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Differential effects of blockade of the HER3-PI3K-Akt pathway by EGFR kinase inhibitors and EGFR monoclonal antibodies on combinations with IGF-1R kinase inhibition

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Background: The receptor for epidermal growth factor (EGFR) can drive growth and survival of tumor cells through activation of the Akt and MAPK pathways. Both neutralizing antibodies and tyrosine kinase domain inhibitors (TKIs) of EGFR have achieved clinical benefit within a broad group of unselected patients, however efficacy is most often associated with stable disease, and current efforts are focused on further improving efficacy through the identification of patient selection biomarkers and the design of combinatorial regimens that enhance efficacy.
Studies have shown that expression of HER3 correlates with sensitivity to the EGFR TKI erlotinib. HER3 is expressed by epithelial tumor cells but not by tumor cells that have undergone an epithelial-mesenchymal transition, and this is supported by retrospective clinical analysis showing heightened benefit for erlotinib in tumors that express the epithelial protein marker E-cadherin. Mechanistically, signaling through the EGFR-HER3 heterodimer can mediate the efficacy for erlotinib against the Akt, but not the MAPK, pathway. We have recently found that kinase inhibition of IGF-1R by OSI-906 can promote activity within the EGFR-HER3-Akt pathway. In a reciprocal manner kinase inhibition of EGFR-HER3 by erlotinib upregulates the phosphorylation state of IGF-1R. This reciprocal receptor cooperativity contributes to the synergistic inhibition of Akt and survival by erlotinib in combination with OSI-906. We have also recently reported that while both erlotinib and the EGFR antibody C225 inhibit EGFR-MAPK, only erlotinib can inhibit EGFR-HER3-Akt in epithelial cells. Since the pharmacologic efficacy for an antibody appears to be mediated solely through downregulation of the MAPK pathway, these data would suggest that antibody sensitivity may be mechanistically more affected by mutations in K-Ras as compared to erlotinib, whose activity is additionally translated through the Akt pathway.
Materials/Methods and Results: We sought to determine if these differing effects on downstream signaling would confer differential cooperativity with OSI-906. Herein we find that C225 does not activate IGF-1R phosphorylation as is observed with erlotinib, suggesting that this element of EGFR/IGF-1R crosstalk is dependent on the EGFR-HER3-Akt pathway but not the EGFR-MAPK pathway. In combination, C225 and OSI-906 promote synergistic growth inhibition, but this is much less when compared to the effects of combining erlotinib with OSI-906. Moreover, the combination of OSI-906 with erlotinib, but not C225, achieves synergistic inhibition of Akt.
Conclusions: Collectively these data indicate that not all biomarker and combination strategies may be similarly applied for both antibody and TKI inhibitors of the EGFR.

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Development and characterization of predictive markers to the IGF-1R inhibitor, PQIP, in colorectal cancer (CRC)

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Background: Signaling through the insulin-like growth factor 1 receptor (IGF-1R) promotes cell growth, migration and survival in several human tumors. IGF-1R is overexpressed in colorectal cancer (CRC) and is associated with a poor prognosis and resistance to chemotherapy. Therefore, the IGF-1R pathway is an attractive target for new therapies against CRC. Although IGF-1R is overexpressed in CRC it is not predictive for response to IGF-1R inhibitors. The goal of this study was to identify predictive markers of sensitivity or resistance to PQIP in order to develop a rational basis for patient selection and combination therapy in CRC.
Methods and Results: A panel of 27 CRC cell lines were exposed to increasing doses of PQIP (0.078 μM-5 μM) and analyzed for inhibition of proliferation using the sulforhodamine B (SRB) assay. Cell lines were designated sensitive (S) or resistant (R) based on IC_{50}'s less than (S) or greater than (R) 1 μM. Therefore, to identify predictive genetic markers, five of the most S and R cell lines, differing in IC_{50} by 10-fold, were subjected to gene array analysis using the Affymetrix U133 plus 2.0 gene chip. ANOVA comparison of gene expression profiles between R and S cell lines resulted in over 100 differentially expressed genes with a global p-value of <0.04, whereas 64 transcripts met the $p < 0.001$ criteria. We initially selected two genes that were more highly expressed in the resistant cell lines and two genes that were more highly expressed in the sensitive cell lines. The specifics of the gene array signature will be presented in the poster. To characterize the potential significance of these genes, we selectively reduced their expression in S and R cell lines using stably transfected shRNAs and examined their effects on the S or R phenotype. We initially examined the effects of one of these genes on two resistant lines, SW480 and HCT116. By RT-PCR we achieved a greater than 80% reduction in gene expression in two separate clones and a nearly complete reduction in protein expression. Both of these transfected cell lines were assayed for proliferation as described above. The transfected cell lines demonstrated enhanced sensitivity to the compound when compared to a scrambled shRNA control. These results indicate that we may have discovered a predictive marker, that when modulated, increases the responsiveness to PQIP. Further experiments are being performed with the remaining three genes to see if a similar effect is observed.
Conclusions: In the current study we demonstrate that we have discovered a potential biomarker for resistance to the IGF-1R inhibitor, PQIP. These results will be validated in vivo using xenografts and human CRC explants, while other potential markers will continue to be explored.

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Amphiregulin and Epiregulin expression in primary colorectal cancer identifies a subgroup of patients that will respond to EGFR inhibition

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Background: Amphiregulin (AR) and Epiregulin (ER) expression is a candidate marker for response prediction in metastatic colorectal cancer patients treated with Cetuximab. (Khambata-ford et al., JCO). However, so far the development of a defined and validated cutoff for ER expression in order to predict response to cetuximab remains absent. Here, we want to develop a cutoff value for Epiregulin expression, instrumental for response prediction, and we validate this cutoff in an external dataset.
Patients and Methods: We determined AR and ER expression in FFPE tissue from 195 patients treated with cetuximab and Irinotecan in clinical trials. Amphiregulin and Epiregulin mRNA expression levels were quantified in duplicate with q-RT PCR and normalized using three reference genes (GAPDH, UBC and RPL13a). Statistical validation of the optimal cut-off is done by "leave-one-out" cross validation. A piecewise log-hazard ratio model with use of restricted cubic splines is used to enable analysis of the expression data as a continuous variable.
The presence of KRAS mutations in codons 12 and 13 were determined by an allelic discrimination assay on a 7500HT Real Time PCR System (Applied Biosystems).
Results: On a smaller dataset of 152 patients, multivariate analysis for survival of Epiregulin expression yielded a Hazard Ratio of 0.67. The optimal cutoff for response prediction (0.5233) was obtained with ROC analysis. In this analysis, an area under the Curve of 0.75, 0.69 and 0.70 was obtained when Epiregulin expression was used respectively for predicting Objective response, Progression free Survival and Overall Survival in Kras wild-type tumors. We will propose a cutoff value in a larger patient population (195 patients) which will be internally validated. This way we estimate the robustness of the obtained cutoff value.
This cutoff value will be consecutively validated in an independent external dataset comprising 170 patients. We will evaluate the performance of the cutoff value in this dataset.
Conclusions: AR and ER mRNA expression in FFPE primary tumors is instrumental in predicting response of subsequent metastatic lesions to therapy with cetuximab combined with irinotecan, especially when combined with Kras mutation state. We will show the external validation of a generated cut-off, a requirement for any biomarker being developed.