.6 mg, 1.5 mg, 3 mg, 6 mg, 12 mg) three patients were treated per cohort.
**Results:** Three patients were enrolled into each of five cohorts of this Phase I study. No Grade 2, 3 or 4 toxicity was noted. Patients expressed significant but transient pain during the administration of electric pulses (lasting a few seconds). Biopsies were done following treatment and showed significant necrosis of melanoma cells within the tumor in the majority of treated lesions. Significant lymphocytic infiltrate was seen in biopsies from patients in cohorts 3, 4 and 5. In addition, IL-12 expression was documented in the tumor samples biopsied but not in serum.
**Conclusion:** This first-in-human phase I study demonstrated the feasibility and efficiency of *in vivo* electrogene delivery in humans. Transient pain during electroporation has been the only toxicity seen at this point in the trial. Expression of IL-12 was documented at the tumor site as well as tumor necrosis and lymphocytic infiltrate. Further evaluation of this method in melanoma and other tumors is warranted by the current trial.

# New drug targets

**341** POSTER

**The discovery of MP529, a potent and selective aurora kinase inhibitor using CLIMB**

H. Vankayalapati, C. Grand, X. Liu, X. Zhang, J. Lamb, M. Lloyd, J. Sunseri, D. Bearss. *SuperGen, Incorporated, Salt Lake City, USA*

Aurora A kinase is a validated target for a number of human malignancies, including pancreas, breast, prostate, ovarian and colorectal cancers. This serine-threonine kinase is of particular interest as of late, due to its important role in proper spindle formation at mitosis. Overexpression of Aurora A leads to dysregulation of the centrosome cycle resulting in the formation of multipolar mitotic spindles. The resulting abnormal mitotic events lead to genomic instability which is an underlying process in tumorigenesis. Previous studies have shown that inhibition of Aurora A kinase in tumor cell lines effectively disrupts mitosis, leading to monopolar spindles, multinucleate cells, growth arrest and eventually cell death. Through the use of our proprietary CLIMB drug discovery process, we have set out to identify and synthesize a new series of Aurora A kinase inhibitors. In traditional small molecule screening, as many as several million compounds may be tested in order to identify the few that interact selectively with a disease-related protein target. CLIMB can achieve similar results by screening as few as several hundred computationally selected compounds. CLIMB screening is based on the clustering of representative chemical structures and pharmacophores that embody our large virtual library of nearly 50 million compound structures. Using CLIMB, the Aurora A kinase crystal structure was used as a substrate for docking to generate a subset of leads based on calculated binding energies. These leads were then screened and ranked using a number of in silico physicochemical and ADMET prediction algorithms to determine which were most likely to be "drug-like". Biochemical enzyme-based assays with recombinant Aurora A kinase have revealed an array of candidates of the substituted (4-p-tolylsulfamoyl-phenyl) amide class, the MP529 series. These compounds exhibit nanomolar activity or better against the Aurora A kinase enzyme, and have been carried forward into ex vivo and in vivo evaluations. In cell-based assays, mitotic markers of Aurora A kinase inhibition are seen, resulting in a reduction of tumor cell growth. Selected compounds from this series have been appraised in an in vivo xenograft context, and have been shown to be effective, while exhibiting a wide therapeutic window and desirable pharmacokinetic properties. The MP529 series represents a novel scaffold which improves upon the pharmacological activities of known Aurora kinase inhibitors.

**342** POSTER

**A phase I dose-escalation and pharmacokinetic (PK) study of XL647, a novel spectrum selective kinase inhibitor, administered orally to patients with advanced solid malignancies (ASM)**

B.I. Sikic[1], H.A. Wakelee[1], A.A. Adjei[2], J. Halsey[1], J.L. Lensing[2], J.D. Dugay[1], L.J. Hanson[2], J.M. Reid[2], J.R. Piens[2]. *[1]Stanford University Medical Center, Oncology Division, Stanford, USA; [2]Mayo Clinic, Oncology Division, Rochester, USA*

**Background:** XL647 is an orally bioavailable small molecule inhibitor of multiple receptor tyrosine kinases involved in tumorigenesis, angiogenesis, and metastasis, including EGFR/ErbB1, ErbB2/HER2, VEGFR2/KDR, and EphB4.
**Methods**: ASM patients (pts) were enrolled in successive cohorts to receive XL647 orally as a single dose on Day 1 with PK sampling, then on Day 4, pts received 5 consecutive daily doses with additional PK sampling. Pts continued treatment with XL647 for 5 consecutive days, followed by a 9-day observation period; cycles were repeated every 14 days. Pharmacodynamic plasma samples were collected from all pts and are undergoing analysis for mechanism-of-action related molecules. Pts were allowed to stay on study in the absence of unacceptable toxicity until evidence of disease progression.
**Results:** A total of 41 pts have been treated across 11 dose levels: 0.06, 0.12, 0.19, 0.28, 0.39, 0.78, 1.56, 3.12, 4.68, and 7.0 mg/kg in liquid formulation, and then at a fixed dose of 350 mg in tablet formulation. One serious adverse event, grade 4 pulmonary embolism, was considered possibly related to study treatment in a pt dosed at 0.28 mg/kg. One pt at 3.12 mg/kg had a dose-limiting toxicity (DLT) of asymptomatic QTc prolongation as assessed by an electrocardiogram, resulting in expansion

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.

**343** POSTER

**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**

H.A. Burris[1], G. Hudes[2], S. Jones[1], J. Cheng[2], D. Spigel[1], M. Mariani[3], H. Macdonald[3], M. Rocchetti[3], B. Laffranchi[3], R.B. Cohen[2]. *[1]Sarah Cannon Cancer Center, Nashville, TN, USA; [2]Fox Chase Cancer Center, Philadelphia, PA, USA; [3]Nerviano Medical Sciences, Nerviano, MI, Italy*

**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.

**344** POSTER

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

D. Matthews. *Exelixis Inc., Drug Discovery, South San Francisco, USA*

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.

**345** POSTER

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

F. Colland, V. Collura, E. Formstecher, X. Jacq, A. Meil, C. Borg-Capra, L. Daviet, P. Guedat, J.C. Rain, R. Delansorne. *Hybrigenics, Paris, France*

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.