

Recent Studies & Advances in Breast Cancer

Chapter 3

Targeted Therapies in Triple Negative Breast Cancer: Current Knowledge and Perspectives

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Abstract

Triple negative breast cancer (TNBC) is a very aggressive type of breast cancer characterized by high cellular heterogeneity, metastatic capacity, and resistance to therapy. This is due to the increased proportion of cancer stem cells (CSCs) partly responsible for the initiation and progression of this type of cancer, and the lack of receptors to target. Therefore, it is a priority to find novel therapies targeting both chemotherapy-sensitive tumor cells and resistant cancer stem cells, to improve the prognosis of these patients. Gene profiling of TNBC revealed several subtypes with unique features, which has been a step forward in the search for new therapeutic modalities. However, treatment options remain limited and ineffective, largely due to delayed tumor detection, accelerated tumor growth, high likelihood of metastasis, and resistance to chemotherapy. In this review, we provide information about current and new-targeted therapies that are under study.

Keywords: Triple negative breast cancer; Targeted therapies; Cancer stem cells; Chemotherapy; Therapeutic targets.

1. Breast Cancer

Breast cancer is one of the most diagnosed cancer in women worldwide, with more than 2.1 million of new cases and 500.000 deaths per year. It is considered a common pathology from developed countries, however, a 69% of deceases attributed to this disease are reported in developing countries. World Health Organization (WHO) estimations indicate that one out of eight women will develop breast cancer by 2020 [1,2].

Breast cancer is considered a group of diseases with a multifactorial etiology and, in most of cases, unknown. While a 5-10% of all breast cancer cases are derived from germline mutations, sporadic breast cancer due to somatic alterations are the most frequent [3]. Hereditary mutations in the tumor suppressor genes *BRCA1* and *BRCA2* are the most important. Indeed, *BRCA1* gene exhibits a dominant pattern and is associated with an increased risk of breast cancer (37-85%) [1,3]. These gene alterations can also be initiated by chemical carcinogens and promoted by environmental and physiological factors. Among these, hormones, diet and life style, age and genre, metabolic and genetic disorders, and preexisting diseases of the breast are well-known inductor factors in breast cancer [1,4].

1.1. Classification

This group of diseases can be classified into different clinical subtypes based on the cellular expression of estrogen receptor (ER), progesterone receptor (PR), as well as human epidermal growth factor 2 (HER2) [5]. Based on this classification the different subtypes are:

Hormone receptor positive or luminal. It represents about 65% of all cases of breast cancers [6]. It can be divided into:

- Luminal A: It is characterized by a higher expression of ER and PR, as well as a low Ki-67 index. This subtype has the best prognosis rate with a 5-year survival of 90%, approximately [7,8].
- Luminal B: It is similar to luminal A and, although less hormone sensitive, is positive for ER and/or PR. It is associated with higher Ki-67 index and its prognosis is slightly less favorable than in luminal A, with a 5-year survival rate of 88% [7,8,9].

HER2 positive. A 10-15% of breast cancers show *ERBB2* oncogene amplified or the HER2 receptor overexpressed, whose activation gives rise to signaling pathways involved in cell differentiation, growth and survival [8]. This type of tumors are associated with poor prognosis markers, such as the alteration of genes involved in angiogenesis and metastatic potential [9]. In addition, patients diagnosed with this type of cancer are usually detected when the disease is advanced [7].

Basal. This subtype includes triple negative breast cancer (TNBC) that is fully described below.

2. Triple Negative Breast Cancer

2.1. Features and clinical relevance

TNBC is a very aggressive subtype of breast cancer and is characterized by the absence of ER, PR and HER2, what makes hormone therapy ineffective on this type of cancer [5,10]. TNBC represents between 15 and 20% of the worldwide-diagnosed breast cancer cases, while is liable for 25% of cancer deaths. It is more prevalent in African, American and Hispanic women at an early age, with association to germline mutations in *BRCAl* [11]. TNBC patients show an aggressive and more destructive clinic, have a high risk of metastasis, a higher rate of relapse and death within 5 years from diagnosis [10]. Compared to other molecular types, TNBC has a poorer prognosis and a higher rate of metastatic relapses, especially to lungs and brain, but not to bone, as occurs in other breast cancers [11,12].

Usually, TNBC tumors are poorly differentiated and, therefore, their diagnosis is difficult and delayed. In addition, metastases derived from these tumors are difficult to recognize, and are often diagnosed as metastases of unknown origin. For this reason, it is necessary to establish metastasis markers with application in the clinical diagnosis for this pathology [12].

The great heterogeneity, at histopathological and molecular levels, of TNBC is a big challenge. Gene profiling of TNBC tumors has evidenced high levels of genetic instability, frequently harboring mutations in TP53 and the alpha catalytic subunit of the phosphoinositol-3 kinase (*PIK3CA*) genes in more than 10% of cases. In addition, this type of cancer frequently shows deletions or epigenetic silencing of the *PTEN* tumor suppressor gene [8]. Mutations in *TP53*, *PIK3CA* and *PTEN* are acquired in early stage of tumor growth [10,13].

Despite of being a very aggressive cancer, a 20% of TNBC cases is associated with a high population of immunomodulatory lymphocytes. Those tumors with more than 50% of tumor-infiltrating lymphocytes (TILs) are correlated with a better prognosis, a higher overall survival (OS) rate, longer metastasis-free survival, less distant relapses, and a greater response to neoadjuvant chemotherapy [14].

The median survival of women with TNBC is less than one year, since most of patients die despite being treated with the aggressive and toxic chemotherapy. The OS of patients with ER/PR positive breast cancer is higher thanks to the availability of targeted therapies. However, metastatic TNBC tumors exhibit a vast heterogeneity, what makes difficult the identification of well-defined molecular targets, this being an obstacle to design effective targeted therapies. Therefore, it is needed to understand the molecular basis of TNBC and its molecular

subtypes to develop more effective and individualized therapies [15].

2.2 Tumor heterogeneity in TNBC

The intratumor heterogeneity in TNBC arises from the coexistence of different neoplastic cell subpopulations that differ in their genetic and phenotypic characteristics, as well as their behavior. These differences are mainly caused by genetic and epigenetic factors, more than hereditary causes, such as an adaptive response or variations in signaling pathways in tumor cells. To understand the cellular origin of cancer and the tumor heterogeneity, two theories were proposed: clonal evolution model and cancer stem cells (CSCs) model [16].

According to the clonal evolution model, tumor heterogeneity is originated by acquired random mutations and clonal selection, where the cellular origin is a phenotypically normal cell that undergoes genetic and/or epigenetic changes [8,17]. However, the CSCs model suggests that tumors are organized hierarchically, like in normal tissues, the apex of this hierarchy is represented by cells with stem cell properties, in addition to high differentiation, proliferation and self-renewal potentials. Both theories are not mutually exclusive, in fact, CSCs can also be genetically unstable units for clonal selection in the genetic evolution of tumors. In that context, multiple CSCs clones with an altered genetic profile give rise to a heterogeneous differentiated progeny [8,16].

TNBC shows a high frequency of CSCs, which are one of the driving forces for tumorigenesis and metastasis, as well as proliferation, aggressiveness and resistance to treatment [18]. As the cancer progresses, the properties and number of CSCs change, leading to high intratumor heterogeneity, which results in different clinical outcomes [19]. This fact is related with the tumor microenvironment, or CSCs niche, which is essential for the production and development of cancer. A certain microenvironment could contribute to the generation of cell plasticity, a very important phenomenon in the metastatic process. Cell plasticity consists on the ability of tissue-derived stem cells to show a phenotypic potential that extends beyond the differentiated-cell phenotypes within the resident tissue [19]. In this sense, breast CSCs may show two distinct phenotypic states: a more proliferative epithelial-like state, characterized by expression of aldehyde dehydrogenase 1 (ALDH1), and another more quiescent and invasive mesenchymal-like state characterized by the expression of $CD44^{+}/CD24^{-/low}$. Both states can generate their epithelial or mesenchymal bulk cell progeny, which secrete signals to reinforce CSCs self-renewal. The mesenchymal bulk cells usually are located at the edge of the tumor, where they have access to circulation, and enabling the formation of micro-metastases in distant tissues [8]. Signals from the tumor microenvironment, as well as epigenetic alterations, can drive the transition of cells between these phenotypes, from epithelial to mesenchymal (EMT), as an example of cell plasticity.

The isolation and characterization of breast CSCs is possible through the analysis of

the surface marker CD44⁺/CD24^{-low} and the ALDH1 activity [20,21]. This fact facilitates the understanding of their behavior and the design of new therapeutic strategies.

2.3. TNBC molecular subtypes

TNBC shows a great different gene expression profiling, therefore, its classification gives rise to several subtypes with a unique biology, a fact what has great potential for personalized medicine.

Based on gene expression profiles in the TNBC tumor tissue, Lehmann et al. [22] proposed a classification on six different subtypes as follows: basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL) and luminal androgen receptor (LAR) [10]. According to this classification, the principal features of each subtype are:

2.3.1. Basal subtypes (BL1 and BL2)

BL1 subtype. In this subtype, an enrichment of the components involved in cell cycle and cell division processes is observed, accompanied by an increase in the DNA damage response pathways (ATR/BRCA). This fact is probable because of the high expression of Ki67 mRNA [14].

BL2 subtype. It is enriched in genes related with growth factor signaling pathways (EGF, NGF, MET, Wnt/ β -catenin, IGF1R), glycolysis and gluconeogenesis. The specific feature of this subtype is the enrichment in growth factor receptors such as epidermal growth factor receptor (EGFR) and tyrosine-protein kinase MET [14].

2.3.2. Immunomodulatory subtype (IM)

This subtype is enriched in factors involved in immunological processes, including the signaling of immune cells and cytokines such as T-helper cells (Th1 and Th2), natural killers, B cell receptors, and dendritic cells. This tumor type is enriched in genes involved in the processing, presentation and antigenic signaling, which could be derived from the tissue microenvironment instead of tumor cells [23].

2.3.3. Mesenchymal (M) and mesenchymal stem-like cell subtypes (MSL)

M subtype. It is enriched in cell motility-related genes and extracellular receptors associated with cell-cell interactions and differentiation [14].

MSL subtype. It is very similar to the mesenchymal subtype, but it also expresses growth factor genes, like EGFR or platelet derived growth factor (PDGF), calcium signaling pathways, G-protein receptor and kinases regulated by extracellular signals 1 and 2. Genes involved in

cell proliferation and claudin are low expressed (probably it is related with the claudin-low subtype) [14,23].

Both subtypes clearly show an enrichment of transforming growth factor β (TGF β) and PI3K/mTOR signaling pathways. This being demonstrated by the higher sensitivity of M and MSL cell types to PI3K/mTOR dual inhibitors [22].

2.3.4. Luminal – AR subtype (LAR)

It is the most different subtype of all. Despite being ER negative, diverse molecular signaling pathways regulated by hormones are present in this subtype. Among them, the synthesis of steroids, the metabolism of porphyrins, and the metabolism of androgens and estrogens are found. Moreover, the androgen receptor (AR) is highly expressed in this subtype [14,23].

3. Therapeutic Approaches in TNBC

TNBC is very difficult to treat because is not a “single” pathology, but a combination of different diseases, as identified by the different subtypes explained above. The classification of this cancer has been an important step in the search of new therapeutic strategies. However, because of the late diagnosis, the high proliferation rates, the aggressiveness and the resistance of conventional therapies, the treatment options are still limited. Here, we show the current therapeutics available for TNBC patients.

3.1. Cytotoxic therapy

The cytotoxic therapy is the main treatment of operable and advanced breast cancer. It includes microtubule inhibitors (paclitaxel, docetaxel, eribulin), anthracyclines (doxorubicin, epirubicin); alkylating agents (cyclophosphamide), antimetabolites (methotrexate, capecitabine, and gemcitabine) and platinum (carboplatin, cisplatin) [24].

The standard adjuvant and neoadjuvant treatments include the combination of anthracycline (doxorubicin or epirubicin) and an alkylating agent (cyclophosphamide), administrated together or sequentially with a taxane (docetaxel or paclitaxel). This treatment has shown a high pathological complete response (PCR) when is used as neoadjuvant therapy [24].

Despite being a beneficial therapy, patients continue to develop resistance. This phenomenon is associated with the high presence of CSCs. Although sensitive cells can be eradicated, there may be remaining cell populations with stem cells properties that originate a new tumor bulk [11].

3.2. Target therapies

As stated above, TNBC is characterized by a high cell and molecular heterogeneity,

making the effectiveness of traditional therapies more difficult. Currently, targeted therapies are gaining ground, and a large variety of them are undergoing clinical trials. The goal is the identification of predictive gene expression patterns or specific mutations that can be used as therapeutic targets. Here, we review the inhibitors of several molecular targets that are studied at *in vitro*, *in vivo* and clinical trial levels [11,24].

3.2.1. Poly ADP-Ribose polymerase (PARP) inhibitors

PARP is a nuclear enzyme essential in DNA damage recognition and repair. PARP inhibitors (PARPi) increase the cytotoxic role of DNA damaging agents [25]. This therapy has been developed for BRCA1 and BRCA2 mutated cancers, which are very common mutations in TNBC. The efficacy in this type of cancer is based on the hypothesis known as “synthetic lethality”. This is the cell death induction when two genes harbor a mutation, but the cell remains viable if that mutation is only present in one gene. Tumors with mutations in BRCA1 have deficiencies in the DNA repair mechanisms; hence, treatment with PARP inhibitors would produce apoptosis and cell death only in mutated BRCA1 cells [24,26,27].

Nowadays, several PARPi have been evaluated in clinical trials for TNBC patients. Iniparib was initially purposed on 2010 in combination with gemcitabine or carboplatin, which phase II clinical trial tripled the clinical benefit rate and improved the median progression-free survival (PFS) [24]. However, a phase III trial did not show an increase of the PFS and OS. Iniparib was tested as a second-line and third-line therapy and showed a better response on these patients [28,29].

Olaparib, an oral PARPi, showed to be safe and efficient in phase I trial in BRCA1/2 mutated TNBC patients. In phase II trials, overall response rates (ORR) on BRCA1/2 mutated patients were of 41%. An ongoing phase III trial is testing olaparib in adjuvant chemotherapy in BRCA-TNBC patients [11,24,29].

Veliparib, a PARP1/2 inhibitor, in combination with cisplatin and vinorelbine on wild-type BRCA-TNBC patients showed an ORR of 53% [11,24]. It has also been tested in combination with dinaciclib, a CDK1 inhibitor, resulting in additive growth inhibition on TNBC cells [11]. PARP inhibitors are clinically approved for ovarian cancer and close to be used against TNBC.

3.2.2. Receptor tyrosine kinase (RTK)

3.2.2.1. EGFR inhibitors

Most of triple negative tumors overexpress EGFR (89%), especially the BL2 subtype. EGFR activation increases the primary tumorigenesis and metastasis, due to the enhancement of cell proliferation, EMT, angiogenesis and cell motility. Furthermore, EGFR nuclear trans-

location is associated with resistance to radio and chemotherapy [30].

The therapies tested against RTK are gefitinib (EGFR inhibitor), erlotinib and lapatinib (tyrosine kinase inhibitor, TKI), and monoclonal antibodies (mAbs), cetuximab and panitumumab [11]. Even when EGFR is overexpressed in TNBC, clinical trials of EGFR inhibitors and mAbs have not been satisfactory [24]. Limited benefits have been shown in clinical trials after the treatment with cetuximab. To date, several phase II clinical trials combining cetuximab with a platinum (cisplatin or carboplatin) have been developed for metastatic and advanced TNBC patients, achieving an improvement against monotherapy [31]. Gefitinib and erlotinib have not shown significant response rates (5%). However, in xenograft models of TNBC, erlotinib inhibits EMT by inducing E-cadherin expression and decreasing vimentin levels [29,32].

Combination of a TKI and mAbs, a new therapeutic modality to improve the EGFR inhibitory response, has been shown to synergistically reduce cell proliferation in TNBC cells. Additionally, the combination of mAbs and chemotherapeutic drugs (ADC) reduces the lack of specificity exhibited by the conventional chemotherapy. For example, the conjugated formed by sacituzumab-govitecan with SN-38, an active metabolite for irinotecan, in a phase II trial induced a durable and early response on pretreated metastatic TNBC [33,34].

The clinical trials using EGFR as therapeutic target are limited, because there are not enough predictive markers, and the selection of appropriated patients remains a challenge. As monotherapy, EGFR inhibition showed disappointing results in TNBC, thus being necessary a different approach [11].

3.2.2.2. Fibroblast growth factor (FGF) inhibitors

FGF signaling is a therapeutic target recently tested in TNBC. Like other factors, such as EGF or transforming growth factor β (TGF β), FGF and its receptors (FGFR) are associated with tumorigenic processes, including EMT. There are five FGFR which activation leads to stimulation of mitogen activated protein kinase (MAPK) and PI3K signaling, therefore promoting proliferation and cell survival [29]. Overexpression of FGFR1 and FGFR2 is present on less than 10% of TNBC cases, and a higher FGFR1 expression is related to a poorer OS [23,27]. There are some inhibitors under preclinical and clinical trials. Alofanib was shown to reduce cell proliferation and growth of patient-derived xenografts by inhibition of FGFR2 [35]. Dovitinib was tested first on a preclinical trial evaluating its effects in both breast cancer cell lines and an FGFR1-amplified xenograft model. The result of this assay was the inhibition of breast cancer cell lines proliferation with amplified FGFR1 and FGFR2. In FGFR1-amplified xenograft model, dovitinib also inhibited tumor growth. After these results, a phase II clinical trial was made in hormone receptor-positive and FGF pathway-amplified breast cancer patients, showing an encouraging clinical activity. Thus, dovitinib demonstrated a higher activity

on FGF pathway amplified patients, being this the reason why it should be tested on TNBC [36,37]. Lucitanib is an FGFR1-3, VEGFR1-3 (vascular endothelial growth factor receptor) and PDGFR α/β inhibitor which is being tested on a phase II clinical trial on metastatic TNBC patients but there are no results yet [11].

3.2.2.3. Antiangiogenic agents

Vascular endothelial growth factor (VEGF) protein family (VEGFA-E), its three receptors (VEGFR1-3), and placental growth factor are involved in tumor angiogenesis. More specifically, VEGFA expression is present in TNBC tumors and is associated with poor prognosis and incidence of distant metastases [38].

Antiangiogenic therapy is based on the combination of monoclonal antibody anti-VEGFA and conventional chemotherapy. Bevacizumab is a mAbs that targets all forms of VEGFA that was initially approved by FDA (Food and Drug Administration) for the treatment of metastatic breast cancer. There are evidences indicating that, added to standard chemotherapy as capecitabine, anthracycline or taxane, bevacizumab improved PCR rates on TNBC patients [39]. However, the phase III clinical trials BEATRICE, RIBBON-1 and RIBBON-2, evidenced that there were no differences in OS. Consequently, FDA finally revoked the approval of bevacizumab for the treatment of breast cancer including TNBC patients [29].

Another antiangiogenic is apatinib, a recently developed drug with effect against VEGFR2. Although it has a limited ORR, is under clinical trial in combination with vinorelbine [40]. Nowadays, additional inhibitors such sunitinib and sorafenib, combined with chemotherapy, paclitaxel and platinum, are under clinical trials in early-stage TNBC patients [29].

Even though the clinical trials showed that anti-VEGF therapies do not have enough benefits to be used in TNBC, new therapies that combine VEGF inhibitors with immunotherapy (anti-programmed cell death-ligand 1, PD-L1) are promising strategies that increase the response rate. This could be interesting because VEGF-A enhances the expression of programmed cell death 1 (PD1) and might be involved in resistance to PD1 blockades [34,41]. This combination will be extensively explained below.

3.2.2.4. N-methyl-N'-nitroso-guanidine human osteosarcoma transforming gene (MET) inhibitors

MET is an RTK involved in proliferation, migration and cell survival signaling. It is highly expressed in TNBC and its tissue expression is correlated with poor OS. MET activates Akt, PI3K, RAS, extracellular signal-related kinase and steroid receptor coactivator (Src) downstream [42]. It was also found a link between EGFR and MET in vitro. Both MET and EGFR targeted therapies are successful in other type of cancer, but they have shown poor

efficacy in monotherapy for TNBC. This is due to the therapeutic resistance originated by a crosstalk between MET and EGFR. A high expression of EGFR causes a low MET inhibitors efficacy, and EGFR inhibitors originate a remain EGFR phosphorylation that correlates with TKI resistance [43]. However, the combination of MET and EGFR inhibitors was shown to reduce tumor cell viability in multiple TNBC cell lines, so this combinatory therapy is being investigated at clinical level [42]. Additionally, MET inhibitors can also be used together with PARPi. This combination therapy showed a synergism on reducing tumor cell growth on TNBC, *in vitro* and *in vivo* preclinical models [44].

3.2.3. Non-receptor tyrosine kinases (NRTKs)

3.2.3.1. PI3K/Akt/mTOR pathway inhibitors

Inhibition of the PI3K/Akt/mTOR signaling pathway has been recognized as a promising targeted therapy in TNBC. This pathway is usually overexpressed in this tumor type and is involved in proliferation and cell survival. Downstream PI3K, mTOR is activated previous Akt phosphorylation. mTOR is composed by two complexes, mTORC1 (regulates protein translation) and mTORC2 (mediates Akt activation). mTOR signaling is regulated by feedback loops, negative of mTORC1 on mTORC2, and positive of mTORC2 on mTORC1 through Akt phosphorylation at Serine 473. Akt is also antagonized by phosphatases, PTEN and INPP4B, whose genes are usually mutated in TNBC [45,4].

Preclinical trials have demonstrated that PI3K inhibition results in transient quiescence of TNBC tumor cells and is effective on M and MSL subtypes. It has also been demonstrated that mTORC1 inhibition sensitizes the BL subtype, what results in cellular quiescence [22]. Everolimus is a mTORC1 inhibitor approved for the treatment of ER-positive breast cancer patients. Nonetheless, it was not shown to be effective in combination with other PI3K/mTOR inhibitors for TNBC [24]. A phase II clinical trial combining everolimus plus chemotherapy did not show an improvement on PCR due to the activation of a feedback on mTORC2 by mTORC1 inhibition and further activation of Akt. This issue is being solved by testing the combination of everolimus and a dual mTORC1/2 inhibitor, which has shown to decrease cell proliferation on TNBC. mTOR inhibitors could be used to improve the antitumor effects of other therapies such as PI3K inhibitors, EGFR-RTKi or PARPi. The combination of PI3K, dual PI3K/mTOR and PARPi are ongoing for TNBC, showing a synergetic action on mouse models with BRCA1 mutation [11,45,47]. Akt inhibitors have been less studied for TNBC, however, a phase I clinical trial with MK-2206 and chemotherapy revealed a high PCR on TNBC compared with control [11].

In summary, monotherapy against this pathway is not quite effective because the tumor cells develop drug resistance through the activation of feedback loops. For this reason, recent researches are focused on combinational therapy and dual inhibitors of PI3K/mTOR.

3.2.3.2. Mitogen-activated protein kinase (MEK) inhibitors

MEK is an element of MAPK signaling transduction which is activated in TNBC due to the overexpression of upstream RTKs, such as EGFR. MAPK regulates some transcription factors linked to cell cycle and tumor survival and its aberrant signaling is more frequent in TNBC [48]. A phase I clinical trial combining trametinib with gemcitabine has shown a complete response for TNBC patients. In addition, it has been observed that tumors treated with MEK inhibitors usually become resistant due to the activation of positive feedback loops on RTKs. The combination of EGFR and MEK inhibitors was found to reduce the cell proliferation in different TNBC cell lines. Furthermore, PI3K/Akt pathway is activated in response to MEK inhibition, and therefore, the rational combination of MEK and PI3K/mTOR inhibitors is under clinical trials in TNBC. For example, the completed phase I clinical trial combining BEZ235 (PI3K/mTOR inhibitor) with MEK162 (MEK1/2 inhibitor) with no results yet [11,47].

3.2.3.3. Src inhibitors

Src is an oncoprotein implicated on multiple pathways regulating angiogenesis, proliferation, invasion and metastasis. Cytoplasmatic Src is enhanced on TNBC cells compared to other breast cancer cells. It could be a good biomarker but, unfortunately, clinical trials with Src inhibitors such dasatinib, saracatinib and bosutinib did not show clinical benefit on TNBC patients. Despite this, combination of dasatinib, cetuximab and cisplatin revealed a decrease on cell proliferation, migration and invasion. Src activity increases EGFR nuclear translocation, what promotes resistance to EGFR inhibitors like cetuximab. Dasatinib is being under a phase II clinical trial on TNBC to determine if could prevent nuclear translocation of EGFR. Based on this, a rational design of combinatorial targeted therapies including dasatinib and cetuximab may be investigated in TNBC patients [11,49].

3.2.4. Epigenetic inhibitors

This therapeutic approach is focused on the inhibition of histone deacetylase (HDAC), miRNAs and proteins such Hsp90 (heat shock protein 90).

3.2.4.1. Histone deacetylase inhibitors (HDAC)

HDACs are a family of enzymes that regulate chromatin remodeling and gene transcription by acetylation and deacetylation of histone lysine residues. These enzymes are involved in tumorigenesis through the inhibition of the expression of different tumor suppressor genes. It has been shown that HDAC inhibitors have preclinical activity in TNBC. Vorinostat has been tested in a phase II trial in combination with carboplatin and nab-paclitaxel, however, PCR rates were not improved. Others clinical trials that combine romidepsin and cisplatin to treat

locally recurrent or metastatic TNBC are ongoing, but there are not results yet [11,24]. These drugs have limitations because their effects are extended to the whole genome, which could be accompanied by silencing other tumor suppressor genes.

3.2.5. Androgen receptor antagonists

This approach is aimed at targeting the LAR subtype (12 – 36% of TNBC cases), which is enriched in androgen receptor [29]. Bicalutamide and enzalutamide are AR inhibitors that reduce cell proliferation, invasion and migration in TNBC cell lines. Additionally, enzalutamide increases tumor necrosis and does not reduce tumor size. In a recent study by blocking LAR localization to the nucleus, enzalutamide reduces tumorigenicity and TNBC progression that can be advantageous for M, MSL and BL2 subtypes because tumor proliferation, metastasis and survival are related to LAR expression [29,50]. Both drugs demonstrated a reduction in PFS and are being combined with other inhibitors to improve the treatment response. Because *PIK3CA* mutation is very frequent in the LAR subtype, combination of AR (enzalutamide) and PI3K inhibitors (taselisib) are being investigated in an ongoing clinical trial in phase Ib/II that has not finished yet. The use of immunotherapy and radiotherapy is also being considered in combination with anti-androgen agents, but they currently are on preclinical assays [11]. To study the increased rates of recurrence after chemotherapy and radiotherapy in TNBC, Speers et al. [51] treated radiotherapy-resistant breast cancer cell lines with AR inhibitors and then evaluated the AR expression. They showed that enzalutamide induced radiation sensitivity in AR+ TNBC cell lines [51]. Future clinical trials could throw light on its effect in patients.

3.2.6. Modulators of the immune system

Immunotherapy is a new research field in TNBC treatment, due to the tumor microenvironment influence in metastasis and cancer progression. This kind of therapy is effective in IM TNBC, which has a strong proportion of TILs. The presence of TILs is correlated with improved OS, increased metastasis-free survival, and decreased distant recurrence [14]. Immunotherapy is based on checkpoint inhibitors as T-lymphocyte antigen 4 (CTLA-4), PD1, PD-L1 and lymphocyte activation gene-3 (LAG-3) [52]. PD1 and PD-L1 are the most studied immunotherapy targets, because a high level of PD-L1 correlates with cancer progression and immune evasion. PD1 is expressed on the surface of T-cells, B-cells, natural killer T-cells, monocytes, and dendritic cells [53]. It provides co-inhibitory signals that reduces cytokine production and controls T cell responses in peripheral tissues via interaction with its ligands. When PD1 binds to PD-L1 expressed on cancer cells, immunomodulatory T cell activity is attenuated, and cancer progression is promoted [52]. Pembrolizumab is a humanized monoclonal IgG4-Kappa isotype, that blocks the interaction between PD1 and its ligands [52]. It has been shown a complete or partial response in 18,5% of TNBC patients in a phase IB study. Currently, a phase III trial in the treatment of metastatic or locally recurrent inoperable TNBC

is in progress [50]. A promising PD1 inhibitor for metastatic TNBC is nivolumab, which has been tested in a phase II clinical trial (TONIC) after administration of induction treatment. The ORR obtained on this study was 22% including 2% PFS, 22% partial response and 1 stable disease lasting more than 24 weeks. The results suggest that with doxorubicin or cisplatin response rate could be higher [54]. This clinical trial is still under study until 2022. An anti-PD-L1 antibody, atezolizumab, is also under study on TNBC patients showing an overall response rate of 33% [11]. It is being investigated in the neoadjuvant and metastatic setting [50].

Cytotoxic therapies and other targeted therapies, such as PARP, VEGF, EGFR, PI3K or MEK inhibitors, are being combined with PD1 and PD-L1 inhibitors to enhance immune response. The application of cytotoxic therapy (chemotherapy or radiotherapy) with checkpoint inhibitors is based on the premise that tumor-associated antigens are released and enhance immune response by killing tumor cell [11]. Recent research shows that chemotherapy, like anthracyclines, use the immune system to kill cancer cells by activating CD8⁺ T cell response and not only have cytotoxic effect [54]. For example, atezolizumab combined with nab-paclitaxel in a phase I clinical trial for 11 metastatic TNBC patients, generated an ORR of 38,3% complete response, 34% partial responses and 44% stable disease. After these results atezolizumab seems to be a promising drug against TNBC, therefore the clinical studies in phase II and III are underway. A phase III clinical study (IMpassion130) of atezolizumab and nab-paclitaxel will be completed on 2020 for untreated metastatic TNBC. NeoTRIPaPDL1 is another phase III study that proposes the triple combination of atezolizumab, nab-paclitaxel and carboplatin, which is estimated to be completed on 2022 [54].

Durvalumab, an anti-PD-L1 mAb combined with an antiangiogenic agent such bevacizumab is an interesting ongoing clinical trial in metastatic TNBC which rationale is that VEGF inhibition could enhance the immunotherapy efficacy [11]. This effect was already observed when bevacizumab was combined with atezolizumab in a phase III trial (IMmotion151) for metastatic renal cell carcinoma, demonstrating that the combination improves the PFS response in patients with PD-L1 positive tumors [41,55]. The research based on PI3K/Akt/mTOR plus anti-PD-L1 inhibitors are being tested because the PI3K/Akt/mTOR signaling upregulated PD-L1 expression in some TNBC cell lines. A phase I clinical trial of INCB050465 (PI3K inhibitor) and pembrolizumab for TNBC patients is ongoing which estimated study completion date is August 2019 [11,54]. There are also other ongoing clinical trials to prove how a PD-L1 antibody and a PARPi can benefit each other. This trial is based on the investigations made by Jiao et al. [56], who demonstrated that the combination of PARPi and PD-L1 or PD-1 blockade is a potential therapeutic approach to treat breast cancer, due to a crosstalk between PARP and immunosuppression. In addition, two clinical trials are currently evaluating the combinations of pembrolizumab with niraparib, and atezolizumab with veliparib, that will be finished on 2019 [11].

4. Future Perspectives

Activating transcription factor 4 (ATF4) belongs to activating transcription factors family/cAMP response element binding-protein (CREB), ATF/CREB. It is widely expressed in mammals in response to cell stress conditions, such as hypoxia, amino acid depletion, oxidative stress and endoplasmic reticulum stress, and is an important factor that determines the cell fate in response to the activation of the integrated stress response [12,13]. It is also expressed in stress-independent cellular conditions downstream the canonical and non-canonical signaling pathways of TGF β [57]. We have reported that ATF4 may be a useful prognostic biomarker and therapeutic target in patients with TNBC. A high expression of ATF4 correlates with a lower overall survival rate and a lower rate of relapse-free survival in patients with breast cancer and TNBC. We demonstrated that ATF4 knockdown leads to a reduction in the survival of tumor cells, metastatic capacity and cells with stem cell characteristics through the TGF β /SMAD2/3/4/ATF4 and TGF β /PI3K/mTORC2/ATF4/Rac1-RhoA signaling pathways. Moreover, our investigations revealed that ATF4 regulates TGF β /SMAD2/3/4 and TGF β /PI3K/mTORC1/2 by feedback loops. Both signaling pathways emerge as a starting point for the development of new targeted therapies for TNBC.

Future perspectives on targeted therapies for TNBC seem to be focused on immunotherapy in combination with conventional and other emerging therapeutic agents. Despite the progress of this therapy, clinical results on TNBC patients are currently limited due to the large number of factors that influence on the antitumor immunity, such as cell heterogeneity. Current research is focused on the use of chimeric antigen receptors-modified T cells (CAR-T cells). CAR-T cells are synthetic molecules that deliver tumoricidal functions to T cells upon recognition of a specific antigen on cancer cell. This is an emerging therapy that has shown benefits on leukemia, but the response in solid cancers such as breast, colon and lung is moderate [58].

In conclusion, development of an effective therapy for TNBC is a challenge for modern medicine, since conventional chemotherapy remains the only available treatment to date.

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