**RESEARCH ARTICLE**

# Therapeutic challenges in quadruple negative breast cancer

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**ABSTRACT**

Recently, breast cancer (BC) continuously ranks first in the incidence rate of malignant neoplasms in women worldwide. Quadruple negative BC (QNBC) is a recently identified subtype of triple negative BC (TNBC) presenting with negative androgen receptor expression. QNBC characterization and treatment is fraught with many challenges. There is cumulative evidence suggesting that QNBC is highly proliferative and immunogenic, rendering it an ideal candidate for cytotoxic chemotherapy and immunotherapy. Several chemotherapeutic agents such as imatinib, cabozantinib, dasatinib, lucitanib, sunitinib, docetaxel, doxorubicin, and cyclophosphamide in QNBC patients are highlighted. Some subtypes and related pathway proteins are preferentially expressed in QNBC and may act as effective therapeutic targets such as acyl-CoA synthetase 4, S-phase kinase associated protein 2, immune checkpoint inhibitors, kinesin family member C1, and epidermal growth factor receptor. Several recent investigations comparing the therapeutic approach to QNBC and TNBC are briefly reviewed. Further more intensive and problem-oriented research in this topic of rising socio-medical importance is needed.

**Key words:** quadruple negative breast cancer, triple negative breast cancer, androgen receptor expression, neoadjuvant chemotherapy, therapeutic targets

## Introduction

In the recent decade, breast cancer (BC) continuously ranks first in the incidence rate of malignant neoplasms in women worldwide. Triple negative BC (TNBC), is more aggressive BC subtype and has a worse prognosis. Quadruple negative BC (QNBC) is characterized by absent expression of several hormone receptors such as estrogen, progesterone, human epidermal growth factor receptor-2 and androgen receptors and represents a clinically relevant BC subtype with the worst prognosis (Huang et al., 2020).

An increasing evidence supports the essential role of the androgen receptor, a nuclear hormone receptor, in BC and nowadays, this receptor is considered a useful prognosic biomarker depending on the context of BC subtypes (You et al., 2022).

The androgen receptor is a member of the steroid hormone receptor family of molecules and a 110 kDa protein residing in the cytoplasm with zinc finger DNA-binding, transcriptional regulation and ligand-binding capabilities (Gelmann, 2002). The concept that the androgen receptor modulates BC growth and progression is currently undeniable (Di Leone et al., 2021). It might act alone or in combination with other effectors participating in intracellular signaling pathways.

Androgen receptor signaling has the potential to regulate DNA repair in QNBC as anti-androgen therapy downregulates DNA repair genes and direct transcriptional targets of this receptor (Qattan et al., 2022).

QNBC tends to be an aggressive basal-like phenotype (Saini et al., 2020) and predominantly exhibits a basal-like molecular subtype (Hon et al., 2016).

Two immune checkpoint inhibitors, programmed death ligand 1 and cytotoxic T-lymphocyte-associated protein 4 along with CD4 expression on T cells are significantly increased in QNBC (Saini et al., 2020).

Acyl-CoA synthetase 4 expression inversely correlates with the expression of estrogen receptors, progesterone receptors, androgen receptors and human epidermal growth factor receptor 2 in both cell lines and tissue samples and may

thus serve as a single biomarker for QNBC status and hormone resistance (Hon et al., 2016).

The immunohistochemical expression of Sry-related high-mobility-group box 10 gene (SOX10) identified in 67% of consecutive TNBC cases is most common in non-apocrine QNBC patients (Rammal et al., 2022).

The QNBC tumour subtype, its molecular and clinical distinctions from other BC subtypes, miRNA dysregulation and function in QNBC are explored and the clinical and translational implications of these dysregulated networks are discussed (Qattan et al., 2022).

QNBC has unique molecular, signaling and expression regulation profiles, particularly those affected by microribonucleic acid (microRNA) regulatory networks (Qattan et al., 2022). MicroRNAs regulate androgen receptor-related targets and pathways that are dysregulated in QNBC, including the immune checkpoint inhibitors, S-phase kinase associated protein 2, engrailed-1, acyl-CoA synthetase 4, and epidermal growth factor receptor.

***Therapeutic opportunities in QNBC***

There is a compelling question of whether regulatory networks specific to QNBC can be leveraged to maximize the efficacy of neoadjuvant therapy (Qattan et al., 2022). Cumulative evidence suggests that QNBC is highly proliferative and immunogenic, rendering it an ideal candidate for cytotoxic chemotherapy and immunotherapy (Bhattarai et al., 2020). QNBCs have worse clinical outcomes even after treatment with adjuvant chemotherapy. Acquired resistance to taxanes is commonly observed. Combining these approaches with agents targeting QNBC biomarkers may enhance treatment response and improve prognosis.

Between January 1, 2015 and June 30, 2019, the application of neoadjuvant chemotherapy in 125 patients at a mean age at diagnosis of 49 years with QNBC results in a pathological complete response in 52 patients (in 41.60% of the cases) (Di Leone et al., 2021). Therapeutic regimens include anthracyclines (epirubicin, 100 mg/m$^2$) and cyclophosphamide (500 mg/m$^2$; triweekly for 4 cycles) and taxanes (docetaxel 70 mg/m$^2$; triweekly for 4 cycles), or carboplatin (100 mg/m$^2$; weekly for 12 cycles). The patients with a luminal androgen receptor phenotype characterized by androgen receptor overexpression have a lower pathological complete response rate than those with QNBC one (25% versus 37.6%). High Ki67 values (>50%) are less frequent in the first than in the second patients' group (in 50% versus 76.8% of the cases). Routine androgen receptor expression assessment in addition to classical biomarkers in TNBCs patients could contribute to better treatment personalization.

In primary BC patients treated with neoadjuvant docetaxel/doxorubicin/cyclophosphamide chemotherapy during the prospective GeparTrio phase-III trial, QNBC patients have a higher chance than those with an androgen receptor-positive TNBC of achieving the pathological complete response (in 25.4% versus 12.8%; $p<0.0001$), poorer disease-free survival (72.5% versus 78.9%; log-rank $p=0.0329$) and overall survival (82.7% versus 88.8%; log-rank $p=0.0234$) (Loibl et al., 2011).

Both U.S. Food and Drug Administration-approved and investigational drugs such as imatinib, cabozantinib, dasatinib, lucitanib, and sunitinib could be of value in QNBC patients (Bhattarai et al., 2020). QNBC patients do not benefit from androgen receptor antagonists such as enzalutamide and bicalutamide (Bhattarai et al., 2020).

***Therapeutic targets in QNBC***

The immunohistochemical analysis of the androgen receptors is a practical assay to determine the most aggressive TNBC subtypes including QNBC and identify tumours that benefit from targeted therapies available (Angajala et al., 2019). Some current efforts to develop alternatives to chemotherapy for QNBC are summarized (Hon et al., 2016). Several subtypes and related pathway proteins are preferentially expressed in QNBC and may serve as effective targets for treatment such as acyl-CoA synthetase 4, S-phase kinase associated protein 2, immune checkpoint inhibitors and epidermal growth factor receptor although QNBC currently lacks a defined targetable pathway (Hon et al., 2016).

The 3-phosphoinositide-dependent protein kinase-1 signaling pathway may represent a viable therapeutic target in QNBC (Bhattarai et al., 2020). The association between S-phase kinase-associated protein 2 and negative androgen receptor status underpins this protein as a potential therapeutic target in QNBC. QNBC expresses unique proteins that may be amenable to use in the development of targeted therapies (Huang et al., 2020). The molecular features of QNBC proteins such as acyl-CoA synthetase 4, S-phase kinase associated protein 2, epidermal growth factor receptor, microRNA signatures and engrailed 1 that may serve as effective targets for QNBC treatment are reviewed. The acyl-CoA synthetase 4 may be a potential target for treatment in QNBC patients (Orlando et al., 2012).

Kinesin family member C1 is a microtubule binding protein that confers the survival of cancer cells with centrosome amplification and represents a promising target for QNBC in women of West African descent (Jinna et al., 2022).

QNBC is insensitive to conventional chemotherapeutic agents and has no efficient treatment targets (Huang et al., 2020). As there are no targeted drugs available or under development for QNBC patients, the treatment options for these patients are restricted to chemotherapy (Saini et al., 2020).



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**Table** 1**.** ***Promising modern therapeutic approaches in QNBC***

| Authors | Therapeutic approaches | | |
|---|---|---|---|
| | Neoadjuvant chemotherapy | Target therapy | Effectiveness |
| Loibl et al., 2011 | docetaxel, cyclophosphamide, doxorubicin | – | various |
| Saini et al., 2020 | chemotherapy only | – | potential |
| Bhattarai et al., 2020 | enzalutamide, bicalutamide | – | absent |
| Bhattarai et al., 2020 | imatinib, lucitanib, cabozantinib, dasatinib, sunitinib | | various |
| Di Leone et al., 2021 | epirubicin, cyclophosphamide, | | median |
| Orlando et al., 2012 | docetaxel, carboplatin | acyl-CoA synthetase 4 | potential |
| Hon et al., 2016 | – | acyl-CoA synthetase 4, S-phase kinase associated protein 2, epidermal growth factor receptor, immune checkpoint inhibitors | potential |
| Bhattarai et al., 2020 | – | gefitinib, erlotinib, cetuximab | various |
| Bhattarai et al., 2020 | – | S-phase kinase associated protein 2 | potential |
| Huang et al., 2020 | – | acyl-CoA synthetase 4, S-phase kinase associated protein 2, epidermal growth factor receptor, microRNA signatures, engrailed 1 | potential |
| Jinna et al., 2022 | – | kinesin family member C1 | potential |

The dynamics of the relatively few reviews and original papers devoted to potential therapeutic approaches in QNBC patients is systematized in Table 1.

***Comparative investigations in TNBC and QNBC***

In a review paper, QNBC is identified against androgen receptor-positive TNBC (Christenson et al., 2018). The difficulties in monitoring the androgen receptor protein concentrations, the new methods for determining the androgen receptor status as well as the role of the androgen receptors in regulation of the immune system are discussed.

Epigenetic modifications such as DNA methylation, histone posttranslational modifications and associated changes in chromatin architecture are implicated in BC pathogenesis (Muhammad et al., 2022). The genes with epigenetic modifications associated with more aggressive TNBC/QNBC pathogenesis and possible interventions are highlighted. The results from advanced literature searches in three data-bases such as *PubMed*/*MEDLINE*, *Scopus* and *Google Scholar* suggest that nine epigenetically altered genes/differentially expressed proteins in addition to the downregulated androgen receptor are related to TNBC aggressiveness and implicated in the TNBC to QNBC transition. Some epigenetic modifications are involved in a more severe disease that is very difficult to control in TNBC patients and could facilitate its transition to the more aggressive QNBC subtype. Restoring the normal expression of these genes by reversing the observed epigenetic alterations through epigenetic reprogramming could be therapeutically beneficial to TNBC and QNBC patients.

The comparative immunohistochemical examination of 29 QNBC and seven TNBC female patients reveals that median tumour-infiltrating lymphocytes are 37.5% and 10%, median CD20 is 20% and 7.5% (p=0.008), mean CD3 is 80.7% and 93.3% (p=0.007), and mean CD8 is 75% and 80.8%, respectively (Elghazawy et al., 2021).

TNBC surpasses other BC subtypes as the most challenging to treat due to its lack of traditional BC biomarkers (Jinna et al., 2022a). Nearly 70% of TNBC patients lack androgen receptor expression. Such an absent expression as a feature of QNBC is established in 45%-88% (Dong et al., 2022), or in 65%-88% (Jinna et al., 2022) of TNBC patients. It is detected in 87 out of 149 Hispanic/Latino females diagnosed with TNBC between 2011 and 2014 in Colombia (in 58.39% of the cases) (Melo-Uribe et al., 2022). It deals with an expression similar to that in populations of European descent.

Of 89 TNBC patients treated with neoadjuvant chemotherapy, 29 patients (32.58%) are TNBC androgen receptor-positive and 60 ones (67.42%) are QNBC (Mohammed et al., 2020). Some 61.7% of QNBC patients are younger than 40 years. QNBC subgroup demonstrates higher pathological complete response rates than TNBC one (60% versus 24%, respectively). Tumour grade III is more common in QNBC patients (in 85%) than in TNBC androgen receptor-positive ones (in 75.9% of the cases). Most patients in both groups, QNBC and TNBC androgen receptor-positive cases, have tumour size between 2 cm and 5 cm (73% and 79%) as well as clinical lymph node involvement (91.7% and 100%, respectively). The Ki-67 expression is higher in QNBC group than in TNBC androgen receptor-positive one (in 86.7% and 65.5%, respectively). Some 60% of QNBC patients and 24% of TNBC androgen receptor-positive ones achieve a

pathological complete response. The higher Ki-67 expression, higher tumour grade and lymph node involvement correlate statistically significantly with the pathological complete response rate in QNBC patients (p=0.02, p=0.04 and p=0.03, respectively). According to the results from the univariate analysis, QNBC, high Ki-67 expression, invasive duct carcinoma pathology type, and presence of lymph node involvement are related to the pathological complete response (odds ratio of 7.960 at 95% confidence interval and p=0.001; odds ratio of 9.212 at 95% confidence interval and p=0.001; odds ratio of 0.244 at 95% confidence interval and p=0.007, and odds ratio of 0.573 at 95% confidence interval and p=0.06, respectively). These results suggest that the androgen receptor expression in TNBC may be applied as a predictive marker for neoadjuvant chemotherapy (Mohammed et al., 2020).

Among 58 TNBC patients treated with neoadjuvant chemotherapy and immunohistochemically evaluated during the period between June 2006 and March 2016, 38 (65.52%) are androgen receptor-negative (Sridhar et al., 2022). There are no statistically significant differences between androgen receptor-positive and androgen receptor-negative patients in terms of the pathological complete response rate (30% versus 26%), median disease-free survival (5.9 years versus 5.2 years) and overall survival (6.2 years versus 5.4 years). There is, however, a statistically significant difference concerning the tumour grade between both groups (p=0.008).

The association of androgen receptor expression with glucose metabolic features is assessed in a total of 608 female TNBC patients (Lee et al., 2022). Maximum standardized uptake value is higher in androgen receptor-negative than in androgen receptor-positive cases (p<0.001) and correlates with lower androgen receptor expression (rho=-0.26; p<0.001). In multivariate analysis, the androgen receptor is a deterministic factor for the low maximum standardized uptake value (p=0.012) and other key clinico-pathological features. According to a recent investigation, high thymidylate synthase concentrations can be a useful therapeutic target in TNBC cases (Siddiqui et al., 2019).

A comparative investigation of 33 QNBC patients at a mean age of 51.53±10.42 years and 20 TNBC ones at a mean age of 51.75±10.22 years is carried out (Bhattarai et al., 2021). Among QNBC samples, it identifies a total of 484 copy number alterations with an average of 25.47±3.73 alterations per sample. Three chromosome instability-25 signature genes such as FOXM1, CNAP1 and RAD51AP1 present with statistically significantly higher copy number alterations in QNBC than in TNBC samples (p=0.0169, p=0.0068 and p=0.02321, respectively). The combined expression of eight microRNAs (miR-1204, miR-1265, miR-1267, miR-23c, miR-548ai, miR-567, miR-613, and miR-943) robustly discriminates QNBCs from TNBCs (area under the curve of 0.946). The high expression levels of miR-548ai, miR-567, miR-1265 and miR-1267 are associated with distant tumour metastases.

Among 224 QNBC patients examined between January 1997 and November 2014, 65 or 80.25% are with primary but 159 or 75.71% of the cases are with recurrent/metastatic disease (Angajala et al., 2019). Bivariate fit analysis demonstrates that QNBC statistically significantly (p<0.03) correlates with younger age at initial biopsy. There are 40 synchronous and 55 asynchronous QNBC cases. QNBC presents with higher concordance than TNBC in both synchronous and asynchronous biopsies. Synchronous and asynchronous QNBC tumours display discordance of 23% and 24%, respectively. QNBC is associated with higher epidermal growth factor receptor, lower phosphatase and tensin homolog and transducin-like enhancer of split 3 than TNBC in metastatic samples.

## Conclusion

The literature dealing with the role of androgen receptor expression and modern therapy of QNBC is relatively scanty. Neoadjuvant chemotherapy is widely used. The role of several chemotherapeutic agents such as imatinib, cabozantinib, dasatinib, lucitanib, sunitinib, docetaxel, doxorubicin, and cyclophosphamide in QNBC patients is outlined. Recently, acyl-CoA synthetase 4, S-phase kinase associated protein 2, immune checkpoint inhibitors, kinesin family member C1, and epidermal growth factor receptor emerge as promising therapeutic targets. United efforts of interdisciplinary teams could ensure effective solutions facing the therapeutic challenges in QNBC.

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