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Review

Proteomics as a Guide for Personalized Adjuvant Chemotherapy in Patients with Early Breast Cancer

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Abstract. *Proteomics allows for better understanding of the function and regulation of cancer cells mediated by intra- and extracellular signaling networks. Integrating such information with clinicopathological characteristics of the tumor may lead to either detection of disease biomarkers useful to differentiate high-from low-risk patients, or to identification of new drug targets. Adjuvant chemotherapy is currently a personalized treatment strategy, especially for breast cancer (BC) patients, and the risk assessment of each patient influences its use because the benefit strictly correlates with the level of risk. Luminal A BCs are endocrine therapy (ET)-sensitive but exhibit low sensitivity to chemotherapy, while luminal B cancers, according to the Ki-67 proliferation rate may require for chemotherapy in addition to ET, and HER2-positive tumors derive benefit from adjuvant chemotherapy containing an anthracycline, a taxane and trastuzumab for one year. Triple-negative BCs have a high degree of genomic instability exhibiting a more aggressive clinical course with respect to other types of BC, and the anthracycline-taxane regimen constitutes the standard approach. Studies considering the use of targeted approaches (drugs), including poly (ADP-ribose) polymerase (PARP-1), vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR) inhibitors, or EFGR and HER2 blockers, are still under evaluation. In the genomic era, promising new targeted-therapies are worthy of further investigation, and mTOR inhibitors have been used for patients with high-risk ER-positive and HER2-negative tumors. In the near future, genetic and molecular profiling of BC will help to better-categorize patients, determine the choice of chemotherapy in low-risk, or intensify the treatment in high-risk cancer patients, eventually revealing new targeted agents.*

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Proteomics allow for a better understanding over the function and regulation of cancer cells mediated by intra- and extracellular signaling networks (1). Integrating such information with clinicopathological characteristics of the tumor may lead to either detection of disease biomarkers useful to differentiate high- from low-risk patients, or to identification of new drug targets (1, 2). Adjuvant chemotherapy is currently a personalized treatment strategy in breast cancer (BC) patients, and the risk assessment of each patient influences its use because the benefit strictly correlates with the level of risk. In this setting, the intrinsic sub-type tumor characterization is crucial because each patient exhibits different outcomes according to molecular biomarkers other than histological characteristics of the tumor. According to the ESMO (European Society for Medical Oncology) guidelines, five main intrinsic sub-types of BC are considered: luminal A, luminal B human epidermal growth factor receptor-2 (HER2)-negative, luminal B HER2-positive, HER2 overexpression, and basal-like (hormone receptor-negative, HER2-negative) (3). However, thanks to genome-expression profiling, additional sub-types with distinct phenotypic features and natural history have been identified, including claudin-low and normal-like sub-types (2).

Traditional and Genomic Characterization of Patients

In BC patients, many predictive and prognostic factors help guide the selection of chemotherapy, including the following factors: (i) high tumor staging and histological grade (G3); (ii) increased rate of Ki-67-positive nuclei (≥20%); (iii) low (≤1%) estrogen receptor (ER) and progesterone receptor (PR)

expression; (iv) HER2 (also named ERBB2) overexpression; and (v) the presence of triple-negative BC (TNBC). In addition, the Nottingham Prognostic Index and updated St. Gallen guidelines are further validating tools on this purpose (4, 5). Axillary node status is still regarded as a prognostic factor because with the presence of ≥4 positive nodes, patients are at increased risk, and chemotherapy needs to be administered (6). Chemotherapy leads to a significant improvement in disease-free survival (DFS) and overall survival (OS) mainly in women aged <50 years compared with those older than 50 (7, 8). However, because comorbidities play a key role for the chemotherapy indication and regimen selection, the biological age of the patient should be taken into consideration, even thought chemotherapy based only on young age (<35 years) is still controversial. Genomic assays, including the 21-gene Recurrence Score (Oncotype DX), the 70-gene test (MammaPrint), and a five-gene test (Mammostrat), are currently available (9-11). These scores represent additional indicators for chemotherapy, but the 21-gene Recurrence Score is the only assay included in the ASCO (American Society of Clinical Oncology) treatment guidelines, as the other tests require further validation (12, 13). In ER^+ node-negative BC treated with adjuvant tamoxifen therapy, the 21-gene Recurrence Score helps to quantify distant recurrence risk and chemotherapy benefit; the assay had an impact on approximately one third of medical oncologists, who changed their treatment regimen according to the test result (14). The suggestion of the 70-gene test signature, that is the first FDA-cleared BC genomic test, represents an independent prognostic factor in node-negative patients (15, 16).

Main Chemotherapeutic Agents and Targeting Drugs

Over the last 50 years, chemotherapy has been the mainstay of adjuvant therapy and its efficacy has improved, representing a strategy primarily based on out-weighing benefits and risks of treatment. A number of chemotherapeutic agents are available to treat patients with early BC. However, only few drugs are suitable for extensive clinical use, including alkylating agents (*e.g.*, cyclophosphamide, cisplatin, carboplatin), anthracyclines (*e.g.*, doxorubicin, epirubicin), anti-metabolites (*e.g.*, fluorouracil), folate inhibitors (*e.g.*, methotrexate), disruptors of microtubule function, such as taxanes (*e.g.*, docetaxel, paclitaxel), topoisomerase inhibitors (*e.g.*, etoposide), and others. Moreover, several molecular targeting drugs are available, almost exclusively for patients with advanced or metastatic disease, or as second- and third-line therapy (Table I).

The current standard-of-care is usually sequential anthracycline *plus* taxane for high risk BC patients, and four cycles of TC (docetaxel-cyclophosphamide) or no chemotherapy for lower-risk ER^+ BCs. In addition, dose-dense adjuvant chemotherapy was shown to improve DFS and OS in node-positive patients (18, 19). We, herein, describe chemotherapy strategies based on BC intrinsic biological sub-types.

BC Sub-types

Luminal A. Luminal A cancers are endocrine therapy (ET)-sensitive but exhibit low sensitivity to chemotherapy (20). A less intensive chemotherapy regimen with AC (doxorubicin-cyclophosphamide), CMF (cyclophosphamide-methotrexate-fluorouracil), or TC may be added to ET, that usually includes selective estrogen receptor modulators (*e.g.*, tamoxifen) and/or aromatase inhibitors. In node-positive BCs, an anthracycline-taxane-based chemotherapy regimen is, however, recommended. In node-negative disease, the chemotherapy selection is more controversial, and may be guided by tumor size (>1 cm), patient age or co-morbidities. Most importantly, the recurrence scores of gene assays such as Oncotype DX may predict chemotherapy benefit in ER^+ node-negative but also node-positive BC cases (12, 21).

Luminal B. Luminal B cancers (HER2-negative) have an increased risk of relapse and may be sensitive to chemotherapy according to the Ki-67 proliferation rate. These patients mostly require adjuvant chemotherapy in addition to ET. The anthracycline-taxane-based regimen is recommended for 6-8 cycles, when chemotherapy is chosen. Dose-dense chemotherapy can be prescribed if ≥4 positive nodes are present. In patients with luminal B cancer and HER2 overexpression ($HER2^+$), chemotherapy plus anti-HER2 therapy is required. In luminal cancer, the use of genomic assays has been increasingly important rather than the surrogate sub-type definition to assess the risk of recurrence and better select the adjuvant chemotherapy.

Triple-negative. Triple-negative BC is a heterogeneous disease with different and complex genetic alterations. However, the clinical significance of these genomic variants is still unclear, and sensitivity to chemotherapy differs among sub-groups. TNBCs are characterized by the lack of ER and PR expression and normal or negative HER2 expression. These tumors exhibit a more aggressive clinical course with respect to other types of BC, but there is an overlap of approximately 20-30% of TNBC with basal-like and BRCA1-related BCs (22, 23). TNBCs are usually poorly-differentiated and have a high degree of genomic instability. A sub-group of TNBCs has a defect in homologous recombination, and most of these are BRCA1-associated (24). Chemotherapy represents the backbone of adjuvant treatment for TNBCs, and the anthracycline-taxane regimen constitutes the standard approach. Platinum-based agents

Table I. *Main molecular targeting in breast cancer.*

Site of inhibition	Target of inhibition	Main drugs approved or under study
Epidermal growth factor receptor (EGFR)	EGFR	Afatinib, gefitinib, lapatinib, erlotinib
	HER2	Trastuzumab
	EGFR and HER2	Lapatinib, cetuximab, pertuzumab, canertinib, neratinib
Vascular endothelial growth factor (VEGF)	VEGF	Bevacizumab
	Multi-kinase	Sunitinib, semaxinib, pazopanib, axitinib, vandetanib
RAS/MEK/ERK Pathway	Farensyltransferase	Tipifarnib
	Raf	Sorafenib
	MEK	Rafametinib, trametinib
Cell-cycle and apoptosis	PI3K/Akt/mTOR pathway	Letrozole
	Cyclin-dependent kinase	Flavopiridol
	Bcl-2 Silencing	Oblimersen
	p53	PRIMA-1
Src-family tyrosine kinases	Src	Dasatinib, saracatinib, bosutinib
HSP 90	HSP 90	Retaspimycin
Insulin-like growth factor	IGF	NDGA
Poly(ADP-ribose) polymerase	PARP-1	Iniparib, niraparib, olaparib, veliparib
Histone deacetylase	HDAC	Valproic acid
Other under study	c-Met	ARQ-197
	Proteosome	Bortezomib, BU-32
	RANKL	Denosumab

Modified from (17) and (28).

showed efficacy in the metastatic setting by inhibiting DNA, conforming DNA adducts (*e.g.*, cisplatin, oxaliplatin) or binding to DNA (*e.g.*, carboplatin) and interfering with cell repair; however, they require further validation in prospective clinical trials in the adjuvant setting (25-27).

Studies considering the use of targeted drugs, including PARP-1 (poly[ADP-ribose] polymerase) inhibitors (*e.g.*, iniparib, niraparib, olaparib, veliparib), VEGF (vascular endothelial growth factor) and multi-kinase inhibitors (*e.g.*, bevacizumab, sunitinib, sorafenib), EGFR (epidermal growth factor receptor) inhibitors (*e.g.*, erlotinib), EGFR and HER2 (*e.g.*, cetuximab) blockers, and androgen receptor-targeting drugs, are still under evaluation (28, 29). A recent meta-analysis showed that in patients with metastatic TNBC, the combination of chemotherapy, bevacizumab, iniparib and sorafenib improves OS, while chemotherapy plus iniparib and cetuxomab improves DFS (30). Lapatinib, a tyrosine kinase reversible inhibitor of HER1 and HER2, exhibits limited effects in pre-surgical treatment of HER2-negative patients (31). In the neoadjuvant treatment of HER2-negative BC, sorafenib, a multikinase inhibitor that targets the VEGF, PDGF-β (platelet-derived growth factor-β) and Raf kinase receptors, added to the anthracycline-taxane-based chemotherapy, had a limited efficacy, by improving progression-free survival but not OS (32).

HER2-positive. The amplification of HER2 gene and the subsequent overexpression of HER2 accounts for approximately 20% of BCs (33). HER2 is a surface receptor at the cell membrane, and the monoclonal antibody against the extracellular domain of HER2 trastuzumab has long been available for clinical use, representing the most successful example of targeted therapy for BC (34). The sub-group of $HER2^+$ patients derives benefit from adjuvant chemotherapy containing an anthracycline, a taxane and trastuzumab for one year. AC→TH (doxorubicin and cyclophosphamide followed by docetaxel plus trastuzumab), TCH (docetaxel-carboplatin-trastuzumab), or anthracyclines followed by trastuzumab are standard treatments. TCH regimen was better tolerated than either AC→T (AC followed by docetaxel) or AC→TH regimens (35). According to a recent systematic review, one year of trastuzumab after adjuvant chemotherapy should be the treatment of choice for $HER2^+$ BCs (36). In addition, patients with small node-negative $HER2^+$ BCs may benefit from chemotherapy plus trastuzumab, but the usefulness of this regimen is still unclear for tumors <1 cm. In the neoadjuvant setting, adding trastuzumab to anthracyclines before FEC (fluorouracil, epirubicin, cyclophosphamide) regimen administration did not provide any advantage compared to sequential FEC followed by trastruzumab and paclitaxel (37).

EGFR inhibitors, including gefitinib and lapatinib, have been used in a limited number of studies, exclusively in the neoadjuvant setting. Combining lapatinib and trastuzumab before surgery significantly improved the pathological complete response rate with respect to lapatinib or trastuzumab alone (38). Lapatinib and trastuzumab exhibit synergistic effects in advanced or metastatic BCs

overexpressing HER2 (39, 40). However, the usefulness of lapatinib and trastuzumab together remains undefined in early BC (41). Two additional HER2-blocking agents have recently become available: emtansine (TDM-1) and pertuzumab. Emtansine is an antibody-drug conjugate that delivers potent cytotoxic effects against $HER2^+$ cancer cells and is also active in patients previously treated with trastuzumab (42, 43). Pertuzumab is an anti-HER2 monoclonal antibody that, in combination with trastuzumab, exhibits a potent dual HER2 blockade (44). Both these new agents are currently under investigation for early-stage BC. In patients with advanced trastuzumab-resistant disease, dual targeting with pertuzumab and trastuzumab together can be an option (45).

Future perspectives

In the genomic era, selecting patients based exclusively on simple clinical and pathological paradigms is not sufficient, since genetic and molecular features are extremely promising in suggesting new targeted-therapies, both in the adjuvant and neoadjuvant setting.

Mammalian target of rapamycin (mTOR) is a protein kinase that belongs to the PIKK (phosphatidylinositol-3OH kinase related kinase) family and regulates several essential cell functions. Both mTOR (*e.g.*, everolimus) and PARP inhibitors have been used for patient with high-risk ER^+ HER2-negative BC and BRCA-mutated TNBC, respectively (46, 47). Everolimus (RAD100) binds to FKBP-12 (FK506-binding protein), reducing tumor cell proliferation, as exhibited by reduction in Ki-67 rate (48). In addition, predictive biomarkers of HER2 blockade for $HER2^+$ BC deserve further study.

We could hypothesize that, in the near future, anti-HER2 agents may substitute for chemotherapy in selected patients, thus avoiding the adverse effects of chemotherapy, including ovary function inhibition. Genetic and molecular profiling of BC will help to better categorize patients, determine the choice of chemotherapy in low-risk patients or intensify the treatment in high-risk cancer patients, eventually adding new targeted agents. BC heterogeneity and inter- or intra-patient variability stimulate intensive research on predictive and prognostic biomarkers that can guide the choice of the most appropriate treatment for each individual early-BC patient.

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