and metastasis. In vitro BAY853474 inhibited MET auto-phosphorylation and MET driven tumor cell proliferation in vitro, which translated into strong inhibition of tumor growth in MET-dependent tumor xenograft models *in vivo*. In preclinical studies the compound demonstrated besides the high selectivity for the MET receptor a favourable physical-chemical, DM/PK and tolerability profile in support of oral administration route for further clinical development. We present representative data supporting pharmacological mode-of-action and efficacy in tumor xenograft models *in vivo*.

**153** POSTER

**Novel pyrazolopyrimidine derivatives as potent mTOR kinase inhibitors with anticancer activities**

S. Chen[1], L. Meng[1], C. Liang[2], J. Ding[1]. [1]*Shanghai Institute of Materia Medica, Division of Anti-Tumor Pharmacology, Shanghai, China;* [2]*Xcovery LLC, Drug Discovery, West Palm Beach, USA*

**Background:** The mammalian target of rapamycin (mTOR),which is deregulated in about 50% of all human malignancies,sits in the center of signaling network regulating cell growth, metabolism, and angiogenesis. mTOR exists in two complexes: the mTOR-raptor complex and the mTOR-rictor complex. Rapamycin and its analogues partially inhibit mTOR through allosteric binding to TORC1 without inhibiting TORC2 and their efficacy is moderate as anticancer agents in the clinic. A few mTOR kinase inhibitors that inhibit both TORC1 and TORC2 have been reported to possess more potent anticancer activities. Herein, we designed and synthesized a series of pyrazolopyrimidine derivatives as novel specific ATP-competitive mTOR kinase inhibitors which inhibit both TORC1 and TORC2 and display potent antitumor activities.
**Materials and Methods:** mTOR kinase activity was measured by an ELISA assay employed purified truncated mTOR protein. Protein levels were detected by Western blot analysis. Cell cycle distribution was assessed by FACS analysis and cell proliferation was evaluated via SRB assay.
**Result:** Compounds specifically inhibited mTOR while sparing a panel of over 400 kinases tested. Further study indicated that the compounds acted in an ATP-competitive manner, blocked mTOR signaling pathway in cell lines with different characteristics such as A549 cells with Kras and LKB1 mutations, MCF-7 cells with PIK3CA mutation and p70S6K1 amplification, and rapamycin resistant HCT116 cells. The compounds inhibited the phosphorylation of TORC1 substrate p70S6K1 and 4EBP1 as well as phosphorylation of the TORC2 substrate AKT and downstream protein GSK3β, which happened within 15 minutes of compound treatment. As a consequence, cells were arrested in G1 phase upon treatment with compound for 24 h or 48 h. Consistent with the reported role of mTOR in regulating autophagy in mammalian cells, we found that the compounds induced autophagy in A549 cells after 24 h of treatment. We further evaluated the anticancer activity of the compounds in a broad panel of tumor cells originating from different tissue types and they displayed potent inhibition of the tumor cell proliferation with $IC_{50}$s ranging from 0.27 mM to 1.17 mM across all cell lines tested.
**Conclusion:** Pyrazolopyrimidine derivatives are a novel class of specific mTOR kinase inhibitors targeting both TORC1 and TORC2. They displayed broad anticancer activities and deserve further evaluation and optimization as new anticancer compounds.

**154** POSTER

**Establishment and evaluation of patient-derived tumor models of adenoid cystic carcinoma: Effects of chemotherapeutics and targeted therapies on human ACC xenografts**

F. Nieves[1], M. Moore[1], M. Mann[1], C. Moskaluk[2], A. Tolcher[3], K. Papadopoulos[3], A. Patnaik[3], J. Kaufman[4], M. Wick[1]. [1]*South Texas Accelerated Research Therapeutics (START), Preclinical Research, San Antonio TX, USA;* [2]*University of Virginia Charlottesville, Pathology/Biochemistry and Molecular Genetics, Charlottesville VA, USA;* [3]*South Texas Accelerated Research Therapeutics (START), Clinical Research, San Antonio TX, USA;* [4]*Adenoid Cystic Carcinoma Research Foundation, Needham MA, USA*

Adenoid cystic carcinoma (ACC) is a rare form of malignant neoplasm defined by a distinct histologic appearance which arises within secretory glands, most commonly the salivary glands of the head and neck. Standard treatment options for this malignancy include resection and local radiation therapy; however, currently no standard of care exists for this cancer type. Several novel therapies have demonstrated single agent or combination activity across a range of in vivo models of human cancer; however, lack of validated ACC models has limited evaluation of these agents in treating this disease.
Previously we and others in collaboration with the Adenoid Cystic Carcinoma Research Foundation (ACCRF) established and evaluated two preclinical models of ACC (ACCx6 and ACCx9) using tumor explants from donor patients implanted into immunocompromised mice. Three additional tumor models (ACCx5M1, ACCX14 and ACCx16) have been established and screened against a panel of 35−40 agents representing each class of approved anticancer agent as well as candidate compounds obtained from academic and pharmaceutical collaborators; follow up studies were also performed evaluating previously untested agents in the ACCx6 and ACCx9 models. Designated endpoints for these studies were a mean control tumor volume of approximately 1−2 $cm^3$ or sixty days following treatment initiation. Treatment with standard chemotherapeutics was ineffective in most screens except for docetaxel which demonstrated statistically significant ($p < 0.05$) tumor growth inhibition in all evaluated models including partial and complete tumor responses towards ACCx16. Activity was reported in ACCx16 and ACC×9 with the tyrosine kinase inhibitor (TKI) sunitinib and sorafenib was active towards ACC×16 but inactive in ACC×5M1. Irinotecan and temsirolimus were active towards ACC×16 but inactive in remaining models. ACC×14 was most insensitive to evaluated chemotherapeutics with only docetaxel reporting activity in this model while ACC×16 was most sensitive in these studies.
Data from these studies demonstrate these low passage models as excellent screening tools to identify potentially useful approved and candidate agents for treatment of ACC. Antitumor activity of docetaxel in these models suggests it as an appropriate combination agent for future studies and activity of TKI agents over several models suggest this class of agents as potentially useful in the treatment of ACC. Additional studies evaluating single agent and combination treatments including docetaxel plus TKI are warranted.

**155** POSTER

**EML4-ALK signaling is required for the maintenance of neoplastic phenotype of non-small cell lung cancer cells: novel strategy for lung cancer tailored therapies**

C. Voena[1], E. Panizza[1], E. Pellegrino[1], L. D'Amico[1], F.E. Boccalatte[1], B. Ruggeri[2], R.D. Polakiewicz[3], E. Medico[4], R. Chiarle[1], G. Inghirami[1]. [1]*University of Torino, Department of Biomedical Science and Human Oncology, Torino, Italy;* [2]*Cephalon Inc, Discovery Research, West Chester PA, USA;* [3]*Cell Signaling Technology Inc., Cell Signaling Technology Inc., Danvers MA, USA;* [4]*Institute for Cancer Research and Treatment (IRCC) University of Torino, The Oncogenomics Center, Torino, Italy*

**Background:** Lung cancer is the most common cancer in the world, and is lethal in 90% of the cases. In non-small cell lung cancer (NSCLC), deregulated receptor tyrosine kinases (RTKs) are among the causal dominant oncogenes. In a subset of NSCLC, the Anaplastic Lymphoma Kinase (ALK) gene has been described to be translocated and fused to EML4. Here, we investigated the ALK oncogenic addiction of human NSCLC and studied the putative co-operative role of other kinases.
**Materials and Methods:** Human lung cancer cell lines H2228, H3122 (EML4-ALK+ cells) and H1395 (negative control) were treated with small molecule ALK (Cephalon) or EGFR inhibitors. Cells were stably transduced with doxycycline inducible shRNA against ALK (A5). Phosphoproteomic and GEP analysis were performed after treatment with ALK inhibitors. Apoptosis was measured by TMRM staining and cell proliferation by iodidum propide staining followed by FACS analysis. Immunocompromised mice were inoculated s.c. and treated with an ALK inhibitor. Tumor growth measuring, bioluminescence imaging and immunohistochemistry were performed.
**Results:** The ectopic expression of EML4-ALK in ALK positive NSCLC cell lines (H2228 and H3122) resulted in the activation of multiple signaling pathways as described for other known ALK fusions. Although EML4-ALK can induce transformation in lung *in vitro* and *in vivo*, ALK inhibition via shRNA or small molecule inhibitors induced only the apoptosis of H3122 cells, whereas in H2228 it caused cell growth arrest. Moreover, the treatment with ALK inhibitors led to tumor regression *in vivo.* Based on Phosphoproteomic analyses we demonstrated that the phosphorylation status of several TKs (EGFR, Met, FGFR, Jak1 or IGFR) was affected by ALK inhibition only in H2228 cell line. Notably, the combined treatment with anti-ALK and EGFR inhibitors resulted in an increased cell death of H2228 cells to values similar to those observed for ALK treated H3122 cells. Finally, GEP analyses showed that known EGFR substrates were specifically down regulated upon the combined treatment in H2228 cells.
**Conclusions:** ALK signaling is required for the maintenance of the neoplastic phenotype of some ALK positive NSCLC cells and its abrogation could represent a novel strategy for the treatment of a well-defined subset of human lung cancer (complete ALK addiction). More importantly, the tumor survival and maintenance of ALK positive neoplastic cells might relay on the concomitant activation of multiple RTKs.