of the NF-κB subunit p65 reversed the increased chemosensitivity of HIF-1α-deficient cells.
Conclusions: In summary, we identified HIF-1α as a potent regulator of p53 and NF-κBactivity under conditions of genotoxic stress. We conclude that *p53* mutations in human tumors hold the potential to confound the efficacy of HIF-1-inhibitors in cancer therapy.

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Anti-angiogenic therapy improves response rate in erlotinib resistant NSCLC xenografts

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Non small cell lung cancer (NSCLC) is one of the leading causes of cancer deaths in the Western world. Combination chemotherapy is the therapeutic option for advanced diseases but with limited efficacy. Targeted therapies entered the clinics and led to a minor improvement of survival. However, many patients do not benefit from cytotoxic agents or targeted therapies. Therefore, reliable markers to select treatments for patients most likely to respond are in urgent need.
In our study, 25 patient-derived NSCLC xenografts were established and characterized. They revealed a high degree of similarity with the original tumor concerning histology, immunohistochemistry as well as gene profiling. The responsiveness to four cytostatics drugs (etoposide, carboplatin, gemcitabine, paclitaxel) and two epidermal growth factor receptor inhibitors (erlotinib and cetuximab) was evaluated in these xenografts according to clinical criteria.
The RNA expression profile of the xenografts was analyzed with the GeneChip HGU133Plus2.0. The data were evaluated statistically with the help of GeneSpring GX 11.0.
Within a class comparison, more than 2500 probe sets were found to be differentially expressed between erlotinib responders (2 xenografts) and non-responders (23 xenografts).
Differentially expressed genes were vascular endothelial growth factor a (VEGFA) and neuropilin and tolloid-like 2 (NETO2). VEGFA was higher expressed in the erlotinib resistant tumors. It may cause a better vascularization and thus result in a better survival of the tumor. The results were validated with TaqMan-PCR. With a combination therapy of erlotinib and bevacizumab the response rates could substantially be improved in the erlotinib resistant tumors.
In conclusion, a differential gene expression pattern was found in our patient derived xenografts allowing the identification of rational combination therapies. The patient-derived xenograft system offers a valuable tool to investigate targeted therapies and biomarker regulations in a clinically related way.

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Targeting glioblastoma stem cells: overcoming temozolomide resistance by ALDH1 inhibition

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Glioblastoma (GBM), the most frequent brain tumor of adults is still associated with a poor prognosis. Despite various efforts to improve postoperative therapeutic regimens in recent times, relapse occurs regularly. The chemoresistance of malignant gliomas might be caused by a barely characterized tumor stem cell subpopulation residing in a specific tumor microenvironment. Besides others, hypoxia may play a critical role in inducing a resistant tumor cell type. While normal brain tissue shows an oxygen partial pressure (pO_2) of 24−27 mmHg, gliomas feature an average pO_2 of only 13 mmHg.
So far, temozolomide (TMZ) is the gold standard of care for newly diagnosed glioblastoma. Recently we could show that aldehyde dehydrogenase 1 (ALDH1) positive glioblastoma cells show brain tumor stem cell capacity. In the current investigation we examined the impact of ALDH1 expression on GBM temozolomide resistance. In vitro cytotoxicity was evaluated by colorimetric MTT- and colony formation assays. Flow cytometry was used to analyze the amount of apoptotic cells. Neurosphere formation in neurobasal medium and differentiation experiments were applied to identify tumor stem cells. Furthermore, ALDH1 expression and temozolomide resistance was correlated with the MGMT status of various established and primary cell lines.
Sensitivity to temozolomide in resistant established and primary glioma cell lines was achieved by inhibition of ALDH1 with 4-diethylaminobenzaldehyde (DEAB). A specific knock down of ALDH1 by siRNA confirmed these findings. Under hypoxic conditions the cytotoxic effect of temozolomide was strongly attenuated but could be restored by ALDH1 inhibition. In the present study we show that ALDH1 is strikingly upregulated under hypoxic conditions, potentially leading to an increase of chemoresistance in gliomas. Hypoxia inducible factors (HIF1 alpha, HIF2 alpha) are involved in the regulation of ALDH1. Since post-therapeutic relapse is most probably due to a stem cell subfraction within the tumor bulk, special interest should be drawn to these cells. After combination therapy of temozolomide and DEAB, glioma cells were no longer able to proliferate in stem cell promoting medium or to form neurospheres; the remaining cells lost their undifferentiated stem cell-like phenotype.
In conclusion we suggest ALDH1 as an important prognostic factor in glioma care. Combination therapy of temozolomide with ALDH1 inhibitors might strongly improve clinical outcome of GBM patients.

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The selective reduction in the production of Bmi-1 protein leads to tumor growth control in multiple tumor models

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Background: Elevated expression of Bmi-1, a polycomb protein (also called PCGF4), is correlated with the chemo- and radio-resistance of a sub-fraction of tumor cells that also demonstrate stem cell characteristics. These stem-like cells are thought to be responsible for tumor recurrence leading to treatment failures in many cancer types. Bmi-1 has been shown to play a significant role in many neoplasias, particularly in glioblastoma where there is compelling evidence that Bmi-1 over-expression in glioblastoma multiforme (GBM) is a key event for tumor growth and intrinsic chemo-resistance.
Results: PTC has identified low molecular weight compounds that potently and selectively inhibit the production of Bmi-1 protein. A subset of these compounds act by targeting the post-transcriptional regulation of Bmi-1 synthesis, which reduces the translation rate of Bmi-1. This occurs both in cancer cells in culture and in various xenograft tumor models. The loss of Bmi-1 expression induced by these molecules leads to the reduction in global levels of mono-ubiquitinated histone 2A and causes either apoptosis or senescence in tumor cells. In murine xenograft tumor models, these compounds reduce intratumor Bmi-1 protein levels by up to 70% and tumor growth by up to 50% when administered orally as single agent therapy. The in vivo evaluation of activity is in progress where lead molecules are used in combination with standard-of-care cytotoxics.
Conclusions: Results from our studies support the hypothesis that targeting the production of the stem cell protein Bmi-1, known to be important for tumor cell survival and resistance, may enhance treatment success and improve patient outcomes.
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An antisense molecule to HER3 sustains growth inhibitory effects in gefitinib resistant cells that are independent of MET overexpression

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Although HER3 is not typically amplified or overexpressed in many tumor cells lines like EGFR or HER2 family members, HER3 is emerging as a critical family member since (1) it is a key link to the PI3K pathway for HER family members, (2) it can heterodimerize with HER1 and HER2, and (3) it can be activated via autocrine signaling by binding its cognate ligand, heregulin. These features help explain why increased activation of HER3 can mediate resistance to HER1 and HER2 inhibitors such as gefitinib, lapatanib, or Herceptin.
We have been attempting to understand the basis of unusual sensitivity of the lung carcinoma cell line, HCC827 to gefitinib (IC_{50} ~10 nM) and acquired resistance mechanisms after the cells were chronically exposed to increasing concentrations of gefitinib in vitro. The resistant cell lines were independently selected and are distinct from that reported to be driven by HER3 hyperactivation associated with MET amplification (Engelman et al., Science. 2007 316: 1039). The cell lines were highly resistant to gefinitib (IC_{50} >10 μM) but unlike past reports, the intracellular pEGFR levels were dramatically reduced while pHER3 and HER3 levels were unchanged when compared to HCC827 parental cells. Furthermore, no alteration in MET expression has been detected. Despite the lack of a HER3 activation signature, the resistant clones were equally or more sensitive to the treatment of an antisense molecule against HER3, designated EZN-3920. The parental HCC827, as well as the resistant cell lines were the most sensitive cells to EZN-3920 compared with 20 other cell lines. Furthermore,