87 POSTER

Correlation analysis utilizing measurements of tumor biomarkers and antibody efficacy against EGFR, IGF1R and VEGFR2 receptors in multiple tumor models

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Preclinical drug development strategies in cancer research frequently utilize a bank of tumor cell lines to establish in vivo subcutaneous (SC) xenograft mouse tumor models for testing a pipeline of drug development candidates. To maximize the use of our own tumor models, selected preclinical biomarkers were evaluated, to aid in the development of novel treatment regimens through a correlation analysis between therapeutic efficacy (T/C%) and biomarker measurements. In addition, to better understand the biology of tumor progression in our models, we have evaluated the correlation between pairs of biomarkers.

Histological biomarkers including Ki-67, pHistone-H3, Apoptag, and cleaved caspase-3 were evaluated in 37 SC xenograft models of a variety of cancer types (n = 3 tumors per model). ELISA biomarkers including HIF-1alpha, human and mouse VEGF, total AKT and ERK2, total IGF-IR and EGFR were evaluated in tumor homogenates from 13 SC models to date. EGFR and IGF-1R receptor expression on the cell lines utilized in the SC xenograft tumor models were analyzed by flow cytometry. Treatment efficacies were evaluated in 15−24 SC models with the target specific monoclonal antibodies IMC-A12 (anti-human IGF-IR), cetuximab (anti-human EGFR), and DC101 (anti-mouse VEGFR2).

Pair-wise correlation analyses of measurements found that the great majority of measurements did not correlate. However, significant correlations did occur such as correlation between EGFR receptor expression in vitro and total EGFR in the tumor measured by ELISA. EGFR FACS also correlated with IGF-IR FACS, but total EGFR and IGF-IR measured by ELISA in tumor protein lysates did not correlate. Interestingly, the efficacy of cetuximab was weaker in models with lower Ki-67 expression, potentially associated with the ability of cetuximab to lower Ki-67 expression in many SC models. Although not well understood, one of the strongest correlations was between IMC-A12 anti-IGF-IR efficacy total ERK2 levels in tumor homogenates.

While some measurements are pending to complete the histology and ELISA biomarker measurements for all 37 SC models, relationships are already standing out as potentially important for developing biomarkers for predicting efficacy and for understanding the mechanism underlying efficacy. Importantly, the biomarker measurements completed on a bank of models, can be utilized repeatedly for all future treatment candidates in the pipeline.

88 POSTER

Development of proximity-based immunoassays for activated HER1, HER2, and HER1-HER2 heterodimers in formalin-fixed, paraffin-embedded (FPPE) cells

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Background: Expression of the ErbB/HER tyrosine kinase receptors in solid tumors has been correlated with poor clinical prognosis of patients and with responses to targeted therapies. With the exception of ErbB2/HER2, sensitive and quantitative detection methods for the ErbB/HER family members are lacking, making treatment choices difficult. Only a subset of patients that overexpress ErbB2/HER2 respond to targeted treatments such as trastuzumab and lapatinib, indicating inherent or acquired resistance. Ligand bound and autophosphorylated ErbB/HER heterodimers are the proposed potent signaling forms of this receptor family, and represent a possible mechanism for determining response to targeted drugs. We have developed and characterized quantitative FFPE assays measuring EGF-dependent increases in activated ErbB/HER receptors in multiple formats: phosphorylated HER1-HER2 heterodimers, phosphorylated HER1, and phosphorylated HER2.

Materials and Methods: Assays for activated HER1 and HER2 receptors in FFPE and cell lysate formats were developed using the VeraTag technology, which requires the proximity of an antibody pair for light-dependent release of a fluorescently labeled tag, followed by capillary electrophoresis quantitation. Assays were verified by co-immunoprecipitation.

Results: We identified a panel of cell lines that differentially express a range of EGF-dependent HER1-HER2 heterodimers using the lysate format of the VeraTag proximity assay, and verified the results by co-immunoprecipitation. This cell line panel was used to develop an assay in FFPE cells that detects a 5−10-fold range of EGF-dependent HER1-HER2 heterodimer signal, using a HER1 pTyr and HER2 antibody pair. HER1-HER2 heterodimer signals in FFPE cells are consistent with expected results from co-immunoprecipitation and VeraTag lysate assays, and display low background with isotype control antibodies. Additionally, three activated HER1 VeraTag FFPE assays were developed which detect a 20-fold or greater range in EGF-dependent phosphotyrosine signal, utilizing a HER1 and site-specific or pan-pTyr-antibodies. Lastly, we developed an activated HER2 assay that detects a 10-fold range in EGF-dependent, HER2-associated phoshotyrosine using a HER2 and pan-pTyr antibody. For all assays, the sensitivity of detection is within the range of amplified receptor levels (>$0.5-1\times10^5$ receptors/cell). We are currently using these assays to measure HER1 and HER2 activation via phosphorylation and heterodimerization in breast, lung and ovarian tumors.

Conclusions: Quantitative and specific VeraTag proximity assays in FFPE cells have been developed for the detection of EGF-activated HER1-HER2 heterodimers, and phosphorylated HER1 and HER2 receptors. These FFPE assays measuring HER activation status will be combined with our existing HER1, HER2, and HER3 FFPE assays, and may have utility in drug development and patient selection for HER targeted therapies.

89 POSTER

Evaluation of peripheral blood cells and hair as surrogate tissues for clinical trial pharmacodynamic assessment of XL147 and XL765, inhibitors of the PI3K signaling pathway

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Background: Identifying and validating surrogate tissues for biomarker development for kinase inhibitors is a challenge in clinical development. Blood and tissue samples obtained from consented pts in Ph 1 clinical trials of inhibitors that target the PI3 kinase (PI3K) signaling pathway were analyzed using conventional or exploratory readouts. Peripheral blood mononuclear cells (PBMCs) and hair are tissue compartments of high interest, owing to accessibility and feasibility of repeated sampling.

Methods: XL147 is a selective oral inhibitor of Class I PI3K isoforms. XL765 is a selective oral inhibitor of Class I PI3K isoforms and mTOR-Raptor/Rictor complexes. These agents are in clinical development for the treatment of pts with solid tumors.

Results: PI3K pathway signaling is robust in PBMCs and requires no exogenous stimulation. Both XL147 and XL765 reproducibly inhibited PI3K pathway signaling in PBMCs, as demonstrated by reduced phosphorylation of AKT, PRAS40, and 4EBP1. However, a number of factors such as fluctuations in pathway signaling, complexity of sample processing, and limited stability of stored lysates make PBMC analysis for this pathway technically challenging. In contrast, hair data were consistently of high quality, presumably reflecting a rapid and simple collection and fixation procedure. Administration of XL147 or XL765 is associated with pharmacodynamic modulation of the PI3K pathway in hair bulbs, as shown by reduction in phosphorylation of multiple pathway readouts (eg AKT, PRAS40, and 4EBP1). Efforts are ongoing to correlate changes in these PI3K pathway readouts from surrogate tissues with effects in matched sequential tumor biopsies.

Conclusions: PBMCs represent a useful, albeit technically challenging, compartment for pharmacodynamic assessment of inhibitors targeting PI3K pathway signaling. Hair bulbs are a convenient tissue for assessing such inhibitors, in light of accessibility, simple handling, and high basal PI3K pathway activity. Emerging technologies for hair processing, such as whole-mount immunostaining, have the potential to alleviate the labor- and time-intensive requirements inherent in conventional immunofluorescent analysis.