

## IGF-2 in Herceptin refractory HER2-positive breast cancer

Human breast cancers have been divided into several subtypes. Among them, HER2-overproducing (HER2-positive) breast cancer is an aggressive one because of its capability to develop life-threatening metastasis. Overproduction of HER2 (or erbB2), a cell surface protein, is observed in about 25% of invasive breast cancers and significantly associated with poor prognosis in breast cancer patients. Increased production of HER2 in breast cancer cells leads to drug resistance and tumor metastasis, which accounts for ~90% of breast cancer deaths. Herceptin (or trastuzumab), a humanized anti-HER2 antibody (Ab), is an effective HER2-targeted therapy for breast cancer patients with HER2-positive tumors. However, resistance to Herceptin-containing treatments frequently occurs after initial response. The resistant tumors likely recur and spread to other organs. Moreover, many metastatic HER2-positive breast cancers do not respond to Herceptin-based treatments. Herceptin resistance is currently a huge clinic problem for successful treatment of HER2-positive breast cancers. To date, we do not have predictive biomarkers for Herceptin response. It is really needed to find useful methods correcting the resistant problem in order to increase survival of the patients with HER2-positive metastatic breast cancer.

We recently discovered that the insulin-like growth factor-1 receptor (IGF-1R)-initiated signaling caused HER2-positive breast cancer cells not responded well to Herceptin. Increased production of one of the IGF-1R ligands, insulin-like growth factor-2 (IGF-2) but not IGF-1 was the key reason for abnormal activation of the IGF-1R signaling, leading to Herceptin resistance. More importantly, we detected higher levels of IGF-2 in the blood of HER2-positive breast cancer patients who had a poor response to Herceptin-containing treatments than that of those patients who had a good response. The tumors obtained from the patients with poor response also displayed higher levels of insulin receptor substrate-1 (IRS1), which is an essential molecule under IGF-1R signaling. Our data suggest that increased production of both IGF-2 and IRS1 induces super activation of the IGF-1R signaling, and thereby makes HER2-positive breast cancers refractory to Herceptin treatments. These novel findings inspire us to believe **that increased blood levels of IGF-2 give rise to poor Herceptin response in HER2-positive breast cancer; and inhibition of the IGF-2/IGF-1R/IRS1 signaling will overcome the resistance and greatly enhance Herceptin-mediated antitumor activity against HER2-positive breast cancer.** We have two areas of study: 1) To figure out the serum levels of IGF-2 that can be used as a reliable biomarker predictive for poor response to Herceptin-containing treatments in HER2-positive breast cancer. 2) To investigate if the IGF-2 blocking Ab Xentuzumab and the histone deacetylase (HDAC) inhibitor (HDACi) entinostat (both Xentuzumab and entinostat are in clinical trials of breast cancer patients) will ultimately enhance the antitumor activity of Herceptin towards Herceptin resistant HER2-positive breast cancer.

For Aim 1 studies, we will utilize *in vivo* mouse models to examine whether the mouse blood levels of IGF-2 increase to a point that can correlate with resistance to Herceptin. We will also measure IGF-2 levels in the blood of the patients with HER2-positive breast cancer who have undergone neoadjuvant Herceptin-containing treatments. We will determine whether the patients with little or no pathological response to the treatments have higher blood levels of IGF-2 than those patients with partial or complete pathological response. For Aim 2 studies, we will take advantage of our Herceptin resistant HER2-positive breast cancer cells (pool2 and HR20) to generate *in vivo* tumor models. With these mouse models, we will test if the combinations Herceptin and Xentuzumab or Herceptin and entinostat, as compared to single drug, will show potent antitumor effects on tumor growth and lung metastasis.

Our proposal is to discover IGF-2 as a useful biomarker predicting Herceptin response for HER2-positive breast cancer patients. We believe that increased levels of IGF-2 in the blood of patients with HER2-positive metastatic breast cancer cause little or no response to Herceptin-containing therapies. Measurement of the blood IGF-2 levels in the patients will tell us the effectiveness of Herceptin treatments. Data generated will not only improve our understanding of why some patients exhibit poor response to Herceptin, they may also help to develop novel drugs/methods to stop progression and shrink existing metastases of HER2-positive breast cancer.