

Abstract

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.

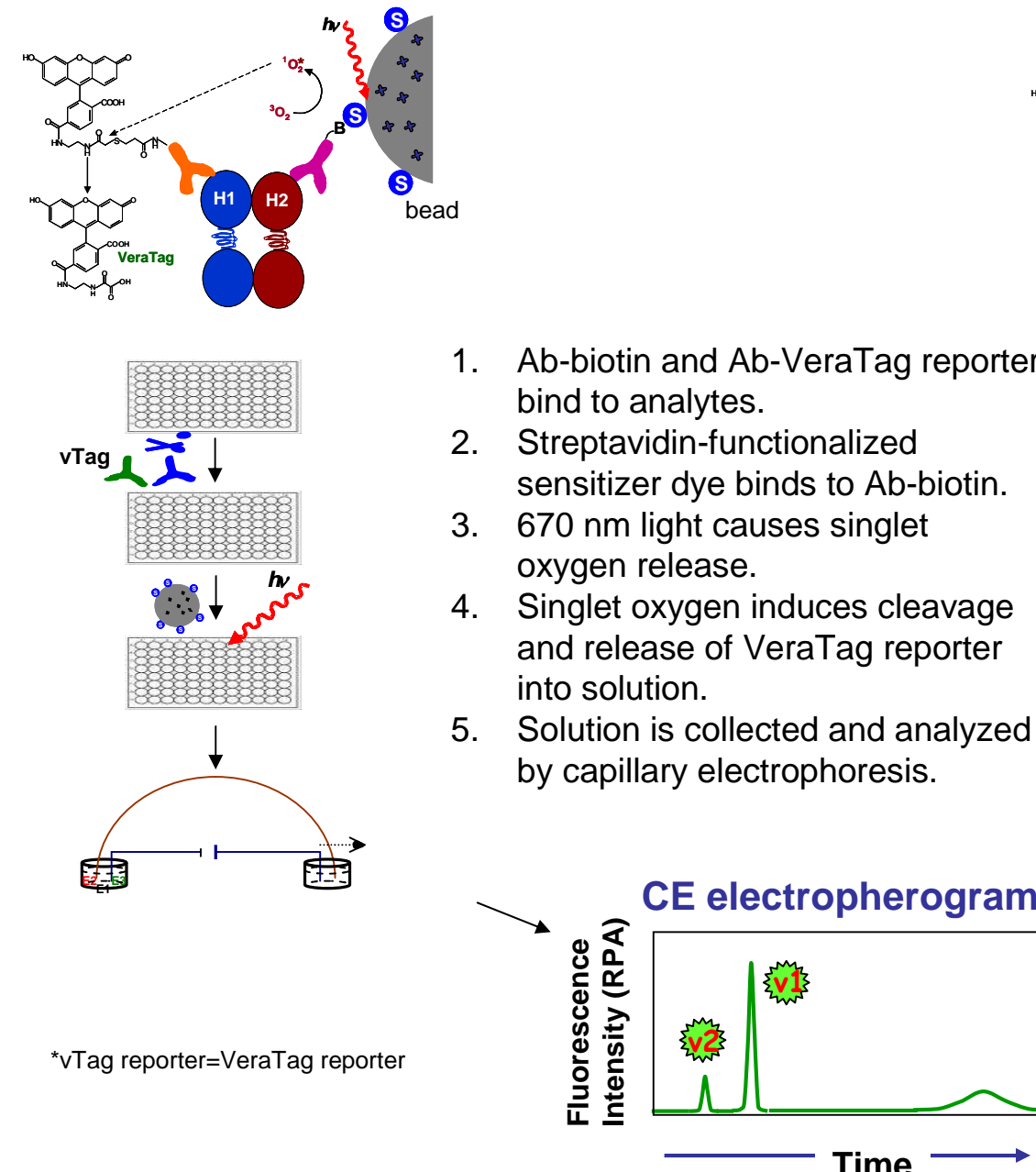
Materials and Methods: Assays for activated HER1 and HER2 receptors in FFPE and cell lysate formats were developed using the VeraTag technology, which requires the proximity of an antibody pair for light-dependent release of a fluorescently labeled tag, followed by capillary electrophoresis quantitation. 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 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 phosphotyrosine 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 x 10⁵ receptors/cell). We are currently using these assays to measure HER1 and HER2 activation via phosphorylation and heterodimerization in breast, lung and ovarian tumors.

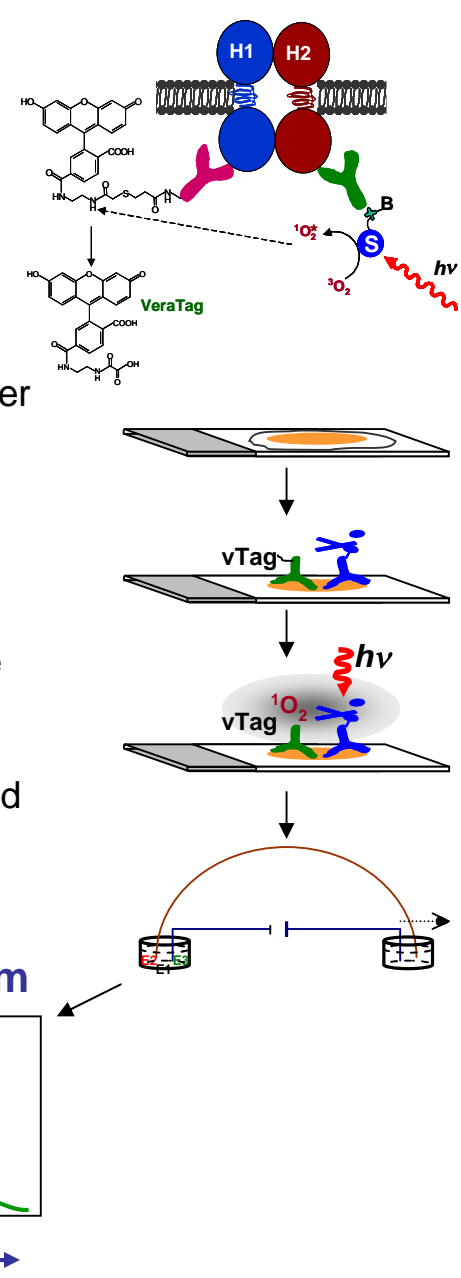
Conclusions: Quantitative and specific VeraTag proximity assays in FFPE cells have been developed for the detection of EGF-activated HER1-HER2 heterodimers, and phosphorylated HER1 and HER2 receptors. These FFPE assays measuring HER activation status will be combined with our existing HER1, HER2, and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

Methods

VeraTag lysate assay



VeraTag FFPE assay

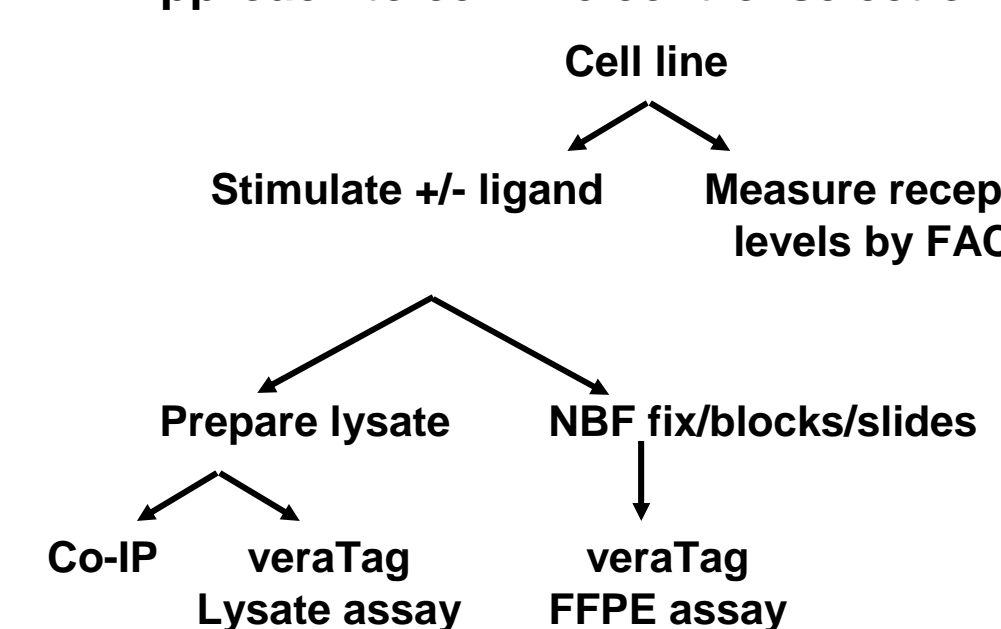


Results

1. Identification and characterization of HER1-HER2 control cells

Cell line controls spanning a range of HER1-HER2 heterodimer expression were selected by VeraTag lysate assays and confirmed by co-immunoprecipitation. HER1-HER2 heterodimers were induced upon stimulation with EGF, while total HER1 and HER2 protein expression levels were unchanged.

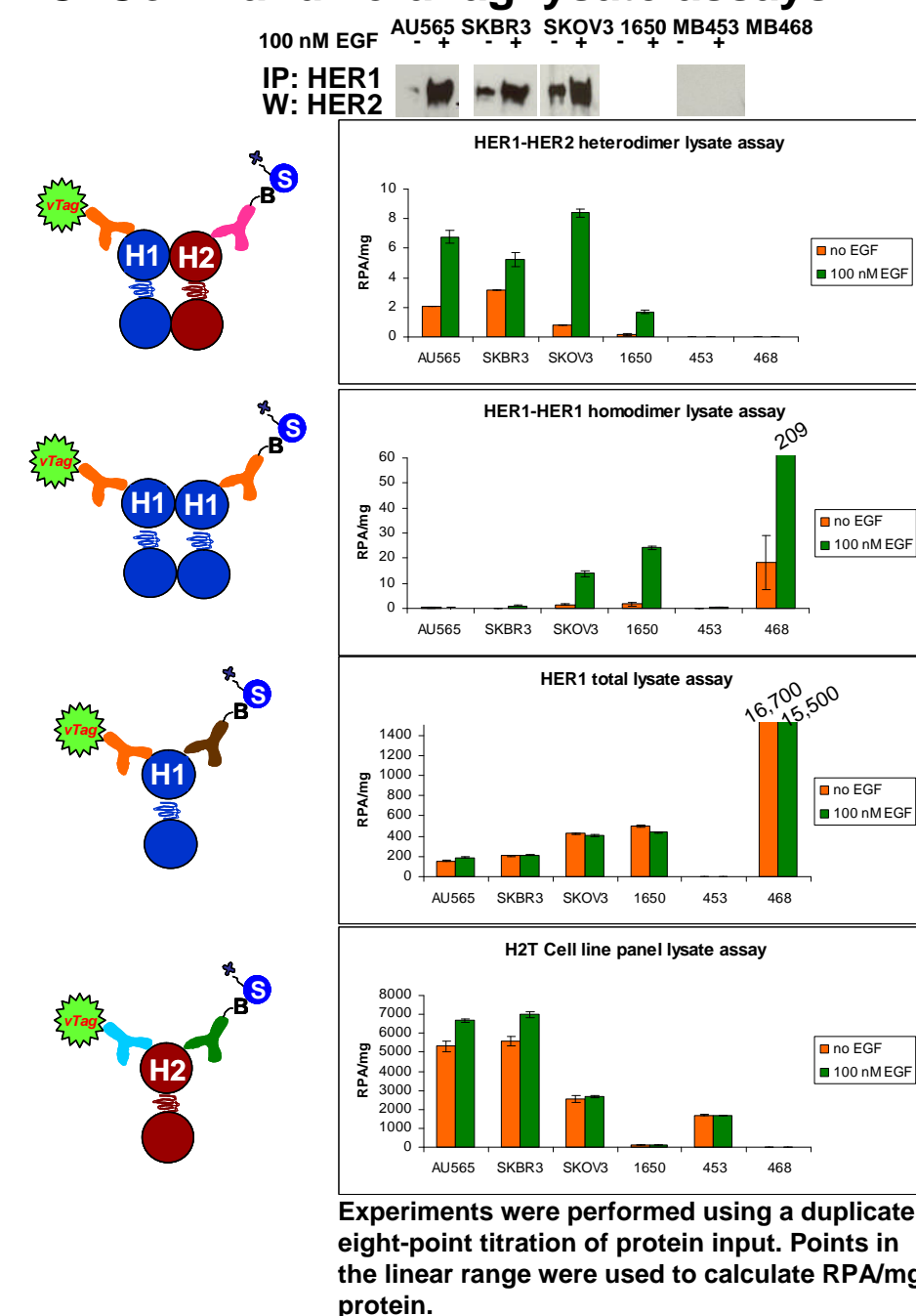
A. Approach to cell line control selection



B. Receptor levels measured by FACS

Cell line	HER1	HER2
AU565	204,560	1,447,688
SKBR3	143,599	1,402,832
SKOV3	387,771	657,080
1650	158,872	53,810
MB453	5,316	292,984
MB468	3,389,807	1,209

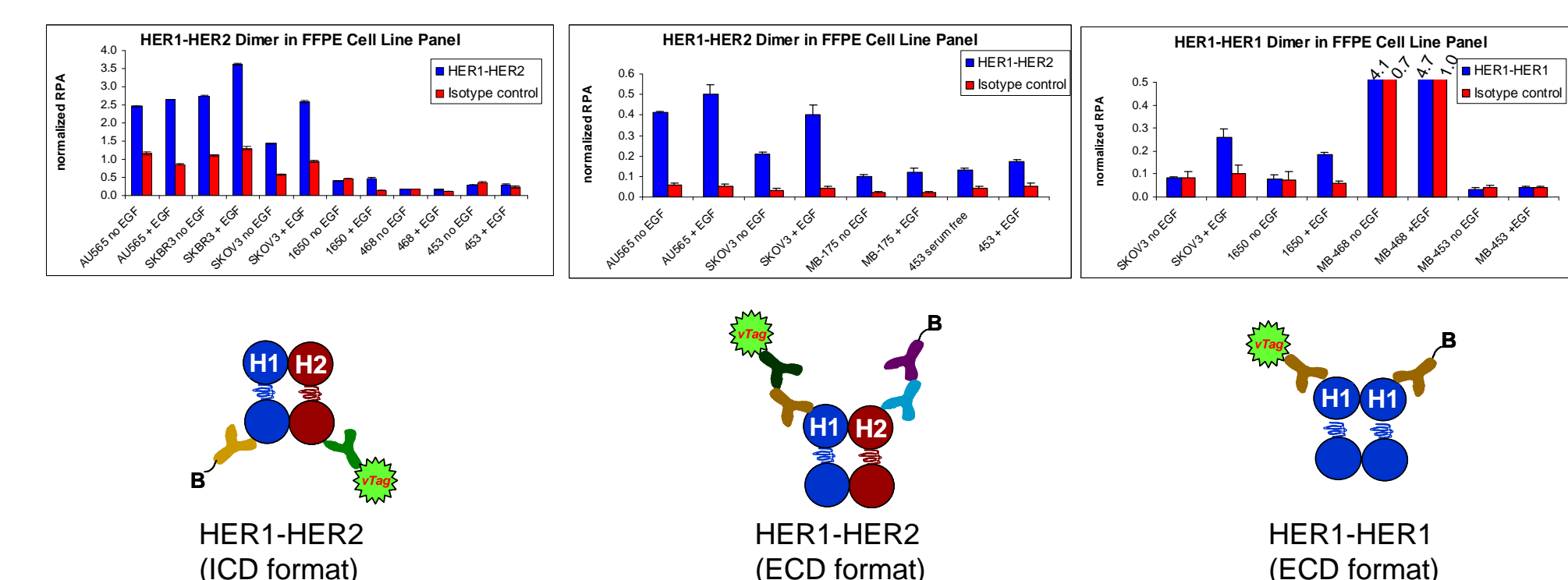
C. Co-IP and veraTag lysate assays



2. HER1-HER2 and HER1-HER1 dimer assays in FFPE cell lines

Cell line controls identified via HER1-HER2 lysate assays were used for the development of FFPE HER1-HER2 heterodimer and HER1-HER1 homodimers assays.

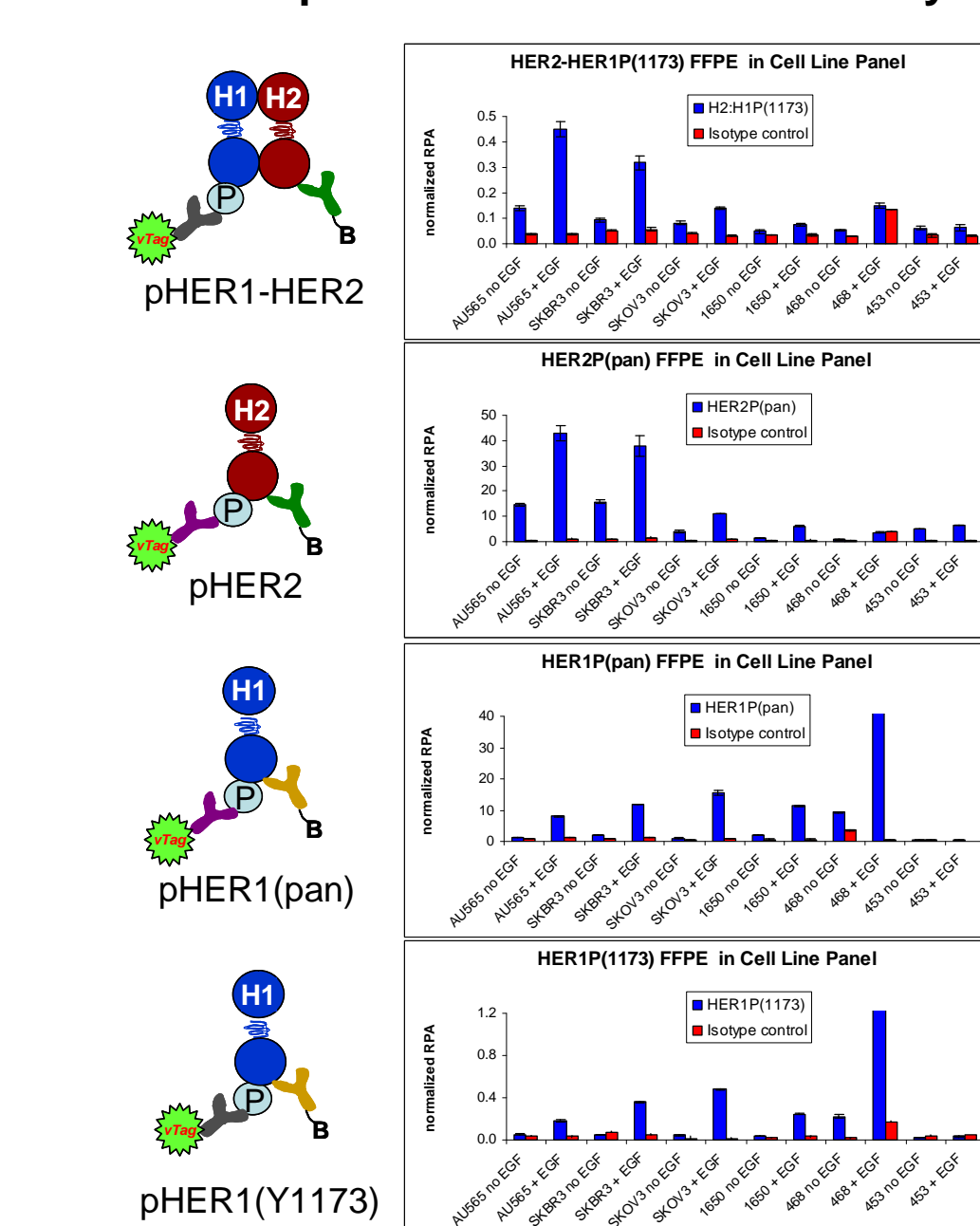
As HER2 levels increase, there is significant ligand-independent HER1-HER2 heterodimer signal. Ligand-dependent and ligand-independent HER1-HER1 homodimers are observed.



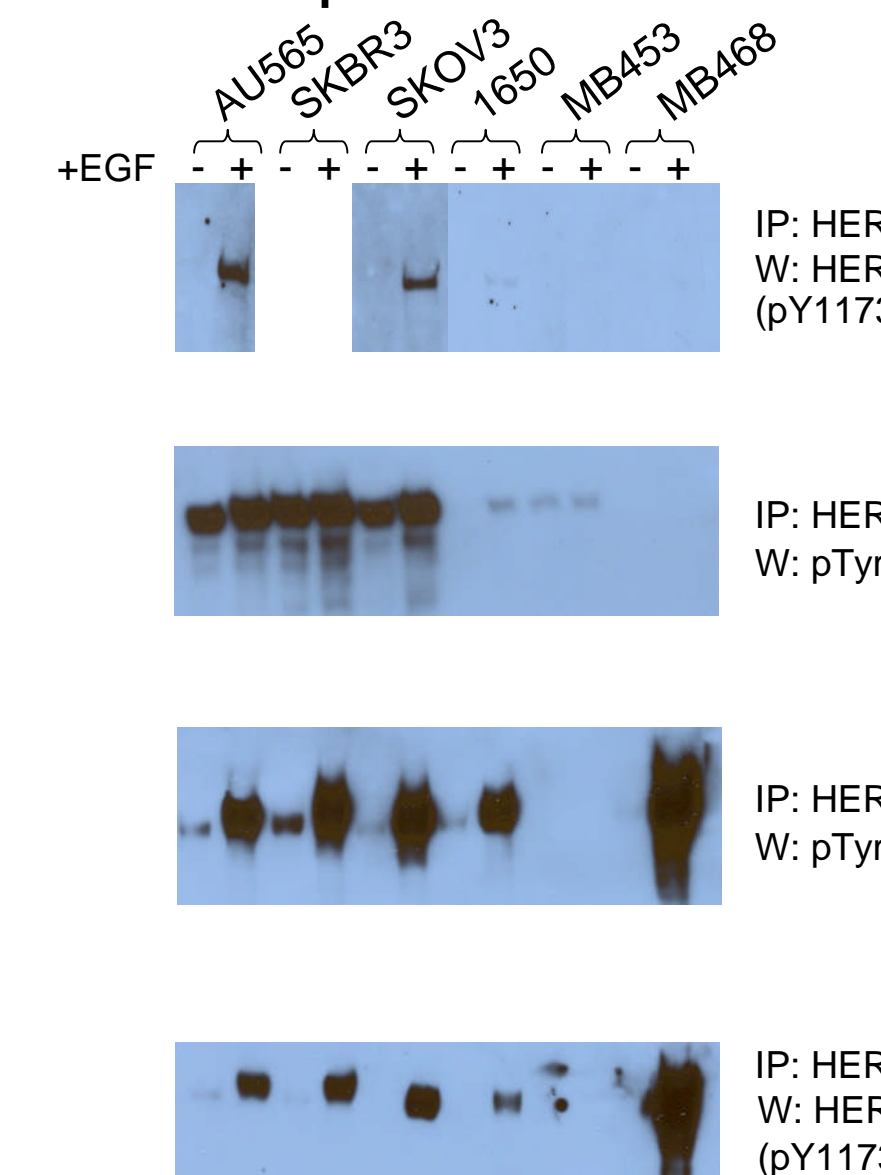
3. Activated HER1, HER2 and HER1-HER2 in FFPE cell lines

Cell line controls identified by VeraTag lysate assays were used for development of FFPE assays measuring activated HER1, HER2 and HER1-HER2 heterodimers. Assay signals in FFPE track with levels expected from lysate assays. FFPE assays were cross-validated by co-immunoprecipitation.

A. Cell line panel in activated FFPE assays



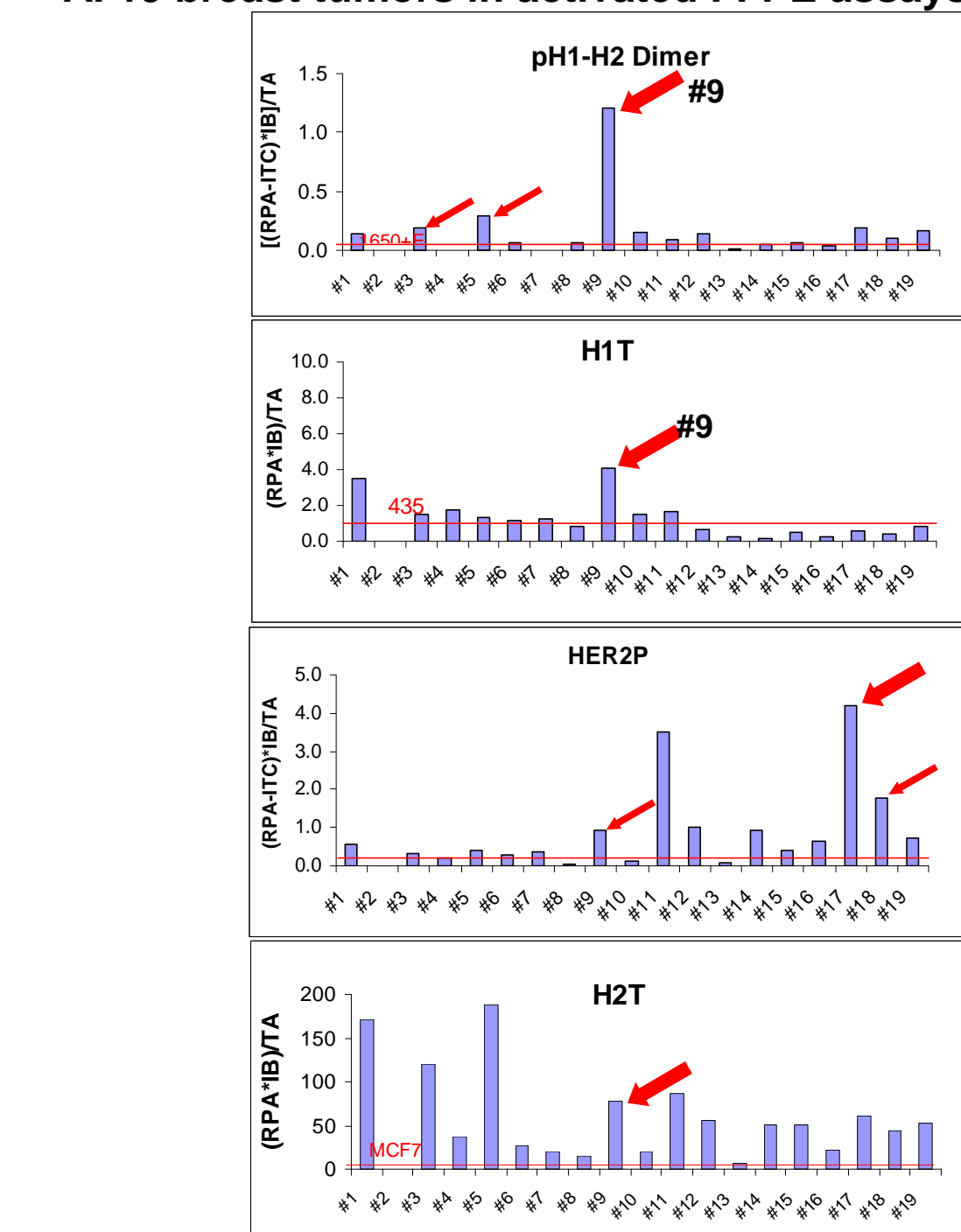
B. Cell line panel in Co-IP/Westerns



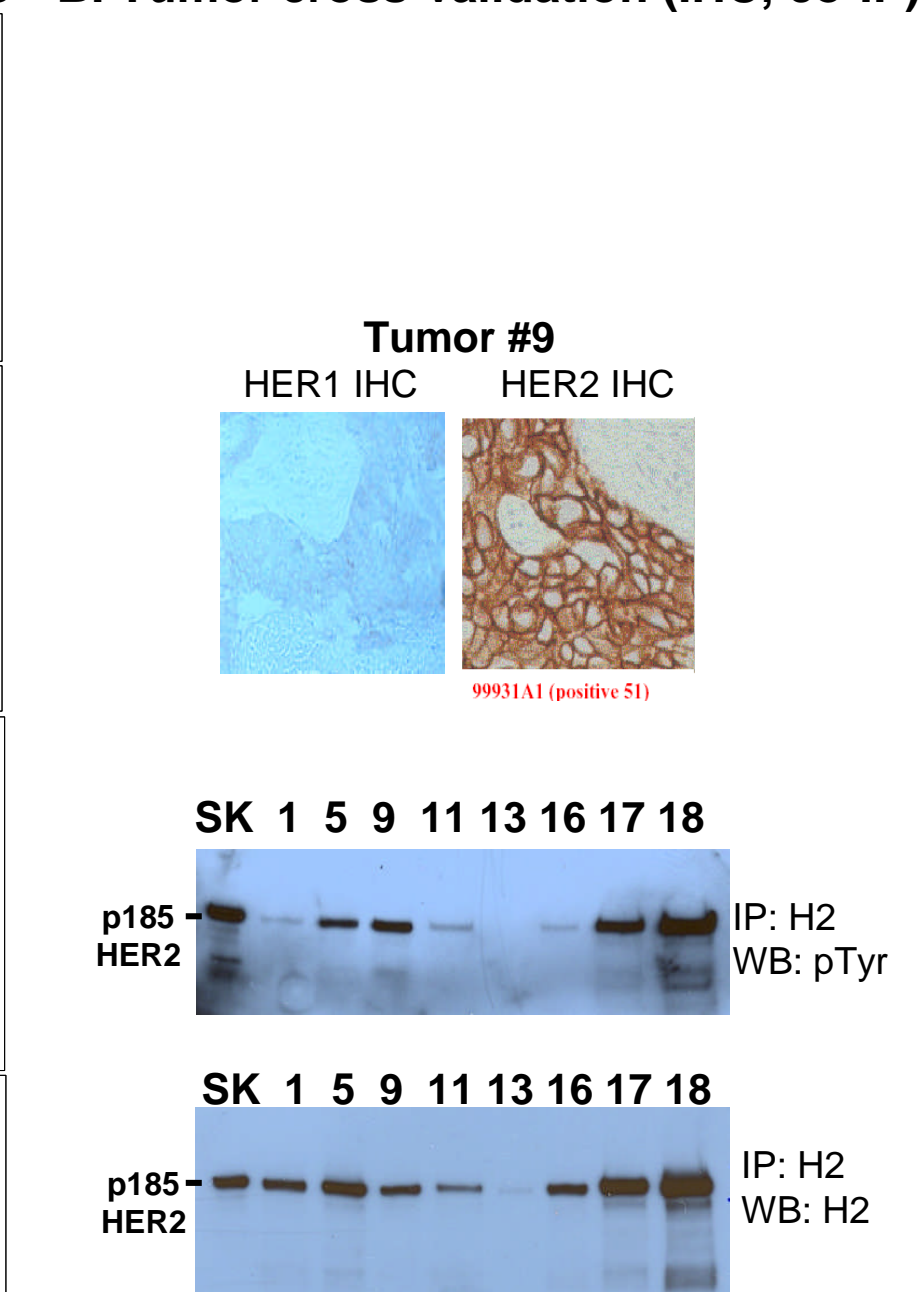
4. Activated HER1-HER2 and HER2 in FFPE breast tumors

3/19 tumors positive (15%) for pHER1-HER2, and 10/19 positive (50%) for pHER2. IHC/Co-IPs indicate HER1 and HER2 in tumor #9, and pHER2 in tumor lysates.

A. 19 breast tumors in activated FFPE assays

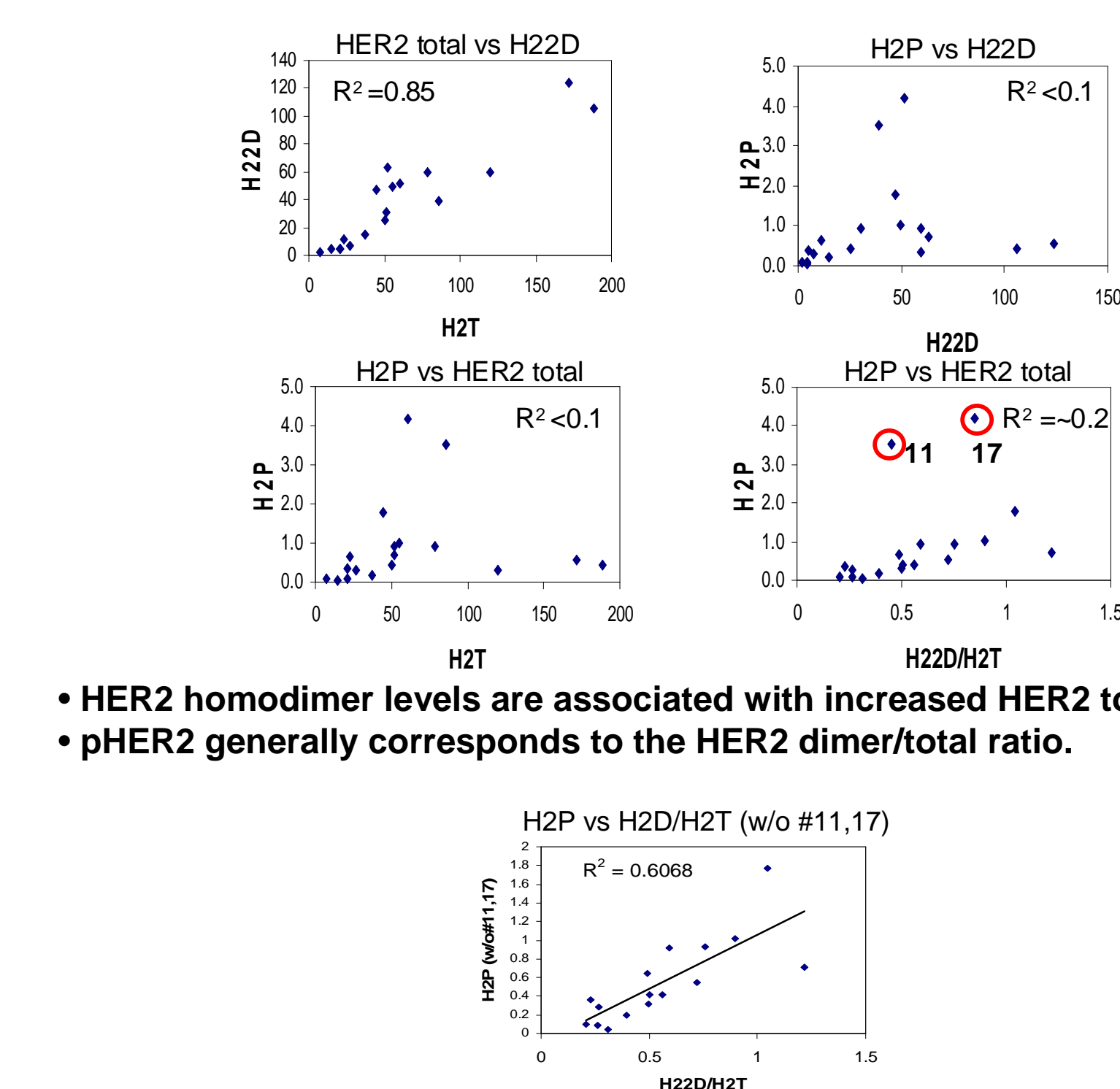


B. Tumor cross-validation (IHC, co-IP)



5. Relationships between activated HER2 and HER2 total

Weak association of HER2 forming homodimers may depend on total HER2. pHER2 may indicate increased activation of HER2 through homodimerization or ligand-dependent heterodimerization



HER2 homodimer levels are associated with increased HER2 total level. pHER2 generally corresponds to the HER2 dimer/total ratio.

Conclusions

To investigate mechanisms of response to HER targeted therapies, we have developed VeraTag lysate and FFPE assays to measure dimerized and/or activated HER1 and HER2: HER1-HER1 homodimers, HER1-HER2 heterodimers, phosphorylated HER1, phosphorylated HER2, and phosphorylated HER1-HER2 heterodimers. These novel VeraTag assays were cross-validated in cell lines using co-immunoprecipitation and Western blotting.

To date, we have profiled over 30 HER2-positive FFPE breast tumors for phospho-HER2, phospho-HER1, and phospho-HER1-HER2 heterodimers. We observed ~50% of the tumors to be positive for phospho-HER2 and ~15% positive for phospho-HER1-HER2 heterodimers.

In the breast tumors assayed here, the measure of phospho-HER2 may indicate the level of HER2 activation, as it generally correlates with the ratio of HER2 dimer/HER2 total.

We anticipate offering a suite of FFPE-based assays including VeraTag dimer and activated assays in addition to our CLIA-validated HER2 total and HER2 homodimer assays, as well as our recently developed FFPE assays for HER1 and HER3 total protein expression. In combination, we anticipate these FFPE assays to provide powerful tools to more accurately predict clinical response to both HER1 and HER2 targeted therapies in tumor biopsy tissue.