tumors transplanted into syngeneic FVB mice, perturbation of HER2/HER3 signaling pathway with EZN-3920 resulted in tumor growth inhibition, reduced expression of HER3, and induction of apoptosis as assessed by immunohistochemical methods. It is concluded that EZN-3920 provides a novel strategy to effectively down modulate HER3-mediated addiction to growth factors. Furthermore, down regulation of HER3 may provide a novel strategy to overcome resistance to HER1 and HER2 targeted therapies.

**145** POSTER

**Utility of microRNA analysis for understanding treatment mechanism of action: Necitumumab +/− gemcitabine/cisplatin in NSCLC models**

S. Samakoglu[1], H. Li[1], D.S. Deevi[1], J.R. Tonra[1]. *[1]ImClone Systems A wholly-owned subsidiary of Eli Lilly and Company, Preclinical Pharmacology, New York NY, USA*

The contribution of mRNA regulation through effects on microRNA (miRNA), to the anti-tumor effects of therapy needs further study. Here, we utilized SABiosciences PCR arrays to identify oppositely regulated miRNA and predicted mRNA targets in A549 and NCI-H1650 non-small cell lung cancer (NSCLC) xenograft tumors growing in nu/nu athymic mice. Necitumumab, a recombinant human IgG1 targeting EGFR, alone or in combination with cisplatin+gemcitabine (cis/gem), inhibited the growth of these tumors. In the A549 model, 34% of 125 human mRNAs that were >2-fold up or down-regulated by necitumumab, cis/gem, or necitumumab+cis/gem ($p < 0.05$ by t-test versus saline control, $n = 3$), were predicted to be targets of at least one of the 16 tumor miRNA affected oppositely by the same respective treatment, indicating potential regulation of mRNA by miRNA in these tumors. In the NCI-H1650 model, cis/gem treatment did not affect the expression levels of the miRNAs evaluated, however 40% of 52 human mRNAs that were >2-fold up or down-regulated by necitumumab or necitumumab+cis/gem treatment were predicted to be targets of at least one of the 5 miRNA affected oppositely by the same respective treatment, again supporting the potential involvement of miRNA in the regulation of mRNA in tumors. The potential value of miRNA analysis for understanding mechanism of action *in vivo* was further highlighted by the observed necitumumab induced increase in mir-15b (18.3 fold, $p = 0.037$), let-7g (6.5 fold, $p = 0.03$) and miR-150 (2.3 fold, $p = 0.026$), that may impact tumor cell apoptosis and cell proliferation through modulation of BCL2 (-46.5 fold, $p = 0.002$), Cyclin D1 (−11.1 fold, $p = 0.0009$) and TP53 (-133.8 fold, $p = 0.0003$) mRNA in NCI-H1650 tumors. In the A549 model necitumumab increased the level of miR-148a (3.44 fold, $p = 0.0034$), miR-148b (2.54 fold $p = 0.006$), and miR-29 (2.1 fold, $p = 0.02$), that may target mRNA for the methyltransferase DNMT3b (−4.5 fold, $p = 0.02$) to restore normal patterns of methylation-silenced tumor suppressor genes such as CDKN2A (66.7 fold, $p = 0.004$), RARA (8.7 fold, $p = 0.006$), RARB (9 fold, $p = 0.007$) and RARG (6.67 fold, $p = 0.001$), contributing towards the treatment induced reduction in tumor growth. To summarize, utilizing necitumumab +/− gem/cis efficacy in NSCLC subcutaneous xenograft tumor models, we demonstrate that miRNA analysis can be an important tool in understanding the mechanism of action underlying antitumor effects of therapy.

**146** POSTER

**Specific MET inhibition using SU11274 impairs cholangiocarcinoma cells proliferation, motility and invasion**

C. Serrate[1], N. Guedj[2], M. Serova[1], D. Garbay[1], I. Bieche[3], M. Riveiro[1], V. Paradis[2], E. Raymond[1], S. Faivre[1]. *[1]Beaujon University Hospital, RayLab Department of Medical Oncology, Clichy, France; [2]Beaujon University Hospital, Department of Pathology, Clichy, France; [3]Beaujon University Hospital, Laboratory of Molecular Genetics, Clichy, France*

**Background:** Cholangiocarcinomas (CCA) are highly malignant tumors of unmet medical needs often displaying aberrant MET signaling. MET activation either by HGF stimulation, MET over-expression or mutations induces cell proliferation, invasion, and angiogenesis, offering opportunities for investigating the potential of novel MET inhibitors such as SU11274.
**Materials and Methods:** SU11274, a pyrrole indolinone, specifically inhibits overexpressed and oncogenic MET activation at nanomolar concentrations. Antiproliferative effects of SU11274 were evaluated in human CCA cell lines (Mz-chA1, Mz-chA2 and SK-ch) using MTT assay. Baseline and phosphorylated (p-) protein levels were assessed by Western blot analysis. mRNA expressions used qRT-PCR. Mobility was investigated by wound-healing and matrigel invasion assays. Cell cycle distribution was studied by FACS analysis.
**Results:** MET-protein and -mRNA expression was detectable in our three CCA cell lines and cells were found responding to HGF stimulation as detected by activation of p-MET$^{Tyr1234/35}$ in all cell lines. MET activation by HGF was associated with increase of p-GAB1, p-ERK1/2, and p-AKT$^{ser473}$ in Mz-chA2 cells. SU11274 displayed antiproliferative effects at concentrations ranging 2−5 μM after 48−72h exposures in our three cell lines without HGF stimulation. Cell cycle analysis of CCA cells exposed to 5μM SU11274 for 72h demonstrated accumulation of cells in G2/M phase. In HGF-stimulated Mz-chA2 and SK-ch cells, SU11274 blocked p-MET and p-GAB1 at MET-specific concentrations ranging 0.5−2 μM that were also shown yielding antiproliferative effects. At these concentrations, SU11274 inhibited HGF-induced downstream MET signaling by reducing p-AKT$^{473}$ and p-ERK1/2. No significant effect of SU11274 on E-cadherin and vimentin expression was observed. SU11274 (5μM) decreased spontaneous HGF-independent cell motility of Mz-chA2 cells and their invasion in matrigel.
**Conclusion:** Inhibition of p-MET by SU11274 inhibited HGF-dependent MET signaling that resulted in inhibition of cell proliferation, motility and invasion in CCA cells. CCA may be an interesting tumor type to evaluate novel MET inhibitors either as single agents and/or in combination with other targeted therapies.

**147** POSTER

**Pharmacological characterization of NMS-P506, a novel second generation HSP90 inhibitor**

G. Fogliatto[1], S. Mantegani[2], N. Amboldi[3], A. De Ponti[3], L. Gianellini[3], M. Guanci[2], M. Paolucci[1], D. Donati[2], E. Pesenti[2], A. Isacchi[1]. *[1]Nerviano Medical Sciences Srl, Biotechnology, Nerviano (Milano), Italy; [2]Nerviano Medical Sciences Srl, Chemistry, Nerviano (Milano), Italy; [3]Nerviano Medical Sciences Srl, Biology, Nerviano (Milano), Italy*

**Background**: the molecular chaperone heat shock protein 90 (HSP90) is essential for the conformational maturation and stability of a variety of key proteins, including kinases, implicated in cancer development and progression.
Prototype geldanamycin derivatives are the most advanced compounds, but their liabilities may ultimately limit clinical applications and justify the development of new non ansamycin drugs. In this perspective, the identification of inhibitors capable of long lasting pharmacodynamic modulation of client proteins in tumour tissues might prove important in order to improve the therapeutic window in the clinics.
**Results:** here we describe the *in vitro* and *in vivo* characterisation of NMS-P506, representative of a novel class of fully synthetic non-ansamycin HSP90 inhibitors. NMS-P506 binds HSP90α with an affinity of 65 pM, and has no significant activity against a broad panel of kinases, as well as other relevant ATPases.
When tested against a panel of tumor cell lines of various tissue origins, NMS-P506 showed widespread antiproliferative activity, with an average $IC_{50}$ of 95 nM. When characterized by Biacore analysis the compound showed a very slow rate of dissociation from HSP90, with a $K_{off}$ of $8.09E^{-5}$ 1/sec, which translates in cells in a long lasting degradation of HSP90-dependent oncoproteins and up regulation of HSP70.
In mice, NMS-P506 had a favourable pharmacokinetic profile with a $t_{1/2}$ of 6 hours in plasma. Selective retention was observed in tumours, with an extended half-life of 139 hours, and relevant concentrations of the compound were found in the brain.
*In vivo*, NMS-P506 showed an excellent anti tumor efficacy in the B-RAF V600E driven A375 melanoma model, as well as in the A2780 ovarian cancer model, resulting in tumor shrinkage after weekly intravenous administrations with a good tolerability profile. This *in vivo* activity was associated with apoptosis induction and prolonged degradation of HSP90 client proteins.
**In conclusion**, we report the characterization of NMS-P506, a new second generation Hsp90 inhibitor, capable of long lasting pharmacodynamic modulation of client proteins in cells and in xenograft tumors. NMS-P506 has a very potent *in vitro* and *in vivo* activity, with a good PK profile and selective retention in tumors, which makes it a candidate for further development. Moreover, the brain penetration makes this compound potentially attractive also for brain tumors or metastases.

**148** POSTER

**Therapeutic targeting of the pro-survival transcription factor CREB sensitizes glioblastomas to temozolomide-based therapy**

J. Graff[1], S.H. Parsons[1], A.M. McNulty[1], M.S. Dowless[1], M. Phong[1], J. Virayah[1], B.W. Konicek[1], G. Tucker-Kellogg[1], L.F. Stancato[1], J.J. Starling[1]. *[1]Eli Lilly and Company, Cancer Growth and Translational Genetics, Indianapolis IN, USA*

**Background**: Glioblastoma multiforme (GBM) is the most common, lethal primary brain tumor in adults. Following debulking surgery, adding temozolomide (TMZ) with, and after, radiation therapy has become the standard of care for newly diagnosed GBM, yielding 15 months median overall survival versus 12 months with radiation alone. Despite this advance, more effective therapeutic options are needed, particularly