

# Triple Negative Breast Cancer: A Review of Common Therapeutic Targets and Current Treatment Options

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## ABSTRACT

Triple negative breast cancer (TNBC) is a subtype of breast cancer which lacks ER, PR, and HER2 expression. It is characterized by poor prognosis and resistance to standard treatment forms for breast cancer. Chemotherapy is still currently the core neo-adjuvant treatment option for patients with TNBC, although it has mixed levels of efficacy on overall survival and many serious side effects. Platinum-based therapies have been used to treat TNBC in conjunction with chemotherapy, but they are not a widely effective treatment due to the heterogeneity of TNBC. For this reason, other novel approaches, particularly those which target molecular components involved in TNBC pathogenesis, are being investigated. Angiogenesis inhibitors, which include monoclonal antibodies or small molecules that inhibit VEGF, have been shown to improve progression-free survival, but have not demonstrated an impact on overall survival. PARP enzyme inhibitors, when combined with chemotherapy and carboplatin for the treatment of TNBC, have demonstrated a significant reduction in risk progression and mortality. However, the majority of PARP inhibitors are still in trials and their effectiveness in clinical settings has yet to be determined. Additional proposed targets for directed therapy against TNBC include cell signalling pathways involving EGFR or PI3K. Overall, issues such as treatment resistance and side effects are important challenges that must be overcome in order to enable improvements in patient prognosis and clinical impact.

## RÉSUMÉ

Le cancer du sein triple négatif (CSTN) est un sous-type de cancer du sein auquel il manque les récepteurs d'œstrogènes (ER), les récepteurs de progestérone (PR) et l'expression de HER2. Il est caractérisé par un pronostic défavorable et une résistance aux traitements standards du cancer du sein. À l'heure actuelle, la chimiothérapie est encore l'option principale de traitement néoadjuvant pour les patients ayant le CSTN, bien qu'elle ait des niveaux variés d'efficacité sur la survie globale, ainsi que de nombreux effets secondaires sérieux. Les thérapies à base de platine ont été utilisées pour traiter le CSTN en conjonction avec la chimiothérapie, mais elles ne sont pas très efficaces étant donné l'hétérogénéité du CSTN. En raison de cela, d'autres approches novatrices, particulièrement celles qui ciblent les composantes moléculaires impliquées dans la pathogenèse du CSTN, font actuellement l'objet d'enquêtes. Les inhibiteurs de l'angiogenèse, dont les anticorps monoclonaux ou les petites molécules inhibant le VEGF, ont démontré la capacité d'améliorer la survie sans progression de la maladie, mais n'ont pas démontré d'impact sur la survie globale. Les inhibiteurs d'enzymes PARP, lorsque combinés avec la chimiothérapie et le carboplatine pour le traitement du CSTN, ont démontré une réduction significative du risque de progression et de la mortalité. Toutefois, la majorité des inhibiteurs PARP subissent encore des essais et leur efficacité clinique reste à être déterminée. D'autres cibles suggérées pour la thérapie dirigée contre le CSTN incluent les voies de signalisation impliquant le EGFR ou le PI3K. Dans l'ensemble, des problèmes tels la résistance au traitement et les effets secondaires sont des défis importants qui doivent être surmontés afin de permettre des améliorations au niveau du pronostic du patient et de l'impact clinique.

## INTRODUCTION

Triple Negative Breast Cancer (TNBC) is a subtype of breast cancer in which tumours lack the estrogen receptor (ER), progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2). TNBC can further be divided into basal-like and non-basal like breast cancer subtypes based on gene expression analysis. Approximately 80% of TNBC falls into the basal-like breast cancer category that lacks steroid receptor expression [1]. Since TNBC tumours lack ER, PR, and HER2, the main treatment forms for breast cancer, such as hormonal or HER2-directed therapy, are significantly less effective [2]. It is due to this key differ-

ence that TNBC is often characterised by poor prognosis, early relapse, and a significantly shorter overall survival rate following recurrences as compared to non-TNBC cancers [3].

Almost all of the classic hallmarks of cancer, including proliferation in the absence of growth signals, insensitivity to anti-growth factors, evasion of apoptosis, infinite replicative potential, invasion, metastasis, and sustained angiogenesis, are linked to abnormalities in the levels, functions, and interactions of proteins and signalling pathways [4]. Recent advancements in technology and biochemical research have identified a number of key proteins and pathways that could be potential therapeutic targets

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in TNBC. Moreover, the heterogeneity of TNBC has fostered the development of personalized forms of treatment that can target the unique tumour phenotypes of individual patients. This review will outline the major concepts and molecular targets of TNBC, the existing treatment options, and the novel technologies that are being explored as future treatments.

## THERAPEUTIC TARGETS IN TNBC

### *Receptor Pathways*

Pathways, such as the tyrosine kinase receptor pathway, play essential roles in initiating processes associated with cell survival and cell proliferation in breast and other epithelial tissues. In normal physiological settings, these pathways are tightly regulated. In TNBC, some pathways such as those involving insulin like-growth factor 1 receptor (IGF1-R), epidermal growth factor receptor (EGFR), rat sarcoma (RAS), phosphatidylinositol 3-kinase (PI3K), and angiogenesis are dysregulated and have become the subject of extensive examination in hopes of identifying novel therapeutics [5]. Most of these pathways involve receptors that have an extracellular ligand-binding region, a trans-membrane region, and a cytoplasmic tyrosine kinase-containing domain, which together function to activate downstream signalling mechanisms [6]. These receptor pathways can be aberrantly activated by a variety of mechanisms such as excessive ligand levels, gain- or loss-of-function mutations, overexpression with or without gene amplification, and gene rearrangements [7]. All of these mechanisms can result in inappropriate activation of the receptor pathways, which can result in cancerous phenotypes.

### *Epidermal Growth Factor Receptors*

EGFRs are a family of growth factor receptors that include HER1 and HER2. Many cancers have mutations that cause overexpression of EGFR, which leads to dysfunctional kinase activity and excessive growth-stimulating secondary messenger activation. Such accelerated proliferation has been consistently linked to an increased risk of disease recurrence and overall shortened patient survival [8].

A pathway associated with EGFR is the IGF pathway, which activates pathways and oncogenic kinases such as PI3K. The triggering of such signalling cascade pathways amplifies IGF-1's effect as a potent mitogen. High levels of IGF-1 and IGF-1R have been observed in breast cancer. Its important pro-oncogenic role, therefore, highlights it as an important culprit in breast cancer growth [9]. The extensive crosstalk that occurs between the signalling pathways associated with IGF-1R and EGFRs support the combination of IGF-1R inhibitors with an anti-EGFR as an effective therapeutic strategy [10].

### *Phosphatidylinositol 3-Kinase (PI3K) Pathway*

One of the oncogenic kinase pathways that can be activated by IGF-1 is the PI3K/Protein Kinase B (AKT) central signalling pathway, which is downstream of many receptor tyrosine kinases. These tyrosine kinases regulate cell growth and proliferation by dephosphorylating PI3K. The phosphorylated PI3K plays a role in activating other oncogenic kinases including AKT1, AKT2, and AKT3 [11]. In many breast cancers, including TNBC, the catalytic domain involved in dephosphorylating PI3K is mutated or under-expressed by methylation, which prevents PI3K dephosphorylation and results in its constitutive activation [11]. Such activation has downstream effects on the mechanistic target of rapamycin (mTOR) complex, which mediates cancerous phenotypes through the suppression of cap-dependant translation inhibitors [12]. This role of the PI3K pathway in breast cancer pathogenesis therefore supports it as another critical target in TNBC cancer therapy.

### *Angiogenesis*

In conjunction with the aberrant activation of signalling pathways, an equally important process in the progression of cancer is the recruitment of blood vessels. Angiogenesis is the process of vessel formation during physiological events such as wound healing or pregnancy. When dysregulated however, angiogenesis has been shown to play a role in tumour growth and spreading and is essential for cancer progression and dissemination [13]. Vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptors (VEGF-R) are the primary proteins responsible for the stimulation of angiogenesis. They play a role in regulating endothelial growth and blood vessel formation by stimulating cellular responses through tyrosine kinase receptor binding (VEGFRs) on the cell surface of breast cancer cells [13]. High levels of VEGF expression correspond to cancers that are fast growing and able to metastasize, and thus VEGF has been implicated with poor prognosis in breast cancer [14,15]. For these reasons, VEGF has been suggested as a suitable target for molecular therapy.

## OVERVIEW OF TREATMENT FOR TNBC

Chemotherapy remains the core neoadjuvant treatment option for patients with TNBC. Due to the aggressive nature of TNBC, however, chemotherapeutic treatment has mixed levels of efficiency and often results in poor outcomes for patients, along with many debilitating side effects and cytotoxicity [2]. Symptoms associated with chemotherapy include vomiting, nausea, diarrhoea, fatigue, anemia, peripheral and central neuropathy, and weight changes. For this reason, identifying correct subtypes of TNBC using specific biomarkers and recognizing possible target options is an important challenge in TNBC treatment. Tumor suppressor genes, DNA repair enzymes, and other molecular path-

ways involved in cancerous phenotypes have been identified as biomarkers that could be used to develop personalized molecular targets for patients with TNBC [13,16]. The main treatment types which currently exist include platinum-based agents, angiogenesis inhibitors, and poly (ADP-ribose) polymerase (PARP) inhibitors.

### *Platinum-Based Agents*

Platinum drugs are responsible for causing cell death by forming chemical cross-links with DNA. These cross-links disrupt essential processes such as DNA replication and transcription that are fundamental for cell growth [17]. Cisplatin, introduced approximately 20 years ago, was the first platinum analogue and is still in use today. They play an important role in the treatment of certain subsets of breast cancer such as TNBC [17]. However, platinum-based drugs are toxic and can be detrimental to nerve and kidney function [18]. Side effects associated with platinating agents include ototoxicity, peripheral neuropathy, myelosuppression, and nephrotoxicity [18]. Another important therapeutic hurdle associated with platinum-based therapy is the formation of platinum resistance in tumours [16]. Nevertheless, outcomes for platinum-containing agents have shown promise when combined with other targeted agents such as bevacizumab, iniparib, and erlotinib. A randomized phase II trial demonstrated promising overall progression-free survival response rates (17% versus 6%) when carboplatin was added to single-agent cetuximab in neo-adjuvant advanced TNBC patients [19]. Overall, while platinum-based therapy is not an effective targeted treatment approach due to the heterogeneity of TNBC, it has been shown to be an important component of adjunct/combination therapies [19].

### *Angiogenesis Inhibitors*

Angiogenesis is an important factor in the growth and spread of cancer. It provides cancerous cells a pathway by which they are able to metastasize and a source of nutrients with which to grow. Specific targeting, in the case of angiogenesis, revolves around anti-VEGF therapy [14,15].

Currently, monoclonal antibodies such as bevacizumab or small molecules that inhibit tyrosine kinases are being explored as a novel approach to TNBC treatment. Angiogenesis inhibitors such as bevacizumab have been shown to improve progression-free survival in aggressive breast cancers. Specifically, a meta-analysis of three phase 3 trials demonstrated that bevacizumab, when used in conjunction with chemotherapy, can increase the median progression-free survival (8.1 months) as compared to those with chemotherapy alone (5.4 months) [20]. However, studies that have measured overall survival response have failed to detect significant improvements upon usage of anti-angiogenic

treatments [21].

There are several limitations and concerns regarding the usage of angiogenesis inhibitors. Recent research has demonstrated an increased risk of bleeding complications such as epistaxis, hemoptysis, gastrointestinal bleeding, and thrombotic events with the use of bevacizumab [22]. Additionally, studies in mouse models have shown that VEGF inhibitors may concomitantly promote invasiveness and metastasis of tumours [23]. One plausible mechanism is tumour hypoxia: the more proficient an angiogenic factor is, the more effectively anti-angiogenic therapy will prevent tumour vessel formation and result in conditions that are hypoxic. This may create a selective pressure on tumour cells to acquire resistance to hypoxic conditions through the process of dedifferentiation, resulting in more aggressive and metastatic cells that are less sensitive to anti-angiogenic treatment [23]. Additionally, tumours may be able to escape such hypoxic conditions by undergoing invasive epithelial mesenchymal transition (EMT). Currently, due to the lack of any substantial improvement in the overall survival rate of patients with TNBC and the side effect profile that has emerged, angiogenesis inhibitors are not administered in adjuvant or metastatic settings.

### *Poly (ADP-Ribose) Polymerase (PARP) Inhibition*

PARP is a DNA-repair enzyme involved in initiating DNA repair by binding to areas where DNA strand breakage has occurred. PARP uses nicotinamide adenine dinucleotide (NAD)<sup>+</sup> as a substrate to generate ADP-ribose polymers, which play a role in initiating a signal to recruit other cellular proteins and factors that mediate an anti-recombinogenic effect [24]. This prevents inappropriate recombination of homologous DNA [24] and control of the homologous repair response, which is important in double-strand break repair. For these reasons, it is a possible target in cancer treatment [25].

PARP inhibitors work by competitively blocking the catalytic domain of the PARP enzyme [25]. PARP inhibition has been shown to be a thousand times more toxic to cancer cells than normal cells, indicating high amounts of specificity [25]. This is most likely because PARP inhibitors exploit tumour cells' defect in homologous recombination. Normal cells can use homologous recombination for repair of double-stranded DNA damage to ensure survival [25]. Some tumours however, lose this ability and ultimately die if a serious mutation occurs which would require homologous recombination repair [25]. For this reason, PARP inhibitors are believed to sensitize cells to DNA-damaging agents by preventing the repair of lethal DNA lesions. PARP inhibitors have a similar side effect profile to other drugs consisting of nausea, vomiting, diarrhoea, and weight loss.

Recent Phase I and II studies that examined the PARP inhibitors

olaparib and veliparib have shown encouraging results in breast cancer, including TNBC, with regards to progression-free survival [24,26]. Furthermore, the PARP inhibitor iniparib, when combined with chemotherapy and carboplatin for the treatment of TNBC, demonstrated a 41% reduction in risk progression and a 43% reduction in mortality with a minimal increase in toxicity [24,27]. As with other targeted therapies, cancers can develop resistance to PARP inhibitors, which can limit their clinical effectiveness and utility [28]. Another concern with regards to PARP inhibitors is their ability to increase the risk of developing new primary malignancies due to their DNA-damaging mechanism of action [27]. Such findings have raised concern, and have emphasized the need to proceed with caution for use in adjuvant settings until more research is done to confirm their utility and safety.

## CONCLUSION

TNBC is a subtype of breast cancer that is associated with poor prognosis and is resistant to the main forms of breast cancer treatment. Molecular therapeutics such as platinating agents, angiogenesis inhibitors, and PARP inhibitors have been suggested as possible solutions to the challenges facing TNBC treatment. TNBCs appear to be responsive to current neoadjuvant chemotherapeutic treatments in combination with platinum agents, yet there is still not enough evidence to support their use as a primary treatment. PARP inhibitors and angiogenesis inhibitors are promising novel therapeutic modalities, but several concerns over side effect profiles and efficacy remain to be resolved. Additionally, research is being undertaken to discover biomarkers that can aid in classifying the different sub-types of TNBC, thereby enabling specific targeting for the unique phenotypes of each individual patient. Advances in gene therapy and the use of genetic modification may also be future therapeutic modalities for targeted treatment of TNBC tumour cells. Overcoming the barriers associated with current TNBC treatment forms, particularly resistance, side effects, and efficacy, are important challenges that will enable improvements in patient prognosis and clinical impact.

## CONFLICT OF INTERESTS

All authors have no conflict of interests to declare.

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