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R1507, a human monoclonal antibody targeting IGF-1R (insulin like growth factor receptor) is effective alone and sensitizes small cell lung cancer cell lines to chemotherapy and radiation in vitro

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Introduction: Insulin like growth factor receptor-1 (IGF-1R) inhibition could be an important therapeutic approach in small cell lung cancer (SCLC), given the activation of multiple autocrine loops in the pathogenesis of this disease, one of them involving the co-expression of IGF-1R and one of its ligands IGF-1 or IGF-2. Besides IGF-1 is a potent stimulator of PI3K-Akt signaling in SCLC.
Materials and Methods: In this study we evaluated the importance of IGF-1R axis in SCLC by assessing IGF-1R expression and Akt activation in human SCLC tissue specimens. In parallel we evaluated *in vitro* the efficacy of R1507, an IgG1 fully human monoclonal antibody directed to IGF-1R. We performed Western blotting to assess the expression of IGF-1R and the effect of R1507 on downstream signaling cascades using three small cell lung cancer cell lines: H69, H146 and H526. The *in vitro* cytotoxicity of R1507 alone and in association with cisplatin or ionizing radiation was evaluated by WST-1 cell proliferation assay and by clonogenic survival. To assess apoptosis induction we evaluated cell cycle distribution. We also tested concomitant targeting of MEK and IGF-1R on H146 cells.
Results: We demonstrated in vitro efficacy of R1507 in two of the three cell lines examined, as well as a synergistic effect with cisplatin and radiotherapy in H526 cell line. Efficacy was dose dependent, becoming more evident at concentrations above 100 nM. The inhibition of PI3K-Akt pathway was correlated with treatment response. The effect of IGF-1R blockage on MAPK pathway showed a high variability among the three cell lines examined. R1507 was able to induce cell cycle arrest in H526 cell line. Concomitant inhibition of MEK and IGF-1R in H146 cell line did not have synergistic effect. IGF-1R was mostly overexpressed (55% showing a more than 1+ expression level) in surgical specimens originating from patients suffering an intervention for limited stage SCLC. We noticed a high level (79%) of concomitant expression of IGF-1R and pAkt in the same samples.
Conclusions: R1507 has a single agent activity and remarkable chemo- and radiosensitizing effect in defined small cancer lung cancer cell lines in vitro. Efficacy is dose-dependent and related to the capacity to inhibit PI3K-Akt signaling pathway.

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Vorinostat induces acetylation of BH3-only gene promoters triggering their expression and leading to apoptosis in mantle cell lymphoma

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Background: Current therapies for the B-cell neoplasm mantle cell lymphoma (MCL) have shown limited efficacy. Recently, Histone Deacetylase Inhibitors (HDACis) have been successfully introduced for the treatment of hematological malignancies. Accordingly, tumor suppressor genes are frequently epigenetically silenced in these entities, due to histone deacetylation in their promoters. Therefore, our purposes were to evaluate the antitumoral properties of the HDACi suberoylanilide hydroxamic acid (Vorinostat; Merck & Co) in MCL, and to describe the molecular mechanisms involved in HDACi signaling in this disorder.
Materials and Methods: 8 MCL cell lines and 10 primary MCL samples were used. HDAC activity was measured using a colorimetric HDAC Assay Kit. Flow cytometry was used to determine sensitivity to vorinostat through measurement of AnnexinV binding, and to analyze apoptosis features. Vorinostat anti-tumoral signaling was evaluated by RQ-PCR determination of gene transcription, western blot analysis and acetyl Histone H4 ChIP assays.
tightenup>**Results:** Vorinostat exhibited a heterogeneous cytotoxic effect among MCL cell lines, with a median LD_{50} of 6.6 μM after 24-hour incubation. Nevertheless, cytotoxicity increased notably after 48 h of exposure to the drug with LD_{50} ranging from 0.4 to 5.3 μM. Interestingly, 7 out of the 10 MCL primary samples tested were extremely sensitive to the compound (with a median LD_{50} of 2.2 μM after 24-hour incubation). Vorinostat increases the acetylation of H3 and H4 histones, as well as inhibits global HDAC activity in just 1 hour of incubation. The drug notably decreases cyclin D1 protein levels while induces upregulation of the proapoptotic BH3-only proteins Bmf, Bim and Noxa, triggering the mitochondria-dependent cell death and activation of the caspases cascade. Acetyl Histone H4 ChIP assays showed that vorinostat increases acetylation of *BMF*, *BIM* and *NOXA* gene promoters, consequently up regulating Bmf, Bim and Noxa mRNA levels.
Conclusions: This study suggests that vorinostat could define an attractive therapeutic approach for the treatment of MCL. We identify *BMF*, *BIM* and *NOXA* as target genes of HDAC inhibitors in MCL cells, promoting the induction of mitochondria-mediated apoptosis.
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Responses of human pancreatic tumour cells to treatment with anti-EGFR mAb ICR62 and the irreversible EGFR/HER1 and HER2 tyrosine kinase inhibitor BIBW2992

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Aberrant expression of epidermal growth factor receptor (EGFR) family members has been reported in a wide range of epithelial tumours and, in some patients, has been associated with poor prognosis and resistance to therapy. Currently, a number of monoclonal antibodies (mAbs) and reversible EGFR and/or HER-2 tyrosine kinase inhibitors (TKIs) have been approved for treatment of human cancers. Of these the reversible EGFR TKI erlotinib, in combination with gemcitabine, has gained FDA approval for the treatment of patients with pancreatic cancer. However, while the EGFR inhibitors improve survival in pancreatic cancer patients, the duration of response is often limited. The aim of this investigation was to evaluate the growth response of a panel of human pancreatic tumour cell lines (Capan-1, Panc-1, FA-6, BxPc-3, PT-45, AsPc-1 and Miapaca-2) to treatment with our anti-EGFR mAb ICR62, BIBW2992 which is an irreversible EGFR/HER1 and HER-2 TKI and gemcitabine, using the SRB colorimetric assay. We reported previously that, at concentrations above 3.2 nM, mAb ICR62 inhibits completely the growth of the EGFR overexpressing DiFi cells *in vitro* and induces apoptosis. However, at maximum concentration of 200 nM used in this study, we found that mAb ICR62 had no effect on growth of the human pancreatic tumour cell lines. Interestingly, of the 7 human pancreatic tumour cell lines examined, BXPC3 cells were highly sensitive to treatment with BIBW2992 with an IC_{50} value of <10 nM. The growth of other human pancreatic tumour cells was also inhibited by BIBW2992 with IC_{50} values ranging from 247 nM (ASPC1) to 821 nM (FA6). Using FACS analysis, we found that the mean fluorescence intensities (MFIs) for EGFR expression in these tumour cell lines ranged from 32 (Miapaca-2) to 184 (PT45). In contrast, the levels of HER-2 expression in these cell lines were much lower and the MFI for HER-2 expression ranged from 11 (Panc-1) to 33 (Miapaca-2). Interestingly, all the human pancreatic cell lines tested were found to be negative for the expression of HER-3 and HER-4. We did not find any clear association between the expression levels of the EGFR family members and the response to treatment with BIBW2992 and ICR62. Taken together, our results presented here underlie the need for further investigation on the anti-tumour activity of the BIBW2992 as a single agent and in combination with gemcitabine and/or other targeted therapies in pancreatic cancer.

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ARQ 087: A potent ATP-independent fibroblast growth factor receptor (FGFR) kinase inhibitor showing in vivo anti-tumor activity in FGFR2-driven tumors

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Dysregulation in the FGFR tyrosine kinase family has been implicated in a number of human cancers, including gastric, breast, endometrial, and bladder carcinomas. We have previously described the discovery of a chemical series of FGFR kinase inhibitors employing a proprietary structure-based design approach. ARQ 087, a lead candidate for clinical development, inhibits FGFR1, 2, and 3 with IC_{50} values in the 2−4 nM range, with FGFR4 being inhibited 10-fold less potently. ARQ 087 displayed inhibition kinetics that were ATP-independent, while showing a 20-fold preference for inactive FGFR2 in biochemical assays. ARQ 087 showed potent inhibition of FGFR2 phosphorylation in KATO III (IC_{50} value = 150 nM) and SNU-16 (IC_{50} value = 45 nM) human gastric carcinoma cells with comparable antiproliferative potencies by MTS assay (KATO III