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Individualized therapy for breast cancer: a decade of improvement

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Breast cancer is the most prevalent female cancer in both the developed and less developed world. According to WHO 2013 estimates, there were over 522 000 female breast cancer deaths in 2012[1]. Breast cancer (1.7 million) was the second commonly diagnosed cancer worldwide after lung cancer (1.8 million), and followed by colorectal cancer (1.4 million)[1]. In less developed regions, breast cancer is the highest cause of cancer death in women; in more developed regions, it is the second highest cause of cancer death, after lung cancer.

Breast cancer incidence is on the rise worldwide. In Asia, the highest incidence is in the Philippines, followed by Singapore, China (including Hong Kong) and other highly populated countries such as Japan, Thailand and India[2-3]. The differences in breast cancer incidence and mortality are highly evident between the east and the west. The mortality of breast cancer is decreasing in Western cohorts, but it is increasing in Asian cohorts[4]. Breast cancer often occurs at younger ages in Asian patients, unlike their Western counterparts (peak age: 45 – 50 years *vs* 65 years)[4-5]. Lower mortality in Western countries may be attributed to the well-established breast cancer screening and preventative programs.

1 Old paradigm - "pre-genomic era"

Less than two decades ago, immunohistochemistry (IHC) was employed to validate the hormone receptor (HR) status of breast tumors. Chemotherapy would generally be given to the females with node positive or negative breast cancers >1 cm, while hormonal therapy such as tamoxifen would be given to ER(+) patients[6]. Chemotherapy and other traditional therapies were predominantly developed to benefit the entire disease population, as opposed to a subgroup of the disease. Chemotherapy is still a effective and biologically active therapy for breast cancer, eradicating all highly proliferative cancer cells. However, its major shortcoming is the toxicity to healthy cells[7]. In some cancers, the inherent cytotoxic characteristics have led to remarkable responses, such as pediatric acute lymphoblastic leukemia. Intensive combinations of cytotoxic chemotherapies have not proven to be effective in other tumors, or all subtypes of breast cancer, where the benefit does not outweigh the toxicities. Moreover, dose-intensity does not always correlate with better response rates, but gives rise to minor or greater adverse effect. Low blood cell counts, hair loss, brittle nails, diarrhea and febrile neutropenia are commonly seen in patients receiving chemotherapeutic agents[7]. Permanent cardiotoxicity damage may be caused when high doses of anthracyclines are given. Other serious long-term effects requiring additional treatment include premature menopause and infertility[8]. Additionally, chemotherapy is not always effective for all stages of breast cancer. In some early breast cancers, such as ER(+) and node-negative tumors, the survival and 5-year recurrence rate are not necessarily improved with chemotherapy[8-9]. According to an overview of randomized trials by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG), females at 50 – 69 years with node-negative disease had a lower risk of recurrence and a smaller absolute benefit from chemotherapy than the females with node-positive disease. In this population, the benefit from addition of chemotherapy is smaller in absolute terms[8-9]. Therefore, whether or not to give chemotherapy entails a complex cost-benefit consideration of the trade-off of the clinical benefit and side effects.

2 Vital paradigm shift and intrinsic breast cancer subtypes

The inherent shortcoming of chemotherapy has given demand to improved therapeutics that maximizes efficacies and minimizes toxicities. Treatment for breast cancer has advanced significantly over the past decade with the discovery of tumor heterogeneity. Gene expression profiling of

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breast cancers has led to classification according to gene signatures[10]. In present clinical practice, all human breast cancers can be divided into 4 major clusters of gene expression: luminal A, luminal B, basal-like (triple negative), and HER-2-enriched. Scientists around the world are working on further sub-classification for individualized treatment. Breast cancer is comprised of various sub-diseases, rather than one disease alone[8-9]. Therefore, no breast cancers are alike. The heterogeneity of breast tumors dictates the diverse features and responses to treatments and outcomes.

Three striking features are inherent of gene expression pattern variations in human breast tumors: great variation of patterns, multidimensional in nature and pervasive order. The variability in gene expression exists from patient to patient. Samples from the same patient showed more similarity than other patient samples, irrespective of surgery and given chemotherapy. Comparisons of samples between different patients also revealed vast differences and similarities, but more importantly, it showed a "biological interpretation" of human breast tumors[10], i. e., the varying expression of gene subsets showed different proliferation rates, signalling pathway activity and cellular composition of tumors. The in-built complexities of tumor cells and the varying responsiveness to treatment are innate to tumor heterogeneity. Despite so, overlaps or commonalities can still be found across the breast cancer subclasses, in the instances of luminal A or B tumors, and dissimilarities as with basal-like, triple negative tumors[11].

3 Subclassification by IHC

The advancement of genetic profiling has provided more information about breast cancer. The gene expression analysis has led to a deeper understanding of how breast cancers develop at the molecular, histological and clinical level. However, due to high costs of genetic profiling and unavailability in clinic, subtype assignment using validated IHC markers have been routinely used instead of differentiating breast cancers into the four subtypes[12]. Variation exists between the panel of markers to be used, but the most commonly used markers are based on IHC expressions of ER, PR, HER-2, in combination with the proliferation marker Ki67.

4 luminal A and B subtypes

Breast tumors in luminal A and B subtypes are characterized by the expression of ER or PR similar to normal luminal breast cells, but differ in the level of proliferative marker Ki67. The luminal A subtype [ER(+) or PR(+) and Ki67<14%] has a higher expression of ER and ER-related genes ESR1, GATA3 and FOXA1[11]. The luminal B subtype [ER(+) or PR(+) and Ki67 ≥ 14%] is characterized by a higher expression of Ki67. Some luminal B tumors also share similarities to that of ER(−) tumors, with increased frequency of tumor protein 53 (TP53) mutations. Overexpression of TP53 has been more commonly found in HER-2-enriched and basal-like tumors. luminal B tumors may also overexpress HER-2, alongside ER/PR[11]. Amongst all subtypes, luminal A has a better prognosis, survival and lower rate of local or regional relapse compared with luminal B, HER-2-enriched and triple negative/basal-like cancers[11-12]. Compared with luminal A, luminal B cancers are more aggressive, with a shorter DFS and OS ($P<0.05$)[10, 13].

ER(+) or PR(+) breast cancers generally respond to and derive the most benefit from endocrine/hormonal therapies. Three types of hormonal therapies are available: (1)selective estrogen receptor modulators (SERMS) (e. g. tamoxifen and raloxifene); (2) aromatase inhibitors (AIs) (e. g. letrozole, anastrozole, exemestane); and (3)selective estrogen down-regulator (SERD) (e. g. fulvestrant).

For most premenopausal females with HR(+) early breast cancer, tamoxifen[14] plus ovarian suppression do not improve DFS compared to tamoxifen alone, while adjuvant exemestane plus ovarian suppression do improve outcomes[14]. As exemplified in the phase Ⅲ clinical trials tamoxifen and exemestane trial (TEXT) and Suppression of Ovarian Function Trial (SOFT), premenopausal females receiving the tamoxifen-ovarian suppression regimen had an estimated 5-year DFS of 86.6%, compared to 84.7% in females who received tamoxifen alone. At 5 years, more patients in the tamoxifen-ovarian suppression group were free from breast cancer recurrence, compared to patients on tamoxifen alone[88.4% (95% *CI*: 86.1−90.3) *vs* 86.4% (95% *CI*: 84.0 − 88.5)]. For females assigned to the exemestane-ovarian suppression arm, 90.9% (95% *CI*: 88.9 − 92.6) remained breast cancer-free 5 years following treatment. Breast cancer recurrence was greater for the females who were previously treated with chemotherapy, who remained premenopausal. For the chemotherapy-treated patient cohort, 5-year DFS was 82.5% (95% *CI*: 78 − 85.6) in patients who received tamoxifen plus ovarian suppression, and 78.0% (95% *CI*: 74.0 − 81.5) in the patients receiving tamoxifen alone. In the exemestane-ovarian suppression group, 5-year DFS was 85.7% (95% *CI*: 0.49−0.87)[14].

The menopausal status of women often change following adjuvant chemotherapy treatment, notably in the younger premenopausal cohort. These females who remain premenopausal are, in fact, at a higher risk of breast cancer recurrence. The addition of

ovarian suppression to tamoxifen was shown to decrease the recurrence risk in this high-risk group of females in SOFT/TEXT trial. Comparatively, exemestane plus ovarian suppression provided a higher freedom to risk of breast cancer recurrence. However, exemestane in combination with ovarian suppression poses more adverse effects to sexual, musculoskeletal and bone-density functions than the tamoxifen-ovarian regimen. Use of hormonal therapies in the adjuvant setting has prolonged OS and DFS, and decreased the metastasis rate to the adjacent breast[15]. The ovarian suppression should not be given in all cases of premenopausal females, for higher risk females who remain premenopausal, ovarian suppression in addition to adjuvant AI was proven to improve outcomes.

In postmenopausal women, large-sample phase Ⅲ clinical trials of endocrine therapies have been conducted to study treatment outcomes and efficacies in HR+, postmenopausal women in both the (neo) adjuvant and metastatic setting[16-17]. In ATLAS study, 10-year adjuvant tamoxifen treatment was proven to be significantly superior to the standard 5-year therapy, reducing risk of recurrence and disease-specific death. Both pre- and postmenopausal women from 36 countries comprise the studied population in this trial. Improved outcomes were demonstrated compared 10-year with 5-year tamoxifen therapy, including reduced risk of breast cancer recurrence (617 *vs* 711 recurrences, P = 0.002), reduced breast cancer mortality (331 *vs* 37, P = 0.01), and decreased overall mortality (633 *vs* 722, P = 0.01). Follow-up results at year 15 showed a lower risk of recurrence for females given 10-year tamoxifen (21.4% *vs* 25.1%). Similarly, breast cancer mortality was reduced (12.2% *vs* 15.0%) in the longer therapy duration group[18]. Ten-year tamoxifen therapy was shown to be beneficial for females with ER+ breast cancers compared with 5-year tamoxifen therapy, although this may not be the current therapy of choice given to patients in the clinical setting.

In spite of improved responses for ER+ tumors with endocrine therapy, approximately half of these patients will develop resistance to therapy. Patients may not respond to treatment *de novo*, at initial presentation and at advanced stages all patients will develop acquired resistance, thus limiting the efficacy to treatment[19]. Endocrine resistance can be explained by the activation of a variety of escape pathways, including the overexpression or amplification of transmembrane growth factor receptors such as HER-2 receptor and the insulin growth factor receptor (IGFR) or the hyperactivation of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/mTOR pathway, one of the most frequently hyperactivated pathways in breast cancer[19]. HER-2 overexpression is associated with worse outcome than HR (+), HER-2 (−) patients to endocrine therapies[19]. The phase Ⅲ BOLERO-2 trial for HR (+)/HER-2-advanced breast cancer demonstrated prolonged PFS after progression on nonsteroidal AIs comparing exemestane therapy with combined therapy of exemestane and everolimus[20-21]. The combination can significantly extended PFS to 7.8 months (3.2 months for exemestane used alone; HR: 0.45, 95% *CI*: 0.38−0.54). The addition of everolimus demonstrated a critical role in resistance to endocrine therapy in AI-resistant tumors[20-21]. Some commonly observed adverse events in the study arm containing exemestane in combination with everolimus were stomatitis, rash, fatigue, diarrhea, nausea, decreased appetite, weight loss, cough, nausea and fatigue of a maximum grade 1/2[20].

An important hallmark of cancer is loss of cycle control. Overactivation of cyclin dependent kinase (CDK) 4/6 in many cancers, including breast cancer, will lead to uncontrollable cancer cell proliferation[22]. CD1 can form a complex with CDK 4/6, key regulators of the cell cycle responsible for triggering cellular progression from G_1 phase to DNA replication during S phase[23]. CD1 is a vital protein in AI-resistant tumors, and the upregulation of upstream promoters of cyclin D1 and downregulation of inhibitors of cyclin D1 lead to endocrine resistance. CDK 4/6 are downstream targets of ER signaling in hormonal receptor positive breast cancers[24]. Inhibition of CDK 4/6 was demonstrated in preclinical studies to halt the growth of breast cancer cell lines in G_1 phase. In the phase Ⅱ PALOMA-1 study, the combination of letrozole and CDK 4/6 dual inhibitor palbociclib led to significantly better outcomes than letrozole alone for ER(+) postmenopausal women (PFS: 20.2 months *vs* 10.2 months, P = 0.0004). Interestingly, the study did not show additional benefit for the combination of palbociclib-letrozole in patients with loss of P16 and/or Cyclin D1 overexpression, biomarkers initially thought to predict the sensitivity to CDK4/6 inhibition. Recently, preliminary data presented during San Antonio Breast Cancer Symposium 2014 on CDK4/6 inhibition by palbociclib in combination with letrozole also showed promising activity in the neoadjuvant setting as well. Adverse effects such as neutropenia, leukopenia, fatigue and anemia were commonly reported in patients given the combination of palbociclib and letrozole[23-24].

Following progression on AI in the metastatic setting (usually tamoxifen), there are few treatment options available for ER(+) women, in which fulvestrant is one possible agent. Fulvestrant is a pure ER antagonist that lowers the markers of hormone sensitivity and proliferation in postmenopausal breast

cancer women. *In vitro* and *in vivo* studies have demonstrated the inhibition of proliferation activity. Two phase Ⅲ trials have demonstrated the noninferiority of fulvestrant *vs* anastrozole for endocrine-sensitive advanced breast cancer for postmenopausal women who progressed on tamoxifen. Treatment with fulvestrant 250 mg in comparison to anastrozole showed similar results in time to progression (TTP), objective response rate (ORR), duration of response, as well as similar safety profile and few withdrawals, which consequently led to the FDA approval[25-26]. The results of *in vivo* studies and pharmacokinetic models have suggested that an increased dose of fulvestrant may lead to improved efficacy. Indeed the phase Ⅲ CONFIRM study for advanced breast cancer demonstrated a longer TTP for women treated with intramuscular injections of fulvestrant 500 mg compared to 250 mg. PFS was also significantly improved in 500 mg *vs* 250 mg arm, corresponding to a 20% decreased risk in progression, and similar ORR of 9.1% and 10.2%, respectively. The safety profile of the higher dose faslodex was comparable to the lower dose. Reported serious adverse events included bronchitis, dyspnea and vomiting that occurred in 2 to 3 patients[27].

More recently, early data of the phase Ⅱ FIRST study showed fulvestrant 500 mg to be superior to anastrazole 1 mg for postmenopausal women in the first-line setting. Females randomized to the fulvestrant 500 mg arm had significantly improved TTP, compared to females receiving anastrozole 1 mg[26]. Median TTP was 23.4 months in fulvestrant group and 13.1 months in anastrozole group (HR: 0.66; 95% *CI*: 0.47 – 0.92; P = 0.01), corresponding to a 34% reduced risk of progression[25-26].

5 HER-2 subtypes

HER-2 tumors are characterized by the overexpression of HER-2 and an absence or low expression of ER and PR, classified respectively as HER-2-enriched [ER(−), PR(−), HER-2(+)] and luminal-HER-2 [ER(+) or PR(+), HER-2(+)]. HER-2 cancers have relatively poor clinical outcomes and high proliferative activity and Ki67, compared to luminal A and luminal B subtypes of cancers. HER-2-overexpressed tumors are associated with a high histological grade and poor prognosis[28]. HER-2 tumors are resistant to endocrine/hormonal therapy, irrespective of hormone status in the tumor, for instance, luminal-HER-2 tumors[28].

HER-2 gene amplification and HER-2 protein overexpression normally occur together, and IHC and fluorescence *in situ* hybridization (FISH) can be used to analyze these changes. However, unlike IHC quantification for endocrine-responsive (luminal A and B) and basal-like/triple negative tumors, reproducibility problems for HER-2 tumors have been reported, which led to a revision of the practices and guidelines from American Society of Clinical Oncology and College of American Pathologists for HER-2 tumor testing[29]. According to the revised recommendation, HER-2 subtype should be determined based on the results—negative, equivocal and positive—of one or more tests for the patients with invasive breast cancer. For HER-2 testing by validated IHC, 0 and 1+ score would be regarded as "negative", 2+ as "equivocal", and 3+ as "positive". For an IHC 2+ or equivocal result, reflex testing should be performed by an alternative assay (FISH or IHC) on the same specimen. If there are technical problems that prevent one or both IHC and FISH from reporting the specimen as negative, equivocal, or positive, the test result should be reported as "indeterminate"[29].

Determining whether a tumor overexpresses HER-2 has proven to be vital for treating this subset of breast cancer, in view of the new targeted therapies available. HER-2(+) breast cancers were once considered as a challenge to treat the triple negative subtype, both of which are associated with high histological grade and poor prognosis[13]. After the introduction of trastuzumab, the targeted therapy for HER-2 (+) breast cancers has markedly improved the prognosis and outcomes. The monoclonal antibody trastuzumab works by binding and blocking HER-2 downstream signaling. Overexpression and amplification of HER-2 are observed in 15% to 25% of all breast cancers; identification of patients with HER-2+ will provide more effective treatment for this group of patients[30]. Moreover, the patients do not experience the same adverse effects associated with chemotherapy, including hair loss, myelosuppression or vomiting. Cardiotoxicity has been reported as a major, common serious adverse event of trastuzumab use and other targeted therapy. Therefore, it is not recommended to use trastuzumab in combination with anthracyclines.

The efficacy and safety of the single agent trastuzumab was studied as a first-line treatment in the metastatic setting by Weigel *et al*[30], and trastuzumab in combination with chemotherapy by Slamon *et al*[31]. In the latter, chemotherapy combinations included doxorubicin plus cyclophosphamide alone or with trastuzumab (for patients not treated with adjuvant anthracycline), or paclitaxel alone or paclitaxel and trastuzumab (if adjuvant treatment included an anthracycline). As the trial demonstrated, adding trastuzumab to chemotherapy led to significantly improved outcomes for disease progression, ORR and longer survival amongst other parameters. Significantly prolonged TTP of 4.6 months

vs 7.4 months ($P<0.001$) for anthracycline and with trastuzumab was observed, respectively. Significant increase was demonstrated in ORR (32% *vs* 50%), $P<0.001$, improved duration of response (6.1 months *vs* 9.1 months), and a longer survival (20.3 months *vs* 25.1 months, $P=0.01$)[31].

Several phase Ⅱ studies (M77001, BCIRG 101 and UCLA-ORN) were also conducted to observe the efficacy and safety of trastuzumab combined with docetaxel, and the same regimen with added platinum salts, respectively by Marty *et al*[32] and Pegram *et al*[33]. In M77001 clinical trial, the addition of trastuzumab to docetaxel provided significant improvements compared to docetaxel alone, including overall response rate (61% *vs* 34%; $P=0.0002$), OS (median: 31.2 months *vs* 22.7 months; $P=0.0325$) and TTP (median: 11.7 months *vs* 6.1 months; $P=0.0001$). The BCIRG 101 (Breast Cancer International Research Group) evaluated docetaxel and trastuzumab with cisplatin, and the UCLA-ORN study assessed the addition of carboplatin to docetaxel and trastuzumab for advanced HER-2-overexpressed breast cancer. Both combination therapies were proven to be feasible and active for HER-2(+) breast tumors. The overall response rate was higher in the BCIRG 101 study: 79% (95% *CI*: 66%–89%) *vs* 58% (95% *CI*: 44%–70%), while median TTP were longer in the UCLA-ORN study: 12.7 months (95% *CI*: 8.6–15.5) *vs* 9.9 months (95% *CI*: 8.3–13.1).

In the large phase Ⅲ BCIRG 007 study comparing trastuzumab and docetaxel *vs* the addition of carboplatin to docetaxel and trastuzumab, it was shown that both arms of chemotherapy were highly active for advanced HER-2(+) breast cancers[34]. Adding carboplatin did not lead to significantly improved outcomes. A comparison of docetaxel and trastuzumab *vs* the same regimen in combination with carboplatin led respectively to TTP of 11.1 months *vs* 10.4 months (HR: 0.914; 95% *CI*: 0.694–1.203; $P=0.57$) and OS of 37.1 *vs* 37.4 months ($P=0.99$) or 72% response rate for both arms. Adverse events were also similar between both arms, with common grades 3 or 4 adverse effects including neutropenia-related complications, thrombocytopenia, anemia, sensory neuropathy, fatigue. In total, there were three death cases: one cardiac death for the patient given docetaxel plus trastuzumab, and two sepsis-related deaths for patients on docetaxel plus trastuzumab and carboplatin[34].

For early-stage HER-2(+) breast cancer, one year trastuzumab application was the standard treatment. In the phase Ⅲ, open-label herceptin adjuvant (HERA) trial of trastuzumab, the administrations of 1- year and 2-year trastuzumab were compared after primary therapy (surgery with or without radiotherapy) and a minimum of 4 cycles of chemotherapy (neoadjuvant or adjuvant). At the first planned interim analysis, DFS was improved for both treatment arms. Trastuzumab following primary therapy led to approximately 50% reduction in the risk of distant recurrence[35]. The final results of the HERA trial have confirmed one year of trastuzumab as the standard care for early-stage HER-2(+) breast cancer[36].

Administration of 52 weeks (1 year) of adjuvant trastuzumab following combination chemotherapy for early HER-2 breast cancer was also studied by Slamon *et al*[31]. In the three-arm comparative study, women were randomly assigned to the following groups: doxorubicin and cyclophosphamide followed by docetaxel (AC-T); AC-T plus 52 weeks of trastuzumab; or docetaxel and carboplatin plus 52 weeks of trastuzumab (TCH). At 65-month median follow-up, the estimated DFS was 75%, 84% and 81% for the AC-T, AC-T plus trastuzumab, and TCH arms respectively. OS was predicted as 87% in AC-T arm, 92% for AC-T plus trastuzumab, and 91% in the TCH arm. The addition of 52 weeks of trastuzumab to chemotherapy has led to a higher predicted DFS and OS.

The development of trastuzumab has been considered one of the major breakthroughs in breast cancer alongside endocrine treatment for luminal cancers, changing the phase of treatment for HER-2(+) tumors and paving the way for more targeted therapies such as lapatinib (second-line therapy)[37]. However, in spite of the remarkable success in outcome and gain in terms of survival from trastuzumab, eventual progression to drug resistance to the monoclonal agent has been documented and vigorously studied[38]. Some proposed pathways thought to be involved in trastuzumab resistance include ATF4, CHEK2, ENAH, ICOSLG and RAD51 as possible biomarkers, and increased expressions of ADAM10 and ADAM17 have also been suggested as a new mechanism for required resistance to trastuzumab[38].

In 2013, additional treatment regimens for HER-2-overexpressed breast cancers were approved by FDA in the metastatic setting. Currently, there are 4 available agents: trastuzumab, lapatinib, pertuzumab and T-DM1. The combination regimen of trastuzumab, pertuzumab and docetaxel was approved for first-line use in metastatic breast cancer based on the pivotal results of the CLEOPATRA trial[39]. The two-arm CLEOPATRA study randomized 808 metastatic breast cancer patients to receive either placebo and trastuzumab and docetaxel (control arm) or pertuzumab plus trastuzumab and docetaxel (pertuzumab arm). The addition of pertuzumab led to significant improvements for patients in PFS (18.5 months in pertuzumab arm *vs* 12.4 months in

control arm, HR for progression or death: 0.62; 95% *CI*: 0.51-0.75; *P*<0.001). Interim analysis results also favored the pertuzumab treatment arm. The ORR was 69.3% in the control arm and 80.2% in the pertuzumab treatment arm, with a similar safety profile for both treatment arms. The pertuzumab arm had more febrile neutropenia and grade 3 diarrhea than the control arm[39].

The safety and efficacy of T-DM1, the drug antibody conjugate of trastuzumab to the cytotoxic molecule, was compared to lapatinib plus capecitabine in the phase Ⅲ EMILIA trial for HER-2+ metastatic patients who were previously treated with trastuzumab and taxane. Patients receiving T-DM1 were found to have fewer side effects than patients in the lapatinib-capecitabine combination treatment, but significantly prolonged PFS. Accelerated approval by FDA was granted for T-DM1 based on the significant outcomes reported. Median PFS was 3.2 months longer in T-DM1-treated patients compared with lapatinib plus capecitabine-treated patients (9.6 months *vs* 6.4 months, HR for progression or death: 0.65; 95% *CI*: 0.55-0.77; *P*<0.001). At the second interim analysis, OS, ORR and other secondary endpoints were significantly higher in T-DM1 treatment arm[40-41].

6 Triple negative subtypes

The triple negative or basal-like subgroup (ER-, PR+, HER-2-) is the final of the four intrinsic subtypes, and constitutes 10%-20% of all breast cancers[28]. As described in the gene expression profiling studies performed by Perou *et al*[10], the basal cluster included keratin 5, keratin 17, calponin 1, caveolin1 and laminin1[10, 42]. Basal-like tumors are characterized by a lack of ER and PR expression, amplification of HER-2 expression and generally a high proliferative activity. The high proliferative factors delineate the aggressiveness underlying the disease and poor prognosis in advanced and metastatic stages. There are currently no known receptors of triple negative breast cancer that can be targeted with novel treatment, leaving limited regimen of chemotherapy as the sole resolution in the treatment. Basal-like cancers were also deemed to be a more aggressive subtype with worst DFS and OS of all other breast cancer subtypes, often affecting younger females[42].

Currently, there are no FDA-approved therapies for the treatment of triple negative breast cancer as most studies combined different subsets of patients. Although recent researches focused on poly ADP ribose polymerase (PARP) inhibitors have been made, they did not follow through with expected results[43-44]. PARP inhibitors were explored for triple negative breast cancer, based on the molecular similarities of triple negative and BRCA1 mutations. Two phase I trials involving the PARP inhibitor veliparib were conducted to explore the optimal dosing schedules for veliparib in addition to a regimen containing carboplatin and paclitaxel for advanced triple negative patients [BRCA1(+) and sporadic]. A standard dose-escalating design was employed in the study. Dose-escalating toxicity was not observed in triple negative breast cancer patients, with 2 of 10 patients and 3 of 9 patients achieving complete response in the first and second trial, respectively. Patients who were treated previously in the adjuvant and metastatic setting or received taxanes had observed responses.

The safety of olaparib, an oral PARP inhibitor, was documented in a phase I trial for triple negative patients with a BRCA1 or BRCA2 mutation. Additionally, adverse event profile, dose-limiting toxicities, along with the pharamacokinetic and pharmacodynamic profiles were determined. Olaparib was discovered with lower toxicities than conventional chemotherapies, with some anticancerous activities in the studied population. Complete and partial responses were observed in BRCA-related mutation carriers, although not all patients in the cohort responded to treatment[43].

Iniparib, another PARP inhibitor, was studied in combination with gemcitabine and capecitabine in a cohort of advanced triple negative breast cancer patients in the phase Ⅱ and Ⅲ setting. Results from the highly anticipated phase Ⅲ trial with 519 recruited patients had not mirrored the success of the phase Ⅱ trial. Statistical significance was not demonstrated with the addition of iniparib, with primary endpoints of PFS and OS[44].

Vivid questions have been left unanswered since all studies combined different treatments with different patient populations. Owing to the varied response of this subgroup of cancer to treatment, gene expression profiles were analyzed to explore at the molecular level if subgroups existed within the basal-like subtypes. Indeed, the basal-like subgroup or triple negative cancers as now commonly referred was discovered to be a heterogeneous disease, subclassified into basal-like, claudin-low, and non-basal-like tumors. Basal-like tumors are characterized by a harbor of TP53 mutations[45]. The claudin-low subgroups can be identified by a low expression of related genes (claudin 3, 4, 7), and a high expression of mesenchymal and stem cell-like biological processes. Androgen receptor is associated with triple negative breast cancer pathogenesis, deemed a prognostic marker of patient outcome in non-basal-like tumors. Within the spectrum of triple negative subgroup, the presence of CD8 T-cells generally corresponds to a better outcome for patients[45].

Molecular characterization further revealed 7 subtypes: basal-like 1, basal-like 2, immune-modulatory, mesenchymal, mesenchymal stem cell line, luminal androgen receptor and unstable tumors[12, 42, 45]. The basal-like 1 and 2 subtypes are characterized by higher cell division pathway components and DNA damage response pathways. An overexpression of gene oncologies in immune cell processes and immune signal transduction pathways are identified for the immune-modulatory subgroup. The presence of epithelial-mesenchymal transition genes are enriched in the mesenchymal and mesenchymal stem cell line subgroup of triple negative breast tumors. Finally, luminal androgen receptor tumors are defined by genes enriched in androgen receptor signaling. The heterogeneous nature of the six subtypes (except unstable tumors) is evident in the response to therapy. For instance, biologically similar basal-like 1 and 2 subtypes are more sensitive to platinum-based treatment; immune-modulatory, mesenchymal and mesenchymal stem cell line are more reactive to PI3K/mTOR inhibitors, and luminal androgen receptor subtype may respond to anti-androgen molecules more apparently[46]. The luminal androgen receptor subtype with a BRCA1/2 mutation also confers platinum sensitivity, unresponsive to chemotherapy.

Immunotherapy may benefit the recurrent and metastatic triple negative breast cancer. Two programmed cell death protein 1 (PD-1) inhibitors, pembrolizumab and MPDL3280A[47], were shown to have anticancerous activity in the recurrent and metastatic setting. In the phase I multicenter trial, the females with recurrent or metastatic triple negative disease were enrolled and given intravenous injections of pembrolizumab every 2 weeks. The endpoints of safety and tolerability, in addition to anticancerous activity, were studied in 32 patients (with sufficient data for analysis in 27 patients). One patient was observed with complete response, 4 with partial response, 7 with stable disease and 12 with progressive disease. The 12 females were identified to have PD-1 protein in their tumors. Nine patients with sufficient data were evaluated for efficacy. One patient had complete response, 2 had partial response and 1 had stable disease.

7 Conclusion

Treatment for breast cancer has been improved significantly for a decade. Research in gene expression profiling has led to a deeper understanding of breast cancer, which is now believed to be comprised of a variety of sub-diseases, rather than one disease. The finding made a decade ago—molecular heterogeneity of breast cancer—was firstly reported in a manner that is still applicable for discussion today. Treatment for breast cancer in the pre-genomic era comprised mostly of chemotherapies for all breast cancer subtypes, and later endocrine therapies for HR sensitive tumors. With the recently approved targeted therapies for HER-2 + breast cancers, tailored strategies for any individual patient can be made. These regimens have revolutionized and given hope to patients with an aggressive form of breast cancer characterized with poor prognosis. Left behind are the triple negative tumors, lacking targeted agents, leaving chemotherapy as the primary treatment option. Molecular subtyping for the triple negative disease has broken down the subtype further which may provide predictions of response to treatment, but until more research is conducted, this group of patients will remain difficult to treat.

【Key words】 Breast neoplasms; Drug therapy; Individualized therapy

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