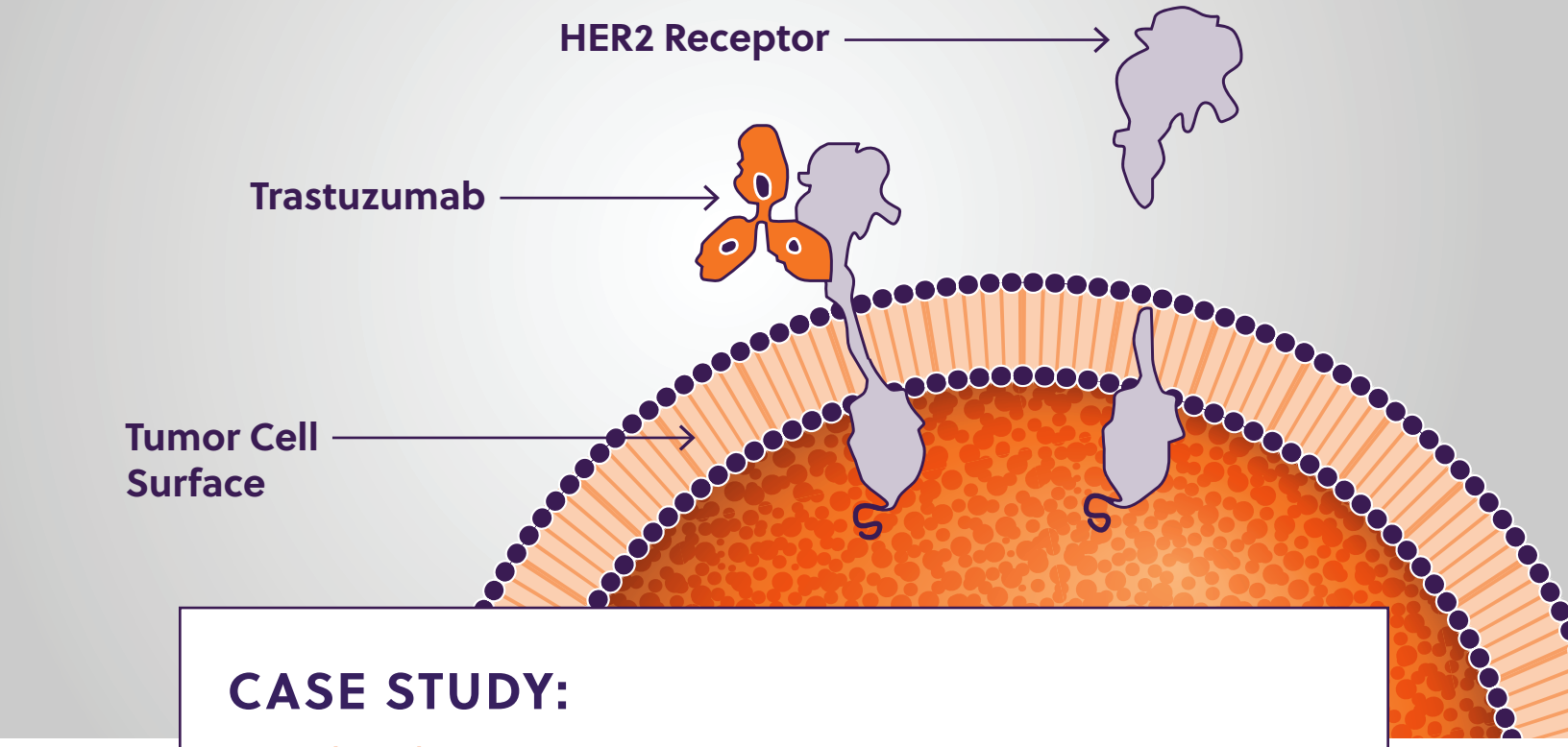

CASE STUDY:

Establishing HER2 as a Companion Diagnostic Biomarker for the Treatment of Breast & Gastric Cancers

More than 20 years ago, pathologist Josef Rüschoff and his team in Kassel, Germany began evaluating the HER2 tissue biomarker in breast cancer samples to help establish a standardized central scoring of the marker in clinical trials. Since then, the group, which is now Discovery's Global Tissue Biomarker Services Europe (formerly Targos Molecular Pathology GmbH), has been instrumental in helping to determine HER2 assay design, evaluation, scoring, and clinical utility for use in breast, esophageal and gastric cancers. Discovery's CLIA-certified, CAP-accredited and GCLP compliant laboratories have participated in hundreds of studies in the last two decades, including the pivotal Phase III global clinical trials that resulted in HER2 assay clearance as a companion diagnostic to Trastuzumab and other HER2-targeted drugs. Our Discovery Biomarker Academy™ with worldwide pathology training capabilities then helped speed the adoption and use of these assays and the guided therapy into clinical routine.

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Evolution of the HER2 Biomarker

BREAST (BC) & GASTRIC CANCER (GC)

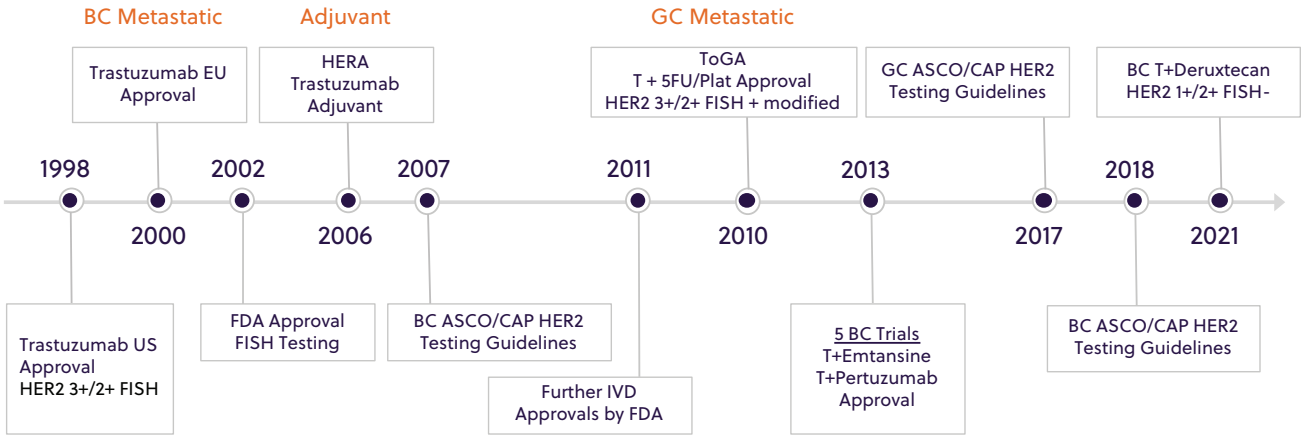

Figure 1: *Timeline of the HER2 biomarker assay evolution. Discovery's Global Tissue Biomarker Services Europe participated in hundreds of global clinical trials to help collect the data submitted to gain clearance for use of HER2 assays in gastric and breast cancer (ToGA and HERA trials), helped define and refine scoring, and trained pathologists globally to ensure standardized clinical use and adoption into clinical routine.*

HER2 is a growth-promoting protein and member of the human epidermal growth factor receptor family. Although it is present on the outside of many cells, overexpression of the HER2 protein or amplification of the HER2 gene (i.e., HER2-positive status) is associated with several solid tumor types and aggressive cancers that can grow and spread more quickly than others (Koeppen et al., 2001; Cancer.org).

UNDERSTANDING EFFICACY WITH ACCURATE HER2 BIOMARKER TESTING

Trastuzumab (Herceptin®) is a recombinant monoclonal antibody against HER2 that was developed by Roche to treat specific types of breast cancer. Used alone or in combination with chemotherapy, it has clinical activity in metastatic breast cancers overexpressing HER2. The efficacy and safety of Trastuzumab was investigated in an international, intergroup, open-label, phase 3 randomized trial known as the Herceptin Adjuvant (HERA) (Breast International Group [BIG] 01-01) Trial (Piccart-Gebhart et al., 2005). The trial included 1,694 women with HER2-positive breast cancer and followed them for at least one year of drug therapy after excision of early-stage breast cancer and completion of chemotherapy. Prior to study randomization, Discovery's Tissue Biomarker Services laboratory in Germany analyzed more than 12,000 patient tumors to confirm HER2-positive status first measured at the participating institutions in the HERA clinical trial. To reduce the risk of false positives that had been observed in community-based assays, it was critical that the HER2-positive status be verified by an expert, high-volume laboratory with rigorous quality control procedures (Paik et al., 2002). Confirmation for this study required immunohistochemical analysis (IHC) (HercepTest™, Dako) indicating increased overexpression and a positive result on fluorescence in situ hybridization (FISH) for HER2 amplification (PathVysion®, Abbott) The HERA trials showed Trastuzumab to significantly improve disease-free survival and reduce the rate of recurrence by almost 50% following adjuvant chemotherapy, among women with HER2-positive breast cancer compared to women who did not receive the drug.

The efficacy of Trastuzumab in early clinical studies underscored the importance of accurately assessing HER2 status in breast cancer patients. Standard validated methods for IHC and FISH are used on formalin-fixed paraffin embedded (FFPE) tumor samples to determine whether patients are eligible for Trastuzumab treatment but early scoring systems such as the HercepTest were validated only to score breast cancer samples (Hoffman et al. 2008).

EXPANDING THE APPLICATION OF HER2 ASSAYS TO NEW INDICATIONS
(Gastric and Esophageal Cancers)

HER2 overexpression, as assessed by IHC and in situ hybridization techniques, can also be identified in a significant number of gastric and esophageal tumors (Gravalos and Jimeno, 2008), but for Trastuzumab therapy to be used in treating gastric cancer, the accuracy of HER2 testing had to be established in tissue from patients with those cancers to determine patient eligibility. Worldwide, gastric cancer is associated with substantial morbidity and mortality, but early screening, identification, and treatment can significantly reduce the incidence of advanced disease. A new validated standard of HER2 testing had to be developed for gastric cancer and was proposed to identify patients for enrollment in the ToGA trial, a multicenter Phase III trial of Trastuzumab in combination with fluoropyrimidine and cisplatin as a first-line therapy. Based on a thorough investigation and application of the HER2 detection methodologies (i.e., IHC and FISH), several refinements were made to the scoring system to address the inherent differences in tumor biology that make HER2-positive identification in gastric cancers more difficult, namely HER2 heterogeneity (<30% of tumor cells staining positive or focal staining) and incomplete membrane staining (Hoffman et al. 2008). Given these differences, international recommendations for HER2 testing in gastric cancer have been published that describe the guideline validation and development of standard IHC testing and the collection practices for multiple tumor biopsy specimens to be used for HER2 diagnostics. Discovery's Global Tissue Biomarker Services Europe helped illustrate potential pitfalls in staining and interpretation, see figure 2, and defined HER2 expression and proper scoring, (Rüschoff et al. 2010).

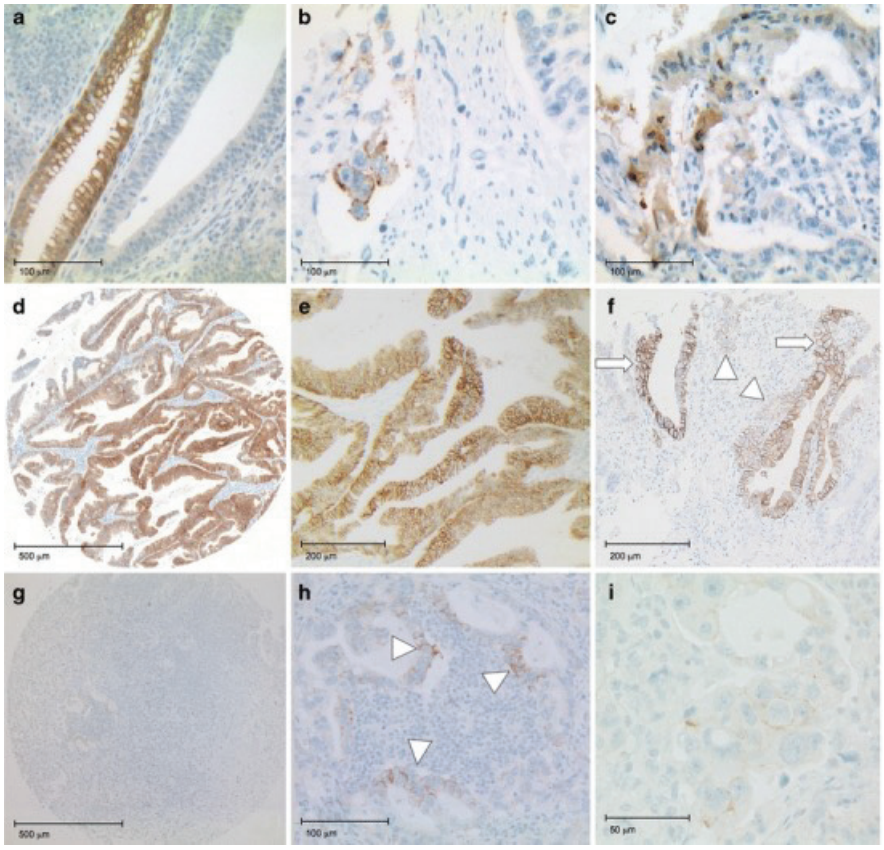

Figure 2: *Photomicrographs of TMA examples.* ***a–c*** *Artifacts leading to potential mis-scoring on IHC:* ***a*** *intestinal metaplasia,* ***b*** *edge artifact at TMA border with granular (not linear) pseudo-membranous staining, and* ***c*** *cytoplasmic as well as nuclear staining.* ***d–h*** *Intensity scoring: d Score 3+ visible by naked eye with membranous staining clearly visible at low magnification (obj. ×5) being either complete, basolateral or lateral (***e***, ×10).* ***f*** *Photomicrograph of TMA sample showing distinction between 2+ and 3+ IHC using 4B5 antibody. Arrows indicate areas with clearly visible membrane staining at low magnification (i.e., 3+), focally in <10% of tumor); arrowheads indicate areas where membrane staining is only visible at ×10 magnification (i.e., 2+).* ***g*** *TMA core suspicious of some focal staining at ×5 which turned out to be a focally specific membranous staining in groups of at least five cells at medium magnification (****h****, ×20; see arrowheads).* **i** *Very weak staining where membranous staining is barely visible and could only be demonstrated using high magnification (****i****, ×40)*

The clinical validation within the ToGA trial performed at Discovery's Global Tissue Biomarker Services Europe laboratory in Kassel, Germany, led by Josef Rüschoff, demonstrated that patients with gastric cancer and high levels of HER2 protein expression in the tumors derived the greatest benefit from Trastuzumab treatment, as measured by significantly improved overall survival compared to patients receiving chemotherapy alone (Bang et al., 2010). Furthermore, a post-hoc analysis of these outcomes showed that this benefit was driven by an increase in overall survival in patients with high expression of HER2 protein compared to patients with low expression. The success of Trastuzumab in treating patients with high HER2-expressing tumors has led to the recommendation that all patients diagnosed with gastric cancer have tumors assessed for HER2 status, and Trastuzumab combined with chemotherapy became a new standard option for patients with HER2-positive advanced gastric or gastro-esophageal cancer (Bang et al. 2010).

> Notably, the method for HER2 scoring in gastric cancer developed by scientists at Discovery's Global Tissue Biomarker Services Europe is recommended by the recent American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines. This evidence-based guidance emphasizes the importance of scoring methods, interpretation, and reporting of results for pathologists, in addition to laboratory quality assurance monitoring, to decrease variability and improve analytic consistency (Bartley et al. 2016).

Starting in 2010, many clinical research groups evaluated whether the HER2 protein on tumor cells can be exploited by other drugs with different modes of actions to inhibit tumor growth and activate the tumor specific immune response. They also sought to expand the HER2 biomarker's clinical utility to broader intended use populations that would include the majority of tumors which show only moderate HER2 expression. Small molecule inhibitors such as Lapatinib, Afatinib and Neratinib or HER2 heterodimer inhibitors, such as Pertuzumab were approved in several indications or subgroups, but all required high HER2 expression, using the same testing algorithm initially established for Trastuzumab.

Next, Trastuzumab was coupled via a chemical linker to anti-tumor,cytotoxic agents into so-called Antibody Drug Conjugates (ADCs). Among others, Trastuzumab-emantasine and Trastuzumab-duocarmazine, were evaluated in clinical studies, again using the HER2 testing expertise at Discovery's Global Tissue Biomarker Services Europe, showed good clinical benefit in combinations with other drugs in patients who failed prior Trastuzumab or Pertuzumab therapy.

Unexpectedly, all drugs still required high HER2 expression, characterized by an IHC score of 3+ or 2+ and FISH+, which limited the use of the biomarker to only ~20% of patients with these characteristics.

UNDERSTANDING THE CLINICAL UTILITY OF HER2 LOW EXPRESSION

More recently, some patients with breast cancers formerly classified as HER2 negative, (IHC Score 1+ or 2+ and FISH-), have had positive clinical outcomes after receiving more potent anti-HER2 agents, such as Trastuzumab-duocarmazine (Modi et al. 2020; Eiger et al. 2021). This is significant because nearly twice as many patients have breast cancers that could be categorized as HER2-low than HER2-positive and under many of the current treatment guidelines these patients may not be eligible for common anti-HER2 therapies (Cronin et al. 2010; Giuliani et al. 2016; Eiger et al. 2021). HER2-low tumor populations can be further subdivided by a distinct molecular signature based on their expression of hormone receptor (HR) that points to genetic, pathological, and prognostic differences (Schettini et al. 2021; Eiger et al. 2021). This heterogeneity among HER2-low tumors necessitates sensitive and reproducible assays to measure low HER2 expression and avoid harmful side effects in HER2 high expressing patients (Schettini et al. 2021).

Finally, the HER2 target and its highly standardized testing were successfully used to target several tumor types with a wide variety of HER2 expression levels and bring clinical benefit and improved survival to a significantly expanded number of patients.

HER2 Biomarker Adoption into Clinical Routine:

DISCOVERY BIOMARKER ACADEMY™: EXPERT PATHOLOGY TRAINING

Fast and standardized testing for HER2 results is extremely important, as these cancers are often rapidly progressing, therefore the laboratories must be well-trained and experienced in reviewing these samples. Given the predictive value of HER2 protein levels for therapeutic response to many drugs, IHC should be the initial testing methodology and fluorescence in situ hybridization or silver in situ hybridization should be used to retest borderline results (Ruschoff et al. 2012). Bright-field methodologies, which are superior to fluorescent methodologies at identifying heterogeneous staining, should also be used. **Due to the more complex nature of accurately identifying HER2-positive gastric cancer samples, specific training is required to avoid underscoring results that lead to false-negatives. Expertise in histology, IHC and microscopy is essential.** Proper techniques and appropriate training can achieve a good concordance between IHC and in situ hybridization results (Ruschoff et al. 2010). Analysis and scoring of HER2 testing results for gastric and gastro–esophageal junction cancer should be performed by a qualified, board-certified pathologist specifically trained in HER2 testing methods and interpretation. As detailed above, quality HER2 testing is critical to ensure accurate diagnoses and appropriate patient care for both breast and gastric cancers. These findings highlight the need to validate the method for determining HER2 status before designing clinical trials aimed at new tumor types.

Discovery provides pathologist and technician training programs through its Discovery Biomarker Academy (formerly Targos Advance Expert Training). Programs are led by experienced pathology faculty including Prof. Josef Rüschoff, Prof. Bharat Jasani, and Prof. Sunil Badve. Using digital slide platforms such as PathXL, and via virtual webinars, a professional team of trainers from leading institutions in Europe, USA, Brazil and Taiwan, offer a sustainable approach for international biomarker standardization and training. To date, this program has trained more than 4.000 international pathologists in the interpretation of FDA-approved testing guidelines.

DISCOVERY BIOMARKER ACADEMY™: GLOBAL BIOMARKER STANDARDIZATION & TRAINING ACTIVITIES

>4,000 pathologists trained since 2005

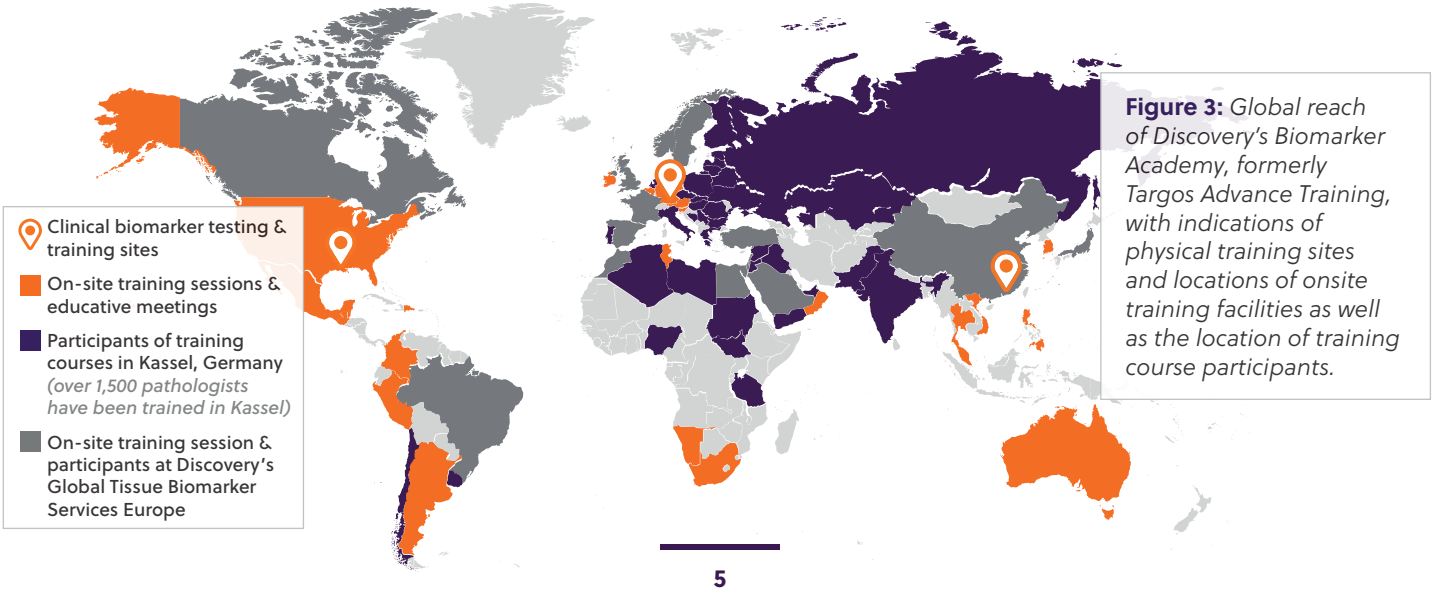

Figure 3: *Global reach of Discovery's Biomarker Academy, formerly Targos Advance Training, with indications of physical training sites and locations of onsite training facilities as well as the location of training course participants.*

One example of early success with the training program was with the evaluation of the HER2 companion diagnostic test. Despite high quality IVD reagents for IHC and ISH, discrepancies in the HER2-based patient selection were often observed between observers and across laboratories. The Discovery Biomarker Academy™, along with the use of standardized reagents, automated staining platforms, and data from ring studies and publications, has helped reduce HER2 testing discrepancies to a minimum. Since 2010, the educational program has cultivated a highly interactive HER2 community and supported the introduction of anti HER2 drugs into clinical routine by providing reference pathology services and acting as the central testing lab -- **reviewing approximately 30,000 samples from hundreds of clinical centers, proposing adapted scoring algorithms and sharing the gained knowledge with the clinical community in training courses.** As a result of this success, within the past decade, immune therapies for new tumor indications targeting the interaction of PD-1 and its ligand PD-L1 have gained market approval. Discovery's Biomarker Academy has continued to evolve its training practices and is now instrumental in global introduction of PD-L1 and other biomarkers to guide new high value therapies.

DISCOVERY'S ToGA TRIAL EXPERIENCE *(Breast (BC) & Gastric Cancer (GC))*

HER2 Testing in > 100 clinical trials

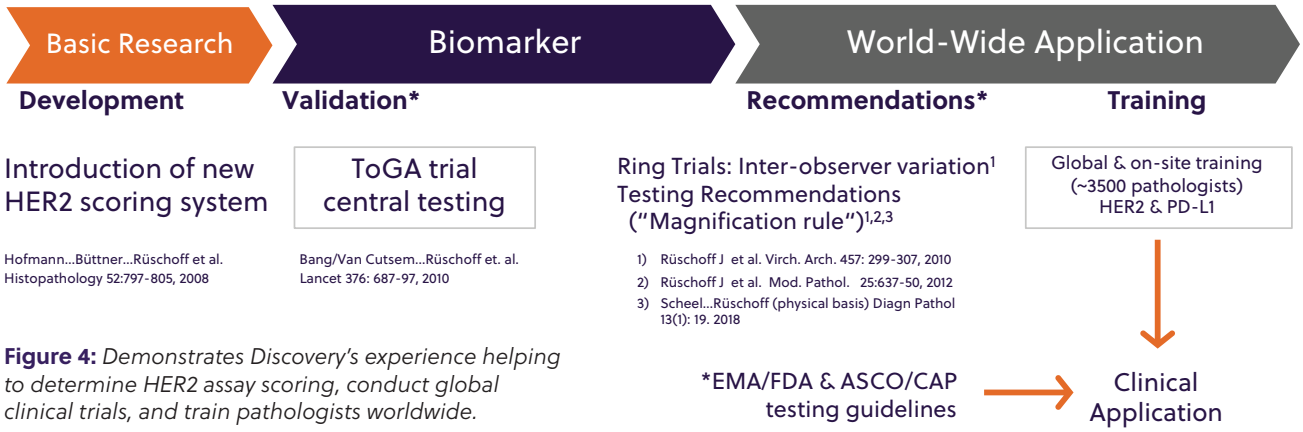

Figure 4: *Demonstrates Discovery's experience helping to determine HER2 assay scoring, conduct global clinical trials, and train pathologists worldwide.*

SUMMARY

The prognostic, diagnostic and predictive value of the HER2 biomarker has been established for breast, gastric, and gastro-esophageal junction cancers. Over the past two decades, therapies such as Trastuzumab, Lapatinib and Trastuzumab-duocarmazine have been developed to treat these cancers, but their use and efficacy depend on accurate classification of the HER2 status of the tumor. Differences in HER2 expression among these cancers and tissue types requires expert analysis to score and interpret, and Discovery's Global Tissue Biomarker Services Europe has led the field in establishing methodological guidelines to meet this need.

The Discovery Biomarker Academy offers global pathology biomarker training services with experts instructing on cutting-edge platforms and current methods for IHC and in situ hybridization. Discovery's Global Tissue Biomarker Services have supported assay development, validation and global clinical trial testing by providing expeditious and high-quality results, which are crucial to the success of drug and companion diagnostic development programs and are fit for any stage of research and development.

BIBLIOGRAPHY

1. Bang, YJ, et al. "Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial." The Lancet, vol. 376, no. 9742, 2010, pp. 687-697.

2. Bartley, A. N., et al. "HER2 Testing and Clinical Decision Making in Gastroesophageal Adenocarcinoma: Guideline From the College of American Pathologists, American Society for Clinical Pathology, and American Society of Clinical Oncology." Arch Pathol Lab Med, vol. 140, no. 12, 2016, pp. 1345-1363.

3. "Cancer.org." Understanding a Breast Cancer Diagnosis, American Cancer Society, 20 September 2019, https://www.cancer.org/cancer/breast-cancer/understanding-a-breast-cancer-diagnosis/breast-cancer-her2-status.html. Accessed 26 June 2021.

4. Cronin, K. A., et al. "Population-based Estimate of the Prevalence of HER-2 Positive Breast Cancer Tumors for Early Stage Patients in the US." Cancer Invest., vol. 28, no. 9, 2010, pp. 963-968.

5. Eiger, D., et al. "The Exciting New Field of HER2-Low Breast Cancer Treatment." Cancers, vol. 13, no. 1015, 2021. The Exciting New Field of HER2-Low Breast Cancer Treatment, https://doi.org/10.3390/cancers13051015.

6. Giuliani, S., et al. "In a cohort of breast cancer screened patients the proportion of HER2 positive cases is lower than that earlier reported and pathological characteristics differ between HER2 3+ and HER2 2+/Her2 amplified cases." Virchows Archiv, vol. 469, 2016, pp. 45-50.

7. Gravalos, C. "HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target." Annals of Oncology, vol. 19, 2008, pp. 1523-1529.

8. Hoffman, M., et al. "Assessment of a HER2 scoring system for gastric cancer: results from a validated study." Histopathology, vol. 52, 2008, pp. 797-805.

9. Koeppen, H. K., et al. "Overexpression of HER2/neu in solid tumours: an immunohistochemical survey." Histopathology, vol. 38, no. 2, 2001, pp. 94-104.

10. Modi, S., et al. "Antitumor Activity and Safety of Trastuzumab Deruxtecan in Patients With HER2-Low-Expressing Advanced Breast Cancer: Results From a Phase Ib Study." J Clin Oncol, vol. 38, no. 17, 2020, pp. 1889-1896.

11. Paik, S., et al. "Real-world performance of HER2 testing--National Surgical Adjuvant Breast and Bowel Project experience." J Natl Cancer Inst., vol. 94, no. 11, 2002, pp. 852-4.

12. Piccart-Gebhart, M. J., et al. "Trastuzumab after Adjuvant Chemotherapy in HER2-Positive Breast Cancer." The New England Journal of MEdicine, vol. 353, no. 16, 2005.

13. Ruschoff, J., et al. "HER2 diagnostics in gastric cancer-guideline validation and development of standardized immunohistochemical testing." Virchows Arch., vol. 457, no. 3, 2010, pp. 299-307.

14. Ruschoff, J., et al. "HER2 testing in gastric cancer: a practical approach." Modern Pathology, vol. 25, 2012, pp. 637-650.

15. Schettini, F., et al. "Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer." NPJ Breast Cancer, vol. 7, no. 1, 2021.

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