Pathway Coding Information

Investigators will be asked to code their abstract based on the TRWG Pathways to Clinical Goals. They will:

1. Identify the pathway(s) most appropriate for the abstract

- Biospecimen-based assessment tool (e.g. serum or tissue biomarker)
- Image-based assessment tool (e.g. PET, MRI, optical)
- Agent intervention (e.g. small molecule drug, inhibitory, antibody)
- Immune response modulation intervention (e.g. vaccine, cytokine)
- Interventive device (e.g. radiation, surgical device)
- Behavioral modification (e.g. nutrition, exercise)

2. Identify the pathway step(s) most appropriate for the abstract (Note: Pathway steps are indicated within the pdf's linked above.)

- Credentialing (scientific validation, need, feasibility)
- Supporting tool (e.g. animal model, cohort, specimen repository)
- Creation of modality (e.g. medicinal chemistry, multi-lab validation)
- Preclinical development (e.g. toxicology, test on phantoms)
- Phase I/II clinical trial

3. Identify the population(s) most appropriate for the abstract

- Individuals at risk for cancer
- Early stage cancer
- Late stage cancer
- Pediatrics
- Minorities and Underserved

4. Identify the organ site(s) most appropriate for the abstract

- Breast
- Brain
- Colorectal
- GI (other than colorectal)
- GU (other than prostate)
- Gynecological
- Head and neck
- Hematopoietic
- Lung
- Prostate
- Skin
- Rare (sarcoma, etc)
- All or most of the above

Tips:

Basic versus Translational Research: Discovery research (fundamental as well as cancer-specific) occurs upstream of the pathways. The pathways start with the credentialing of a basic research discovery to determine if it is broadly applicable, important, and/or feasible enough to invest in its translation. For example, the discovery and characterization of a gene and determination of oncogenic activity in mice is discovery research. Early translational research, on the other hand, could involves modulating the gene in a large panel of cancer cell lines to determine if it is applicable to a particular form of cancer.

Translational versus Clinical Research: The pathways terminate in Phase I/II clinical trials, which are part of early translational research. Phase III clinical trials, by contrast, are considered to be part of late translational research (per the President's Cancer Panel definition). Correlative studies to clinical trials (including Phase III) that ask specific questions about cohort selection, response markers, etc. are considered early translational research.

More than one pathway: There are cases where both an assessment tool and an interventive device are developed concurrently, for example a biomarker of response to a particular intervention/treatment. The intervention pathways may include an assessment tool as a supporting tool in the pathway. If the intervention is novel and is being co-developed with an assessment tool, the intervention pathway is most relevant. If use of the assessment tool is novel and the intervention is well established, the assessment tool pathway is most relevant.

Continuum: Cancer research is a continuum, so distinctions between steps in a pathway are not always clear. There may be no right or wrong answer. The pathway grids are being used as tools to form multi-investigator/multi-disciplinary groups that have the potential to produce a product that completes an entire translational pathway.

Example 1

Multiplexed In Situ Analysis of Protein Expression (AQUATM) To Predict Breast Cancer Prognosis and Response to Trastuzumab

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HER2 belongs to a family of tyrosine kinase receptors, including EGFR, HER3, and HER4, that homo- and hetero-dimerize to activate distinct programs of proliferation, survival, migration, and angiogenesis. In breast cancer, this family also demonstrates cross-talk with the hormone receptors for estrogen (ER) and progesterone (PR). Trastuzumab is the primary and most effective antibody-based targeted therapy in advanced breast cancer, and recently evidence of its

effectiveness has been extended to the adjuvant setting. In this study, we quantitatively assessed the expression of ER, PR, and the four HER family proteins in four subcellular compartments using automated quantitative analysis (AQUA®), a novel, immunofluorescence-based quantitative method of measuring protein expression in situ with the accuracy and precision of an ELISA assay. We compared expression patterns in breast cancer specimens with patient survival in one cohort and with response to trastuzumab therapy in a second cohort.

Individual, pairwise, and multivariate logistic models were constructed with the data from expression of the six proteins in the BC Cancer Agency trastuzumab-treated cohort (n = 152). Prediction error estimates for dichotomized outcome (complete/partial response vs. stable/progressive disease) were assessed with leave-one-out cross-validation and the corresponding intervals with bootstrap resampling. Using continuous AQUA scores, both low estrogen receptor expression (p = .027) and high HER2/neu (p = .0007) expression were significantly associated with favorable clinical response. A multivariate model including ER, HER2, EGFR, and HER4 had a misclassification estimate of 31.8% (95% CI, 18.9-44.7), which was superior to any one protein, IHC, or FISH for prediction of trastuzumab response.

In summary, using AQUA, we found that combined analysis of ER, PR, and ErbB family members improved prognostic classification, and we developed a multivariate logistic model that optimized prediction of trastuzumab response in the metastatic setting. As trastuzumab treatment is extended to patients in the adjuvant setting, development of prediction methods such as these will be essential to accurately identifying patients who will respond favorably to treatment.

Keywords: HER2; quantitative immunofluorescence; trastuzumab

Step 1: TRWG Developmental Pathways

Please select at least **one** below.

	ASSESSMENT		INTERVENTIVE			
	Biospecimen	**Imaging**	**Agents**	**Immune**	**Devices**	**Behavioral**
Credentialed discovery	☐	☐	☐	☐	☐	☐
Supporting Tools	☐	☐	☐	☐	☐	☐
Creation of Modality	☒	☐	☐	☐	☐	☐
Preclinical development	☐	☐	☐	☐	☐	☐
Phl/II Clinical trials	☐	☐	☐	☐	☐	☐

Step 2: Select Your Population

Please select at least **one** below.

- ☐ At Risk
- ☒ Early disease
- ☐ Late disease
- ☐ Pediatric
- ☐ Minorities & Underserved

Step 3: Select Your Organ Sites

Please select at least **one** below.

- ☒ Breast
- ☐ Brain
- ☐ Colorectal
- ☐ Gastrointestinal (other than CR)
- ☐ Genitourinary (other than prostate)
- ☐ Gynecologic
- ☐ Head and Neck
- ☐ Hematopoietic
- ☐ Lung
- ☐ Prostate
- ☐ Skin
- ☐ Rare (Sarcoma, etc)
- ☐ All or most organ sites

Rationale: The modality being developed is "the use of AQUA measurement of ER, Pr, and the four HER family proteins together with a mathematical model to predict breast cancer prognosis and response to trastuzumab". This is a biospecimen-based assessment tool. AQUA itself is a component of the modality, and its basic technical characteristics have already been validated/credentialed. This fits the "creation of modality" step best since it is being tested in several retrospective cohorts, although it is noted that it represents two different through related modalities – one for prediction of survival, the other for prediction of response to trastuzumab.

Example 2

Strategies for Overcoming Trastuzumab Resistance Caused by PTEN Deficiency

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ErbB2 overexpression is found in ~30% of human breast cancers and leads to a very aggressive, highly metastatic breast cancer phenotype and poor patient survival. The ErbB2-targeting antibody trastuzumab (Herceptin) has shown remarkable therapeutic efficiency in some patients with ErbB2-overexpressing breast cancer. However, the overall trastuzumab response rate is limited and the causes of trastuzumab resistance are poorly understood. We found that PTEN activation contributes to the antitumor activity of trastuzumab and that reduced PTEN expression in ErbB2-overexpressing human breast cancer cells confers trastuzumab resistance in vitro and in xenograft models. More importantly, in a small cohort of patients with ErbB2-overexpressing breast cancers treated by trastuzumab plus taxanes, those with PTEN-deficient tumors had significantly worse response than those with normal levels of PTEN (see reference). Thus we hypothesized that PTEN deficiency in an ErbB2-overexpressing breast tumor is a sensitive and specific predictor of trastuzumab resistance and that combination treatment with trastuzumab and agents targeting PI3K pathways will overcome trastuzumab resistance caused by PTEN loss. Cell culture and xenograft models were used to test a panel of clinically applicable, small-molecule inhibitors of the Akt/mTOR signal transduction pathway, a critical pathway downstream of ErbB2, and to identify compounds with the ability to restore trastuzumab sensitivity to PTEN-defi cient cells. When trastuzumab was combined with the Akt inhibitor triciribine, breast cancer cell growth was inhibited and apoptosis was induced. In a xenograft model, combination therapy with trastuzumab and triciribine dramatically decreased the average tumor size. The combination of trastuzumab and the mTOR inhibitor RAD001 also slowed breast cancer cell growth in vitro and in vivo.

A Phase I/II clinical trial was initiated to test the efficacy of combining the mTOR inhibitor RAD001 with trastuzumab in patients with resistance to trastuzumab-based therapy for metastatic breast cancer. Of six patients treated to date, four exhibited stable disease for >5 months, as defined by RECIST criteria. Our findings demonstrate that combining trastuzumab with inhibitors of the PI3K/Akt/mTOR pathway is a clinically applicable strategy, and that combinations of trastuzumab with triciribine or RAD001 are promising regimens for rescue of trastuzumab resistance caused by PTEN loss.

Reference Nagata Y et al. Cancer Cell 6:117, 2004.

Keyword: breast cancer, PTEN, combination therapy

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Supporting Tools	☐	☐	☐	☐	☐	☐
Creation of Modality	☐	☐	☐	☐	☐	☐
Preclinical development	☐	☐	☐	☐	☐	☐
PhI/II Clinical trials	☐	☐	☒	☐	☐	☐

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Rationale: The modality being developed is a therapeutic agent combination that is useful in patients with trastuzumab resistance caused by PTEN loss. This is an agent-based interventive modality. The first paragraph explains the discovery research that led to the concept for this modality. The second paragraph is in vitro and in vivo work that credentials the concept, and the last paragraph describes Phase I/II trials for the combination. Thus, two steps of the Agents pathway are indicated.