Review

Molecular Classification and Future Therapeutic Challenges of Triple-negative Breast Cancer

NIKOLAOS GARMPI^{1,2*}, CHRISTOS DAMASKOS^{2*}, ANNA GARMPI^{3*}, KONSTANTINOS NIKOLETTOS^{2*},
DIMITRIOS DIMITROULIS¹, EVANGELOS DIAMANTIS⁴, PARASKEVI FARMAKI⁵,
ALEXANDROS PATSOURAS⁶, ERRIKA VOUTYRITSA², ATHANASIOS SYLLAIOS⁷,
CONSTANTINOS G. ZOGRAFOS⁷, EFSTATHIOS A. ANTONIOU^{1,2}, NIKOS NIKOLETTOS⁸,
ALKIVIADIS KOSTAKIS⁹, KONSTANTINOS KONTZOGLOU^{1,2}, DIMITRIOS SCHIZAS⁷ and AFRODITI NONNI¹⁰

¹Second Department of Propedeutic Surgery, Laiko General Hospital, Medical School,
National and Kapodistrian University of Athens, Athens, Greece;

²N.S. Christeas Laboratory of Experimental Surgery and Surgical Research,
Medical School, National and Kapodistrian University of Athens, Athens, Greece;

³First Department of Propedeutic Internal Medicine, Laiko General Hospital,
Medical School, National and Kapodistrian University of Athens, Athens, Greece;

⁴Department of Endocrinology and Diabetes Center, G. Gennimatas General Hospital, Athens, Greece;

⁵First Department of Pediatrics, Agia Sofia Children's Hospital, Medical School,
National and Kapodistrian University of Athens, Athens, Greece;

⁶Second Department of Internal Medicine, Tzanio General Hospital, Piraeus, Greece;

⁷First Department of Surgery, Laiko General Hospital, Medical School,
National and Kapodistrian University of Athens, Athens, Greece;

⁸Obstetric - Gynecologic Clinic, Medical School, Democritus University of Thrace, Alexandroupolis, Greece;

⁹Biomedical Research Foundation (BRFAA), Academy of Athens, Athens, Greece;

¹⁰First Department of Pathology, Medical School, National and Kapodistrian University of Athens, Athens, Greece

Abstract. Triple-negative breast cancer (TNBC) is an extremely diverse group of breast tumors, with aggressive clinical behavior, higher rates of distant recurrence and worse overall survival compared to other types of breast cancers. The genetic, transcriptional histological and clinical heterogeneity of this disease has been an obstacle in the progression of targeted therapeutic approaches, as a ubiquitous TNBC marker has not yet been discerned. In

terms of that, current studies focus on the classification of TNBC tumors in subgroups with similar characteristics in order to develop a treatment specialized for each group of patients. To date, a series of gene expression profiles analysis in order to identify the different molecular subtypes have been used. Complementary DNA microarrays, PAM50 assays, DNA and RNA sequencing as well as immunohistochemical analysis are some of the methods utilized to classify TNBC tumors. In 2012, the Cancer Genome Atlas (TCGA) Research Network conducted a major analysis of breast cancers using six different platforms, the genomic DNA copy number arrays, DNA methylation, exome sequencing, messenger RNA arrays, microRNA sequencing and reverse-phase protein arrays, in order to assort the tumors in homogenous subgroups. Since then, an increasing number of breast cancer data sets are being examined in an attempt to distinguish the classification with biological interpretation and clinical implementation. In this review, the progress in molecular subtyping of TNBC is discussed, providing a brief insight in novel TNBC biomarkers and therapeutic strategies.

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*These Authors contributed equally to this study.

Correspondence to: Dr. Christos Damaskos, MD, MSc, Ph.D., Second Department of Propedeutic Surgery, Laiko General Hospital, National and Kapodistrian University of Athens, Medical School, 17 Agiou Thoma Street, Athens, 11527, Greece. Tel: +30 6948467790, e-mail: x_damaskos@yahoo.gr

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In 2005, Brenton *et al.*, mentioned a group of basal-like breast cancers with “triple-negative receptor status”, not responding to conventional targeted therapies (hormonal or anti-HER2), which is commonly named as Triple-Negative Breast Cancer (TNBC) (1). TNBC is a diverse and heterogeneous type of carcinoma characterized by the absence of estrogen and progesterone receptors (ER and PR respectively), as well as lack of HER2 gene amplification (Figure 1) (2). TNBC represent 10-20% of all types of breast cancers (3). These carcinomas occur more frequently in younger women (<50 years old), in women harboring mutations in *BRCA1* gene (more than 75% of *BRCA1* carcinomas are triple-negative) and women with specific extraction or economic status (more common to African-American and Hispanic origin) (4-7). The median time to death for patients with TNBC is estimated at 4.2 years in comparison with the 6 years of other breast cancers, while survival after diagnosis of TNBC patients is 10 years at most, compared to 18 years in patients with other breast cancers (8). Furthermore, tumor’s distant recurrence risk in patients with TNBC is maximized in 1-3 years after surgery and remains steady afterwards, while the likelihood of metastasis within 5 years of diagnosis is significantly increased in TNBC compared to other types of cancers (9). The median survival time to death after relapse is only 9 months, although disease-free patients for more than 8 years are less likely to die because of breast cancer (10). Importantly, women with TNBC have higher rates of pathological complete response (pCR) following neoadjuvant chemotherapy, with favorable lasting outcome and lower percentages of recurrence. However, in the case of remaining disease after the therapy, TNBC patients display decreased overall survival (11, 12).

Histologically, TNBC are mainly high-grade invasive carcinomas of no special type (approximately 90% of the cases), also referred as invasive ductal carcinomas-not otherwise specified (IDC-NOS) (13, 14). However, numerous high-grade TNBC have special histological features and can be classified as apocrine, medullary or metaplastic breast carcinomas (MBC) (15). IDCs-NOS are large and highly malignant tumors (16), with pushing invasive border configuration, dense lymphocytic infiltration, nuclear pleomorphism and high mitotic rates (8, 17). Apocrine carcinomas are extremely rare (<1%), exhibit nuclear pleomorphism and are characterized by a lymphocytic environment as well as presence of androgen receptors (AR) (18-20). The presence of AR could imply prognostic value, but so far, the data are rather uncertain and not conclusive (21, 22). Medullary breast carcinomas usually display basal-like characteristics, with well-defined borders, syncytial growth and brisk lymphocytic infiltrate and have been related to better prognosis (23). In fact, the lymphocytic infiltrate of these carcinomas displays prognostic value for

Figure 1. Definition of triple-negative breast cancer (TNBC). TNBC is characterized by the absence of estrogen and progesterone receptors (ER and PR respectively), as well as lack of HER2 gene amplification. HER2: Human epidermal growth factor receptor 2.

chemotherapy-treated women with TNBC (24, 25). MBC consist a group of high-grade, heterogeneous lesions with poor prognosis, including carcinomas with either claudin-low (CLDN-1) or basal-like characteristics (26).

On the other hand, a few TNBC are low-grade, less aggressive carcinomas and can be divided into two subgroups, the salivary gland-like breast tumors with unique genetic alterations and the low-grade triple-negative breast neoplasia family whose expression patterns resemble those of high-grade TNBC (27). Nevertheless, it is important to mention that all subsets of low-grade TNBC can progress to high-grade tumors and during this process, the salivary gland-like subset maintains the differences from the conventional high-grade TNBC (28-30), whereas the low-grade TNBC family is rather similar to conventional high-grade TNBC (31).

The salivary gland-like tumors do not harbor recurrent TP53 mutations, carry specific genes' expression profile and they represent mostly two subsets; the rare adenoid cystic carcinomas (AdCC) (less than 0.1% of all breast cancers) and the secretory carcinomas (less than 0.15% of all breast cancers) (32). The former show favorable prognosis, have basal-like features and harbor the MYB-NFIB fusion gene due to the t(6;9)(q22-23;p23-24) genetic translocation (33), whereas the latter display excellent clinical outcome, abundant intra- and extracellular secretion of eosinophilic material and harbor the ETV6-NTRK3 fusion gene as a result of the t(12;15)(p13;q25) translocation (34). As far as

the low-grade TN breast neoplasia family is concerned, it includes microglandular adenosis (MGA), atypical MGA (AMGA) and acinic cell carcinoma (ACC) (35). MGA/AMGA are clonal neoplastic lesions expressing the S100 protein, display specific genetic alterations (*e.g.* 5q losses or 8q gains) and carry mutations of TP53 (80%) and other genes (BRCA1, PI3K pathway genes, receptor tyrosine kinases genes) (36, 37). ACCs show good prognosis with rare local or distant recurrences, different genomic alterations as well as TP53 (80% of cases) and PIK3CA (10% of cases) mutations (38).

To date, there are considerably restricted prognostic biomarkers and Food and Drug Administration (FDA)-approved therapies for TNBC cases (39, 40). The complexity of histopathological and molecular features of these carcinomas along with the diverse clinical response, point out the necessity of the TNBC classification in subgroups with similar characteristics so as to develop new targeted treatments.

Materials and Methods

A review of the literature was performed through the PubMed database and Cochrane library aiming to identify articles regarding molecular classification of TNBC. Specifically, our research used keywords, separately and in numerous combinations, such as triple-negative breast cancer, classification, molecular, treatment and prognosis. Furthermore, we double-checked the references from all articles in order to include any other eligible studies. The initial research identified 39 articles. No duplicates were found. These articles were screened and 30 were excluded for various reasons. Some of them were only abstracts, whereas others were not completely relevant to the topic. It should be mentioned that publications in non-English language were also excluded. The full-text articles assessed for eligibility were 8 and 2 of them were excluded because they were reviews of the classifications. Finally, 6 articles were included. The inclusion process is shown in Figure 2.

Results

Molecular subtypes. In 2000, Perou *et al.* analyzed the gene expression patterns of 65 human breast tumors and distinguished four clusters, the ER+/luminal-like, basal-like, *Erb*-B2+ (or HER2+) and normal breast (2). Subsequently, another intrinsic subtype of breast cancer, the CLDN-1 tumors, with low expression of luminal markers, high expression of genes related to the epithelial to mesenchymal transition, immune response markers and cancer stem-cell like characteristics, was described. Clinically, most CLDN-1 tumors are triple-negative IDCs with poor prognosis and different response to chemotherapy if compared to luminal or basal-like tumors. However, the majority (50-75%) of TNBC exhibit basal-like characteristics and a smaller part can be correlated with any of the aforementioned subgroups (41). Furthermore, basal-like breast carcinomas display histological

and immunohistochemical similarities to myoepithelial cells of the breast (42). The identification of basal-like carcinomas requires molecular analysis of genes, however, more commonly, it is used the immunohistochemical expression profile of specific markers such as cytokeratins 5/6,14 and 17, cyclin E, P-cadherin, epidermal growth factor receptor (EGFR) and receptor tyrosine kinase (C-kit) (43, 44). Particularly, EGFR and C-kit are overexpressed in 77% of basal-like breast cancers (45). Importantly, 80% of basal-like tumors harbor mutated TP53, whereas there is a 9% having PI3KC mutations (46).

The molecular heterogeneity of TNBC, the poor prognosis and the absence of targeted therapeutic strategies highlight the requirement to classify this diverse disease. Recently, a number of studies concentrated on the investigation of the gene expression profile of TNBC with the use of high-throughput technology tools (47). In 2011, a gene expression database analysis led Lehmann *et al.*, to the identification of six reproducible subtypes of TNBC: Basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL) and luminal androgen receptor (LAR) subtype. Another type of unstable carcinomas (UNS), was also proposed (48).

The BL1 subtype consists of genes involved in cellular proliferation as well as DNA repair. The database analysis revealed an augmented expression of genes related to cell cycle and DNA damage response [neuroblastoma-RAS (NRAS), MYC, polo-like kinase 1 (PLK1), checkpoint kinase 1 (CHEK1), metalloprotease/disintegrin-like 1 (MDC1), breast cancer gene (BRAC) *etc.*] (48, 49). On the other hand, in subtype BL2 there are genes involved in the signaling pathways of growth factors (EGFR, nerve growth factor (NGF), receptor tyrosine kinase MET, Wnt/ β -catenin and insulin-like growth factor 1 (IGF1R) pathways *etc.*), glycolysis, gluconeogenesis and in the expression of myoepithelial factors [tumor protein 63 (TP63) and membrane metalloendopeptidase (MME) *etc.*] (50). The IM subtype includes genes associated to immunological processes and cascades (cytokine pathways, mechanisms of antigen processing, nuclear factor- κ B, tumor necrosis factor (TNF) and JAK/STAT signaling *etc.*) (51). The other two subtypes, M and MSL, are described by genes related to cellular differentiation, migration and growth, as well as extracellular matrix processes. However, the MSL subtype differs because of the decreased expression of cell-cycle genes and the resemblance of its gene expression profile to that of mesenchymal stem cells [HOX, NGF receptor (NGFR), vascular cell adhesion protein 1 (VCAM1) *etc.*] (52, 53). Finally, the LAR subtype displays a luminal-like expression profile with increased presence of AR and molecules involved in AR pathways (54). It is, also, enriched with genes involved in steroid synthesis, androgen/estrogen and porphyrin metabolism (48). The LAR TNBC subtype has been also linked to molecular apocrine type of breast cancer (55).

Figure 2. PRISMA flow diagram for the current study.

Cytokeratins can be also criteria for breast cancer characterization as basal-like or luminal (56). Particularly, TNBC subtypes either highly express basal cytokeratins (UNS, BL1, BL2 and M), or luminal cytokeratins (LAR) (57, 58).

As far as the intrinsic molecular classification is concerned, the majority of TNBC have basal-like molecular subtype, whereas the M and MSL subtype are mostly normal breast-like. However, it is still controversial if the intrinsic classification is appropriate for TNBC, considering the variety of results in BL2, M and MSL analysis. On the other hand, the vast percentage of LAR TNBC (82%) are luminal A or B (48). A later study conducted to investigate the correlation among PAM50 breast cancer intrinsic subtypes (2) and TNBC subtypes (59), analyzed data from 374 TNBC

cases. The results indicated a high percentage of BL1 (99%), BL2 (95%), IM (84%) and M (97%) subtypes exhibiting basal-like phenotype, verifying previous studies (60). On the other hand, half of the MSL TNBC resemble to the basal-like intrinsic subclass, while 28% is normal-like and 14% luminal B. Finally, LAR molecular subtype mostly pertains to HER2 (74%) group, whereas a 14% is luminal B (61). Intriguingly, a cluster of TNBC lacked luminal-specific markers and expressed CLDN-1-related genes (62).

Recently, Burstein *et al.*, proposed 4 stable subtypes of the TNBC with differential prognoses, through mRNA expression analysis and DNA profiling. These subtypes are the luminal/androgen receptor (LAR), the mesenchymal (MES), the basal-like immune suppressed (BLIS) and the basal-like

immune-activated subtype (BLIA). The aforementioned subtypes are described by distinguishing RNA and DNA indicators that could be used in treatment strategies. The LAR subtype expresses steroid-related genes (androgen receptor (AR), progesterone receptor (PGR), GATA3 *etc.*). Interestingly, gene expression patterns of the LAR TNBC subgroup showed that in 1% of these ER-negative tumors (as shown by immunohistochemistry) occurs ER activation, as a result of ER low expression. The MES subtype is enriched with genes implicated in cell proliferation, DNA damage response as well as with osteocyte- and adipocyte-specific markers [osteoglycin (OGN), adiponectin-C1Q and collagen domain containing (ADIPOQ) *etc.*]. The basal-like immune suppressed subgroup of TNBC, as implied by its name, displays low expression of genes related to immune cell processes. On the contrary, basal-like immune activated cluster is characterized by augmentation of immune cell-specific pathways (63).

Furthermore, a comprehensive study conducted by Le Du *et al.* gathered published data about TNBC and suggested 5 potential clinically applicable molecular subtypes of TNBC. These are: 1) the basal-like TNBC (BL-TNBC), which appears to be the most common group (25-80% based on the characterization), 2) the mesenchymal-like TNBC (ML-TNBC) 3) the immunomodulatory TNBC (I-TNBC), 4) the luminal/apocrine TNBC (LA-TNBC) and 5) the HER2-enriched TNBC (HER2e-TNBC) (64). The first cluster of TNBC is described by the defects in DNA repair pathways or by the up-regulation of growth factor cascades, as previously reported, mentioning the Lehmann's division to BL1 and BL2 subtypes (48). The BL-TNBC display higher rates of post-chemotherapy pCR, (65), but it seems that these rates refer to Lehman's BL1 subtype (51% compared to BL2 with 0%), indicating the possibility of grouping BL2 cluster with other molecular subtypes (66). The ML-TNBC (that is mesenchymal, mesenchymal stem-like, CLDN-I carcinomas) display elevated expression of mesenchymal indicators as well as up-regulation of cancer stem cell-related genes (48, 62). PAM50 analysis, in which CLDN-I cluster is not included, assort MSL to normal-like tumors and the M to basal-like (67). I-TNBC highly express genes associated with immune cell signaling cascades and functions. Despite the diversity of tumor characterization, it is certain that there is a cluster of cancers with increased immune cells infiltration (CLDN-I tumors and Burstein's BLIA molecular subtype) (68, 69). In the LA-TNBC subtype (LAR, luminal A-B, molecular apocrine) there are high levels of androgen receptors leading to up-regulation of steroid-regulated pathways. Finally, the HER2-enriched (low HER2 expression-no HER2 amplification) TNBC subgroup (6-8% of TNBC), shows quite resemblance to the LA TNBC and its separation on account of a unique clinical behavior is yet controversial (2, 10, 70, 71).

Reassessment of previous TNBC grouping by Lehmann *et al.* led to lessening the number of subtypes from 6 to 4, based on the cell types ambulating in the tumor environment. The redefined subgroups are the BL1, BL2, M and LAR TNBC, whereas the other two (IM and MSL) subtypes were merged with the above, as their initial classification proved to be rather the result of the tumors' neighboring diverse cellular composition. Particularly, the gene expression portrait of the former subtype referred as IM was proposed to be by virtue of the immune cells surrounding the tumor, rather than a tumor-specific indicator. Likewise, the MSL-cluster characteristics were correlated to the stromal mesenchymal tissue in the adjacent environment of the tumors (72).

Interestingly, a different classification of the TNBC based on the presence or absence of AR, has been recently proposed. Therefore, the two major subclasses are the AR+ TNBC, expressing AR, and the quadruple-negative breast cancer (QNBC) which lacks the androgen receptor (73). Several ongoing clinical trials with AR-targeting agents indicate the significance of the above grouping (74). On the other hand, a comparative study about the inflammatory and non-inflammatory TNBC, revealed no correlation between the molecular subtype of the TNBC and its inflammatory status (75).

In 2019, Prado-Vázquez *et al.* described two different molecular classifications which were based to cellular type and immune activity. The initial classification included 4 subgroups: 1) CLDN-low, 2) CLDN-high, 3) basal-like and 4) LAR group and was performed according to the stem cell cancer hypothesis (76, 77). This classification is related to the differentiation process and especially the initiation of carcinogenesis. This means that the LAR and the CLDN-low type will have the higher and lower cell differentiation rate respectively. As far as the clinical presentation is concerned, the CLDN-low and LAR tumors had a bigger size and higher infiltration rate than the other two groups. The basal-like group had a small size and did not demonstrate lymph node infiltration. The CLDN-high type was large in size but did not show node infiltration. The CLDN-low tumors were related more to haptoglobin, whereas the CLDN-high subtype was related to chemokine. The LAR type demonstrated less cellular adhesion, chemokine activity and disorders of G₁/S transition cell cycle. The basal-like type had higher regulation of actin cytoskeleton and cell adhesion (76).

An immune classification was added in the cellular aiming to associate the cellular types to prognosis. The tumors with positive immune activity had better prognosis than the ones with negative immune activity. This was significantly important to the LAR subtype tumors in which the immune positive group demonstrated better survival rates. The CLDN-high tumors with positive immune activity had also a better prognosis. However, these results did not appear in the other two groups.

Table I. Molecular subtypes of triple negative breast cancer as classified by different research groups and their characteristics.

Study	Molecular subtypes	Characteristics
Lehmann <i>et al.</i> , 2011 (48)	BL1	Genes related to cellular proliferation and DNA repair.
	BL2	Growth factors, glycolysis, gluconeogenesis, myoepithelial factors.
	IM	Immunological processes and cascades (cytokine pathways, mechanisms of antigen processing and presentation).
	M	Cellular differentiation, migration and growth, extracellular matrix processes.
	MSL	Cellular differentiation, migration and growth, extracellular matrix processes.
		Decreased expression of cell cycle genes, resemblance of gene expression profile to mesenchymal stem cells.
	LAR	Steroid synthesis, androgen/estrogen and porphyrin metabolism.
		AR and molecules implicated in AR pathways.
Burstein <i>et al.</i> , 2015 (63)	UNS	Correlation to basal-like intrinsic molecular subtype.
	LAR	Steroid-related genes
	M	Cell proliferation, DNA damage response, osteocyte- and adipocyte-specific markers.
	BLIS	Low expression of genes related to immune cell processes.
Du <i>et al.</i> , 2015 (64)	BLIA	High expression of genes related to immune cell processes.
	BL TNBC	Defects in DNA repair pathways or upregulation of growth factor cascades.
	MSL	Mesenchymal indicators, upregulation of cancer stem cell-related genes.
	IM	Genes associated with immune cell signaling cascades and functions.
	LA	Androgen receptors, upregulation of steroid-regulated pathways.
	HER2-e	Resemblance to the LA-TNBC but unique clinical behavior.
Barton <i>et al.</i> , 2015 (73)	AR+	Response to AR targeted therapies.
	QNBC (AR-)	No response to AR targeted therapies
Lehmann <i>et al.</i> , 2016 (72)	BL1	IM and MSL subtypes coalesced with any of these subtypes.
	BL2	IM: Initial gene expression portrait by virtue of the immune cells surrounding the tumor.
	M	MSL: Characteristics correlated to the stromal mesenchymal tissue in the adjacent environment of the tumors.
	LAR	
Prado-Vázquez <i>et al.</i> , 2019 (76)	CLDN-l	Higher haptoglobin activity.
		No difference in prognosis.
	CLDN-h	Higher chemokine activity.
		Better prognosis in the immune positive group.
	BL	Higher regulation of actin cytoskeleton and cell adhesion.
		No difference in prognosis between immune positive and negative groups.
	LAR	Less cellular adhesion, chemokine activity and disorders of G ₁ /S transition cell cycle.
		The immune positive group has a better prognosis.

BL1: Basal-like 1. BL2: Basal-like 2. IM: Immunomodulatory. M: Mesenchymal. MSL: Mesenchymal stem-like. LAR: Luminal androgen receptor. UNS: Unstable. BLIS: Basal-like immune suppressed. BLIA: Basal-like immune-activated. BL: Basal-like. LA: Luminal/apocrine. HER2-e: HER2-enriched. AR+: Androgen receptor positive. AR-: Androgen receptor negative. TNBC: Triple-negative breast cancer. QNBC: Quadruple-negative breast cancer. CLDN-l: Claudin-low. CLDN-h: Claudin-high.

Table I summarizes all the proposed classifications of TNBC and Figure 3 describes their similarities and their differences.

Therapeutic strategies. Therapeutically, the absence of specific targets in TNBC makes chemotherapy (anthracyclines, taxanes) the only established approach in TNBC treatment, usually with the addition of platinum agents (66, 78). The pathological complete response (pCR) rates, differ amongst TNBC subtypes, with BL1 having the highest rate, while BL2 and LAR displaying the lowest (66). Unfortunately, women who do not achieve pCR have worse outcomes compared to patient with other types of breast cancers (79). Generally, non-

metastatic TNBC develop better response to neoadjuvant chemotherapy, in contrast to other types of breast cancers. However, there is a diverse response among the different molecular subtypes. During the last decade, a growing number of different therapeutic approaches is under research so as to develop subtype-specific treatments.

Approximately 75% of breast tumors containing the BRCA1 mutation are triple-negative and sensitive to double-stranded DNA breaks. As a result, platinum agents (carboplatin, cisplatin, epirubicin) are widely used in clinical trials, as the DNA damage they cause cannot be repaired by the endogenous homologous recombination mechanism (80). Platinum-based chemotherapy has been shown to increase

Figure 3. Similarities and differences between the molecular classification of triple-negative breast cancer. BL: Basal-like. BL1: Basal-like 1. BL2: Basal-like 2. BLIS: Basal-like immune suppressed. BLIA: Basal-like immune-activated. IM: Immunomodulatory. M: Mesenchymal. MSL: Mesenchymal stem-like. LAR: Luminal androgen receptor. UNS: Unstable. LA: Luminal/apocrine. HER2-e: HER2-enriched. AR+: Androgen receptor positive. QN: Quadruple-negative breast cancer. CLDN-l: Claudin-low. CLDN-h: Claudin-high.

pCR rate in TNBC patients, whereas patients with metastatic TNBC and BRCA mutations have a better response to carboplatin and docetaxel (12, 81, 82). However, the results are contradictory when it comes to disease-free survival (DFS), overall survival (OS), as well as metastatic progression (83). Furthermore, ongoing pre-clinical data and clinical trials on PARP inhibitors seem to be promising in basal-like TNBC (84-86). Regarding the mesenchymal TNBC, there are no data from clinical trials and so far, there is little progress in specific targeting (64).

At the same time, immune-based therapy, is also being investigated by several groups (87, 88). The immune cells that infiltrate the tumors (TILs) are of great importance in TNBC clinical features as well as in the tumor's response to treatments (89). The immune-based subtype in the classification of Prado-Vázquez can possibly provide information concerning response to immunotherapy (76). Paradoxically, higher TILs are correlated to worse clinical characteristics, but improved adjuvant and neoadjuvant chemotherapy response (66). Notably, IM TNBC display promising response to pembrolizumab, an immune checkpoint inhibitor (90).

Furthermore, next-generation sequencing revealed some of the most frequent mutations in metastatic TNBC [TP53, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), phosphatase and tensin homolog (PTEN)] as well as an AR protein amplification (AR+ TNBC), indicating new promising therapeutic strategies (91). Currently, it is estimated that approximately 30% of TNBC, including non-LAR subtypes, are AR-dependent, highlighting enzalutamide as a potential targeted chemotherapy in diverse TNBC subtypes (73). Moreover, clinical trials using bicalutamide and enobosarm (AR-inhibitors) report relatively high rates of clinical benefits in AR+ TNBC (92-94). Notably, Garmpis *et al.*, suggested that histone deacetylase inhibitors (HDACi) display some promising *in vitro* and *in vivo* results, but clinical trials are preliminary (95).

Several signaling pathways play an important role in different TNBC subtypes, suggesting potential targets (64). Damaskos *et al.*, reported the PI3K/AKT/mTOR pathway,

poly ADP-ribose polymerase-1 (PARP-1) inhibitors, tyrosine kinase inhibitors, anti-androgens and anti-angiogenic factors as possible therapeutic tools against TNBC (96). One particular target is EGFR, which is overexpressed in ML, BL and HER2e TNBC and has been shown to have beneficial results when combined with chemotherapy (97, 98). Recently, it has been proposed that the circulating tumor DNA (ctDNA) could be either used as a biomarker for early-phase TNBC diagnosis, or as a way of «monitoring» the tumor clones resistant to chemotherapy in order to redefine the treatment (99).

On the basis of these data, novel targeted approaches should be developed for TNBC. There is no known particular target for TNBC up to now. However, TNBC is a type of breast cancer with challenging biological features and the scientific community's increasing understanding of these features leads us to improve clinical outcomes. A promising future goal is the application of valuable biomarkers that can predict therapies for TNBC. Concerning the chemosensitive TNBC seem to respond to cytotoxic agents such as anthracyclines and taxanes. Moreover, platinum derivatives lead to promising results. Preliminary results with AR inhibitors and PARP inhibitors show hopeful results and can be potential therapeutic tools against TNBC. Anti-angiogenic drugs and EGFR inhibitors are also promising therapeutic tools and up to date results indicate that they may play a significant role TNBC therapy. Recently, clinical studies among patients with breast matrix-producing carcinomas (MPCs), which are rare and usually triple-negative, have been reported (100). Patients with TNBC received anthracycline- and taxane-based neoadjuvant chemotherapy (NAC), but they showed a poor response to standard NAC. A future target could be further studies in order to improve the treatment of this rare but aggressive tumor. At the same time, the investigation of the prognostic value of tumor-infiltrating lymphocytes (TILs) in systemically untreated and early-stage TNBC led to promising results (101). More specifically, the clinical studies suggest that a low TIL level ($\leq 10\%$) was correlated to beneficial results in patients with T1a/b or N0 disease. Therefore, patients with a low TIL

level, even in the T1a/bN0 subset display promising response to adjuvant chemotherapy regardless of the status or the tumor size. Finally, it is known that the radiation therapy (RT) represents a therapeutic strategy in breast cancer. It has been proposed that there are cell lines at a transcriptional level which demonstrate resistance to RT (102). Taking all these into consideration, we are able to better investigate all the mechanisms involved in RT as a therapeutic tool in TNBC in order to develop upgraded targeted therapies.

Conclusion

TNBC are undoubtedly one of the most diverse breast cancer categories. New technologies have facilitated researchers to analyze a large amount of data sets so as to compare different TNBC and categorize them in stable subgroups based on their similarities. The ongoing molecular classification of TNBC is crucial for the understanding of the genetic targets characterizing these tumors and provides potential prognostic and therapeutic approaches. Obviously, the identification of unique subtype-specific genetic targets is the highest ambition for a successful TNBC therapy.

Conflicts of Interest

All the Authors declare that there are no conflicts of interest regarding this study.

Authors' Contributions

NG, CD and AN designed the study. NG, CD, AG and KN wrote the article. NG, CD, AG, KN, ED, PF, AP, EV, AS and CGZ collected the data. NN and KK offered scientific advice. DD, EAA, AK and DS revised the manuscript. AN was the supervisor.

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