From linear ‘dogma’ and trastuzumab–emtansine to future transcriptional circuitry-based drug discovery for breast cancer

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Almost six decades have been spent by researchers and clinicians, after the DNA double helix discovery, on the simple transcription concept of a single gene–phenotype linear approach. All currently available drugs, including targeted agents, against a single genetic abnormality [1], are based on this idea established by Crick in 1958 [2]. However, the transcriptional process is far more complicated [3], with recent evidence for transcriptional circuitry driving genes and cell function. This knowledge has been enabled by high-throughput sequencing technologies and computational network approaches [4,5]. In this article, we summarize the latest success paradigm of the trastuzumab–emtansine conjugate (T-DMI) and other new combinations of monoclonal antibodies (mAbs) and tyrosine kinase inhibitors (TKIs) for human EGF receptor 2 (HER2)-positive breast cancer, all based on the central ‘dogma’ of linear experimentation. In addition, we highlight the future next-generation, molecular networks-based therapeutics in breast cancer.

Genetic & genomic advances

Despite advances in research, breast cancer remains a major health problem, with approximately 1.4 million new cases of breast cancer diagnosed worldwide [6]. Apart from established surgery, radiotherapy, systemic cytotoxic chemotherapy and tamoxifen or aromatase inhibitors, targeted therapy represents significant progress in the multimodal treatment of breast cancer.

Trastuzumab

Results from Phase III randomized controlled trials (RCTs) and meta-analyses have proven that this mAb blocking the ligand-binding extracellular part of the HER2 receptor provides a significant clinical response and survival benefit in HER2-positive female breast cancer patients [7]. In the adjuvant setting, early Phase III trials with 1- or 2-year follow-up showed that this antibody added to chemotherapy reduces the relative risk of recurrence by 50% and increases the overall survival (OS) rate. A few months ago, the HERA Phase III trial released longer follow-up

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results [7]. There were 471 reported events of disease-free survival (DFS) in 1552 patients in the 1-year trastuzumab group and 570 events in 1697 patients in the control group (30.3 vs 33.6%) at a median 8-year follow-up. Interestingly, the differences in DFS and OS remain significant at 8 years [7]. Therefore, trastuzumab is able to provide a significant benefit in terms of survival. However, we have to highlight that, despite this long-term statistically important benefit, the clinical response rate and absolute survival benefit is small (3.3% DFS). This tumor nonresponsiveness to chemotherapy plus trastuzumab is reflected by a 70% DFS rate at 8 years in the adjuvant setting and approximately 25 months median OS in the metastatic setting. In the potentially curable disease, the HERA trial at a median 8-years follow-up reports that among women who received trastuzumab, recurrence was observed in 367 women in the 1-year treatment group vs 367 women in the 2-years treatment group [7,8]. These high relapse rates with 1 or 2 years treatment with trastuzumab with no significant difference between the two groups reveal, the high intrinsic and acquired resistance rates and the temporary efficacy of trastuzumab in a substantial proportion of HER2-positive women treated with trastuzumab and chemotherapy. Once longer treatment with trastuzumab does not overcome temporary effect without increasing efficacy, research is focused in other directions to improve clinical response and survival.

Trastuzumab–emtansine conjugate

Nowadays, an alternative is offered by the single agent T-DM1. T-DM1 is an antibody drug conjugate that incorporates the HER2-targeted anti-tumor capacities of trastuzumab with the cytotoxic properties of the microtubule inhibitory agent DM1 [9].

Three well-conducted Phase II single-arm studies of T-DM1 have been published to date, with promising results [10–12]. On the basis of these positive results, the EMILIA trial – a Phase III RCT – has recently been reported [13]. Among almost 1000 HER2-positive women enrolled who had prior treatment with trastuzumab and taxane-based chemotherapy, 496 patients were randomly assigned to the lapatinib plus capecitabine group and 495 to the T-DM1 group. The median progression-free survival (PFS) was 9.6 months in the T-DM1 group versus 6.4 months in the lapatinib plus

capecitabine group (hazard ratio [HR]: 0.65; 95% CI: 0.55–0.77; $p < 0.001$). In addition, the median OS was 30.9 versus 25.1 months (HR for death from any cause: 0.68; 95% CI: 0.55–0.85; $p < 0.001$). Analyzing these exciting data, we can conclude that the EMILIA study is a positive Phase III trial providing strong evidence for the efficacy and safety of T-DM1. In this study, the crucial finding was that T-DM1 extended life by 6 months. This represents an important achievement considering that most available targeted drugs added to chemotherapy in the treatment of various types of cancer provide only a PFS benefit but no OS prolongation in the metastatic setting. In summary, T-DM1 provides high therapeutic potential, but even with this single agent, the median OS is approximately 31 months [13].

Conclusion & future perspective

Now that the clinical benefit and limitations of T-DM1 have been assessed, rational research is now exploring how to expand indications of T-DM1 and to develop new more effective drug combinations. Progress in this area has led to three Phase III trials on T-DM1, which are currently underway. The MARIANNE study is comparing the efficacy and safety of single-agent T-DM1 with T-DM1 plus pertuzumab with trastuzumab plus a taxane for the first-line treatment of HER2-positive, metastatic or locally recurrent breast cancer [14]. Moreover, the TH3RESA study is evaluating the efficacy and safety of T-DM1 compared with treatment of the physician's choice in patients with metastatic or unresectable locally advanced or recurrent HER2-positive breast cancer [101]. Furthermore, the KATHERINE study will evaluate T-DM1 versus trastuzumab as adjuvant therapy for patients with HER2-positive primary breast cancer who have residual tumor present pathologically in the breast or axillary lymph nodes following preoperative therapy. The estimated enrollment is 1484 patients and the first results are expected soon [102].

Given the presence of several or even multiple genetic aberrations in the same patient, one of the top research aims is the combination of two or more antibodies and TKIs that may act synergistically. A Phase III study, the CLEOPATRA trial, examined the activity of combined trastuzumab and docetaxel chemotherapy with or without pertuzumab in patients with HER2-positive metastatic breast cancer. The median PFS was 18.5 months in the pertuzumab group

and 12.4 months in the control group (HR: 0.62–0.51 to 0.75; $p < 0.001$). Moreover the OS rates are in favor of the trastuzumab–pertuzumab group. Based on this trial, 1 year ago the US FDA approved pertuzumab (PERJETA™; Genentech/Roche, CA, USA) in combination with trastuzumab and docetaxel for the treatment of HER2-positive metastatic breast cancer [15]. Moreover, the combination of an antibody with a TKI showed promising results in various types of cancers and can perhaps represent an important therapeutic option. In the breast cancer setting, a Phase III study of lapatinib and trastuzumab showed superior PFS and OS rates compared with lapatinib in patients with advanced EGF receptor-positive cancer who had progressed after having previously been treated with trastuzumab (HR: 0.73–0.57 to 0.93; $p = 0.008$). The same results were obtained in the neoadjuvant setting. Dual therapy with trastuzumab and lapatinib, however, shows important benefit for patients with HER2-positive breast cancer [16].

• Transcriptional network-based targets

More recent and emerging genome-scale data on both a personal mutational landscape from patient-derived sampling analysis [14], as well as basic science discoveries on transcriptome architecture exploration [17], shape now the new era of the next-generation genome diagnostics-based therapeutics will target tumor heterogeneity.

Breakthrough sequencing technologies nowadays reveal the importance not only of protein-coding sequences for identifying intragenetic variation, but also noncoding, regulatory natural variants that affect transcription and gene expression, ensuring biodiversity in human evolution [18]. Genome-wide association studies have shown that most (88%) disease-associated variants in susceptibility loci are within the non-coding region of the human genome. Therefore, mapping both protein-coding and noncoding of genomes using next-generation sequencing technologies, including whole-genome sequencing, whole-exome sequencing and RNA sequencing, appears essential for understanding oncogenesis, tumor growth and metastasis. Therefore, large-scale genomic studies are underway to explore interindividual genetic variation as a cause of diversity in phenotypes and interpatient tumor genetic and genomic mutational heterogeneity in both coding and noncoding DNA and RNA sequences to gain a better understanding of tumorigenesis and tumor progression [19].

However, the current high-throughput sequencing-based confirmation of the previous concept of clonally mutation evolution, interpatient and perhaps intratumor heterogeneity dramatically increases the challenge in how to predict tumor responsiveness to different therapeutics. However, this is not a unique problem to reach robust personalized treatment. Beyond a patient's mutational landscape assessment, the next challenge of to understand how this genomic aberration affects individual genome function. Evidence now leads to the consensus that personalized clinical medicine is much more complex than previously thought. Understanding the transcriptional circuitry that drives gene expression regulation represents a daunting challenge, which requires innovative developments in both technologies and methods.

New and exciting research efforts are emerging in the exploration of genome-wide molecular mechanisms regulating gene expression. Now, in the postencyclopedia of DNA elements era, core technologies including high-throughput sequencing and arrays, as well as living-cell imaging techniques including visualization of interacting molecules using biosensors coupled with novel computational approaches, allow the study of spatiotemporal dynamics of molecular interactions, transcriptional circuitry activity and gene expression regulation [20]. As we are now moving from the standard, linear approach of simplified single gene transcription-dominated human biology and medicine over the past approximately 60 years to a much more complex transcriptional network driving gene regulation, the new postencyclopedia of DNA elements age of clinical genomics integrating systems biology approach is now beginning. The aim of this patient-derived sampling analysis is biospecimen-based comprehensive cancer genome architecture assessment [20]. International megagenomic studies that are currently underway [103,104] will not only complete the genetic mutations catalog for each cancer type, but also aim to provide information on how genome-scale mutational and epigenetic landscapes affect transcriptional regulatory networks, gene function and cancer cell aggressive metastatic capacity or nonmetastatic behavior. This rational networks-based approach combining advances in genome science and network biology provides high expectations for the future development of robust biomarkers and more effective therapeutics.

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In summary, single-agent T-DM1 has become a new standard second-line treatment for relapsed or refractory HER2-positive breast cancer. The potential efficacy of T-DM1 as a first-line treatment in the metastatic and adjuvant setting, as well as the role of new mAbs and TKIs in various combinations for the treatment of HER2-positive breast cancer, will be determined in current and new Phase III RCTs. However, the most hope can be derived from the application of breakthrough sequencing mapping and visualizing technologies coupled with powerful new computational methods, beyond the current linear approach-based therapeutics. Exploring how mutational and functional cancer genome heterogeneity affects genes expression and cell function represents a fascinating field

for whole-genome molecular characterization of cancer. This is one of the most the most rational ways to discover novel structural and functional genome-scale targets, paving the way for discovering highly effective drugs that will disrupt cancer's molecular networks.

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