Razavi Int J Med. 2015 May; 3(2): e24992. DOI: 10.5812/rijm.3(2)2015.24992

Published online 2015 May 2. **Review Article**

Triple Negative Breast Cancer: Molecular Classification, Prognostic Markers and Targeted Therapies

Narges Jafarzadeh [1]; Hami Ashraf [1,2]; Fahimeh Khoshroo [1]; Alireza Sepehri Shamloo [3]; Farzad Bidouei [1]; Kamran Ghaffarzadehgan [1,*]

[1]Department of Research and Education, Razavi Hospital, Mashhad, IR Iran
[2]Zakaria Idea and Innovation Support and Leading Center, Tehran, IR Iran
[3]Student Research Committee, Mashhad University of Medical Sciences, Mashhad, IR Iran

*Corresponding author: Kamran Ghaffarzadehgan, Department of Research and Education, Razavi Hospital, Mashhad, IR Iran. Tel: +98-5116004594, Fax: +98-5116668883, E-mail: kghafar46@gmail.com.

Received: November 10, 2014; **Revised:** February 1, 2015; **Accepted:** February 17, 2015

Context: Triple negative breast cancer (TNBC) is a heterogeneous group of diseases that is negative for esterogen receptor (ER) progesteron receptor (PR) and human epidermal growth factor receptor 2 (HER2). This type of breast cancer is typically high-grade carcinomas, although low-grade tumors occur. The aim of this review is to focus on molecular classification and features, prognostic markers and targeted therapies of triple negative breast cancer.

Evidence Acquisition: We searched using electronic databases Pubmed/Medline, Dare, Scopus, Embase, and Cochrane Database of Systematic Reviews with terms of 'Triple negative breast cancer', 'Breast cancer', 'Molecular classification', 'Immunohistochemical markers', 'Molecular features, 'Targeted therapy', and 'Prognostic marker'.

Results: It seems that TNBC itself can be subdivided into immunomodulatory, mesenchymal, mesenchymal stem-like, luminal androgen receptor, and distinct basal-like subtypes that differ substantially from basal-like tumors. There are several prognostic makers for TNBC including EGFR and ALDH1, Lysyl Oxidase-Like 2 protein (LOXL2), Synuclein gamma (SNCG), LDHB (Lactate Dehydrogenase B). The antiangiogenic agents, EGFR inhibitors, and PARP inhibitors are new therapeutic Implications and potent factors to targeted therapies of TNBC.

Conclusions: Only a few clinical trials are performed on TNBC patients because this disease has a low incidence. Therefore, it seems larger scale clinical trials are needed to be conducted in the future.

Keywords: Triple Negative Breast Neoplasms; Molecular Targeted Therapy; Classification; Biological Markers

1. Context

Breast cancer, the most commonly diagnosed cancer among the females in both developing and developed countries, is a significant global public health issue and the major cause of cancer-related death (1). Breast cancer is a heterogeneous disease with varied morphological appearances, molecular features, behavior, and response to therapy (2). Advances in molecular biology lead to improved methods in breast cancer diagnostics and therapeutic strategies. The advent of genomics technologies has increased understanding of breast cancer as several different biologically and molecularly distinct diseases.

The basal-like intrinsic breast cancer subtype represents about 15% of invasive ductal breast cancers. These tumors are frequently estrogen receptor (ER)-negative, estrogen receptor (PR)-negative, human epidermal growth factor receptor 2 (HER2)-negative, cytokeratin 5/6 CK5/6-positive, and/or epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 1 (HER1)-positive by Immunohistochemistry (IHC) (3). Claudin-low breast cancer subtype is characterized by overexpression of genes associated with epithelial to mesenchymal transition (EMT). The majority of claudin-low breast cancers without expression of luminal differentiation markers are HER2 and hormone-receptor-negative by IHC method (4). So, that are named are considered ER/PR and HER2/neu negative that named triple negative breast cancer (TNBC) (5).

TNBCs account for 15% - 20% of all breast cancers which are characterized by a typically ductal histology, high grade, high proliferation and mitotic rates. They are significantly more aggressive than other subtypes of breast cancer and disproportionately affect younger premenopausal women, with a higher mortality rate among African-American women (6). Noticeably, significant overlap can be seen between BRCA1 associated breast cancer and TNBC, particularly in younger women (7). TNBC is associated with poor prognosis, a higher risk of the local recurrence rate (LRR), poor Disease-Free Survival (DFS) and cancer-specific survival (CSS) (8). The major part of TNBC includes invasive ductal carcinomas, however other phenotypes such as metaplastic, atypical medullary and ad-

enoid cystic are also seen. Other characteristics of these tumors are over expression of EGFR, P53 mutations, C-myc amplification and cytogenetic abnormalities. Several risk factors associated with TNBC compared to hormone receptor positive tumors have been identified including younger age at menarche, higher parity, earlier age at first full term pregnancy, shorter duration of breast feeding and also, higher Body Mass Index (BMI) and Waist to Hip Ratio (WHR) in pre-menopausal women (9). Furthermore, studies have indicated using oral contraceptive more than 1 year increases the risk of TNBC in women under 45 years of age up to 2.7-fold. The significant risk of this type of breast cancer can be seen in relation to longer oral contraceptive duration and fewer years since the last issue (10).

TNBC represents an important clinical challenge since this cancer does not respond to endocrine therapy or other available targeted therapies (11). To date, there have been fewer advances in the treatment of TNBC compared to other subtypes of cancer. Although these tumors respond to conventional chemotherapy, which is toxic and affects a wide range of dividing cells, the approach has met with mixed success (12). The aim of this review is to focus on molecular classification and features, prognostic markers and targeted therapies of triple negative breast cancer.

2. Evidence Acquisition

We conducted our research using electronic databases [PubMed/Medline (1966-September 2014), Dare (1966-September 2013), Scopus (1965- September 2014), and Embase (1965- September 2014)]. Additionally, review articles from Cochrane Database of Systematic Reviews were evaluated. In some cases, the similar studies which were suggested by data bases were obtained for further data. The main data search terms were: 'Triple negative breast cancer', 'Breast cancer', 'Molecular classification', 'Immunohistochemical markers', 'Molecular features, 'Targeted therapy', and 'Prognostic marker'. Studies, which were published in any other languages than English, were excluded from the review. 1293 studies were screened and finally seventy studies were included.

3. Results

3.1. Molecular Classification of TNBC

IHC markers, ER, PR, and HER2, are routinely used in clinical practice to classify breast tumors and thereby determine potential courses of therapy. In this regard, three subtypes of breast tumors with different biologic behaviors were discovered including hormone-receptor-positive, triple negative, and HER 2/neu-positive breast cancers. Management approaches and natural histories of these subtypes are different.

More detailed molecular characterization of breast cancers are performed by Genome-wide expression profiling and hierarchical clustering to identify additional subtypes. At least 7 different biologic subtypes including luminal A, luminal B, luminal C, HER2-enriched, basal-like, claudin-low, and normal breast-like were identified. Basal-like group that is largely TNBC, expresses basal epithelial cell layer proteins including cytokeratins 5, 6 (CK5/6) and EGFR. They are considered ER/PR and HER2/neu negative "triple negative" due to low expression of the luminal and HER2 gene clusters. Only 77% of basal- like breast cancers are triple-negative, with 71% - 91% of TNBC being basal- like. Thus, triple negative (TN) and basal breast cancer are not synonymous (4). Nevertheless, many of the clinical features of the basal-like and TNBC phenotypes such as shorter relapse-free and overall survival times compared with other types of breast cancers, a tendency toward visceral versus bone metastasis (13), and over-representation in BRCA1 mutation carriers are similar (14).

In addition, claudin-low group is also comprised largely of TNBC (71%), characterized by a lack of expression of luminal differentiation markers, enrichment for epithelial-to-mesenchymal transition markers, immune response genes and cancer stem cell-like features (15). In brief, Triple-negative carcinoma is diagnosed by immunohistochemistry and is characterized by tumors that do not express Progesterone Receptor (PR), Estrogen Receptor (ER) or overexpress human epidermal growth factor receptor 2 (HER2) (16, 17).

Defining the negativeness of ER, PR and HER2 should be based on the most recent guidelines by American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP). According to these guidelines, ER and PR are considered positive when at least 1% of the tumor cells nuclei is positive and in the case of internal and external positive control (18).

One of the most important points in interpretation of ER and PR is the internal control of the normal tissue. This means that all the fixations and processing should have been performed on it like a tumoral tissue.

New studies have indicated that TNBC itself can be subdivided into immunomodulatory, mesenchymal, mesenchymal stem-like, luminal androgen receptor, and distinct basal-like subtypes that differ substantially from basal-like tumors (19). So it seems for a better classificationof TNBC, further researches are needed.

3.2. Molecular Features of TNBC

TNBC is either a team noun or not the usage is not determined. An assemblage of different breast cancers that are still poorly understood at the molecular level. Molecular profiling of TNBC using gene expression assays conferring a clear understanding of the heterogeneous nature of these tumors (18). TNBC shows different mutational profiles; tumor suppressor P53 mutation which leads to overexpression or loss-of-function has been reported in almost 75% of TNBC tumors, whilst the MYC oncogene was ranked second with 40% of TNBC cases (20, 21). Likewise, mutations in retinoblastoma (pRb) and p16, and

G1/S cell cycle regulators are No article : the other characteristics of TNBC. CGH array data and gene expression platforms revealed genes that are recurrently amplified and consistently overexpressed in a subgroup of TNBC tumors including fibroblast growth factor receptor 2 FGFR2 (10q26.3), mitotic spindle checkpoint protein BUB3 (10q26.3), RAS oncogene family member RAB20 (13q34), protein kinase C super family member PKN1 (19p13), and Notch family member NOTCH3 (19p13.12). Noticeably, copy number gains of tyrosine kinase receptors and upregulation of signal transduction kinases downstream of receptors (e.g. RAF1, PIK3C2G) has been reported (16). TNBC tumors are consisted of mosaic cancer cells with significant genetic aberrations. These tumors show genetic aberrations such as losses on 1p, 2q, 3p, 4p, 5q, 8p, 9q, 16q, 17p, 19p, and 23p; and gains on 1p, 3q, 6p, 9p, 7q, 8p, 10p, and 12p (16, 17). Regarding genetically unstable and complex patterns of genetic aberrations of TNBCs, comprehensive molecular pathology analyses of primary tumors are required to develop therapeutic strategies and select appropriate targeted therapy for the individual tumors (22).

Studies have demonstrated that microRNAs (miRNAs) are responsible for a large proportion of TNBC heterogeneity. miRNAs are small in size, regulatory molecules that function at post-transcriptional level by regulating almost 50% of human protein-coding genes (23). They are involved in a variety of cellular process such as differentiation and growth which are all of them are dysregulated in tumourigenesis. A list of miRNAs has been presented that they act as key regulators of genes involved in invasion and metastasis. Therefore, these regulatory molecules are considered as a potential target for therapy intervention.

Recent reports of expression level of miRNAs in triple-negative breast cancer identified them as a novel candidate prognostic and diagnostic biomarkers and therapeutic targets (24). The expression pattern of different miRNAs has been associated with particular pathological characteristics of breast cancer, i.e. ER, PR, and her2/neu expression, tumor stage, and lymph node status (25). Hence, it has been suggested that miRNA profiling is more valuable and accurate than mRNA profiling (26). In 2009, a publication in breast cancer research by Lowery and colleagues identified miRNA signature can predict ER, PR, and HER2/neu status with a median accuracy of 100%. The Her2/neu signature comprised of five miRNAs (miR-520d, miR-181c, miR-302c, miR-376b, miR-30e) that can discriminate cases with 100% accuracy (26). In a recent paper (2014) it was reported that miR-210 is overexpressed in TNBC tissue (23). miR-210 increases the hypoxia-inducible factor 1α (HIF1α) through enhancement of HIF levels. Response to hypoxia is a distinctive molecular feature of TNBC (27, 28). Interestingly, miRNAs are detectable in circulating tumor cells in serum and plasma of TNBC patients (29). Circulating miRNA level can be applied for serial disease monitoring as a minimally invasive biomarker (30).

3.3. Prognostic Markers of TNBC

The TNBC is clinically heterogenous and lacks established certain prognostic markers. Nevertheless, several markers such as HER1, ALDH1, LOXL2, Ki-67, SNCG and LDHB are identified as prognostic or predictive markers and provide valuable prognostic information. Molecular markers are likely to play an increasing role as targets for the systemic therapy of breast cancer.

Human epidermal growth factor receptor 1 (HER1 or EGFR) is a receptor tyrosine kinase belonging to the HER family of transmembrane receptors that have an important role in cell proliferation, migration and by following activation of intracellular pathways can protect cells against apoptosis (12). HER1 expression is higher (up to 80%) in TNBC. Monoclonal antibodies including cetuximab, panitumumab and/or synthetic Tyrosine Kinase Inhibitors (TKIs) can target HER1. Using cetuximab in breast cancer did not have good results probably because of the activation of down-stream signaling pathways (31).

Aldehyde dehydrogenase 1 (ALDH1), a cytosolic enzyme responsible for the metabolism of intracellular aldehydes, is one of the most consistently used biomarkers to identify the breast cancer stem cell groups (32). Some studies have indicated that stem-like features like innate chemoresistance and clonal capacity have been related with ALDH1 expression in tumor cells (33). Therefore, ALDH1 expression may serve as a marker of highly clonogenic, chemoresistant stem-like cells that form the basis for recurrent disease in locally advanced breast cancer (34).

Lysyl Oxidase-Like 2 protein (LOXL2) a member of the lysyl oxidase (LOX) family, have a highly conserved carboxy terminal catalytic domain required for the oxidative deamination of peptidyl-lysine residues in substrates to generate reactive aldehyde groups that initiate covalent inter and intramolecular crosslinks (35). Higher amount of LOXL2 can be seen in TNBC than non-TNBC tumors. Studies showed that higher expression of LOXL2 was associated with poor outcome and silencing of LOXL2 resulted in a marked decrease in migratory ability and invasion capacity (36).

Synuclein gamma (SNCG), previously identified as BC-specific gene 1, appears as an independent predictive marker for recurrence and metastasis in BC (37). 34.3% of TNBC showed moderate to strong positive SNCG expression and SNCG-positive TNBC is more likely to have a more aggressive phenotype than SNCG-negative TNBC. In addition, researches indicated tumor size was significantly associated with SNCG expression. Patients whose tumors expressed SNCG had significantly shorter DFS and a higher probability of death when compared with those whose tumors did not express SNCG (38).

Lactate Dehydrogenase B (LDHB) utilized by cancer cells to bypass oxidative phosphorylation and produce lactate from pyruvate has an essential role in TNBC (39). LDHB is a predictive factor for prognosis of the TNBC groups with a high degree of power the prognosis (40).

Cyclooxygenase-2 (COX-2), a proinflammatory enzyme, contributes to catalyze the conversion of arachidonic acid to prostaglandins and thromboxanes. Tumor cells induce expression of COX-2 and its regulation is performed by transcriptional and translational processes mediated by cytokines, growth factors and oncogenes (41). COX-2 overexpression is caused to stimulate epithelial cell proliferation and angiogenesis, inhibit apoptosis, increase multidrug resistance and enhance cell motility and invasion. So it is a key factor in tumorigenesis (42). COX-2 is associated with TNBC and high tumor grade. Because of aggressiveness of nature of TNBC, evaluation of COX-2 expression in TNBC patients may be valuable prognostic marker and can help to TNBC patient's recognition with higher risk of recurrence (43).

Ki-67 is a proliferation marker that is identified as an independent predictive and prognostic factor in early breast cancer (44). High Ki-67 expression is associated with better Response to chemotherapy but with poor prognosis that is similar to the TNBC features (45). TNBCs had a poorer survival rate, regardless of a higher response rate to neoadjuvant chemotherapy (46). TNBC with high Ki-67 expression was associated with a more aggressive clinical feature despite a higher pCR rate. Ki-67 is able to be used for further classification of TNBC into two subtypes with different prognosis (47).

3.4. Triple-Negative and BRCA1 Mutation Carriers

Breast cancer in patients with BRCA1 mutation includes individual characteristics which are not evident even in patients with BRCA-2 (12). Most of these tumors express phenotypic characteristics of Triple- negative or Basal- like.

90% of tumors occurring in patients with BRCA1 mutation are TNBC or basal- like phenotype. Tumors with BRCA2 mutation are hormone receptor positive most of the time. Regarding the role of BRCA1 mutation in DNA repair (methods which identify any disorder in DNA repair); it is possible to use these methods for TNBC patients who respond to anti DNA therapies such as anthracyclines, platinums and poly (ADP-ribose) polymerase inhibitors.

There are many similarities between tumors with BRCA1 mutation carriers and tumors with sporadic BLBC, including higher frequency of P53 mutation, high grade, triple negative and expression of basal creatinine. Also, these tumors are located in one class in gene expression studies (23).

All in all, the significant characteristics of BRCAness include basal- like phenotype, ER-, EGFR expression, C-MYC amplification, and TP53 mutation, loss of RAD51- focus formation, genomic instability and sensitivity to DNA-cross-linking agents (23, 25).

3.5. Targeted Therapies of TNBC

In general, triple negative breast cancer is associated with a worse prognosis. the selection of chemotherapy is based on the traditional parameters used for breast cancer; although there are currently no evidence-based preferred regimes for TNBC. The only current systemic treatment for TNBC is chemotherapy, so alternative targeted therapies are essentially needed to improve the prognosis for TNBC patients. Presence of p53, cytokeratins, HER1, and other molecular alterations are factors that may be useful to predict therapeutic response and make decision for patient management.

Almost 60% of TNBC express EGFR (48). Expression of EGFR in breast cancer is associated with poor disease outcome. The EGFR over-expression in most TNBC has provided a rationale for trials of the anti-EGFR monoclonal antibody cetuximab; that it usually combines with platinum (49). Also, inhibition of the tyrosine kinase domain of EGFR was investigated by erlotinib in combination with docetaxel and carboplatin in patients with metastatic TNBC (5).

The mammalian target of rapamycin (mTOR), a serine/threonine kinase is an important effector downstream of the phosphatidylinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway. It plays roles in many cellular processes including cell growth, survival, and invasion. Results of studies have indicated phospho-mTOR (p-mTOR) is active form of mTOR. p-mTOR is detected at nuclear level and is expressed more frequently in triple negative (TN) human breast cancers compared with non-TN cancers (50). These findings may suggest mTOR as a more important player in the progression of TNBC and could be considered as a new target for tumor therapy of this subtype. Torin1 is a novel ATP-competitive inhibitor of mTOR. It has been reported Torin1 inhibits cell proliferation more effectively than rapamycin (51).

It seems TOP2A gene that encode topoisomerase II alpha, is a molecular target for anthracyclines so it is a predictive marker of response to anthracycline therapy (52). Microarray expression studies indicate that is a significant down regulation in PTEN and TOP2A in a subgroup of TNBC which might partly explain observed differences in response to chemotherapy in TNBC. TNBC patients treatment with Adjuvant anthracycline shows that patients with low expression of TOP2A protein have poor response (3).

To get the oxygen and nutrients they need to grow and spread, tumors create new blood vessels through a process called angiogenesis. The Vascular Endothelial Growth Factor (VEGF) is a key regulatory protein of angiogenesis. Patients with TNBC have high levels of intratumoral VEGF compared with non-TNBC patients. In addition, a higher proportion of TNBC tumors was found to have a gain in the VEGFA gene compared with non-TNBC tumors (34% versus 6%). Taken together, these results suggest that TNBC could present higher sensitivity to antiangiogenic inhibition (53). It is targeted for monoclonal antibody therapy in several solid tumors. Targeted therapy with bevacizumab, a humanized anti VEGF monoclonal

antibody, in these tumors indicates improvement in progression free survival in combined with chemotherapy in the treatment of TNBC (2).

BRCA1 is a gene involved in homologous DNA repair whose mutations mutations are seen in TNBC. BRCA1 mutations are caused to DNA repair by like base excision repair pathway using Poly (ADP-ribose) polymerase (PARP). Following BRCA1 mutations, PARP inhibition leads to an accumulation of unrepaired DNA damage that induces cell death. Nonetheless, inhibition of PARP is not effective on cancers without the BRCA1 mutation. The association of BRCA1 and triple negative status may be potentially exploited with therapeutic benefit by the combination of PARP inhibitors and chemotherapy. PARP inhibitor olaparib suggests antitumor effective-ness in cancers associated with the BRCA1 or BRCA2 mutation (54). Results of a recent randomized phase II study with the PARP inhibitor BSI-201 in combination with carboplatin and gemcitabine in metastatic TNBC showed significantly improved clinical benefit rate, progression free survival and overall survival (55).

In several subtypes of breast cancer including TNBC have been shown poorer prognosis correlated with overexpression of the heat shock protein (HSP) 90 isoforms (56). The data demonstrate that Hsp90 inhibitors are useful therapeutic targets against TNBC. Hsp90 inhibitors prevent the protein folding function of the chaperone protein Hsp90, resulting in the degradation of client proteins (57).

The v-src sarcoma viral oncogene homolog (Src) is a proto-oncogene involved in signaling that contributes in the control of several biological functions such as cell proliferation, cell differentiation, migration, angiogenesis and survival. Therefore, it seems that src plays a key role in tumor formation and progression that is considered as a potential new target for the cancer therapy (58). One of the effective and safe SRC-family kinase inhibitors is isdasatinib that has confirmed its preclinical anti-proliferative, anti-metastatic, and anti-osteoclastic activity against TNBC (59). Furthermore, studies have shown that dasatinib in combination with other agents such as cisplatin and FUDR indicate substantial synergy in TNBC cell lines (60).

The Androgen Receptor (AR), a member of the steroid hormone receptor family, is expressed in more than 70% of breast cancers and has been implicated in breast cancer pathogenesis. Preclinical studies have shown a subset of TNBC that exhibits AR-dependent, estrogen receptor-independent cell growth. These studies have indicated that expression of the AR in ER-/PR- tumors varies widely from 9 to 50%, so AR + TNBC comprises a small percentage (~2%) of all breast cancers (61). Early clinical trials have demonstrated clinical benefit with the use of the AR antagonist, bicalutamide, for the treatment of patients with AR+, estrogen receptor/progesterone receptor negative metastatic breast cancer (62).

The checkpoint proteins 1 and 2 (Chk1 and Chk2) play a role in cell cycle arrest when induced induced by double strand breaks antitumor activity. Also, combination of Hsp90 inhibitors including tanespimycin and trastuzumab exhibite antitumor activity in patients with breast cancer (63).

The Insulin Growth Factor 1 Receptor (IGF1R) plays a role in growth, invasion, and metastasis in breast cancer patients and its overexpression can be seen in 50% - 75% of TNBCs (64). Over-expressing IGF1R develops tumor formation and metastasis and induces chemo-resistance to the cancer cells (65, 66). Studies have suggested that tumor cells are sensitized to DNA damaging agents the inhibition of Chk1 and Chk2. Several clinical trials using Chk1 and Chk2 in combination with genotoxic agents including gemcitabine, irinotecan, and cisplatin in different types of solid tumors including TNBC are ongoing (67).

Results of studies have shown Fibroblast growth factor receptors (FGFR2) that bind to members of the fibroblast growth factor family of proteins which are amplified in a subgroup of patients with TNBC (68). Inhibition of FGFR2 in cell lines with FGFR2 amplification decreases cell proliferation. In this regard, several clinical trials are currently underway that FGFR inhibitors target FGFRs in patients with TNBC (69).

4. Conclusions

TNBC is a heterogeneous group of breast cancers with various histology, molecular profile and response to treatment. Loss of expression of the estrogen receptor, progesterone receptor, and a lack of over-expression of the human epidermal growth factor receptor 2 are major features of TNBC so this cancer does not respond to endocrine therapy. Therefore TNBC is a highly aggressive and metastatic disease with with a very poor overall prognosis.

Diagnosis of triple negative disease has currently important implications for Choosing systemic therapies. Novel targeted therapies including antiangiogenic agents, EGFR inhibitors, and PARP inhibitors are promising areas of research. New researches have focused on sensitivity to platinum agents and the utility of newer targeted therapy directed against other receptors in both the neoadjuvant and adjuvant setting. Only a few clinical trials have been performed on TNBC patients because this disease has a low incidence. Therefore, it seems that larger scale clinical trials are needed to be conducted in the future.

Authors' Contributions

Study concept and design: Narges Jafarzadeh, Kamran Ghaffarzadehgan, Farzad Bidouei, Hami Ashraf. Interpretation of data: Narges Jafarzadeh, Kamran Ghaffarzadehgan, Alireza Sepehri Shamloo, Fahimeh Khoshroo. Drafting of the manuscript: Alireza Sepehri Shamloo, Fahimeh Khoshroo, Narges Jafarzadeh and Farzad Bidouei. Critical revision of the manuscript for important intellectual content: Hami Ashraf, Kamran Ghaffarzadehgan.

References

1. Redig AJ, McAllister SS. Breast cancer as a systemic disease: a view of metastasis. *J Intern Med.* 2013;**274**(2):113–26.
2. Rakha EA, Reis-Filho JS, Baehner F, Dabbs DJ, Decker T, Eusebi V, et al. Breast cancer prognostic classification in the molecular era: the role of histological grade. *Breast Cancer Res.* 2010;**12**(4):207.
3. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA.* 2006;**295**(21):2492–502.
4. Oakman C, Viale G, Di Leo A. Management of triple negative breast cancer. *Breast.* 2010;**19**(5):312–21.
5. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res.* 2007;**13**(15 Pt 1):4429–34.
6. Perou CM. Molecular stratification of triple-negative breast cancers. *Oncologist.* 2010;**15 Suppl 5**:39–48.
7. Dawood S. Triple-negative breast cancer: epidemiology and management options. *Drugs.* 2010;**70**(17):2247–58.
8. Lara-Medina F, Perez-Sanchez V, Saavedra-Perez D, Blake-Cerda M, Arce C, Motola-Kuba D, et al. Triple-negative breast cancer in Hispanic patients: high prevalence, poor prognosis, and association with menopausal status, body mass index, and parity. *Cancer.* 2011;**117**(16):3658–69.
9. Brady-West DC, McGrowder DA. Triple negative breast cancer: therapeutic and prognostic implications. *Asian Pac J Cancer Prev.* 2011;**12**(8):2139–43.
10. Dolle JM, Daling JR, White E, Brinton LA, Doody DR, Porter PL, et al. Risk factors for triple-negative breast cancer in women under the age of 45 years. *Cancer Epidemiol Biomarkers Prev.* 2009;**18**(4):1157–66.
11. Bosch A, Eroles P, Zaragoza R, Vina JR, Lluch A. Triple-negative breast cancer: molecular features, pathogenesis, treatment and current lines of research. *Cancer Treat Rev.* 2010;**36**(3):206–15.
12. Carey L, Winer E, Viale G, Cameron D, Gianni L. Triple-negative breast cancer: disease entity or title of convenience? *Nat Rev Clin Oncol.* 2010;**7**(12):683–92.
13. Rodriguez-Pinilla SM, Sarrio D, Honrado E, Hardisson D, Calero F, Benitez J, et al. Prognostic significance of basal-like phenotype and fascin expression in node-negative invasive breast carcinomas. *Clin Cancer Res.* 2006;**12**(5):1533–9.
14. Foulkes WD, Stefansson IM, Chappuis PO, Begin LR, Goffin JR, Wong N, et al. Germline BRCA1 mutations and a basal epithelial phenotype in breast cancer. *J Natl Cancer Inst.* 2003;**95**(19):1482–5.
15. Prat A, Parker JS, Karginova O, Fan C, Livasy C, Herschkowitz JI, et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. *Breast Cancer Res.* 2010;**12**(5):R68.
16. Turner N, Lambros MB, Horlings HM, Pearson A, Sharpe R, Natrajan R, et al. Integrative molecular profiling of triple negative breast cancers identifies amplicon drivers and potential therapeutic targets. *Oncogene.* 2010;**29**(14):2013–23.
17. Andre F, Job B, Dessen P, Tordai A, Michiels S, Liedtke C, et al. Molecular characterization of breast cancer with high-resolution oligonucleotide comparative genomic hybridization array. *Clin Cancer Res.* 2009;**15**(2):441–51.
18. Rody A, Karn T, Liedtke C, Pusztai L, Ruckhaeberle E, Hanker L, et al. A clinically relevant gene signature in triple negative and basal-like breast cancer. *Breast Cancer Res.* 2011;**13**(5):R97.
19. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest.* 2011;**121**(7):2750–67.
20. Linn SC, Van 't Veer LJ. Clinical relevance of the triple-negative breast cancer concept: genetic basis and clinical utility of the concept. *Eur J Cancer.* 2009;**45 Suppl 1**:11–26.
21. Turner N, Moretti E, Siclari O, Migliaccio I, Santarpia L, D'Incalci M, et al. Targeting triple negative breast cancer: is p53 the answer? *Cancer Treat Rev.* 2013;**39**(5):541–50.
22. Purrington KS, Slager S, Eccles D, Yannoukakos D, Fasching PA, Miron P, et al. Genome-wide association study identifies 25 known breast cancer susceptibility loci as risk factors for triple-negative breast cancer. *Carcinogenesis.* 2014;**35**(5):1012–9.
23. Radojicic J, Zaravinos A, Vrekoussis T, Kafousi M, Spandidos DA, Stathopoulos EN. MicroRNA expression analysis in triple-negative (ER, PR and Her2/neu) breast cancer. *Cell Cycle.* 2011;**10**(3):507–17.
24. de Rinaldis E, Gazinska P, Mera A, Modrusan Z, Fedorowicz GM, Burford B, et al. Integrated genomic analysis of triple-negative breast cancers reveals novel microRNAs associated with clinical and molecular phenotypes and sheds light on the pathways they control. *BMC Genomics.* 2013;**14**:643.
25. Chen JQ, Russo J. ERalpha-negative and triple negative breast cancer: molecular features and potential therapeutic approaches. *Biochim Biophys Acta.* 2009;**1796**(2):162–75.
26. Lowery AJ, Miller N, Devaney A, McNeill RE, Davoren PA, Lemetre C, et al. MicroRNA signatures predict oestrogen receptor, progesterone receptor and HER2/neu receptor status in breast cancer. *Breast Cancer Res.* 2009;**11**(3):R27.
27. Bernardi R, Gianni L. Hallmarks of triple negative breast cancer emerging at last? *Cell Res.* 2014;**24**(8):904–5.
28. Chen X, Iliopoulos D, Zhang Q, Tang Q, Greenblatt MB, Hatziapostolou M, et al. XBP1 promotes triple-negative breast cancer by controlling the HIF1alpha pathway. *Nature.* 2014;**508**(7494):103–7.
29. Criscitiello C, Sotiriou C, Ignatiadis M. Circulating tumor cells and emerging blood biomarkers in breast cancer. *Curr Opin Oncol.* 2010;**22**(6):552–8.
30. Heneghan HM, Miller N, Lowery AJ, Sweeney KJ, Newell J, Kerin MJ. Circulating microRNAs as novel minimally invasive biomarkers for breast cancer. *Ann Surg.* 2010;**251**(3):499–505.
31. Shiu KK, Tan DS, Reis-Filho JS. Development of therapeutic approaches to 'triple negative' phenotype breast cancer. *Expert Opin Ther Targets.* 2008;**12**(9):1123–37.
32. Jackson B, Brocker C, Thompson DC, Black W, Vasiliou K, Nebert DW, et al. Update on the aldehyde dehydrogenase gene (ALDH) superfamily. *Hum Genomics.* 2011;**5**(4):283–303.
33. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell.* 2007;**1**(5):555–67.
34. Ohi Y, Umekita Y, Yoshioka T, Souda M, Rai Y, Sagara Y, et al. Aldehyde dehydrogenase 1 expression predicts poor prognosis in triple-negative breast cancer. *Histopathology.* 2011;**59**(4):776–80.
35. Csiszar K. Lysyl oxidases: a novel multifunctional amine oxidase family. *Prog Nucleic Acid Res Mol Biol.* 2001;**70**:1–32.
36. Jiao Q, Wu A, Shao G, Peng H, Wang M, Ji S, et al. The latest progress in research on triple negative breast cancer (TNBC): risk factors, possible therapeutic targets and prognostic markers. *J Thorac Dis.* 2014;**6**(9):1329–35.
37. Gluz O, Liedtke C, Gottschalk N, Pusztai L, Nitz U, Harbeck N. Triple-negative breast cancer--current status and future directions. *Ann Oncol.* 2009;**20**(12):1913–27.
38. Wu K, Huang S, Zhu M, Lu Y, Chen J, Wang Y, et al. Expression of synuclein gamma indicates poor prognosis of triple-negative breast cancer. *Med Oncol.* 2013;**30**(3):612.
39. McCleland ML, Adler AS, Shang Y, Hunsaker T, Truong T, Peterson D, et al. An integrated genomic screen identifies LDHB as an essential gene for triple-negative breast cancer. *Cancer Res.* 2012;**72**(22):5812–23.
40. Dennison JB, Molina JR, Mitra S, Gonzalez-Angulo AM, Balko JM, Kuba MG, et al. Lactate dehydrogenase B: a metabolic marker of response to neoadjuvant chemotherapy in breast cancer. *Clin Cancer Res.* 2013;**19**(13):3703–13.
41. Singh-Ranger G, Salhab M, Mokbel K. The role of cyclooxygenase-2 in breast cancer: review. *Breast Cancer Res Treat.* 2008;**109**(2):189–98.
42. Hoellen F, Kelling K, Dittmer C, Diedrich K, Friedrich M, Thill M. Impact of cyclooxygenase-2 in breast cancer. *Anticancer Res.* 2011;**31**(12):4359–67.
43. Mosalpuria K, Hall C, Krishnamurthy S, Lodhi A, Hallman DM, Baraniuk MS, et al. Cyclooxygenase-2 expression in non-metastatic triple-negative breast cancer patients. *Mol Clin Oncol.* 2014;**2**(5):845–50.
44. Urruticoechea A, Smith IE, Dowsett M. Proliferation marker Ki-67 in early breast cancer. *J Clin Oncol.* 2005;**23**(28):7212–20.
45. Petit T, Wilt M, Velten M, Millon R, Rodier JF, Borel C, et al. Com-

parative value of tumour grade, hormonal receptors, Ki-67, HER-2 and topoisomerase II alpha status as predictive markers in breast cancer patients treated with neoadjuvant anthracycline-based chemotherapy. *Euro J of Cancer.* 2004;**40**(2):205–11.
46. Rhee J, Han SW, Oh DY, Kim JH, Im SA, Han W, et al. The clinicopathologic characteristics and prognostic significance of triple-negativity in node-negative breast cancer. *BMC Cancer.* 2008;**8**:307.
47. Keam B, Im SA, Lee KH, Han SW, Oh DY, Kim JH, et al. Ki-67 can be used for further classification of triple negative breast cancer into two subtypes with different response and prognosis. *Breast Cancer Res.* 2011;**13**(2):R22.
48. Siziopikou KP, Ariga R, Proussaloglou KE, Gattuso P, Cobleigh M. The challenging estrogen receptor-negative/ progesterone receptor-negative/HER-2-negative patient: a promising candidate for epidermal growth factor receptor-targeted therapy? *Breast J.* 2006;**12**(4):360–2.
49. Carey LA, Dees EC, Sawyer L, Gatti L, Moore DT, Collichio F, et al. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. *Clin Cancer Res.* 2007;**13**(8):2329–34.
50. Rakha EA, Reis-Filho JS, Ellis IO. Basal-like breast cancer: a critical review. *J Clin Oncol.* 2008;**26**(15):2568–81.
51. Populo H, Lopes JM, Soares P. The mTOR signalling pathway in human cancer. *Int J Mol Sci.* 2012;**13**(2):1886–918.
52. Burgess DJ, Doles J, Zender L, Xue W, Ma B, McCombie WR, et al. Topoisomerase levels determine chemotherapy response in vitro and in vivo. *Proc Natl Acad Sci U S A.* 2008;**105**(26):9053–8.
53. Sharpe R, Pearson A, Herrera-Abreu MT, Johnson D, Mackay A, Welti JC, et al. FGFR signaling promotes the growth of triple-negative and basal-like breast cancer cell lines both in vitro and in vivo. *Clin Cancer Res.* 2011;**17**(16):5275–86.
54. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N Engl J Med.* 2009;**361**(2):123–34.
55. O'shaughnessy J, Osborne C, Pippen J, Yoffe M, Patt D, Monaghan G, et al., editors. Efficacy of BSI-201, a poly (ADP-ribose) polymerase-1 (PARP1) inhibitor, in combination with gemcitabine/ carboplatin (G/C) in patients with metastatic triple-negative breast cancer (TNBC): results of a randomized phase II trial.; ASCO Annual Meeting Proceedings.; 2009; p. 3.
56. Chiosis G, Caldas Lopes E, Solit D. Heat shock protein-90 inhibitors: a chronicle from geldanamycin to today's agents. *Curr Opin Investig Drugs.* 2006;**7**(6):534–41.
57. Patel HJ, Modi S, Chiosis G, Taldone T. Advances in the discovery and development of heat-shock protein 90 inhibitors for cancer treatment. *Expert Opin Drug Discov.* 2011;**6**(5):559–87.
58. Kim LC, Song L, Haura EB. Src kinases as therapeutic targets for cancer. *Nat Rev Clin Oncol.* 2009;**6**(10):587–95.
59. Finn RS, Bengala C, Ibrahim N, Roche H, Sparano J, Strauss LC, et al. Dasatinib as a single agent in triple-negative breast cancer: results of an open-label phase 2 study. *Clin Cancer Res.* 2011;**17**(21):6905–13.
60. Tryfonopoulos D, Walsh S, Collins DM, Flanagan L, Quinn C, Corkery B, et al. Src: a potential target for the treatment of triple-negative breast cancer. *Ann Oncol.* 2011;**22**(10):2234–40.
61. Niemeier LA, Dabbs DJ, Beriwal S, Striebel JM, Bhargava R. Androgen receptor in breast cancer: expression in estrogen receptor-positive tumors and in estrogen receptor-negative tumors with apocrine differentiation. *Mod Pathol.* 2010;**23**(2):205–12.
62. Shah PD, Gucalp A, Traina TA. The role of the androgen receptor in triple-negative breast cancer. *Womens Health (Lond Engl).* 2013;**9**(4):351–60.
63. Modi S, Stopeck A, Linden H, Solit D, Chandarlapaty S, Rosen N, et al. HSP90 inhibition is effective in breast cancer: a phase II trial of tanespimycin (17-AAG) plus trastuzumab in patients with HER2-positive metastatic breast cancer progressing on trastuzumab. *Clin Cancer Res.* 2011;**17**(15):5132–9.
64. Grunstein M. Histone acetylation in chromatin structure and transcription. *Nature.* 1997;**389**(6649):349–52.
65. Jones RA, Campbell CI, Gunther EJ, Chodosh LA, Petrik JJ, Khokha R, et al. Transgenic overexpression of IGF-IR disrupts mammary ductal morphogenesis and induces tumor formation. *Oncogene.* 2007;**26**(11):1636–44.
66. Bolderson E, Richard DJ, Zhou BB, Khanna KK. Recent advances in cancer therapy targeting proteins involved in DNA double-strand break repair. *Clin Cancer Res.* 2009;**15**(20):6314–20.
67. Ashwell S, Zabludoff S. DNA damage detection and repair pathways--recent advances with inhibitors of checkpoint kinases in cancer therapy. *Clin Cancer Res.* 2008;**14**(13):4032–7.
68. Turner N, Pearson A, Sharpe R, Lambros M, Geyer F, Lopez-Garcia MA, et al. FGFR1 amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer. *Cancer Res.* 2010;**70**(5):2085–94.
69. Sharpe R, Pearson A, Herrera-Abreu MT, Johnson D, Mackay A, Welti JC, et al. FGFR Signaling Promotes the Growth of Triple-Negative and Basal-Like Breast Cancer Cell Lines Both In Vitro and In Vivo. *Clinical Cancer Research.* 2011;**17**(16):5275–86.