mice, ranging from 0.5 to 1. The plasma clearance of GDC-0941 was similar in the four strains of mice. Exposure following PO dosing was also comparable in the wild type and all knockout mice. Following administration of GDC-0941 to Mdr1a/b(−/−)/Bcrp1(−/−) mice, the PI3K pathway was markedly inhibited in the brain for up to 6 hours post-dose, with a 60% suppression of the pAkt signal, while no effect on the PI3K pathway was detected in the brain of wild type mice. GDC-0941 was efficacious in the U87 glioblastoma orthotopic model that contained compromised blood–brain barriers (BBB) but inactive in a neurosphere-derived model with non-compromised BBB. Additionally, in the Fo1282 breast cancer brain metastasis model, GDC-0941 treatment increased survival benefit and decreased phospho-S6 ribosomal protein levels indicative of PI3K pathway suppression *in vivo*.
Conclusions: These findings show concerted effects of Pgp and Bcrp1 in restricting GDC-0941 access and pathway modulation in the brain of non-tumor bearing mice. Anti-tumor activity of GDC-0941 is observed in orthotopic brain tumor and metastasis models that contain compromised blood–brain barriers.

134 POSTER

Biological characterization of ETP-46321 a potent and selective phosphoinositide-3-kinase inhibitor with antitumor activity

J.R. Bischoff[1], J. Pastor[1], D. Cebrián[1], S. Martínez[1], M. Lorenzo[1], T. Merino[1], J. Fominaya[1], P. Pizcueta[1], A. Rodríguez Lopez[1], T.G. Granda[1]. [1]*Spanish National Cancer Research Centre, Experimental Therapeutics, Madrid, Spain*

The phosphoinositide-3-kinase (PI3K) signaling pathway is activated in a variety of solid and non-solid tumors. In many instances this is due to either activating mutations in the catalytic subunit of PI3Kα, p110α or inactivating mutations or deletions of the tumor suppressor PTEN. In addition, the PI3K pathway is activated by mutations in certain receptor tyrosine kinases as well as by mutation of the oncogene KRAS. These data provide a strong rationale for the discovery of PI3K inhibitors for treatment for cancer. Following a rational design strategy, we identified the fused imidazole derivative ETP-46321 as a potent inhibitor of PI3K (e.g., K_i = 2.4 nM *vs.* p110α, K_i = 549 nM *vs*. p110β, K_i = 14 nM *vs.* p110δ, and K_i = 153 nM *vs*. p110γ, respectively). ETP-45321 also inhibits three oncogenc mutants of p110α: p110α E542K K_i = 1.9 nM, p110α E545K K_i = 1.8 nM and p110α H1047R K_i = 2.4 nM. The compound does not significantly inhibit the related PIKK family members such as mTOR, DNA PK or ATR (K_i's >10 μM), or an additional 280 protein kinases that were screened. The compound blocks PI3K signaling, induces cell cycle arrest and inhibits VEGF-dependent sprouting of HUVEC cells. ETP-46321 has a pharmacokinetic profile suitable for oral dosing in mice (%F = 95%, Cl = 0.56 L/hr/kg; Vds = 0.016 L). Analysis of xenograft tumor tissue after acute dosing reveals a reduction in P-Akt levels. Once a day treatment with ETP-46321 of mice with human tumor xenografts with ETP-46321 results in tumor growth delay and is well tolerated. In a mouse model of lung cancer induced by expression of an oncogenic mutant KRAS, treatment with ETP-46321 results in tumor growth delay and a significant PET response. These and combination data will be presented.

135 POSTER

Pediatric Preclinical Testing Program (PPTP) stage 1 evaluation of JNJ-26481585, a second generation histone deacetylase inhibitor

M.A. Smith[1], J.M. Maris[2], S.T. Keir[3], R.B. Lock[4], H. Carol[4], E.A. Kolb[5], M.H. Kang[6], C.P. Reynolds[6], I. Hickson[7], P.J. Houghton[8]. [1]*National Cancer Institute, Cancer Therapy Evaluation Program, Bethesda, USA;* [2]*Children's Hospital Philadelphia, Division of Oncology, Philadelphia, USA;* [3]*Duke University Medical Center, Dept of Surgery, Durham, USA;* [4]*Children's Cancer Institute Australia, Leukemia Biology Program, Randwick, Australia;* [5]*A.I. duPont Hospital for Children, Dept of Oncology, Wilmington, USA;* [6]*Texas Tech University Health Sciences Center, Cancer Center, Lubbock, USA;* [7]*Johnson & Johnson, Oncology Research, Turnhoutseweg, Belgium;* [8]*Nationwide Children's Hospital, Center for Childhood Cancer, Columbus, USA*

Background: JNJ-26481585 is a 'second-generation' HDAC inhibitor with prolonged pharmacodynamic response *in vivo*. The agent has demonstrated superior efficacy compared to both standard of care agents and 'first generation' HDAC inhibitors in adult cancer preclinical models. The activity of JNJ-26481585 was evaluated against the *in vitro* and *in vivo* panels of the Pediatric Preclinical testing Program (PPTP).
Methods: JNJ-26481585, which was provided by Johnson & Johnson Pharmaceutical Research and Development, was tested against the PPTP *in vitro* panel (n = 23) at concentrations ranging from 1.0 nM to 10 mM and was tested against the PPTP *in vivo* panel using a dose of 5 mg/kg (solid tumor) or 2.5 mg/kg [acute lymphoblastic leukemia (ALL)] administered by the intraperitoneal route daily for 21 days. Three measures of antitumor activity were used: 1) response criteria modeled after the clinical setting; 2) treated to control (T/C) tumor volume at day 21; and 3) a time to event (4-fold increase in tumor volume) measure based on the median EFS of treated and control lines (intermediate activity required EFS T/C > 2, and high activity additionally required a net reduction in median tumor volume at the end of the experiment).
Results: JNJ-26481585 demonstrated potent cytotoxic activity, with T/C% values approaching 0% for all of the cell lines at the highest concentration tested. The median EC_{50} value for the PPTP cell lines was 2.2 nM, with a range from <1 nM (MOLT-4) to 19 nM (NB-EBc1). JNJ-26481585 was well tolerated and induced significant differences in EFS distribution compared to control in 20 of 31 (65%) of the evaluable solid tumor xenografts and in 5 of 8 (63%) of the evaluable ALL xenografts. JNJ-26481585 induced tumor growth inhibition meeting criteria for intermediate EFS T/C activity (EFS T/C > 2) in 5 of 30 (17%) evaluable solid tumor xenografts. Intermediate activity for the EFS T/C metric occurred most frequently in the glioblastoma panel (2 of 4) and was also observed for one xenograft in the rhabdoid, Ewing, and rhabdomyosarcoma panels. An objective response was observed in 1 of 31 solid tumor xenografts, Rh28 in the rhabdomyosarcoma panel that achieved a maintained complete remission (MCR). For the ALL panel, two xenografts (both T-cell ALL xenografts) achieved CR and MCR, respectively, and a third xenograft achieved stable disease (SD).
Conclusions: The activity signals observed for JNJ-26481585 against the PPTP preclinical models warrant follow-up. *In vivo* activity signals for rhabdomyosarcoma, glioblastoma, and T-cell ALL are particularly noteworthy, with further exploration of the preclinical activity of JNJ26481585 for T-cell ALL being a high priority.

136 POSTER

Activity of the Cdc7 inhibitor NMS-1116354 as single agent and in combination in breast cancer models

A. Montagnoli[1], D. Ballinari[1], A. Ciavolella[1], S. Rainoldi[1], M. Menichincheri[1], E. Pesenti[1], A. Galvani[1], A. Isacchi[1], J. Moll[1]. [1]*Nerviano Medical Sciences, Oncology, Nerviano (Milano), Italy*

NMS-1116354 is a potent ATP competitive oral inhibitor of the Cdc7 kinase (IC_{50} <3 nM). Consistently with the inhibition of this enzyme, treated cells show inhibition of phosphorylation of serine 40 in the Mcm2 protein and an impairment of DNA replication. While this event leads to apoptotic tumor cell death, in normal cells it induces a reversible cell cycle arrest. In addition, NMS-1116354 induces the down regulation of the pro-survival proteins Mcl-1 and XIAP expression thus exacerbating the effects of the Cdc7 inhibition on tumor growth.
NMS-1116354 has potent antiproliferative activity against a wide panel of tumor cell lines with IC_{50} values ranging between 0.1 and 3 μM. Oral administration of NMS-1116354 demonstrated significant antitumor activity in various tumor animal models as well as in disseminated human leukemia models. Particularly, strong tumor regressions were obtained in breast cancer models. In combination studies, NMS-1116354 exhibited synergistic effects when administered with Irinotecan, Gemcitabine, Erlotinib, Bortezomib and with other approved antineoplastic drugs. The combination with Docetaxel in triple negative breast cancer animal models gave tumor free animals lasting for >4 months.
The phase I clinical trials to evaluate the safety of orally administered NMS-1116354 as single agent with different schedules in cancer patients are ongoing. The results of the combination studies open a possible path for its clinical development in combination with approved drugs.

137 POSTER

Preclinical characterization of ACTB-1010, an orally activity Aurora kinase inhibitor

A. Burd[1], L. Kunkel[2], A. Fattaey[1]. [1]*ACT Biotech, Research and Development, South San Francisco CA, USA;* [2]*ACT Biotech, Clinical Development, South San Francisco CA, USA*

Introduction: Aurora Kinases A and B are dysregulated in a number of human cancers and are essential to the regulation and function of mitosis and cytokinesis. ACTB-1010 is an oral kinase inhibitor targeting both Aurora Kinase A and B. ACTB-1010 was selected for development based on the nanamolar potency against targeting Aurora Kinase A and B, without cross reactivity to other major kinases such as VEGF, FGF, KIT and FLT3. We investigated the preclinical efficacy and mechanism of action of ACTB-1010.
Results: ACTB-1010 inhibits both Aurora Kinase A (IC_{50} = 1.6 nM) and Aurora Kinase B (IC_{50} = 9 nM) and is highly active in cell-based mechanistic assays. Human tumor cell lines treated with ACTB-1010 demonstrate a phenotype consistent with Aurora B inhibition including enlarged nuclei

and >2N DNA content and inhibition of histone H3 phosphorylation. This translates to potent *in vivo* activity with dose-dependent tumor growth inhibition in various tumor models including HL60 human acute myelogenous leukemia, HCT-116 human colon carcinoma and H460 human. *In vivo* dose and schedule optimization studies demonstrated tumor growth inhibition without myelosuppression, a common side effect of Aurora Kinase inhibitors currently in development.
Conclusion: ACTB-1010 is a potent and selective Aurora Kinase inhibitor with reduced off target side inhibition of other kinases and eliminates unwanted side effects such as myelosuppression. The selectivity of ACTB-1010, allows optimal inhibition of Aurora kinase A and B that may be developed as a therapeutic for multiple tumor types.

138 POSTER

Combination of PI3K inhibitor BAY 80-6946 with allosteric MEK inhibitor BAY 86-9766 (RDEA119) and with erlotinib for the treatment of non-small cell lung cancer

N. Liu[1], F. Puehler[1], A. Haegebarth[1], A. Scholz[1], J. Hoffmann[2], D. Mumberg[1], K. Ziegelbauer[1]. *[1]Bayer Schering Pharma AG, GDD-TRG-Oncology, Berlin, Germany; [2]EPO-Berlin GmbH, EPO-Berlin GmbH, Berlin, Germany*

Lung cancer is the leading cause of cancer-related deaths worldwide. Non-small cell lung cancer (NSCLC) accounts for about 85% of all lung cancer cases. We evaluated the anti-tumor activity of the combination of BAY 80-6946, a highly selective pan class I PI3K inhibitor with the allosteric MEK inhibitor, BAY 86-9766, as well as with erlotinib, in NSCLC cell lines and tumor models. A set of 40 well characterized human lung tumor cell lines were profiled with BAY 80–6946 and BAY 86-9766 in tumor cell proliferation assays. Eight NSCLC tumor cell lines representing various clinically relevant somatic mutations and different sensitivities to each agent were selected for further studies. *In vitro* combination of BAY 80-6946 (PI3K) and BAY 86-9766 (MEK) demonstrated a strong synergy resulting in lowering both the IC_{50}s (combination Index/CI: 0.12–0.59) and even more dramatically the IC_{90}s (CI: 0.07–0.34). More importantly, neither BAY 86-9766 nor BAY 80-6946 induced significant tumor cell apoptosis in the eight NSCLC cell lines tested, while the combination of the both agents led to a significant activation of caspases (3/7) in 4 out of 8 cell lines tested. Interestingly the combination of BAY 80-6946 (PI3K) with erlotinib showed a differential profile of synergy compared to the combination with BAY 86-9766 (MEK), with the strongest synergy observed in erlotinib sensitive NSCLC cell lines. *In vivo*, BAY 80-6946 (i.v.) demonstrated high to low anti-tumor efficacy (T/C: 0.13–0.69) in 11 human NSCLC xenograft tumor models (1 tumor cell line and 10 patient-derived primary tumors) in mice. Combining BAY 80-6946 (PI3K) with BAY 86-9766 (MEK) demonstrated strongest activity in KRAS mutated and erlotinib-resistant primary NSCLC xenograft tumor model, while combining BAY 80-6946 (PI3K) with erlotinib further enhanced tumor growth inhibition and prolonged the time to tumor regrowth in erlotinib active (T/C < 0.5), carboplatin- and etoposid-resistant patient-derived NSCLC models. In conclusion: (1) combination of BAY 80-6946 (PI3K) and BAY 86–9766 (MEK) is a promising approach for the treatment of NSCLC, especially for tumors with KRAS mutations; (2) BAY 80-6946 (PI3K) can further enhance the anti-tumor efficacy of erlotinib in preclinical tumor models responsive to erlotinib. Currently BAY 80-6946, a selective pan class I PI3K investigational agent is being evaluated in a phase I study in solid tumors.

139 POSTER

Inhibition of osteolysis by CSF-1R antagonist in MM.1S orthotopic multiple myeloma model

S. Palakurthi[1], S. Grondine[1], N. Gingles[2], X. Rong[1], D. Lawson[1], P. Hall[1], Y. Cao[1], K. Wu[1], C. Reimer[1]. *[1]AstraZeneca R&D, Cancer Bioscience, Boston Massachusetts, USA; [2]AstraZeneca, Translational Science, Macclesfield, United Kingdom*

Multiple myeloma (MM) is a B-cell malignancy characterized by excess abnormal plasma cells in the bone marrow (BM), myeloma bone disease (MBD), characterized by diffuse osteopenia, focal lytic lesions, bone fractures and pain causing major discomfort to the patients. In patients with MBD, bisphophonates show reduced skeletal complications when dosed by intravenous infusion but show minimal beneficial effects when dosed by oral route, indicating a paucity of oral agents that ameliorate MBD and improve the quality of life. CSF-1R signaling axis is important for bone homeostasis and CSF-1 directly contributes to osteoclastogenesis, as both mice that fail to express functional CSF-1 and the mice lacking *csf1r*, exhibit osteopetrotic phenotype due to reduction in osteoclasts. We hence reasoned that an orally available CSF-1R antagonist, may have potential beneficial effects in MBD.

We established and validated a mouse MM model by intravenous injection of luciferase tagged MM.1S cells in to SCID mice. The MM model presents with the characteristic features of bone marrow infiltration and progressive MBD, leading to hind limb paralysis caused due to vertebral bone destruction. At the early signs of BM infiltration, the mice were randomized into various treatment groups and monitored for tumor load, as measured by either IVIS imaging or MM markers expression such as, CD138 in the bone marrow or serum IgG levels. Progression of MBD was evaluated either by trabecular volume measurement in the femero tibial joint by microCT method or by measurement of TRAP5b, serum bone resorption marker levels. In this study, CSF-1R antagonist was treated by po route at a well tolerated dose, shown to inhibit CSF-1R in vivo as demonstrated by its ability to decrease the expression of F4/80 macrophage marker. We report here that CSF-1R antagonist caused significant increase in bone density as measured by trabecular volume and reduction of osteolytic lesions in the femero tibial joint, there by, leading to decreased incidence and time of onset of hind limb paralysis with overall improvement in the mobility of mice. This was consistent with the ability of CSF-1R antagonist to cause inhibition of serum TRAP5b levels. However, CSF-1R antagonist treatment had negligible effects on tumor growth per se, showed minimal effects on mean overall survival of mice, indicating that CSF-1R- signaling axis does not have a direct role in either the survival of myeloma cells or their infiltration in to the BM. We conclude that targeting CSF-1R by a small molecule inhibitor reversed osteoclastic activity, ameliorated MBD, with negligible antimyeloma effects in an in vivo disseminated orthotopic MM model. The data from this report supports setting the stage for testing CSF-1R inhibitors in human MM clinical trials in combination either with current standards of care or novel agents under investigation − potentially offering an alternative to bisphosphonates with an oral route dosing option.

140 POSTER

Combination of PI3K inhibitor BAY 80-6946 and allosteric MEK inhibitor BAY 86-9766 (RDEA119), a promising approach for the treatment of colorectal cancers

N. Liu[1], A. Haegebarth[1], F. Puehler[1], A. Scholz[1], J. Hoffmann[2], R. Dubowy[3], H. Krissel[4], S. Wilhelm[5], D. Mumberg[1], K. Ziegelbauer[1]. *[1]Bayer Schering Pharma AG, GDD-TRG-Oncology, Berlin, Germany; [2]EPO-Berlin GmbH, EPO-Berlin GmbH, Berlin, Germany; [3]Bayer HealthCare Pharmaceuticals, Global Clinical Pharmacology, Montville NJ, USA; [4]Bayer Schering Pharma AG, Global Clinical Development, Berlin, Germany; [5]Bayer HealthCare Pharmaceuticals, GMA Oncology, Montville NJ, USA*

Despite the introduction of new chemotherapeutic and targeted agents, the prognosis for patients with metastatic CRC (mCRC) remains poor. Particularly, patients with mutant KRAS tumors have limited treatment options after failure of standard chemotherapy. We report our preclinical studies on the combination of a highly selective pan class I PI3K inhibitor, BAY 80-6946 and an allosteric MEK inhibitor, BAY 86-9766 (RDEA119) in CRC models. A set of 32 well characterized CRC tumor cell lines were profiled with BAY 80-6946 (PI3K) and BAY 86-9766 (MEK). Seven cell lines with various clinic-relevant genetic alterations were selected for further studies. Interestingly, combining BAY 86-9766 (MEK) and BAY 80-6946 (PI3K) inhibitors demonstrated a significant synergy not only in lowering cellular IC_{50}s (CI: 0.21–0.90), but also more dramatically in decreasing the concentration needed to reach complete tumor growth inhibition (CI at IC_{90}: 0.02–0.18) in the CRC cell lines with either KRAS or BRAF mutations. Neither BAY 86-9766 (MEK) nor BAY 80-6946 (PI3K) were able to induce apoptosis in Colo205 ($BRAF^{mut}$) and HCT116 ($KRAS^{mut}$ and $PIK3CA^{mut}$) tumor cells whereas the combination of these two compounds led to the activation of caspase 3/7 with CI<0.2. *In vivo* profiling of BAY 86–9766 (MEK) in 18 different patient-derived CRC xenografts demonstrated strong to moderate efficacy with a median treatment/control (T/C) value of 0.13 (0.05–0.46). Three CRC tumor models with differential sensitivities to BAY 86-9766 (MEK) were further evaluated in the combination studies with BAY 80-6946 (PI3K). BAY 86-9766 was dosed at 25 (MTD) and 12.5 mg/kg, po, QD with two dosing regimens for BAY 80-6946 (i.v., Q2D and weekly), respectively. The combination of MEK (p.o.) and PI3K (i.v.) at both dosing schedules significantly enhanced anti-tumor efficacy in all 3 patient-derived CRC xenograft models and 4 CRC tumor cell line derived xenograft models. In addition, an increase in number of animals with tumor shrinkage (PRs) was observed in HCT116 ($KRAS^{mut}$ and $PIK3CA^{mut}$) xenograft model and complete tumor growth inhibition was also observed in two cetuximab-resistant patient derived primary CRC models (carrying KRAS and PIK3CA mutations, respectively). In conclusion these promising preclinical data suggest that future clinical trials are warranted to examine the efficacy of BAY 80-6946 (PI3K) in combination with BAY 86-9766 (MEK) in mCRC patients, especially those with mutant KRAS tumors.