Study on *CYP2D6* and *ABCB* polymorphisms with respect to Tamoxifen adjuvant treatment in ER and PR breast cancer patients

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By

SMT. TANUMA MISTRY

Department of Life Science & Biotechnology

Faculty Council of Science

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Dr. Vilas D. Nasare
PhD
Senior Scientific Officer
Pathology and Cancer Screening
vilas.dr@gmail.com
9899396176

Chittaranjan National Cancer Institute
37, S. P. Mukherjee Road, Kolkata-700026,W.B. India
(An autonomous body under the Ministry of Health
&Family Welfare, Government of India,
Tel: (033)2475-9313/8057/Fax: +91-33-2475-7606
Website: www.cnci.org.in

CERTIFICATE FROM THE SUPERVISOR

This is to certify that the thesis entitled "**Study on *CYP2D6* and *ABCB* polymorphisms with respect to Tamoxifen adjuvant treatment in ER and PR breast cancer patients**" submitted by **Smt. Tanuma Mistry**, who got her name registered on **12th October, 2020 (Index No. 28/20/Life Sc./27)** for the award of Ph.D. (Science) degree of Jadavpur University is absolutely based upon her own work under the supervision **Dr. Vilas D. Nasare** and that neither her thesis nor any part of the thesis has been submitted for any degree/diploma or any other academic award anywhere before.

Nasare

Dr. Vilas D. Nasare

Signature of the Supervisor

Vilas D. Nasare, Ph.D.
Senior Scientific Officer
Pathology & Cancer Screening
Chittaranjan National Cancer Institute
37, S.P. Mukherjee Road,
Kolkata - 700 026, INDIA

Dedicated with love and gratitude to

My beloved parents

Late DILIP KUMAR MISTRY

Smt. NILIMA MISTRY

My dear brother, sister-in-law and nephew

Sri. ATANU MISTRY

Smt. ESHITA MISTRY

Sri. IRAJ MISTRY

My loving and supportive husband

Sri. SHANU KUMAR

Their love, sacrifices, and unwavering support have been the foundation of my achievements. This work is a tribute to them.

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TANUMA MISTRY

PREFACE

Breast cancer remains one of the most prevalent malignancies worldwide, with its incidence steadily rising despite of advancements in early detection and therapeutic interventions. The treatment modalities—including surgery, radiation, targeted therapy, hormone therapy and immunotherapy—were integral to breast cancer management; however, chemotherapy continued to be a cornerstone. The standard chemotherapeutic regimens (anthracycline-taxane) and hormone therapy (tamoxifen) often face limitations due to inter-individual variability in drug metabolism & transport, therapeutic response, and toxicity. Recent clinical trials were exploring novel combinatorial approaches and precision oncology strategies to optimize efficacy while minimizing adverse effects. Major obstacles in chemotherapy were unpredictable drug metabolism, rapid efflux leading to suboptimal therapeutic outcomes and severe toxicities. Body mass index (BMI) has emerged as a crucial risk factor influencing chemotherapy outcomes, with high BMI being well-established as a predictor of poor prognosis. However, the impact of low BMI has often been overlooked, despite its potential association with altered drug pharmacokinetics, increased toxicity, and poorer clinical outcomes. Cytochrome P450 (*CYP*) enzymes and ATP-binding cassette (*ABC*) transporters, particularly their genetic polymorphisms, play a crucial role in modulating the pharmacokinetics and pharmacodynamics of key chemotherapeutic agents. Understanding these polymorphic variations holds promise for personalized medicine, allowing for tailored treatment strategies based on genetic profiles. This study, aims to elucidate the clinical efficacy of chemotherapy and polymorphisms of *ABC* and *CYP* gene variants, as well as the impact of low BMI, with the goal of identifying potential clinical factors to improve breast cancer management, enhance therapeutic precision, minimize adverse effects, and improve quality of life, overall survival and treatment efficacy.

Standard chemotherapy (anthracycline-taxane-based) and adjuvant hormone therapy (tamoxifen) is primarily metabolized and transported via *CYP* and *ABC* transporter genes, facilitating drug metabolism, efflux, and intracellular retention. These processes ultimately induce apoptosis through microtubule stabilization, DNA intercalation, binding with estrogen receptors, etc. A comprehensive literature review indicates that polymorphisms in these genes significantly alter drug metabolism and enhance rapid efflux, potentially leading to suboptimal therapeutic responses and increased toxicity. Therefore, clinically relevant single nucleotide polymorphisms (SNPs) in *CYP2C9, CYP2C19, CYP3A4, CYP2D6***,** and *ABCB1, ABCC2* were

selected for analysis. Their impact was evaluated across BMI stratifications to assess associations with chemotherapy efficacy, safety profiles, quality of life, and survival outcomes.

The methodology employed in this study encompassed clinical assessments, conducted at multiple time intervals to monitor disease progression, treatment response, and adverse drug reactions. Histopathological evaluation and immunohistochemistry (IHC) were performed to characterize tumor subtypes, receptor status, and molecular markers, providing critical insights into disease heterogeneity and therapeutic implications. Genotypic analysis was carried out using PCR-RFLP. In addition, anthropometric assessments (BMI, MUAC, SFT), patients' QoL, objective response rates, progression-free survival (PFS), and overall survival (OS), proportional hazards regression models were employed to identify independent prognostic factors influencing treatment outcomes.

The majority of patients in this study were diagnosed between the age 41 to 60, predominantly from rural backgrounds, with limited access to basic education, unemployment, and lower socio-economic status. Notably, a significant proportion of these patients lacked a family history of cancer. A striking observation was the high prevalence of low BMI, affecting 53.38% of the cohort. Most patients were diagnosed at stage II with grade II tumors, with infiltrating ductal carcinoma being the most frequently observed histological subtype. The objective response rate (ORR) was achieved by 50.67% of patients. BMI stratification demonstrated a statistically significant association with treatment response and estrogen receptor status of the tumor (***p<0.0001***). QoL assessments revealed improvements across chemotherapy groups and clinical response; however, patients with low BMI exhibited significantly poorer QoL compared to those with normal BMI. Among chemotherapy regimens, the AC-T regimen demonstrated superior clinical efficacy, with improved survival outcomes compared to FEC and TAC regimens. Genetic analysis revealed that patients with low BMI and also carrying the homozygous mutant AA variant in *ABCC2* and *CYP2C19* genes were more likely to be non-responders to treatment. Survival analysis further indicated that patients with low BMI, the AA genotype in *ABCC2,* and the GA genotype in *CYP2C19* exhibited the lowest OS **(*p<0.05*).** Additionally, PFS was significantly reduced in patients harboured the following genotypes in *ABCC2* (AA), *ABCB1* (CC), *CYP2C9* (CT), *CYP2C19* (AA), and *CYP2D6* (AA) genes**.** Cox proportional hazards regression identified several clinical and genetic parameters associated with higher hazard ratios (HR>1; ***p<0.05*)**, indicated an increased risk of mortality. These included tumor expression of ER, patients' menopausal status, patients received TAC and AC-T regimens, and the presence of the CA genotype in *ABCC2* and GA genotype in *CYP2C19***.**

These findings underscore the individual and combine influence of BMI and pharmacogenetic variations on treatment response, QoL and survival outcomes, in breast cancer patients. Even though, this study need to be validated in larger sample with multi-center cohort. However, the present study findings thereof have crucial impact on determining personalized therapeutic strategy.

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- 5Fu 5-fluorouracil
- *ABC* ATP-Binding Cassette
- AC-T Anthracycline, Cyclophosphamide - Taxane
- AJCC American Joint Committee on Cancer
- *ATM* Ataxia-Telangiectasia Mutated
- BC Breast Cancer
- *BCRP* Breast Cancer Resistance Protein
- BCT Breast Conserving Therapy
- BMI Body Mass Index
- *BRCA1* Breast Cancer Gene 1
- *BRCA2* Breast Cancer Gene 2
- CECT Contrast Enhanced Computed Tomography
- *CHEK2* Checkpoint Kinase 2
- CI Confidence Intervals
- CNV Copy Number Variants
- CRs Complete Responders
- CT scan Computed Tomography scan
- CTCAE Common Terminology Criteria for Adverse Events
- *CYP* Cytochrome P450
- DAB 3,3'- Diaminobenzidine
- DFS Disease Free Survival
- DNA Deoxyribonucleic Acid
- dNTP Deoxynucleotide Triphosphate
- DPX Dibutylphthalate Polystyrene Xylene
- EDTA Ethylenediaminetetra Acetic Acid
- ER Estrogen Receptor
- ESAS The Edmonton Symptom Assessment System
- FACIT-Sp-12 Functional Assessment of Chronic Illness Therapy - Spiritual Well-Being-12
- FACT-B Functional Assessment of Cancer Therapy - Breast

- FDG-PET Fluorodeoxyglucose-Positron Emission Tomography
- FEC 5-fluorouracil, Epirubicin, Cyclophosphamide
- FFPE Formalin Fixed Paraffin Embedded
- FISH Fluorescence In Situ Hybridization
- *GAPDH* Glyceraldehyde-3-Phosphate Dehydrogenase
- Gy Gray
- HER2/neu Human Epidermal Growth Factor Receptor-2
- HIV Human Immune Deficiency Virus
- HR Hazard Ratios
- HRP Horse Radish Peroxidase
- IHC Immunohistochemistry
- MANOVA Multivariate Analysis of Variance
- MDR Multidrug Resistance
- MRI Magnetic Resonance Imaging
- MRM Modified Radical Mastectomy
- MUAC Mid-Upper Arm Circumference
- NBF Neutral Buffered Formalin
- NCBI National Center for Biotechnology Information
- NCI National Cancer Institute
- NIH National Institutes of Health
- NRs Non-Responders
- OR Odds Ratios
- ORR Objective/Overall Response Rate
- OS Overall Survival
- PAGE Polyacrylamide Gel Electrophoresis
- PBS Phosphate Buffered Saline
- PCR Polymerase Chain Reaction
- pCR Pathological Complete Response
- PD Progressive Disease
- PFS Progression-Free Survival
- P-gp P-glycoprotein
- PR Progesterone Receptor

- PRs Partial Responders
- *PTEN* Phosphatase and Tensin Homolog Deleted on Chromosome 10
- QoL Quality of Life
- RECIST Response Evaluation Criteria in Solid Tumors
- RFLP Restriction Fragment Length Polymorphism
- SD Standard Deviations
- SDi Stable Disease
- SE Standard Error
- SERD Selective Estrogen Receptor Degraders
- SERM Selective Estrogen Receptor Modulators
- SFT Skin Fold Thickness
- SNP Single Nucleotide Polymorphisms
- SPSS Statistical Package for the Social Sciences
- TAC Taxane, Anthracycline, Cyclophosphamide
- TB Tuberculosis
- UICC Union for International Cancer Control
- UM Ultra-rapid Metabolizer
- WBC White Blood Cells

CHAPTER 1
INTRODUCTION

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INTRODUCTION

Cancer is a multifactorial diseases characterized by uncontrolled proliferation of abnormal cells, which can invade surrounding tissues and metastasize to distant sites (Brown et al., 2023). Breast cancer (BC) remains a significant global health challenge, accounting for 46.8% of total diagnosed cancer cases in women, with 2,296,840 new cases and 666,103 deaths reported in 2022 (Ferlay et al., 2024). In Asia, BC had the highest incidence (985,817) but was the second leading cause of cancer-related deaths (315,309). However, as per the recent statistics data, in India, BC recorded top position in terms of incidence (192,020) and mortality (98,337) among women (Ferlay et al., 2024). The most common symptoms encountered by the BC patients were palpable lump in the breast or armpit often painless, occasionally with a sensation of pain (mastalgia) along with changes in nipples (discharge/ retraction sometimes serous or bloody) and skin (as peau d'orange), localized erythema, breast asymmetry, and axillary lymphadenopathy, indicating possible metastasis (Waks and Winer, 2019). This heterogeneous disease, hence required systemic management includes chemotherapy, radiation, hormonal therapy, targeted therapy, and immunotherapy**,** aimed at eradicating micro-metastatic disease, preventing recurrence, and improving survival outcomes. The current standard of care for BC involves a combination of surgical staging and the strategic administration of chemotherapy played a pivotal role in reduce recurrence risk and enhance patient survival. Recent advancements have introduced more effective agents in both preoperative (neo-adjuvant) and postoperative (adjuvant) settings, enhancing treatment efficacy (Curigliano et al., 2023; Leclerc et al., 2016). The optimization of chemotherapy regimens remains an active area of research and clinical investigation. Currently, anthracycline-taxane-based agents were administered sequentially to maximize therapeutic efficacy (Bonneterre et al., 2005; Von Minckwitz and Loibl, 2015; Henderson et al., 2003). The primary recommended chemotherapy for the patients were (1) FEC regimen: 5-fluorouracil 500 mg/m2 i.v. at day 1, epirubicin 60 mg/m2 i.v. at day 1 and cyclophosphamide 500 mg/m2-i.v. dose at day 1; (Coombes et al., 1996), (2) TAC regimen: docetaxel 75 mg/m2 i.v. at day 1; doxorubicin 50 mg/m2 i.v. at day 1; cyclophosphamide 500 mg/m2 i.v. at day 1; (Martín et al., 2006) (3) AC-T regimen: doxorubicin 50 mg/m2 i.v. at day 1; cyclophosphamide 500 mg/m2 i.v. at day 1 for four cycle followed by paclitaxel 175 mg/m2 by IV infusion day 1 every 21 days for 4 cycles (Mamounas et al., 2005), in tri-weekly manner for six cycles. Due to high toxicity and poor quality of life

(QoL) outcome from FEC and TAC regimen, the clinician preferred sequential anthracycline-taxane (AC-T) as a new standard of care (Shao et al., 2012; Jemal et al., 2011; Fujii et al., 2015). In terms of surgery, the clinicians often prioritize mastectomy and breast-conserving surgery as the primary surgical treatment options. Hormone receptor-positive (ER and PR-positive) BC patients (Luminal A and B) exhibit a better prognosis compared to other subtypes, as demonstrated by the ATLAS trial. The trial data showed that these patients, who received 5 years of tamoxifen followed by an additional 5 years of hormone therapy, experienced improved outcomes (Davies et al., 2013). However, in low- to middle-income countries like India, mastectomy is more commonly favored by clinicians due to various socio-economic and healthcare system factors (Deepa et al., 2020). Despite of the improvement patients till faced toxicity which affect their clinical outcome, QoL and survival. Anthropometric factors (height, weight, and BMI) have been linked to an increased risk of BC (Lin et al., 1971; Li et al., 1997). In the low socio-economic settings, the patients often faced low level of nutrition which resulted in low BMI, an important anthropometric parameter often overlooked. Notably, overweight and obesity have been definitively identified as risk factors, with a 1.2–1.4 times higher risk in post-menopausal women (Renehan et al., 2008; Munsell et al., 2014) and a 0.8 times higher risk in pre-menopausal women (Munsell et al., 2014; Schoemaker et al., 2018). However, nutritionally neglected patients were often missed, underscoring the importance of promptly assessing BMI status. There is a critical need to better understand how underweight conditions impact on QoL and treatment outcomes due to higher toxicity faced by standard chemotherapy among BC patients. Despite this, there remained a significant gap in research on the challenges faced by underweight BC patients with low BMI and poor nutritional status, particularly in the context of the Indian healthcare system.

Single nucleotide polymorphisms (SNPs) are unique genetic alterations in cancer cells that can disrupt certain drug transportation and metabolism pathways, leading to treatment resistance and failure (Yang et al., 2022). These variations can affect the pharmacokinetics of chemotherapeutic agents, potentially reducing their effectiveness or contributing to multidrug resistance, thereby compromising therapeutic outcomes. Loss-of-function SNPs in transporter (*ABC*) and metabolizer (*CYP*) genes may reduce drug efflux and substrate elimination rates, leading to increased intracellular drug accumulation and potential cytotoxicity. In contrast, gain-of-function of *ABC* and *CYP* SNPs can enhance drug efflux or accelerate drug metabolism, reducing intracellular drug concentrations and efficacy, contributing to treatment failure (Loscocco et al., 2021). Polymorphisms in *ABC* and *CYP* genes have been extensively

studied (Chang et al., 2009; Abdul Aziz et al., 2018; Gutierrez-Rubio et al., 2015; Shimada et al., 2009; Chen et al., 2007; Mokhosoev et al., 2024), findings showed that such polymorphisms lead to increased toxicity, drug resistance, and diminished efficiency. Clinical efficacy influenced not only by pharmacogenetic factors but also by anthropometric parameters such as BMI. Studies have demonstrated that high BMI correlates with treatment response and survival outcomes (Chen et al., 2022; Raman et al., 2016; Singh et al., 2011; Usiskin et al., 2019). Therefore, this study aims to assess the clinical efficacy of anthracycline-taxane chemotherapy and polymorphisms of *ABC* and *CYP* gene variants, as well as the impact of low BMI, with the goal of identifying potential clinical pathways to improve BC management.

CHAPTER 2

REVIEW OF LITERATURE

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2.1 Statistics of breast cancer

Breast cancer stands as the most frequently diagnosed cancer worldwide, presenting an enduring challenge to global public health. In 2024, BC accounted for approximately 11.5% (2,296,840) of all cancer diagnoses, culminating in a troubling 2.29 million new cases. Within India, it maintains its status as the most prevalent cancer, comprised 13.6% (192,020) of all cancer diagnoses and the leading cause of cancer related death (98,337) among women (Ferlay et al., 2024).

2.2 Risk factors

The risk of developing BC was influenced by various factors, including gender (women being more susceptible), age (particularly between 41 to 60 years in India), genetic mutations (*BRCA1*, *BRCA2*, *PALB2*, *ATM*, *CHEK2*, *PTEN*), and hormonal factors (early onset of menstruation and late menopause), family history of BC (first-degree relatives), exposure to radiation, reproductive aspects (nulliparity, late pregnancy, a lack of breast-feeding), prolonged use of hormone replacement therapy and lifestyle factors (obesity and insufficient physical activity) associated with a higher risk of BC (**Figure 2.1**) ("Breast Cancer Risk Factors and Prevention Methods | American Cancer Society," n.d.; Harbeck et al., 2019; National Cancer Institute, 2021; Clarke et al., 2012).

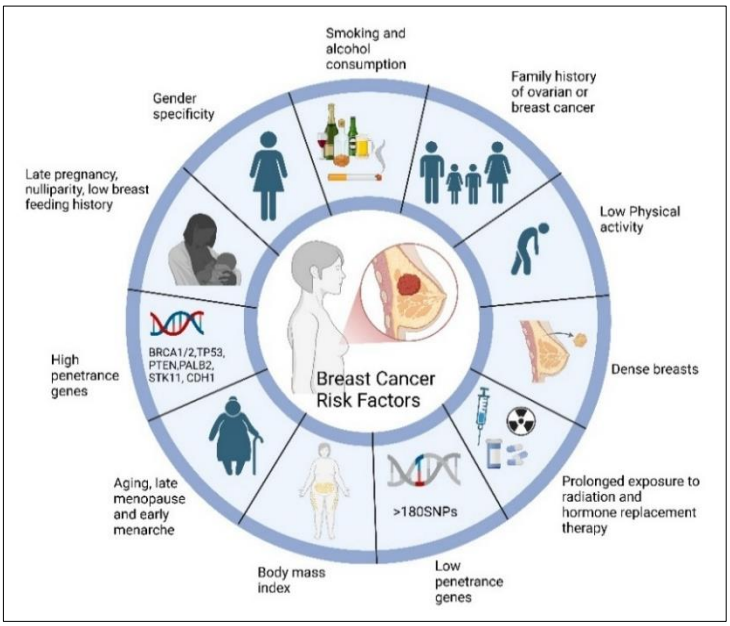


Figure 2.1 Risk factors of breast cancer

2.3 Signs & symptoms

Signs and symptoms of BC could differ, but frequently encompass the existence of a palpable lump in the breast or armpit region. Additional symptoms may include persistent mastalgia, tenderness, itching, burning sensation, nipple discharge, swelling, erythema, nipple retraction, fungating breast, sometimes with foul smell and bleeding, etc. These changes could lead to alterations in breast size, shape, or texture, such as dimpling or puckering of the skin, scaling of nipples, etc. ("Breast Cancer Signs and Symptoms | Most Common Symptoms | American Cancer Society," n.d.; Harbeck et al., 2019**;** Mistry et al., 2024b). Early detection through regular self-examinations, clinical screenings, and imaging techniques like mammography, is crucial for timely diagnosis and effective management. Raising awareness about these symptoms could help in prompt medical consultation and improve treatment outcomes and overall prognosis.

2.4 Staging and grading

BC was subdivided in 4 stages-Stage I, II, III and IV. Whereas stage 0 considered as a non-invasive stage (Amin et al., 2017). American Joint Committee on Cancer (AJCC) (8^{th} edition) in association with Union for International Cancer Control (UICC) published the TNM staging system in 2017, reflected the latest advancements in BC staging (Amin et al., 2017; Brierley J.D. et al., 2017). This system categorized cancer based on the size of the primary tumor (T), presence of lymph node involvement (N), and presence of distant metastasis (M), providing a comprehensive assessment of the extent of disease throughout the body (**Table 2.1**). However, TNM staging was invaluable for predicting cancer recurrence, and estimation of prognosis, it was primarily focused on disease spread and might have overlooked other important factors such as comorbidities and treatment-related risks.

Table 2.1 AJCC staging of breast cancer (Amin et al., 2017)

Stage		Tumor (T)	Nodes (N)	Metastasis (M)	Additional Features
0		Tis	N0	M0	Non-invasive
I	IA	T1	N0	M0	Tumor (≤20 mm) in greatest dimension. No lymph node or metastasis involvement.
	IB	T0	N1mi	M0	Micro-metastases approximately 200 cells, (>0.2mm to ≤2mm) in lymph nodes.
		T1	N1mi	M0	
II	IIA	T0	N1	M0	No distant metastasis; nodes have tumor cells.
		T1	N1	M0	
		T2	N0	M0	
	IIB	T2	N0ŧŧ/ N1*	M0	Tumor (2-5 cm), 4-9 axillary nodes. No metastasis but larger tumor or node spread. ŧŧ AJCC 8th edition. *NCCN guidelines 2025.
		T3	N1ŧŧ/ N0*	M0	
III	IIIA	T0	N2	M0	Tumor (>5 cm) Larger tumor or extensive node involvement.
		T1	N2	M0	
		T2	N2	M0	
		T3	N1	M0	
		T3	N2	M0	
	IIIB	T4	N0	M0	Tumor (invasion into chest wall/skin) Includes inflammatory BC.
		T4	N1	M0	
		T4	N2	M0	
	IIIC	Any T	N3	M0	Node (10+ nodes or clavicular nodes). Extensive lymph node involvement.
IV		Any T	Any N	M1	Metastasis to distant organs like bones or lungs.

T0: No evidence of primary Tumor; Tis: Tumor in situ; T1-T4: Tumor size increases as the number rises; TX: Primary tumor cannot be assessed; N0: No regional lymph node involvement; N1-N3: Increasing node involvement; mi- Micro-metastases; NX- Regional Lymph Node cannot be assessed; M0: No distant metastasis; M1: Distant metastasis present; MX- Distant metastasis cannot be assessed.

BC grading was a critical pathological assessment that evaluated the aggressiveness and behavior of cancer cells which further helps to assess tumor's growth rate and potentiality of spread, guide treatment decisions and predict patients' clinical outcomes. It was involved in examining the microscopic features of tumor cells, including their size, shape, and how closely they resemble normal breast tissue. BC categorized into three histological grades (Amin et al., 2017)**.** Typically, BC was graded on a scale from 1 to 3, with higher grades indicated more abnormality and severity of tumor characteristics (**Table 2.2**).

Table 2.2 Histological grades of breast cancer

Grade	Description	Key Features	SBR Score*
Grade I	Well-differentiated	Tumor cells look similar to normal breast cells, slow-growing, organized structure.	3-5
Grade II	Moderately differentiated	Tumor cells appear abnormal, faster-growing, moderate loss of organization.	6-7
Grade III	Poorly differentiated	Tumor cells look very abnormal, rapid growth, little to no organized structure.	8-9
GX	Grade cannot be assessed.		

*Scarff-Bloom-Richardson (SBR) grading system; Nottingham Score: Tubule Formation: 1: >75% tubule formation; 2: 10–75% tubule formation; 3: <10% tubule formation; Nuclear Pleomorphism: Variation in the size and shape of the tumor cell nuclei; 1: Small, uniform nuclei resembling normal cells; 2: Moderately variable nuclei; 3: Large, irregular nuclei; Mitotic Rate: Number of dividing cells in a given area of the tumor; 1: Low mitotic activity; 2: Moderate mitotic activity; 3: High mitotic activity; * Combine score.*

2.4.1 Clinical significance of BC staging and grading

BC staging, a crucial process in diagnosis and management. It helps to determine the extent of the disease, predict disease prognosis, treatment outcomes, survival, and helps in selecting appropriate systemic therapies, monitor disease progression, standardized patient care, and also aids in patient counselling. Similarly, grading is also a critical part of pathology that evaluates degree of abnormality of the cancer cells. Grading system helps in predicting the invasiveness of the tumor cells, determination of disease prognosis, decision in treatment approach, selection of systemic therapies, helps to understand the risk of metastasis and recurrence (Rakha et al., 2008).

2.5 Histological subtypes

Histological subtypes of BC were determined by examining tumor samples under a microscope to identify the type of cells and tissue patterns involved. These subtypes helps to differentiate between various forms of cancer, their behavior and response to treatment. This classification was critical for understanding the prognosis and selecting appropriate treatment strategies (**Figure 2.2)**. Different subtypes exhibit distinct characteristics, influencing tumor behavior, response to therapy, and overall patient outcomes (**Table 2.3)**. For instance, hormone receptor-positive subtypes, such as invasive ductal carcinoma and invasive lobular carcinoma, respond well to hormone therapy, while HER2-positive cancers benefit from targeted treatments like *trastuzumab*. In contrast, triple-negative breast cancer lacks hormone receptors and HER2 expression, made chemotherapy the primary treatment option. Understanding these subtypes helps to predict tumor aggressiveness, recurrence risk, and survival rates, ultimately guiding personalized treatment approaches and improve patient management.

Table 2.3 Different histological subtypes of breast cancer (Gomes Do Nascimento and Otoni, 2020)

Histological subtypes	Features
Invasive Ductal Carcinoma (IDC)	• Tumor cells are pleomorphic, with protruding nucleoli and numerous mitoses. • Areas of necrosis and calcifications can be detected in more than half of the cases.
Invasive Lobular Carcinoma (ILC)	• The classic form of the ILC is characterized by the presence of small tumor cells with little atypia, uniformly distributed throughout the stroma in a concentric pattern. • Among pleomorphic ILC, tumor cells have a hyperchromatic and eccentric nucleus, prominent mitoses and apocrine. • Histiocytic or signet ring cells can be observed and they are more likely to have TP53 mutations.
Mucinous Carcinoma	• This special subtype, also known as colloid or mucinous carcinoma. • It has abundant extracellular mucin surrounding small clusters of tumor cells with various growth patterns and mild nuclear atypia and associated with a favorable prognosis.
Metaplastic Carcinoma	• These aggressive tumors often involve lymph nodes and are poorly differentiated and heterogeneous. • They contain ductal carcinoma cells mixed with squamous, spindle, or mesenchymal elements like chondroid, bone, and epithelial cells.
Apocrine Carcinoma	• This subtype, features at least 90% apocrine-differentiated tumor cells, • It is typically high-grade with a poor prognosis, and affects a wide age range, but is more common in postmenopausal women.
Cribriform Carcinoma	• Microscopically, it features islands of uniform low-grade atypia cells, a cribriform pattern in 90% of the tumor, • It is often associated with DCIS without clear stromal invasion.
Tubular Carcinoma	• This well-differentiated subtype, often linked to premalignant lesions, feature prominent tubules (>90%) that are angled, • Oval or elongated in shape, with disorganized arrangement and open lumens covered by a single epithelial layer, usually without necrosis or mitosis.
Neuroendocrine Carcinoma	• Morphologically, it shows an infiltrative growth pattern with solid aggregates of tumor cells in alveolar, trabecular, or rosette patterns, and peripheral palisades. • Neoplastic cells vary in size and generally have fine eosinophilic granular cytoplasm.
Medullary Carcinoma	• It is well-circumscribed with large pleomorphic tumor cells, a syncytial growth pattern, frequent mitoses, and prominent lymphoplasmacytic infiltrate. • Spindle cell metaplasia and giant tumor cells are also common.

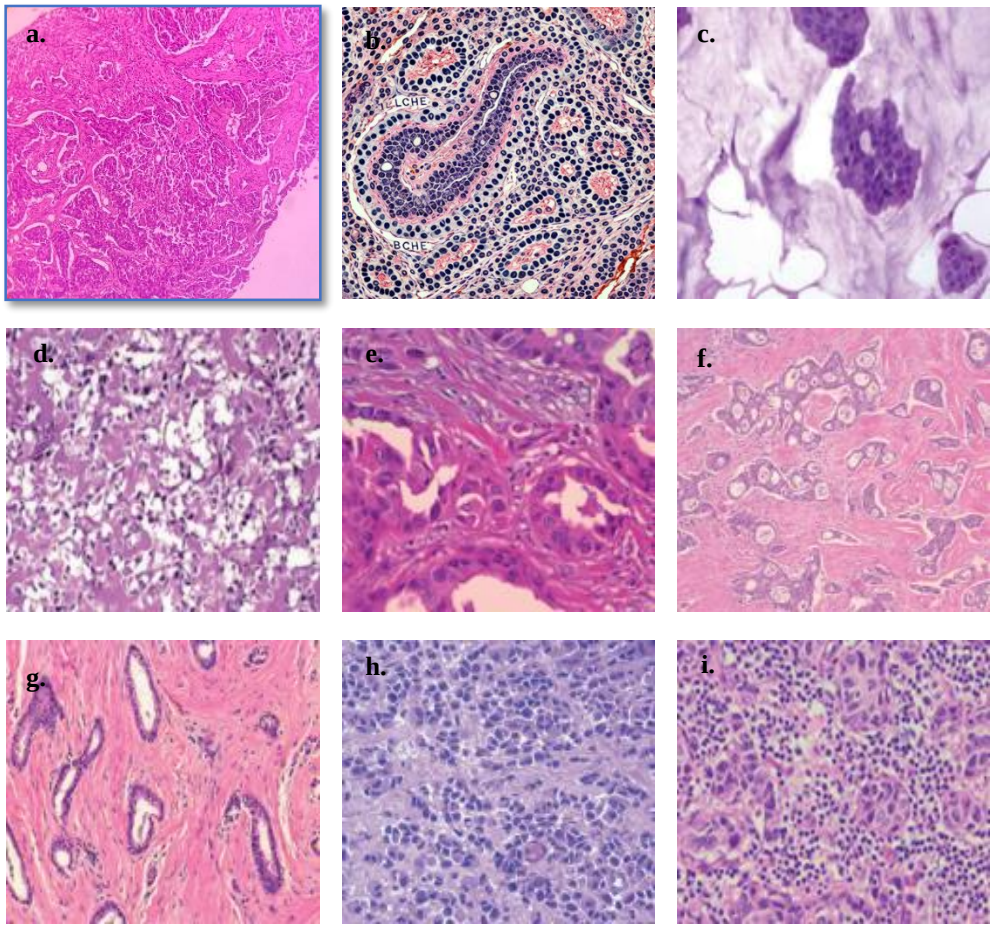


Figure 2.2 Histological subtypes of breast tumor

a. Invasive Ductal Carcinoma (IDC), b. Invasive Lobular Carcinoma (ILC), c. Mucinous Carcinoma, d. Metaplastic Carcinoma, e. Apocrine Carcinoma, f. Cribriform Carcinoma. g. Tubular Carcinoma, h. Neuroendocrine Carcinoma, i. Medullary Carcinoma (Gomes Do Nascimento and Otoni, 2020)

2.6Molecular subtypes of BC

From a molecular perspective, intrinsic subtypes had been identified through global studies of gene expression profiles. Currently, four molecular subgroups were widely recognized and well established in clinical practice: Luminal A, Luminal B, HER2+, and triple negative breast cancer (Nath et al., 2022; Mistry et al., 2024a). Their probable prognosis and expected response to treatment was described below **(Table 2.4)**.

Table 2.4 Classification of molecular subtypes of BC and ongoing treatment options
(Gomes Do Nascimento and Otoni, 2020)

Molecular subtypes	Luminal A	Luminal B		HER2 Positive	TNBC
		HER2/neu -	HER2/neu +		
Biomarkers	ER+ PR+ HER2- Ki-67 low	ER+ PR- HER2- Ki-67 high	ER+ PR-/+ HER2+ Ki-67 low/high	ER- PR- HER2+ Ki-67 high	ER- PR- HER2- Ki-67 high
Frequency of Cases (%)	40–50	20–30		15–20	10–20
Histological Grade	Grade I	Grade II		Grade III	Grade III
Prognosis	Good	Intermediate		Poor	Poor
Response to Therapies	Endocrine	Endocrine Chemotherapy	Endocrine Chemotherapy Target Therapy	Target Therapy Chemotherapy	Chemotherapy PARP Inhibitors

2.7 Treatment approach of BC

The treatment approach for BC was highly individualized and typically involved a multidisciplinary team of healthcare professionals, including oncologists, surgeons, radiation therapist, and other specialists (Wang and Wu, 2023) (**Figure 2.3**). The primary treatment modalities for BC included surgery, chemotherapy, radiation therapy sometimes hormone therapy, targeted therapy, and immunotherapy also, which might be used alone or in combination depending on the type and stage of the cancer, as well as the patient's overall health and preferences (Burguin et al., 2021; Mutebi et al., 2020**;** Tong et al., 2018**;** Liu et al., 2024). Surgical interventions, such as lumpectomy or mastectomy, were frequently employed as primary treatment strategies to achieve tumor resection with clear margins. To mitigate the risk of recurrence, adjuvant therapies—comprising chemotherapy and radiation therapy—were often recommended to eliminate residual malignant cells. Targeted therapies, such as HER2-directed agents, were designed to inhibit oncogenic pathways in tumors harboring specific genetic alterations, while immunotherapy harnesses the host immune system to recognize and eliminate malignant cells. With advancements in molecular profiling and genetic testing, personalized treatment strategies were increasingly being implemented, enabling the optimization of therapeutic regimens based on the unique molecular characteristics of an individual's tumor. For hormone receptor-positive BC, endocrine therapy plays a pivotal role by targeting estrogen signaling pathways to suppress tumor progression. This approach includes selective estrogen receptor modulators (SERMs), aromatase inhibitors, and selective estrogen receptor degraders (SERDs), which are utilized across both early and advanced disease stages to reduce recurrence and improve survival (Osborne and Schiff, 2011). Tamoxifen, a SERM, is one of the most widely used drugs in endocrine therapy. It acts by

competitively binding to estrogen receptors, preventing estrogen from stimulating tumor growth. Tamoxifen is effective in premenopausal and postmenopausal women and is used in both adjuvant and metastatic settings. Its benefit include reducing the risk of recurrence for contralateral breast cancer, and improving overall survival. However, it is associated with some side effects, including a higher risk of endometrial cancer and thromboembolic events (Jordan, 2003). Additionally, supportive care measures, including pain management, nutritional support, and psychosocial support, play a vital role in optimizing patients' clinical outcomes and QoL throughout the treatment journey (Mistry et al., 2024a).

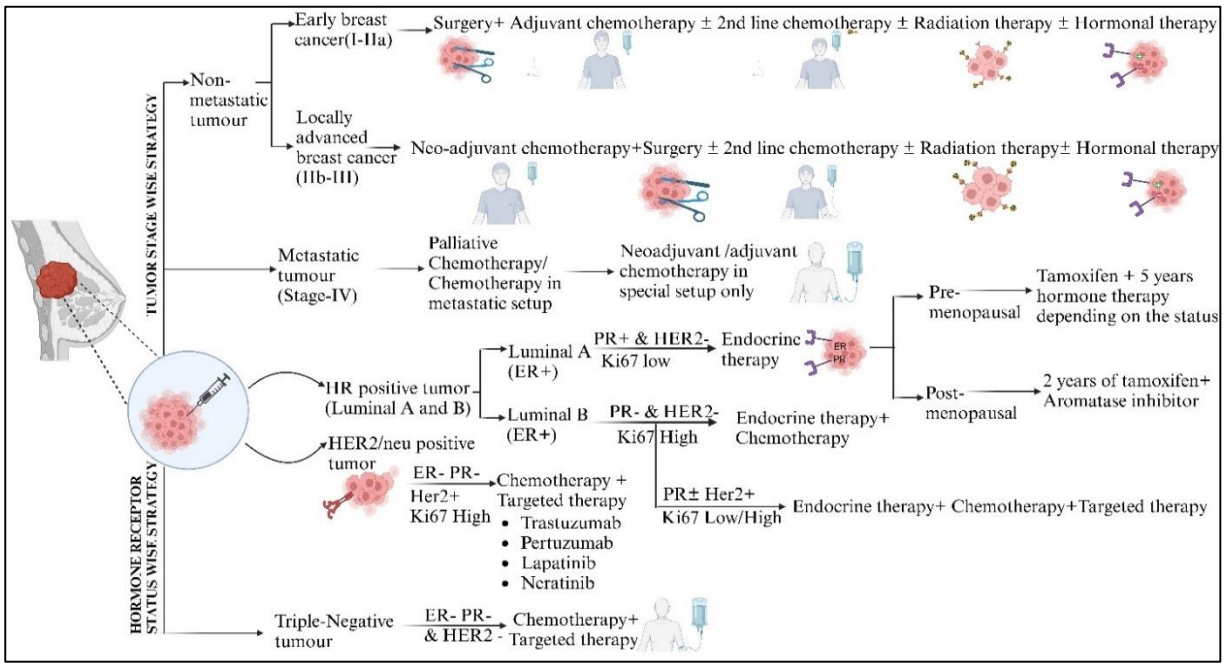


Figure 2.3 Treatment strategies for breast cancer patients

2.8 Chemo-induced toxicity

Chemotherapy, while effective in treating cancer, however, often associated with significant adverse effects that can impact patient outcomes and QoL. Anthracycline and taxane based chemotherapies were main treatment options for BC and could induce significant toxicities. Anthracyclines, like doxorubicin, often cause cardiotoxicity (Ewer and Ewer, 2010), leading to long-term heart issues. They could also cause myelosuppression, resulting in reduced blood cell counts and increased infection risk (Minotti et al., 2004). Taxanes, such as paclitaxel, were known for causing neurotoxicity, presenting as peripheral neuropathy, along with myelosuppression and hypersensitivity reactions (Cavaletti and Marmiroli, 2010; Cidon and Ballesteros, 1995). Both drug classes could lead to gastrointestinal disturbances, alopecia, and fatigue, impacting patients' QoL during and after treatment (Fisusi and Akala, 2019).

Additionally, toxicities such as fever, anemia, diarrhea, and constipation were also reported among BC patients (Mistry et al., 2024c).

2.9 Quality of Life

QoL among cancer patients had been enormously studied throughout the last decade as it was a primary endpoint of treatment outcome besides overall survival. QoL in BC patients was profoundly influenced by various factors, including treatment modalities, psychological well-being, socio-demographic characteristics, etc. A recent study highlighted that both positive and negative psychological states significantly impact QoL in postoperative BC patients, emphasizing the need for comprehensive mental health support during recovery (Zhao et al., 2024). Additionally, research had been identified resilience as a mediating factor in QoL outcomes, suggested that interventions aimed at enhancing resilience could improve overall well-being in BC population (Faroughi et al., 2023). Furthermore, a systematic review and meta-analysis underscored the importance of addressing global QoL issues in BC care, advocating for personalized treatment plans that consider individual patients' need to optimize health-related QoL (Javan Biparva et al., 2023).

2.10 Role of BMI in QoL

Anthropometric factors, including height, weight, and BMI, had been reported to be associated with a higher risk of BC (Lin et al., 1971; Li et al., 1997). Significantly, overweight and obesity definitively identified as risk factors for BC, with postmenopausal women facing a 1.2–1.4 times higher risk (Renehan et al., 2008; Munsell et al., 2014) and pre-menopausal women experienced 0.8 times increased risk (Munsell et al., 2014; Schoemaker et al., 2018).

Heterogeneous Indian population often grapple with health issues stemmed from the risk factors such as unhealthy diet/undernourishment, manifested in low BMI, with lower mid-upper arm circumference (MUAC) and skin fold thickness (SFT). As patients battle therapeutic side effects like toxicities, therapy failures, and swift illness development (Muscaritoli et al., 2017), neglected nutrition exposes them to negative repercussions and a poor prognosis (Chen et al., 2022). This nutritional deficiency significantly amplifies cancer mortality rates, with malnourished patients facing a 2 to 5 times higher risk compared to the well-nourished counterparts (Muscaritoli et al., 2017; Chen et al., 2022; Genet, 2020). Moreover, the treatment side effects indirectly impact food consumption and nutrient absorption. As a result, the detection of dietary inadequacies using conventional BMI-based screening approaches has become more challenging (Basaran et al., 2011; Trédan et al., 2010). Besides BMI, the

measurement of MUAC and SFT represented as another non-interventional approach for evaluating nutritional status (Mistry et al., 2024c). The measurement of the supra-iliac skinfold was pivotal for determining SFT which was widely employed caliper testing method to ascertain a patient's body fat percentage. Although numerous studies have reported the impact of obesity on QoL of BC patients (Leach et al., 2023; Shaikh et al., 2020; Dieli-Conwright et al., 2018; Juan et al., 2018; Connor et al., 2013).

2.11 Role of BMI in treatment response

BMI also plays a critical role in modulating treatment response in BC patients. High BMI had been associated with a poorer prognosis and reduced treatment efficacy, potentially due to alterations in drug metabolism and distribution. Studies had demonstrated that obesity could impact the pharmacokinetics of chemotherapy, leading to suboptimal drug dosing and heightened toxicity risks (Connor et al., 2013; Dieli-Conwright et al., 2018; Connor et al., 2013). Additionally, elevated BMI was linked to increase circulating levels of estrogen and insulin, which might contribute to tumor progression and resistance to hormone therapies (Barone et al., 2022; Gunter et al., 2009; Rubinstein et al., 2021). Conversely, patients with normal BMI tend to exhibit better treatment responses and improved overall survival rates. However, the impact of low BMI and poor nutritional status on chemotherapy outcomes remains underexplored. A comprehensive understanding of treatment responses across different BMI categories is essential for optimizing personalized therapeutic strategies and enhancing clinical outcomes in BC management (Mistry et al., 2024c).

2.12 Development of adversity and delayed in chemotherapy response due low BMI

Cancer patients experienced an average delay of 29.4 weeks in their treatment (Jassem et al., 2014). The negative impacts were more pronounced in the patients who had a lower nutritional status. Consequently, this had an adverse effect on the treatment outcomes. Patients who had a lower BMI and insufficient nutritional status (MUAC and SFT) sometimes faced challenges in completing their chemotherapy treatment beyond the 2^{nd} or 3^{rd} cycles. As a result, they experienced delay in the administration of chemotherapy (Gangane et al., 2017; Chintamani et al., 2011; Jassem et al., 2014; Mehrotra and Yadav, 2022), which negatively impacted the overall response to treatment and long-term survival prospects.

2.13 Treatment failure/ variations in individual drug response

Treatment failure in BC patients could arise from multiple factors, posing a substantial challenge. One common cause is the development of resistance to conventional therapies such as chemotherapy, hormonal therapy, or targeted therapies. Cancer cells might acquire genetic mutations or undergo molecular changes that render them less responsive to treatment, allowing the disease to progress. Additionally, incomplete eradication of tumor cells during primary treatment could lead to recurrence or metastasis over time. Tumor heterogeneity, defined as different cancer cell populations within the same tumor exhibit different characteristics, further complicates treatment success (Polyak, 2011). Addressing these complexities required a multifaceted approach involved personalized treatment strategies, continuous monitoring of disease progression, and the advancement of novel therapeutic interventions. Among these approaches, targeting microtubule dynamics and DNA integrity remains central to chemotherapeutic efficacy. Taxane agents (docetaxel/paclitaxel) bind to β-tubulin subunits within microtubules, stabilizing their structure and preventing depolymerization. This disruption of microtubule dynamics inhibits mitotic progression, leading to cell cycle arrest and apoptosis (Crown et al., 2004). Notably, docetaxel exhibits approximately twice the potency of paclitaxel in inhibiting tubulin depolymerization. Similarly, anthracycline (doxorubicin/epirubicin) intercalates into DNA, obstructing the function of topoisomerase II and thereby hindering the relaxation of supercoiled DNA necessary for replication and transcription. Additionally, doxorubicin generates free radicals, causing oxidative damage to DNA and cellular membranes (van Rossum et al., 2018). Likewise, alkylating agent, cyclophosphamide underwent metabolic activation to form phosphoramide mustard, which creates inter strand DNA cross-links. These cross-links disrupt DNA replication and transcription, ultimately triggering apoptosis in rapidly dividing cells (van Rossum et al., 2018). However, pyrimidine analog 5Fluorouracil, metabolized into active nucleotides that inhibit thymidylate synthase, leading to depletion of thymidine triphosphate and subsequent disruption of DNA synthesis. Incorporation of its metabolites into RNA also interferes with RNA processing and function (Anampa et al., 2015) (**Figure 2.4)**.

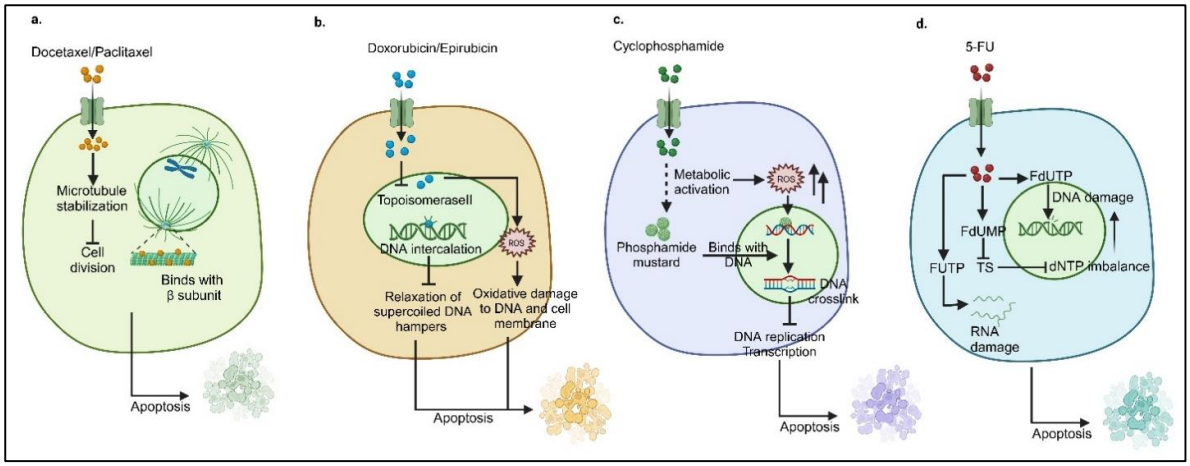


Figure 2.4 Mechanistic pathways of key chemotherapeutic agents

Illustration depicting the molecular mechanisms of action of key chemotherapeutic agents, including a. Taxanes (docetaxel/paclitaxel), b. Anthracyclines (doxorubicin), c. Alkylating agents (cyclophosphamide), and d. Antimetabolites (5-fluorouracil), highlighting their roles in disrupting cellular processes to induce apoptosis in cancer cells.

Despite the efficacy of these chemotherapeutic agents, their success was often hindered by multidrug resistance (MDR) mechanisms, including variations in transporter genes, could contribute to treatment failure (Skinner et al., 2023). These transporter proteins, such as P-glycoprotein (P-gp) encoded by the *ABCB1* gene, play crucial roles in pumping out anticancer drugs from cancer cells, thereby reducing their effectiveness. Genetic variations in these transporter genes could lead to overexpression or altered function of these proteins, resulted in decreased intracellular drug concentrations and resistance to chemotherapy agents. This phenomenon poses a significant challenge in the management of BC, as it limits the efficacy of commonly used chemotherapeutic agents. Understanding the genetic basis of *MDR* and transporter gene variations is essential for developing strategies to overcome treatment resistance and improve outcomes for BC patients (Tulsyan et al., 2016). Research efforts focused on identifying novel therapeutic targets and developing targeted therapies to circumvent MDR mechanisms offer promise in addressing this aspect of treatment failure in BC (Robinson and Tiriveedhi, 2020). The management of BC challenges might arise from genetic variations in metabolizer genes that influence the metabolism and effectiveness of anticancer medications. Genetic polymorphisms in genes the encode drug-metabolizing enzymes, including cytochrome P450 (*CYP*) enzymes, could modify the pharmacokinetics and bioavailability of chemotherapeutic drugs often use in BC treatment (Khan et al., 2018) (**Figure 2.5**). Alterations in these metabolizer genes could result in enhanced or diminished enzymatic activity, influence drug activation, detoxification, or elimination. As a result, people with specific genetic variations may encounter inadequate drug exposure, which could result in treatment failure or heightened

toxicity (Hlaváč et al., 2020). Comprehending the influence of metabolizer gene variants on pharmacokinetics is crucial for individualized therapeutic strategies in BC. Pharmacogenomic testing identifies patients susceptible to treatment failure due to hereditary variables, allowing clinicians to customize therapy regimens and enhance therapeutic outcomes. Furthermore, current research focused on clarifying the intricate relationship among genetic variation, drug metabolism, and treatment response shows potential on enhancing the efficacy and safety of BC (Lim et al., 2011; Mistry et al unpublished work).

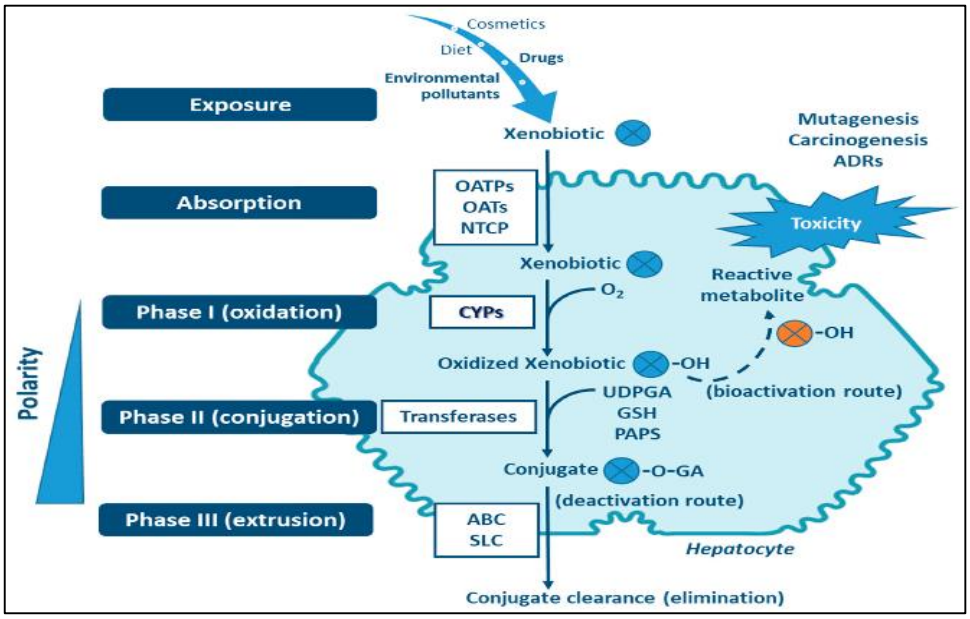


Figure 2.5 Overview of enzymatic biotransformation of xenobiotics (Esteves et al., 2021)

This figure illustrates the metabolism and elimination of xenobiotics such as drugs and pollutants in hepatocytes. The process involves three phases: Phase I (oxidation) by CYP enzymes, generating oxidized metabolites, some of which may be toxic; Phase II (conjugation) by transferases, increasing polarity for detoxification or bioactivation; and Phase III (extrusion) via ABC and SLC transporters for clearance. The balance between bioactivation and detoxification determines the toxic or therapeutic effects of xenobiotics.

2.14 Role of transporter and metabolizing genes in treatment failure

Pharmacogenomics was revolutionized by uncovering the intricate genetic blueprint behind individual responses to drugs. This cutting-edge field delves into differential response to treatment. By leveraging advancements in genomics, proteomics, transcriptomics, and metabolomics, researchers were unraveling the genetic variations that dictate drug efficacy and safety (Mizzi et al., 2016; Ahmed et al., 2016). Drug response was influenced not just by the pharmacokinetics and pharmacodynamics, but also by genetic polymorphisms in drug-metabolizing enzymes and transporter molecules. These variations, unique to each individual, could profoundly impact how drugs were absorbed, distributed, metabolized, and eliminated, paving the way for personalized treatment strategies. Genome sequencing and mutation

analysis were crucial in pharmacogenomics. These allowed to analyze the genetic characteristics of both patients and tumors. Such insights are vital, especially when it comes to anti-cancer medications, as genetic differences could alter drug metabolism, transport, retention, and even penetration into tumor tissues (Aboul-Soud et al., 2021). The diversified human genome containing over 14 million SNPs and genetic variations that occur approximately every 300–1000 nucleotides (Gorlov et al., 2008). These SNPs were key to understanding why individuals respond differently to drugs, environmental factors, and diseases. Numerous studies had shown that various factors cause medication response differences and affect them directly or indirectly (Ingelman-Sundberg et al., 2018).

ATP-binding cassette (*ABC*) transporters play a crucial role in drug efflux, impacting drug bioavailability and treatment efficacy. As key membrane transporters, *ABC* proteins, such as P-glycoprotein (*ABCB1*), actively pump drugs out of the cells, reducing intracellular drug concentrations (Tsukamoto et al., 2019). SNPs in *ABC* transporter genes could significantly alter drug transport activity, leading to variable therapeutic responses. Loss-of-function SNPs in *ABC* genes may reduce drug efflux, increasing intracellular drug accumulation and potential toxicity. Conversely, gain-of-function SNPs enhance drug efflux, lowering intracellular drug concentrations and contributing to treatment failure (Loscocco et al., 2021). These variations could affect the pharmacokinetics of chemotherapeutic agents, rendering them less effective or leading to multidrug resistance, ultimately compromising therapeutic outcomes.

Most *CYPs*, responsible for drug metabolism, were present in the liver and intestines. As the cytochrome P450 (*CYP*) superfamily metabolizes 90% of clinically used medicines, toxins, and carcinogens, its mutations have the greatest therapeutic impact (Song et al., 2021). In pharmacology, *CYP* variations were categorized into two groups: loss-of-function variants and gain-of-function variants (Zanger and Schwab, 2013). Loss-of-function variants decrease the rate at which substances were eliminated from the body, resulting in higher plasma concentrations. SNPs in *CYP* genes often impact splicing and expression, rather than transcription or protein structure (Sadee et al., 2011). Conversely, gain-of-function variants enhance the rate of substance clearance, leading to lower drug concentrations. Polymorphisms of *CYP* genes could be caused by copy number variants (CNV) that increase the number of functional gene copies or promoter variants and amino acid variants that accelerate substrate breakdown (Johansson and Ingelman-Sundberg, 2008). These genetic variation affect metabolism of important chemotherapeutic agents resulted in drug accumulation, increase toxicity and treatment failure.

2.14.1 Independent effect of *ABC* gene in treatment response

ATP-binding cassette (*ABC*) transporter genes play pivotal role in BC, significantly impacting drug resistance and disease progression. These genes encode a large family of proteins that harness the energy from ATP hydrolysis to transport diverse substrates across cellular membranes (Gottesman et al., 2002). In BC, the overexpression of specific *ABC* transporter genes, including *ABCB1 (MDR1), ABCC1 (MRP1), ABCC2 (MRP2),* and *ABCG2 (BCRP),* had been strongly linked to multidrug resistance (MDR) (Sharom, 2008). These transporters act as molecular gatekeepers, actively efflux chemotherapeutic agents out of cancer cells. This reduces intracellular drug accumulation, rendering treatments such as doxorubicin, paclitaxel, and other commonly used chemotherapeutics less effective (Robey et al., 2018) **(Figure 2.6)**. The role of *ABC* transporters in mediating drug resistance underscores the need for targeted strategies to overcome MDR, ensuring improved therapeutic outcomes in BC patients. Targeting *ABC* transporter-mediated drug resistance offers promising potential for enhancing treatment outcomes and improving the prognosis of BC patients. Investigating the regulatory mechanisms governing these transporters and developing inhibitors or alternative therapeutic strategies to mitigate their effects remain active areas of research in combating BC (Muriithi et al., 2020).

Genetic polymorphisms in drug transporters, significantly impact the ability of medications to be eliminated from the body, thereby influencing their efficacy and toxicity. A key example was, P-glycoprotein (P-gp), a transporter belong to the *ABC* family, which was responsible for pumping xenobiotics and other foreign compounds out of cells (Wolking et al., 2015). The *ABCB1/MDR-1* gene encodes P-gp, and mutations in this gene could alter the efficiency of various therapies, leading to resistance to certain chemotherapeutic regimens such as 5-fluorouracil (5-Fu) (Hoffmeyer et al., 2000) (**Figure 2.6**).

There were three primary polymorphisms in the *ABCB1/MDR-1* gene that affect P-gp functionality. The first involved a single nucleotide polymorphism (SNP) in exon 21, causing the mutation of three amino acids and ultimately enhancing P-gp activity. The second occurs in exon 26, reducing P-gp expression in the duodenum, thereby affecting drug absorption. The third polymorphism, located in exon 12, does not directly influence P-gp expression but may still impact drug transporter function (Kim et al., 2001). Each of these polymorphisms affects the performance of chemotherapeutic drugs in distinct ways.

Another crucial member of the *ABC* transporter family was *BCRP* (Breast Cancer Resistance Protein). A SNP in exon 5 of the *BCRP* gene has been linked to reduced *BCRP* levels and decreased drug clearance (Zhang et al., 2006; Bertholee et al., 2017). For instance, bicalutamide, an anti-androgen used to treat prostate cancer, was expelled from cells via P-gp and *BCRP*. SNPs in *BCRP* could lead to elevated plasma levels of bicalutamide, resulting in increased chemotherapy toxicity (Bertholee et al., 2017).

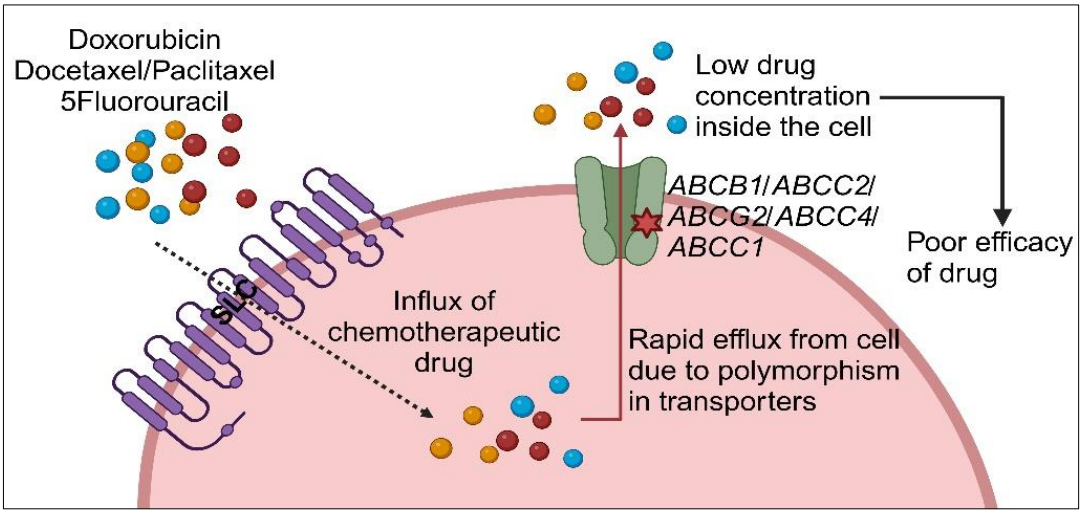


Figure 2.6 Schematic illustration of chemotherapeutic drug efflux modulated by *ABC* gene polymorphism

The figure demonstrates the impact of ABC transporter gene polymorphisms on the intracellular retention and efficacy of chemotherapeutic drugs. Key drugs such as doxorubicin, docetaxel/paclitaxel, and 5-fluorouracil entered the cancer cells through influx mechanisms. However, polymorphisms in ABC transporters, including ABCB1, ABCC1, ABCC2, ABCC4, and ABCG2, enhance the rapid efflux of these drugs from the cell. This leads to a reduced intracellular drug concentration, thereby diminishing their effects and resulting in poor therapeutic efficacy. Such transporter-mediated drug resistance poses a significant challenge in cancer treatment, affecting patient response to chemotherapy.

Tamoxifen and its active metabolite endoxifen, the substrates of P-glycoprotein **(***ABCB1***),** which might limit their intracellular concentrations by maintaining sub-therapeutic levels (**Figure 2.7**). At high doses, tamoxifen is used to treat brain metastases, where saturation of blood-brain barrier drug transporters likely allows tamoxifen and its metabolites to penetrate the brain effectively. Variations in *ABC* genes (C3435T, G2677T/A in *ABCB1*, C421A in *ABCG2*, and C-24T in *ABCC2*) influence transporter expression or function, affecting tamoxifen plasma levels, intracellular accumulation, therapeutic outcomes, and toxicity (Sensorn et al., 2013; Kiyotani et al., 2010; Teft et al., 2011)**.**

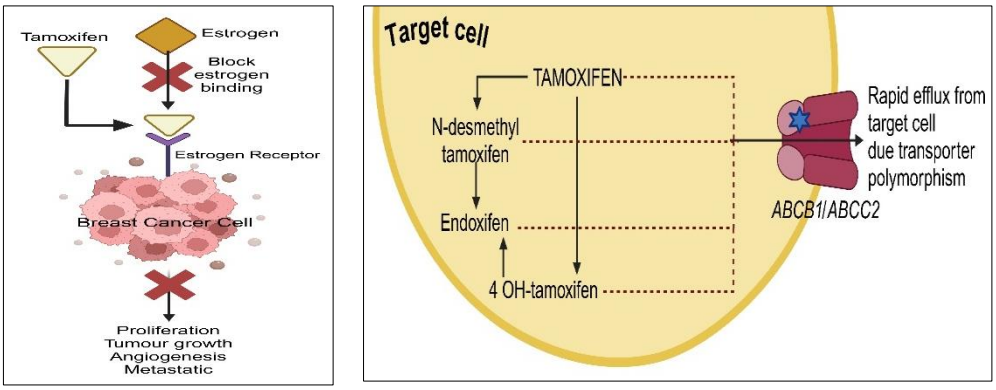


Figure 2.7 Mechanism of tamoxifen in controlling breast cancer proliferation and its impairment by *ABC* gene polymorphism-induced rapid efflux

In the first panel, tamoxifen acts as a selective estrogen receptor modulator (SERM), competing with estrogen to bind estrogen receptors in breast cancer cells, thereby blocking estrogen-driven proliferation, angiogenesis, and metastasis. In the second panel, tamoxifen underwent metabolism to form active metabolites such as 4-hydroxy-tamoxifen and endoxifen, which were crucial for its therapeutic efficacy. However, polymorphisms in ABC transporters (e.g., ABCB1 and ABCC2) lead to rapid efflux of tamoxifen and its metabolites from target cells, reducing their intracellular concentration and potentially compromising therapeutic effectiveness.

2.14.2 Combine role of BMI and *ABC* transporters

Both pharmacogenetic and anthropometric factors were anticipated to act as crucial regulators of treatment, QoL and survival outcomes of BC patients. SNP in *ABC* genes, which facilitates drug transport across cellular membranes were shown to be associated with tumor non-responsiveness (Chang et al., 2009; Chaturvedi et al., 2013; Hlaváč et al., 2020). SNPs within these genes could disrupt their normal function, leading to altered drug disposition (cytotoxicity), bioavailability, and resistance (Woo et al., 2003). Polymorphism in the *ABCB1* gene particularly C1236T, G2677T/A, C3435T were extensively studied (Chang et al., 2009; Tulsyan et al., 2014; Gutierrez-Rubio et al., 2015). While C58626A locus of *ABCC2* gene was relatively less explored. BMI serves as a crucial anthropometric parameter influencing variances in chemotherapy response in a wide range of cancers. Distinct BMI categories, showed correlation with patient's treatment response and survival outcomes (Singh et al., 2011; Usiskin et al., 2019). The combined effect of BMI and *ABC* gene polymorphism remained unexplored, highlighting lacunae that need to be addressed. Exploring the association of pharmacogenetic and anthropometric factors, independently and in a combined fashion, with the differential treatment outcomes of non-metastatic BC patients receiving anthracycline-taxane regimen is urgently required. Correlating the genetic impact of *ABC* gene polymorphisms and BMI with treatment response and survival outcome in BC patients need to be addressed. (refer to **Table 2.5**)

2.14.3 Independent effect of *CYP* gene in treatment response

Cytochrome P450 (*CYP*) genes play a significant role in BC through their involvement in drug metabolism and hormone biosynthesis (Shimada et al., 2009). The *CYP* gene family encodes enzymes that metabolize a wide range of substrates, including chemotherapeutic agents and endogenous hormones like estrogens, which were critical in the pathogenesis and progression of BC. Polymorphisms in *CYP* genes, such as *CYP2D6, CYP2C9, CYP2C19,* and *CYP3A*4, could lead to variations in enzyme activity, influencing the efficacy and toxicity of BC treatments. Understanding the impact of *CYP* polymorphisms was crucial for personalized medicine approaches, enabling the optimization of treatment regimens based on an individual's genetic makeup and improving clinical outcomes for BC patients.

CYP polymorphisms significantly influence the metabolism and therapeutic outcomes of cyclophosphamide, docetaxel, paclitaxel, etc. Cyclophosphamide, a prodrug that require a bioactivation by *CYP* enzymes to form its active metabolites (**Figure 2.8**). These metabolites, including 4-hydroxycyclophosphamide, mediate its cytotoxic effects. *CYP* polymorphisms significantly influence the drug's activation and detoxification, affecting efficacy and toxicity *CYP2C19, CYP3A4, CYP2C9* also contributes to the hydroxylation of cyclophosphamide (Seredina et al., 2012).

Docetaxel and paclitaxel were primarily metabolized by *CYP3A4* and *CYP3A5* into active metabolites (van Eijk et al., 2019). Variations in these enzymes affect drug clearance, which could influence therapeutic outcomes and the risk of side effects. Polymorphisms such as *CYP3A4* (in the promoter region) result in reduced enzyme expression and activity (Mulder et al., 2021). Decreased *CYP3A4* activity slows docetaxel clearance, leading to higher plasma concentrations and an increased risk of toxicity, such as neutropenia and peripheral neuropathy (Pratt et al., 2023). Patients with reduced *CYP3A4* or *CYP3A5* activity were at higher risk of severe side effects due to slower drug clearance (Frederiks et al., 2015) (**Figure 2.8**). Genotyping for *CYP3A4* and *CYP3A5* polymorphisms could be guided for dose adjustments to balance efficacy and minimize toxicity (Sim et al., 2018; Yin et al., 2017)**.**

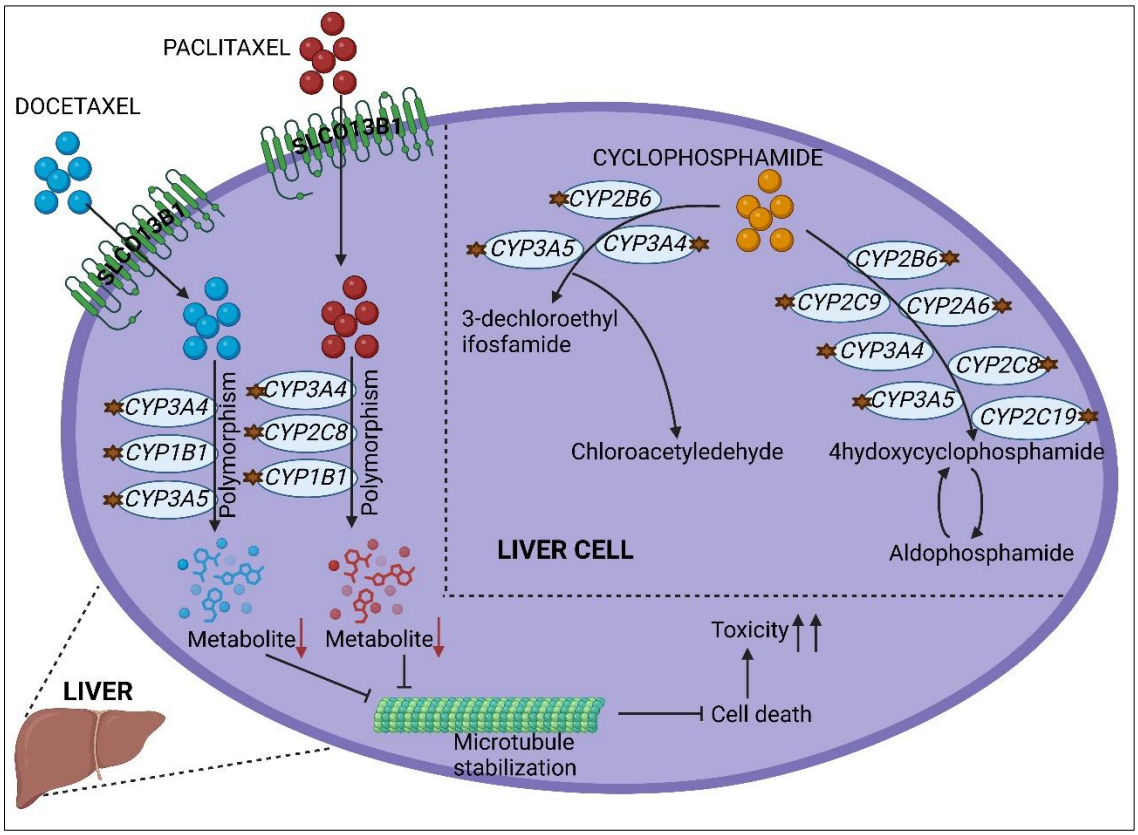


Figure 2.8 Variants of *CYP* gene involved in the metabolism of paclitaxel, docetaxel, and cyclophosphamide

The metabolic pathways of key chemotherapeutic agents highlighting the role of CYP3A4, CYP3A5, CYP2C8, and CYP1B1 in the metabolism of paclitaxel and docetaxel. Genetic polymorphisms in these enzymes influence drug metabolism, affecting therapeutic efficacy and toxicity. Cyclophosphamide metabolism is mediated by CYP2B6, CYP3A4, CYP3A5, CYP2C9, CYP2A6, CYP2C8, and CYP2C19, leading to the formation of active cytotoxic metabolites (aldophosphamide) or toxic byproducts (chloroacetaldehyde). Variations in CYP gene activity impact drug activation, detoxification, and patient response to chemotherapy.

The therapeutic efficacy of tamoxifen largely depends on its metabolic activation to form more potent anti-estrogenic metabolites, primarily endoxifen and 4-hydroxytamoxifen. This biotransformation was predominantly mediated by cytochrome P450 (*CYP*) enzymes (**Figure 2.9**). For instance, polymorphisms in *CYP2D6* affect the metabolism of tamoxifen, a commonly used hormone therapy in estrogen receptor-positive BC, by altering its conversion to active metabolites, thereby impacting therapeutic outcomes (Mokhosoev et al., 2024). Variations in *CYP1A1* and *CYP1B1*, involved in estrogen metabolism, could also affect BC risk by modifying the balance of estrogen metabolites, some of which were carcinogenic (Chen et al., 2007). Personalized treatment approaches based on genotyping could enhance clinical outcomes for patients underwent tamoxifen therapy. Polymorphisms in the *CYP2D6* gene lead to alteration in enzyme activity levels, categorizing individuals into different metabolizer phenotypes: poor, intermediate, extensive and ultra-rapid metabolizer. Reduced *CYP2D6* activity correlates with lower endoxifen levels, potentially compromising tamoxifen's

effectiveness in preventing cancer recurrence (Abraham et al., 2010). While decreased metabolism may reduce certain side effects, it could also lead to sub-therapeutic efficacy. Conversely, increased metabolism in ultra-rapid metabolizers might elevate the risk of adverse effects due to higher active metabolite levels. These enzymes were involved in the formation of primary tamoxifen metabolites, such as N-desmethyl tamoxifen and 4-hydroxy tamoxifen. Polymorphisms in these genes could influence tamoxifen metabolism, albeit to a lesser extent than *CYP2D6* (Sidibe et al., 2024). These enzymes participate in tamoxifen's metabolic pathways, and genetic variations might affect drug clearance and efficacy (Boucenna et al., 2022).

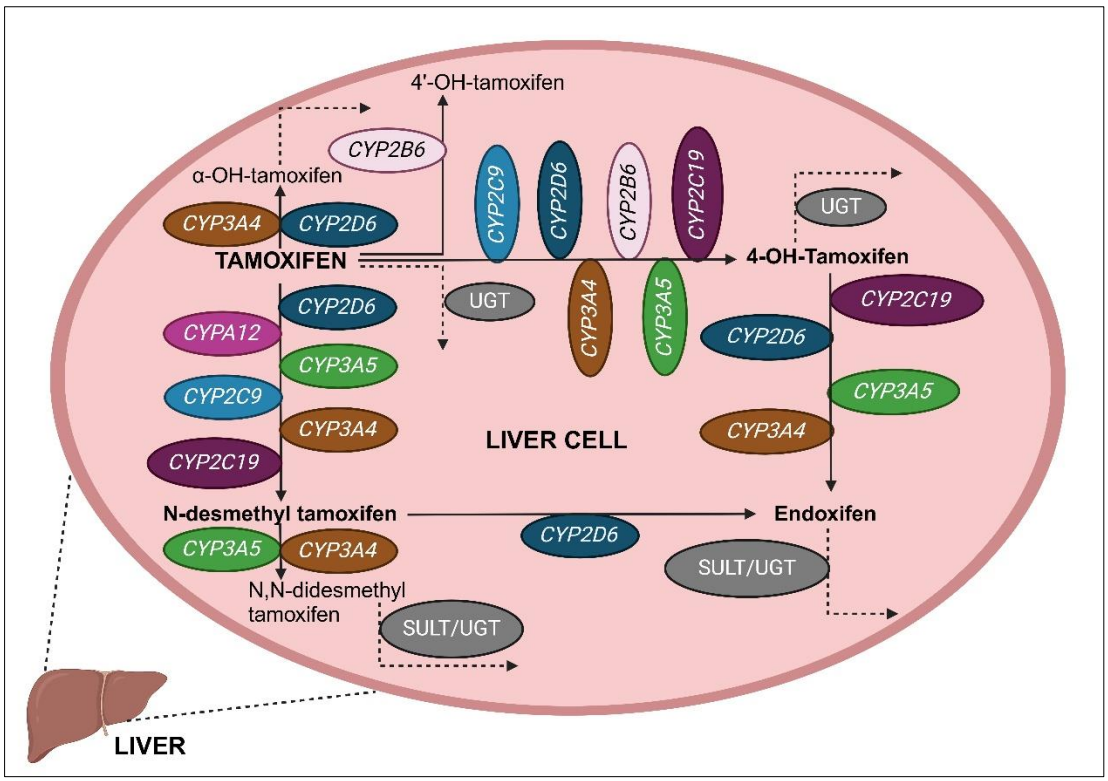


Figure 2.9 *CYP* genes involved in the metabolism of tamoxifen

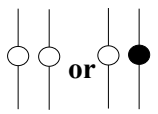
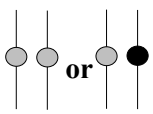
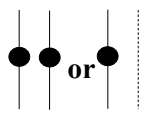
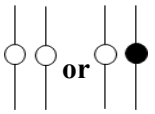
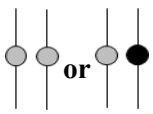
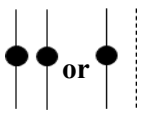
Tamoxifen underwent sequential metabolism primarily through CYP2D6 and CYP3A4, converting it into its active metabolites, 4-hydroxytamoxifen and endoxifen, which exhibit higher anti-estrogenic potency. Additional enzymes such as CYP2C9, CYP2C19, and CYP2B6 contribute to the metabolism, influencing drug clearance and therapeutic efficacy. Genetic polymorphisms in these CYP genes can lead to inter-individual variability in tamoxifen metabolism, potentially affecting treatment outcomes in breast cancer patients.

2.14.4 Combine role of BMI and *CYP* metabolizer genes

Cytochrome P450 (*CYP*) polymorphisms and BMI play a combined crucial role in influencing BC outcomes and treatment responses. *CYP* enzymes, particularly those in the *CYP2D6* family, were instrumental in metabolizing various chemotherapeutic drugs and hormonal therapy drugs like tamoxifen (Zembutsu et al., 2017; Jin et al., 2005). Polymorphisms in these enzymes could lead to variations in drug metabolism, affecting therapeutic efficacy and toxicity profiles.

Concurrently, BMI, a marker of body fat composition, was linked to BC prognosis, with higher BMI often associated with poorer outcomes due to factors like altered hormone levels and inflammation (Zangouri et al., 2025). However, the impact of low BMI in nutritionally challenged patients remains underexplored. The interplay between *CYP* polymorphisms and BMI variation, further complicate treatment outcomes, as both obesity and low BMI may influence drug metabolism, distribution, and clearance (Kong et al., 2025). Understanding the combined effects of genetic and physiological factors was crucial for optimizing personalized treatment strategies and improving clinical outcomes in BC patients. (**Table 2.5**)

Table 2.5 Influence of genotype-based classification of polymorphic genes and their clinical response across different BMI classes of BC patients

BMI	Transporter gene (*ABC*)			Metabolizer gene (*CYP*)			Chemotherapy response
	wt/wt	wt/mt	mt/mt	wt/wt	wt/mt	mt/mt	
							
Low	CRs/PRs	PRs/NRs	NRs	CRs/PRs	PRs/NRs	NRs	
Normal	CRs	CRs/PRs	NRs	CRs	CRs/PRs	NRs	
High	CRs/PRs	PRs	NRs	CRs/PRs	PRs	NRs	

Note: CRs complete responders; PRs partial responders; NRs non responders; wt wild type; mt mutant type; Null variants (mutant type) are represented by black circles; decreased-function (heterozygous) variants by gray circles; and fully functional variants (wild type) by white circles; the dash line indicates a whole-gene deletion.

2.15 Research lacunae and rationale of the study

This review of the literature reveals that, no previous study has examined the impact of transporter and metabolizer gene polymorphisms on clinical response and quality of life (QoL) in non-metastatic BC patients, specifically from the low BMI group receiving standard chemotherapy. This study highlights opportunities to improve clinical efficacy, safety, and QoL by exploring the pharmacogenetic associations of *ABC* and *CYP* genes in nutritionally neglected non-metastatic BC patients, particularly from a rural, socio-economic Indian context. The findings of this study is expected to aid in designing safer and more effective dosing regimens, reducing drug toxicity, and enhancing therapeutic efficacy and QoL in BC patients. It is anticipated that combining genetic evaluation with nutritional assessment would enable the development of optimized management strategies for BC, minimizing severe adverse drug reactions.

CHAPTER 3

AIM & OBJECTIVES

CHAPTER 3

AIM & OBJECTIVES

Aim and objectives of work

This study aims to investigate the pharmacogenetic influence of metabolizer and transporter gene polymorphisms on the clinical outcomes of BC patients (clinical efficacy, toxicity, QoL, overall survival, etc.) from varying hormonal receptor profiles, with a particular focus on the low BMI.

The aim of the work had been achieved through the following objectives:

Objective 1: Assessment the immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER2/neu) in BC patients.

Objective 2: Evaluation of the effects of the single nucleotide polymorphisms in *ABCB1, ABCC2* genes and their allele frequencies in response to the transport of tamoxifen and anthracycline-taxane chemotherapy (FEC/TAC/AC-T) in ER and PR positive BC patients.

Objective 3: Determination of association among *CYP2D6, CYP2C19, CYP2C9, CYP3A4* genes and their allele frequencies with respect to tamoxifen and anthracycline-taxane chemotherapy (FEC/TAC/AC-T) metabolism in ER and PR positive BC patients.

Objective 4: Assessment the clinical efficacy and quality of life in ER and PR positive BC patients in response to tamoxifen adjuvant therapy and anthracycline-taxane chemotherapy (FEC/TAC/AC-T).

Objective 5: Evaluation of the impact of low BMI on treatment response, quality of life, and genetic predisposition to altered drug transport, metabolism and therapy outcomes among BC patients.

Objective 6: Analysis of data for prediction and clinical management of breast cancer.

CHAPTER 4

MATERIALS & METHODS

CHAPTER 4

MATERIALS & METHODS

4.1 Study design

This non-randomized, prospective study evaluated the pharmacogenetic impact of metabolizer and transporter gene polymorphisms on the clinical efficacy of anthracycline-taxane based chemotherapy and tamoxifen adjuvant therapy in BC patients from different hormonal receptor profiles, with a particular focus on the low BMI group. This single-centered study included 148 (93% power) histopathologically confirmed breast carcinoma patients (AJCC stages I–III), treated at the surgical outpatient department of CNCI, tertiary cancer hospital in Eastern India. Surgical procedures, chemotherapy, and radiation therapy were took place at the Departments of Surgical, Medical, and Radiation Oncology respectively. Subsequent follow-up and data analysis occurred at the Department of Pathology and Cancer Screening. The study dataset comprised of socio-demographics (***Annexure-III***), clinico-pathological, gynecological and anthropometric parameters along with chemo-induced toxicity, QoL, survival outcome and pharmacogenetic analysis of *ABC* and *CYP* polymorphisms (**Figure 4.1**).

4.2 Ethics & informed consent

This study adhered to the guidelines outlined in the Declaration of Helsinki and obtained approval from the Institutional Ethical Committee [CNCI-IEC-DL-2020-6] (***Annexure-I***). Prior to participation, all patients provided written informed consent in their preferred language. For the illiterate patients, the study details were explained to them in their local language, either Bengali or Hindi, to ensure their understanding before consenting. Subsequently, a thumb impression was taken in the presence of a witness, a guardian, and the responsible physician (***Annexure-II***).

4.3 Patients eligibility

4.3.1 Inclusion criteria

The breast cancer patients were selected based on the following inclusion criteria:

1. Female patients aged 18–80 years.
2. Histopathologically proven newly diagnosed breast cancer.
3. Chemotherapy and hormone therapy naïve.
4. Must not have received prior radiotherapy.

5. Satisfactory bone marrow function, hepatic function, neurological function, kidney and cardiac function.
6. Normal platelet count (≥100,000/μL) along with blood coagulation parameters.
7. Not taking medicines likely to alter enzymes concerned.

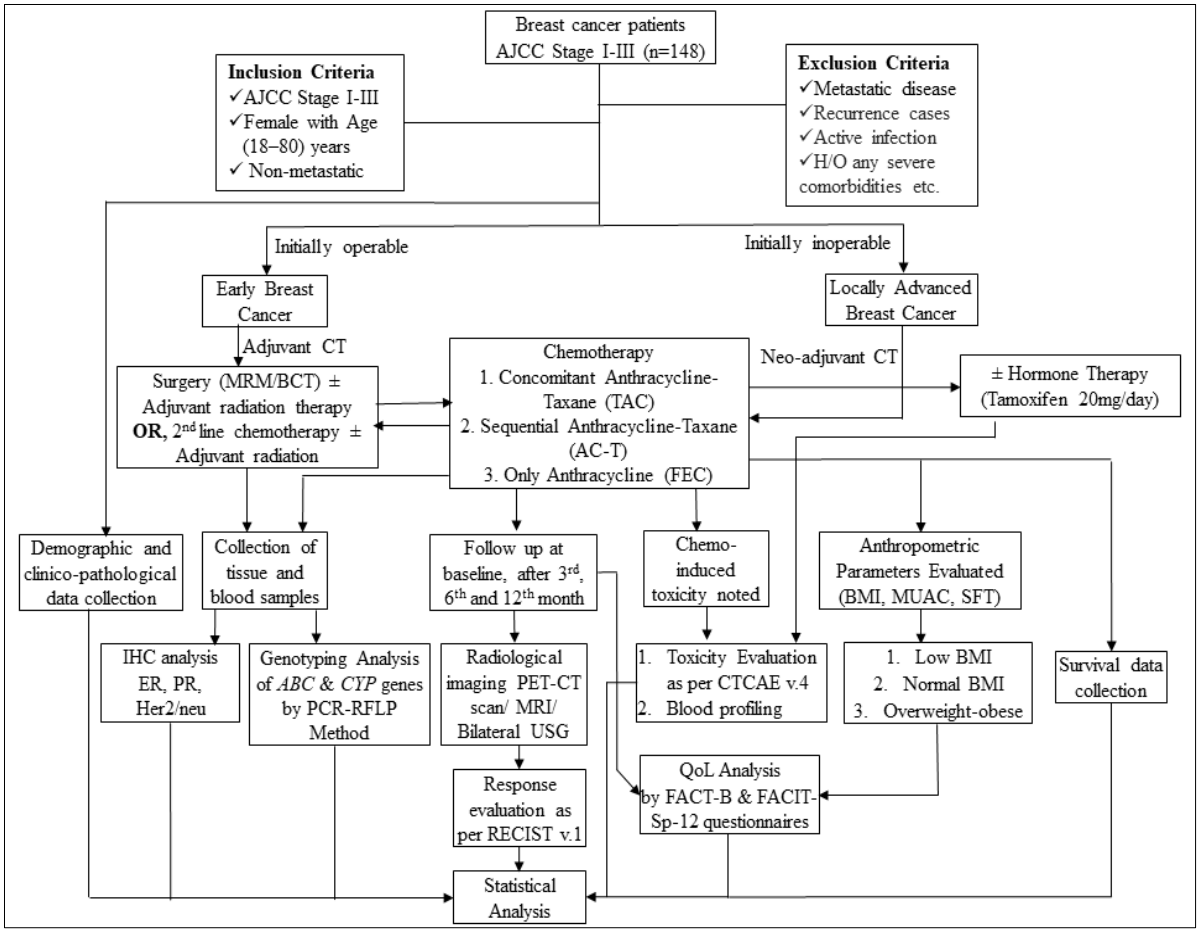


Figure 4.1 Schematic representation of study design

4.3.2 Exclusion criteria

1. Male BC patients.
2. Patients who had previously received a diagnosis of BC (recurrence cases), underwent radiotherapy and chemotherapy, or undergone total mastectomy.
3. Infections like tuberculosis (TB), human immune-deficiency virus (HIV), or any other autoimmune diseases and non-resolving active bacterial infections requiring parenteral antibiotic treatment.
4. Drug hypersensitivity and uncontrolled diabetes, cardiac failure, myocardial infarction in recent past, psychiatric illness and any other conditions.

5. In addition, patients having WBC count<2500 mm^3, neutrophil count <1500 mm^3 or platelet count <100 x 10^3 mm^3 were also excluded.
6. Patients with a history of deep vein thrombosis (blood clots in veins) or pulmonary embolism, elevated triglyceride levels, cataracts, stroke, clinically significant proteinuria, were also excluded from the study.
7. Pregnant or breast- feeding women and women who were at risk of becoming pregnant during the study period were not included.

4.4 Anthropometric measurements

Before chemotherapy administration, patients' height, weight, and BMI were recorded according to WHO standards: underweight/low BMI (<18.5 kg/m²), normal BMI (18.5 kg/m² to 22.9 kg/m²), or overweight to obese (≥23 kg/m²) ("Global Strategy on Diet, Physical Activity and Health - 2004," n.d.). Nutritional status was assessed through non-invasive methods, measuring mid upper arm circumference (MUAC) and skinfold thickness (SFT). MUAC, measured between the shoulder's tip and elbow midpoint (Jung et al., 2022), categorized patients into upper (>26.5 cm), mid (23.5-26.5 cm) and lower (<23.5 cm) groups. SFT involved skin folds, excluding muscle or fascia measured by clinician. This process repeated at least twice to address experimenter bias, with the final score based on the average of repetitions (Wells and Fewtrell, 2006).

4.5 Treatment procedure

4.5.1 Surgical approach and chemotherapy selection

Initially, operable patients underwent directly into adjuvant chemotherapy, with consideration for either breast conserving therapy (BCT) or modified radical mastectomy (MRM). In contrast, initially inoperable patients received neo-adjuvant chemotherapy. These patients were advised one of three chemotherapeutic regimens administered tri-weekly for six cycles: 1. FEC regimen (5-fluorouracil 500 mg/m^2 i.v. on day 1, epirubicin 60 mg/m^2 i.v. on day 1, and cyclophosphamide 500mg/m^2 i.v. on day 1 (Coombes et al., 1996); 2. Concomitant TAC regimen (Docetaxel 75mg/m^2 i.v. on day 1, Doxorubicin 50mg/m^2 i.v. on day 1, and Cyclophosphamide 500mg/m^2 i.v. on day 1 (Martín et al., 2006); 3. Sequential AC followed by Taxane regimen (Doxorubicin 50mg/m^2 i.v. on day 1, Cyclophosphamide 500mg/m^2 i.v. on day 1 for four cycles followed by Paclitaxel 175 mg/m^2 by IV infusion on day 1 every 21 days for 4 cycles) (Mamounas et al., 2005). The selection of the chemotherapy regimen was based on

institutional guidelines and clinician discretion. Pre-medication was advised to mitigate potential adverse events of chemotherapy, following the physician's recommendations.

4.5.2 Radiation therapy

Radiotherapy was tailored to patients based on tumor size and condition, typically administered at a standard dose of 50 Gray (Gy) in 25 daily fractions over 5 per weeks. The dose could be adjusted, allowing extended fractionation, such as 45 Gy in 25 daily fractions with additional 10 Gy boost doses in 5 fractions, or 50.4 Gy in 28 daily fractions (Provincial Health Services Authority, 2022). Post-mastectomy patients received radiation therapy if they met criteria like tumor size >5cm, any positive lymph nodes, or centrally located tumors with high-risk features. Adjuvant radiation therapy was standard for mastectomy patients with inadequate lymph node dissection and those underwent Breast Conserving Therapy (BCT).

4.5.3 Hormonal therapy

Pre-menopausal women were prescribed 20mg/day of tamoxifen for a duration of 5 years, followed by an additional 5 years of hormone therapy tailored to their menopausal status, as indicated by the ATLAS trial data (Davies et al., 2013). In the case of post-menopausal women, the approach involved switch therapy, comprised 2 years of tamoxifen followed by an Aromatase Inhibitor, as determined by the clinician's preference (***Annexure-V***). Patients with Her2-positive status received *trastuzumab* as part of their treatment plan.

4.6 Clinical response evaluation and categorization

Tumor burden, disease recurrence, and progression were assessed at baseline, after the 3rd and 6th cycles of chemotherapy using a combination of radiological techniques (whole-body CT scan, thorax imaging, breast MRI) and physical examinations following primary treatment completion, as per the protocol. In addition to CT scans, bilateral breast and axilla ultrasonography, bilateral diagnostic mammography, and thorax-abdomen CECT scans were performed when indicated. FDG-PET scans were reserved for patients with a high likelihood of micro-metastasis (Cardoso et al., 2020). Radiological and clinical examination were done to detect recurrence in adjuvant chemotherapy. The response to neo-adjuvant chemotherapy was categorized as complete responders (CRs), partial responders (PRs), and non-responders (NRs) based on RECIST (Response Evaluation Criteria in Solid Tumors) criteria version 1 (Eisenhauer et al., 2009).

- **Complete Response (CR):** Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10mm.
- **Partial Response (PR):** At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
- **Progressive Disease (PD):** At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study
- **Stable Disease (SDi):** Neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

BC patients who exhibited either a complete or partial response were collectively categorized under the Objective/Overall Response Rate (ORR) group. In contrast, patients with stable disease, progressive disease, those receiving palliative care, and non-evaluable patients were grouped together as non-responders (NRs).

4.7 Toxicity and symptom burden assessment

Toxicities linked with each chemotherapy regimen were evaluated utilizing standardized toxicity grading systems, specifically the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE v4) (Dueck et al., 2015).

The Edmonton Symptom Assessment System (ESAS) was used to evaluate symptom burden in BC patients. Participants rated on the severity of ten common symptoms, including pain, fatigue, nausea, anxiety, etc. on a numeric scale from 0 (none) to 10 (worst possible). The total symptom distress score was calculated to assess overall burden (Bruera et al., 1991).

4.8 Quality of Life assessments

Patients underwent individual assessments at four crucial time points: baseline (before chemotherapy), during the 3rd and 6th month of chemotherapy, and post-chemotherapy at the 12th month. Functional Assessment of Cancer Therapy - Breast (FACT-B) questionnaire tailored for BC patients to measure their QoL and Spiritual well-being was evaluated using the Functional Assessment of Chronic Illness Therapy - Spiritual Well-Being 12 (FACIT-Sp-12) questionnaire (Brady et al., 1997). The questionnaire encompassed 49 questions across six domains: spiritual, emotional, social, functional, physical well-being, and a specific set of questions designed for BC patients under the 'additional concern' domain (***Annexure-IV***).

4.9 Clinical endpoint

- **Progression-Free Survival (PFS):** It was defined as the time from randomization until first evidence of disease progression or death (Lebwohl et al., 2009).
- **Overall Survival (OS):** It was measured from the date of randomization/diagnosis till death (Lebwohl et al., 2009).
- **Disease-Free Survival (DFS):** It was defined as the time from randomization until evidence of disease recurrence (Kilickap et al., 2018).

Patients lost to follow-up, including those who did not returned for the last 6 months and unreachable by phone, were considered events in survival analysis. All patients were tracked for four years to determine OS.

- **Hazard Risk evaluation**

The hazard ratio was measured using the survival analysis to compare the rate of an event occurred between two groups over time. Hazard ratio (HR) reports the difference: HR < 1 indicates a lower risk, while HR > 1 indicates a higher risk of the event compared to the control group. A hazard ratio (HR) of 1 means there was no difference in risk between the two groups being compared—the event occurs at the same rate in both groups.

4.10 Collections of blood and tissue samples

For genetic analysis, 5 ml venous blood samples were collected, from each recruited patients in EDTA-coated vials to isolate genomic DNA from peripheral leukocytes. To minimize discomfort, blood samples were collected either during pre-operative preparation or during routine biochemical tests. Post-surgery, breast tumor samples (500mg-1g) were collected in 1X PBS (phosphate buffered saline) and 10% NBF (neutral buffered formalin) for histopathological analysis. Trained medical practitioners supervised the sample collection (**Figure 4.2)**.

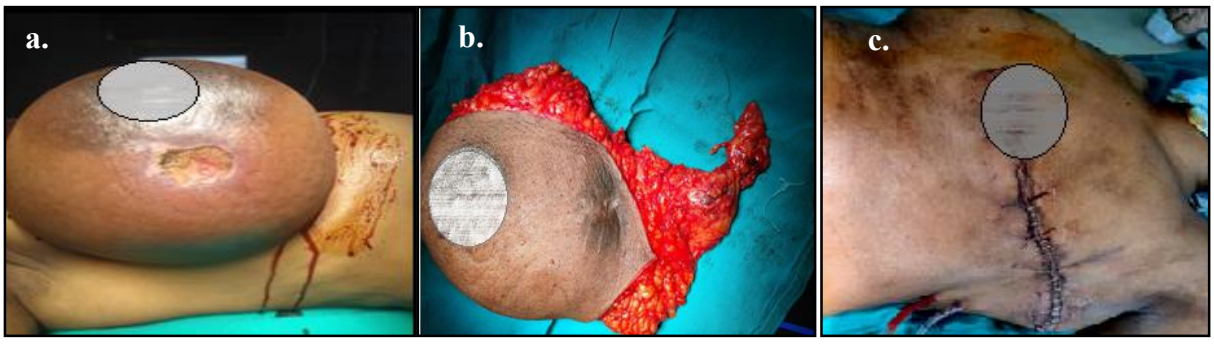


Figure 4.2 Breast tumor during different times of surgical interventions
a. Breast tumor before surgery; b. Surgically resected Modified Radical Mastectomy (MRM) specimen; c. Vacant chest-wall after removal of breast tumor

4.11 Histopathological analysis

Breast carcinoma tissues were fixed in 10% formalin and then was dehydrated in graded alcohol, acetone and xylene followed by paraffin embedding. After the paraffin blocks were prepared 5 μm thin sections were cut in the microtome and pasted on poly-L-Lysine coated slides. For histopathological analysis the slides were deparaffinized, rehydrated and then stained with hematoxylin (5mins) and eosin (2mins). The color was developed under running tap water. The slides were then cleared with alcohol, xylene and mounted with DPX (Fischer et al., 2008).

4.12 Immunohistochemistry (IHC)

Formalin fixed tissues were used in preparation of paraffin block. FFPE (Formalin Fixed Paraffin Embedded) tissue sections were used for immunohistochemical staining for ER, PR and HER2/neu protein. Their expression was performed with commercially available IHC Select- HRP/DAB Kit Millipore protocol. Deparaffinization was done by 4 Xylene changes, graded alcohol (100% to 30%) and then distilled water. Then the slides were heated in 0.01M citrate buffer, pH 6.0 in microwave oven for 10 minutes to retrieve antigen, followed by endogenous peroxidase blocking with 3% hydrogen peroxidase in water for 10 minutes. Non-specific binding was inhibited by applying blocking reagent provided in the kit for 5 mins and slides were not completely washed down. Monoclonal antibodies against ER (1:100), PR (1:100) and HER2/neu (1:150) was applied and kept at 4ºC overnight in a humid chamber. The next step was to apply secondary antibody followed by Streptavidin HRP (Horseradish Peroxidase) sequentially and incubation for 10 minutes in each step. Then the chromogen DAB (3,3'- Diaminobenzidine) was freshly prepared in a solution for application to the sections (10 mins in dark) and then counterstained with Meyer's haematoxylin for 1 min. The slides were then dehydrated with graded alcohol and xylene and mounted with DPX (McGrogan et al., 2014; Stahl et al., 2017). Analysis of immunostaining was performed using a Zeiss microscope and scored according to the system. The presence of nuclear or cytoplasmic immunolocalization for each antibody, *i.e.,* for ER, PR and HER2/neu was scored as: 3+, strong. 2+, moderate; 1+, weak; For Her2/neu with a 2+ score considered as equivocal results underwent the FISH test and a 1+ score, considered as negative Her2/neu status (Rhodes et al., 2000).

4.13 Molecular evaluation

4.13.1 Quantification of genomic DNA

The genomic DNA was isolated from collected blood samples using the phenol-chloroform extraction method (Sambrook et al., 2012). The samples were loaded on a 1 % agarose gel. λ DNA digested with *Hind*III used as a marker. DNA quantification was done to check the concentration of extracted genomic DNA. The size and quantity of DNA fragments was estimated by it.

4.13.2 Selection of transporter and metabolizing genes

PharmGKB, a NIH-funded resource accessible at https://www.pharmgkb.org/, is dedicated to exploring the impact of human genetic variation on medication response. It collects, curates, and shares information on clinically relevant gene-drug associations and genotype-phenotype relationships. This included flagged SNPs like rs1128503, rs2032582, rs1045642 in *ABCB1*; rs145008610 *ABCC2*; rs1799853, rs4917639 in *CYP2C9*; rs4244285 in *CYP2C19*; rs2740574 in *CYP3A4* and rs3892097, rs1065852 in *CYP2D6* gene were sourced from the National Center for Biotechnology Information (NCBI) SNP database (dbSNP) (Chang et al., 2009; Abdul Aziz et al., 2018**;** Gutierrez-Rubio et al., 2015**;** Shimada et al., 2009; Chen et al., 2007**;** Mokhosoev et al., 2024) (**Figure 4.3**).

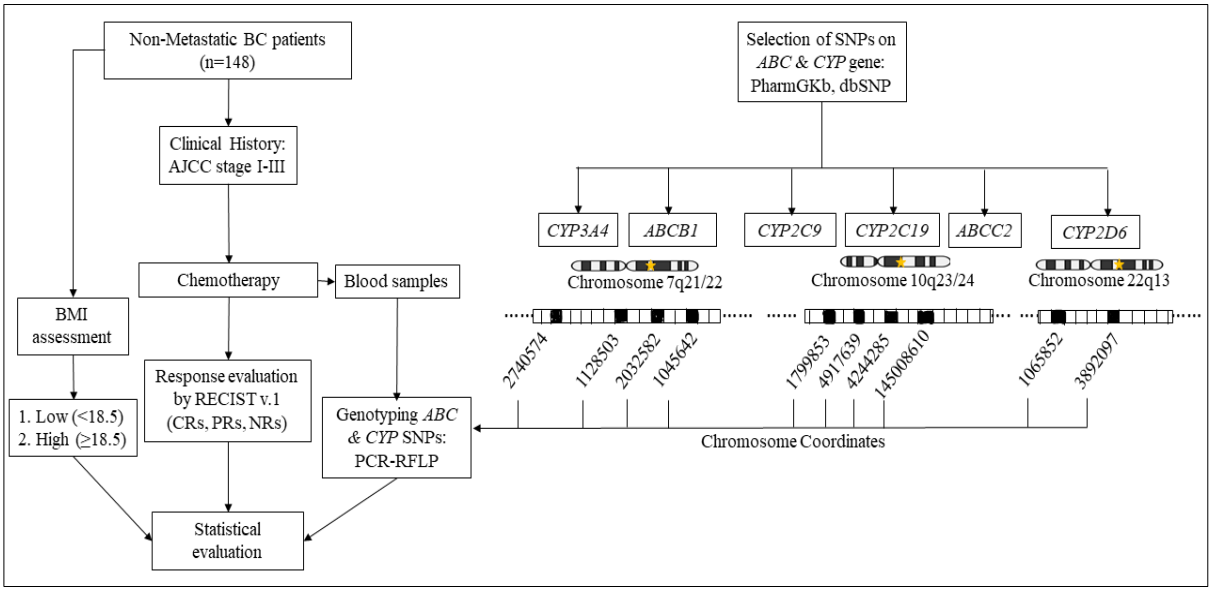


Figure 4.3 Selection of flagged SNPs in transporter and metabolizing genes

4.13.3 PCR amplification

A reaction mixture of 25µl was prepared using the specified reagents and their respective working concentrations. Polymerase Chain Reaction (PCR) reactions (25 µl) utilized 50–500 ng of DNA, including 2.5 µl of PCR reaction buffer, 1 µl of 2.5mM dNTPs, 10 pmol of respective primers, and 0.3 U/µl of Taq polymerase (APS Lab, Pvt. Limited) (**Table 4.1**). The amplification process occurred in a BIO-RAD C1000 Touch Thermal Cycler. *GAPDH*, being a constitutive gene, was crucial for ensuring the optimization and suitability of the obtained DNA for subsequent applications.

Table 4.1 Reagents required for PCR amplification

Reagents	Working Concentration	Total 25 µl reaction
dNTP mix	25mM	1.0 µl
Taq Buffer	1X	2.5 µl
Forward primer	0.2 µM	0.25 µl
Reverse Primer	0.2 µM	0.25 µl
Taq polymerase	3 units /µl	0.25 µl
Distilled Water	-	20.75 µl
Template DNA	(50-500ng/µl)	1.0 µl

A negative control was established by including all the reagents in the PCR setup except for the template DNA. The thermal profile for the PCR was configured as per the specifications outlined in **Table 4.2**.

Table 4.2 Different steps of thermal cycler reaction

Steps	Temperature	Time
1.Initial denaturation	94°C	11 minutes
2.Denaturation	94°C	30 seconds
3.Annealing	50°-60°C	45 seconds
4.Extension	72°C	30 seconds
5.Final Extension	72°C	1 minutes

Steps 2 to 4 were repeated for 34 cycles. The PCR products were stored at 4°C for further use.

4.13.4 Genotyping of Transporter and Metabolizing genes

Genotyping of transporter genes (*ABCB1* and *ABCC2*) and metabolizing genes (*CYP3A4, CYP2C9, CYP2C19 and CYP2D6*) was carried out through PCR coupled Restriction Fragment Length Polymorphism (RFLP). This involved using specific forward and reverse primer

sequences, relevant enzymes, and band patterns (**Table 4.3**). The genomic alterations analysis (polymorphism) of the amplified products were custom-digested by restriction enzymes selected from the NEBcutter tool ("NEBcutter 3.0," n.d.), then electrophoresed on a 12% Native Polyacrylamide Gel Electrophoresis (PAGE) and analyzed with a 50bp DNA ladder for precise characterization and visualized under Chemi-doc. Identification and classification of *ABCB1, ABCC2, CYP2C9, CYP2C19, CYP3A4* and *CYP2D6* genotypes were based on the observed DNA fragments. Various cutting patterns post-digestion were outlined in **Table 4.4**.

Table 4.3 The transporter and metabolizer genes with their primer sequences, annealing temperature and amplicon size

Sl. No.	Genes/ Accession No.	Primer Sequence	Annealing Temperature	Amplicon size (bp)
1.	*ABCB1* (rs1128503)	Forward 5'TGTGTCTGTGAATTGCCTTGA3' Reverse 5'CATCTCACCATCCCCTCTGT3'	55°C/45sec	181
2.	*ABCB1* (rs1045642)	Forward 5'TGTTTTCAGCTGCTTGATGG3' Reverse 5'GCATGTATGTTGGCCTCCTT3'	56°C/45sec	194
3.	*ABCB1* (rs2032582)	Forward 5'TGTTGTCTGGACAAGCACTGA3' Reverse 5'GTCCAAGAACTGGCTTTGCT3'	56°C/45sec	239
4.	*ABCC2* (rs145008610)	Forward 5'CCGTATCAGGTTTGCCAGTT3' Reverse 5'CCTCCCACCGCTAATATCCA3'	54°C/45sec	191
5.	*CYP2C9* (rs1799853)	Forward 5'AATTTTGGGATGGGGAAGAG3' Reverse 5'CCGCTTCACATGAGCTAACA3'	55°C/45sec	213
6.	*CYP2C9* (rs4917639)	Forward 5'TGGGCACTTGGATTACTTTCA3' Reverse 5'TCACAGGGTCAGGAGTTTGA3'	53°C/45sec	199
7.	*CYP2C19* (rs4244285)	Forward 5'CAACCAGAGCTTGGCATATTG3' Reverse 5'TAAAGTCCCGAGGGTTGTTG3'	54.5°C/45sec	210
8.	*CYP3A4* (rs2740574)	Forward 5'CTGGGTTTGGAAGGATGTGT3' Reverse 5'TGTTACTGGGGAGTCCAAGG3'	55°C/45sec	223
9.	*CYP2D6* (rs3892097)	Forward 5'AAGAAGTCGCTGGAGCAGTG3' Reverse 5'CACGGCTTTGTCCAAGAGA3'	54°C/45sec	188
10.	*CYP2D6* (rs1065852)	Forward 5'TATGGGGCTAGAAGCACTGG3' Reverse 5'ACCTGGTCGAAGCAGTATGG3'	56°C/45sec	320
11.	*GAPDH*	Forward 5'GACAGTCAGCCGCATCTTCT3' Reverse 5'GCGCCCAATACGACCAAATC3'	56°C/45sec	196

Table 4.4 Table shows different genes with their respective restriction enzymes along with band patterns

Sl. No.	Genes/ Accession No.	Restriction Enzymes	Expected patterns to be observed after enzyme digestion
1.	*ABCB1* (rs1128503)	*Hpy*188I	CC- 181 CT- 181, 120, 61 TT- 120, 61
2.	*ABCB1* (rs1045642)	*Hpy*188III	CC- 194 CT- 194, 162, 32 TT- 162, 32
3.	*ABCB1* (rs2032582)	*Bse*YI	GG- 239 GT/GA- 239, 194, 45 TT/AA/AT- 194, 45
4.	*ABCC2* (rs145008610)	*Hpy*188I	CC- 191 CA- 191, 139, 52 AA- 139, 52
5.	*CYP2C9* (rs1799853)	*Hpy*CH4III	CC- 213; CT- 213, 179, 34 TT- 179, 34
6.	*CYP2C9* (rs4917639)	*Hpy*166II	AA- 199 AC- 199, 154, 45 CC- 154, 45
7.	*CYP2C19* (rs4244285)	*Bst*NI	GG- 210 GA- 210, 155, 55 AA- 155, 55
8.	*CYP3A4* (rs2740574)	*Mse*I	AA-223 AG-223, 159, 64 GG-159, 64
9.	*CYP2D6* (rs1065852)	*Sma*I	CC-188 CT- 188, 133, 59 TT- 133, 59
10.	*CYP2D6* (rs3892097)	*Bst*NI	GG- 302 GA- 302, 160, 160 AA- 160, 160

4.14 Statistical Analysis

Descriptive statistics were utilized to assess the frequency, percentage, mean values, standard deviations (SD), and standard error (SE) for patients' socio-demographic, clinico-pathological, and gynecological characteristics, along with symptoms and chemotherapy-induced toxicities. Cross-tabulations using the chi-square (χ^2) test examined the associations between clinico-pathological characteristics across different BMI classes and the immunohistochemical (IHC) expression of ER, PR, and HER2/neu. IHC scoring was validated through three independent iterations for accuracy. A heat-map was generated based on the frequency distribution of

clinico-pathological characteristics and their corresponding χ^2 values. Differential treatment responses within and between BMI groups were analyzed using the χ^2 test, while proportion comparisons were conducted using the Standard Normal Deviate (Z) test. Mean differences between groups were assessed using an unpaired t-test. The ESAS was analyzed using both χ^2 tests and descriptive statistics (mean±SD). A two-way multivariate analysis of variance (MANOVA) was performed to evaluate the effects of clinical response, chemotherapy mode, and BMI group on QoL domains, considering time as a repeated measure and QoL domains vs. groups as a fixed factor. Greenhouse-Geisser and Wilk's Lambda significance values (***p<0.05***) were used, with results reported as mean±SD.

Genotype frequency distribution among patients was analyzed using descriptive statistics, while allele frequencies were determined using the Hardy-Weinberg equilibrium. Associations between genetic models of transporter and metabolizer genotypes and chemotherapy response groups—stratified by BMI classes—were evaluated by calculating Odds Ratios (OR) and 95% Confidence Intervals (CI).

Kaplan-Meier survival analysis (log-rank test) was performed over a 48-month follow-up to assess the impact of BMI classes, chemotherapy regimens, and genotype groups on survival outcomes. Additionally, Cox regression analysis was conducted to estimate HR for death risk factors, considering patient baseline characteristics and genotypes as potential confounders. All statistical analyses were performed using IBM SPSS Statistics (Version 26.0; IBM Corporation, Armonk, NY, USA) and GraphPad Prism (Version 5.0; GraphPad Software, Inc., now part of Dotmatics, California, USA) with ***p<0.05*** considered statistically significant.

CHAPTER 5

RESULTS

CHAPTER 5

RESULTS

5.1 Clinical Evaluation

5.1.1 Socio-demographic details of the patients

A total of 148 patients were enrolled in this prospective study. The predominant age group among the participants was 41-60 years (60.81%), with the mean age recorded at (49.68 ± 11.42) years. A notable portion of the participants (68.9%) did not have formal education, and the majority originated from rural setup (70.9%) dispersed throughout diverse regions of the state. Even though, majority of the patients impoverished low socio-economic status (Rs.2000-5000/-; 67.58%) but most of the patients did not have any family history of cancer (85.81%).

The anthropometric parameters revealed that the mean body weight was (53.945±10.90) kg, and the MUAC was (22.762±1.92) cm. Majority of patients had low BMI (53.38%), while others had normal (38.51%) and only a few were classified as overweight or obese (8.11%). Notably, individuals with a low BMI also exhibited low MUAC (<23.5 cm; 52.7%) and poor SFT (11.45±6.4; 54.4%) **(Table 5.1)**.

Table 5.1 Socio-demographic distribution of BC patients

Characteristics (n=148)		**Frequency (%)**
Age (Years)	20-40	35 (23.64)
	41-60	**90 (60.81)**
	61-80	23 (15.55)
Education	**Illiterate**	**102 (68.9)**
	Primary/Secondary School	46(31.1)
Occupation	**Housewife/ Unemployed**	**134(90.54)**
	Self- employed/ Business/ Professional/Desk job	6(4.05)
	Others	8(5.41)
Monthly income of family (INR)	<2000	24(16.21)
	Rs2000< Rs ≥5000/-	**100(67.58)**
	Rs5000< Rs ≥10000/-	20(13.51)
	>Rs10000/-	4(2.7)
Religion	Hindu	93(62.83)
	Muslim	53(35.82)
	Others	2(1.35)
Setup	**Rural**	**105(70.9)**
	Urban	43(29.1)
Marital Status	Unmarried	4(2.7)
	Married	**116(78.4)**
	Widowed	28(18.9)
Family History of Cancer	**No family history of cancer**	**127(85.81)**
	1stdegree relatives with cancer history	21(14.18)

Anthropometric parameters		
BMI Class (kg/m^2)	**Low BMI (<18.5)**	**79(53.38)**
	Normal BMI (18.5-22.9)	57 (38.51)
	Overweight to Obese class (≥23)	12 (8.11)
MUAC (cm)	**Lower MUAC group (<23.5)**	**78 (52.70)**
	Mid MUAC group (23.5-26.5)	59 (39.86)
	Upper MUAC group (>26.5)	11 (7.43)
n= Number of Patients; Bold lettering denotes highest percentage; BMI-Body Mass Index; MUAC- Mid Upper Arm Circumference; INR-Indian Rupees.		

5.1.2 Clinico-pathological and gynaecological aspects of BC patients

During the time of diagnosis, the most common initial symptoms reported by patients were a palpable lump (98%), redness (50%), mastalgia (50%) and itching (48%), etc. (**Figure 5.1**). The majority of diagnosed patients were at AJCC stage II (54.05%) with grade II tumors (83.79%), primarily falling within the tumor size range between >2cm to ≤5cm (50%; T2). A high proportion had at least one positive lymph node metastasis (71.6%; N1), contributing to an overall positive lymph node status (81.8%). The affected breast site distribution was relatively equal. Infiltrating ductal carcinoma (IDC) was the most common histological subtype (94.6%). Most patients had an ECOG score of 1 (82.8%), underwent modified radical mastectomy (MRM, 88.51%), and received one of the following anthracycline-taxane based chemotherapies (FEC/TAC/AC-T). Majority patients received adjuvant chemotherapy (72.98%), whereas few underwent neo-adjuvant chemotherapy (27.02%). Treatment response of the patients revealed a majority of the cohort was non-responders to the therapy (49.33%). However, the gynaecological history of the patients showed high prevalence of early menarche (≤12 years; 72.7%) and menopause (<45 years; 36.4%). Additionally, the majority were post-menopausal (56.75%) and had a well breast feeding history (66.89%) (**Table 5.2**).

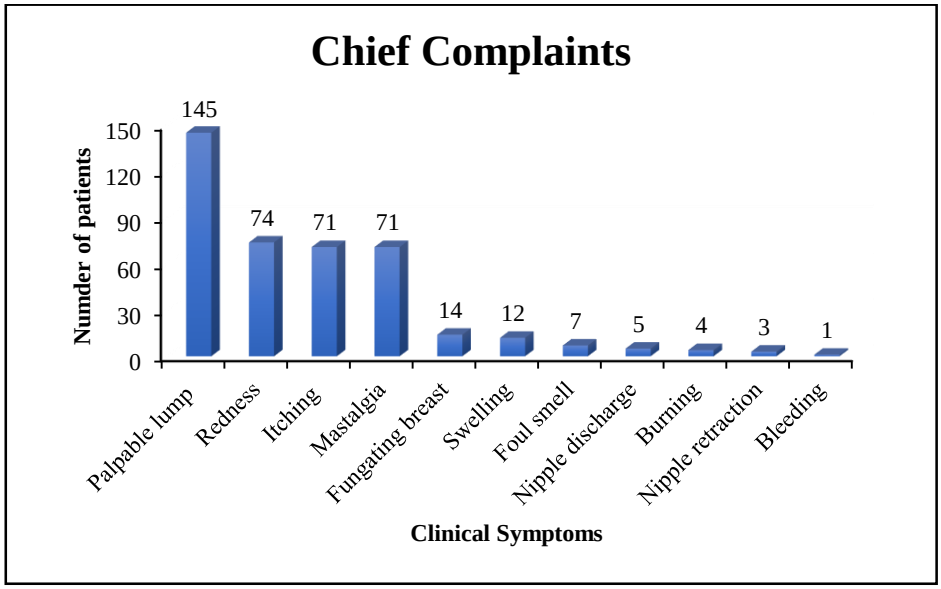


Figure 5.1 Frequency distribution of baseline symptoms

Table 5.2 Clinico-pathological and gynaecological characteristics of BC patients

Characteristics(n=148)		Frequency (%)
AJCC Stage	Stage I	20(13.51)
	Stage II	**80(54.05)**
	Stage III	48(32.43)
Grade	Grade I	9(6.08)
	Grade II	**124(83.79)**
	Grade III	15(10.13)
Size of tumor mass (pre-treatment)	T1 (≤2cm)	2(1.4)
	T2(>2cm to ≤5cm)	**74(50.0)**
	T3 (>5cm)	31(20.9)
	T4 (Direct extension to the chest wall or skin)	34(23.0)
	TX	7(4.7)
Regional Lymph node metastasis	N0	24(16.2)
	N1	**106(71.6)**
	N2	10(6.8)
	N3	8(5.4)
Lymph Node Status	**Positive**	**121(81.8)**
	Negative	27(18.2)
Breast Site	Left	71(47.97)
	Right	77(52.03)
Tumor Histology	**IDC**	**140(94.6)**
	ILC	8(5.4)
ECOG score at the time of study entry	Asymptomatic: Fully active (Score 0)	20(13.52)
	Symptomatic: Restricted in physically strenuous activity (Score 1)	**122(82.8)**
	Symptomatic: 50% IB bed during the day (Score 2)	6(5.08)
Treatment Modality	Surgery + Radiotherapy + Chemotherapy	21(14.18)
	Chemotherapy	3(2.02)
	Chemotherapy + Surgery	54(36.49)
	Surgery + Chemotherapy	66(44.6)
	Surgery + Chemotherapy + Radiotherapy + Hormone therapy	4(2.71)
Surgery	BCS	2(1.35)
	MRM	**131(88.51)**
	Toilet Mastectomy	9(6.09)
	Palliative Mastectomy	4(2.7)
	No surgery	2(1.35)
Treatment Regimen	FEC	33(22.30)
	TAC	50(33.78)
	AC followed by Paclitaxel (AC-T)	65(43.92)
Tamoxifen	Yes	12(9.11)
	No	136(90.89)

Therapy	**Adjuvant Chemotherapy**	**108(72.98)**
	Neo-adjuvant Chemotherapy	40(27.02)
Clinical Response[¥]	Complete Responders	57(38.51)
	Partial Responders	18(12.16)
	Non-responders	**73(49.33)**
Gynaecological History		
Age at menarche (Years)	Never had menses	3(2.5)
	<=12	**88(72.7)**
	>12	33(24)
Age at menopause (Years)	**<45**	**44(36.4)**
	≥45	26(21.5)
	Not attained	51(42.1)
Menopausal status	Pre-menopausal	64(43.24)
	Post-menopausal	**84(56.75)**
Breast Feeding History	**Well**	**99 (66.89)**
	Moderate	8 (5.40)
	Poor	8 (5.40)
	Never	6 (4.05)
	Unknown	27 (18.24)

n= Number of Patients; Bold lettering denotes highest percentage; AJCC-American Joint Committee on Cancer; IDC- Infiltrating Ductal Carcinoma; ILC- Infiltrating Lobular Carcinoma; ECOG-Eastern Cooperative Oncology Group; BCS-Breast Conserving Surgery; MRM-Modified Radical Mastectomy; FEC-5fluorouracil + Epirubicin + Cyclophosphamide; TAC- Docetaxel + Doxorubicin + Cyclophosphamide; AC-T- Doxorubicin + Cyclophosphamide + Paclitaxel.

5.1.3 Stratification of clinico-pathological characteristics across BMI categories

A cohort of non-metastatic BC (n=148) were stratified by BMI classes. The low BMI (<18.5) group exhibited the highest patients in the age range 41-60 (51.1%) and AJCC Stage II (54.3%) with Grade II tumors (51.61%) were most prevalent, and IDC (52.1%) was the most frequent histological subtype and mostly underwent MRM (52.67%). Similarly, in the normal BMI group (18.5-22.9), the 41-60 age group remained predominant (45.55%), with AJCC Stage II (39.5%) and Grade II tumors (41.1%) also being the most common, and IDC (40%) was again the most prevalent histological subtype, underwent MRM (40.45%). However, no significant association was observed between the BMI groups in terms of these clinico-pathological parameters.

Based on treatment responses, patients in the low BMI group had the highest proportion of NRs (83.56%), whereas the normal BMI group contained the majority of CRs (75.43%) ***(p<0.0001)***. Correspondingly, the lower MUAC (<23.5 cm) group was predominant (83.33%) in the low BMI category, while in the normal BMI, the mid MUAC (23.5-26.5) cm class was the most prevalent (74.57%), and the MUAC groups also varied significantly across BMI categories (***p<0.0001***) **(Table 5.3)**.

Table 5.3 Stratification of clinico-pathological characteristics among different BMI classes

Characteristics (n=148)		BMI Class (kg/m^2) Frequency (%)			
		Low (<18.5) (n=79)	**Normal (18.5-22.9) (n=57)**	**Overweight/ Obese (≥23) (n=12)**	***p-value****
Age (Years)	20-40	21 (60)	10 (28.6)	4 (11.4)	*0.372*
	41-60	**46 (51.1)**	**36 (45.55)**	8 (3.33)	
	61-80	12 (52.17)	11 (39.13)	-	
AJCC Stage	Stage I	9 (45)	10 (50)	1 (5)	*0.491*
	Stage II	**44 (54.3)**	**32 (39.5)**	5 (6.2)	
	Stage III	26 (55.3)	15 (31.9)	6 (12.5)	
Grade	I	7 (77.8)	1 (11.1)	1 (11.1)	*0.424*
	II	**64 (51.61)**	**51 (41.1)**	9 (7.3)	
	III	8 (53.3)	5 (33.3)	2 (13.3)	
Tumor histology	**IDC**	**73 (52.1)**	**56 (40)**	11 (7.9)	*0.297*
	ILC	6 (75)	1 (12.5)	1 (12.5)	
Surgery	BCS	2 (100)	-	-	-
	MRM	**69 (52.67)**	**53 (40.45)**	9(.87)	
	Toilet Mastectomy	5 (55.56)	3 (37.5)	1 (12.5)	
	Palliative Mastectomy	1 (25)	2 (50)	1(25)	
	No surgery	2 (100)	-	-	
Chemotherapy	FEC	18 (54.54)	12 (36.36)	3 (9.09)	*0.997*
	TAC	26 (52)	20 (40)	4 (8)	
	AC-T	**35 (53.85)**	25 (38.46)	5 (7.69)	
Treatment Response	**Complete Responders**	10 (17.54)	**43 (75.43)**	4 (7.01)	***<0.00001***
	Partial Responders	8 (44.44)	4 (22.22)	6 (33.33)	
	Non-Responders	**61 (83.56)**	10 (13.69)	2(2.73)	
MUAC (cm)	**Lower group (<23.5)**	**65 (83.33)**	10 (12.82)	3(3.85)	***<0.0001***
	Mid group (23.5-26.5)	9 (15.25)	**44 (74.57)**	6 (10.16)	
	Upper group (>26.5)	5 (45.45)	3 (27.27)	3 (27.27)	

*n= Number of Patients Bold lettering denotes p value ≤ 0.05 and highest percentage; *Pearson Chi-square.*

5.1.4 Treatment response distribution across BMI groups

After identifying a significant correlation between treatment responses and BMI categories, further analysis was conducted to understand the individual effects within each BMI class. Interestingly, the chemotherapy responses (CRs, PRs, and NRs) exhibited a highly significant difference (**χ^2= 75.39; Z-score= 6.17**) within the low BMI (***p=4.259^{e-14}***) and the normal BMI group (***p=2.2^{e-16}***). However, in the obese category, the difference was not statistically significant (*p=0.716*) (**Figure 5.2**).

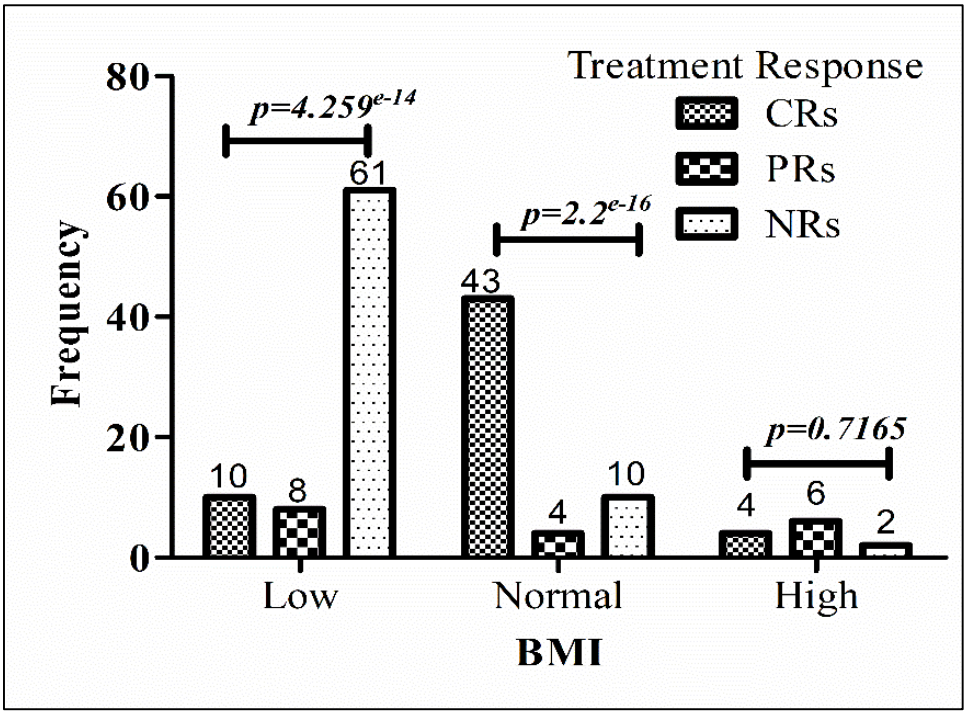


Figure 5.2 Comparison of treatment responses in non-metastatic BC patients stratified with respect to BMI

Note: Chi-square (χ^2=75.39) test showed that there was significant association between BMI and treatment response (p<0.0001). Thus, LVEF was more or less equally distributed over the number of risk factors. NRs and PRs were significantly higher that CRs among the patients with underweight (Low BMI) (Z=6.17; p<0.0001).

5.2 Histopathological study of breast tumor tissue

5.2.1 Association of clinical parameters with ER, PR, and HER2/neu

The histological subtypes of the BC samples were confirmed using H&E staining, and the molecular expression of ER, PR, and Her2/neu in the patient cohort was validated by IHC which revealed (54.73%, 45.27%) were ER & PR positive, respectively, while (45.27% & 54.73%) were ER and PR negative. Furthermore, (34.46%) tested positive for Her2/neu with a 3+ score, and (12.16%) expressed +2 score with equivocal results underwent the FISH test for further confirmation. Additionally, (53.38%) demonstrated a 1+ score, indicating a negative Her2/neu status. (**Figure 5.3**).

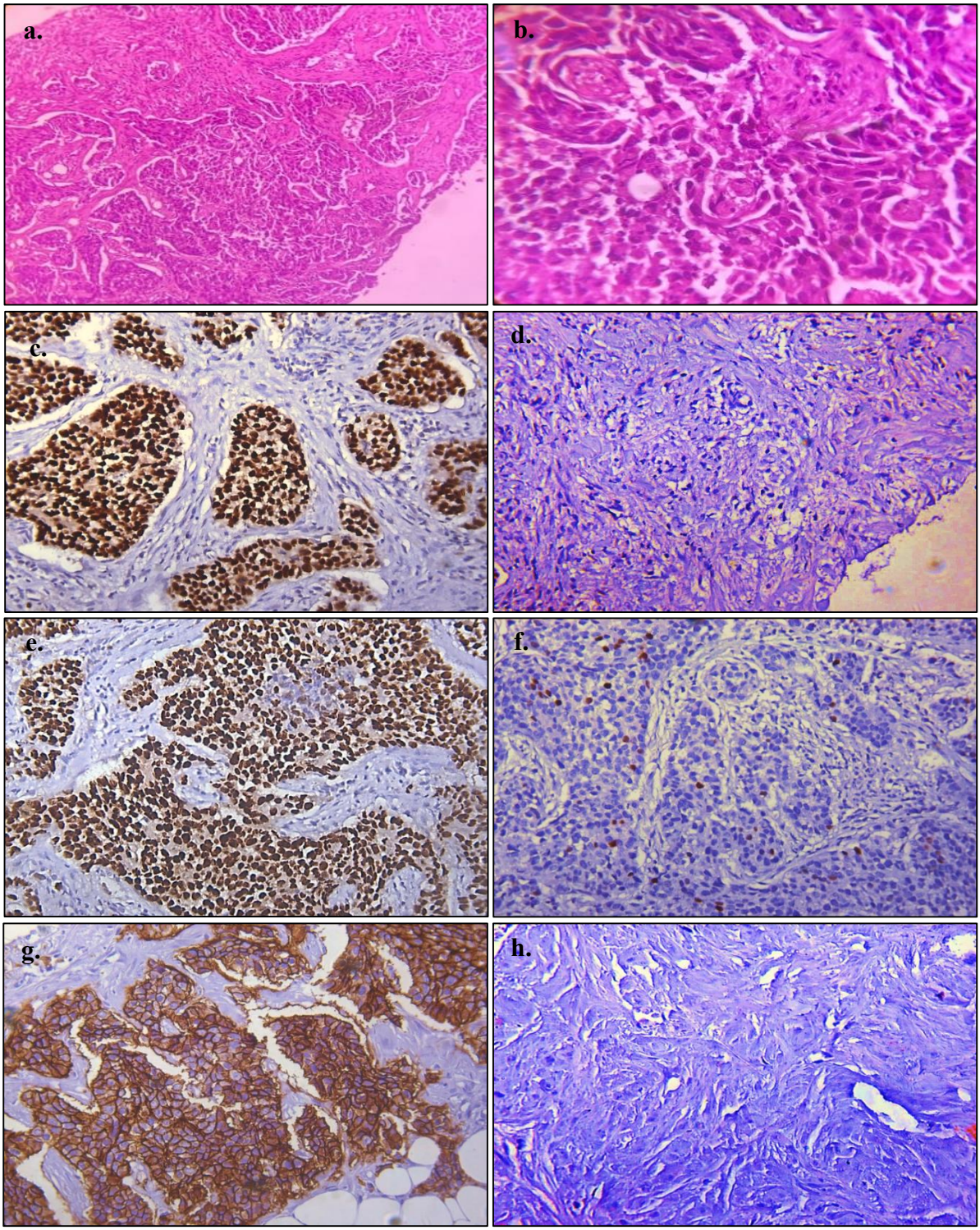


Figure 5.3 Hematoxylin-Eosin staining of breast tumor tissue and Immunohistochemistry of ER, PR, HER2/neu

(a & b)- Infiltrating Ductal Carcinoma 10X & 20X; (c & d)- Nuclear expression of ER (+ve/-ve); (e & f)- Nuclear expression of PR (+ve/-ve);(g & h)- Cytoplasmic expression of Her2/neu (+ve/-ve).

The association study of ER, PR, Her2 expression in breast tumor with clinical parameters revealed that, the positive ER expression was predominant in CRs (64.9%), whereas negative

expression of ER was higher among NRs (56.2%) (***p=0.031***). Likewise, BMI was also significantly associated with ER expression, where normal BMI patients (50.6%) were predominant in ER positive group, and low BMI patients (67.2%) were predominant in ER negative group (***p=0.004***). However, no significant associations were observed for ER, PR, and Her2/neu in any other clinical parameters: AJCC stage, tumor grade, size of tumor mass, lymph node status, regional lymph node metastasis, menopausal status, etc. (*p>0.05*) (**Table 5.4)**.

Table 5.4 Association between clinical parameters with expression of ER, PR and HER2/neu

Clinical parameters (n=148)	ER		PR		HER2/neu		
Expression pattern Frequency (%)	**Positive**	**Negative**	**Positive**	**Negative**	**Strong (Score 3+)**	**Equivocal (Score 2+)**	**Weak (Score 1+)**
	81(54.73)	67(45.27)	67(45.27)	**81(54.73)**	51(34.46)	18(12.16)	**79(53.38)**
AJCC Stage							
Stage I (n=20)	7(35)	13(65)	9(45)	11(55)	5(25)	3(15)	12(60)
Stage II (n=80)	**47(58.75)**	**33(41.25)**	**35(43.7)**	**45(56.3)**	**33(41.3)**	**12(15)**	**35(43.7)**
Stage III (n=48)	27(56.25)	21(43.75)	23(47.9)	25(52.1)	13(27.1)	4(8.3)	32(66.6)
p-value	*0.303*		*0.915*		*0.262*		
Tumor grade							
Grade 1 (n=9)	3(33.3)	6(66.7)	3(33.3)	6(66.7)	2(22.2)	1(11.1)	6(66.7)
Grade 2 (n=124)	**72(58.1)**	**52(41.9)**	**61(49.2)**	**63(50.8)**	**45(36.3)**	**16(12.9)**	**63(50.8)**
Grade 3 (n=15)	6(40)	9(60)	3(20)	12(80)	4(26.7)	1(6.7)	10(66.7)
p-value	*0.171*		*0.076*		*0.706*		
Size of tumor mass (pre-treatment)							
T1 (n=2)	1(50)	1(50)	-	2(100)	-	1(50)	1(50)
T2(n=74)	**41(55.4)**	**33(44.6)**	**35(47.3)**	**39(52.7)**	**31(41.9)**	**10(13.5)**	**33(44.6)**
T3(n=31)	15(48.4)	16(51.6)	13(41.9)	18(58.1)	11(35.5)	2(6.5)	18(58.1)
T4(n=34)	20(58.8)	14(41.2)	17(50)	17(50)	6(17.6)	3(8.8)	25(73.5)
TX(n=7)	4(57.1)	3(42.9)	2(28.6)	5(71.4)	3(42.9)	2(28.6)	2(28.6)
p-value	*0.941*		*0.556*		*0.068*		
Lymph node status							
Positive (n=121)	**67(55.3)**	**54(44.7)**	**55(45.5)**	**66(54.5)**	**42(34.7)**	**17(14.1)**	**62(51.2)**
Negative (n=27)	14(51.8)	13(48.2)	12(44.4)	15(55.6)	9(33.3)	1(3.7)	17(63)
p-value	*0.577*		*0.776*		*0.232*		
Regional lymph node metastasis							
N0(n=24)	11(45.8)	13(54.2)	10(41.7)	4(58.3)	8(33.3)	1(4.2)	5(62.5)
N1(n=106)	**56(52.8)**	**50(47.2)**	**48(45.2)**	**8(54.8)**	**37(34.9)**	**16(15.1)**	**3(50)**
N2(n=10)	8(80)	2(20)	5(50)	(50)	5(50)	1(10)	(40)
N3(n=8)	6(75)	2(25)	4(50)	(50)	1(10)	-	(70)
p-value	*0.268*		*0.973*		*0.204*		
Menopausal status							
Pre-menopausal (n=64)	32(50)	32(50)	25(39.1)	39(60.9)	22(34.4)	9(14.1)	33(51.6)
Post-menopausal (n=84)	**49(58.3)**	**35(41.7)**	**42(50)**	**42(50)**	**29(34.5)**	**9(10.7)**	**46(54.8)**
p-value	*3.313*		*0.185*		*0.817*		
Treatment response							
CRs(n=57)	**37(64.9)**	20(35.1)	26(45.6)	31(54.4)	22(38.6)	5(8.8)	30(52.6)
PRs(n=18)	12(66.7)	6(33.3)	8(44.4)	10(55.6)	5(27.8)	2(11.1)	11(61.1)
NRs(n=73)	32(43.8)	**41(56.2)**	**33(45.2)**	**40(54.8)**	**24(32.9)**	**11(15.1)**	**38(52.1)**
p-value	***0.031***		*0.996*		*0.761*		
BMI							
Low (n=79)	34(42)	**45(67.2)**	**33(49.3)**	**6(56.8)**	**25(49)**	**12(66.7)**	**42(53.2)**
Normal (n=57)	**41(50.6)**	16(23.9)	31(46.3)	6(32.1)	20(39.2)	6(33.2)	31(39.2)
High (n=12)	6(7.4)	6(9)	3(4.5)	(11.1)	6(11.8)	-	6(7.6)
p-value	***0.004***		*0.117*		*0.571*		

n= Number of Patients; Bold lettering denotes p value ≤ 0.05 and highest percentage; Pearson Chi-square test; ER-Estrogen receptor; PR-Progesterone Receptor; Her2- Human Epidermal Growth Factor Receptor-2; BCS-Breast Conserving Surgery; MRM-Modified Radical Mastectomy.

5.3 Common chemotherapy-induced toxicities among patients

The majority of patients experienced grade 1-2 toxicity anemia (13.51%), nausea (14.18%), vomiting (10.81%), constipation (10.81%), mucositis (7.4%), etc., while a few patients encountered grade 3-4 toxicity, such as anemia (1.35%), diarrhea (1.35%), and constipation (1.35%). Notably, almost all patients experienced hair loss (alopecia; 79.72%), as a side effect of the chemotherapy. Scalp cooling was done to reduce alopecia as per clinician discretion. Adequate premedication with dexamethasone, 5-HT_3 antagonist, NK1 receptor blocker, and hydration was used to lessen the toxicity **(Table 5.5)**.

Table 5.5 Grades of common toxicities experienced by the patients

Sl. No.	Adverse Effects (n=148)	Grade 1-2	Grade 3-4
1.	Alopecia (n=118)	118 (79.72%)	-
2.	Anemia (n=22)	20 (13.51%)	2 (1.35%)
3.	Antharlgia (n=11)	11 (7.4%)	-
4.	Diarrhea (n=11)	9 (6.08%)	2 (1.35%)
5.	Fever (n=21)	21 (14.18%)	-
6.	Mucositis (n=11)	11 (7.4%)	-
7.	Nausea (n=21)	21 (14.18%)	-
8.	Vomiting (n=16)	16 (10.81%)	-
9.	Constipation (n=18)	16 (10.81%)	2 (1.35%)

5.4 Assessment of symptoms burden by ESAS Scale

BC patients were commonly reported with major physical and psychological symptom burdens such as pain, nausea, depression, anxiety, etc. which were assessed via ESAS questionnaire at baseline and after 12th month. There was a notable variance observed in the mean difference of the symptoms score after 12th month (***p<0.05***) (pain, fatigue, drowsiness, nausea, loss of appetite, shortness of breath, depression, anxiety, well-being, etc.). Global Distress Score (GDS), Physical Health Score (PHS), Psychological Symptom Score (PSS) also reduced significantly (***p<0.05***) (**Table 5.6**).

Table 5.6 Comparison of ESAS mean score at baseline and post-chemotherapy

Symptoms (n=148) (Physical, Global and Psychological distress)	Baseline Mean±SD	12th month Mean±SD	*p-value*
Pain	1.09±1.874	0.29±0.860	***<0.05***
Fatigue	3.07±1.668	1.93±1.605	
Drowsiness	3.20±1.672	2.03±1.417	
Nausea	0.28±0.813	0.14±0.408	
Appetite	1.89±1.009	1.24±0.747	
Shortness of breath	1.07±0.845	0.59±0.703	
Depression	2.25±1.422	1.49±1.138	
Anxiety	1.62±1.045	0.81±0.821	
Wellbeing	2.00±1.774	0.92±0.886	
Constipation	0.31±0.858	0.00±0.000	
GDS (Global Distress Score)*	14.00±8.227	7.95±4.980	
PHS (Physical Health Score)#	9.07±5.391	5.22±3.527	
PSS (Psychological Symptom Score)$	3.22±2.336	1.96±1.625	

** GDS includes the sum of core 9 symptoms (Except constipation); # PHS comprises the sum of pain, fatigue, drowsiness, nausea, appetite, and shortness of breath; $ PSS referred sum of anxiety and depression; p<0.05 for all symptoms at Baseline and 12th month difference using paired t-test.*

5.5 Quality of Life assessment

5.5.1 Impact of treatment response and therapy on the QoL in BC Patients

The two-way repeated-measures analysis of variance (ANOVA) examined the QoL scores across various domains as assessed by the FACT-B and FACIT-Sp-12 questionnaires categorized by treatment response (CRs, PRs, NRs) and mode of therapy (adjuvant, neo-adjuvant). Notably, QoL scores demonstrated a statistically significant difference (***p<0.05***) in the spiritual, social, emotional, and functional domains throughout the follow-up period in both treatment response and therapy groups, as determined by Wilk's Lambda and the Greenhouse-Geisser test (***p<0.05***). However, the physical domain was not significantly correlated in any of the group (*p>0.05*); but additional concern was significant in treatment response only ***(p<0.05***) (**Table 5.7 & 5.8**).

Table 5.7 QoL domains at various time intervals among treatment response group

QoL Domains	Group (n=148)	Baseline	3rd Month	6th Month	12th Month	*p-value*ǂ (Within Group)
Spiritual	CRs (n=57)	31.27±6.043	31.00±5.742	31.65±6.196	33.40±7.054	***<0.0001***
	PRs (n=18)	24.87±3.357	25.38±3.204	24.50±2.976	24.62±4.406	
	NRs (n=73)	23.67±6.241	24.73±6.017	20.87±5.854	17.93±5.824	
*p-value** (Between group)	***<0.0001***					
Social	CRs (n=57)	14.57±3.358	16.15±3.388	17.49±3.832	17.85±3.748	***<0.0001***
	PRs (n=18)	14.62±3.292	13.62±2.669	14.25±3.240	16.38±3.378	
	NRs (n=73)	13.87±3.642	12.27±3.369	11.60±3.334	13.73±4.183	
*p-value** (Between group)	***<0.0001***					
Emotional	CRs (n=57)	9.44±3.289	8.91±3.382	7.92±3.066	8.08±3.635	***0.015***
	PRs (n=18)	13.38±4.502	13.38±2.875	14.25±4.367	14.62±5.208	
	NRs (n=73)	14.60±4.137	14.33±3.994	14.07±4.877	13.47±4.422	
*p-value** (Between group)	***0.013***					
Functional	CRs (n=57)	12.89±4.002	14.48±3.930	15.01±3.577	15.79±3.786	***0.010***
	PRs (n=18)	11.88±3.682	10.50±3.251	12.00±2.330	12.50±2.000	
	NRs (n=73)	11.60±3.888	10.40±2.823	12.33±3.109	12.13±2.356	
*p-value** (Between group)	***0.004***					
Physical	CRs (n=57)	13.40±3.397	12.24±2.963	11.05±2.913	10.19±2.535	*0.915*
	PRs (n=18)	14.12±4.454	12.12±3.227	11.12±3.182	10.62±3.777	
	NRs (n=73)	12.47±3.796	11.20±3.028	10.33±3.132	9.20±3.121	
*p-value** (Between group)	*0.855*					
Additional Concern	CRs (n=57)	8.8±2.225	8.893±2.46	8.42±2.41	7.89±2.68	***0.021***
	PRs (n=18)	13.78±0.83	14.44±0.88	14.66±1.32	15.22±2.33	
	NRs (n=73)	18.18±1.93	18.31±3.64	17.75±5.14	18.75±4.90	
*p-value** (Between group)	***0.029***					

*Complete Responders (CRs); Partial Responders (PRs) & Non-Responders (NRs); All values are expressed as Mean±SD; * Multivariate analysis (Wilk's Lambda); ǂ Greenhouse-Geisser was significant (p<0.05).*

Table 5.8 QoL domains at various time intervals among adjuvant and neo-adjuvant chemotherapy group

QoL Domains	Group	Baseline	3rd month	6th Month	12th Month	*p-value*ǂ (Within Group)
Spiritual	ACT (n=108)	31.20±6.187	31.01±6.017	31.46±6.685	33.08±7.565	***0.004***
	NACT (n=40)	26.97±6.018	27.36±4.980	25.82±6.292	25.82±8.361	
*p-value** (Between group)	***0.037***					
Social	ACT (n=108)	14.51±3.287	16.04±3.266	17.54±3.709	17.91±3.608	***<0.0001***
	NACT (n=40)	14.21±3.507	13.97±3.584	13.76±3.913	15.52±4.251	
*p-value** (Between group)	***<0.0001***					
Emotional	ACT (n=108)	9.61±3.306	9.04±3.374	8.03±3.056	8.07±3.631	***0.015***
	NACT (n=40)	12.79±4.768	12.42±4.352	12.48±4.976	12.33±4.998	
*p-value** (Between group)	***0.007***					
Functional	ACT (n=108)	12.93±4.008	14.50±3.918	15.08±3.557	15.93±3.739	***0.012***
	NACT (n=40)	11.73±3.494	11.39±3.297	12.76±3.326	13.00±2.905	
*p-value** (Between group)	***0.004***					
Physical	ACT (n=108)	13.35±3.450	12.15±3.019	11.04±2.968	10.14±2.614	*0.937*
	NACT (n=40)	13.09±3.503	11.73±2.886	10.61±2.749	9.79±2.848	
*p-value** (Between group)	*0.968*					
Additional Concern	ACT (n=108)	9.19±2.72	9.14±2.73	8.70±2.75	8.22±3.19	*0.206*
	NACT (n=40)	15.21±3.01	15.36±4.00	14.96±4.96	15.27±5.40	
*p-value** (Between group)	*0.249*					

Adjuvant Chemotherapy (ACT); Neo-adjuvant chemotherapy (NACT); All values are expressed as Mean±SD; Multivariate analysis (Wilk's Lambda); ǂGreenhouse-Geisser was significant ($p<0.05$).*

5.5.2 Impact of BMI on Quality of Life

BMI emerged as a significant factor in our study; hence, we analyzed the QoL of the patients based on their BMI profile. ANOVA showed significant differences in QoL scores within the functional (***p=0.012; p=0.040***) and social (***p=0.043, p=0.015***) domains. Similarly, the emotional domain exhibited significant differences only within the group (Greenhouse-Geisser test; ***p=0.025***). No statistically significant differences (*p>0.05*) were detected at baseline in the mean QoL domain scores, including physical, spiritual, and additional concerns, among diverse BMI groups (**Figure 5.4**). Notably, the low BMI group showed a deterioration in scores, indicated poor QoL, whereas the normal BMI patients exhibited an improvement in scores, suggested betterment in QoL.

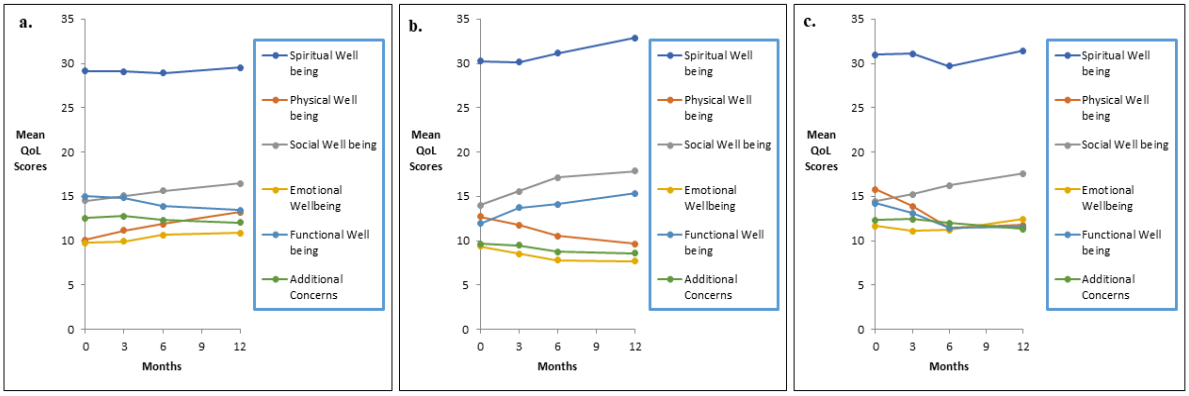


Figure 5.4 Trends in overall QoL mean score of BMI groups among breast cancer patients; a. Underweight/ Low BMI group, b. Normal BMI group, c. Overweight-Obese group

Note: Spiritual, physical and additional concern p>0.05; social, functional domain both the group p<0.05; emotional domain within the group only p<0.05. Within group Multivariate analysis (Wilk's Lambda). Between group ǂ Greenhouse-Geisser Mauchly's sphericity.

5.6 Translational relevance of the SNPs

5.6.1 Genotyping of transporter (*ABC*) and metabolizer (*CYP*) genes

The genomic DNA was quantified by gel electrophoresis on 1% agarose (**Figure 5.5. a**). *GAPDH* gene served as a positive reference for the PCR assays conducted for genotyping the chosen SNPs **(Figure 5.5. b)**. All SNPs were genotyped using the PCR-RFLP approach, as illustrated in the subsequent figures: *ABCC2* (**Figure 5.5 c-d)**, *ABCB1* **(Figure 5.6)**, *CYP2C9* & *CYP2C19* **(Figure 5.7)**, and *CYP3A4* & *CYP2D6* (**Figure 5.8)**.

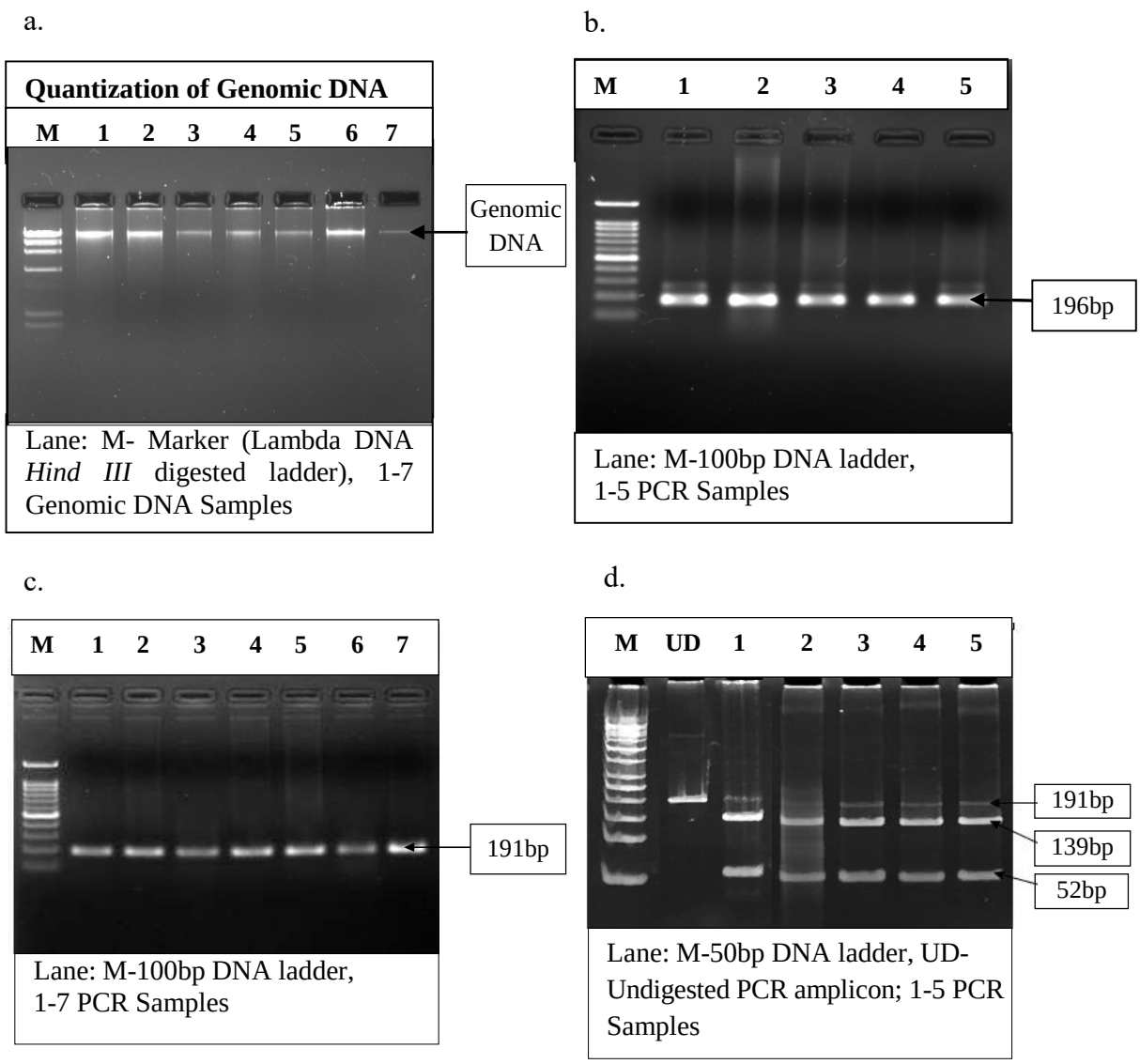


Figure 5.5 a. Quantization of genomic DNA using 1% Agarose gel; b. PCR of *GAPDH* gene on 2% agarose; (c & d) PCR-RFLP of *ABCC2* gene (rs145008610) on 2%agarose gel and 12% native PAGE

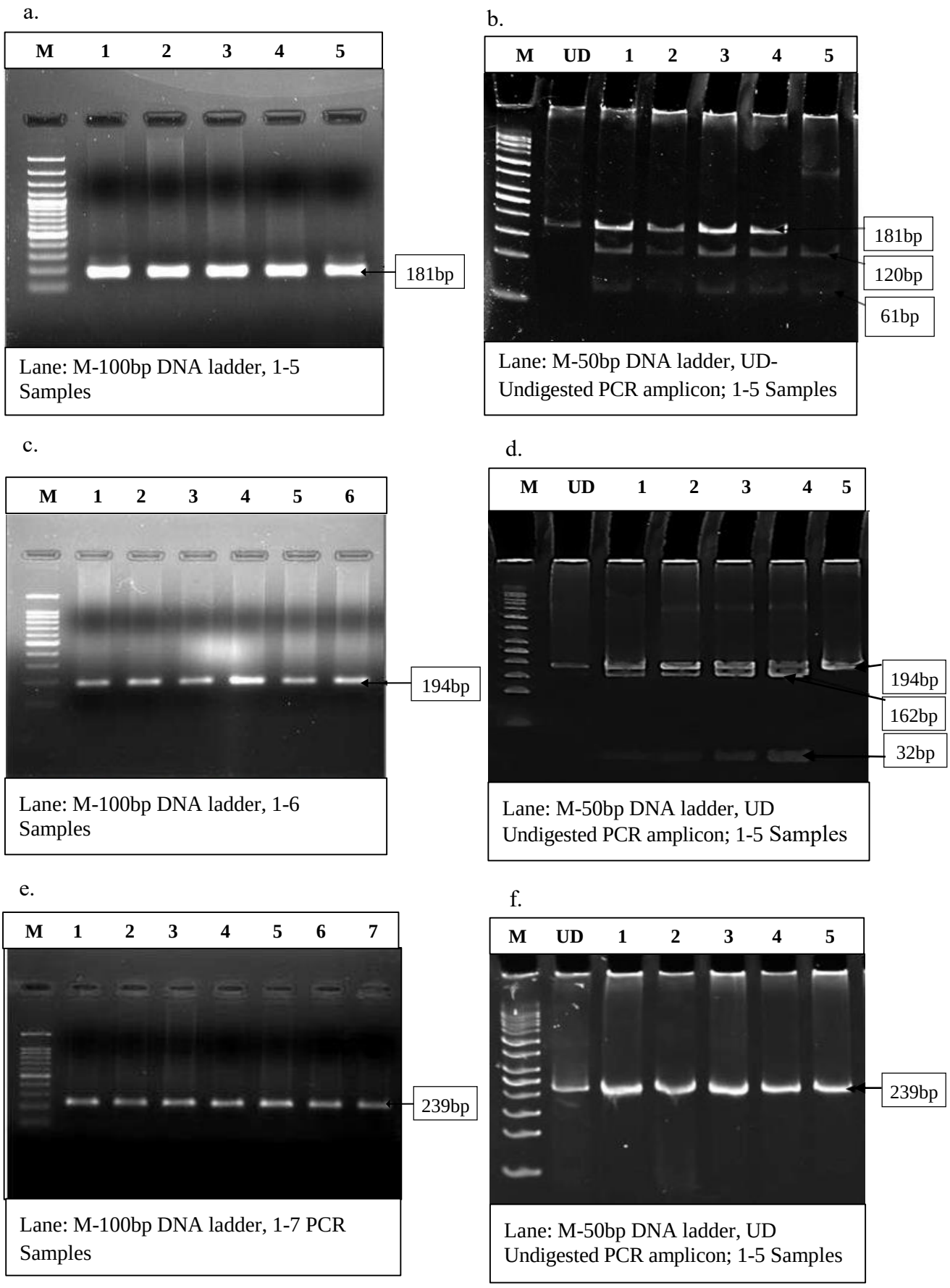


Figure 5.6 a. PCR-RFLP of *ABCB1* gene on 2% agarose gel and 12% native PAGE
(a & b) rs1128503, (c & d) rs1045642, (e & f) rs2032582

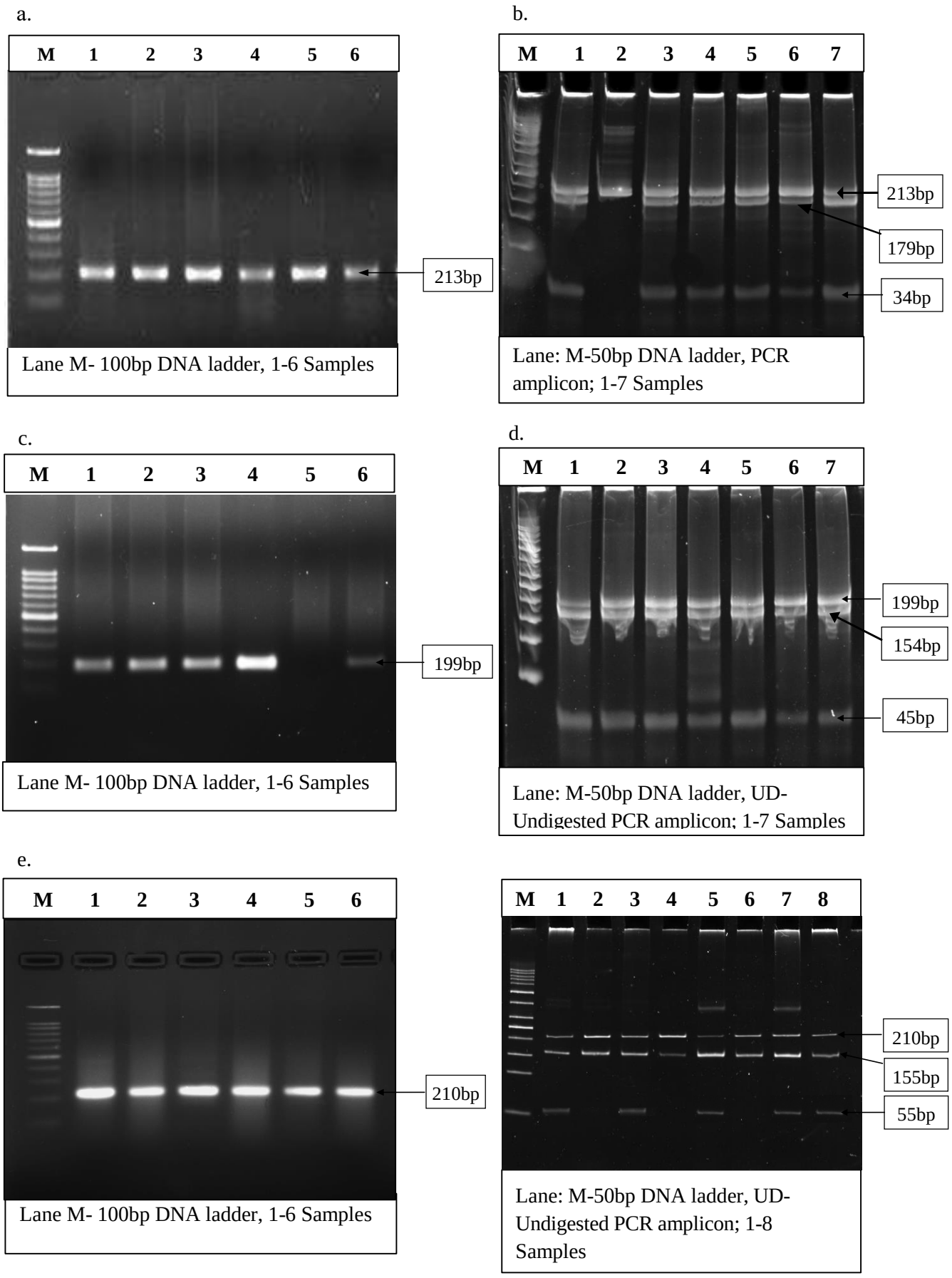


Figure 5.7 PCR-RFLP of *CYP2C9* and *CYP2C19* gene on 2% agarose gel and 12% native PAGE
(a & b) rs1799853 CYP2C9, (c & d) rs4917639 CYP2C9, (e & f) rs4244285 CYP2C19 gene

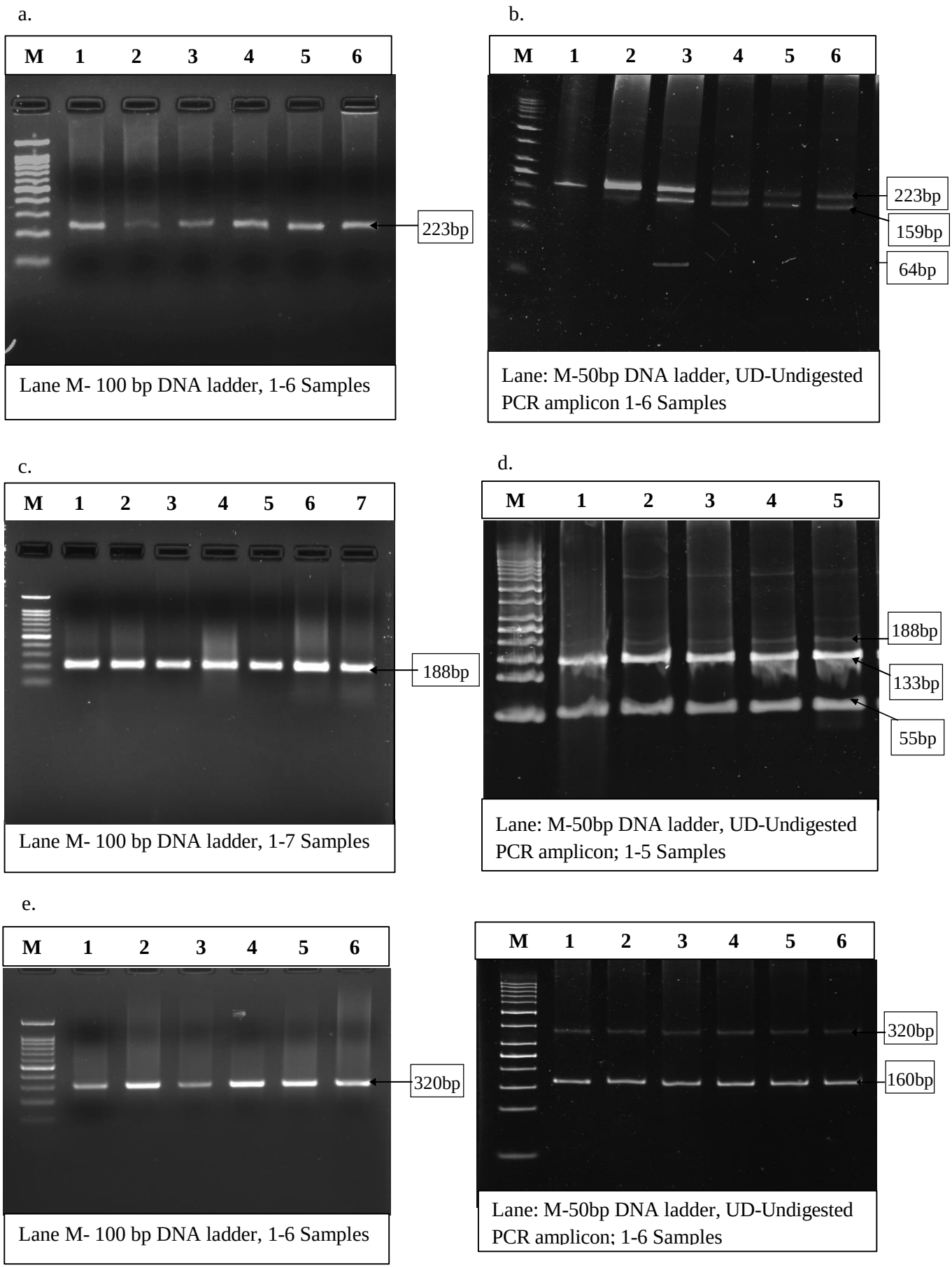


Figure 5.8 PCR-RFLP of *CYP3A4* and *CYP2D6* gene on 2% agarose gel and 12% native PAGE
(*a & b) rs2740574 in CYP3A4, (c & d) rs1065852 in CYP2D6, (e & f) rs3892097 CYP2D6 gene*

The genotype and allele frequencies of the chosen SNPs along with their detailed chromosome number, variant position, type, amino acid change, in the overall study population were examined. The findings indicated the following genotypes represented the highest percentage: CT (C1236T; 43.9%), CC (C3435T; 44.6%), GG (G2677T/A; 100%) in *ABCB1* gene; CA (C58626A; 38.5%) in the *ABCC2* gene; CC (C430T; 92.5%), AA (A32621C; 89.2%) in *CYP2C9*; GG (G681A; 45.6%) in *CYP2C19*; AA (A392G; 79.1%) in *CYP4A4*; and CC (C100T; 43.9%) in *CYP2D69*. This study identified *ABCB1* (G2677T/A) as monomorphic; hence, this SNP excluded from subsequent analysis. The minor allele frequency (MAF) of the SNPs were as follows: T (C1236T; 0.44), (C3435T; 0.35) in *ABCB1*; A (C58626A; 0.47) in *ABCC2*; T (C430T; 0.03), C (A32621C; 0.06) in *CYP2C9*; A (G681A; 0.38) in *CYP2C19*; A (A392G; 0.13) in *CYP4A4*; and T (C100T; 0.38), A (G1846A; 0.43) in *CYP2D6* gene. The genotype distribution of all investigated SNPs conformed to Hardy-Weinberg Equilibrium (HWE*; $p>0.05$*) **(Table 5.9)**.

Table 5.9 Genotype and allelic frequency distribution of clinically relevant polymorphisms in transporter genes and drug metabolizing enzymes

Genes/ (n=148)	Accession No./ Variants	Chr. No.	Variant position	Variant Type	Amino acid change	Genotype Frequency (%)*		Allele Frequency#	
ABCB1	(rs1128503)/ (C1236T)	7	87550285	Synonymous variant	G>G	CC	51(34.5)	**C**	**T**
						CT	**65(43.9)**	167 (0.56)	129 (0.44)
						TT	32(21.6)		
	(rs1045642)/ (C3435T)	7	87509329	Missense variant	I>I I>M	**CC**	**66(44.6)**	**C**	**T**
						CT	62(41.9)	194 (0.65)	102 (0.35)
						TT	20(13.5)		
	(rs2032582)/ (G2677T/A)	7	87531302	Missense variant	S>A S>T	**GG**	**148 (100)**	**G**	**T/A**
						GT/GA	-	296 (1)	-
						TT/AA/AT	-		
ABCC2	(rs145008610)/ (C58626A)	10	99836223	Missense variant	L>M	CC	50(33.8)	**C**	**A**
						CA	**57(38.5)**	157(0.53)	139(0.47)
						AA	41(27.7)		
CYP2C9	(rs1799853)/ (C430T)	10	94942290	Missense variant	R>S R>C	**CC**	**137 (92.5)**	**C**	**T**
						CT	11 (7.5)	285 (0.97)	11 (0.03)
						TT	-		
	(rs4917639)/ (A32621C)	10	94965778	Intronic variant	-	**AA**	**132 (89.2)**	**A**	**C**
						AC	16 (10.8)	280 (0.94)	16 (0.06)
						CC	-		
CYP2C19	(rs4244285)/ (G681A)	10	94781859	Synonymous variant (Splicing defect)	P>P	**GG**	**66 (45.6)**	**G**	**A**
						GA	50 (33.8)	182 (0.62)	114 (0.38)
						AA	32 (21.6)		
CYP3A4	(rs2740574)/ (A392G)	7	99784473	2kb upstream variant	-	**AA**	**117 (79.1)**	**A**	**G**
						AG	23 (15.5)	257 (0.87)	39 (0.13)
						GG	8 (5.4)		
CYP2D6	(rs1065852)/ (C100T)	22	42130692	Missense variant	P>S P>A	**CC**	**65 (43.9)**	**C**	**T**
						CT	52 (35.1)	182 (0.62)	114 (0.38)
						TT	31 (21)		
	(rs3892097)/ (G1846A)	22	42128945	Splice acceptor variant	-	**GG**	**58 (39.2)**	**G**	**A**
						GA	53 (35.8)	169 (0.57)	127 (0.43)
						AA	37 (25)		

*Chr- Chromosome; *Genotype frequency denoted as Frequency and percentage (%); #Allele frequency denoted as total allele number & Frequency; Bold lettering denotes highest percentage.*

5.6.2 Impact of BMI on clinico-pathological characteristics and treatment response

Before understanding the correlation of genetic polymorphism with BMI, we combined the normal BMI and overweight/obese groups due to the limited number of patients (n=12) in the obese category. The frequency distribution of clinico-pathological parameters was tested among this low (<18.5) and high (≥18.5) BMI group. The heat map analysis showed the low BMI group had the highest proportion of NRs (83.56%), whereas the high BMI group contained the majority of CRs (82.46%) (***p<0.0001***). Similarly, positive expression ER was predominant in the high BMI group (58.03%), whereas negative estrogen receptors were higher in low BMI patients (67.16%) (***p=0.002***) (**Figure 5.9**).

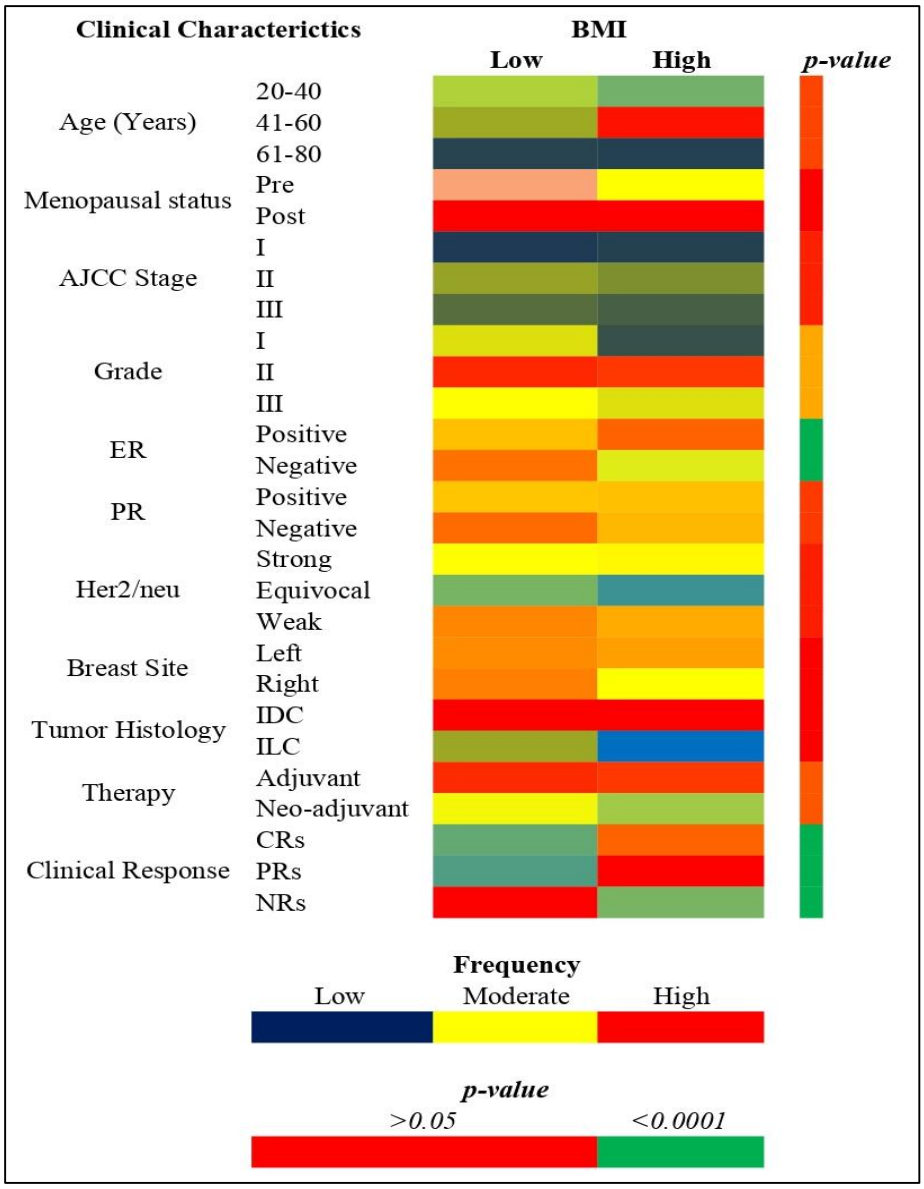


Figure 5.9 Heat map showing frequency distribution of clinico-pathological characteristics among low and high BMI group

The mean difference in BMI classes, analyzed using the unpaired t-test, showed significant variation within the response groups: ORR (OR 5.77, 95% CI (3.868-7.672); ***p<0.0001***) and NRs (OR 10.00, 95% CI (8.524-11.475); ***p<0.0001***). Additionally, the overall frequency distribution differed significantly between the chemotherapy response groups (OR 16.097, 95% CI (7.126-36.359); ***p<0.0001***) as determined by the Chi-square test (**Table 5.10**).

Table 5.10 Chemotherapy response in BC patients stratified by low and high BMI groups

Chemotherapy Response	BMI (Kg/m^2) n (%)		Mean Difference (95% CI) *p-value*$^{\Psi}$	OR (95%CI) χ^2 *p-value**
	<18.5	**≥18.5**		
ORR (n=75)	18 (24) (17.53±0.73)	57 (76) (23.30±4.01)	5.77 (3.868-7.672) ***<0.0001***	**16.097** (7.126-36.359) ***<0.0001***
NRs (n=73)	61 (83.56) (16.66±1.33)	12 (16.44) (26.66±5.08)	10.00 (8.524-11.475) ***<0.0001***	

All values in numbers (%) and (Mean±SD); OR-Odds Ratio; ORR-Objective Response Rate; NRs- Non-responders; $^{\Psi}$ Unpaired t-test;* Chi square-test; Bold lettering denotes p value < 0.05 and higher odds ratio.

5.6.3 Effect of *ABC* gene polymorphisms on chemotherapy response and BMI

As BMI significantly associated with treatment response groups, we conducted an association test between BMI, response and genotype variations. *ABC* transporters were crucial in chemotherapy transport by regulating drug efflux, influencing drug absorption, distribution, resistance, and overall treatment efficacy. *ABCB1* genotype distribution across response and BMI groups at both loci (C1236T and C3435T) revealed no significant associations in any genetic model (p>0.05). However, the frequency of the reference allele 'C' was higher at both loci of the *ABCB1* gene across treatment response groups (C1236T-OR 1.020, *p=0.930*; C3435T-OR 0.870, *p=0.572*) and BMI categories (C1236T-OR 0.795, *p=0.330*; C3435T-OR 1.090, *p=0.723*).

In contrast, the *ABCC2* gene polymorphism (C58626A) exhibited a strong independent association across all three genetic models with both in treatment response: dominant model (OR 2.954, ***p=0.003***), recessive model (OR 5.723, ***p<0.0001***), and codominant model (χ^2 20.219; ***p<0.0001***) and in BMI: dominant model (OR 3.343, ***p=0.001***), recessive model (OR 3.810, ***p=0.001***), and codominant model (χ^2 15.711; ***p<0.0001***). Interestingly, the allele frequency distribution at this locus was unique where, the frequency of the 'A' allele was notably higher among non-responders (NRs, 60.9%) compared to overall response rate group (ORR, 33.3%). Similarly, the frequency of the 'A' allele was elevated in the low BMI group (58.8%) compared to the high BMI group (33.3%) among the BC patient cohort (Treatment response: OR 3.122, ***p<0.0001***; BMI: OR 0.349, ***p<0.0001***). (**Table 5.11**)

Table 5.11 Distribution of *ABC* genotypes and its independent effects on chemotherapeutic response and BMI using genetic models

Models		Dominant Model			Recessive Model			Co-dominant Model				Allelic Association		
***ABCB1* (rs1128503) C1236T**														
Factors		**CC vs (CT+TT) n(%)**		**OR (95% CI) *p-value***	**(CC+CT) vs TT n(%)**		**OR (95%CI) *p-value***	**CC vs CT vs TT n(%)**			**Pearson χ^2 *p-value***	**C n(%)**	**T n(%)**	**OR (95%CI) *p-value***
Response	ORR (n=75)	24 (32)	51 (68)	0.802 (0.407-1.581) *0.523*	61 (81.3)	14 (18.7)	**1.426** (0.649-3.315) *0.376*	24 (32)	37 (49.3)	14 (18.7)	1.896 *0.388*	85 (56.7)	65 (43.3)	**1.020** (0.644-1.616) *0.930*
	NRs (n=73)	27 (37)	46 (63)		55 (75.3)	18 (24.7)		27 (37)	28 (38.4)	18 (24.7)		82 (56.1)	64 (43.8)	
BMI	<18.5 (n=79)	27 (34.2)	52 (65.8)	0.974 (0.494-1.921) *0.938*	58 (73.4)	21 (26.6)	0.524 (0.232-1.184) *0.117*	27 (34.2)	31 (39.2)	21 (26.6)	2.777 *0.249*	85 (53.7)	73 (46.2)	0.795 (0.501-1.261) *0.330*
	≥18.5 (n=69)	24 (34.8)	45 (65.2)		58 (84.1)	11 (15.9)		24 (34.8)	34 (49.3)	11 (15.9)		82 (59.4)	56 (40.6)	
***ABCB1* (rs1045642) C3435T**														
Factors		**CC vs (CT+TT) n(%)**		**OR (95% CI) *p-value***	**(CC+CT) vs TT n(%)**		**OR (95%CI) *p-value***	**CC vs CT vs TT n(%)**			**Pearson χ^2 *p-value***	**C n(%)**	**T n(%)**	**OR (95%CI) *p-value***
Response	ORR (n=75)	32 (42.7)	43 (57.3)	0.854 (0.446-1.633) *0.632*	64 (85.3)	11 (14.7)	0.818 (0.317-2.108) *0.677*	32 (42.7)	32 (42.7)	11 (14.7)	0.298 *0.862*	96 (64)	54 (36)	0.870 (0.538-1.407) *0.572*
	NRs (n=73)	34 (46.6)	39 (53.4)		64 (87.7)	9 (12.3)		34 (46.6)	30 (41.1)	9 (12.3)		98 (67.2)	48 (32.8)	
BMI	<18.5 (n=79)	36 (45.6)	43 (54.4)	**1.088** (0.568-2.085) *0.798*	69 (87.3)	10 (12.7)	**1.168** (*0*.456-3.003) *0.745*	36 (45.6)	33 (41.8)	10 (12.7)	0.143 *0.931*	105 (66.5)	53 (33.5)	**1.090** (0.674-1.763) *0.723*
	≥18.5 (n=69)	30 (43.5)	39 (56.5)		59 (85.5)	10 (14.5)		30 (43.5)	29 (42)	10 (14.5)		89 (64.4)	49 (35.6)	
***ABCC2* (rs145008610) C58626A**														
Factors		**CC vs (CA+AA) n(%)**		**OR (95% CI) *p-value***	**(CC+CA) vs AA n(%)**		**OR (95%CI) *p-value***	**CC vs CA vs AA n(%)**			**Pearson χ^2 *p-value***	**C n(%)**	**A n(%)**	**OR (95%CI) *p-value***
Response	ORR (n=75)	34 (45.3)	41 (54.7)	**2.954** (1.442-6.051) ***0.003***	66 (88)	9 (12)	**5.723** (2.481-13.204) ***<0.0001***	34 (45.3)	32 (42.7)	9 (12)	20.219 ***<0.0001***	100 (66.7)	50 (33.3)	**3.122** (1.941-5.022) ***<0.0001***
	NRs (n=73)	16 (21.9)	57 (78.1)		41 (56.2)	32 (43.8)		16 (21.9)	25 (34.2)	32 (43.8)		57 (39.1)	89 (60.9)	
BMI	<18.5 (n=79)	17 (21.5)	62 (78.5)	**3.343** (1.636-6.833) ***0.001***	48 (60.8)	31 (39.2)	**3.810** (1.698-8.550) ***0.001***	17 (21.5)	31 (39.2)	31 (39.2)	15.711 ***<0.0001***	65 (41.2)	93 (58.8)	0.349 (0.217-0.562) ***<0.0001***
	≥18.5 (n=69)	33 (47.8)	36 (52.2)		59 (85.5)	10 (14.5)		33 (47.8)	26 (37.7)	10 (14.5)		92 (66.7)	46 (33.3)	

ORR-Objective Response Rate; NRs- Non-responders; OR- Odds Ratio; Bold lettering denotes p value < 0.05 and higher odds ratio.

5.6.4 Combined effect of BMI and *ABC* gene polymorphisms on treatment response

To understand the combine effect of BMI and genetic polymorphism on chemotherapy response, we stratified the treatment response groups among BMI classes. The *ABCB1* gene locus (C3435T) showed no significant association with the combined effect of BMI and treatment response (*p>0.05*). However, dominant model of C1236T locus showed significant association for dominant model in both low BMI (OR 3.235, ***p=0.030***) and high BMI (OR 0.065, ***p<0.001***) and also in codominant model of high BMI group (***p<0.0001***). The potential

role of the C1236T locus remained unclear until now but was clarified through survival analysis later.

However, a distinct result was observed for the *ABCC2* gene (C58626A). In the low BMI group, a significant correlation with gene polymorphism was identified across all genetic models: dominant model (OR 3.245, ***p=0.041***), recessive model (OR 7.250, ***p=0.012***), and codominant model (χ^2=8.657, ***p=0.013***). Conversely, in the high BMI group, no significant association was observed across any genetic model (*p>0.05*) (**Table 5.12)**. This indicate the role of low BMI and abcc2 variation in the response. This highlights the potential role of low BMI and *ABCC2* variation in treatment response.

Table 5.12 Combined effects of chemotherapeutic response and BMI on *ABC* gene applying different genetic models

Models	**Dominant Model**				**Recessive Model**			**Co-dominant Model**			
***ABCB1* (rs1128503) C1236T**											
BMI	**Response**	**CC vs (CT+TT) n(%)**		**OR (95% CI) *p-value***	**(CC+CT) vs TT n(%)**		**OR (95% CI) *p-value***	**CC vs CT vs TT n(%)**			**Pearson χ^2 *p-value***
<18.5 (n=79)	ORR (n=18)	10 (55.6)	8 (44.4)	**3.235** (1.093-9.576) ***0.030***	14 (77.8)	4 (22.2)	**1.352** (0.390-4.693) *0.634*	10 (55.6)	4 (22.2)	4 (22.2)	5.505 *0.082*
	NRs (n=61)	17 (27.9)	44 (72.1)		44 (72.1)	17 (27.9)		17 (27.9)	27 (44.3)	17 (27.9)	
≥18.5 (n=69)	ORR (n=57)	14 (24.6)	43 (75.4)	0.065 (0.013-0.334) ***<0.0001***	47 (82.5)	10 (17.5)	0.427 (0.049-3.697) *0.428*	14 (24.6)	33 (57.9)	10 (17.5)	15.313 ***<0.0001***
	NRs (n=12)	10 (83.3)	2 (16.7)		11 (91.7)	1 (8.3)		10 (83.3)	1 (8.3)	1 (8.3)	
***ABCB1* (rs1045642) C3435T**											
BMI	**Response**	**CC vs (CT+TT) n(%)**		**OR (95% CI) *p-value***	**(CC+CT) vs TT n(%)**		**OR (95% CI) *p-value***	**CC vs CT vs TT n(%)**			**Pearson χ^2 *p-value***
<18.5 (n=79)	ORR (n=18)	7 (38.9)	11 (61.1)	0.702 (0.240-2.053) *0.517*	15 (83.3)	3 (16.7)	0.648 (0.149-2.815) *0.561*	7 (38.9)	8 (44.4)	3 (16.7)	0.564 *0.754*
	NRs (n=61)	29 (47.5)	32 (52.5)		54 (88.5)	7 (11.5)		29 (47.5)	25 (41)	7 (11.5)	
≥18.5 (n=69)	ORR (n=57)	25 (43.9)	32 (56.1)	**1.094** (0.310-3.861) *0.889*	49 (86)	8 (14)	**1.225** (0.226-6.653) *0.814*	25 (43.9)	24 (42.1)	8 (14)	0.059 *0.971*
	NRs (n=12)	5 (41.7)	7 (58.3)		10 (83.3)	2 (16.7)		5 (41.7)	5 (41.7)	2 (16.7)	
***ABCC2* (rs145008610) C58626A**											
BMI	**Response**	**CC vs (CA+AA) n(%)**		**OR (95% CI) *p-value***	**(CC+CA) vs AA n(%)**		**OR (95% CI) *p-value***	**CC vs CA vs AA n(%)**			**Pearson χ^2 *p-value***
<18.5 (n=79)	ORR (n=18)	7 (38.9)	11 (61.1)	**3.245** (1.012-10.406) ***0.041***	16 (88.9)	2 (11.1)	**7.250** (1.533-34.278) ***0.012***	7 (38.9)	9 (50)	2 (11.1)	8.657 ***0.013***
	NRs (n=61)	10 (16.4)	51 (83.6)		32 (52.5)	29 (47.5)		10 (16.4)	22 (36.1)	29 (47.5)	
≥18.5 (n=69)	ORR (n=57)	27 (47.4)	30 (52.6)	**1.111** (0.320-3.860) *0.868*	50 (87.7)	7 (12.3)	0.420 (0.091-1.935) *0.255*	27 (47.4)	23 (40.4)	7 (12.3)	1.741 *0.419*
	NRs (n=12)	6 (50)	6 (50)		9 (75)	3 (25)		6 (50)	3 (25)	3 (25)	

BMI-Body Mass Index; OR- Odds Ratio; ORR-Objective Response Rate; NRs- Non-Responders; Bold lettering denotes p value <0.05 and higher odds ratio.

5.6.5 Effect of *CYP* gene polymorphisms on chemotherapy response and BMI

CYP enzymes play a crucial role in chemotherapy metabolism by influencing drug activation, detoxification, efficacy, and toxicity through genetic polymorphisms. To investigate the potential influence of *CYP* gene polymorphisms on chemotherapy response and BMI, we conducted a comprehensive genetic association analysis.

Analysis of the *CYP2C9* (C430T), *CYP2C19* (G681A) genes exhibited a strong independent association across treatment response and BMI. The genetic association of *CYP2C9* (C430T) gene with treatment response: dominant and codominant (OR 5.113, ***p=0.025***) and in BMI: dominant and codominant model (OR 0.232, ***p=0.049***). Recessive model did not show any difference as no patients showed homozygous recessive (TT) genotype in this locus. The genetic association of *CYP2C19* (G681A) gene was significant with treatment response: dominant model (OR 11.119, ***p<0.0001***), recessive (OR 0.063, ***p<0.0001***), and codominant (χ^2 48.229, ***p<0.0001***) and in BMI: dominant (OR 0.356, ***p=0.002***), recessive (OR 5.515, ***p<0.0001***) and codominant model (χ^2 15.183, ***p=0.001***). Moreover, the *CYP3A4* (A392G) gene exhibited a significant association with BMI: dominant model (OR 2.523, ***p=0.025***), recessive (OR 0.363, *p=0.208*), and codominant (χ^2 11.812, ***p=0.003***); however, no significant association was observed between this locus and treatment response (*p>0.05*). Other SNPs (*CYP2C9*, G681A; *CYP2D6*, C100T) did not exhibit any association with these covariates. Interestingly, allelic association analysis of the *CYP2C19* gene revealed a higher frequency of the 'G' allele in the ORR group, whereas the 'A' allele was more prevalent in the NRs group **(Table 5.13)**.

Table 5.13 Distribution of *CYP* genotypes and its independent effects on chemotherapeutic response and BMI using genetic models

Models		Dominant Model			Recessive Model			Co-dominant model				Allelic Association		
***CYP2C9* (rs1799853) C430T**														
Factors		**CC vs (CT+TT) n(%)**		**OR (95% CI) *p-value***	**(CC+CT) vs TT n(%)**		**OR (95%CI) *p-value***	**CC vs CT vs TT n(%)**			**Pearson χ^2 *p-value***	**C n(%)**	**T n(%)**	**OR (95%CI) *p-value***
Response	**ORR (n=75)**	73 (97.3)	2 (2.7)	**5.113** (1.069-24.635) ***0.025***	75 (100)	-	-	73 (97.3)	2 (2.7)	-	5.020 ***0.025***	148 (98.7)	2 (1.3)	**4.861** (1.032-22.897) ***0.045***
	NRs (n=73)	64 (87.7)	9 (12.3)		73 (100)	-		64 (87.7)	9 (12.3)	-		137 (93.8)	9 (6.2)	
BMI	**<18.5 (n=79)**	70 (88.6)	9 (11.4)	0.232 (0.048-1.114) ***0.049***	79 (100)	-	-	70 (88.6)	9 (11.4)	-	3.862 ***0.049***	149 (94.3)	9 (5.6)	0.024 (0.051-1.146) *0.074*
	≥18.5 (n=69)	67 (97.1)	2 (2.9)		69 (100)	-		67 (97.1)	2 (2.9)	-		136 (98.5)	2 (1.5)	
***CYP2C9* (rs4917639) A32621C**														
Factors		**AA vs (AC+CC) n(%)**		**OR (95% CI) *p-value***	**(AA+AC) vs CC n(%)**		**OR (95%CI) *p-value***	**AA vs AC vs CC n(%)**			**Pearson χ^2 *p-value***	**A n(%)**	**C n(%)**	**OR (95%CI) *p-value***
Response	**ORR (n=75)**	69 (92)	6 (8)	**1.825** (0.627-5.312) *0.264*	75 (100)	-	-	69 (92)	6 (8)	-	**1.246** *0.264*	144 (96)	6 (4)	**1.764** (0.624-4.987) *0.28*
	NRs (n=73)	63 (86.3)	10 (13.7)		73 (100)	-		63 (86.3)	10 (13.7)	-		136 (93.2)	10 (6.8)	
BMI	**<18.5 (n=79)**	71 (89.9)	8 (10.1)	**1.164** (0.412-3.286) *0.774*	79 (100)	-	-	71 (89.9)	8 (10.1)	-	0.082 *0.774*	150 (94.9)	8 (5.1)	**1.153** (0.421-3.160) *0.780*
	≥18.5 (n=69)	61 (88.4)	8 (11.6)		69 (100)	-		61 (88.4)	8 (11.6)	-		130 (94.3)	8 (5.7)	
***CYP2C19* (rs4244285) G681A**														
Factors		**GG vs (GA+AA) n(%)**		**OR (95% CI) *p-value***	**(GG+GA) vs AA n(%)**		**OR (95%CI) *p-value***	**GG vs GA vs AA n(%)**			**Pearson χ^2 *p-value***	**G n(%)**	**A n(%)**	**OR (95%CI) *p-value***
Response	**ORR (n=75)**	53 (70.7)	22 (29.3)	**11.119** (5.103-24.228) ***<0.0001***	72 (96)	3 (4)	**15.818** (4.548-55.015) ***<0.0001***	53 (70.7)	19 (25.3)	3 (4)	48.229 ***<0.0001***	125 (83.3)	25 (16.7)	**7.807** (4.535-13.438) ***<0.0001***
	NRs (n=73)	13 (17.8)	60 (82.2)		44 (60.3)	29 (39.7)		13 (17.8)	31 (42.5)	29 (39.7)		57 (39.1)	89 (61.9)	
BMI	**<18.5 (n=79)**	26 (32.9)	53 (67.1)	0.356 (0.182-0.695) ***0.002***	53 (67.1)	26 (32.9)	0.194 (0.074-0.507) ***0.0008***	26 (32.9)	27 (34.2)	26 (32.9)	15.183 ***0.001***	79 (50)	79 (50)	0.339 (0.207-0.557) ***<0.0001***
	≥18.5 (n=69)	40 (58)	29 (42)		63 (91.3)	6 (8.7)		40 (58)	23 (33.3)	6 (8.7)		103 (74.6)	35 (25.4)	
***CYP3A4* (rs2740574) A392G**														
Factors		**AA vs (AG+GG) n(%)**		**OR (95% CI) *p-value***	**(AA+AG) vs GG n(%)**		**OR (95%CI) *p-value***	**AA vs AG vs GG n(%)**			**Pearson χ^2 *p-value***	**A n(%)**	**G n(%)**	**OR (95%CI) *p-value***
Response	**ORR (n=75)**	57 (76)	18 (24)	0.686 (0.308-1.527) *0.355*	71 (94.7)	4 (5.3)	**1.029** (0.247-4.278) *0.969*	57 (76)	14 (18.7)	4 (5.3)	1.137 *0.566*	128 (85.3)	22 (14.7)	0.766 (0.389-1.511) *0.442*
	NRs (n=73)	60 (82.2)	13 (17.8)		69 (94.5)	4 (5.5)		60 (82.2)	9 (12.3)	4 (5.5)		129 (88.3)	17 (11.7)	
BMI	**<18.5 (n=79)**	68 (86.1)	11 (13.9)	**2.523** (1.109-5.724) ***0.025***	73 (92.4)	6 (7.6)	0.363 (0.071-1.862) *0.208*	68 (86.1)	5 (6.3)	6 (7.6)	11.812 ***0.003***	141 (89.3)	17 (10.7)	**1.573** (0.797-3.101) *0.191*
	≥18.5 (n=69)	49 (79)	20 (29)		67 (97.1)	2 (2.9)		49 (71)	18 (26.1)	2 (2.9)		116 (84.1)	22 (15.9)	

ORR-Objective Response Rate; NRs- Non-responders; OR- Odds Ratio; Bold lettering denotes p value <0.05 and higher odds ratio.

5.6.4 Combined effect of BMI and *CYP* metabolizer gene polymorphisms on treatment response

The individual loci exhibited significant associations with specific covariates, their significance was lost when analyzed combination manner across all loci (*CYP2C9, CYP3A4, CYP2D6*, etc.), except for *CYP2C19*, which retained its association in the collective analysis also. A significant correlation between *CYP2C19* polymorphism and chemotherapy response was observed across all genetic models. The low BMI group demonstrated a significantly reduced odds ratio, whereas the high BMI group showed an opposite trend. Low BMI:

dominant (OR 9.6, ***p<0.0001***), recessive (OR 1.473, ***p=0.001***), and codominant model (χ^2=18.897, ***p<0.0001***) and High BMI: dominant (OR 0.298, ***p<0.0001***), recessive (OR 0.167, ***p=0.027***), and codominant model (χ^2=20.428, ***p<0.0001***). (**Table 5.14**). These findings suggest that BMI plays a crucial role in modulating the genetic influence of *CYP2C19* on chemotherapy response.

Table 5.14 Combined effects of chemotherapeutic response and BMI on *CYP* gene applying different genetic models

Models		**Dominant Model**			**Recessive Model**			**Co-dominant Model**			
***CYP2C9* (rs1799853) C430T**											
BMI	**Response**	**CC vs (CT+TT) n(%)**		**OR (95% CI) *p-value***	**(CC+CT) vs TT n(%)**		**OR (95%CI) *p-value***	**CC vs CT vs TT n(%)**			**OR (95%CI) *p-value***
<18.5 (n=79)	ORR (n=18)	17 (94.4)	1 (5.6)	**2.566** (0.299-22.017) *0.375*	18 (100)	-	-	17 (94.4)	1 (5.6)	-	**2.566** (0.299-22.017) *0.375*
	NRs (n=61)	53 (86.9)	8 (13.1)		61 (100)	-		53 (86.9)	8 (13.1)	-	
≥18.5 (n=69)	ORR (n=57)	56 (98.2)	1 (1.8)	**5.091** (0.296-87.675) *0.217*	57 (100)	-	-	56 (98.2)	1 (1.8)	-	**5.091** (0.296-87.675) *0.217*
	NRs (n=12)	11 (91.7)	1 (8.3)		12 (100)	-		11 (91.7)	1 (8.3)	-	
***CYP2C9* (rs4917639) A32621C**											
BMI	**Response**	**AA vs (AC+CC) n(%)**		**OR (95% CI) *p-value***	**(AA+AC) vs CC n(%)**		**OR (95%CI) *p-value***	**AA vs AC vs CC n(%)**			**OR (95%CI) *p-value***
<18.5 (n=79)	ORR (n=18)	18 (100)	-	**1.151** (1.044-1.269) ***0.015***	18 (100)	-	-	18 (100)	-	-	**1.151** (1.044-1.269) ***0.015***
	NRs (n=61)	53 (86.9)	8 (13.1)		61 (100)	-		53 (86.9)	8 (13.1)	-	
≥18.5 (n=69)	ORR (n=57)	51 (89.5)	6 (10.5)	**1.70** (0.299-9.666) *0.546*	57 (100)	-	-	51 (89.5)	6 (10.5)	-	**1.70** (0.299-9.666) *0.546*
	NRs (n=12)	10 (83.3)	2 (16.7)		12 (100)	-		10 (83.3)	2 (16.7)	-	
***CYP2C19* (rs4244285) G681A**											
BMI	**Response**	**GG vs (GA+AA) n(%)**		**OR (95% CI) *p-value***	**(GG+GA) vs AA n(%)**		**OR (95%CI) *p-value***	**GG vs GA vs AA n(%)**			**Pearson χ^2 *p-value***
<18.5 (n=79)	ORR (n=18)	13 (72.2)	5 (27.8)	**9.60** (2.892-31.864) ***<0.0001***	18 (100)	-	**1.473** (1.404-2.164) ***0.001***	13 (72.2)	5 (27.8)	-	18.897 ***<0.0001***
	NRs (n=61)	13 (21.3)	48 (78.7)		35 (57.4)	26 (42.6)		13 (21.3)	22 (36.1)	26 (42.6)	
≥18.5 (n=69)	ORR (n=57)	40 (70.2)	17 (29.8)	0.298 (0.200-0.444) ***<0.0001***	54 (94.7)	3 (5.3)	0.167 (0.029-0.958) ***0.027***	40 (70.2)	14 (24.6)	3 (5.3)	20.428 ***<0.0001***
	NRs (n=12)	-	12 (100)		9 (75)	3 (25)		-	9 (75)	3 (25)	
***CYP3A4* (rs2740574) A392G**											
BMI	**Response**	**AA vs (AG+GG) n(%)**		**OR (95% CI) *p-value***	**(AA+AG) vs GG n(%)**		**OR (95%CI) *p-value***	**AA vs AG vs GG n(%)**			**Pearson χ^2 *p-value***
<18.5 (n=79)	ORR (n=18)	16 (88.9)	2 (11.1)	**1.385** (0.271-7.077) *0.695*	16 (88.9)	2 (11.1)	0.561 (0.094-3.348) *0.522*	16 (88.9)	-	2 (11.1)	1.876 *0.391*
	NRs (n=61)	52 (85.2)	9 (14.8)		57 (93.4)	4 (6.6)		52 (85.2)	5 (8.2)	4 (6.6)	
≥18.5 (n=69)	ORR (n=57)	41 (71.9)	16 (28.1)	**1.281** (0.338-4.854) *0.715*	55 (96.5)	2 (3.5)	0.965 (0.918-1.014) *0.510*	41 (71.9)	14 (24.6)	2 (3.5)	0.752 *0.687*
	NRs (n=12)	8 (66.7)	4 (33.3)		12 (100)	-		8 (66.7)	4 (33.3)	-	

BMI-Body Mass Index; ORR-Objective Response Rate; NRs- Non-Responders; OR- Odds Ratio; Bold lettering denotes p value<0.05 and higher odds ratio.

5.7 Survival Analysis

Kaplan-Meier survival analysis is a non-parametric statistical method used to estimate survival probabilities over time and compare survival distributions between different patient groups. Among the entire study cohort, 19 patients (12.8%) were died, while 129 patients (87.2%) were survived up to 48months follow up. Kaplan-Meier (KM) analysis after 48 months revealed that the patient population did not achieve median overall survival time (mean OS 42.97±1.076 months; *p=0.065*) **(Figure 5.10)**. Low BMI group demonstrated the shortest predicted OS time of 22 months (log rank ***p<0.0001)*** **(Figure 5.11)**.

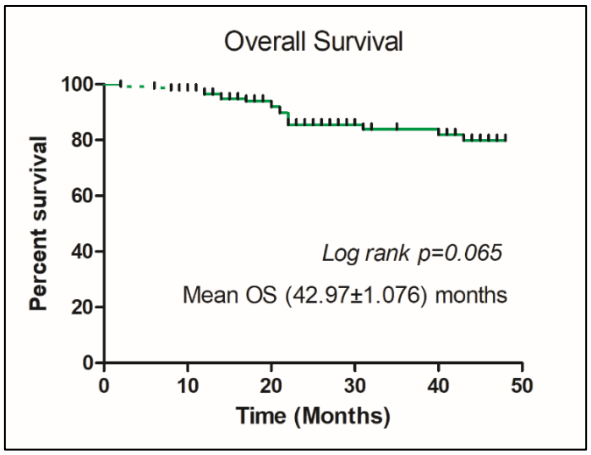


Figure 5.10 Overall survival in the entire patient cohort

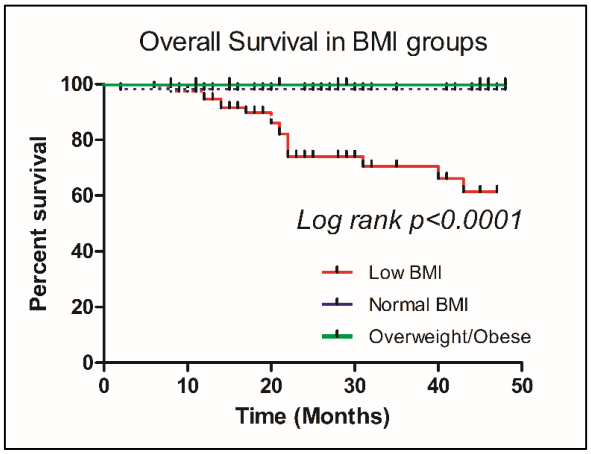


Figure 5.11 Overall survival among BMI classes

5.7.1 Progression-Free, Overall, and Disease-Free Survival across different treatment regimens (FEC, TAC, AC-T)

The entire cohort received sequential and concomitant anthracycline-taxane (AC-T/TAC) and FEC chemotherapy. Their differential survival outcomes were tested among treatment regimen groups. The PFS by KM analysis showed that the estimated mean (± S.E.) survival times were similar across regimens: FEC (43.806 ± 1.934 months), TAC (43.986 ± 1.523 months), and AC-T (43.427 ± 2.152 months) (*p=0.830*). In contrast, PFS for the neo-adjuvant chemotherapy (NACT) group significantly correlated with survival (***p<0.0001***), with a median PFS of 40 ±

6.401 months. OS data revealed mean survival times of (40 ± 2.818), (43.5 ± 1.516) and (43.6 ± 2.077) months for FEC, TAC, and AC-T regimen respectively (*p=0.703*). Similarly, KM analysis for DFS showed mean survival times of (37 ± 3.579) months for FEC, (38 ± 2.010) months for TAC, and (39 ± 2.432) months for AC-T (*p=0.534*). Although, a significant relationship was found between treatment response and DFS (***p<0.0001***) (**Figure 5.12**).

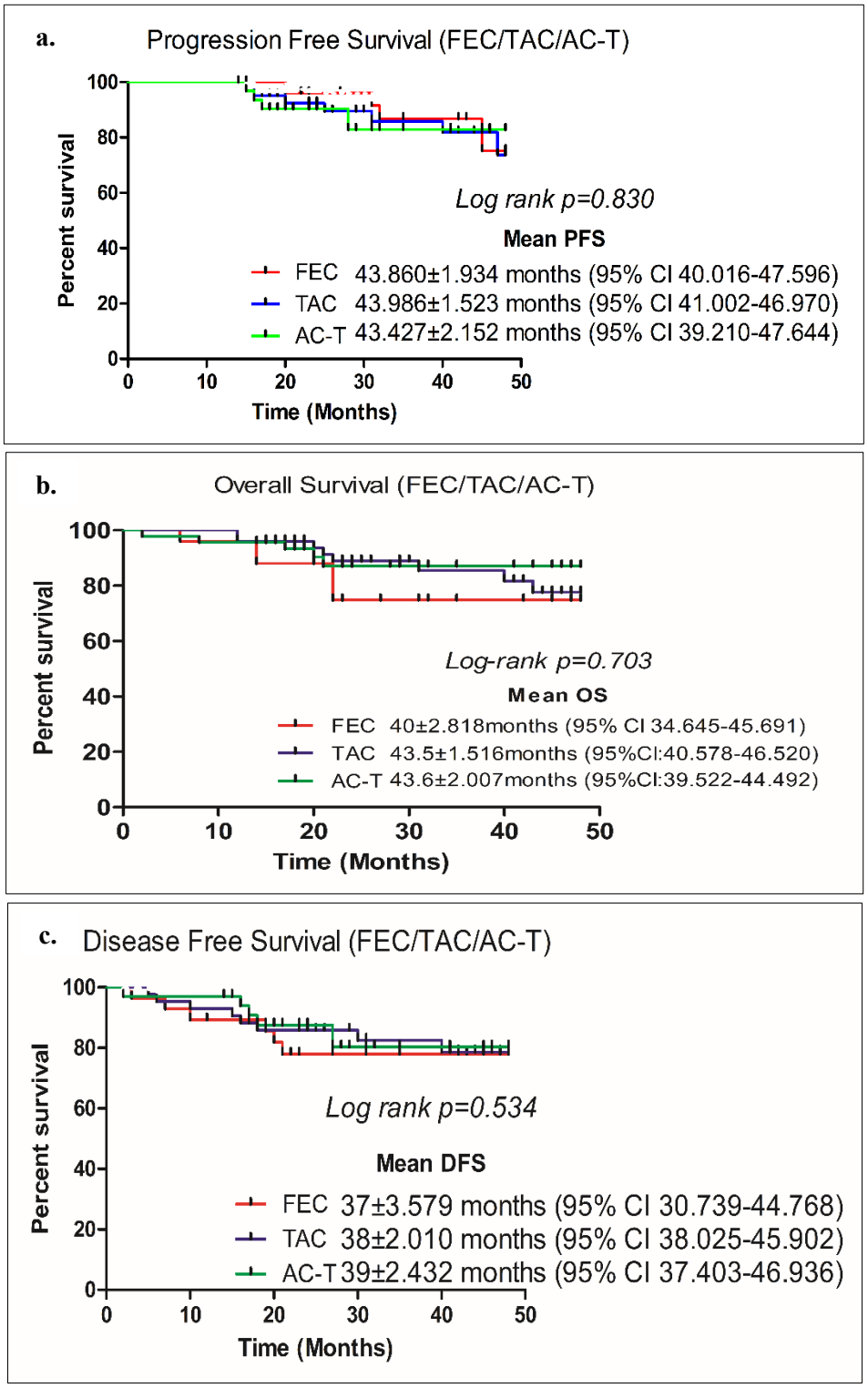


Figure 5.12 Kaplan- Meier survival analysis of different treatment regimen (FEC/TAC/AC-T)
a. Progression Free Survival; b. Overall Survival; c. Disease Free Survival.

5.7.2 Impact of *ABC* gene polymorphism on survival outcome

Kaplan-Meier was conducted to assess the independent effects of transporter genotype on survival outcome. PFS and OS across different SNPs were illustrated in **Figure 5.13**. A significant difference in PFS among the different genotypes was observed at the *ABCB1* (C1236T) and *ABCC2* (C58626A) loci (***p<0.05***), where significantly lower PFS time was found in the CC (45 months) and AA (39 months) genotypes at the *ABCB1* (C1236T) and *ABCC2* (C58626A) loci, respectively (**Figure 5.13 a-c**). Highest number of patients reported with disease progression in *ABCC2* gene showing CC genotype (38.1%). However, OS data revealed a significant difference only at the *ABCC2* gene, where the AA genotype exhibited the shortest OS time (39 months; ***p=0.032***). No significant differences were observed for the other loci (*p>0.05*) in transporter gene. (**Figure 5.13 d-f**). The highest percentage of death was observed in AA genotype of *ABCC2* loci (21.7%).

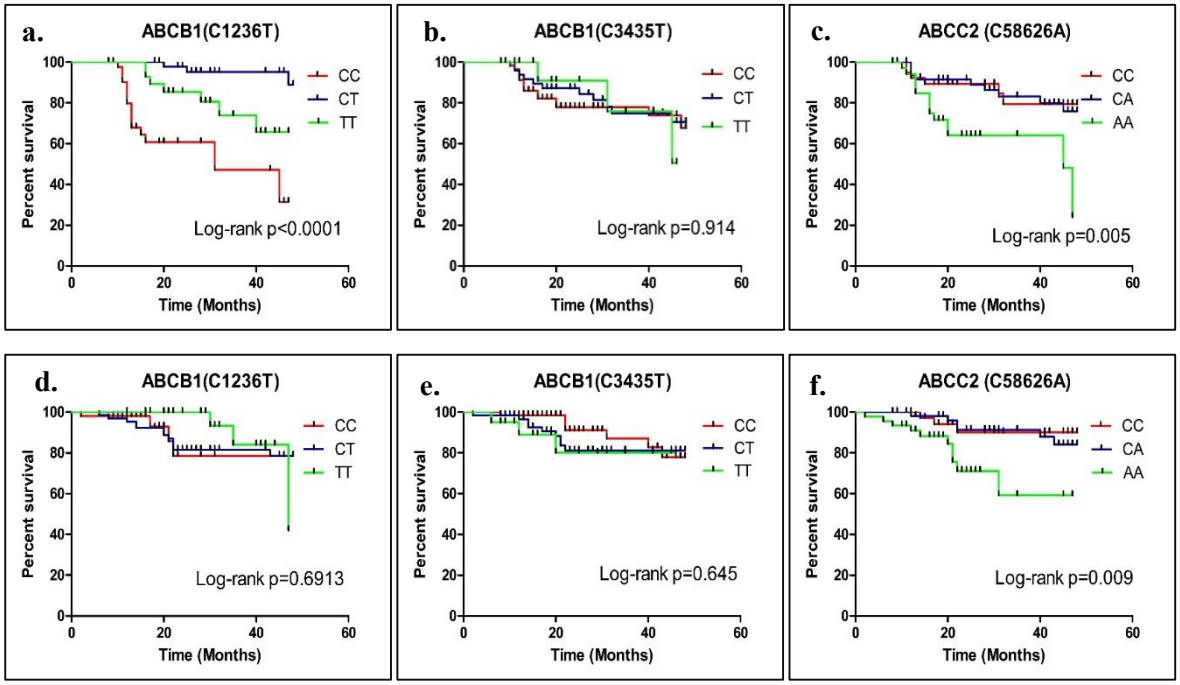


Figure 5.13 Kaplan-Meier survival curves illustrating the impact of *ABC* transporter gene polymorphisms in BC patients

(a-c) Progression Free Survival, (d-f) Overall Survival

5.7.3 Impact of *CYP* gene polymorphism of survival outcome

Kaplan-Meier survival was performed to assess PFS and OS in relation to *CYP* polymorphism. A statistically significant association with PFS was observed for the *CYP2C9* (C430T), *CYP2C19* (A392G), and *CYP2D6* (G1846A) polymorphisms (***p<0.05***). Specifically, patients harbored the CT genotype at *CYP2C9* (C430T; 27months), AA genotype at *CYP2C19* (G681A;

32 months) and AA genotype at *CYP2D6* (G1846A; 14 months) exhibited a markedly reduced PFS. In contrast, no significant correlation was identified in other *CYP* loci analyzed (**Figure 5.14 a–f**).

Likewise, OS analysis across different *CYP* genotypic groups revealed a statistically significant association at a single locus (***p<0.039***), where the shortest OS duration (39 months) was observed in heterozygous (AG) carriers of *CYP2C19* (G1846A). All other loci showed no significant correlation with OS (*p>0.05*) (**Figure 5.14 g–l**).

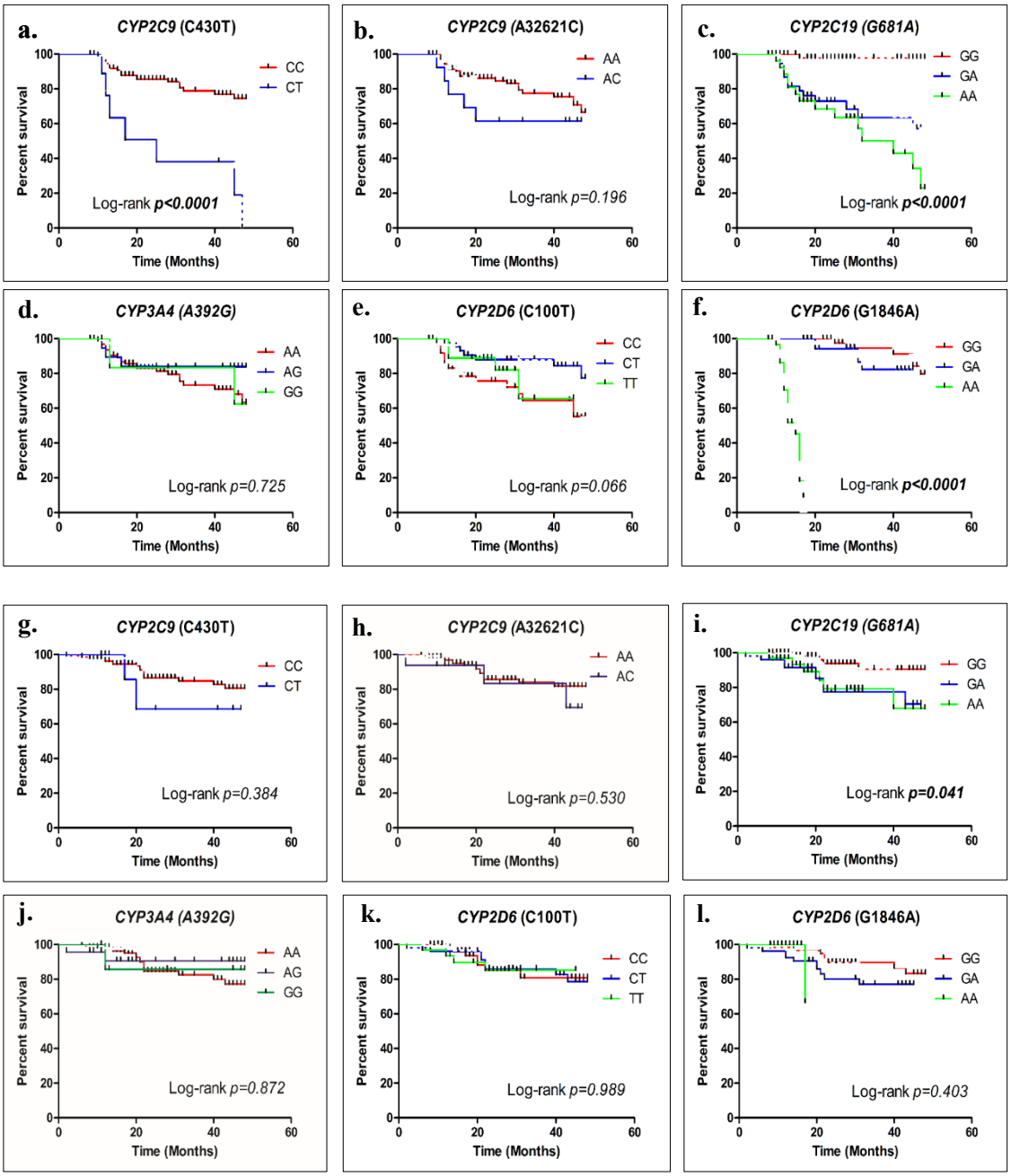


Figure 5.14 Kaplan-Meier survival curves depicting the association between *CYP* polymorphisms in BC patients

(a-f) Progression Free Survival and (g-l) Overall Survival

5.8 Cox-regression Analysis

Cox regression analysis is a semi-parametric statistical method used to assess the impact of multiple covariates on survival time, estimating hazard ratios while accounting for censored data.

5.8.1 Hazard Risk analysis of clinical parameters

Cox regression analyses on the baseline risk factors revealed that the TAC regimen (HR 1.585; 95% CI 0.441-5.694) and the AC-T regimen (HR 1.077; 95% CI 0.320-3.626) had a higher hazard ratio for overall survival (*p>0.05*). However, HR for ER expression (HR 1.447; 95% CI 0.545-3.841), menopausal status (HR 1.300; 95% CI 0.400-4.228) and menopausal age (HR 1.090; 95% CI 0.715-1.662) associated with significant predictive value for poor survival (HR>1) (**Figure 5.15**). Hence, this factors might contribute to an increased hazard risk for poor survival.

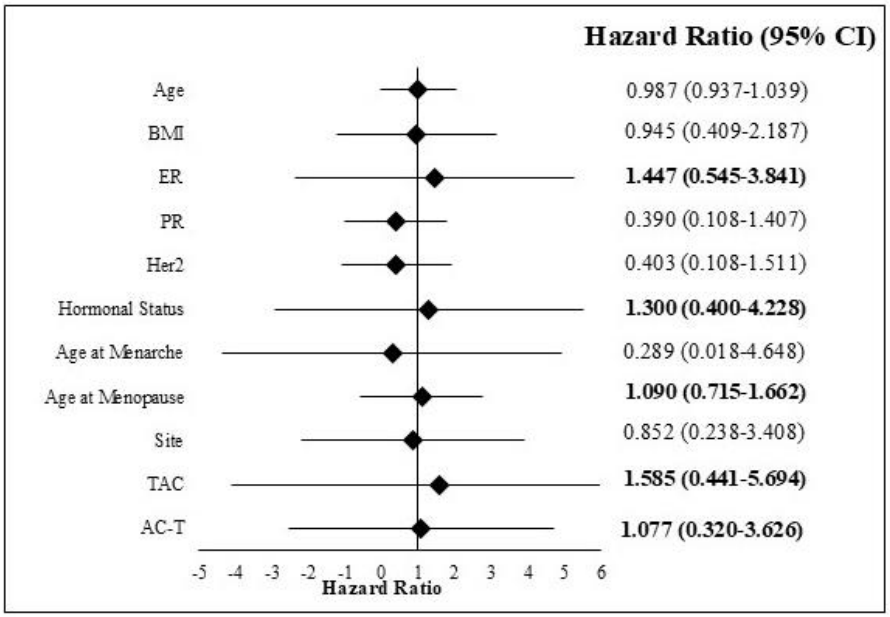


Figure 5.15 Survival hazard ratio of baseline risk factors

5.8.2 Hazard Risk analysis of *ABC* gene polymorphism

Cox regression analysis for all SNPs indicated a lower risk of mortality (HR<1), except for the homozygous TT genotype at the C1236T locus, which showed an increased hazard independently without BMI addition (HR=1.818). However, this association was not statistically significant (*p>0.05*). When genotypes were analyzed in combination with BMI, the CT and TT genotypes of the C1236T SNP and the AA genotype of the C58626A SNP were associated with an increased risk of death (HR>1*)*. In contrast, the CT genotype of the C3435T

SNP, when combined with BMI, was significantly associated with a reduced risk of mortality (HR<1, ***p<0.05***), suggesting a potential protective effect on survival.

The impact of genotype on OS was assessed using univariate analysis, both before and after incorporating BMI as a covariate. Univariate analysis for *ABCB1* genotypes across both SNPs (C1236T and C3435T) ranged between 42–45 months, whereas for *ABCC2* (C58626A), the OS range was slightly lower, at 39–45 months. However, when genotypes were analyzed in combination with BMI, the following genotypes were associated with relatively lower survival: the CT genotype for the C1236T SNP (21 months; ***p<0.0001***), the TT genotype for the C3435T SNP (22 months; ***p<0.0001***), and the AA genotype of the C58626A SNP (22 months; ***p=0.003***) (**Table 5.15**).

Table 5.15 Univariate and multivariate analysis of overall survival across *ABC* genotypic groups stratified by pre- and post-BMI covariate in breast cancer patients

Genotype Groups		Kaplan-Meier Survival (Univariate)			Cox Regression Survival (Multivariate)		
		Mean OS	**95% CI**	***p-value***	**Hazard Risk**	**95% CI**	***p-value***
***ABCB1* (C1236T)**	CC	45.05	(42.26- 47.84)	*0.235*	ref	ref	*ref*
	CT	42.17	(39.10-45.24)		0.748	(0.166-3.366)	*0.706*
	TT	44.01	(40.82-47.20)		**1.818**	(0.512-6.46)	*0.355*
	CT&BMI	**21.00**	-	***<0.0001***	**1.745**	(0.385-7.901)	*0.47*
	TT&BMI	40.00	-		**2.507**	(0.70-8.948)	*0.157*
***ABCB1* (C3435T)**	CC	45.11	(42.96-47.26)	*0.691*	ref	ref	*ref*
	CT	42.98	(39.93-46.03)		0.603	(0.156-2.334)	*0.464*
	TT	42.30	(36.35-48.24)		0.856	(0.232-3.164)	*0.816*
	CT&BMI	43.00	-	***<0.0001***	0.134	(0.023-0.774)	***0.025***
	TT&BMI	**22.00**	-		0.179	(0.032-1.011)	*0.051*
***ABCC2* (C58626A)**	CC	45.73	(43.26-48.20)	***0.032***	ref	ref	*ref*
	CA	45.47	(43.39-47.56)		0.258	(0.071-0.938)	***0.04***
	AA	39.75	(35.26-44.24)		0.369	(0.133-1.019)	*0.054*
	CA&BMI	43.00	-	***0.003***	0.689	(0.168-2.811)	*0.604*
	AA&BMI	**22.00**	-		**1.963**	(0.766-5.031)	*0.16*

Bold lettering denotes p value<0.05 and HR>1.

5.8.3 Hazard Risk analysis of *CYP* gene polymorphism

Multivariate analysis for *CYP* gene across CT (C430T), AC (A32621C), GA & AA (G681A), CT & TT (C100T) and GA & AA (G1846A) genotypes exhibited elevated hazard ratios with respect to OS. However, only the GA (G681A) genotype showed a statistically significant association among all genotypes (***p=0.039***). Furthermore, when genotypes were analyzed in combination with BMI, a higher hazard ratio was observed for the following genotypes: GA

and AA (G681A), AG (A392G), CT (C100T) and GA (G1846A), although this association was not statistically significant *(p>0.05*) (**Table 5.16**).

Table 5.16 Univariate and multivariate analysis of overall survival across *CYP* genotypic groups stratified by pre- and post-BMI covariate in breast cancer patients

Genotype Groups		Kaplan-Meier Survival (Univariate)			Cox Regression Survival (Multivariate)		
		Mean OS	**95% CI / ‖Percentile75%**	***p-value***	**Hazard Risk**	**95% CI**	***p-value***
***CYP2C9* (C430T)**	CC	43.21	(41.07-45.35)	*0.384*	ref	ref	*ref*
	CT	38.08	(27.76-48.40)		**1.480**	(0.32-6.80)	*0.615*
	TT	-	-		-	-	-
	CT&BMI	22.00	±3.260‖	**-**	0.895	(0.19-4.20)	*0.888*
	TT&BMI	-	-		-	-	-
***CYP2C9* (A32621C)**	AA	43.13	(40.92-45.34)	*0.530*	ref	ref	*ref*
	AC	41.02	(34.15-47.90)		**1.108**	(0.30-4.01)	*0.876*
	CC	-	-		-	-	-
	AC&BMI	22.00	±5.884‖	*0.547*	0.929	(0.28-4.14)	*0.923*
	CC&BMI	**-**	-		-	-	-
***CYP2C19* (G681A)**	GG	45.66	(43.47-47.85)	***0.039***	ref	ref	*ref*
	GA	**39.79**	(35.50-44.08)		**3.786**	(1.13-12.64)	***0.031***
	AA	40.97	(35.57-45.82)		**1.838**	**(**0.64-0.07)	*0.253*
	GA & BMI	22.00	±4.724‖	*0.241*	**2.717**	(0.77-9.49)	*0.117*
	AA & BMI	22.00	±7.805‖		**1.140**	**(**0.40-3.19)	*0.803*
***CYP3A4* (A392G)**	AA	42.75	(40.37-45.12)	*0.872*	ref	ref	*ref*
	AG	44.18	(39.14-49.23)		0.861	(0.19-3.84)	*0.844*
	GG	42.85	(33.52-52.18)		0.645	(0.73-5.70)	*0.693*
	AG & BMI	22.00	±4.708‖	*0.938*	**1.039**	(0.21-5.05)	*0.962*
	GG & BMI	22.00	-		0.659	(0.73-5.91)	*0.710*
***CYP2D6* (C100T)**	CC	42.79	(39.26-46.31)	*0.989*	ref	ref	*ref*
	CT	43.16	(39.87-46.53)		**1.055**	(0.37-2.99)	*0.919*
	TT	40.43	(36.26-44.60)		**1.092**	(0.35-3.39)	*0.879*
	CT&BMI	22.00	±5.385‖	*0.928*	**1.107**	(0.34-3.59)	*0.866*
	TT&BMI	22.00	±10.404‖		0.815	(0.25-2.56)	*0.726*
***CYP2D6* (G1846A)**	GG	44.49	(42.08-46.91)	*0.396*	ref	ref	*ref*
	GA	38.66	(35.10-42.21)		**1.852**	(0.725-4.729)	*0.198*
	AA	17.00	(17.00-17.00)		0.743	(0.087-6.308)	*0.785*
	GA&BMI	20.00	±4.055‖	*0.230*	**2.146**	(0.837-5.504)	*0.112*
	AA&BMI	17.00	-		0.672	(0.080-5.632)	*0.714*

Bold lettering denotes p value<0.05 and HR>1; ‖Genotype with BMI stratification does not showed 95% CI, hence all values are expressed as ±standard error at percentiles (75%).

CHAPTER 6

DISCUSSION

CHAPTER 6

DISCUSSION

BC remains one of the most prevalent malignancies worldwide, exhibited diverse clinical presentations and treatment responses. The heterogeneity in therapeutic outcomes among BC patients influenced by multiple factors, including demographic characteristics, clinical attributes, and genetic variability. The management of BC primarily relied on surgery, chemotherapy, and radiation therapy. While anthracycline-taxane-based chemotherapy was well established as a first-line treatment option, most of the safety-efficacy data for these regimens reported from high-income countries, leaving gaps in understanding their impact in low- and middle-income countries. Previously, FEC (5-fluorouracil, epirubicin, cyclophosphamide) and TAC (docetaxel, doxorubicin, cyclophosphamide) regimens were commonly used in BC management. However, due to poor survival outcomes and high toxicity, clinicians have adopted sequential AC-T (doxorubicin, cyclophosphamide followed by taxane) chemotherapy. While Indian studies have described the symptoms and treatment outcomes for recurrent BC and survival post-chemotherapy, the clinical presentation and outcomes of the sequential AC-T regimen as a front-line treatment remain inadequately documented. Furthermore, hormone receptor-positive BC often develop resistance to endocrine therapies such as tamoxifen and aromatase inhibitors, limiting their long-term efficacy.

This study presented a comprehensive analysis of the clinical efficacy, safety profile, toxicity outcome, QoL metrics, and survival distributions of eastern Indian BC patients received concomitant and sequential anthracycline-taxane chemotherapeutic regimens, along with the impact of genetic polymorphisms in chemotherapy related transporters and metabolizing enzymes. Thus, this study might contributed to the shift toward personalized or precision medicine underscores the importance of tailoring treatments based on the genetic and molecular profile of an individual's cancer.

In the present study, BC patients predominantly belonged to the 41–60 age group. This finding aligns with another Indian study where the 41–50 age group was mostly affected (Chopra et al., 2014). Majority of the patients were from rural backgrounds, had low socioeconomic status, and lacked formal education. Interestingly, these patients also exhibited nutritional neglect, as indicated by anthropometric parameters such as low BMI, MUAC, and SFT. Previous studies on body mass of BC patients was primarily discussed about the high BMI group, as obesity was well-established independent risk factors for the development of BC (Tzenios et al., 2024;

Cao et al., 2024; Chan et al., 2014). However, women in low socio-economic countries, who often suffer from poor nutrition, were also at risk of developing BC. Our study was the first to identify low BMI as an independent risk factor for poor clinical outcomes. This factor not extensively studied worldwide and should not be overlooked. Low BMI patients, characterized by inadequate nutritional status as indicated by low MUAC and SFT, often struggle to complete chemotherapy beyond the 2nd or 3rd cycle. This results in delays in chemotherapy administration (Gangane et al., 2017; Chintamani et al., 2011; Jassem et al., 2014) (Mehrotra and Yadav, 2022), ultimately compromised the treatment response and long-term survival outcomes.

In the present study, the majority of patients presented with commonly reported symptoms such as a palpable breast lump, mastalgia (breast pain), redness, and itching. Other Indian studies have also identified breast and armpit lumps accompanied by pain as the most frequently observed symptoms (Prusty et al., 2020). Likewise, another study from the USA by Galipeau and colleagues reported breast lumps and pain as the most detectable symptoms (Galipeau et al., 2019).

Considerable number of the patients were diagnosed at stages II–III (52.89%, 38.01%) with T2 and N1 being the predominant tumor sizes although it was reported that, 70% BC was diagnosed at early stage (I–II) in high-income countries while <50% early BC was diagnosed in lower-middle income countries (Anderson et al., 2021). Other South Asian studies also suggested that the patients were diagnosed in stage III (Chaturvedi et al., 2013; Sathishkumar et al., 2022). However, the affected breast site was nearly equal in our study while past studies leaned toward left breast's higher cancer susceptibility (Amer, 2014). IDC was the most common histological subtype in the present study. Other studies also reported IDC as a most common BC subtype (Zeliha et al., 2020; Tulsyan et al., 2014).

Considering the importance of different BMI categories, as discussed above, crucial clinico-pathological parameters were stratified based on the varying BMI groups. No significant correlation were noticed in clinico-pathological parameters in terms of BMI-based stratification; however, chemotherapy non-responders were significantly higher in the low BMI group, whereas complete responders were more prevalent in the normal BMI group. Additionally, the low BMI group had a higher proportion of low MUAC, while the high BMI group had a greater percentage of mid-range MUAC. The chemotherapy response across different BMI groups showed a significant distribution.

The expression of breast tumor biomarkers ER, PR, HER2/neu varied among the patients and influence the treatment decisions and prognosis. A study conducted at a tertiary care center in Patna reported that 46.29% of cases were ER-positive, 31.48% were PR-positive, and 37.5% exhibited HER2 overexpression. Additionally, TNBC characterized by the absence of ER, PR, and HER2 expression, constituted 30% of the cases (Sinha et al., 2018). This result was nearly similar to our study and the association of these biomarkers with clinical parameters revealed, ER status had a significant association with both treatment response and BMI groups. Patients with positive ER status exhibited a complete response to chemotherapy, whereas those with negative ER status showed poorer responses. Interestingly, low BMI patients predominantly expressed positive ER status, while normal BMI patients had a higher prevalence of ER-negative tumors. Different observations were reported by Onitilo et al., who found a significant association between ER status and clinical parameters such as age, tumor stage, grade, size, and lymph node involvement (Onitilo et al., 2009). Additionally, a study by Hai-long Chen et al reported a better response to neo-adjuvant chemotherapy among patients with low ER-positive tumors (Chen et al., 2023). Present study findings suggested that ER expression of breast tumor might be crucial in predicting treatment response in association with BMI profile of the patients.

Although standard anthracycline-taxane chemotherapy was the first line treatment option for BC patients, however it was associated with significant toxicities, including cardiotoxicity from anthracyclines and neuropathy from taxanes. During chemotherapy, BC patients in our study experienced toxicities such as alopecia, anemia, nausea, and vomiting, but the frequency was relatively low, possibly due to the pre-medications prescribed by clinicians. Hatam *et al*. reported, chemotherapy-induced toxicity (such as amenorrhea, anaemia, nail discoloration, febrile neutropenia, hyperpigmentation, neurologic toxicity, and edema), the TAC arm exhibited higher toxicity compared to the FAC arm (Hatam et al., 2011). As per the USON9735 trial, four cycles of TC were superior to AC in BC patients (Batra et al., 2020). The majority of these patients had hormone receptor-positive (71.2%), and three-fourths had grade III tumours, with a remarkable OS rate of 95.5%. Ferreira *et al*. studied a prospective Phase II trial of neoadjuvant treatment of Stage IIB/III TNBC with cyclophosphamide, doxorubicin, and cisplatin; where they reported very few grade ≥3 toxicity such as nausea (16.3%), vomiting (14.0%), and neutropenia (9.3%) (Ferreira et al., 2018). In this study, Grade 2–3 toxicity was detected in a limited patient subset, potentially linked to the administration of standard pre-medication. This might be attributed to the preventive measures provided to the patients, which

likely contributed to the enhanced well-being among the survival group. The anthracycline-taxane chemotherapy-induced toxicities were relatively low, which suggests that effective supportive care could significantly enhance patient tolerance and overall well-being during treatment.

At the time of diagnosis, patients often experienced significant physical burdens from the tumor and severe psychological distress. In the present study, symptom burden during chemotherapy was assessed using the ESAS scale, which showed a significant reduction. Similarly, Gabriel Lopez et al. reported an improvement in psychosocial symptoms among patients who returned for scheduled follow-ups; however, the decline in symptom scores was not clinically significant (Lopez et al., 2017). Different results was observed by other researchers where symptom burden was increased and fatigue, anxiety depression was indicated as a predictor of poor QoL (Hamer et al., 2017; Chow et al., 2019).

Chemotherapy-induced toxicity and symptom burden played a vital role in shaping patients' QoL outcomes as well as clinical efficacy (Hatam et al., 2011). In the present study, QoL responses were assessed based on treatment strategy, chemotherapy response, and patients' BMI status. The QoL assessment demonstrated significant improvements in QoL mean scores across different domains (social, functional, spiritual, and emotional, etc.) concerning treatment strategy and chemotherapy response. These findings highlight the positive impact of treatment strategy and chemotherapy response on overall QoL, emphasizing the need for a comprehensive approach to patient care that addresses both physical and psychosocial well-being. QoL was also assessed in relation to BMI to evaluate its influence on patients' well-being, as nutritional status plays a critical role in treatment tolerance, overall health, and recovery outcomes. However, low BMI patients experienced poorer QoL scores compared to normal and high BMI patients.

The physical well-being of BC patients significantly affects their QoL and was closely linked to nutritional status. Higher physical scores on the FACT-B questionnaire indicated poor QoL, with underweight patients (<18 kg/m²) showed increased physical scores during and after chemotherapy due to treatment-related toxicities. Poor physical functioning was associated with symptoms like pain, diarrhea, vomiting, nausea, and constipation, leading to delayed chemotherapy and negatively impacting treatment outcomes and overall survival (OS) (Gangane et al., 2017; Chintamani et al., 2011; Jassem et al., 2014; Mehrotra and Yadav, 2022). Notably, BC patients experienced an average treatment delay of 29.4 weeks (Jassem et al., 2014). Our analysis highlighted that patients with low BMI and poor nutritional markers

(MUAC, SFT) faced difficulties completing chemotherapy beyond the second or third cycles. Both low BMI and obese patients reported significantly lower functional scores compared to normal BMI patients, reflecting reduced work capacity, sleep quality, and overall functioning. Rural women (70.9%), often experiencing nutritional neglect, were particularly vulnerable. Financial constraints further exacerbated these issues, with 67.58% of patients from low socio-economic backgrounds (monthly income ≤5000 INR), limiting access to proper nutrition (Bellanger et al., 2020). Underweight patients had the lowest social support scores, heavily relying on family for emotional and financial support during treatment. Emotionally, patients with lower BMI experienced higher distress due to body image concerns (e.g., chemotherapy-induced alopecia in 79.33% and mastectomy in 88.4% of low-income patients). Higher emotional scores indicated poorer QoL, influenced by factors like BMI, income, and treatment type (Fox et al., 2020).

Spiritual well-being, vital in the Indian context, declined significantly in low BMI patients compared to those with normal BMI. Devotional practices provided emotional resilience, though fear of social stigma, especially in rural areas, hindered early diagnosis and treatment-seeking behaviors (Forouzi et al., 2017; Jafari et al., 2013). Concerns about family reputation, marriage prospects, and misconceptions linking cancer to immoral behavior were common barriers. Addressing nutritional support, emotional well-being, and social stigma was crucial for improving BC outcomes in India.

FACT-B and FACIT-Sp 12 questionnaires were used to evaluate the QoL among the concerned cohort had been proved as helpful tool for evaluating QoL in Indian BC patients, with multiple studies confirmed its relevance, validity, and reliability in the Indian setting (Pandey et al., 2002; (Kaur et al., 2023; Amarsheda and Bhise, 2021; Bichoo et al., 2021; Deepa et al., 2020; Paswan et al., 2015; Gandhi et al., 2020). Hence, BMI might be considered an important parameter for evaluating QoL among BC patients. In low- and middle-income countries, where patients often suffer from nutritional deficiencies, we recommend including dietary assessment questions in the additional concern domain of the FACT-B questionnaire to better address the impact of nutrition/ BMI factors on treatment outcomes and QoL.

Following clinical evaluation, a pharmacogenetic analysis was conducted to identify genetic determinants influencing treatment response. SNPs from *ABCB1, ABCC2, CYP2D6, CYP3A4, CYP2C9,* and *CYP2C19* were selected from the dbSNP database based on their functional significance in drug metabolism and transport. These genes play a crucial role in modulating

the pharmacokinetics of chemotherapeutic agents, potentially impacting drug efficacy, toxicity, and overall treatment outcomes in BC patients.

In the Indian heterogenenous population, the genotype frequency of transporter and metabolizer genes exhibited notable variations among the patient population. In this study, the most prevalent genotypes for the *ABCB1* gene were CT, CC, and GG at the C1236T, C3435T, and G2677T/A loci, respectively. The allelic distribution among transporter genes also varied, with C, C, and G alleles being more frequent at the aforementioned loci. While the genotype distribution at the C1236T locus aligns with findings from other studies, different results were observed for the C3435T and G2677T/A loci, as a study from North India reported CT, CT, and GT as the most frequent genotypes at the C1236T, C3435T, and G2677T/A loci, respectively (Chaturvedi et al., 2013). In contrast, a European study reported higher frequencies of CT, CT, and GG genotypes ((Lévy et al., 2013), while a Bangladeshi study identified CC, CT, and TT as the predominant genotypes at these loci (Parvin et al., 2021). For the *ABCC2* gene, C58626A locus exhibited CA genotype as highest frequency. This specific locus was not studied well throughout the world, to the best of our knowledge this was the first time to report this locus data. However other loci of this *ABCC2* were extensively studied mentioned varied genotype distribution (Cao et al., 2020; Zan et al., 2021; Hjorth et al., 2023).

The anthropometric factor BMI plays important role in chemotherapy response, survival outcome, QoL among various cancers which needs to be addressed highlighting the need to consider it while determining treatment strategies. *ABC* gene extensively studied to unveil its implications in toxicity, treatment response, and survival outcomes (Choi et al., 2015). Despite extensive investigation, a definitive correlation was not established. However, in this study, a significant association in *ABCC2* SNP across all the genetic models in both treatment response: dominant (***p=0.003***), recessive (***p<0.0001***), and codominant (***p<0.0001***) and in BMI: dominant (***p=0.001*)**, recessive (***p=0.001***), and codominant (***p<0.0001***). Odds ratio (>1) was also higher in these models, indicated the odds of the events was higher towards the risk factors. Other studies reported, certain SNPs in *ABCB1* gene was reported to be independently associated with BC prognosis and treatment response (Hlaváč et al., 2020; Chaturvedi et al., 2013; Chang et al., 2009; Lévy et al., 2013). Furthermore, a higher abundance of the 'A' allele was detected among NRs (60.9%) compared to ORR (33.3%), indicated a higher correlation for tumor non-responsiveness among the 'A' allele carried patients (OR 3.122, ***p<0.0001***). Likewise, 'A' allele was also higher in low BMI patients (58.8%) compare to higher BMI group (33.3%). Although, no significant association was observed for *ABCB1* SNPs. The combine impact of BMI and

response revealed, the recessive model (CC+CA vs AA) exhibited the association at 7.25-fold (***p=0.012***) where as dominant model (CC vs CA+AA) denoted 3.245 fold (***p=0.041***). Moreover, codominant model (CC vs CA vs AA) showed low BMI patients carrying of AA genotype more prone to treatment non-responsiveness (***p=0.013***). These findings suggest that low BMI independently and jointly with 58626AA genotype exhibited a worst treatment response compared to higher BMI group (≥18.5).

The cytochrome P450 (*CYP*) superfamily was responsible for the metabolism of approximately 60–70% of chemotherapeutic drugs, involving key enzymes such as *CYP1A2, CYP1B1, CYP2B6, CYP2C9, CYP2C19, CYP3A4*, and *CYP3A5*. Several pharmacogenetic studies have highlighted the impact of polymorphic variants in metabolizer genes, where variant alleles contribute to differences in drug efficacy, toxicity and overall poor clinical outcome (Rodriguez-Antona and Ingelman-Sundberg, 2006).

In the present study, specific SNPs within the *CYP* superfamily, focusing on *CYP2C9, CYP2C19, CYP3A4,* and *CYP2D6* were analyzed. The metabolizer genes also exhibited varied genotype frequency, CC, AA, GG, AA, CC, GG were in higher percentage among the following loci C430T, A32621C (*CYP2C9),* G681A (*CYP2C19),* A392G (*CYP3A4),* C100T, G1846A (*CYP2D6)* respectively. The allelic distribution in metabolizer gene also showed varied distribution C, A, G, A, C, G were higher frequency in the aforementioned loci the minor alleles were T, C, A, G, T, A, accordingly. A Russian study demonstrated similar result where, CC, GG, GG genotypes were higher in percentage in C430T, G681A, A392G loci respectively (Seredina et al., 2012). Rafał Świechowski reported AG as a common genotype whereas Kus et al reported GG as most common among Turkish population in A392G gene (Świechowski et al., 2021; Kus et al., 2016).

In the present study, the number of BC patients who received tamoxifen therapy was limited (*n*=12) and restricted the ability to establish a robust correlation between tamoxifen metabolism and *CYP2D6* polymorphisms. The metabolizer variants *CYP2D6*10* and *CYP2D6*4* were specifically involved in tamoxifen bioactivation but did not influence the metabolism of chemotherapeutic agents. Due to the small sample size for tamoxifen receiving patients, statistical power was insufficient to derive meaningful associations, and further analysis was not pursued. Hence, these two SNPs were tested for their survival effect only.

CYP2C9 gene variants like *CYP2C9*2* and *CYP2C9*3* might alter drug clearance, impacted patients' response and survival outcomes (Seredina et al., 2012; Mwinyi et al., 2014). In this

study, *CYP2C19* gene, both loci—C430T and A32621C—did not exhibit the homozygous mutant genotypes (TT and CC, respectively) in this cohort. Consequently, the genetic association analyses for these loci yielded similar results under both dominant and codominant models. Interestingly, the C430T locus demonstrated a significant association with chemotherapy response, showing a 5-fold increased risk for poor chemo-response in both dominant and codominant models (***p<0.05***). An Indian study showed the association of (681G>A) SNP of Adriamycin based chemotherapy induced (Gudur et al., 2024). However, present study exhibited a significantly lower risk was observed in relation to BMI groups (***p<0.05***). In contrast, the A32621C locus displayed a non-significant association, with an increased risk of 1.8-fold to chemo-response and 1.1-fold for BMI across dominant genetic models (*p>0.05*). The combined effect of the treatment response stratified by BMI at the C430T and A32621C loci exhibited similar outcomes. The C430T locus showed an increased risk, with a 2.5-fold and 5.0-fold higher risk in the dominant and codominant models, respectively. The A32621C locus demonstrated a 1.1-fold and 1.7-fold higher risk to non-responsiveness towards chemotherapy. However, the associations were not statistically significant (*p>0.05*). Hence, these two loci (C430T & A32621C) of *CYP2C9* gene did not have any significant role for predicting chemotherapy response. Moreover, the allelic association exhibited higher frequency of wild type allele C and A in the loci C430T & A32621C of *CYP2C9* respectively (*p>0.05*). A study conducted among Russian BC patients, showed an association of *CYP2C9*2* polymorphism with resistance to fluorouracil, cyclophosphamide and Adriamycin based chemotherapy in a neo-adjuvant setting (Seredina et al., 2012). The findings of our study, suggested that the *CYP2C9* polymorphisms might not play a major role in predicting chemotherapy response in Indian heterogeneous population, though further studies with larger cohorts were needed to validate these results.

Among the selected *CYP* genes, *CYP2C19* exhibits the highest enzymatic activity. The *CYP2C19*2 and CYP2C19*3* variants were the most commonly studied polymorphisms (Beelen et al., 2013; Helsby et al., 2021). Several studies have reported the associations between *CYP2C19* polymorphisms and therapeutic outcomes in response to various chemotherapy drugs (Seredina et al., 2012; Gudur et al., 2024). In this study, we investigated the G681A locus, which showed a significant correlation with treatment response, demonstrating an 11.1-fold and 15.8-fold higher risk to poor chemotherapy response (***p<0.05***). Interestingly, in the BMI analysis, a significantly lower risk was observed across all genetic models (***p<0.05***). Moreover, the frequency of the vaiant allele (A) was significantly higher in

NRs, while the wild type allele (G) was more prevalent in the ORR group (***p<0.0001***). Hence, our study demonstrate that, variant "A" allele might be associated with poor chemotherapy response of BC patients in the Eastern Indian cohort. Another Indian study identified the G681A variant as a significant biomarker associated with poor outcomes in BC patients undergoing adjuvant therapy (Kalra et al., 2018). A study from UK Research group reported the influence of pharmacogenetics on chemotherapy response highlighted the role of the *CYP2C19*2* (G681A) splice variant in affecting treatment efficacy and toxicity in breast cancer patients (Bray et al., 2010). In our study, the combined impact of BMI and treatment response at this locus revealed a significantly higher risk to worst response in the low BMI group across all models and a lower risk in the high BMI group (***p<0.05***). Amongst all dominant model in low group showed highest risk 9.6-fold (***p<0.05***). Notably, in the codominant model, the homozygous mutant AA genotype was more frequent among NRs with low BMI, whereas the homozygous dominant GG genotype was predominant in the ORR group with high BMI (***p<0.05*).** A meta-analysis examined the effect of BMI on pathological complete response (pCR) rates following neoadjuvant chemotherapy in operable breast cancer. The study found that increased BMI was associated with lower pCR rates, suggesting that BMI influences chemotherapy efficacy (Wang et al., 2021). Hence, in this study, BC patients suffer from low BMI and tends to carry homozygous recessive genotype AA to exhibit poor chemotherapy response. However, the homozygous wild type genotype GG carrying patients with high BMI (≥18.5) might exhibit complete to partial response in this cohort.

The metabolic activity of *CYP3A4*1B* was variable. Some studies have reported that polymorphisms at this locus were associated with increased promoter activity, potentially leading to enhanced *CYP3A4* expression and accelerated drug metabolism. This might result in reduced drug efficacy due to lower plasma drug concentrations. Few studies identified variant allele G carrying individual exhibited poor response (Keshava et al., 2004; Kuehl et al., 2001). Our study showed reference allele A at higher frequency. Only the recessive model showed 1.0 fold higher risk among treatment response group (*p>0.05*). But, BMI group showed 2.5 fold higher risk in dominant model (***p=0.025***). When combine effect was tested for BMI and treatment response, there was no strong association observed (*p>0.05).* The allelic distribution denoted that, the wild type allele A was higher in both response and BMI subgroups (*p>0.05*). These findings suggest that while the *CYP3A4*1B* polymorphism might not be strongly associated with treatment response considering their combined effect, indicate the need for further studies to clarify its clinical significance.

The present study exhibited OS time of 48 months when BMI added as a covariate the lowest OS was observed in low BMI group (41.93 months). However, others studies suggested obesity was associated with poor survival outcome. Patients received different chemotherapy was also compared for differential survival outcome, where TAC regimen exhibited lowest survival time followed by FEC and AC-T. The results of our study are consistent with previous findings from the NASBP B-30 trial, which compared TAC, AT, and AC-T regimens. The NASBP B-30 trial demonstrated that AC-T had a significant advantage over TAC in terms of DFS (HR = 0.83; ***p=0.006***), but not in OS (HR=0.86; *p=0.086*) for both ER-positive and ER-negative tumors. (Swain et al., 2009) However, when AC-T was compared with AT, a significant improvement in OS (HR=0.83; ***p=0.034***) was observed. Batra et al. conducted the USON9735 trial in 2020 and reported that four cycles of TC were superior to AC in BC patients, especially those with hormone receptor-positive tumors (71.2%) and Grade III tumors (75%), with a 95.5% OS rate (Batra et al., 2020). This study also supports this observation, DFS AC-T regimen exhibited a slight advantage over concomitant anthracycline-taxane (TAC) and anthracycline alone (FEC). Mean DFS times were: FEC 37 months, TAC 38 months, and AC-T 39 months with non-significant (*p=0.534*) association. OS was significantly associated with higher tumor grade (***p=0.008***) and poor chemotherapy response (***p=0.001***) indicating its prognostic value in predicting survival outcomes. Remarkably, the FEC regimen exhibited the worst OS, whereas patients undergoing either sequential (AC-T) or concomitant (TAC) anthracycline-taxane therapies demonstrated similar OS results. The mean OS was 40 months for FEC, 43.5 months for TAC, and 43.6 months for AC-T. This suggested that FEC as least favorable prognosis, there was no significant distinction in OS between patients receiving anthracycline and taxane therapies in either sequential or concomitant administration. Concluding that the survival distributions were not equal among the different treatment regimen groups. Mackey et al., in BCIRG-005 randomized trial studied the differential outcome of AC-T versus TAC as a sequential to concurrent combination in node positive and non-metastatic BC patients. The result of this trial, revealed that, in terms of OS, TAC (OS 78.0%) arm was not superior over AC-T (OS 79.9%) arm (*p=0.506*) (Mackey et al., 2016). In a similar study by van Rossum et al. in 2020, the comparison between adjuvant, dose-dense anthracycline-taxane (ddAC) and TAC among high risk BC where no significant difference was observed on RFS and OS after 6 cycles with 7 years' median follow-up (van Rossum et al., 2018). DFS analysis in this study showed, suggests that the achievement of disease-free status was notably shorter for NRs compared to the overall population across different treatment regimens. AC-T regimen exhibited a slight advantage over the others. Although a significant relationship was found

between treatment response and DFS (***p<0.0001***), patients who did not respond to chemotherapy (NRs group) experienced a median DFS only at 6 ± 2.449 months.

Effect of SNP among *ABC* and *CYP* gene also exhibited different PFS and OS in our study. The CC (C1236T*)*, TT (C3435T) and AA (C58626A*)* genotypes associated with lowest PFS. Whereas TT (C1236T), AA (C58626A) associated with lower OS individually and when BMI included as a covariate: CT (C1236T*)*, the TT (C3435T), and the AA (C58626A) (***p<0.05***) showed worst outcome. However, multivariate test showed increased risk (HR>1) to survival typically when genotypes were analyzed in combination with BMI: CT and TT genotypes of the C1236T SNP and the AA genotype of the C58626A SNP (*p>0.05*). Interestingly, for the C3435T SNP, the CT genotypes combined with BMI were significantly associated with a lowered hazard risk (HR<1; ***p<0.05***), suggesting 87% reduction in risk of death; might have protective role in survival, consistent with previous findings that link certain genetic variants to improved clinical outcomes. To our knowledge, no studies documented these findings regarding C58626A SNP prior to ours. However, other findings reported that, the C1236T polymorphism has been linked to a poor response irrespective of BMI stratification (Chaturvedi et al., 2013). Although, among triple-negative BC patients, TT genotypes of C3435T associated with chemo-resistance (Lévy et al., 2013). Similar trends have been noted among Turkish women, where 3435TT was identified as a potential risk factor (Zeliha et al., 2020).

In addition, the influence of metabolizer gene polymorphisms on overall survival was also studied. Among the genotypes analyzed, *CYP2C9, CYP3A4*, and *CYP2D6* did not demonstrate any statistically significant association with survival outcomes consistent with previous findings that suggested these polymorphisms might have limited impact on clinical outcomes in certain cancers (Zanger and Schwab, 2013; Ingelman-Sundberg et al., 2007). However, for *CYP2C19*, heterozygous individuals (GA) exhibited the shortest median survival time (***p=0.039***), aligned with studies that indicate *CYP2C19* polymorphisms could influence drug metabolism and therapeutic response (Swen et al., 2011; Scott et al., 2013**)**. Stratification based on BMI revealed a uniform median survival of 22 months across all SNPs, with no significant differences observed (*p>0.05*). These findings suggested that the survival data in this study may be immature due to a limited follow-up period (van Schaik, 2008). Notably, all SNPs, except for *CYP3A4*, were associated with an increased HR (>1). Furthermore, after BMI stratification, the elevated HR (>1) persisted for *CYP2C19* (GA, AA), *CYP3A4* (AG), *CYP2D6* (CT; C100T) and (GA; G1846A) genotypes (*p>0.05*) in continuation with previous reports

linked these polymorphisms to altered drug metabolism and treatment efficacy (Rodriguez-Antona and Ingelman-Sundberg, 2006).

This study was limited by inherent biases due to its small sample size and single-center design. Increasing the sample size in future studies will enhance statistical robustness also helps to identify the pharmacogenetic effect of the SNPs. Additionally, the short follow-up period may have led to immature survival data. Future research should include larger, multi-center cohorts, transethnic analyses, and cost-effectiveness evaluations, especially for low- to middle-income countries. Nonetheless, these findings offer valuable insights for BC treatment strategies in the Indian context.

CHAPTER 7

CONCLUSION

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Over the past decades, breast cancer chemotherapy had been underwent significant advancements, with ongoing research refined optimal treatment strategies. Currently, anthracycline-taxane–based regimens were widely established as first-line therapy. However, the majority of safety and efficacy data reported from high-income countries, created a research gap regarding their impact in low- and middle-income settings, highlighting the need for region-specific studies. Moreover, BMI assessment was a critical risk factor yet often overlooked in BC management, particularly among patients with low BMI. This was especially relevant in the heterogeneous Indian population, where rural patients' QoL and treatment outcome with low BMI remain understudied. Additionally, analyzing *ABC* and *CYP* gene polymorphisms was essential for understanding drug resistance and metabolism affecting clinical outcome, further emphasizing the need for comprehensive, personalized treatment strategies. Therefore, it was utmost importance to study clinical relevance of this cohort with an emphasis on pharmacogenetic effect.

1. The clinical efficacy of chemotherapeutic drugs revealed that, prognosis of the Indian BC patients treated with anthracycline and taxane chemotherapy either sequentially or concomitantly was better in terms of survival and efficacy. Although, the AC-T regimen does not improve DFS and OS significantly; however, the observed trend toward DFS (39 months) and OS (43 months) was improved along with reduced toxicity.
2. A statistically significant disparity in the QoL domains- including social, functional, and emotional well-being—was observed between low and normal BMI patients over the 12-month follow-up period. Patients with lower BMI and compromised nutritional status exhibited diminished QoL, suboptimal therapeutic response and reduced overall survival (22 months).
3. *ABC* gene polymorphism revealed that low BMI factor, both independently and in conjunction with the AA genotype in the *ABCC2* gene, was associated with the worst chemotherapy response and unfavorable survival outcomes. Furthermore, the presence of the "A" allele at this locus was significantly linked to non-responders. This genotype was also correlated with compromised PFS (40 months) and OS (39 months), along with an elevated hazard ratio (HR>1), indicating a higher risk of adverse outcomes.

4. Conversely, patients carrying the heterozygous CT and CA genotype at the C3435T and *C*58626A loci in *ABCB1 & ABCC2 gene* respectively demonstrated a potential protective effect, as exhibited a lower hazard ratio (HR<1), suggesting improved treatment response and survival benefits (***p<0.05***). This finding implies that the 3435CT, 58626CA variants might be modulate drug transport efficiency, contributing to better therapeutic outcomes.
5. *CYP* polymorphism revealed that low BMI patients carrying the homozygous recessive genotype (AA) at the *CYP2C19* gene in G681A locus exhibited the poorest chemotherapy response, with the lowest PFS (38 months) and increased HR (>1). However, patients with the heterozygous GA genotype demonstrated the shortest overall survival duration, accompanied by an increased HR (>1), indicating an adverse clinical outcomes. These findings underscore the critical influence of *CYP2C19* polymorphisms on chemotherapy efficacy and survival in BC patients with low BMI.
6. The patients with low BMI and ER positive tumors exhibited a diminished response to chemotherapy, potentially due to altered drug metabolism, hormonal influence, and differences in adipose tissue composition affecting endocrine signaling.

Clinical efficacy of chemotherapeutic drugs among Indian heterogeneous population was very similar with western world. AC-T regimen suggested as a preferred option in specific patient cohorts as a first line chemotherapy showed better efficacy, safety profile and survival outcome Indian BC cancer patients. BMI might act as a modulating factor in chemotherapy response, reinforcing the need for nutritional assessment and intervention. Providing sufficient nutritional support is essential, as it may help to improve QoL, reduce the negative effects of chemotherapy, increase adherence to treatment, and boost long-term treatment outcomes. Hence it is recommended to get guidance from a certified nutritionist prior to commencing any treatment. Polymorphisms in transporter genes play a pivotal role in multidrug resistance, while variations in metabolizer genes significantly impact drug efficacy. The loss- or gain-of-function mutations in these genes critically influence overall treatment response, often leading to reduced therapeutic effectiveness and poorer clinical outcomes. Hence, these genetic factors may serve as predictive markers in chemotherapy response evaluation in relation to BMI. Therefore, genetic counseling is recommended before initiating any treatment regimen to optimize therapeutic outcomes. Moreover, BC tumor biomarker ER expression may have significant implications to predict treatment response.

BIBLIOGRAPHY

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Abdul Aziz, A.A., Md Salleh, M.S., Mohamad, I., Bhavaraju, V.M.K., Yahya, M.M., Zakaria, A.D., Gan, S.H., Ankathil, R., 2018. Genotypes and haplotypes of ABCB1 contribute to TAC chemotherapy response in Malaysian triple negative breast cancer patients. Meta Gene 16, 21–27. https://doi.org/10.1016/J.MGENE.2018.01.002

Aboul-Soud, M.A.M., Alzahrani, A.J., Mahmoud, A., 2021. Decoding variants in drug-metabolizing enzymes and transporters in solid tumor patients by whole-exome sequencing. Saudi J. Biol. Sci. 28, 628–634. https://doi.org/10.1016/J.SJBS.2020.10.052

Abraham, J.E., Maranian, M.J., Driver, K.E., Platte, R., Kalmyrzaev, B., Baynes, C., Luccarini, C., Shah, M., Ingle, S., Greenberg, D., Earl, H.M., Dunning, A.M., Pharoah, P.D.P., Caldas, C., 2010. CYP2D6 gene variants: association with breast cancer specific survival in a cohort of breast cancer patients from the United Kingdom treated with adjuvant tamoxifen. Breast Cancer Res. 12, R64. https://doi.org/10.1186/BCR2629

Ahmed, S., Zhou, Z., Zhou, J., Chen, S.Q., 2016. Pharmacogenomics of Drug Metabolizing Enzymes and Transporters: Relevance to Precision Medicine. Genomics. Proteomics Bioinformatics 14, 298–313. https://doi.org/10.1016/J.GPB.2016.03.008

Amarsheda, S.B., Bhise, A.R., 2021. Association of Fatigue, Quality of Life and Functional Capacity in Breast Cancer Patients Receiving Adjuvant Chemotherapy. Asian Pacific J. Cancer Care 6, 59–64. https://doi.org/10.31557/APJCC.2021.6.1.59-64

Amer, M.H., 2014. Genetic factors and breast cancer laterality. Cancer Manag. Res. 6, 191–203. https://doi.org/10.2147/CMAR.S60006

Amin, M.B., Greene, F.L., Edge, S.B., Compton, C.C., Gershenwald, J.E., Brookland, R.K., Meyer, L., Gress, D.M., Byrd, D.R., Winchester, D.P., 2017. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA. Cancer J. Clin. 67, 93–99. https://doi.org/10.3322/CAAC.21388

Anampa, J., Makower, D., Sparano, J.A., 2015. Progress in adjuvant chemotherapy for breast cancer: An overview. BMC Med. 13, 1–13. https://doi.org/10.1186/S12916-015-0439-8/TABLES/4

Anderson, B.O., Ilbawi, A.M., Fidarova, E., Weiderpass, E., Stevens, L., Abdel-Wahab, M., Mikkelsen, B., 2021. The Global Breast Cancer Initiative: a strategic collaboration to strengthen health care for non-communicable diseases. Lancet. Oncol. 22, 578–581. https://doi.org/10.1016/S1470-2045(21)00071-1

Barone, I., Caruso, A., Gelsomino, L., Giordano, C., Bonofiglio, D., Catalano, S., Andò, S., 2022. Obesity and endocrine therapy resistance in breast cancer: Mechanistic insights and perspectives. Obes. Rev. 23. https://doi.org/10.1111/OBR.13358

Basaran, G., Turhal, N.S., Cabuk, D., Yurt, N., Yurtseven, G., Gumus, M., Teomete, M., Dane, F., Yumuk, P.F., 2011. Weight gain after adjuvant chemotherapy in patients with early

breast cancer in Istanbul Turkey. Med. Oncol. 28, 409–415. https://doi.org/10.1007/S12032-010-9463-X/TABLES/5

Batra, A., Hannouf, M.B., Alsafar, N., Lupichuk, S., 2020. Four cycles of docetaxel and cyclophosphamide as adjuvant chemotherapy in node negative breast cancer: A real-world study. Breast 54, 1–7. https://doi.org/10.1016/j.breast.2020.08.002

Beelen, K., Opdam, M., Severson, T.M., Koornstra, R.H.T., Vincent, A.D., Hauptmann, M., Van Schaik, R.H.N., Berns, E.M.J.J., Vermorken, J.B., Van Diest, P.J., Linn, S.C., 2013. CYP2C19*2 predicts substantial tamoxifen benefit in postmenopausal breast cancer patients randomized between adjuvant tamoxifen and no systemic treatment. Breast Cancer Res. Treat. 139, 649. https://doi.org/10.1007/S10549-013-2568-0

Bellanger, M., Barry, K., Rana, J., Regnaux, J.P., 2020. Cost-Effectiveness of Lifestyle-Related Interventions for the Primary Prevention of Breast Cancer: A Rapid Review. Front. Med. https://doi.org/10.3389/fmed.2019.00325

Bertholee, D., Maring, J.G., van Kuilenburg, A.B.P., 2017. Genotypes Affecting the Pharmacokinetics of Anticancer Drugs. Clin. Pharmacokinet. 56, 317–337. https://doi.org/10.1007/S40262-016-0450-Z

Bichoo, R.A., Mishra, A., Lal, P., Gyan, C., Agarwal, G., Agarwal, A., Mishra, S.K., 2021. Quality of Life (QoL) in Postmenopausal Breast Cancer Patients Receiving Adjuvant Hormonal Therapy. Indian J. Surg. 83, 461–467. https://doi.org/10.1007/S12262-021-02766-6/METRICS

Bonneterre, J., Roché, H., Kerbrat, P., Brémond, A., Fumoleau, P., Namer, M., Gaudier, M.J., Schraub, S., Fargeot, P., Chapelle-Marcillac, I., 2005. Epirubicin Increases Long-Term Survival in Adjuvant Chemotherapy of Patients With Poor-Prognosis, Node-Positive, Early Breast Cancer: 10-Year Follow-Up Results of the French Adjuvant Study Group 05 Randomized Trial. J. Clin. Oncol. 23, 2686–2693. https://doi.org/10.1200/JCO.2005.05.059

Boucenna, A., Boudaoud, K., Hireche, A., Rezgoune, M.L., Abadi, N., Filali, T., Satta, D., 2022. Influence of CYP2D6, CYP2C19 and CYP3A5 polymorphisms on plasma levels of tamoxifen metabolites in Algerian women with ER+ breast cancer. Egypt. J. Med. Hum. Genet. 23, 1–11. https://doi.org/10.1186/S43042-022-00332-7/FIGURES/4

Brady, M.J., Cella, D.F., Mo, F., Bonomi, A.E., Tulsky, D.S., Lloyd, S.R., Deasy, S., Cobleigh, M., Shiomoto, G., 1997. Reliability and validity of the functional assessment of cancer therapy- breast quality-of-life instrument. J. Clin. Oncol. 15, 974–986. https://doi.org/10.1200/JCO.1997.15.3.974

Bray, J., Sludden, J., Griffin, M.J., Cole, M., Verrill, M., Jamieson, D., Boddy, A. V, 2010. Influence of pharmacogenetics on response and toxicity in breast cancer patients treated with doxorubicin and cyclophosphamide. https://doi.org/10.1038/sj.bjc.6605587

Breast Cancer Risk Factors and Prevention Methods | American Cancer Society [WWW Document], n.d. URL https://www.cancer.org/cancer/types/breast-cancer/risk-and-prevention.html (accessed 2.19.25).

Breast Cancer Signs and Symptoms | Most Common Symptoms | American Cancer Society [WWW Document], n.d. URL https://www.cancer.org/cancer/types/breast-cancer/screening-tests-and-early-detection/breast-cancer-signs-and-symptoms.html (accessed 2.19.25).

Brierley J.D., Gospodarowicz M.K., Wittekind C., 2017. TNM Classification of Malignant Tumours, 8 th edition due December 2016. Union Int. Cancer Control 1–272.

Brown, J.S., Amend, S.R., Austin, R.H., Gatenby, R.A., Hammarlund, E.U., Pienta, K.J., 2023. Updating the Definition of Cancer. Mol. Cancer Res. 21, 1142. https://doi.org/10.1158/1541-7786.MCR-23-0411

Bruera, E., Kuehn, N., Miller, M.J., Selmser, P., Macmillan, K., 1991. The Edmonton Symptom Assessment System (ESAS): A Simple Method for the Assessment of Palliative Care Patients. https://doi.org/10.1177/082585979100700202 7, 6–9.

Burguin, A., Diorio, C., Durocher, F., 2021. Breast Cancer Treatments: Updates and New Challenges. J. Pers. Med. 11. https://doi.org/10.3390/JPM11080808

Cao, J., Li, J., Zhang, Z., Qin, G., Pang, Y., Wu, M., Gu, K., Xu, H., 2024. Interaction between body mass index and family history of cancer on the risk of female breast cancer. Sci. Reports 2024 141 14, 1–9. https://doi.org/10.1038/s41598-024-54762-x

Cao, K., Ren, G., Lu, C., Wang, Y., Tan, Y., Zhou, J., Zhang, Y., Lu, Y., Li, N., Chen, X., Zhao, D., 2020. ABCC2 c.-24 C>T single-nucleotide polymorphism was associated with the pharmacokinetic variability of deferasirox in Chinese subjects. Eur. J. Clin. Pharmacol. 76, 51–59. https://doi.org/10.1007/S00228-019-02775-1Cardoso, F., Paluch-Shimon, S., Senkus, E., Curigliano, G., Aapro, M.S., André, F., Barrios, C.H., Bergh, J., Bhattacharyya, G.S., Biganzoli, L., Boyle, F., Cardoso, M.J., Carey, L.A., Cortés, J., El Saghir, N.S., Elzayat, M., Eniu, A., Fallowfield, L., Francis, P.A., Gelmon, K., Gligorov, J., Haidinger, R., Harbeck, N., Hu, X., Kaufman, B., Kaur, R., Kiely, B.E., Kim, S.B., Lin, N.U., Mertz, S.A., Neciosup, S., Offersen, B. V., Ohno, S., Pagani, O., Prat, A., Penault-Llorca, F., Rugo, H.S., Sledge, G.W., Thomssen, C., Vorobiof, D.A., Wiseman, T., Xu, B., Norton, L., Costa, A., Winer, E.P., 2020. 5th ESO-ESMO international consensus guidelines for advanced breast cancer (ABC 5). Ann. Oncol. 31, 1623–1649. https://doi.org/10.1016/J.ANNONC.2020.09.010

Cardoso, F., Paluch-Shimon, S., Senkus, E., Curigliano, G., Aapro, M.S., André, F., Barrios, C.H., Bergh, J., Bhattacharyya, G.S., Biganzoli, L., Boyle, F., Cardoso, M.J., Carey, L.A., Cortés, J., El Saghir, N.S., Elzayat, M., Eniu, A., Fallowfield, L., Francis, P.A., Gelmon, K., Gligorov, J., Haidinger, R., Harbeck, N., Hu, X., Kaufman, B., Kaur, R., Kiely, B.E., Kim, S.B., Lin, N.U., Mertz, S.A., Neciosup, S., Offersen, B. V., Ohno, S., Pagani, O., Prat, A., Penault-Llorca, F., Rugo, H.S., Sledge, G.W., Thomssen, C., Vorobiof, D.A., Wiseman, T., Xu, B., Norton, L., Costa, A., Winer, E.P., 2020. 5th ESO-ESMO international consensus guidelines for advanced breast cancer (ABC 5). Ann. Oncol. 31, 1623–1649. https://doi.org/10.1016/J.ANNONC.2020.09.010

Cavaletti, G., Marmiroli, P., 2010. Chemotherapy-induced peripheral neurotoxicity. Nat. Rev. Neurol. 6, 657–666. https://doi.org/10.1038/NRNEUROL.2010.160

Chan, D.S.M., Vieira, A.R., Aune, D., Bandera, E. V., Greenwood, D.C., McTiernan, A., Navarro Rosenblatt, D., Thune, I., Vieira, R., Norat, T., 2014. Body mass index and survival in women with breast cancer-systematic literature review and meta-analysis of 82 follow-up studies. Ann. Oncol. Off. J. Eur. Soc. Med. Oncol. 25, 1901–1914. https://doi.org/10.1093/ANNONC/MDU042

Chang, H., Rha, S.Y., Jeung, H.C., Im, C.K., Ahn, J.B., Kwon, W.S., Yoo, N.C., Roh, J.K., Chung, H.C., 2009. Association of the ABCB1 gene polymorphisms 2677G>T/A and 3435C>T with clinical outcomes of paclitaxel monotherapy in metastatic breast cancer patients. Ann. Oncol. Off. J. Eur. Soc. Med. Oncol. 20, 272–277. https://doi.org/10.1093/ANNONC/MDN624

Chaturvedi, P., Tulsyan, S., Agarwal, G., Lal, P., Agarwal, S., Mittal, R.D., Mittal, B., 2013. Influence of ABCB1 genetic variants in breast cancer treatment outcomes. Cancer Epidemiol. 37, 754–761. https://doi.org/10.1016/j.canep.2013.04.012

Chen, C., Huang, Y., Li, Y., Mao, Y., Xie, Y., 2007. Cytochrome P450 1A1 (CYP1A1) T3801C and A2455G polymorphisms in breast cancer risk: a meta-analysis. J. Hum. Genet. 52, 423–435. https://doi.org/10.1007/S10038-007-0131-8

Chen, H. long, Huang, F. bo, Chen, Q., Deng, Y. chuan, 2023. Impact of estrogen receptor expression level on response to neoadjuvant chemotherapy and prognosis in HER2-negative breast cancers. BMC Cancer 23, 1–9. https://doi.org/10.1186/S12885-023-11368-2/TABLES/4

Chen, L., Qi, Y., Kong, X., Su, Z., Wang, Z., Wang, X., Du, Y., Fang, Y., Li, X., Wang, J., 2022. Nutritional Risk Index Predicts Survival in Patients With Breast Cancer Treated With Neoadjuvant Chemotherapy. Front. Nutr. 8. https://doi.org/10.3389/fnut.2021.786742

Chintamani, Tuteja, A., Khandelwal, R., Megha, T., Bamal, R., Jain, S., Narayan, N., Srinivas, S., Kumar, Y., 2011. Patient and provider delays in breast cancer patients attending a tertiary care centre: a prospective study. JRSM Short Rep. 2, 1–4. https://doi.org/10.1258/shorts.2011.011006

Choi, J.R., Kim, J.O., Kang, D.R., Shin, J.Y., Zhang, X.H., Oh, J.E., Park, J.Y., Kim, K.A., Kang, J.H., 2015. Genetic variations of drug transporters can influence on drug response in patients treated with docetaxel chemotherapy. Cancer Res. Treat. 47, 509–517. https://doi.org/10.4143/crt.2014.012

Chow, S., Wan, B.A., Pidduck, W., Zhang, L., DeAngelis, C., Chan, S., Yee, C., Drost, L., Leung, E., Sousa, P., Lewis, D., Lam, H., Chow, R., Lock, M., Chow, E., 2019. Symptoms Predictive of Overall Quality of Life Using the Edmonton Symptom Assessment Scale in Breast Cancer Patients Receiving Radiotherapy. Clin. Breast Cancer 19, 405–410. https://doi.org/10.1016/J.CLBC.2019.05.007

Cidon, E.U., Ballesteros, A., 1995. Paclitaxel (taxol). N. Engl. J. Med. 332, 291–297. https://doi.org/10.1056/NEJM199504133321507Chang, H., Rha, S.Y., Jeung, H.C., Im, C.K., Ahn, J.B., Kwon, W.S., Yoo, N.C., Roh, J.K., Chung, H.C., 2009. Association of the ABCB1 gene polymorphisms 2677G>T/A and 3435C>T with clinical outcomes of

paclitaxel monotherapy in metastatic breast cancer patients. Ann. Oncol. 20, 272–277. https://doi.org/10.1093/annonc/mdn624

Clarke, C.A., Keegan, T.H.M., Yang, J., Press, D.J., Kurian, A.W., Patel, A.H., Lacey, J. V., 2012. Age-Specific Incidence of Breast Cancer Subtypes: Understanding the Black–White Crossover. JNCI J. Natl. Cancer Inst. 104, 1094–1101. https://doi.org/10.1093/JNCI/DJS264

Connor, A.E., Baumgartner, R.N., Boone, S.D., Pinkston, C.M., Baumgartner, K.B., 2013. Obesity, Ethnicity, and Quality of Life Among Breast Cancer Survivors and Women Without Breast Cancer: The Long-Term Quality of Life Follow-Up Study. Ann. Epidemiol. 23, 582–583. https://doi.org/10.1016/J.ANNEPIDEM.2013.06.017

Coombes, R.C., Bliss, J.M., Wils, J., Morvan, F., Espié, M., Amadori, D., Gambrosier, P., Richards, M., Aapro, M., Villar-Grimalt, A., McArdle, C., Pérez-López, F.R., Vassilopoulos, P., Pinto Ferreira, E., Chilvers, C.E.D., Coombes, G., Woods, E.M., Marty, M., 1996. Adjuvant Cyclophosphamide, Methotrexate, and Fluorouracil Versus Fluorouracil, Epirubicin, and Cyclophosphamide Chemotherapy in Premenopausal Women with Axillary Node-Positive Operable Breast Cancer: Results of a Randomized Trial. J. Clin. Oncol. 14, 35–45. https://doi.org/10.1200/JCO.1996.14.1.35

Chopra, B., Kaur, V., Singh, K., Verma, M., Singh, S., Singh, A., 2014. Age shift: Breast cancer is occurring in younger age groups - Is it true? Clin. Cancer Investig. J. 3, 526–529. https://doi.org/10.4103/2278-0513.142652

Crown, J., O'Leary, M., Ooi, W.-S., 2004. Docetaxel and Paclitaxel in the Treatment of Breast Cancer: A Review of Clinical Experience. Oncologist 9, 24–32. https://doi.org/10.1634/THEONCOLOGIST.9-SUPPL_2-24

Curigliano, G., Burstein, H.J., Gnant, M., Loibl, S., Cameron, D., Regan, M.M., Denkert, C., Poortmans, P., Weber, W.P., Thürlimann, B., Aebi, S., Al-Foheidi, M., André, F., Anikusko, M., Badwe, R., Barrio, A. V., Barrios, C., Bergh, J., Bonnefoi, H., Bretel Morales, D., Brucker, S., Caldas, C., Cardoso, F., Cardoso, M.J., Carey, L., Chia, S., Coles, C., Cortes, J., de Boniface, J., Delaloge, S., DeMichele, A., Fastner, G., Fitzal, F., Francis, P., Gamal, H., Gentilini, O., Gradishar, W., Gulluoglu, B., Harbeck, N., Heil, J., Huang, C.S.H., Huober, J., Jiang, Z., Kaidar-Person, O., Kok, M., Lee, E.S., Loi, S., Martin, M., Meattini, I., Morrow, M., Partridge, A., Penault-Llorca, F., Piccart, M., Pierce, L., Regan, M., Reis-Filho, J., Rubio, I., Rugo, H., Rutgers, E., Saura, C., Senkus, E., Shao, Z., Singer, C., Spanic, T., Thuerlimann, B., Toi, M., Tolaney, S., Turner, N., Tutt, A., Vrancken Peeters, M.J., Watanabe, T., Weber, W., Wildiers, H., Xu, B., 2023. Understanding breast cancer complexity to improve patient outcomes: The St Gallen International Consensus Conference for the Primary Therapy of Individuals with Early Breast Cancer 2023, in: Annals of Oncology. Elsevier, pp. 970–986. https://doi.org/10.1016/j.annonc.2023.08.017

Davies, C., Pan, H., Godwin, J., Gray, R., Arriagada, R., Raina, V., Abraham, M., Medeiros Alencar, V.H., Badran, A., Bonfill, X., Bradbury, J., Clarke, M., Collins, R., Davis, S.R., Delmestri, A., Forbes, J.F., Haddad, P., Hou, M.F., Inbar, M., Khaled, H., Kielanowska,

J., Kwan, W.H., Mathew, B.S., Mittra, I., Müller, B., Nicolucci, A., Peralta, O., Pernas, F., Petruzelka, L., Pienkowski, T., Radhika, R., Rajan, B., Rubach, M.T., Tort, S., Urrútia, G., Valentini, M., Wang, Y., Peto, R., 2013. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. Lancet (London, England) 381, 805–816. https://doi.org/10.1016/S0140-6736(12)61963-1

Deepa, K. V, Gadgil, A., Löfgren, J., Mehare, S., Bhandarkar, P., Roy, N., 2020. Is quality of life after mastectomy comparable to that after breast conservation surgery? A 5-year follow up study from Mumbai, India. Qual. Life Res. 29, 683–692. https://doi.org/10.1007/s11136-019-02351-1

Dieli-Conwright, C.M., Courneya, K.S., Demark-Wahnefried, W., Sami, N., Lee, K., Sweeney, F.C., Stewart, C., Buchanan, T.A., Spicer, D., Tripathy, D., Bernstein, L., Mortimer, J.E., 2018. Aerobic and resistance exercise improves physical fitness, bone health, and quality of life in overweight and obese breast cancer survivors: A randomized controlled trial. Breast Cancer Res. 20. https://doi.org/10.1186/s13058-018-1051-6

Dueck, A.C., Mendoza, T.R., Mitchell, S.A., Reeve, B.B., Castro, K.M., Rogak, L.J., Atkinson, T.M., Bennett, A. V., Denicoff, A.M., O'Mara, A.M., Li, Y., Clauser, S.B., Bryant, D.M., Bearden, J.D., Gillis, T.A., Harness, J.K., Siegel, R.D., Paul, D.B., Cleeland, C.S., Schrag, D., Sloan, J.A., Abernethy, A.P., Bruner, D.W., Minasian, L.M., Basch, E., 2015. Validity and reliability of the us national cancer institute's patient-reported outcomes version of the common terminology criteria for adverse events (PRO-CTCAE). JAMA Oncol. 1, 1051–1059. https://doi.org/10.1001/jamaoncol.2015.2639

Eisenhauer, E.A., Therasse, P., Bogaerts, J., Schwartz, L.H., Sargent, D., Ford, R., Dancey, J., Arbuck, S., Gwyther, S., Mooney, M., Rubinstein, L., Shankar, L., Dodd, L., Kaplan, R., Lacombe, D., Verweij, J., 2009. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur. J. Cancer 45, 228–247. https://doi.org/10.1016/j.ejca.2008.10.026

Esteves, F., Rueff, J., Kranendonk, M., 2021. The Central Role of Cytochrome P450 in Xenobiotic Metabolism—A Brief Review on a Fascinating Enzyme Family. J. Xenobiotics 11, 94. https://doi.org/10.3390/JOX11030007

Ewer, M.S., Ewer, S.M., 2010. Cardiotoxicity of anticancer treatments: what the cardiologist needs to know. Nat. Rev. Cardiol. 7, 564–575. https://doi.org/10.1038/NRCARDIO.2010.121

Faroughi, F., Fathnezhad-Kazemi, A., Sarbakhsh, P., 2023. Factors affecting quality of life in women with breast cancer: a path analysis. BMC Womens. Health 23, 1–9. https://doi.org/10.1186/S12905-023-02755-9/TABLES/3

Ferlay, J., Ervik, M., Lam, F., Laversanne, M., Colombet, M., Mery, L., Piñeros, M., Znaor, A., Soerjomataram, I., Bray, F., 2024. Global Cancer Observatory: Cancer Today [WWW Document]. Int. Agency Res. Cancer. URL https://gco.iarc.who.int/today (accessed 4.27.24).

Ferreira, A.R., Metzger-Filho, O., Sarmento, R.M.B., Bines, J., 2018. Neoadjuvant Treatment of Stage IIB/III Triple Negative Breast Cancer with Cyclophosphamide, Doxorubicin, and Cisplatin (CAP Regimen): A Single Arm, Single Center Phase II Study (GBECAM 2008/02). Front. Oncol. 7. https://doi.org/10.3389/FONC.2017.00329

Fischer, A.H., Jacobson, K.A., Rose, J., Zeller, R., 2008. Hematoxylin and eosin staining of tissue and cell sections. CSH Protoc. 2008. https://doi.org/10.1101/PDB.PROT4986

Fisusi, F.A., Akala, E.O., 2019. Drug Combinations in Breast Cancer Therapy. Pharm. Nanotechnol. 7, 3–23. https://doi.org/10.2174/2211738507666190122111224

Forouzi, M.A., Tirgari, B., Safarizadeh, M.H., Jahani, Y., 2017. Spiritual Needs and Quality of Life of Patients with Cancer. Indian J. Palliat. Care 23, 437. https://doi.org/10.4103/IJPC.IJPC_53_17

Fox, R.S., Ancoli-Israel, S., Roesch, S.C., Merz, E.L., Mills, S.D., Wells, K.J., Sadler, G.R., Malcarne, V.L., 2020. Sleep disturbance and cancer-related fatigue symptom cluster in breast cancer patients undergoing chemotherapy. Support. Care Cancer 28, 845–855. https://doi.org/10.1007/s00520-019-04834-w

Frederiks, C.N., Lam, S.W., Guchelaar, H.J., Boven, E., 2015. Genetic polymorphisms and paclitaxel- or docetaxel-induced toxicities: A systematic review. Cancer Treat. Rev. 41, 935–950. https://doi.org/10.1016/J.CTRV.2015.10.010

Fujii, T., Le Du, F., Xiao, L., Kogawa, T., Barcenas, C.H., Alvarez, R.H., Valero, V., Shen, Y., Ueno, N.T., 2015. Effectiveness of an Adjuvant Chemotherapy Regimen for Early-Stage Breast Cancer: A Systematic Review and Network Meta-analysis. JAMA Oncol. 1, 1311–1318. https://doi.org/10.1001/JAMAONCOL.2015.3062

Galipeau, N., Klooster, B., Krohe, M., Tang, D.H., Revicki, D.A., Cella, D., 2019. Understanding key symptoms, side effects, and impacts of HR+/HER2- advanced breast cancer: qualitative study findings. J. patient-reported outcomes 3. https://doi.org/10.1186/S41687-019-0098-1Gangane, N., Khairkar, P., Hurtig, A.K., Sebastián, M.S., 2017. Quality of life determinants in breast cancer patients in central rural India. Asian Pacific J. Cancer Prev. 18, 3325–3332. https://doi.org/10.22034/APJCP.2017.18.12.3325

Gandhi, A., Samuel, S.R., Vijaya Kumar, K., Prakash Saxena, P.U., Mithra, P., 2020. Effect of a Pedometer-based Exercise Program on Cancer Related Fatigue and Quality of Life amongst Patients with Breast Cancer Receiving Chemotherapy. Asian Pac. J. Cancer Prev. 21, 1813–1818. https://doi.org/10.31557/APJCP.2020.21.6.1813

Gangane, N., Khairkar, P., Hurtig, A.K., Sebastián, M.S., 2017. Quality of life determinants in breast cancer patients in central rural India. Asian Pacific J. Cancer Prev. 18, 3325–3332. https://doi.org/10.22034/APJCP.2017.18.12.3325

Genet, S., 2020. Assessment of Malnutrition among Female Breast Cancer Patients using Biochemical Markers. J Oncol Res Treat 2020, 148.

Global Strategy on Diet, Physical Activity and Health - 2004 [WWW Document], n.d. URL https://www.who.int/publications/i/item/9241592222 (accessed 9.21.23).

Gomes Do Nascimento, R., Otoni, K.M., 2020. Histological and molecular classification of breast cancer: what do we know? Mastology 30, 20200024. https://doi.org/10.29289/25945394202020200024

Gorlov, I.P., Gorlova, O.Y., Amos, C.I., 2008. Relative effects of mutability and selection on single nucleotide polymorphisms in transcribed regions of the human genome. BMC Genomics 9, 1–11. https://doi.org/10.1186/1471-2164-9-292/TABLES/8

Gottesman, M.M., Fojo, T., Bates, S.E., 2002. Multidrug resistance in cancer: role of ATP-dependent transporters. Nat. Rev. Cancer 2, 48–58. https://doi.org/10.1038/NRC706

Gudur, R.A., Bhosale, S.J., Gudur, A.K., Kale, S.R., More, A.L., Datkhile, K.D., 2024. The Effect of CYP2C19*2 (rs4244285) and CYP17 (rs743572) SNPs on Adriamycin and Paclitaxel based Chemotherapy Outcomes in Breast Cancer Patients. Asian Pac. J. Cancer Prev. 25, 1977–1986. https://doi.org/10.31557/APJCP.2024.25.6.1977

Gunter, M.J., Hoover, D.R., Yu, H., Wassertheil-Smoller, S., Rohan, T.E., Manson, J.E., Li, J., Ho, G.Y.F., Xue, X., Anderson, G.L., Kaplan, R.C., Harris, T.G., Howard, B. V., Wylie-Rosett, J., Burk, R.D., Strickler, H.D., 2009. Insulin, insulin-like growth factor-I, and risk of breast cancer in postmenopausal women. J. Natl. Cancer Inst. 101, 48–60. https://doi.org/10.1093/JNCI/DJN415

Gutierrez-Rubio, S.A., Quintero-Ramos, A., Durán-Cárdenas, A., Franco-Topete, R.A., Franco-Topete, R.A., Castro-Cervantes, J.M., Oceguera-Villanueva, A., Jiménez-Pérez, L.M., Balderas-Peña, L., Morgan-Villela, G., Del-Toro-Arreola, A., Daneri-Navarro, A., 2015. 1236 C/T and 3435 C/T polymorphisms of the ABCB1 gene in Mexican breast cancer patients. Genet. Mol. Res. 14, 1250–1259. https://doi.org/10.4238/2015.FEBRUARY.13.3

Hamer, J., McDonald, R., Zhang, L., Verma, S., Leahey, A., Ecclestone, C., Bedard, G., Pulenzas, N., Bhatia, A., Chow, R., DeAngelis, C., Ellis, J., Rakovitch, E., Lee, J., Chow, E., 2017. Quality of life (QOL) and symptom burden (SB) in patients with breast cancer. Support. Care Cancer 25, 409–419. https://doi.org/10.1007/S00520-016-3417-6

Harbeck, N., Penault-Llorca, F., Cortes, J., Gnant, M., Houssami, N., Poortmans, P., Ruddy, K., Tsang, J., Cardoso, F., 2019. Breast cancer. Nat. Rev. Dis. Prim. 2019 51 5, 1–31. https://doi.org/10.1038/s41572-019-0111-2

Hatam, N., Ahmadloo, N., Ahmad Kia Daliri, A., Bastani, P., Askarian, M., 2011. Quality of life and toxicity in breast cancer patients using adjuvant TAC (docetaxel, doxorubicin, cyclophosphamide), in comparison with FAC (doxorubicin, cyclophosphamide, 5-fluorouracil). Arch. Gynecol. Obstet. 284, 215–220. https://doi.org/10.1007/S00404-010-1609-8

Henderson, I.C., Berry, D.A., Demetri, G.D., Cirrincione, C.T., Goldstein, L.J., Martino, S., Ingle, J.N., Cooper, M.R., Hayes, D.F., Tkaczuk, K.H., Fleming, G., Holland, J.F.,

Duggan, D.B., Carpenter, J.T., Frei, E., Schilsky, R.L., Wood, W.C., Muss, H.B., Norton, L., 2003. Improved outcomes from adding sequential Paclitaxel but not from escalating Doxorubicin dose in an adjuvant chemotherapy regimen for patients with node-positive primary breast cancer. J. Clin. Oncol. 21, 976–983. https://doi.org/10.1200/JCO.2003.02.063

Helsby, N., Yong, M., Burns, K., Findlay, M., Porter, D., 2021. Cyclophosphamide bioactivation pharmacogenetics in breast cancer patients. Cancer Chemother. Pharmacol. 88, 533–542. https://doi.org/10.1007/S00280-021-04307-0

Hjorth, C.F., Damkier, P., Stage, T.B., Feddersen, S., Hamilton-Dutoit, S., Ejlertsen, B., Lash, T.L., Bøggild, H., Sørensen, H.T., Cronin-Fenton, D., 2023. The impact of single nucleotide polymorphisms on return-to-work after taxane-based chemotherapy in breast cancer. Cancer Chemother. Pharmacol. 91, 157–165. https://doi.org/10.1007/S00280-022-04499-Z

Hlaváč, V., Václavíková, R., Brynychová, V., Koževnikovová, R., Kopečková, K., Vrána, D., Gatěk, J., Souček, P., 2020. Role of genetic variation in ABC transporters in breast cancer prognosis and therapy response. Int. J. Mol. Sci. 21, 1–17. https://doi.org/10.3390/ijms21249556

Hoffmeyer, S., Burk, O., Von Richter, O., Arnold, H.P., Brockmöller, J., Johne, A., Cascorbi, I., Gerloff, T., Roots, I., Eichelbaum, M., Brinkmann, U., 2000. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc. Natl. Acad. Sci. U. S. A. 97, 3473–3478. https://doi.org/10.1073/PNAS.97.7.3473

Ingelman-Sundberg, M., Mkrtchian, S., Zhou, Y., Lauschke, V.M., 2018. Integrating rare genetic variants into pharmacogenetic drug response predictions. Hum. Genomics 12. https://doi.org/10.1186/S40246-018-0157-3

Jafari, N., Zamani, A., Farajzadegan, Z., Bahrami, F., Emami, H., Loghmani, A., 2013. The effect of spiritual therapy for improving the quality of life of women with breast cancer: A randomized controlled trial. Psychol. Health Med. 18, 56–69. https://doi.org/10.1080/13548506.2012.679738

Jassem, J., Ozmen, V., Bacanu, F., Drobniene, M., Eglitis, J., Lakshmaiah, K.C., Kahan, Z., Mardiak, J., Pieńkowski, T., Semiglazova, T., Stamatovic, L., Timcheva, C., Vasovic, S., Vrbanec, D., Zaborek, P., 2014. Delays in diagnosis and treatment of breast cancer: a multinational analysis. Eur. J. Public Health 24, 761–767. https://doi.org/10.1093/EURPUB/CKT131

Javan Biparva, A., Raoofi, S., Rafiei, S., Pashazadeh Kan, F., Kazerooni, M., Bagheribayati, F., Masoumi, M., Doustmehraban, M., Sanaei, M., Zarabi, F., Raoofi, N., Beiramy Chomalu, Z., Ahmadi, B., Seyghalani Talab, F., Sadat Hoseini, B., Asadollahi, E., Mir, M., Deylami, S., Zareei, M., Sanaei, H., Dousti Nia Kakavand, F., Koohestani, H., Nasiri, M., Vali, N., Ghashghaee, A., 2023. Global quality of life in breast cancer: systematic review and meta-analysis. BMJ Support. Palliat. Care 13, e528–e536. https://doi.org/10.1136/BMJSPCARE-2022-003642

Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E., Forman, D., 2011. Global cancer statistics. CA. Cancer J. Clin. 61, 69–90. https://doi.org/10.3322/CAAC.20107

Jin, Y., Desta, Z., Stearns, V., Ward, B., Ho, H., Lee, K.H., Skaar, T., Storniolo, A.M., Li, L., Araba, A., Blanchard, R., Nguyen, A., Ullmer, L., Hayden, J., Lemler, S., Weinshilboum, R.M., Rae, J.M., Hayes, D.F., Flockhart, D.A., 2005. CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. J. Natl. Cancer Inst. 97, 30–39. https://doi.org/10.1093/JNCI/DJI005

J. Sambrook, T. Maniatis, E. F. Fritch, 2012. Molecular Cloning This is a free sample of content from Molecular Cloning: A Laboratory Manual, 4th edition. Click here for more information or to buy the book. Cold Spring Harb. 4th.

Johansson, I., Ingelman-Sundberg, M., 2008. CNVs of human genes and their implication in pharmacogenetics. Cytogenet. Genome Res. 123, 195–204. https://doi.org/10.1159/000184709

Jordan, V.C., 2003. Tamoxifen: a most unlikely pioneering medicine. Nat. Rev. Drug Discov. 2, 205–213. https://doi.org/10.1038/NRD1031

Juan, X., Zheng, T., Qinglong, D., Jiwei, W., Jinming, Y., 2018. Being slightly overweight is associated with a better quality of life in breast cancer survivors. Sci. Rep. 8, 3022. https://doi.org/10.1038/s41598-018-20392-3

Jung, E.H., Hiratsuka, Y., Suh, S.Y., Won, S.H., Choi, S.E., Kang, B., Lee, S.W., Kim, Y.J., Suh, K.J., Kim, J.W., Kim, S.H., Kim, J.W., Lee, K.W., 2022. Association between mid-upper arm circumference and functional status in patients with advanced cancer. Clin. Nutr. Open Sci. 45, 72–79. https://doi.org/10.1016/j.nutos.2022.08.002

Kalra, S., Kaur, R.P., Ludhiadch, A., Shafi, G., Vashista, R., Kumar, R., Munshi, A., 2018. Association of CYP2C19*2 and ALDH1A1*1/*2 variants with disease outcome in breast cancer patients: results of a global screening array. Eur. J. Clin. Pharmacol. 74, 1291–1298. https://doi.org/10.1007/S00228-018-2505-6

Kaur, N., Mahapatra, P.P., Chakraborty, S., 2023. Prevalence, Characteristics, and Correlates of Fatigue in Indian Breast Cancer Survivors: A Cross-Sectional Study. Asian J Cancer 12, 55–61. https://doi.org/10.1055/s-0042-1749319

Keshava, C., McCanlies, E.C., Weston, A., 2004. CYP3A4 Polymorphisms—Potential Risk Factors for Breast and Prostate Cancer: A HuGE Review. Am. J. Epidemiol. 160, 825–841. https://doi.org/10.1093/AJE/KWH294

Khan, B.A., Robinson, R., Fohner, A.E., Muzquiz, L.A.I., Schilling, B.D., Beans, J.A., Olnes, M.J., Trawicki, L., Frydenlund, H., Laukes, C., Beatty, P., Phillips, B., Nickerson, D., Howlett, K., Dillard, D.A., Thornton, T.A., Thummel, K.E., Woodahl, E.L., 2018. Cytochrome P450 Genetic Variation Associated with Tamoxifen Biotransformation in American Indian and Alaska Native People. Clin. Transl. Sci. 11, 312–321. https://doi.org/10.1111/CTS.12542

Kilickap, S., Demirci, U., Karadurmus, N., Dogan, M., Akinci, B., Sendur, A.N., 2018. Endpoints in oncology clinical trials. JBUON 23, 1–6.

Kim, R.B., Leake, B.F., Choo, E.F., Dresser, G.K., Kubba, S. V., Schwarz, U.I., Taylor, A., Xie, H.G., McKinsey, J., Zhou, S., Lan, L. Bin, Schuetz, J.D., Schuetz, E.G., Wilkinson, G.R., 2001. Identification of functionally variant MDR1 alleles among European Americans and African Americans. Clin. Pharmacol. Ther. 70, 189–199. https://doi.org/10.1067/MCP.2001.117412

Kiyotani, K., Mushiroda, T., Imamura, C.K., Hosono, N., Tsunoda, T., Kubo, M., Tanigawara, Y., Flockhart, D.A., Desta, Z., Skaar, T.C., Aki, F., Hirata, K., Takatsuka, Y., Okazaki, M., Ohsumi, S., Yamakawa, T., Sasa, M., Nakamura, Y., Zembutsu, H., 2010. Significant effect of polymorphisms in CYP2D6 and ABCC2 on clinical outcomes of adjuvant tamoxifen therapy for breast cancer patients. J. Clin. Oncol. 28, 1287–1293. https://doi.org/10.1200/JCO.2009.25.7246

Kong, Y.H., Huang, J.Y., Ding, Y., Chen, S.H., Li, Q.S., Xiong, Y., 2025. The effect of BMI on survival outcome of breast cancer patients: a systematic review and meta-analysis. Clin. Transl. Oncol. 27. https://doi.org/10.1007/S12094-024-03563-9

Kuehl, P., Zhang, J., Lin, Y., Lamba, J., Assem, M., Schuetz, J., Watkins, P.B., Daly, A., Wrighton, S.A., Hall, S.D., Maurel, P., Relling, M., Brimer, C., Yasuda, K., Venkataramanan, R., Strom, S., Thummel, K., Boguski, M.S., Schuetz, E., 2001. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat. Genet. 2001 274 27, 383–391. https://doi.org/10.1038/86882

Kus, T., Aktas, G., Kalender, M., Demiryurek, A.T., Ulasli, M., Oztuzcu, S., Sevinc, A., Kul, S., Camci, C., 2016. Polymorphism of CYP3A4 and ABCB1 genes increase the risk of neuropathy in breast cancer patients treated with paclitaxel and docetaxel. Onco. Targets. Ther. 9, 5073. https://doi.org/10.2147/OTT.S106574

Leach, M.J., Barber, G., Monacella, S., Jamieson, P., Trinh, T., Vo, N., Schmidt, U., Byrne, A., Ristevski, E., 2023. Physical activity, obesity, and quality of life among rural Australian cancer survivors: a cross-sectional study. Support. Care Cancer 31, 3. https://doi.org/10.1007/s00520-023-07691-w

Lebwohl, D., Kay, A., Berg, W., Baladi, J.F., Zheng, J., 2009. Progression-free survival: gaining on overall survival as a gold standard and accelerating drug development. Cancer J. 15, 386–394. https://doi.org/10.1097/PPO.0B013E3181B9C5EC

Leclerc, A.F., Jerusalem, G., Devos, M., Crielaard, J.M., Maquet, D., 2016. Multidisciplinary management of breast cancer. Arch. Public Heal. https://doi.org/10.1186/s13690-016-0163-7

Lévy, P., Gligorov, J., Antoine, M., Rezai, K., Lévy, E., Selle, F., Saintigny, P., Lokiec, F., Avenin, D., Beerblock, K., Lotz, J.P., Bernaudin, J.F., Fajac, A., 2013. Influence of ABCB1 polymorphisms and docetaxel pharmacokinetics on pathological response to neoadjuvant chemotherapy in breast cancer patients. Breast Cancer Res. Treat. 139,

421–428. https://doi.org/10.1007/S10549-013-2545-7

Li, C.I., Malone, K.E., White, E., Daling, J.R., 1997. Age when maximum height is reached as a risk factor for breast cancer among young U.S. women. Epidemiology 8, 559–565. https://doi.org/10.1097/00001648-199709000-00014

Lim, J.S.L., Chen, X.A., Singh, O., Yap, Y.S., Ng, R.C.H., Wong, N.S., Wong, M., Lee, E.J.D., Chowbay, B., 2011. Impact of CYP2D6, CYP3A5, CYP2C9 and CYP2C19 polymorphisms on tamoxifen pharmacokinetics in Asian breast cancer patients. Br. J. Clin. Pharmacol. 71, 737–750. https://doi.org/10.1111/J.1365-2125.2011.03905.X

Lin, T.M., Chen, K.P., Macmahon, B., 1971. Epidemiologic characteristics of cancer of the breast in Taiwan. Cancer 27, 1497–1504. https://doi.org/10.1002/1097-0142(197106)27:6<1497::AID-CNCR2820270634>3.0.CO;2-C

Liu, B., Zhou, H., Tan, L., Siu, K.T.H., Guan, X.Y., 2024. Exploring treatment options in cancer: Tumor treatment strategies. Signal Transduct. Target. Ther. 9. https://doi.org/10.1038/S41392-024-01856-7

Lopez, G., Liu, W., McQuade, J., Lee, R.T., Spelman, A.R., Fellman, B., Li, Y., Bruera, E., Cohen, L., 2017. Integrative Oncology Outpatient Consultations: Long-Term Effects on Patient-Reported Symptoms and Quality of Life. J. Cancer 8, 1640–1646. https://doi.org/10.7150/JCA.18875

Loscocco, F., Visani, G., Ruzzo, A., Bagaloni, I., Fuligni, F., Galimberti, S., Di Paolo, A., Stagno, F., Pregno, P., Annunziata, M., Gozzini, A., Barulli, S., Gabucci, E., Magnani, M., Isidori, A., 2021. Clinical Relevance of ABCB1, ABCG2, and ABCC2 Gene Polymorphisms in Chronic Myeloid Leukemia Patients Treated With Nilotinib. Front. Oncol. 11, 672287. https://doi.org/10.3389/FONC.2021.672287

Mackey, J.R., Pieńkowski, T., Crown, J., Sadeghi, S., Martin, M., Chan, A., Saleh, M., Sehdev, S., Provencher, L., Semiglazov, V., Press, M.F., Sauter, G., Lindsay, M., Houé, V., Buyse, M., Drevot, P., Hitier, S., Bensfia, S., Eiermann, W., 2016. Long-term outcomes after adjuvant treatment of sequential versus combination docetaxel with doxorubicin and cyclophosphamide in node-positive breast cancer: BCIRG-005 randomized trial. Ann. Oncol. Off. J. Eur. Soc. Med. Oncol. 27, 1041–1047. https://doi.org/10.1093/ANNONC/MDW098

Mamounas, E.P., Bryant, J., Lembersky, B., Fehrenbacher, L., Sedlacek, S.M., Fisher, B., Wickerham, D.L., Yothers, G., Soran, A., Wolmark, N., 2005. Paclitaxel after doxorubicin plus cyclophosphamide as adjuvant chemotherapy for node-positive breast cancer: Results from NSABP B-28. J. Clin. Oncol. 23, 3686–3696. https://doi.org/10.1200/JCO.2005.10.517

Martín, M., Lluch, A., Seguí, M.A., Ruiz, A., Ramos, M., Adrover, E., Rodríguez-Lescure, Á., Grosse, R., Calvo, L., Fernandez-Chacón, C., Roset, M., Antón, A., Isla, D., del Prado, P.M., Iglesias, L., Zaluski, J., Arcusa, A., López-Vega, J.M., Muñoz, M., Mel, J.R., 2006. Toxicity and health-related quality of life in breast cancer patients receiving adjuvant docetaxel, doxorubicin, cyclophosphamide (TAC) or 5-fluorouracil,

doxorubicin and cyclophosphamide (FAC): impact of adding primary prophylactic granulocyte-colony stimulating factor to the TAC regimen. Ann. Oncol. 17, 1205–1212. https://doi.org/10.1093/ANNONC/MDL135

Mehrotra, R., Yadav, K., 2022. World Journal of Clinical Oncology Breast cancer in India: Present scenario and the challenges ahead . World J Clin Oncol 13, 209–218. https://doi.org/10.5306/wjco.v13.i3.209

Minotti, G., Menna, P., Salvatorelli, E., Cairo, G., Gianni, L., 2004. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol. Rev. 56, 185–229. https://doi.org/10.1124/PR.56.2.6

Mistry, T., Nath, A., Pal, R., Ghosh, S., Mahata, S., Kumar Sahoo, P., Sarkar, S., Choudhury, T., Nath, P., Alam, N., Nasare, V.D., 2024a. Emerging Futuristic Targeted Therapeutics: A Comprising Study Towards a New Era for the Management of TNBC. Am. J. Clin. Oncol. Cancer Clin. Trials 47, 132–148. https://doi.org/10.1097/COC.0000000000001071

Mistry, T., Nath, P., Alam, N., Nasare, V.D., 2024b. Four Year Clinical Outcomes: Evaluating the Efficacy of Concomitant and Sequential Anthracycline-taxane Chemotherapy in Indian Breast Cancer Patients – A Regional Cancer Center Study. Journal of Current Oncological Trends 1, 110–119. https://doi.org/10.4103/JCOT.JCOT_18_24

Mistry, T., Pal, R., Ghosh, S., Choudhury, T., Mandal, S., Nath, P., Alam, N., Nasare, V.D., 2024. Impact of Low BMI and Nutritional Status on Quality of Life and Disease Outcome in Breast Cancer Patients: Insights From a Tertiary Cancer Center in India. Nutr. Cancer 76, 596–607. https://doi.org/10.1080/01635581.2024.2347396

Mizzi, C., Dalabira, E., Kumuthini, J., Dzimiri, N., Balogh, I., Başak, N., Böhm, R., Borg, J., Borgiani, P., Bozina, N., Bruckmueller, H., Burzynska, B., Carracedo, A., Cascorbi, I., Deltas, C., Dolzan, V., Fenech, A., Grech, G., Kasiulevicius, V., Kádaši, L., Kučinskas, V., Khusnutdinova, E., Loukas, Y.L., Macek, M., Makukh, H., Mathijssen, R., Mitropoulos, K., Mitropoulou, C., Novelli, G., Papantoni, I., Pavlovic, S., Saglio, G., Setric, J., Stojiljkovic, M., Stubbs, A.P., Squassina, A., Torres, M., Turnovec, M., Van Schaik, R.H., Voskarides, K., Wakil, S.M., Werk, A., Zompo, M. Del, Zukic, B., Katsila, T., Lee, M.T.M., Motsinger-Rief, A., Leod, H.L.M., Van Der Spek, P.J., Patrinos, G.P., 2016. A European Spectrum of Pharmacogenomic Biomarkers: Implications for Clinical Pharmacogenomics. PLoS One 11. https://doi.org/10.1371/JOURNAL.PONE.0162866

Mokhosoev, I.M., Astakhov, D. V., Terentiev, A.A., Moldogazieva, N.T., 2024. Human Cytochrome P450 Cancer-Related Metabolic Activities and Gene Polymorphisms: A Review. Cells 13. https://doi.org/10.3390/CELLS13231958

Mulder, T.A.M., van Eerden, R.A.G., de With, M., Elens, L., Hesselink, D.A., Matic, M., Bins, S., Mathijssen, R.H.J., van Schaik, R.H.N., 2021. CYP3A4∗22 Genotyping in Clinical Practice: Ready for Implementation? Front. Genet. 12. https://doi.org/10.3389/FGENE.2021.711943

Munsell, M.F., Sprague, B.L., Berry, D.A., Chisholm, G., Trentham-Dietz, A., 2014. Body mass index and breast cancer risk according to postmenopausal estrogen-progestin use and hormone receptor status. Epidemiol. Rev. 36, 114–136. https://doi.org/10.1093/epirev/mxt010

Muriithi, W., Macharia, L.W., Heming, C.P., Echevarria, J.L., Nyachieo, A., Filho, P.N., Neto, V.M., 2020. ABC transporters and the hallmarks of cancer: roles in cancer aggressiveness beyond multidrug resistance. Cancer Biol. Med. 17, 253–269. https://doi.org/10.20892/J.ISSN.2095-3941.2019.0284

Muscaritoli, M., Lucia, S., Farcomeni, A., Lorusso, V., Saracino, V., Barone, C., Plastino, F., Gori, S., Magarotto, R., Carteni, G., Chiurazzi, B., Pavese, I., Marchetti, L., Zagonel, V., Bergo, E., Tonini, G., Imperatori, M., Iacono, C., Maiorana, L., Pinto, C., Rubino, D., Cavanna, L., Di Cicilia, R., Gamucci, T., Quadrini, S., Palazzo, S., Minardi, S., Merlano, M., Colucci, G., Marchetti, P., Fioretto, L., Cipriani, G., Barni, S., Lonati, V., Frassoldati, A., Surace, G.C., Porzio, G., Martella, F., Altavilla, G., Santarpia, M.C., Pronzato, P., Levaggi, A., Contu, A., Contu, M., Adamo, V., Berenato, R., Marchetti, F., Pellegrino, A., Violante, S., Guida, M., 2017. Prevalence of malnutrition in patients at first medical oncology visit: The PreMiO study. Oncotarget 8, 79884–79896. https://doi.org/10.18632/oncotarget.20168

Mutebi, M., Anderson, B.O., Duggan, C., Adebamowo, C., Agarwal, G., Ali, Z., Bird, P., Bourque, J.M., DeBoer, R., Gebrim, L.H., Masetti, R., Masood, S., Menon, M., Nakigudde, G., Ng'ang'a, A., Niyonzima, N., Rositch, A.F., Unger-Saldaña, K., Villarreal-Garza, C., Dvaladze, A., El Saghir, N.S., Gralow, J.R., Eniu, A., 2020. Breast cancer treatment: A phased approach to implementation. Cancer 126 Suppl 10, 2365–2378. https://doi.org/10.1002/CNCR.32910

Mwinyi, J., Vokinger, K., Jetter, A., Breitenstein, U., Hiller, C., Kullak-Ublick, G.A., Trojan, A., 2014. Impact of variable CYP genotypes on breast cancer relapse in patients undergoing adjuvant tamoxifen therapy. Cancer Chemother. Pharmacol. 73, 1181–1188. https://doi.org/10.1007/S00280-014-2453-5

Nath, A., Mitra, S., Mistry, T., Pal, R., Nasare, V.D., 2022. Molecular targets and therapeutics in chemoresistance of triple-negative breast cancer. Med. Oncol. 39, 1–33. https://doi.org/10.1007/S12032-021-01610-X/FIGURES/5

National Cancer Institute, 2021. Breast Cancer Prevention (PDQ®): Patient Version. PDQ Cancer Inf. Summ. 1–12.

NEBcutter 3.0 [WWW Document], n.d. URL https://nc3.neb.com/NEBcutter/ (accessed 2.19.25).

Onitilo, A.A., Engel, J.M., Greenlee, R.T., Mukesh, B.N., 2009. Breast cancer subtypes based on ER/PR and Her2 expression: comparison of clinicopathologic features and survival. Clin. Med. Res. 7, 4–13. https://doi.org/10.3121/CMR.2009.825

Osborne, C.K., Schiff, R., 2011. Mechanisms of endocrine resistance in breast cancer. Annu. Rev. Med. 62, 233–247. https://doi.org/10.1146/ANNUREV-MED-070909-182917

Pandey, M., Thomas, B.C., Ramdas, K., Eremenco, S., Nair, M., 2002. Quality of life in breast cancer patients: validation of a FACT-B Malayalam version. Qual. Life Res. 11, 87–90. https://doi.org/10.1023/a:1015083713014

Parvin, M.N., Aziz, M.A., Rabbi, S.N.I., Al-Mamun, M.M.A., Hanif, M., Islam, Md Saiful, Islam, Mohammad Safiqul, 2021. Assessment of the Link of ABCB1 and NR3C1 gene polymorphisms with the prednisolone resistance in pediatric nephrotic syndrome patients of Bangladesh: A genotype and haplotype approach. J. Adv. Res. 33, 141–151. https://doi.org/10.1016/J.JARE.2021.02.001

Paswan, S.S., Kataria, K., Parshad, R., Srivastava, A., Seenu, V., Mishra, B., 2015. Feasibility of fast track discharge in breast cancer patients undergoing definitive surgery and impact on quality of life: A prospective study from tertiary care center in India. J. Surg. Oncol. 111, 265–269. https://doi.org/10.1002/JSO.23817

Polyak, K., 2011. Heterogeneity in breast cancer. J. Clin. Invest. 121, 3786–3788. https://doi.org/10.1172/JCI60534

Pratt, V.M., Cavallari, L.H., Fulmer, M.L., Gaedigk, A., Hachad, H., Ji, Y., Kalman, L. V., Ly, R.C., Moyer, A.M., Scott, S.A., van Schaik, R.H.N., Whirl-Carrillo, M., Weck, K.E., 2023. CYP3A4 and CYP3A5 Genotyping Recommendations: A Joint Consensus Recommendation of the Association for Molecular Pathology, Clinical Pharmacogenetics Implementation Consortium, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, European Society for Pharmacogenomics and Personalized Therapy, and Pharmacogenomics Knowledgebase. J. Mol. Diagn. 25, 619–629. https://doi.org/10.1016/J.JMOLDX.2023.06.008

Provincial Health Services Authority, 2022. BC Cancer [WWW Document]. URL http://www.bccancer.bc.ca/ (accessed 1.2.23).

Prusty, R.K., Begum, S., Patil, A., Naik, D.D., Pimple, S., Mishra, G., 2020. Knowledge of symptoms and risk factors of breast cancer among women: A community based study in a low socio-economic area of Mumbai, India. BMC Womens. Health 20, 1–12. https://doi.org/10.1186/S12905-020-00967-X/FIGURES/5

Rakha, E.A., El-Sayed, M.E., Lee, A.H.S., Elston, C.W., Grainge, M.J., Hodi, Z., Blamey, R.W., Ellis, I.O., 2008. Prognostic significance of Nottingham histologic grade in invasive breast carcinoma. J. Clin. Oncol. 26, 3153–3158. https://doi.org/10.1200/JCO.2007.15.5986

Raman, R., Mott, S.L., Schroeder, M.C., Phadke, S., El Masri, J., Thomas, A., 2016. Effect of Body Mass Index– and Actual Weight–Based Neoadjuvant Chemotherapy Doses on Pathologic Complete Response in Operable Breast Cancer. Clin. Breast Cancer 16, 480–486. https://doi.org/10.1016/j.clbc.2016.06.008

Renehan, A.G., Tyson, M., Egger, M., Heller, R.F., Zwahlen, M., 2008. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet 371, 569–578. https://doi.org/10.1016/S0140-6736(08)60269-X

Rhodes, A., Jasani, B., Barnes, D.M., Bobrow, L.G., Miller, K.D., 2000. Reliability of immunohistochemical demonstration of oestrogen receptors in routine practice: interlaboratory variance in the sensitivity of detection and evaluation of scoring systems. J. Clin. Pathol. 53, 125. https://doi.org/10.1136/JCP.53.2.125

Robey, R.W., Pluchino, K.M., Hall, M.D., Fojo, A.T., Bates, S.E., Gottesman, M.M., 2018. Revisiting the role of ABC transporters in multidrug-resistant cancer. Nat. Rev. Cancer 18, 452–464. https://doi.org/10.1038/S41568-018-0005-8

Robinson, K., Tiriveedhi, V., 2020. Perplexing Role of P-Glycoprotein in Tumor Microenvironment. Front. Oncol. 10. https://doi.org/10.3389/FONC.2020.00265

Rodriguez-Antona, C., Ingelman-Sundberg, M., 2006. Cytochrome P450 pharmacogenetics and cancer. Oncogene 2006 2511 25, 1679–1691. https://doi.org/10.1038/sj.onc.1209377

Rubinstein, M.M., Brown, K.A., Iyengar, N.M., 2021. Targeting obesity-related dysfunction in hormonally driven cancers. Br. J. Cancer 125, 495. https://doi.org/10.1038/S41416-021-01393-Y

Sadee, W., Wang, D., Papp, A.C., Pinsonneault, J.K., Smith, R.M., Moyer, R.A., Johnson, A.D., 2011. Pharmacogenomics of the RNA world: structural RNA polymorphisms in drug therapy. Clin. Pharmacol. Ther. 89, 355–365. https://doi.org/10.1038/CLPT.2010.314

Sathishkumar, K., Chaturvedi, M., Das, P., Stephen, S., Mathur, P., 2022. Cancer incidence estimates for 2022 & projection for 2025: Result from National Cancer Registry Programme, India. https://doi.org/10.4103/ijmr.ijmr_1821_22

Schoemaker, M.J., Nichols, H.B., Wright, L.B., Brook, M.N., Jones, M.E., O'Brien, K.M., Adami, H.O., Baglietto, L., Bernstein, L., Bertrand, K.A., Boutron-Ruault, M.C., Braaten, T., Chen, Y., Connor, A.E., Dorronsoro, M., Dossus, L., Eliassen, A.H., Giles, G.G., Hankinson, S.E., Kaaks, R., Key, T.J., Kirsh, V.A., Kitahara, C.M., Koh, W.P., Larsson, S.C., Linet, M.S., Ma, H., Masala, G., Merritt, M.A., Milne, R.L., Overvad, K., Ozasa, K., Palmer, J.R., Peeters, P.H., Riboli, E., Rohan, T.E., Sadakane, A., Sund, M., Tamimi, R.M., Trichopoulou, A., Ursin, G., Vatten, L., Visvanathan, K., Weiderpass, E., Willett, W.C., Wolk, A., Yuan, J.M., Zeleniuch-Jacquotte, A., Sandler, D.P., Swerdlow, A.J., 2018. Association of Body Mass Index and Age with Subsequent Breast Cancer Risk in Premenopausal Women. JAMA Oncol. 4, e181771–e181771. https://doi.org/10.1001/jamaoncol.2018.1771

Scott, S.A., Sangkuhl, K., Stein, C.M., Hulot, J.S., Mega, J.L., Roden, D.M., Klein, T.E., Sabatine, M.S., Johnson, J.A., Shuldiner, A.R., 2013. Clinical Pharmacogenetics Implementation Consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update. Clin. Pharmacol. Ther. 94, 317–323. https://doi.org/10.1038/CLPT.2013.105

Sensorn, I., Sirachainan, E., Chamnanphon, M., Pasomsub, E., Trachu, N., Supavilai, P., Sukasem, C., Pinthong, D., 2013. Association of CYP3A4/5, ABCB1 and ABCC2 polymorphisms and clinical outcomes of Thai breast cancer patients treated with

tamoxifen. Pharmgenomics. Pers. Med. 6, 93–98. https://doi.org/10.2147/PGPM.S44006

Seredina, T.A., Goreva, O.B., Talaban, V.O., Grishanova, A.Y., Lyakhovich, V. V., 2012. Association of cytochrome P450 genetic polymorphisms with neoadjuvant chemotherapy efficacy in breast cancer patients. BMC Med. Genet. 13, 45. https://doi.org/10.1186/1471-2350-13-45

Shaikh, H., Bradhurst, P., Ma, L.X., Tan, S.Y., Egger, S.J., Vardy, J.L., 2020. Body weight management in overweight and obese breast cancer survivors. Cochrane Database Syst. Rev. 2020. https://doi.org/10.1002/14651858.CD012110.PUB2

Shao, N., Wang, S., Yao, C., Xu, X., Zhang, Yunjian, Zhang, Yuanyuan, Lin, Y., 2012. Sequential versus concurrent anthracyclines and taxanes as adjuvant chemotherapy of early breast cancer: A meta-analysis of phase III randomized control trials. The Breast 21, 389–393. https://doi.org/10.1016/J.BREAST.2012.03.011

Sharom, F.J., 2008. ABC Multidrug Transporters: Structure, Function and Role in Chemoresistance. Pharmacogenomics 9, 105–127. https://doi.org/10.2217/14622416.9.1.105

Shimada, N., Iwasaki, M., Kasuga, Y., Yokoyama, S., Onuma, H., Nishimura, H., Kusama, R., Hamada, G.S., Nishimoto, I.N., Iyeyasu, H., Motola, J., Laginha, F.M., Kurahashi, N., Tsugane, S., 2009. Genetic polymorphisms in estrogen metabolism and breast cancer risk in case-control studies in Japanese, Japanese Brazilians and non-Japanese Brazilians. J. Hum. Genet. 54, 209–215. https://doi.org/10.1038/JHG.2009.13

Sidibe, M., Tazzite, A., Jouhadi, H., Dehbi, H., 2024. Impact of CYP2D6, CYP2C9/19, CYP3A4, UGT, and SULT Variability on Tamoxifen Metabolism in Breast Cancer Treatment. https://doi.org/10.1177/25898892231223300. https://doi.org/10.1177/25898892231223300

Sim, S., Bergh, J., Hellström, M., Hatschek, T., Xie, H., 2018. Pharmacogenetic impact of docetaxel on neoadjuvant treatment of breast cancer patients. Pharmacogenomics 19, 1259–1268. https://doi.org/10.2217/PGS-2018-0080

Singh, P., Kapil, U., Shukla, N.K., Deo, S.V.S., Dwivedi, S.N., 2011. Association of overweight and obesity with breast cancer in India. Indian J. Community Med. 36, 259–262. https://doi.org/10.4103/0970-0218.91326

Sinha, D.K., Kumari, Anita, Pankaj, S., Kumari, S., Choudhary, V., Kumari, Anjili, Kumari, J., Nazneen, S., Kumar, S., 2018. To Evaluate the Incidence of ER, PR and Her2/Neu Status in Newly Diagnosed Breast Cancer Patients at a Tertiary Care Centre. Indian J. Gynecol. Oncol. 16, 1–5. https://doi.org/10.1007/S40944-018-0225-6/METRICS

Skinner, K.T., Palkar, A.M., Hong, A.L., 2023. Genetics of ABCB1 in Cancer. Cancers (Basel). 15. https://doi.org/10.3390/CANCERS15174236

Song, Y., Li, C., Liu, G., Liu, R., Chen, Y., Li, W., Cao, Z., Zhao, B., Lu, C., Liu, Y., 2021. Drug-Metabolizing Cytochrome P450 Enzymes Have Multifarious Influences on

Treatment Outcomes. Clin. Pharmacokinet. 60, 585–601. https://doi.org/10.1007/S40262-021-01001-5

Swain, S., Jeong, J., Geyer, C., Costantino, J., Pajon, E., Fehrenbacher, L., Atkins, J., Polikoff, J., Vogel, V., Erban, J., Livingston, R., Perez, E., Mamounas, E., Ganz, P., Land, S., Wolmark, N., 2009. NSABP B-30: definitive analysis of patient outcome from a randomized trial evaluating different schedules and combinations of adjuvant therapy containing doxorubicin, docetaxel and cyclophosphamide in women with operable, node-positive breast cancer. Cancer Res. 69, 75–75. https://doi.org/10.1158/0008-5472.SABCS-75

Swen, J.J., Nijenhuis, M., De Boer, A., Grandia, L., Maitland-Van Der Zee, A.H., Mulder, H., Rongen, G.A.P.J.M., Van Schaik, R.H.N., Schalekamp, T., Touw, D.J., Van Der Weide, J., Wilffert, B., Deneer, V.H.M., Guchelaar, H.J., 2011. Pharmacogenetics: from bench to byte--an update of guidelines. Clin. Pharmacol. Ther. 89, 662–673. https://doi.org/10.1038/CLPT.2011.34

Świechowski, R., Jeleń, A., Pietrzak, J., Gałecki, P., Szmajda-Krygier, D., Balcerczak, E., 2021. The influence of CYP2C19*2 and CYP3A5*3 variants on the development of depression and effectiveness of therapy: A preliminary study. Biomed. Pharmacother. 142. https://doi.org/10.1016/J.BIOPHA.2021.112055

Teft, W.A., Mansell, S.E., Kim, R.B., 2011. Endoxifen, the active metabolite of tamoxifen, is a substrate of the efflux transporter P-glycoprotein (multidrug resistance 1). Drug Metab. Dispos. 39, 558–562. https://doi.org/10.1124/DMD.110.036160

Tong, C.W.S., Wu, M., Cho, W.C.S., To, K.K.W., 2018. Recent Advances in the Treatment of Breast Cancer. Front. Oncol. 8. https://doi.org/10.3389/FONC.2018.00227

Trédan, O., Bajard, A., Meunier, A., Roux, P., Fiorletta, I., Gargi, T., Bachelot, T., Guastalla, J.P., Lallemand, Y., Faure, C., Pérol, D., Bachmann, P., 2010. Body weight change in women receiving adjuvant chemotherapy for breast cancer: A French prospective study. Clin. Nutr. 29, 187–191. https://doi.org/10.1016/j.clnu.2009.08.003

Tsukamoto, M., Yamashita, M., Nishi, T., Nakagawa, H., 2019. A Human ABC Transporter ABCC4 Gene SNP (rs11568658, 559 G > T, G187W) Reduces ABCC4-Dependent Drug Resistance. Cells 8. https://doi.org/10.3390/CELLS8010039

Tulsyan, S., Chaturvedi, P., Singh, A.K., Agarwal, G., Lal, P., Agrawal, S., Mittal, R.D., Mittal, B., 2014. Assessment of clinical outcomes in breast cancer patients treated with taxanes: Multi-analytical approach. Gene 543, 69–75. https://doi.org/10.1016/j.gene.2014.04.004

Tulsyan, S., Mittal, R.D., Mittal, B., 2016. The effect of ABCB1 polymorphisms on the outcome of breast cancer treatment. Pharmgenomics. Pers. Med. 9, 47. https://doi.org/10.2147/PGPM.S86672

Tzenios, N., Tazanios, M.E., Chahine, M., 2024. The impact of BMI on breast cancer - an updated systematic review and meta-analysis. Medicine (Baltimore). 103, E36831. https://doi.org/10.1097/MD.0000000000036831

Usiskin, I., Li, F., Irwin, M.L., Cartmel, B., Sanft, T., 2019. Association between pre-diagnosis BMI, physical activity, pathologic complete response, and chemotherapy completion in women treated with neoadjuvant chemotherapy for breast cancer. Breast Cancer 26, 719–728. https://doi.org/10.1007/s12282-019-00974-3

van Eijk, M., Boosman, R.J., Schinkel, A.H., Huitema, A.D.R., Beijnen, J.H., 2019. Cytochrome P450 3A4, 3A5, and 2C8 expression in breast, prostate, lung, endometrial, and ovarian tumors: relevance for resistance to taxanes. Cancer Chemother. Pharmacol. 84, 487–499. https://doi.org/10.1007/S00280-019-03905-3

van Rossum, A.G.J., Kok, M., van Werkhoven, E., Opdam, M., Mandjes, I.A.M., van Leeuwen – Stok, A.E., van Tinteren, H., Imholz, A.L.T., Portielje, J.E.A., Bos, M.M.E.M., van Bochove, A., Wesseling, J., Rutgers, E.J., Linn, S.C., Oosterkamp, H.M., 2018. Adjuvant dose-dense doxorubicin-cyclophosphamide versus docetaxel-doxorubicin-cyclophosphamide for high-risk breast cancer: First results of the randomised MATADOR trial (BOOG 2004-04). Eur. J. Cancer 102, 40–48. https://doi.org/10.1016/J.EJCA.2018.07.013

van Schaik, R.H.N., 2008. CYP450 pharmacogenetics for personalizing cancer therapy. Drug Resist. Updat. 11, 77–98. https://doi.org/10.1016/J.DRUP.2008.03.002

Von Minckwitz, G., Loibl, S., 2015. Evolution of adjuvant chemotherapy for breast cancer. Lancet (London, England) 385, 1812–1814. https://doi.org/10.1016/S0140-6736(14)62348-5

Waks, A.G., Winer, E.P., 2019. Breast Cancer Treatment: A Review. JAMA - J. Am. Med. Assoc. https://doi.org/10.1001/jama.2018.19323

Wang, H., Zhang, S., Yee, D., Basu, S., Beckwith, H., Potter, D., Blaes, A., 2021. Impact of body mass index on pathological complete response following neoadjuvant chemotherapy in operable breast cancer: a meta-analysis. Breast Cancer 28, 618–629. https://doi.org/10.1007/S12282-020-01194-W/METRICS

Wang, J., Wu, S.G., 2023. Breast Cancer: An Overview of Current Therapeutic Strategies, Challenge, and Perspectives. Breast cancer (Dove Med. Press. 15, 721–730. https://doi.org/10.2147/BCTT.S432526

Wells, J.C.K., Fewtrell, M.S., 2006. Measuring body composition. Arch. Dis. Child. https://doi.org/10.1136/adc.2005.085522

Wolking, S., Schaeffeler, E., Lerche, H., Schwab, M., Nies, A.T., 2015. Impact of Genetic Polymorphisms of ABCB1 (MDR1, P-Glycoprotein) on Drug Disposition and Potential Clinical Implications: Update of the Literature. Clin. Pharmacokinet. 54, 709–735. https://doi.org/10.1007/S40262-015-0267-1

Woo, J.S., Lee, C.H., Shim, C.K., Hwang, S.J., 2003. Enhanced oral bioavailability of paclitaxel by coadministration of the p-glycoprotein inhibitor KR30031. Pharm. Res. 20, 24–30. https://doi.org/10.1023/A:1022286422439

Yang, W., Zhang, T., Song, X., Dong, G., Xu, L., Jiang, F., 2022. SNP-Target Genes Interaction

Perturbing the Cancer Risk in the Post-GWAS. Cancers (Basel). 14. https://doi.org/10.3390/CANCERS14225636

Yin, B., Li, Y., Lu, P., Wang, H., Pei, K., Liang, J., Liu, H., Liu, X., Xiao, J., 2017. Review Article Advances in pharmacogenomic studies of docetaxel-a review. Int J Clin Exp Med 10, 7450–7456.

Zan, X., Yue, G., Hao, Y., Sima, X., 2021. A systematic review and meta-analysis of the association of ABCC2/ABCG2 polymorphisms with antiepileptic drug responses in epileptic patients. Epilepsy Res. 175. https://doi.org/10.1016/J.EPLEPSYRES.2021.106678

Zanger, U.M., Schwab, M., 2013. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol. Ther. 138, 103–141. https://doi.org/10.1016/J.PHARMTHERA.2012.12.007

Zangouri, V., Balaneji, S.S., Golmoradi, R., Kafili, E., Bologhi, S., Mousavi, S.A., Hesar, A.A., Amestejani, M., 2025. Effects of BMI on prognosis, disease-free survival and overall survival of breast cancer. BMC Cancer 2025 251 25, 1–21. https://doi.org/10.1186/S12885-025-13638-7

Zeliha, K.P., Dilek, O., Ezgi, O., Halil, K., Cihan, U., Gul, O., 2020. Association between ABCB1, ABCG2 carrier protein and COX-2 enzyme gene polymorphisms and breast cancer risk in a Turkish population. Saudi Pharm. J. 28, 215–219. https://doi.org/10.1016/j.jsps.2019.11.024

Zembutsu, H., Nakamura, S., Akashi-Tanaka, S., Kuwayama, T., Watanabe, C., Takamaru, T., Takei, H., Ishikawa, T., Miyahara, K., Matsumoto, H., Hasegawa, Y., Kutomi, G., Shima, H., Satomi, F., Okazaki, M., Zaha, H., Onomura, M., Matsukata, A., Sagara, Y., Baba, S., Yamada, A., Shimada, K., Shimizu, D., Tsugawa, K., Shimo, A., Tan, E.Y., Hartman, M., Chan, C.W., Lee, S.C., Nakamura, Y., 2017. Significant Effect of Polymorphisms in CYP2D6 on Response to Tamoxifen Therapy for Breast Cancer: A Prospective Multicenter Study. Clin. Cancer Res. 23, 2019–2026. https://doi.org/10.1158/1078-0432.CCR-16-1779

Zhang, W., Yu, B.N., He, Y.J., Fan, L., Li, Q., Liu, Z.Q., Wang, A., Liu, Y.L., Tan, Z.R., Fen-Jiang, Huang, Y.F., Zhou, H.H., 2006. Role of BCRP 421C>A polymorphism on rosuvastatin pharmacokinetics in healthy Chinese males. Clin. Chim. Acta. 373, 99–103. https://doi.org/10.1016/J.CCA.2006.05.010

Zhao, J., Yang, D.S., Liu, Y.Q., Wu, Y.K., Chen, C., Li, J.T., Wu, R.G., 2024. Characteristics of positive and negative effects on the quality of life of breast cancer patients. BMC Psychiatry 24, 1–15. https://doi.org/10.1186/S12888-024-06311-Z/FIGURES/5

Annexure

ANNEXURE I

INSTITUTIONAL ETHICS COMMITTEE

Chittaranjan National Cancer Institute
An autonomous body under the Ministry of
Health & Family Welfare, Govt. Of India
37, S.P.Mukherjee Road, Kolkata-700026, WB, India
Tel: 2475-9313/8057, Fax: +91-33-2475-7606
Web: www.cnci.org.in

IEC Ref: CNCI-IEC-DL-2020-6 Date: 10.12.2020

Dr. Vilas Nasare, CNCI

Sub.: IEC decision on review of the project submitted for approval.

Protocol Title: **Study on CYP2D6 & ABCB polymorphism with respect to tamoxifen adjuvant treatment in ER and PR receptor breast cancer patients**

Study Site: Chittaranjan National Cancer Institute, Kolkata

The Chairman and members of Institutional Ethics Committee (IEC) reviewed the project and other related documents submitted by you for the proposed study entitled "**Study on CYP2D6 & ABCB polymorphism with respect to tamoxifen adjuvant treatment in ER and PR receptor breast cancer patients.**"

The following documents were reviewed:

1. Model form to be filled by the Principal Investigator (PI) for submission to Institutional Ethics Committee (IEC)
2. Project with Summary
3. Informed Consent Sheets
4. Administrative Approval
5. Approval from Academic Committee

The committee should be informed:

I. About the progress of the study annually
II. Any changes in the protocol and patient information/ informed consent documents, prior to their implementation

The Project is approved by IEC.

Final report of the study shall have to be submitted to the IEC in all cases, even when the study is abandoned for any reason(s).

Yours Sincerely,

Member Secretary 10/12/2020
IEC, CNCI
Member Secretary
Institutional Ethics Committee
Chittaranjan National Cancer Institute
37, S. P. Mukherjee Road, Kolkata-700026

Chairman 10/12/2020
IEC, CNCI
Chairman
Institutional Ethics Committee
Chittaranjan National Cancer Institute
37, S. P. Mukherjee Road, Kolkata-700026

ANNEXURE II

Chittaranjan National Cancer Institute
37 S. P. Mukherjee Road, Kolkata -700026

Study on *CYP2D6* and *ABCB* polymorphisms with respect to tamoxifen adjuvant treatment in ER and PR breast cancer patients

PROPOSED FORMAT FOR INFORMED CONSENT FORM

(This form will be translated in local language after sanctioning of the project)

Subject Information Sheet

Introduction:

You are being invited to take part in a research study being conducted at Chittaranjan National Cancer Institute, 37 S. P. Mukherjee Road, Kolkata-700026**.** Your participation in this study is voluntary, which means you can decide whether or not you want to be in the study. If you don't want to be in the study, you will not be prevented from receiving any medical care or other benefits that you are entitled to.

Before you agree to be in this study, it is important that you read the following information completely and ask as many questions as necessary to the study doctor or nurse to be sure that you understand what you will be asked to do. This subject information sheet provides you with detailed information about the study.

Prior to being enrolled in the study, you will be required to personally sign and date the Subject Information Sheet and Informed Consent Form provided at the end of this document. A copy of the signed Informed Consent Form will be provided to you.

Ask your study doctor Dr. Neyaz Alam, Dr. Partha Nath Co-Supervisors of the project, if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

- To assess the immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER2/neu) in BC patients.
- To evaluate the effects of the single nucleotide polymorphisms in *ABCB1, ABCC2* genes and their allele frequencies in response to tamoxifen metabolisms and

anthracycline-taxane chemotherapy (FEC/TAC/AC-T) transport and metabolism in ER and PR positive BC patients.

- To determine the correlation among *CYP2D6, CYP2C19, CYP2C9, CYP3A4* genes and their allele frequencies with respect to tamoxifen metabolisms and anthracycline-taxane chemotherapy (FEC/TAC/AC-T) transport and metabolism in ER and PR positive BC patients.
- To assess the clinical efficacy and quality of life in ER and PR positive BC patients in response to tamoxifen therapy and anthracycline-taxane chemotherapy (FEC/TAC/AC-T).
- To evaluate the impact of low BMI on treatment response, quality of life, and genetic predisposition to altered drug transport, metabolism and therapy outcomes among BC patients.
- Analysis of data for prediction and clinical management of breast cancer.

What will be happen during the study?

Before you join this study, you will be assessed against a few criteria for selection as a study participant. You will be considered for the study, if you fulfill these criteria. Once the study doctor or the staff has answered all questions, you will be asked to read, sign and date this consent form given. Below is a detailed description of what will happen to you during the study.

Study Procedure:

For this study, about 500mg - 1gm of tumor will be taken from the surgical specimen and 5ml of blood will be taken from the respective patients after taking proper consent in patient consent form. Following tests will be done in the samples: Histology, Immunohistochemistry with *in-vitro* diagnostic (IVD) approved antibodies exclusively for patient service and Molecular analysis.

Why should I participate in this study?

You have been chosen to participate in the study because of the following reasons: You have been diagnosed with breast cancer and you will be given adjuvant therapy consisting of Tamoxifen. This therapy has precipitated many adverse drug reactions. This study will focus on the quality of life of the patients and reduce adverse effect of drug toxicity based on drug response. This study may help in determining the judicious use the dose of tamoxifen to be administered. Hence, genotyping may be an important tool in determining the effective dose of tamoxifen in patients subjected to tamoxifen adjuvant therapy.

How many other people like me will be participating in the study?

The total number of breast cancer patients who will be participating in the study will be 140.

What are the possible risks / discomforts?

There is no known risk / discomfort of the patients in taking 5ml blood. The tumor sample will be taken from the surgical specimen after surgery of the tumor for therapy.

What are the potential benefits?

The aim of this study is to understand the molecular mechanism related to drug metabolism generated by adverse drug reactions during the drug response treatment in breast cancer patients. The knowledge that will accrue from this project will be helpful for improvement of quality of life and reduce adverse effect of drug toxicity based on drug response. You may benefit from satisfaction of participating in research study that may provide new information about the disease which may help you or other patients in the future for treatment, predictive and therapeutic measures of the disease.

Compensation: You will not be charged for any tests of the study.

If I take part what are my responsibilities?

Your primary responsibilities include visiting the hospital as per treatment schedule, follow study staff instructions. You will not be allowed to take certain medications and treatments while you are participant in this study. Please check with your doctor for drugs prohibited for usage during the study. The usage of other chemotherapy agents that do not fall under standard regimen also will not be permitted during the treatment period.

Will my taking part in the study be kept confidential?

Confidentiality of your medical records will be maintained to the extent permitted by law. Authorized representatives of Indian Council of Medical Research, Govt. of India, independent auditors and / or regulatory bodies and monitors will be able to have access to your original medical records for the verification of clinical study procedures. No information will be disclosed to anyone, other than may be required by law. Your identity will not be revealed.

What will happen to the results of the research study?

All information collected during the research study will be kept in a written report, where you will not be mentioned by name, only your initials, date of birth and a study number individual to you. Your confidentiality will be protected.

Contact Details:

If you have any questions about this study or you experience a side effect, illness or injury that you believe results from this study you may contact the following study doctors:

Dr. Neyaz Alam
MBBS, MS
Specialist Grade I, Associate Professor
Head, Department of Surgical Oncology

Dr. Partha Nath,
MBBS, Chief Medical Officer
Department Of Medical Oncology

Chittaranjan National Cancer Institute, Kolkata
37 S. P. Mukherjee Road, Kolkata 700 026
Telephone No.: 33-2476 5101/02/04, Extn. 345 and Extn.343
Fax: 91-33-2475-7606

Informed Consent Form

Project Title: Study on *CYP2D6* and *ABCB* polymorphisms with respect to tamoxifen adjuvant treatment in ER and PR receptor breast cancer patients

1. **Name of the Participant:** Tanuma Mistry Regd. No.-
2. **Name of the Principal Investigator:** Dr. Vilas D. Nasare Senior;
   Scientific Officer Grade-I, Dept. of Pathology and Cancer Screening.
3. **Name of the Institution:** Chittaranjan National Cancer Institute; 37, P. Mukherjee Road, Kolkata 700 026
4. **Co-Investigator:** Dr. Neyaz Alam, Dr. Partha Nath
5. **Name of Institute:** Chittaranjan National Cancer Institute, Kolkata
6. **Documentation of the informed consent:**
   I, ---------- have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am 18 years of age and exercising my free power of choice, hereby give my consent to be included as a participant in the project entitled "Study on *CYP2D6* and *ABCB* polymorphisms with respect to tamoxifen adjuvant treatment in ER and PR receptor breast cancer patients".

I. I have read and understood this consent form and the information provided to me.
II. I have had the consent document explained to me.
III. I have been explained the nature of the study.
IV. My rights and responsibilities have been explained to me by the investigator.
V. I have informed the investigator of all the treatments I am taking or have been taken in the past ---------- months including any *desi* (alternative) treatments.
VI. I hereby give permission to the investigator to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Government agencies and ethics committee. I understand that they may inspect my original records.
VII. My identity will be kept confidential if my data are publicly presented.
VIII. I have had my questions answered to my satisfaction.
IX. I have decided to be in the research study.

I am aware that if I have any questions during this study, I should contact the Principal Investigator/ Co-Investigators & Study Doctors of the project. By signing this consent form I attest the information in this document.

I will be given a copy of this consent document.

Participant's (or legal representative if participant incompetent) signature / thumb impression:

Name and signature of impartial witness (required for illiterate patients):

Date: Time: Place:

Address and Contact number of the impartial witness:

Name and signature of the PI & Co-PI:

Name: Date:

Signature:

ANNEXURE III

Title of the project: Study on *CYP2D6* and *ABCB* polymorphisms with respect to tamoxifen adjuvant treatment in ER and PR receptor breast cancer patients.

Chittaranjan National Cancer Institute. 37, S.P. Mukherjee Road, Kolkata -700026

Demographic Information

1.	ID Number
2.	Date of registration (Day/Month/Year)
3.	Name First Name Surname
4.	Contact No/ Mobile no.
5.	Address
6.	Setup 1-Urban; 2-Rural State
7.	Occupation (Self) 1. Student; 2- Self-employed; 3. Labour or manual worker or unskilled worker; 4 -Farmer; 5- professional; 6. Desk (office) job
8.	What is your age ?.....…….years
9.	Gender ( )1- Male; 2- Female
10.	Marital Status ( )1- Married; 2 –Unmarried; 3 – divorced; 4- widowed
11.	If Employed,1- Self-employed ; 2- Laborer or manual worker; 3- professional; 4- Family own business 5-Farmers; 6- Retired
12.	What is your religion? 1- Hindu; 2- Muslim; 3- Christian; 4- Buddhist; 5- Others
13.	Do you use tobacco 1-Yes; 2-No;
14.	If yes Duration of tobacco use
15.	If yes just occasional use ( ) If chronic Duration of tobacco use ---- Months----- Years
16.	If you ever have taken any contraceptive pills?1- Yes ( ) 2- No ( )

ANNEXURE IV

Quality of life

Please circle or mark one number per line to indicate your response as it applies to the baseline, 3rd month, 6th month and 12th month of treatment.

	SPIRITUAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
1	Do you feel peaceful?	0	1	2	3	4
2	Do you feel that you have any reason for living?	0	1	2	3	4
3	Has your life been productive?	0	1	2	3	4
4	Do You have trouble of feeling peace of mind?	0	1	2	3	4
5	Do you feel a sense of purpose in your life?	0	1	2	3	4
6	Are you able to reach down deep into Yourself for comfort?	0	1	2	3	4
7	Do you feel a sense of harmony within yourself?	0	1	2	3	4
8	Does your life lack meaning and purpose?	0	1	2	3	4
9	Do you find comfort in your faith or spiritual beliefs?	0	1	2	3	4
10	Do you find strength in your faith or spiritual beliefs?	0	1	2	3	4
11	Does your illness strengthen your faith or spiritual beliefs?	0	1	2	3	4
12	Do you think that whatever happens to your illness, your other things will be okay?	0	1	2	3	4

Reference:

https://www.facit.org/measures/facit-sp-12 FACITSp-12 breast cancer: For patients with breast cancer

Please circle or mark one number per line to indicate your response as it applies to the baseline, 3rd month, 6th month and 12th month of treatment.

	PHYSICAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
1	Do You feel or have a lack of energy?	0	1	2	3	4
2	Do you have nausea?	0	1	2	3	4
3	Do you have trouble meeting the needs of your family because of your physical condition?	0	1	2	3	4
4	Do you have pain?	0	1	2	3	4
5	Are you bothered with the side effects of treatment?	0	1	2	3	4
6	Do you feel ill?	0	1	2	3	4
7	Are you forced to spend time in bed?	0	1	2	3	4
8	Do you feel pain in certain parts of your body?	0	1	2	3	4
9	Do you feel short of breath?	0	1	2	3	4
10	Do you feel pain in bones?	0	1	2	3	4
11	Do you feel fatigued?	0	1	2	3	4
12	Do you have mouth sores?	0	1	2	3	4
13	Are you bothered about hair loss?	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
1	Do you feel close to your friends?	0	1	2	3	4
2	Are you getting emotional support from your family?	0	1	2	3	4
3	Do your friends support you?	0	1	2	3	4
4	Has your family accepted your illness?	0	1	2	3	4
5	Are you satisfied with family communication about your illness?	0	1	2	3	4
6	Do you feel, close to your partner (or the person who is your main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box ☐ and go to the next section.*					
7	Are you satisfied with your sex life?	0	1	2	3	4

	EMOTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
1	Do you feel sad?	0	1	2	3	4
2	Are you satisfied with how you are coping with your illness?	0	1	2	3	4
3	Are you losing hope in the fight against your illness?	0	1	2	3	4
4	Do you feel nervous?	0	1	2	3	4
5	Do you worry about dying?	0	1	2	3	4
6	Do you worry that your condition might get worse?	0	1	2	3	4

	FUNCTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
1	Are you able to work (including work at home)	0	1	2	3	4
2	Is your work (include work at home) fulfilling?	0	1	2	3	4
3	Are you able to enjoy your life?	0	1	2	3	4
4	Have you accepted your illness?	0	1	2	3	4
5	Are you sleeping well?	0	1	2	3	4
6	Are you enjoying the things that you usually do for fun?	0	1	2	3	4
7	Are you contented with the quality of your life right now?	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the <u>baseline, 3rd month, 6th month and 12th month</u> of treatment.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
1	I have been short of breath	0	1	2	3	4
2	I am self-conscious about the way I dress	0	1	2	3	4
3	One or both of my arms are swollen or tender	0	1	2	3	4
4	I feel sexually attractive	0	1	2	3	4
5	I am bothered by hair loss	0	1	2	3	4
6	I worry that other members of my family might someday get the same illness I have	0	1	2	3	4
7	I worry about the effect of stress on my illness	0	1	2	3	4
8	I am bothered by a change in weight	0	1	2	3	4
9	I am able to feel like a woman	0	1	2	3	4
10	I have certain parts of my body where I experience pain	0	1	2	3	4

Reference:

http://www.facit.org/FACITOrg/Questionnaires FACT-breast cancer: For patients with breast cancer

ANNEXURE V

Tamoxifen treatment Symptoms

During treatment baseline, 3rd month, 6th month and 12th month did you have any of the following symptoms?

Symptoms	Did not have	(If yes), how often did you have it?	(If Yes), how severe was it usually?	(If yes), how much did it distress or bother you
		Rarely Occasionally Frequently Almost Constantly	No AE Mild Moderate Severe Life Threatening Death	Not at all A little bit Somewhat Quite a bit Very much
Amenorrhea				
Fluid retention				
Hot flash				
Nausea				
Vaginal discharge				
Vaginal hemorrhage				
Weight loss				
Skin changes				
Infection				
Sepsis				
Alopecia				
Constipation				
Cough				
Diarrhoea				
Edema				
Menstrual disease				
Oligomenorrhea				
Ostalgia				
Vomiting				
Increased serum aspartate aminotransferase				

Reference:

https://www.drugs.com/sfx/tamoxifen-side-effects.htm

Publications

LIST OF PUBLICATIONS

❖ First Author Publications

1. **Mistry T,** Nath P, Alam N, Nasare VD. Four Year Clinical Outcomes: Evaluating the Efficacy of Concomitant and Sequential Anthracycline-taxane Chemotherapy in Indian Breast Cancer Patients – A Regional Cancer Center Study. ***Journal of Current Oncological Trends***. 2025 Feb 12:110-119. Epub ahead of print. Vol 1(2). doi: 10.4103/JCOT.JCOT_18_24.
2. **Mistry T,** Pal R, Ghosh S, Choudhury T, Mandal S, Nath P, Alam N, Nasare VD. Impact of Low BMI and Nutritional Status on Quality of Life and Disease Outcome in Breast Cancer Patients: Insights from a Tertiary Cancer Center in India. ***Nutrition and Cancer***. 2024 Jun 5:1-12. PMID: 38836498. Vol 76(7). doi: 10.1080/01635581.2024.2347396.
3. **Mistry T,** Nath A, Pal R, Ghosh S, Sahoo PK, Mahata S, Sarkar S, Choudhury T, Nath P, Alam N, Nasare VD. Emerging futuristic targeted therapeutics: A comprising study towards a new era for the management of TNBC. ***American Journal of Clinical Oncology***. 47(3): p 132-148, March 2024. doi: 10.1097/COC.0000000000001071.

Submitted Article

1. **Mistry T,** Sengupta S, Kumar S, Thakur N, Nath P, Alam N, Nasare VD. Low BMI and *ABCC2* Genotype Associated with Poor Clinical Response among Sequential Anthracycline-Taxane Chemotherapy Receiving Breast Cancer Patients: A Tertiary Care Hospital-Based Study. Submitted on 20/01/2025. Manuscript id: GENEJOURNAL-D-25-00339; Current Status Under review. ***Gene***.

❖ Co-Author Publications

1. Lathwal E, Kumar S, Sharma V, Sharma A, **Mistry T,** Nasare VD. Design and synthesis of propargyloxy functionalized pyrazole-based aurones: exploring their potent anticancer properties against AGS and MCF-7 cell lines. ***Chemistry & biodiversity.*** 2024 Dec 06. Doi 10.1002/cbdv.202402186.
2. Sahoo PK, **Mistry T,** Sarkar S, Ghosh D, Mahata S, Pal R, Ghosh S, Roy A, Bucha H, Mandal S, Datta S, Nasare VD. Polymorphism of transporter genes contributes to variations in treatment outcomes and adverse events in platinum based-chemotherapy- treated oral cancer patients. ***Precision Oncogenomics***. 2024 Apr. 1(1). doi:10.1080/28354311.2023.2292798.
3. Sahoo PK, Bhowmick AK, Sarkar S, Mahata S, Pal R, **Mistry T,** Ghosh S, Choudhury T, Kumar RS, Mandal S, Datta S, Nath P, Mukherjee KK, Nasare VD. Outcome of 3-year follow up with induction vs first line chemotherapy in oral cancer patients: An observational hospital-based study. ***Journal of Cancer Research and Therapeutics***. 2024 Jan 22. PMID: 38261440. 20(3):p 1006-1012, Apr–Jun 2024. doi: 10.4103/jcrt.jcrt_2179_22.
4. Mahata S, Sahoo PK, Pal R, Sarkar S, **Mistry T,** Ghosh S, Nasare VD. PIM1/STAT3 axis: a potential co-targeted therapeutic approach in triple-negative breast cancer. ***Medical Oncology***. 2022 May 15;39(5):74. PMID: 35568774. doi: 10.1007/s12032-022-01675-2.
5. Sarkar S, Sahoo PK, Pal R, **Mistry T**, Mahata S, Chatterjee P, Vernekar M, Mandal S, Bera T, Nasare VD. Assessment of quality of life among advanced ovarian cancer patients in a tertiary care hospital in India. ***Supportive Care in Cancer***. **2022** Apr;30(4):3371-3378. Epub 2022 Jan 6. PMID: 34988703. doi: 10.1007/s00520-021-06735-3.
6. Nath A, Mitra S, **Mistry T,** Pal R, Nasare VD. Molecular targets and therapeutics in chemoresistance of triple- negative breast cancer. ***Medical Oncology***. 2021 Nov 23;39(1):14. PMID: 34812991. doi:10.1007/s12032-021-01610-x.
7. Sahoo PK, Sarkar S, Ghosh D, Mahata S, Pal R, **Mistry T,** Ghosh S, Roy A, Bucha H, Mandal S, Nasare VD. Premalignant and malignant lesions of oral cavity in eastern India: a hospital-based study. ***European Journal of Cancer Prevention***. 2020 Nov 27. doi: 10.1097/CEJ.0000000000000640.
8. Sarkar S, Sahoo PK, Mahata S, Pal R, Ghosh D, **Mistry T,** Ghosh S, Bera T, Nasare VD. Mitotic checkpoint defects: en route to cancer and drug resistance. ***Chromosome Research***. **2021** Jun;29(2):131-144. Epub 2021 Jan 6. PMID: 33409811**.** doi: 10.1007/s10577-020-09646-x.

❖ List of Presentations in National/International Conferences/ Workshops

Thesis Abstracts

1. **Mistry T,** Sengupta S, Kumar SK, Thakur N, Chakraborty P, Nath P, Alam N, Nasare VD. Impact of BMI and *ABCC2* Genotype in Predicting Chemotherapy Response among Non-Metastatic Breast Cancer Patients Receiving Sequential Anthracycline-Taxane Treatment: Insights from an Indian Cohort. **44th IACR-2025. 44th Annual Meeting of the Indian Association for Cancer Research.** Organised by Chittaranjan National Cancer Institute in association with West Bengal Chapter, Kolkata, January 16-18, 2025. Page 412-413.

2. **Mistry T,** Nath P, Alam N, Nasare VD. Assessing *ABCB1* Transporter Gene (C1236T, G2677T/A, C3435CT) for Predicting Chemotherapy-Induced Toxicity in Breast Cancer Patients: A Comparative Study of Anthracycline and Taxane Regimens. **13th EZOS-2024 13th East Zonal Oncology Symposium.** Organised by Saroj Gupta Cancer Centre and Research Institute, Kolkata, February 10, 2024.

3. **Mistry T**, Pal R, Ghosh S, Nath P, Alam N, Nasare VD. Predicting toxicity following cancer chemotherapy by detecting transporter gene *ABCB1* (C1236T, G2677T/A, C3435CT) polymorphism in breast cancer patients receiving chemotherapy with anthracycline and taxane either sequentially or concomitantly. **ESMO Asia Congress 2023.** ***Annals of Oncology***. 2023 Nov, Volume 34: Supplement 4, 1482. DOI: 10.1016/j.annonc.2023.10.177.

4. **Mistry T**, Ghosh S, Mahata S, Sahoo PK, Pal R, Sarkar S, Choudhury T, Alam N, Mandal S, Nasare VD. *ABCB1* polymorphism in tamoxifen treated ER & PR positive breast cancer patients. **INCD-2022 5th International Conference on Nutraceuticals for Cancer and Other Chronic Disease.** Organized by Department of Zoology, University of Delhi, Delhi, October 7-9, 2022, OP-34 Page 112.

5. **Mistry T**, Ghosh S, Sahoo PK, Mahata S, Pal R, Sarkar S, Choudhury T, Alam N, Mandal S, Nasare VD. Single nucleotide polymorphisms of *ABCB1* (rs1128503) and *ABCC2* (rs145008610) genes and its clinical impact in ER & PR positive breast cancer patients in a tertiary care hospital of India. **13th European Breast Cancer Conference**. ***European Journal of Cancer*.** 2022; 175:S79. DOI: 10.1016/S0959-8049(22)01565-9.

Other Conferences Presentations

1. Ghosh S, **Mistry T,** Pal R, Sahoo PK, Mahata S, Sarkar S, Choudhury T, Banerjee R, Chakrabarti J, Mukherjee KK, Alam N, Mandal S, Nasare VD. Role of Sorcin in the development of multidrug resistance against 5-Flurouracil, Leucovorin, oxaliplatin and Docetaxel (FLOT) chemotherapeutic regimens in the Gastric cancer subtypes **INCD2022 5th International Conference on Nutraceuticals for Cancer and Other Chronic Disease,** Organized by Department of Zoology, University of Delhi, Delhi, October 7-9, 2022, OP33, Page 111. OP 33, Page 111.

2. Sahoo PK, **Mistry T,** Ghosh S, Mahata S, Sarkar S, Pal R, Choudhury T, Datta S, Mandal S, Bhowmick AK, Mukherjee KK, Nasare VD. Clinical implications of drug-metabolizing genes CYP3A4 and GSTP1 single nucleotide polymorphisms in oral cancer patients undergoing induction chemotherapy JCO Global Oncology. 2023 Aug, 9(Supplement_1):95-95. **ASCO Breakthrough: A Global Summit for Oncology Innovators.** doi: 10.1200/GO.2023.9.Supplement_1.95.

3. Ghosh S, **Mistry T,** Pal R, Sahoo PK, Mahata S, Sarkar S, Choudhury T, Banerjee R, Chakrabarti J, Mukherjee K, Alam N, Nasare VD. A soluble resistance related calcium binding protein (sorcin) up-regulates in the multi-drug resistant advanced gastric cancer patients. Annals of Oncology. 2023 July, Volume: 34. **25th Annual World Congress on Gastrointestinal Cancer ESMO 2023**. doi: 10.1016/j.annonc.2023.04.061.

4. Sarkar S, Sahoo PK, **Mistry T**, Pal R, Ghosh S, Choudhury T, Mahata S, Vernekar M, Bera T, Mukherjee KK, Nath P, Nasare VD. BUB1 (2530C>T) polymorphism and expression affects chemotherapy response and predicts poor prognosis in advanced epithelial ovarian cancer Annals of Oncology. 2022 Nov, 33:S1508-S1509. **ESMO Asia Congress 2022.** doi: 10.1016/j.annonc.2022.10.223.

5. Ghosh S, Pal R, **Mistry T,** Sahoo PK, Mahata S, Sarkar S, Choudhury T, Banerjee R, Chakrabarti J, Mukherjee K, Alam N, Mandal S, Nasare VD. Effect of overexpression of ERK1/2 and MDR1 on chemoresistance in advanced gastric cancer patients. Journal of Clinical Oncology. 2023 June, 41(16_suppl):e16051-e16051. **American Society of Clinical Oncology (ASCO) 2023**. doi: 10.1200/JCO.2023.41.16_suppl.e16051.

6. Pal R, Ghosh S, **Mistry T,** Mahata S, Sarkar S, Sahoo PK, Choudhury T, Vernekar M, Mukherjee KK, Nasare VD. Micro-RNAs as a biomarker for the prognosis of ovarian cancer patients receiving first-line chemotherapy. International Journal of Gynaecology

and Obstetrics. 2023 Oct; Volume 163 S1: 100-512. **XXIV FIGO World Congress of Gynecology and Obstetrics.** doi: 10.1002/ijgo.15058.

7. Sahoo PK, Sarkar S, Mahata S, Pal R, **Mistry T,** Ghosh S, Choudhury T, Datta S, Mondal S, Bhowmick AK, Nasare VD. Association of CYPA (986T>A) and GSTP(788G>A) with its clinical impact in oral cancer patients undergoing induction chemotherapy **INCD2022 5th International Conference on Nutraceuticals for Cancer and Other Chronic Disease,** Organized by Department of Zoology, University of Delhi, Delhi, October 7-9,2022, OP11, Page 100. OP 11, Page 100.

8. Pal R, Mahata S, Sahoo PK, Sarkar S, **Mistry T,** Ghosh S, Choudhury T, Vernekar M, Nath P, Mukherjee KK, Nasare VD. MicroRNAs as the potential biomarkers for the monitoring of Ovarian Cancer patients **INCD 2022 5th International Conference on Nutraceuticals for Cancer and Other Chronic Disease,** Organized by Department of Zoology, University of Delhi, Delhi, October 7-9, 2022, PP22, Page 127.

9. Sahoo, P. K., Sarkar, S., Mahata, S., Pal, R**., Mistry, T.,** Ghosh, S., Choudhury, T., Bhowmick, A. K., Mukherjee, K. K., Datta, S., & Nasare,V. D. (2022). Clinical Efficacy and Quality of Life of Oral Cancer Patients Treated With Paclitaxel/Cisplatin/5-FU Vs Paclitaxel/Carboplatin Chemotherapeutic Regimens in a Tertiary Cancer Center in Eastern India, ***Journal of the National Comprehensive Cancer Network***, 20(3.5), CLO22-081-CLO22-081. Retrieved Apr 7, 2022.

10. Sarkar S, Sahoo PK, Ghosh S, Mahata S, Pal R, **Mistry T,** Choudhury T, Vernekar M, Chatterjee P, Nath P, Mukherjee KK, Nasare VD. Clinical outcomes of paclitaxel-carboplatin chemotherapy with clinical impact of mitotic checkpoint proteins in advanced ovarian cancer patients **INCD2022 5th International Conference on Nutraceuticals for Cancer and Other Chronic Disease.** Organized by Department of Zoology, University of Delhi, Delhi, October 7-9,2022, IL-8 Page 46.

ACHIEVEMENTS

1. Awarded "2nd Best Poster Award" at 13th East Zonal Oncology Symposium 2024 organised by Saroj Gupta Cancer Centre and Research Institute; India, held on 10th February 2024

2. Awarded "SERB International Travel Support (ITS) Scheme" by SERB- DST, Govt. of India for presenting poster at ESMO Asia Congress 2023, Singapore, held on 1st-3rd 2023 December 2023

3. Awarded "CSIR-Foreign Travel Grant" by CSIR Govt. of India for presenting poster at EBCC-13 Conference, Barcelona, Spain held on 16-18th November 2022.

Tanuma Mistry.

TANUMA MISTRY

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Impact of Low BMI and Nutritional Status on Quality of Life and Disease Outcome in Breast Cancer Patients: Insights From a Tertiary Cancer Center in India

Tanuma Mistry[a,b], Ranita Pal[a], Sushmita Ghosh[a], Trisha Choudhury[a], Syamsundar Mandal[c], Partha Nath[d], Neyaz Alam[e] and Vilas D. Nasare[a]

[a]Department of Pathology & Cancer Screening, Chittaranjan National Cancer Institute, Kolkata, India; [b]Department of Life Science & Biotechnology, Jadavpur University, Kolkata, India; [c]Department of Epidemiology and Biostatistics, Chittaranjan National Cancer Institute, Kolkata, India; [d]Department of Medical Oncology, Chittaranjan National Cancer Institute, Kolkata, India; [e]Department of Surgical Oncology, Chittaranjan National Cancer Institute, Kolkata, India

ABSTRACT

This study investigates the impact of Body Mass Index (BMI) on Quality of Life (QoL) and treatment outcomes in breast cancer (BC) patients, particularly focusing on underweight individuals with compromised nutritional status. A nonrandomized prospective study comprising 121 newly diagnosed patients across various BMI categories utilized FACT-B & FACIT-Sp-12 questionnaires. Follow-ups occurred at baseline, during (3rd and 6th), and after (12th month) anthracycline-taxane chemotherapy, either sequentially or concomitantly. Patients with low BMI (<18.5 kg/m^2; 53.7%) exhibited significantly poorer QoL, marked by compromised nutritional indicators (low MUAC and SFT). Repeated measures ANOVA identified significant correlations between BMI groups in functional, social, and emotional QoL aspects ($p < 0.05$), with no notable differences in other domains. A Chi-square ($\chi 2$) test underscored a significant link between BMI and treatment response ($p < 0.0001$), showing higher rates of non-responders among underweight patients ($p = 4.259e^{-14}$). The study advocates pretreatment consultation with a dietitian as standard care for Indian BC patients, offering complimentary nutritional support for improved QoL outcomes and treatment responses.

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BMI; Breast cancer; Quality of Life; Chemotherapy delay; Chemotherapy response; Survival

Introduction

Breast cancer (BC) remains a substantial and persistent obstacle to worldwide public health. In 2024, BC accounted for nearly 11.6% of all diagnosed cancer cases, resulting in a worrying 2.3 million new cases (1).

The rural Indian population frequently faces health challenges related to insufficient nutrition, resulting in low Body Mass Index (BMI), reduced Mid-Upper Arm Circumference (MUAC), and thin Skin Fold Thickness (SFT). Malnourished patients had a significantly higher risk of cancer death, with a 2–5 times greater risk compared to well-nourished individuals (2–4). Patients who have therapeutic adverse effects such as toxicities, therapy failures, and rapid disease progression (2), faced the risk of unfavorable consequences and a poor prognosis due to inadequate nutrition (3).

Identification of nutritional deficiencies using traditional BMI-based screening methods became more challenging (5, 6). In addition to BMI, the assessment of MUAC and SFT served as an alternative non-interventional method for evaluating nutritional status. The measurement of the supra-iliac skinfold was crucial in estimating SFT, which was a commonly used caliper testing procedure to determine a patient's body fat percentage.

An anthropometric factor, including height, weight, and BMI, had been associated with a higher risk of BC (7, 8). Significantly, being overweight and obese were definitively identified as risk factors for BC, with a 1.2–1.4 times greater risk in post-menopausal women (9, 10) and a 0.8 times higher risk in pre-menopausal women (10, 11). Although numerous studies have reported the impact of obesity on QoL in BC patients (12–16).

CONTACT Vilas D. Nasare vilas.dr@gmail.com Department of Pathology and Cancer Screening, Chittaranjan National Cancer Institute, 37, S.P. Mukherjee Road, Kolkata 700026, India

Several researchers have proposed that incorporating nutritional assistance, such as the Mediterranean diet, could be a viable therapeutic approach for BC patients (17–22). However, it should be noted that implementing this diet in poor socio-economic nations is currently not feasible. Furthermore, research studies have highlighted various aspects of QoL among Indian BC patients. Their daily functioning and overall well-being can be significantly affected by physical symptoms such as pain, exhaustion, and side effects from treatments (23–26).

Highlighting the critical need for promptly assessing BMI status, especially with a strong focus on understanding how underweight conditions affect QoL and treatment outcomes among the BC population, there is a significant lack of research investigating the difficulties experienced by underweight BC patients, who have a low BMI and inadequate nutritional status. This lack of understanding is especially evident in the Indian healthcare system. To the best of our knowledge, there is scant evidence accessible in the Indian scenario as well as the global sector that directly explains the relation between low BMI and QoL domains that affect the treatment response and survival outcome.

Materials and Methods

Study Design

A total of 152 patients initially participated, but finally 121 patients were recruited in this study, which was conducted in collaboration with the Department of Surgical Oncology, Medical Oncology and Pathology & Cancer Screening, Chittaranjan National Cancer Institute (CNCI) Kolkata in India, during the time frame between August 2019 to October 2022. We monitored these patients until August 2023 (Figure 1).

Inclusion and Exclusion Criteria

The study included 121 female volunteers who were newly diagnosed with histologically verified invasive breast carcinoma (AJCC Stages I–III) and were aged 18 years or older. Patients with adequate bone marrow function, platelet counts (≥100,000/μL), blood coagulation parameters, and hepatic and neurological compliances were included in the study. All the registered patients were initiated cytotoxic therapy at baseline, and they were all enrolled within 3 weeks after being diagnosed.

Patients were excluded from the study ($n = 31$) based on the specified exclusion criteria. Patients under the age of 18 and who had a prior diagnosis and treatment of radiotherapy, chemotherapy and total mastectomy were excluded. Pregnant or breast-feeding women and those who were at risk of getting pregnant during the study period were excluded. Patients with a prior medical history venous or pulmonary blood clots (deep vein thrombosis), liver disease, high triglycerides, cataract history or stroke, acute hepatitis, active infection, uncontrolled diabetes, or autoimmune disease were also excluded from this study. Among the 31 excluded patients, a few lost follow up due to the following reasons. After receiving primary

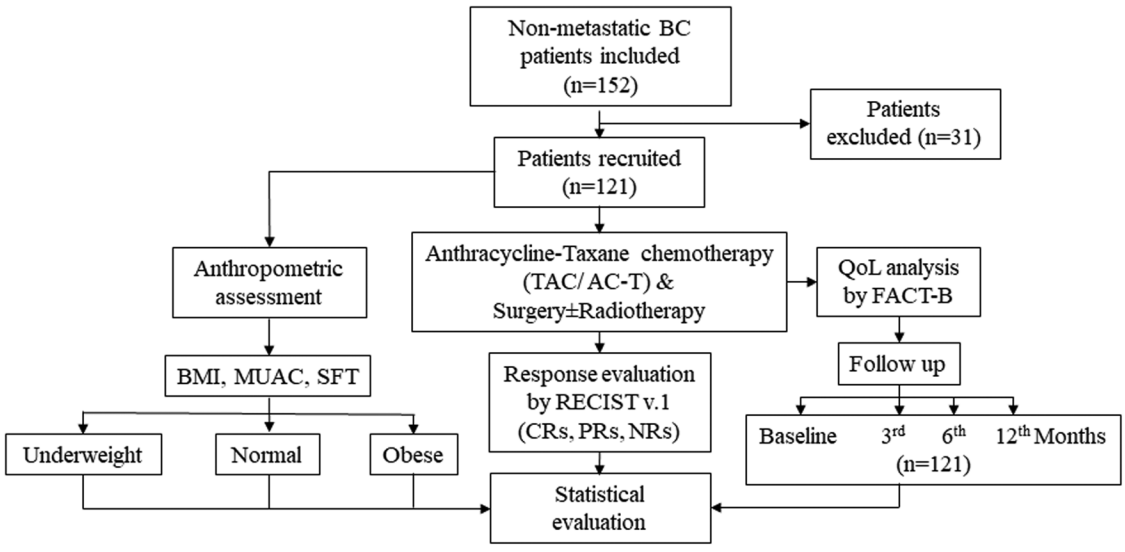

Figure 1. Schematic representation of study cohort. BC: Breast Cancer; TAC: Taxane-Anthracycline-Cyclophosphamide; AC-T: Anthracycline-Cyclophosphamide followed by Taxane; QoL: Quality of Life; FACT-B: Functional Assessment of Cancer Therapy—Breast; BMI: Body Mass Index; MUAC: Mid-Upper Arm Circumference; SFT: Skin Fold Thickness; RECIST: Response Evaluation Criteria In Solid Tumors; CRs: Complete Responders; PRs: Partial Responders; NRs: Non-Responders.

treatment management from this institute, they chose to pursue treatment outside the primary facility, closer to their localities, to access governmental health facilities like Swasthya Sathi Yojana and the Health Ministers Cancer Patient Fund (HMCPF). Additionally, mortality due to various causes, including COVID-19, heart failure, dengue, and other ailments, causes the patients not to attend further appointments.

Treatment

During the initial phase of patient enrollment, the concurrent administration of Antracycline-Taxane (TAC) ($n = 75$) was a commonly utilized chemotherapeutic regimen. However, midway through the study period, the NCCN guidelines began advocating for the sequential administration of Antracycline-Taxane (AC-T) ($n = 46$) as the preferred approach for treating BC patients. Chemotherapy was recommended to the study population in the following manner- 1. TAC regimen: comprised of Docetaxel 75 mg/m^2 i.v. at day 1; Doxorubicin 50 mg/m^2 i.v. at day 1; Cyclophosphamide 500 mg/m^2 i.v. at day 1 (27), repeated every 21 days. 2. AC-T regimen: Doxorubicin 50 mg/m^2 i.v. at day 1; Cyclophophamide 500 mg/m^2 i.v. at day 1 for four cycles followed by Paclitaxel 175 mg/m^2 by IV infusion on day 1 every 21 days for four cycles (28).

The choice of chemotherapy regimen was determined in accordance with institutional protocols and at the discretion of clinicians. Patients were advised to undergo pre-medications, including NK1 receptor antagonists, H2 blockers/PPIs, dexamethasone, and 5HT3 antagonists, as deemed necessary by their physicians to alleviate potential adverse effects associated with chemotherapy.

Radiotherapy was tailored to eligible patients, with doses adjusted based on tumor size and conditions. Typically, the standard radiotherapy regimen involved 50 Gray (Gy) over 25 daily fractions (5 fractions/week) spanned over 5 week. However, flexibility existed in the treatment approach, with variations such as extended fractionation either 45 Gy administered in 25 daily fractions with supplementary boost doses of 10 Gy over 5 fractions or an alternative regimen of 50.4 Gy given across 28 daily fractions (29).

Ethical Approval

The study was approved by the Institutional Ethical Committee, CNCI, Kolkata [IEC Ref. CNCI-IEC-DL-2020-6], adhering to the Declaration of Helsinki. Written informed consent, provided in the patients' preferred language (Bengali/Hindi), was obtained from all participants.

Data collection

Anthropometric Measurements

Before the administration of chemotherapy, the height and weight of each patient were recorded, and their BMI was measured individually. Patients were categorized as per WHO standard into the following BMI categories: underweight/low BMI class (<18.5 kg/m^2), normal BMI class (18.5–22.9 kg/m^2), or overweight/obese class (≥23 kg/m^2) (30).

The evaluation of nutritional status was conducted through an easy, rapid, and noninvasive procedure, which included the measurement of MUAC and SFT. We measured the MUAC at the midpoint between the shoulder's most prominent tip and the elbow (31). The upper MUAC group was categorized as individuals with a MUAC measurement exceeding 26.5 cm, whereas the lower group consisted of those with measurements falling below 23.5 cm. During SFT measurements, the clinician securely holds a fold of the patient's skin between the thumb and index finger, raising it to measure two components: the thickness of the skin and that of the subcutaneous fat-excluding any muscle or fascia (32). In order to overcome the influence of experimenter bias, the process was iterated a minimum of two times, and the final skinfold score was determined based on the average of these repetitions.

QoL Assessment

All the recruited patients ($n = 121$) were assessed exclusively during the chemotherapy treatment period individually at four key time points: before chemotherapy (baseline), during chemotherapy at the 3rd and 6th months, and after completion of chemotherapy at the 12th month. They were stratified based on their response to chemotherapy and their anthropometric characteristics (BMI). We analyzed the QoL of BC patients using the Functional Assessment of Cancer Therapy - Breast (FACT-B) questionnaire tailored for them, and spiritual well-being was assessed by the Functional Assessment of Chronic Illness Therapy-Spiritual Well-Being 12 (FACIT-Sp-12) questionnaire (33). This questionnaire was designed in six different domains, comprised of a total 49 questions in the following sections: physical, functional, social, emotional and spiritual well-being. A distinct set of questions was

designed typically for BC patients under the 'additional concern' domain.

Clinical Response

Tumor assessment and monitoring occurred at baseline, following the 3rd and 6th cycles of chemotherapy. Chemotherapy response was evaluated using clinical, radiological, and pathological data, and patients were categorized as complete responders (CRs), partial responders (PRs), or non-responders (NRs) in accordance with RECIST criteria version 1. Patients demonstrating stable or progressive disease were grouped as non-responders (34). The pattern of treatment response was evaluated after each cycle of chemotherapy and before giving the next cycle. Radiological assessments such as ultrasonography, computed tomography scans were employed following four cycles of chemotherapy. Pathological stages were determined by expert histopathologists. Different grades of toxicity in patients caused by chemotherapy were recorded in Common Toxicity Criteria (CTC) (CTCAE v4) according to the National Cancer Institute (NCI) (35).

Statistical Analysis

Descriptive statistical analyses were used to analyze the frequency, mean value, and corresponding standard deviation (S.D.) of demographic and clinical characteristics. Two-way repeated-measures analysis of variance (ANOVA) was applied, taking time as a repeated factor and QoL domains and BMI group as fixed factors. We considered multivariate (Wilk's Lambda test) analysis and Greenhouse-Geisser significance values ($p < 0.05$). A chi-square (χ^2) test was performed to find the associations of treatment responses among different BMI groups. Kaplan-Meier survival analysis was monitored at 48 months to understand the effect of BMI on survival outcomes. All these statistical analyses were done using Statistical Package for the Social Sciences (SPSS v.26.0), and $p < 0.05$ was taken to be statistically significant.

Results

A cohort of 121 individuals diagnosed with non-metastatic BC had an average age of 49.2 ± 11.66 years, body weight was (53.945 ± 10.90) kg, and mean MUAC of (22.762 ± 1.92) cm. When patients were stratified by BMI, 53.7% of the entire sample had a low BMI, while (38.8%) fell within the normal BMI range. Only (7.4%) were classified as overweight or obese. A substantial majority of the whole study population (57.85%) were between the age group 41 to 60 years. Within this age category, the low BMI group exhibited the highest 51.4%. Patients with a low BMI were primarily from families with a very low socio-economic status (2000≤ INR ≤5000) (48.78%), residing predominantly in rural areas (51.8%), with a high proportion characterized as illiterate (53.6%) and unemployed/homemakers (52.3%). Additionally, irrespective of BMI stratification, the majority did not have a familial background of cancer. However, stage III (56.5%) and grade II (54.9%) tumors were most commonly observed during the time of primary diagnosis. It was worth noting that patients with a low BMI were also found to have poor SFT (11.45 ± 6.4; 54.4%) cm. Low BMI group showed the majority number of NRs (90.2%), while normal BMI group exhibited mostly CRs (82%). The Chi-square (χ^2= 75.39; Z-score= 6.17) test underscored a significant association between BMI and treatment response ($p < 0.0001$). The lower MUAC (<23.5) cm group was predominated (81.69%) in low BMI category, while in the normal BMI, the mid MUAC (23.5–26.5) cm class was the most prevalent (72.34%) and the MUAC groups also varied significantly across BMI categories ($p < 0.0001$) (Table 1). The chemotherapy responses (CRs, PRs, and NRs) showed a highly significant difference when compared within the group (low BMI; $p = 4.259e^{-14}$ and normal BMI; $p = 2.2e^{-16}$). However, in the obese category, the difference was not significant ($p = 0.716$) (Figure 2).

Two-way repeated-measures analysis of variance (ANOVA) that examined the QoL scores across various domains as assessed by the FACT-B and FACIT-Sp-12 questionnaires categorized by different BMI groups. Notably, QoL scores demonstrated a statistically significant difference ($p < 0.05$) in the functional and social domains throughout the follow up period (baseline, 3rd, 6th, and 12th months), as determined by Wilk's Lambda and the Greenhouse-Geisser test. Similarly, there was a significant difference observed in the emotional domain within the group ($p = 0.025$) while other domains were not statistically significant ($p > 0.05$) (Table 2) and these QoL mean scores were graphically represented in Figure 3 at 4 time points for all the six domains among the different BMI groups. The majority of patients experienced grade 1-2 toxicity, with alopecia (79.33%), anemia (14.1%), and fever (14%) being the most commonly seen side effects. However, a small number of patients encountered grade 3-4 toxicity, including anemia (1.6%), diarrhea (1.6%), and constipation (1.6%) (Table 3). The Kaplan-Meier exhibited the lowest

Table 1. Socio-demographic and clinical characteristics of breast cancer patients.

	BMI class (kg/m^2) Frequency (%)			
Characteristics ($n=121$)	Low (<18.5) ($n=65$)	Normal (18.5-22.9) ($n=47$)	Overweight/Obese (≥23) ($n=9$)	p-value
Age (Years)				
20–40	18 (58.1)	9 (29)	4 (12.9)	0.405
41–60	**36 (51.4)**	29 (41.4)	5 (7.1)	
61–80	11 (55)	9 (45)	–	
Education				
Illiterate	**45 (53.6)**	33 (39.3)	6 (7.1)	0.922
Primary/Secondary School	19 (52.8)	14 (38.9)	3 (8.3)	
Graduate and above	1 (100)	–	–	
Occupation				
Homemaker/Unemployed	**56 (52.3)**	42 (39.3)	9 (8.4)	0.816
Self- employed/ Business	2 (66.7)	1 (33.3)	–	
Others	7 (63.6)	4 (36.4)	–	
Monthly income of family (INR)				
<2000	12 (60)	6 (30)	2 (10)	0.228
2000≤ to ≤5000	**40 (48.78)**	37 (45.12)	5 (6.1)	
5000< to ≤10000	12 (75)	3 (18.75)	1 (6.25)	
>10000	1 (33.33)	1 (33.33)	1 (33.33)	
Religion				
Hindu	43 (55.8)	30 (39)	4 (5.2)	0.727
Muslim	21 (50)	16 (38.1)	5 (11.9)	
Others	1 (50)	1 (50)	–	
Setup				
Rural	**44 (51.8)**	34 (40)	7 (8.2)	0.761
Urban	21 (58.3)	13 (36.1)	2 (5.6)	
Marital Status				
Married	51 (53.7)	37 (38.9)	7 (7.4)	0.997
Widowed	14 (53.8)	10 (38.5)	2 (7.7)	
Family History of Cancer				
No family history of cancer	54 (51.9)	41 (39.4)	9 (8.7)	0.372
1st degree relatives with cancer history	11 (64.7)	6 (35.3)	–	
AJCC Stage				
Stage I	4 (36.4)	7 (63.6)	–	0.150
Stage II	35 (54.7)	26 (40.6)	3 (4.7)	
Stage III	26 (56.5)	14 (30.4)	6 (13)	
Grade				
I	4 (66.7)	1 (16.7)	1 (16.7)	0.552
II	56 (54.9)	39 (38.2)	7 (6.9)	
III	5 (38.5)	7 (53.8)	1 (7.7)	
Tumor histology				
IDC	58 (52.3)	45 (40.5)	8 (7.2)	0.358
ILC	3 (75)	–	1 (25)	
DCIS	4 (66.7)	2 (33.3)	–	
ECOG score at the time of study entry				
Fully active	11 (55)	9 (45)	–	0.364
Restricted in physically strenuous activity	54 (53.5)	38 (37.6)	9 (8.9)	
Surgery				
BCS	3 (100)	–	–	0.648
MRM	57 (53.3)	42 (39.3)	8 (7.5)	
Toilet Mastectomy	4 (50)	3 (37.5)	1 (12.5)	
Palliative Mastectomy	1 (33.3)	2 (66.7)	–	
Chemotherapy				
TAC	39 (52)	32 (42.67)	4 (5.33)	0.363
AC-T	26 (56.52)	15 (32.61)	5 (10.87)	
Chemoradiotherapy				
Yes	49 (59.76)	31 (37.8)	2 (2.44)	0.571
No	16 (49.02)	16 (49.02)	7 (17.95)	
Treatment response				
Complete responders	5 (10)	**41 (82)**	4 (8)	**<0.0001**
Partial responders	5 (50)	2 (20)	3 (30)	
Non-responders	**55 (90.2)**	4 (6.6)	2 (3.3)	
MUAC (cm)				
Lower MUAC group (<23.5)	**58 (81.69)**	13 (18.31)	–	**<0.0001**
Mid MUAC group (23.5–26.5)	7 (14.9)	**34 (72.34)**	6 (12.76)	
Upper MUAC group (>26.5)	–	–	3 (100)	
ER* ($n=88$)				
Positive	24 (47.1)	22 (43.1)	5 (9.8)	0.302
Negative	20 (54.1)	13 (35.1)	4 (10.8)	
PR* ($n=88$)				
Positive	20 (47.6)	19 (45.2)	3 (7.1)	0.199
Negative	24 (52.2)	16 (34.8)	6 (13)	

(*Continued*)

Table 1. Continued.

Characteristics ($n=121$)	BMI class (kg/m^2) Frequency (%) Low (<18.5) ($n=65$)	Normal (18.5-22.9) ($n=47$)	Overweight/Obese (≥23) ($n=9$)	p-value
Her2ǂ ($n=88$)				
Positive (Score 3+)	11 (45.8)	9 (37.5)	4 (16.7)	0.348
Equivocal (Score 2+)	2 (50)	2 (50)	–	
Negative (Score 1+)	31 (51.7)	24 (40)	5 (8.3)	

Note: n= Number of Patients; BMI-Body Mass Index; INR- Indian rupees; AJCC-American Joint Committee on Cancer; IDC-Infiltrating Ductal Carcinoma; ILC-Infiltrating Lobular Carcinoma; DCIS-Ductal Carcinoma in-Situ; ECOG-Eastern Cooperative Oncology Group; BCS-Breast Conserving Surgery; MRM-Modified Radical Mastectomy; TAC: Taxane-Anthracycline-Cyclophosphamide; AC-T: Anthracycline-Cyclophosphamide followed by Taxane; MUAC-Mid upper arm circumference; *ER and PR positive, that is, >1% stained cells considered as positive. ǂHer2/neu score 3+ is considered as positive, score 2+ on IHC considered as equivocal; 1+ score considered as Her2/neu negative; Bold lettering denotes p-value ≤ 0.05 and highest percentage.

Table 2. Mean QoL domain scores in different BMI group at various time intervals.

QoL domains	Group (BMI)	Before chemotherapy Baseline	During treatment 3rd month	6th Month	After Chemotherapy 1 Year	p-value‡ (within group)
Physical	Low (n=65)	10.06±3.366	11.14±2.917	11.85±2.850	13.22±2.487	0.191
(Scale 0–52)	Normal (n=47)	12.72±3.354	11.77±2.868	10.53±2.865	9.62±2.575	
	Obese (n=9)	15.78±2.587	13.89±2.848	11.44±2.068	11.78±3.308	
p-value* (Between group)	0.083					
Functional	Low (n=65)	15.02±4.366	14.85±4.321	13.88±3.846	13.42±4.185	**0.040**
(Scale 0–28)	Normal (n=47)	11.94±3.332	13.70±3.464	14.13±3.480	15.32±3.142	
	Obese (n=9)	14.22±3.358	13.11±4.275	11.44±3.018	11.56±4.265	
p-value* (Between group)	**0.012**					
Social	Low ($n=65$)	14.52±3.428	15.06±3.724	15.62±4.175	16.45±3.754	**0.015**
(Scale 0–28)	Normal ($n=47$)	13.98±3.378	15.57±3.275	17.11±3.941	17.83±4.045	
	Obese ($n=9$)	14.44±3.358	15.22±3.528	16.22±3.346	17.56±3.321	
p-value* (Between group)	**0.043**					
Emotional	Low ($n=65$)	9.75±4.013	9.91±3.924	10.62±4.234	10.85±4.202	**0.025**
(Scale 0–24)	Normal ($n=47$)	9.38±3.193	8.53±3.314	7.79±3.127	7.70±3.432	
	Obese ($n=9$)	11.67±5.874	11.11±5.519	11.22±5.826	12.44±6.930	
p-value* (Between group)	0.082					
Spiritual	Low ($n=65$)	29.12±6.419	29.11±5.794	28.91±7.249	29.55±9.287	0.075
(Scale 0–48)	Normal ($n=47$)	30.23±6.394	30.17±5.858	31.15±6.221	32.91±6.396	
	Obese ($n=9$)	31.00±5.612	32.11±5.754	29.67±.557	31.44±7.844	
p-value* (Between group)	0.114					
Additional Concern	Low ($n=65$)	12.54±4.217	12.77±4.589	12.32±5.047	12.06±5.643	0.725
(Scale 0–40)	Normal ($n=47$)	9.62±3.254	9.49±3.329	8.77±2.987	8.57±3.354	
	Obese ($n=9$)	12.33±3.279	12.44±3.321	12.00±3.841	11.33±4.770	
p-value* (Between group)	0.865					

Note: All values are expressed as mean±SD; *Multivariate analysis (Wilk's Lambda); ǂGreenhouse-Geisser; Mauchly's sphericity was significant ($p<0.05$); Bold lettering denotes p-value ≤ 0.05.

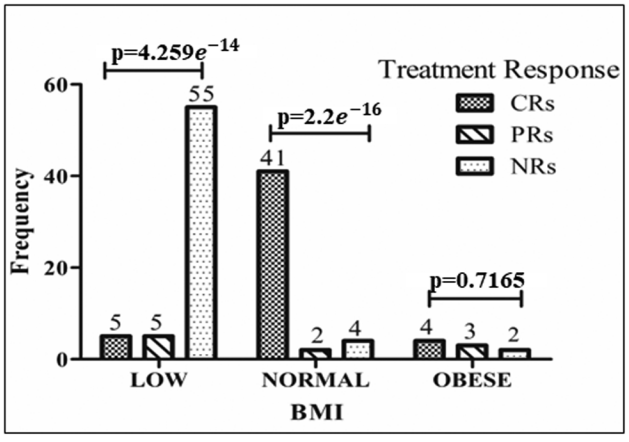

Figure 2. Comparison of treatment responses in non-metastatic BC patients stratified with respect to BMI. CRs: Complete Responders; PRs: Partial Responders; NRs: Non-Responders; BMI: Body Mass Index.

survival time (estimated 22 months S.E 5.225 with 75%) in low BMI group and overall survival analysis was significantly correlated with the BMI groups (log rank $p<0.0001$) (Figure 4).

Discussion

Assessing the QoL had a significant impact within the cancer-battling community. This study examines the influence of BMI on the QoL and treatment outcomes of BC patients, with a specific focus on individuals who are underweight and have poor nutritional status.

In this study, BC patients were diagnosed mostly among individuals aged 41–60 years. Stage III and Grade II tumors were most commonly detected. These

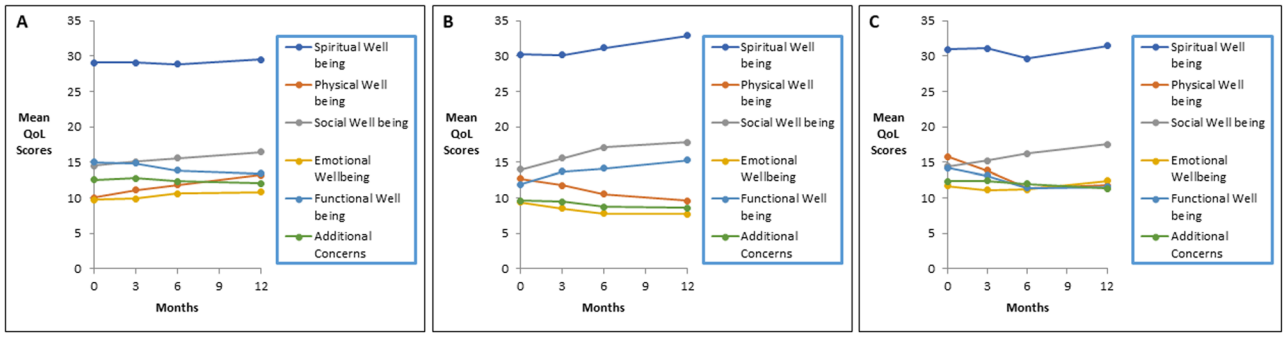

Figure 3. Trends in overall QoL mean score of different BMI groups among breast cancer patients. A: Underweight/Low BMI group; B: Normal BMI group; C: Overweight/Obese group; QoL: Quality of Life.

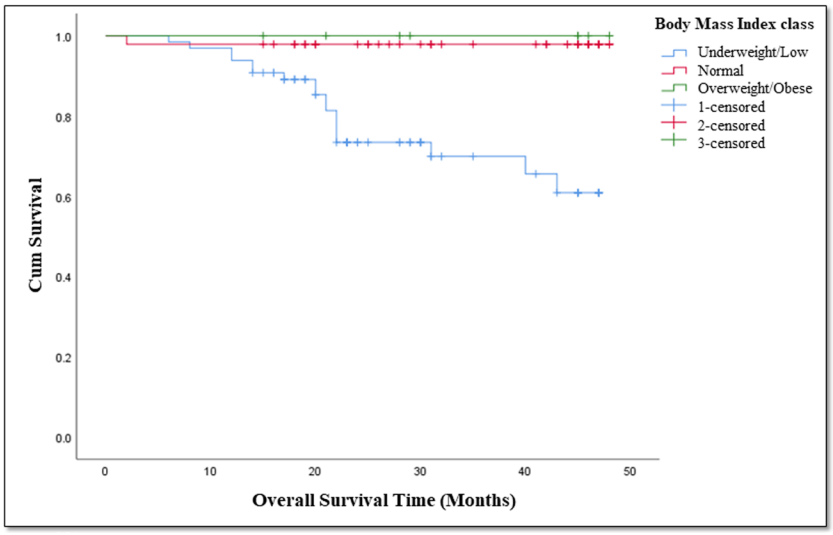

Figure 4. Overall survival among different BMI classes.

Table 3. Grades of common Side effects experienced by the patients ($n = 121$).

Sl. No.	Adverse Effects	Grade 1-2	Grade 3-4
1.	Alopecia ($n = 96$)	96 (79.33%)	–
2.	Anemia ($n = 19$)	17 (14.1%)	2 (1.6%)
3.	Antharlgia ($n = 9$)	9 (7.4%)	–
4.	Diarrhea ($n = 9$)	7 (5.8%)	2(1.6%)
5.	Fever ($n = 17$)	17 (14%)	–
6.	Mucositis ($n = 9$)	9 (7.4%)	–
7.	Nausea ($n = 17$)	17 (14%)	–
8.	Vomiting ($n = 13$)	13 (10.7%)	–
9.	Constipation ($n = 15$)	13 (10.7%)	2(1.6%)

results were consistent with previous research conducted in India (36, 37). Other South Asian studies also suggested that the patients were diagnosed in stage III (38, 39). The anthropometric factor, BMI was a significant factor in assessing the effectiveness of chemotherapy and the OS rates among the patients with different types of cancer. Incorporating BMI into the development of a treatment plan was of utmost importance. A majority of the study group, precisely 53.7%, had a low BMI (<18.5), emphasizing the impact of nutritional health on the efficacy of treatment.

The QoL of Indian BC patients was assessed using the FACT-B multidimensional questionnaire. This questionnaire had been proved as helpful tool for evaluating QoL in Indian BC patients, with multiple studies confirmed its relevance, validity, and reliability in the Indian setting (40–46).

The physical well-being of BC patients significantly affects their QoL, as it was closely linked to their nutritional status. Greater physical scores on the FACT-B questionnaire were indicative of a poor QoL. The results of our study showed increasing trend in physical score among patients who were underweight (<18 kg/m^2) both during and after chemotherapy treatment. The rise in physical well-being scores occurred as a result of the unfavorable effects of treatment and the toxicities caused by the drugs. There was a correlation between higher levels of symptoms and worsen physical well-being. A higher score signified a diminished capacity to carry out physical tasks. Pain mostly impacted the individual's physical wellbeing. Most individuals reported with a drop in their QoL and income

level as a result of reduced physical functioning. In addition, adverse effects such as diarrhea, vomiting, nausea, constipation had a direct impact on the patients' QoL, resulted in inconsistent follow-up and delays in chemotherapy (47–50). These factors, in turn, had a negative effect on treatment outcomes and posed a risk to the OS of the patients. BC patients experienced an average delay of 29.4 weeks in their treatment (49). The negative impacts were more pronounced in cancer patients who had a lower nutritional status, as determined by our analysis. Therefore, the American Dietetic Association advised that cancer patients should undergo a standardized nutrition review. However, the adoption of this practice in India was restricted.

The functional well-being questionnaire assessed various aspects such as work capacity, sleep quality, acceptance of sickness, and so on. These factors were directly linked to the nutritional health of the patients. Approximately, 70.2% of the participants in this study were from rural areas. In Indian society, rural women often experienced nutritional neglect, which resulted in a lower nutritional status. Consequently, this had an adverse effect on the treatment outcomes. Patients who had a lower BMI and insufficient nutritional status (MUAC and SFT) sometimes faced challenges in completing their chemotherapy treatment beyond the second or third cycles. As a consequence, they experienced delay in the administration of chemotherapy (47–50), which negatively impacted the overall response to treatment and long-term survival prospects. Both patients with low BMI and obesity reported with a significant decrease in their functional mean scores, suggested a lower QoL in compare to the normal BMI group.

The cost-effectiveness of diet-related therapy has posed challenges for families in providing nutritional care to patients (51). Approximately 84.29% of the patients at this specific regional hospital came from low socio-economic backgrounds, with a monthly income of ≤5000 INR. Consequently, a considerable proportion of individual faced the challenges of insufficient nourishment. The underweight group exhibited the lowest average social score in comparison to the normal and obese categories. In India, a woman's family frequently offered crucial assistance during her struggle against BC and her progression through the illness and its therapy. Therefore, it was clear that Indian BC patients relied heavily on their care-givers, husbands, and relatives for both emotional and social support, as well as for the financial costs associated with treatment.

The emotional well-being of a patient continued to closely connected with their social and family life. Greater emotional scores were associated with decreased QoL. Patients were queried about their apprehensions of mortality, anxiety, feelings of despair, and fluctuating optimism in their struggle against their sickness, within the realm of emotions. These characteristics were influenced by anthropometric and socio-demographic elements such as BMI, household income, as well as treatment-related factors like chemotherapy, surgery, and so on (52). Among the financially disadvantaged patients, the majority (88.4%) underwent mastectomy, a surgical intervention to remove the breast. Additionally, 79.33% experienced chemotherapy-induced alopecia, a common adverse effect characterized by hair loss, often resulting in body image concerns. As a result, there was a rise in emotional challenges, particularly among individuals with lower BMI levels.

Devotional thoughts have a significant impact in overall QoL (53, 54), specifically in the Indian cultural context, especially when it comes to spiritual well-being. This dimension encompasses various aspects like mental serenity, sense of purpose, efficacy in life, contentment, balance, spiritual beliefs, faith, and inner strength derived from spirituality. Patients with a low BMI had a notable decline in their spiritual ratings in comparison to those with a normal BMI, suggesting a downward trend ($p > 0.05$). Conversely, individuals with a normal BMI have a gradual increase in their spiritual assessments.

BC is a disease that predominantly impacts women as it is closely linked to female physiology. In certain nations such as India, physicians tend to show a preference for mastectomy over Breast Conserving Therapy (BCT) (44). Another factor deterring early care-seeking was the fear of social stigma and seclusion. Women worry not only about the threat of cancer and its potential impact on their family's reputation but also the potential challenges, such as difficulties in their daughter's wedding. Moreover, there was a widespread misconception associated with cancer, especially in private body parts like the breast and genitals, with "bad" and "immoral" behavior (55). This behavior was more evident in rural India. Addressing this social stigma was an urgent priority, as it not only hinders early diagnosis but also affects women's willingness to seek treatment for BC symptoms (50).

A Kaplan–Meier survival analysis revealed a statistically significant (log rank $p < 0.0001$) correlation among different BMI groups. Due to relatively short follow-up period, none of the groups achieved the median survival time. However, the underweight category exhibited the highest number of deaths events (18/65).

This study had a few limitations, including a small sample size and a single-center distribution. To overcome these limitations, we used standardized data

collection methods, performed rigorous statistical analyses, and adjusted for confounding variables when required. However, due to the limited follow-up time, OS might not have matured sufficiently for a comprehensive assessment. Nevertheless, the format of the present study and the findings thereof have a crucial impact on determining the strategy of BC treatment in the Indian scenario.

Conclusion

The QoL among cancer patients has been extensively studied over the past decade. It was considered as a crucial measure of therapeutic outcome, alongside overall survival. Observations have shown that Indian BC patients with a lower BMI and poorer nutritional health were more likely to experience unfavorable disease outcomes and a poor QoL. Hence, it is recommended to get guidance from a certified nutritionist prior to commencing any treatment. Providing sufficient nutritional assistance is essential, as it can help improve QoL, reduce the negative effects of chemotherapy, increase adherence to treatment, and boost long-term treatment outcomes.

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Authors' Contributions

Study concepts by TM, PN, VDN; Study Design by TM, VDN; Data acquisition by TM, PN, NA, RP, SG, TC; Data analysis and interception by TM, VDN; Statistical analysis TM, SSM, VDN, Manuscript preparation TM, VDN; Manuscript editing All authors; Manuscript review by All authors.

Ethical Approval

The study protocol was reviewed and approved by Institutional Ethical Committee and was conducted according to Good Clinical Practice under the Declaration of Helsinki [Reference id: CNCI-IEC-DL-2020-6].

Data Availability and Material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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References

1. Ferlay J, Ervik M, Lam F, Laversanne M, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F. Gobal Cancer Observatory: cancer Today. Lyon, France: International Agency for Research on Cancer 2024; https://gco.iarc.who.int/today, accessed [27 Apr 2024].
2. Muscaritoli M, Lucia S, Farcomeni A, Lorusso V, Saracino V, Barone C, Plastino F, Gori S, Magarotto R, Carteni G, et al. Prevalence of malnutrition in patients at first medical oncology visit: the PreMiO study. Oncotarget [Internet]. 2017 [cited 2023 Sep 19];8(45):79884–96. 10.18632/oncotarget.20168
3. Chen L, Qi Y, Kong X, Su Z, Wang Z, Wang X, Du Y, Fang Y, Li X, Wang J, et al. Nutritional risk index predicts survival in patients with breast cancer treated with neoadjuvant chemotherapy. Front Nutr. 2022;8(January) 8:786742. 10.3389/fnut.2021.786742
4. Genet S. Assessment of malnutrition among female breast cancer patients using biochemical markers. J Oncol Res Treat. 2020;2020(2):148.
5. Basaran G, Turhal NS, Cabuk D, Yurt N, Yurtseven G, Gumus M, Teomete M, Dane F, Yumuk PF. Weight gain after adjuvant chemotherapy in patients with early breast cancer in Istanbul Turkey. Med Oncol. 2011; [cited 2023 Sep 20] 28(2):409–15. https://link.springer.com/article/10.1007/s12032-010-9463-x 10.1007/s12032-010-9463-x
6. Trédan O, Bajard A, Meunier A, Roux P, Fiorletta I, Gargi T, Bachelot T, Guastalla J-P, Lallemand Y, Faure C, et al. Body weight change in women receiving adjuvant chemotherapy for breast cancer: a French prospective study. Clin Nutr. 2010; [cited 2023 Sep 20] 29(2):187–91. http://www.clinicalnutritionjournal.com/article/S0261561409001678/fulltext 10.1016/j.clnu.2009.08.003
7. Lin TM, Chen KP, Macmahon B. Epidemiologic characteristics of cancer of the breast in Taiwan. Cancer [Internet]. 1971; [cited 2023 Sep 15] 27(6):1497–504. https://acsjournals.onlinelibrary.wiley.com/doi/pdf/10.1002/1097-0142(197106)27:6%3C1497::AID-CNCR2820270634%3E3.0.CO;2-C 10.1002/1097-0142(197106)27:6<1497::AID-CNCR2820270634>3.0.CO;2-C
8. Li CI, Malone KE, White E, Daling JR. Age when maximum height is reached as a risk factor for breast cancer among young U.S. women. Epidemiology. 1997; [cited 2023 Sep 15] 8(5):559–65. https://pubmed.ncbi.nlm.nih.gov/9270959/ 10.1097/00001648-199709000-00014
9. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a sys-

tematic review and meta-analysis of prospective observational studies. Lancet. 2008; [cited 2023 Sep 12] 371(9612):569–78. http://www.thelancet.com/article/S0140673608602 69X/fulltext 10.1016/S0140-6736(08)60269-X
10. Munsell MF, Sprague BL, Berry DA, Chisholm G, Trentham-Dietz A. Body mass index and breast cancer risk according to postmenopausal estrogen-progestin use and hormone receptor status. Epidemiol Rev. 2014; [cited 2023 Sep 12] 36(1):114–36. 10.1093/epirev/mxt010
11. Schoemaker MJ, Nichols HB, Wright LB, Brook MN, Jones ME, O'Brien KM, Adami H-O, Baglietto L, Bernstein L, Bertrand KA, et al. Association of body mass index and age with subsequent breast cancer risk in premenopausal women. JAMA Oncol. 2018; [cited 2023 Sep 13] 4(11):e181771–e181771. https://jamanetwork.com/journals/jamaoncology/fullarticle/2685650 10.1001/jamaoncol.2018.1771
12. Leach MJ, Barber G, Monacella S, Jamieson P, Trinh T, Vo N, Schmidt U, Byrne A, Ristevski E. Physical activity, obesity, and quality of life among rural Australian cancer survivors: a cross-sectional study. Support Care Cancer. 2023 [cited 2023; Sep 12] 31(4):222. 10.1007/s00520-023-07691-w.
13. Shaikh H, Bradhurst P, Ma LX, Tan SY, Egger SJ, Vardy JL. Body weight management in overweight and obese breast cancer survivors [Internet]. Cochrane Database Syst Rev. 2020 [cited 2023 Sep 12];(12):1–44. 10.1002/14651858.CD012110.pub2
14. Dieli-Conwright CM, Courneya KS, Demark-Wahnefried W, Sami N, Lee K, Sweeney FC, Stewart C, Buchanan TA, Spicer D, Tripathy D, et al. Aerobic and resistance exercise improves physical fitness, bone health, and quality of life in overweight and obese breast cancer survivors: a randomized controlled trial. Breast Cancer Res. 2018; [cited 2024 Apr 2]20(1):124. https://pubmed.ncbi.nlm.nih.gov/30340503/ 10.1186/s13058-018-1051-6
15. Juan X, Zheng T, Qinglong D, Jiwei W, Jinming Y. Being slightly overweight is associated with a better quality of life in breast cancer survivors. Sci Rep. 2018;8(1):3022.
16. Connor AE, Baumgartner RN, Boone SD, Pinkston CM, Baumgartner KB. Obesity, ethnicity, and quality of life among breast cancer survivors and women without breast cancer: the long-term quality of life follow-up study. Ann Epidemiol. 2013; 23(9):582–3. 10.1016/j.annepidem.2013.06.017
17. Porciello G, Montagnese C, Crispo A, Grimaldi M, Libra M, Vitale S, Palumbo E, Pica R, Calabrese I, Cubisino S, et al. Mediterranean diet and quality of life in women treated for breast cancer: a baseline analysis of DEDiCa multicentre trial. PLoS One [Internet]. 2020 [cited 2024 Apr 3];15(10):e0239803. 10.1371/journal.pone.0239803
18. Montagnese C, Porciello G, Vitale S, Palumbo E, Crispo A, Grimaldi M, Calabrese I, Pica R, Prete M, Falzone L, et al. Quality of life in women diagnosed with breast cancer after a 12-month treatment of lifestyle modifications. Nutrients [Internet]. 2021; [cited 2024 Apr 313(1):1–15. 10.3390/nu13010136
19. Bu Y, Qu J, Ji S, Zhou J, Xue M, Qu J, Sun H, Liu Y. Dietary patterns and breast cancer risk, prognosis, and quality of life: a systematic review. Front Nutr. 2022 [cited 2024 Apr 3];9:1057057. 10.3389/fnut.2022.1057057
20. Vitale S, Palumbo E, Polesel J, Hebert JR, Shivappa N, Montagnese C, Porciello G, Calabrese I, Luongo A, Prete M, et al. One-year nutrition counselling in the context of a Mediterranean diet reduced the dietary inflammatory index in women with breast cancer: a role for the dietary glycemic index. Food Funct. 2023; [cited 2024 Apr 314(3):1560–72. https://pubmed.ncbi.nlm.nih.gov/36655860/ 10.1039/d2fo02198f
21. Barchitta M, Maugeri A, Magnano San Lio R, Quattrocchi A, Degrassi F, Catalano F, Basile G, Agodi A. The effects of diet and dietary interventions on the quality of life among breast cancer survivors: a cross-sectional analysis and a systematic review of experimental studies. Cancers (Basel). 2020; [cited 2024 Apr 312(2):322. 10.3390/cancers12020322
22. Mantzorou M, Tolia M, Poultsidi A, Vasios GK, Papandreou D, Theocharis S, Kavantzas N, Troumbis AY, Giaginis C. Adherence to Mediterranean diet and nutritional status in women with breast cancer: what is their impact on disease progression and recurrence-free patients' survival? Curr Oncol. 2022;[cited 2024 Apr 3] 29(10):7482–97. 10.3390/curroncol29100589
23. Prusty RK, Begum S, Patil A, Naik DD, Pimple S, Mishra G. Knowledge of symptoms and risk factors of breast cancer among women: a community based study in a low socio-economic area of Mumbai, India. BMC Womens Health [Internet]. 2020;20(1):106 [cited 2022 Dec 12]. 10.1186/s12905-020-00967-x.
24. Holzner B, Kemmler G, Greil R, Kopp M, Zeimet A, Raderer M, Hejna M, Zöchbauer S, Krajnik G, Huber H, et al. The impact of hemoglobin levels on fatigue and quality of life in cancer patients. Ann Oncol. 2002;13(6):965–73. 10.1093/annonc/mdf122
25. Adamsen L, Quist M, Andersen C, Møller T, Herrstedt J, Kronborg D, Baadsgaard MT, Vistisen K, Midtgaard J, Christiansen B, et al. Effect of a multimodal high intensity exercise intervention in cancer patients undergoing chemotherapy: randomised controlled trial. BMJ. 2009 [cited 2022 Dec 12];339(7726):b3410. https://www.jstor.org/stable/25672957 10.1136/bmj.b3410
26. Kim CJ, Kang DH, Smith BA, Landers KA. Cardiopulmonary responses and adherence to exercise in women newly diagnosed with breast cancer undergoing adjuvant therapy. Cancer Nurs. 2006; [cited 2022 Dec 14] 29(2):156–65. https://pubmed.ncbi.nlm.nih.gov/16565627/ 10.1097/00002820-200603000-00013
27. Martín M, Lluch A, Seguí MA, Ruiz A, Ramos M, Adrover E, Rodríguez-Lescure A, Grosse R, Calvo L, Fernandez-Chacón C, et al. Toxicity and health-related quality of life in breast cancer patients receiving adjuvant docetaxel, doxorubicin, cyclophosphamide (TAC) or 5-fluorouracil, doxorubicin and cyclophosphamide (FAC): impact of adding primary prophylactic granulocyte-colony stimulating factor to the TAC regimen. Ann Oncol. 2006;17(8):1205–12. 10.1093/annonc/mdl135
28. Mamounas EP, Bryant J, Lembersky B, Fehrenbacher L, Sedlacek SM, Fisher B, Wickerham DL, Yothers G, Soran A, Wolmark N, et al. Paclitaxel after doxorubicin plus cyclophosphamide as adjuvant chemotherapy for node-positive breast cancer: results from NSABP

B-28. J Clin Oncol. 2005;23(16):3686–96. 10.1200/JCO.2005.10.517
29. Provincial Health Services Authority. BC Cancer [Internet]. 2022. [cited 2023 Jan 2]. http://www.bccancer.bc.ca/.
30. Global Strategy on Diet, Physical Activity and Health [Internet]. 2004. [cited 2023 Sep 21]. https://www.who.int/publications/i/item/9241592222.
31. Jung EH, Hiratsuka Y, Suh S-Y, Won S-H, Choi S-E, Kang B, Lee SW, Kim YJ, Suh KJ, Kim J-W, et al. Association between mid-upper arm circumference and functional status in patients with advanced cancer. Clin Nutr Open Sci [Internet]. 2022 [cited 2023 Sep 13];45:72–9. 10.1016/j.nutos.2022.08.002.
32. Wells JCK, Fewtrell MS. Measuring body composition [Internet]. Arch Dis Child. 2006 [cited 2023 Oct 4];91(7):612–7. http://www. 10.1136/adc.2005.085522
33. Brady MJ, Cella DF, Mo F, Bonomi AE, Tulsky DS, Lloyd SR, Deasy S, Cobleigh M, Shiomoto G. Reliability and validity of the functional assessment of cancer therapy-breast quality-of-life instrument. J Clin Oncol. 1997; 15(3):974–86. 10.1200/JCO.1997.15.3.974
34. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228–47. 10.1016/j.ejca.2008.10.026
35. Dueck AC, Mendoza TR, Mitchell SA, Reeve BB, Castro KM, Rogak LJ, Atkinson TM, Bennett AV, Denicoff AM, O'Mara AM, et al. Validity and reliability of the us national cancer institute's patient-reported outcomes version of the common terminology criteria for adverse events (PRO-CTCAE). JAMA Oncol. 2015;1(8):1051–9. Nov 1 10.1001/jamaoncol.2015.2639
36. Tulsyan S, Chaturvedi P, Singh AK, Agarwal G, Lal P, Agrawal S, Mittal RD, Mittal B. Assessment of clinical outcomes in breast cancer patients treated with taxanes: multi-analytical approach. Gene. 2014; 543(1):69–75. 10.1016/j.gene.2014.04.004
37. Abdul Aziz AA, Md Salleh MS, Mohamad I, Bhavaraju VMK, Yahya MM, Zakaria AD, Gan SH, Ankathil R. Genotypes and haplotypes of ABCB1 contribute to TAC chemotherapy response in Malaysian triple negative breast cancer patients. Meta Gene. 2018; 16:21–7. 10.1016/j.mgene.2018.01.002
38. Chaturvedi P, Tulsyan S, Agarwal G, Lal P, Agarwal S, Mittal RD, Mittal B. Influence of ABCB1 genetic variants in breast cancer treatment outcomes. Cancer Epidemiol. 2013; 37(5):754–61. 10.1016/j.canep.2013.04.012
39. Sathishkumar K, Chaturvedi M, Das P, Stephen S, Mathur P. Cancer incidence estimates for 2022 & projection for 2025: result from National Cancer Registry Programme. India. Indian J Med Res. 2022; [cited 2024 Mar 27] 156(4&5):598–607. 10.4103/ijmr.ijmr_1821_22
40. Pandey M, Thomas BC, Ramdas K, Eremenco S, Nair M. Quality of life in breast cancer patients: validation of a FACT-B Malayalam version. Qual Life Res. 2002;11(2):87–90. https://sci-hub.se/https://pubmed.ncbi.nlm.nih.gov/12018741/ 10.1023/a:1015083713014
41. Kaur N, Mahapatra PP, Chakraborty S. Prevalence, characteristics, and correlates of fatigue in indian breast cancer survivors: a cross-sectional study. South Asian J Cancer. 2023; [cited 2024 Mar 27]12(1):55–61. 10.1055/s-0042-1749319.
42. Amarsheda SB, Bhise AR. Association of fatigue, quality of life and functional capacity in breast cancer patients receiving adjuvant chemotherapy. Asian Pac J Cancer Care. 2021; [cited 2024 Apr 3] 6(1):59–64. https://www.waocp.com/journal/index.php/apjcc/article/view/628 10.31557/apjcc.2021.6.1.59-64
43. Bichoo RA, Mishra A, Lal P, Gyan C, Agarwal G, Agarwal A, Mishra SK. Quality of life (QoL) in postmenopausal breast cancer patients receiving adjuvant hormonal therapy. Indian J Surg. 2021; [cited 2024 Apr 3]83(S2):461–7. https://link.springer.com/article/10.1007/s12262-021-02766-6 10.1007/s12262-021-02766-6
44. Deepa KV, Gadgil A, Löfgren J, Mehare S, Bhandarkar P, Roy N. Is quality of life after mastectomy comparable to that after breast conservation surgery? A 5-year follow up study from Mumbai, India. Qual Life Res. 2020;[cited 2022 Dec 12]29(3):683–92. 10.1007/s11136-019-02351-1.
45. Paswan SS, Kataria K, Parshad R, Srivastava A, Seenu V, Mishra B. Feasibility of fast track discharge in breast cancer patients undergoing definitive surgery and impact on quality of life: a prospective study from tertiary care center in India. J Surg Oncol. 2015; [cited 2024 Apr 3]111(3):265–9. https://onlinelibrary.wiley.com/doi/full/10.1002/jso.23817 10.1002/jso.23817
46. Gandhi A, Samuel SR, Vijaya Kumar K, Prakash Saxena PU, Mithra P. Effect of a Pedometer-based Exercise Program on Cancer Related Fatigue and Quality of Life amongst Patients with Breast Cancer Receiving Chemotherapy. Asian Pac J Cancer Prev. 2020; [cited 2024 Apr 3]21(6):1813–8. https://pubmed.ncbi.nlm.nih.gov/32592382/ 10.31557/APJCP.2020.21.6.1813
47. Gangane N, Manvatkar S, Ng N, Hurtig A-K, San Sebastián M, Anshu. Prevalence and risk factors for patient delay among women with breast cancer in rural india. Asia Pac J Public Health. 2016 [cited 2023 Sep 12];28(1):72–82. Available from: https://pubmed.ncbi.nlm.nih.gov/26658324/ 10.1177/1010539515620630
48. Chintamani, Tuteja A, Khandelwal R, Tandon M, Bamal R, Jain S, Narayan N, Srinivas S, Kumar Y . Patient and provider delays in breast cancer patients attending a tertiary care centre: a prospective study. JRSM Short Rep. 2011 [cited 2023 Sep 12];2(10):76.https://pubmed.ncbi.nlm.nih.gov/22046495/ 10.1258/shorts.2011.011006
49. Jassem J, Ozmen V, Bacanu F, Drobniene M, Eglitis J, Lakshmaiah KC, Kahan Z, Mardiak J, Pieńkowski T, Semiglazova T, et al. Delays in diagnosis and treatment of breast cancer: a multinational analysis. Eur J Public Health. 2014; [cited 2023 Sep 12] 24(5):761–7. https://europepmc.org/article/med/24029456 10.1093/eurpub/ckt131
50. Mehrotra R, Yadav K. World Journal of Clinical Oncology Breast cancer in India: present scenario and the challenges ahead. World J Clin Oncol. 2022;13(3):209–18. 10.5306/wjco.v13.i3.209
51. Bellanger M, Barry K, Rana J, Regnaux JP. Cost-effectiveness of lifestyle-related interventions for the primary prevention of breast cancer: a rapid review

[Internet]. Front Med (Lausanne). 2020; [cited 2023 Sep 12]. 6:325. https://pubmed.ncbi.nlm.nih.gov/32117999/ 10.3389/fmed.2019.00325
52. Fox RS, Ancoli-Israel S, Roesch SC, Merz EL, Mills SD, Wells KJ, Sadler GR, Malcarne VL. Sleep disturbance and cancer-related fatigue symptom cluster in breast cancer patients undergoing chemotherapy. Support Care Cancer. 2020; Feb 1 [cited 2023 Sep 1228(2):845–55. Available fromhttps://pubmed.ncbi.nlm.nih.gov/31161437/ 10.1007/s00520-019-04834-w
53. Forouzi MA, Tirgari B, Safarizadeh MH, Jahani Y. Spiritual needs and quality of life of patients with cancer. Indian J Palliat Care. 2017; [cited 2024 Apr 16] 23(4):437–44. 10.4103/IJPC.IJPC_53_17
54. Jafari N, Zamani A, Farajzadegan Z, Bahrami F, Emami H, Loghmani A. The effect of spiritual therapy for improving the quality of life of women with breast cancer: A randomized controlled trial. Psychol Health Med. 2013; [cited 2024 Apr 16] 18(1):56–69. https://www.tandfonline.com/doi/abs/10.1080/13548506.2012.679738 10.1080/13548506.2012.679738
55. Women and Cancer in India [Internet]. [cited 2023 Sep 13]. https://www.newsecuritybeat.org/2018/07/women-cancer-india/.

Original Article

Four Year Clinical Outcomes: Evaluating the Efficacy of Concomitant and Sequential Anthracycline-taxane Chemotherapy in Indian Breast Cancer Patients – A Regional Cancer Center Study

Tanuma Mistry[1,2*], Partha Nath[3*], Neyaz Alam[4], Vilas D. Nasare[1]

[1]Department of Pathology and Cancer Screening, Chittaranjan National Cancer Institute, Kolkata, West Bengal, India, [2]Department of Life Science and Biotechnology, Jadavpur University, Kolkata, West Bengal, India, [3]Department of Medical Oncology, Chittaranjan National Cancer Institute, Kolkata, West Bengal, India, [4]Department of Surgical Oncology, Chittaranjan National Cancer Institute, Kolkata, West Bengal, India

*Tanuma Mistry and Partha Nath as equal contributors

Abstract

Background: Breast cancer (BC) continues to be one of the most significant challenges in global public health, contributing to approximately 11.6% of all cancer cases worldwide. While anthracycline-taxane-based regimens have been established as a first-line treatment option, the majority of safety and efficacy data for these therapies originate from the high-income countries, leaving gaps in understanding their impact in low- and middle-income settings. **Objective:** This study aims to report on clinical efficacy, toxicity, quality of life (QoL), and survival outcome of the following chemotherapeutic regimens: Anthracycline only (5-fluorouracil-epirubicin–cyclophosphamide), concomitant Docetaxel + Doxorubicin + Cyclophosphamide (TAC), and sequential Doxorubicin + Cyclophosphamide followed by Taxane (AC-T), among Indian BC patients in a high-volume tertiary cancer center. **Materials and Methods:** This study was conducted among histopathologically diagnosed 121 BC patients who received one of the aforementioned neo-adjuvant chemotherapies (NACTs) followed by surgery or surgery followed by adjuvant chemotherapy ± adjuvant radiation therapy ± hormone therapy. Clinical data, including patient demographics, tumor characteristics, treatment response, toxicity, QoL, and survival data, were collected and analyzed. **Results:** Stage II–III (52.89%, 38.01%) with Grade II (84.3%) tumor was more frequently diagnosed. NACT group was categorized: Complete (12.5%), partial (34.37%), and nonresponders (NRs) (53.13%). Few patients faced chemo-induced high-grade toxicities alopecia (79.33%), anemia (1.6%), diarrhea (1.6%), and constipation (1.6%). QoL among BC patients noticed to be decreased significantly ($P < 0.05$) with chemotherapy response during treatment but improved after the completion of chemotherapy (12th month). A significant association was found between progression-free survival (PFS) in NACT and disease-free survival (DFS) in NRs group ($P < 0.0001$). Although no significant difference was observed when three regimens were compared for PFS, overall survival, and DFS ($P > 0.05$). TAC regimen (hazard ratio [HR] 1.585; 95% confidence interval [CI]: 0.441–5.694) and AC-T regimen (HR 1.077; 95% CI: 0.320–3.260) showed higher HRs ($P > 0.05$). **Conclusions:** This study was robust and not significantly different in the subgroup. All three regimens showed varying tolerance with minimal high-grade toxicity and comparable clinical efficacy and safety. While the AC-T regimen did not significantly improve survival; however, a positive trend was observed, suggesting its potential as a preferred option for certain patient cohorts, aligning with findings from Western studies. These results support the evidence-based optimization of chemotherapy regimens for BC management in the resource-limited settings.

Keywords: Breast cancer, clinical efficacy, concomitant/sequential anthracycline-taxane chemotherapy, survival outcome

Introduction

Breast cancer (BC) remains one of the most prevalent and formidable challenges to global public health, accounting for about 11.6% of all feasible cancer cases, summed up to about 2.3 million new cases as recorded in 2022.[1] The survival rate of BC had been steadily improved over time, likely due to the emergence of innovative therapeutic strategies, such as immune-based therapies, CRISPR technology, RNA-based

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Address for correspondence: Dr. Vilas D. Nasare, Department of Pathology and Cancer Screening, Chittaranjan National Cancer Institute, 37, S.P. Mukherjee Road, Kolkata - 700 026, West Bengal, India.
E-mail: vilas.dr@gmail.com
Dr. Neyaz Alam, Department of Surgical Oncology, Chittaranjan National Cancer Institute, 37, S.P. Mukherjee Road, Kolkata - 700 026, West Bengal, India.
E-mail: drneyazonco1@gmail.com

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approaches, and other advanced treatments.[2-4] The certain symptoms were often observed in BC patients at the time of diagnosis, such as a breast lump, mastalgia, nipple discharge, or retraction,[2] which had a significant impact on treatment adherence, quality of life (QoL), and ultimately, clinical outcomes.

The multidisciplinary treatment options for BC include surgery, chemotherapy, radiation therapy, hormonal therapy, immunotherapy, etc.[5] Clinicians typically prioritized mastectomy and breast-conserving surgery as the most preferred surgical treatment procedures. In a low- to middle-income country like India, mastectomy usually preferred by the clinicians.[6] Over the past decades, chemotherapy-based treatment options evolved enormously; chemotherapy played crucial role in the risk reduction of recurrence and improved survival rates among the patients. The optimal chemotherapy regimen continued to evolve and determined the most effective approach remained a topic of ongoing research and clinical investigation. Now-a-days, anthracyclines taxanes-based agents were sequentially administered to the patients.[7-9] Standard combinational chemotherapy showed better efficacy and good overall survival (OS) with few drug-related adversities such as anemia, neutropenia, fever, diarrhea, constipation, alopecia, and arthralgia.[10] These toxicities not only affect patients' QoL but also had the potential to impact treatment efficacy, treatment duration, and overall clinical outcomes.

The clinical efficacy of 5-fluorouracil-epirubicin–cyclophosphamide (FEC) and docetaxel–doxorubicin–cyclophosphamide (TAC) as the first-line chemotherapy in BC was well established, but most of its safety and efficacy data are from the high-income countries. Indian studies previously described the symptoms and outcomes of treatment for recurrent BC, survival after chemotherapy.[11-13] However, the clinical presentation of AC-T (Doxorubicin–cyclophosphamide followed by Paclitaxel) regimen and outcomes of front-line treatment of BC were not well documented. Therefore, this study aimed to present a comprehensive, 4-year interim study on the clinical outcomes of three commonly used chemotherapy regimens for BC: FEC, TAC, and AC-T.

Materials and Methods

Study design

This prospective cohort study spanned 4 years with three groups of regimens (FEC, TAC, and AC-T) among 121 histopathologically confirmed breast carcinomas (AJCC stages I–III) included in the study (93% power), all of whom sought treatment at the surgical outpatient department. This single-center study was conducted at the exclusive regional cancer hospital of Eastern India, taking into account the diverse cancer landscape of the region.

The recruitment of patients occurred from August 2019 to August 2023 through nonrandomized sampling. Patients from varied socioeconomic backgrounds and a wide geographic expanse were included, ensuring a comprehensive representation. Data were collected primarily from nonmetastatic cancers without any local recurrence patient population to maintain the homogeneity of the data. Eligible patients underwent surgical procedures, chemotherapy, and radiotherapy at the department of surgical oncology, medical oncology, and radiation oncology, respectively. Subsequent patient follow-up and data analysis were carried out at the department of pathology and cancer screening.

Female patients aged 18–80 years who met the specific inclusion criteria were recruited for this study. The inclusion criteria encompassed patients underwent chemotherapy, radiotherapy, or hormone therapy, without the use of medications likely to influence enzyme activity, and with satisfactory bone marrow function, hepatic function, neurologic function, platelet count (≥100,000/μL), and blood coagulation parameters. Patients who had previously diagnosed with BC and underwent radiotherapy, chemotherapy, or total mastectomy were excluded from participation in this study. In addition, patients with a history of deep-vein thrombosis (blood clots in veins) or pulmonary embolism, liver disease, elevated triglyceride levels, cataracts, stroke, acute hepatitis, active bacterial or viral infection requiring parenteral antibiotic treatment, uncontrolled diabetes, nonhealing wounds of significant severity, clinically significant proteinuria, cardiovascular complications, or autoimmune diseases were also excluded from the study [Figure 1].

Ethics and informed consent

This study adhered to the guidelines outlined in the Declaration of Helsinki and obtained approval from the Institutional Ethical Committee (IEC Ref. CNCI-IEC-DL-2020-6). Before participation, all patients provided written informed consent in their preferred language.

Treatment

Initially, patients who were deemed operable underwent adjuvant chemotherapy directly. Surgical resection, either breast conserving therapy (BCT) or modified radical mastectomy (MRM) was taken into consideration. In contrast, initially inoperable patients received neo-adjuvant chemotherapy (NACT). Patients were recommended one of the following chemotherapeutic regimens in a tri-weekly manner for six cycles: (1) FEC regimen: Comprised of 5-fluorouracil 500 mg/m^2 i.v. at day 1, epirubicin 60 mg/m^2 i.v. at day 1 and cyclophosphamide 500 mg/m^2-i.v. dose at day 1;[14] (2) TAC regimen: Comprised of docetaxel 75 mg/m^2 i.v. at day 1; Doxorubicin 50 mg/m^2 i.v. at day 1; cyclophosphamide 500 mg/m^2 i.v. at day 1;[10] (3) AC-T regimen: doxorubicin 50 mg/m2 i.v. at day 1; cyclophosphamide 500 mg/m2 i.v. at day 1 for four cycle followed by paclitaxel 175 mg/m2 by IV infusion day 1 every 21 days for 4 cycles.[15] Decision of chemotherapy regimen selection was made as per the institutional guidelines and clinician discretion. Premedication was recommended to the patients as per the physician to mitigate potential adverse events of chemotherapy.

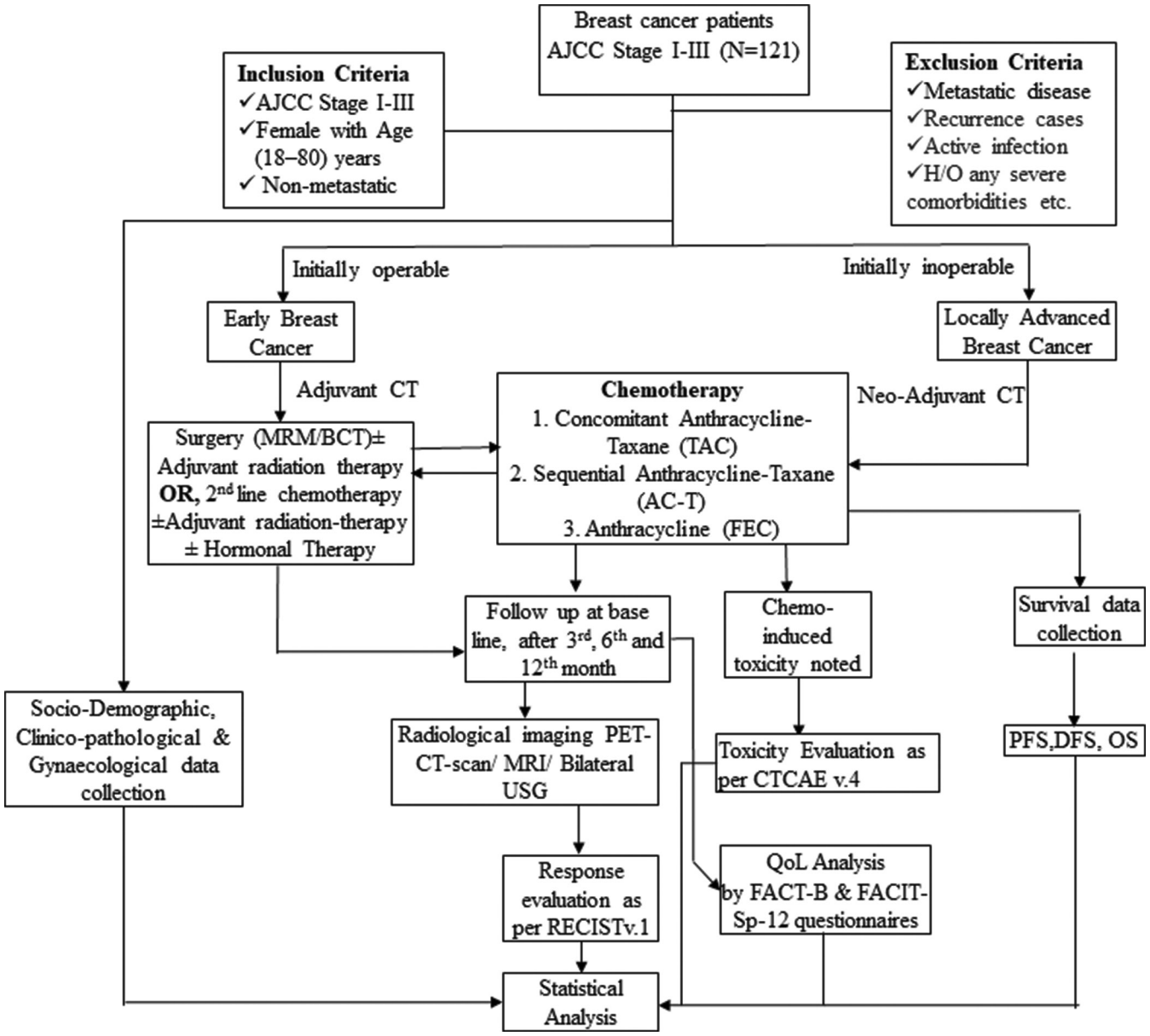

Figure 1: Schematic representation of study cohort. AJCC: American joint committee on cancer, CT: Chemotherapy, MRM: Modified radical mastectomy, BCT: Breast conserving therapy, FEC- 5fluorouracil + Epirubicin + Cyclophosphamide, TAC- Docetaxel + Doxorubicin + Cyclophosphamide, AC-T- Doxorubicin + Cyclophosphamide followed by Taxane, PET-CT: Positron emission tomography and computed tomography, MRI: Magnetic resonance imaging, USG: Ultrasound sonography, CTCAE: Common terminology criteria for adverse events, RECIST: Response evaluation criteria in solid tumors, FACT-B Functional assessment of cancer therapy-breast, FACIT Sp-12-Functional assessment of chronic illness therapy-spiritual well-being

Radiotherapy was administered to patients with varying doses depending on the tumor size and condition. The standard radiotherapy dose was 50 Gray (Gy) delivered in 25 daily fractions (5 fractions/week) over a span of 5 weeks. However, the radiotherapy regimen could be adjusted, considering extended fractionation as either 45 Gy in 25 daily fractions with additional boost doses of 10 Gy in 5 fractions or 50.4 Gy in 28 daily.[16] For postmastectomy patients, radiation therapy was recommended on the basis of the following criteria: Tumor size >5 cm; presence of any number of positive lymph node; centrally located tumor with high-risk features. Mastectomy patients with inadequate lymph node dissection and the patients under BCT all received adjuvant radiation therapy as a standard of care.

Premenopausal women received 5 years of 20 mg/day tamoxifen plus another 5 years of hormone therapy depending on the menopausal status as per the ATLAS trial data.[17] Regarding, postmenopausal women, the data for the collected patients were on switch therapy, i.e., 2 years of tamoxifen followed by aromatase inhibitor as preferred by the clinician. Her 2-positive patients received trastuzumab.

Clinical response and categorization

The assessment of tumor burden, disease recurrence, and progression was conducted at baseline and after the 3rd and 6th cycles of chemotherapy using a combination of radiological imaging techniques (including whole-body computed

tomography [CT] scan, thorax imaging, and breast magnetic resonance imaging), along with physical examinations following the completion of the primary treatment as per the protocol. Apart from the CT scan, we also used bilateral ultrasound of breast and axilla, bilateral diagnostic mammography along with contrast-enhanced CT scan of the thorax and abdomen whenever indicated. Fluorodeoxyglucose-positron emission tomography scan was only done in patients with high chance of micro-metastasis.[18] Radiological and clinical examinations were done to detect recurrence in adjuvant chemotherapy. Clinical, radiological, and pathological assessments were used to analyse the response of NACT and patients were categorized into complete responders (CRs), partial responders (PRs), and nonresponders (NRs) as per the response evaluation criteria in solid tumours version 1, (RECIST v.1).[19] Patients demonstrated stable disease, progressive disease, were grouped as NRs.

Clinical endpoints and toxicity assessment

Toxicities associated with each chemotherapy regimen were assessed using standardized toxicity grading systems, National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE v4). Toxicity data, including hematological, gastrointestinal, cardio, and neurotoxicity, and other chemotherapy-related adverse events, were collected at regular intervals during and after chemotherapy administration.[20]

Quality of life assessment and survival analysis

QoL was evaluated by the Functional Assessment of Cancer Therapy-Breast (FACT-B) and Functional Assessment of Chronic Illness Therapy-Spiritual Well-Being-12 (FACIT Sp-12) questionnaires from baseline up to the 3rd, 6th, and 12th months of the treatment.[21]

Kaplan–Meier (KM) survival analysis was employed to estimate the progression-free survival (PFS), OS, and disease-free survival (DFS) for each chemotherapy regimen, and Cox proportional hazards models were used to assess the association between baseline risk factors for potential confounding variables.

Statistical analysis

Descriptive statistical analysis was performed to calculate the means with corresponding standard deviations and standard error. KM survival analysis was employed to estimate survival time, and Cox proportional hazards models were used to assess the association between the baseline risk factors for potential confounding variables. IBM SPSS Statistics (Version 26.0; IBM Corporation, Armonk, New York, USA.) was use to analyze all the statistical data and $P < 0.05$ was taken to be statistically significant.

Results

Patient and treatment characteristics

One hundred and twenty-one BC patients were diagnosed with an overall mean age at diagnosis of 49.2 ± 11.66 years and body weight 53.945 ± 10.90 kg. Predominance of patients (57.9%) belonged to the age group of 41–60 years. A significant proportion of patients had low literacy (69.4%) and came from the rural backgrounds (70.2%). Very few patients were reported with alcohol, smoking, and tobacco-related consumption [Table 1].

The majority of patients were diagnosed with Stage II (52.89%) and Stage III (38.01%) BC, with a predominance of moderately differentiated Grade II tumors (84.3%). Tumor sizes ranging from 2 to 5 cm was most commonly observed (42.1%), and 88.4% of patients showed positive lymph node metastasis. Infiltrating ductal carcinoma (IDC) was the most common histological tumor subtype, accounting for 90.9% of cases. Adjuvant chemotherapy was provided to 73.6% of the patient cohort, with NACT being administered to the remaining 26.4% of patients. Within the patient population, 52.9% exhibited estrogen receptor (ER) positivity, and 47.1% demonstrated PR positivity. Among the patients, 31.4% tested positive for Her2/neu with a 3+ score, while 14% received equivocal

Table 1: Sociodemographic distribution of breast cancer patients

Characteristics (*n*=121)	Frequency (%)
Age (years)	
20–40	31 (25.6)
41–60	**70 (57.87)**
61–80	20 (16.53)
Education	
Illiterate	**84 (69.4)**
Primary/secondary school	36 (29.8)
Graduate and above	1 (0.8)
Occupation	
Student	1 (0.8)
Homemaker/unemployed	**107 (88.4)**
Self-employed/business	3 (2.5)
Professional/desk job	3 (2.5)
Laborer	5 (4.1)
Retired	2 (1.7)
Religion	
Hindu	77 (63.6)
Muslim	42 (34.7)
Others	2 (1.6)
Setup	
Rural	**85 (70.2)**
Urban	36 (29.8)
Marital status	
Unmarried	2 (1.7)
Married	95 (78.5)
Widowed	24 (19.8)
Family history of cancer	
No family history of cancer	**104 (86)**
1st degree relatives with cancer history	17 (14)
History of addiction	
Addictions (quid, tobacco, pan, alcohol, and bidi)	19 (15.7)
No addiction	**104 (85.9)**

Bold lettering denotes highest percentage. *n*: Number of patients

results and were subsequently subjected to the FISH test. In addition, 54.5% of patients demonstrated a 1+ score, indicated as Her2/neu negative. The hormonal status analysis revealed that the majority of patients were postmenopausal (57.9%) and experienced early menarche at the age of 12 years or younger (72.7%). The right breast was the most common site of cancer occurrence (52.1%), and the majority of patients underwent MRM (88.4%) [Table 2]. Surprisingly, a significant proportion of patients had no history of addiction (85.9%). The most prevalent initial symptoms reported by patients were palpable lump (94.2%), mastalgia (22.3%), swelling (8.2), etc. [Figure 2].

Adverse events and quality of life

Majority of patients experienced Grade 1–2 toxicity such as alopecia (79.33%), anemia (14.1%), nausea (14%), vomiting (10.7%), constipation (10.7%), mucositis (7.4%), etc., while a few patients encountered Grade 3–4 adverse effects, such as anemia (1.6%), diarrhea (1.6%), and constipation (1.6%). Notably, almost all patients experienced hair loss (alopecia) as a side effect of the chemotherapy. Scalp cooling was done to reduce alopecia as per the clinician discretion. Adequate premedication with dexamethasone, 5-HT_3 antagonist, NK1 receptor blockers, hydration was used to lessen the toxicity (date not shown).

FACT-B questionnaire implies significant improvement ($P < 0.05$ both within and between the groups) in QoL with chemotherapy arms (ACT/NACT) at the 12th month across all four domains among spiritual ($P = 0.004$; $P = 0.037$) social ($P < 0.0001$, $P < 0.0001$), emotional ($P = 0.015$, $P = 0.007$), functional ($P = 0.012$, $P = 0.004$), except physical well-being ($P = 0.937$, $P = 0.968$), and additional concern ($P = 0.206$, $P = 0.249$) [Table 3]. Overall mean score among the domains demonstrated moderate to good QoL at the end of the study [Figure 3].

Survival outcome

Among the entire study cohort, 19 patients (15.7%) were died, while 102 patients (84.3%) were censored. KM survival analysis after 48 months revealed that the patient population did not achieve any median survival values. The

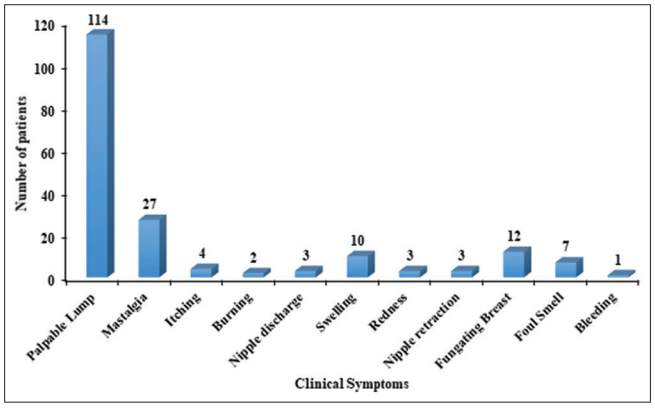

Figure 2: Baseline symptoms reported by the breast cancer patients

Table 2: Clinicopathological and gynecological characteristics of breast cancer patients

Characteristics ($n=121$)	Frequency (%)
AJCC stage	
Stage I	11 (9.1)
Stage II	**64 (52.89)**
Stage III	46 (38.01)
Grade	
Grade I	6 (5)
Grade II	**102 (84.3)**
Grade III	13 (10.7)
Size of tumor mass (pretreatment)	
T1 (≤2 cm)	3 (2.48)
T2 (>2–≤5 cm)	**51 (42.15)**
T3 (>5 cm)	27 (22.31)
T4 (direct extension to the chest wall or skin)	40 (33.06)
TX	1 (0.8)
Regional lymph node metastasis	
N0	17 (14)
N1	**85 (70.2)**
N2	16 (13.2)
N3	3 (2.5)
Lymph node status	
Positive	**107 (88.4)**
Negative	14 (11.6)
Site of cancer	
Left	58 (47.9)
Right	63 (52.1)
Tumor histology	
IDC	**110 (90.9)**
ILC	4 (3.3)
DCIS	6 (5)
Mucinous carcinoma	1 (0.8)
ECOG score	
Fully active	20 (16.5)
Restricted in physically strenuous activity	101 (83.5)
Treatment modality	
Surgery + radiotherapy + chemotherapy	18 (14.86)
Chemotherapy	2 (1.66)
Chemotherapy + surgery	44 (36.36)
Surgery + chemotherapy	54 (44.62)
Surgery + chemotherapy + radiotherapy + hormone therapy	3 (2.5)
Surgery	
BCS	1 (0.8)
MRM	**107 (88.4)**
Toilet mastectomy	8 (6.5)
Palliative mastectomy	3 (2.5)
No surgery	2 (1.6)
Treatment regimen	
FEC	33 (27.27)
TAC	50 (41.32)
AC-T	38 (31.40)
Chemotherapy	
Adjuvant	**89 (73.6)**
Neoadjuvant	32 (26.4)

Contd...

Table 2: Contd...

Characteristics (*n*=121)	Frequency (%)
Clinical response (*n*=32) (NRs include, progressive disease, stable disease, and incomplete response)¥	
CRs	4 (12.5)
PRs	11 (34.37)
NRs	17 (53.13)
Gynecological history	
Hormonal status	
ER*	
Positive	**64 (52.9)**
Negative	57 (47.1)
PR	
Positive	57 (47.1)
Negative	**64 (52.9)**
Her2/neu¤	
Positive (score 3+)	38 (31.4)
Equivocal (score 2+)	17 (14)
Negative (score 1+)	**66 (54.5)**
Menopausal status	
Premenopausal	51 (42.1)
Postmenopausal	70 (57.9)
Age at menarche	
Never had menses	3 (2.5)
≤12	**88 (72.7)**
>12	29 (24)
Unknown	4 (3.3)
Age at menopause	
<45	44 (36.4)
≥45	26 (21.5)
Not attained	51 (42.1)
Breastfeeding history	
Well	**99 (81.8)**
Moderate	8 (6.6)
Poor	8 (6.6)
Never	6 (5)

n= Number of Patients; Bold lettering denotes highest percentage; AJCC: American Joint Committee on Cancer, IDC: Infiltrating Ductal Carcinoma, ILC: Infiltrating Lobular Carcinoma, DCIS: Ductal Carcinoma In-Situ, ECOG: Eastern Cooperative Oncology Group, BCS: Breast Conserving Surgery, MRM: Modified Radical Mastectomy, FEC: 5fluorouracil + Epirubicin + Cyclophosphamide, TAC: Docetaxel + Doxorubicin + Cyclophosphamide, AC-T: Doxorubicin + Cyclophosphamide followed by Taxane, ¥ Objective Response Rate (ORR): 46.87%, CRs: Complete Responders, PRs: Partial Responders, NRs: Non-Responders, ER-Estrogen receptor, PgR-Progesterone Receptor, Her2/neu: Human Epidermal Growth Factor Receptor-2, *Low ER positive i.e. >1% stained cells considered as positive, ¤Her2/neu score 3+ is considered as positive, score 2+ on IHC considered as equivocal and recommended for FISH test, 1+ score considered as Her2/neu negative, IHC: Immunohistochemistry

Log-rank (Mantel-Cox) test indicated no significant differences in PFS, OS, or DFS patterns among the three regimens [Figure 4]. For PFS, KM analysis showed that the estimated mean (± SE.) survival times were similar across regimens: FEC (43.806 ± 1.934 months), TAC (43.986 ± 1.523 months), and AC-T (43.427 ± 2.152 months) (P = 0.830). In contrast, PFS for the NACT group significantly correlated with survival (P < 0.0001), with a median PFS of 40 ± 6.401 months. OS data revealed mean survival times of (40 ± 2.818), (43.5 ± 1.516) and (43.6 ± 2.077) months for FEC, TAC, and AC-T regimen, respectively. Similarly, KM analysis for DFS showed mean survival times of 37 ± 3.579 months for FEC, 38 ± 2.010 months for TAC, and 39 ± 2.432 months for AC-T (P = 0.534). Although a significant relationship was found between treatment response and DFS (P < 0.0001). The TAC regimen (hazard ratio [HR] 1.585) and the AC-T regimen (HR 1.077) demonstrated a higher HR (P > 0.05). However, HR for ER expression, hormonal status, and age at menopause exhibited the significant predictive value for poor survival (HR > 1) [Figure 5].

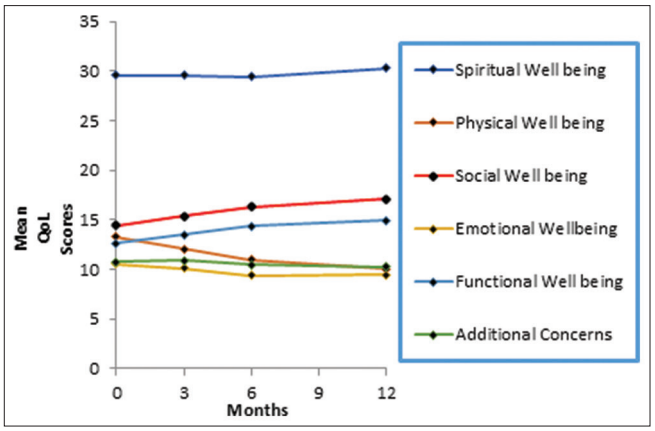

Figure 3: Trends in overall quality of life in breast cancer patients. QoL: Quality of life

Discussion

This observational study aimed to evaluate the efficacy of anthracycline, concomitant anthracycline-taxane, and sequential anthracycline-taxane chemotherapy among Indian BC patients over a 4-year period along with a brief interim report on demographic characteristics, clinical attributes, chemotherapy-induced toxicity, QoL, and survival distribution from a high-volume tertiary cancer center.

Majority of the patients were diagnosed with Stages II–III (52.89%, 38.01%); although it was reported that, 70% BC was diagnosed at early Stage (I–II) in high-income countries while <50% early BC was diagnosed in lower-middle income countries.[22] A notable 69.4% of patients exhibited limited literacy backgrounds, a critical parameter requiring attention in the Indian context. Consequently, the majority of chemotherapy treatments were administered on a daycare basis under clinician supervision, with support from literate individuals who followed postchemotherapy instructions from clinicians. Thus, education played a pivotal role in addressing this factor in Indian population. In this study, 46.87% of patients who underwent NACT achieved an objective response rate (ORR). Among the treatment regimens, AC-T demonstrated the highest ORR (25%), surpassing FEC (9.3%) and TAC (12.5%). This

Table 3: Quality of life domains at various time intervals among adjuvant and neo-adjuvant chemotherapy group

QoL domains	Group	Baseline	3rd month	6th month	12th month	P[ł] (within group)
Spiritual	ACT (*n*=89)	31.20±6.187	31.01±6.017	31.46±6.685	33.08±7.565	**0.004**
	NACT (*n*=32)	26.97±6.018	27.36±4.980	25.82±6.292	25.82±8.361	
*P** (between group)	**0.037**					
Social	ACT (*n*=89)	14.51±3.287	16.04±3.266	17.54±3.709	17.91±3.608	**<0.0001**
	NACT (*n*=32)	14.21±3.507	13.97±3.584	13.76±3.913	15.52±4.251	
*P** (between group)	**<0.0001**					
Emotional	ACT (*n*=89)	9.61±3.306	9.04±3.374	8.03±3.056	8.07±3.631	**0.015**
	NACT (*n*=32)	12.79±4.768	12.42±4.352	12.48±4.976	12.33±4.998	
*P** (between group)	**0.007**					
Functional	ACT (*n*=89)	12.93±4.008	14.50±3.918	15.08±3.557	15.93±3.739	**0.012**
	NACT (*n*=32)	11.73±3.494	11.39±3.297	12.76±3.326	13.00±2.905	
*P** (between group)	**0.004**					
Physical	ACT (*n*=89)	13.35±3.450	12.15±3.019	11.04±2.968	10.14±2.614	0.937
	NACT (*n*=32)	13.09±3.503	11.73±2.886	10.61±2.749	9.79±2.848	
*P** (between group)	0.968					
Additional concern	ACT (*n*=89)	9.19±2.72	9.14±2.73	8.70±2.75	8.22±3.19	0.206
	NACT (*n*=32)	15.21±3.01	15.36±4.00	14.96±4.96	15.27±5.40	
*P** (between group)	0.249					

*Multivariate analysis (Wilk's Lambda), [ł]Greenhouse-Geisser was significant ($P<0.05$). Bold lettering $P<0.05$. All values are expressed as mean±SD. The mean scores of QoL domains were significant within-subject effect and between the groups, except physical and additional concern domain using the multivariate analysis. ACT: Adjuvant chemotherapy, NACT: Adjuvant chemotherapy, QoL: Quality of life, SD: Standard deviation

highlights AC-T's strong efficacy and substantial reduction in tumor burden in the NACT group[23] site of breast is a controversial parameters to discuss, while past studies leaned toward left breast's higher cancer susceptibility,[24] our research suggests a near-equal incidence for left and right site. Although there is no significant difference of risk for the site of cancer occurrence (HR – 0.852; 95% confidence interval [CI], 0.238–3.408; $P = 0.801$).

The results of our study are consistent with previous findings from the NASBP B-30 trial, which compared TAC, AT, and AC-T regimens. The NASBP B-30 trial demonstrated that AC-T had a significant advantage over TAC in terms of DFS (HR = 0.83; $P = 0.006$), but not in OS (HR = 0.86; $P = 0.086$) for both ER-positive and ER-negative tumors.[25] However, when AC-T was compared with AT, a significant improvement in OS (HR = 0.83; $P = 0.034$) was observed. Batra *et al*. (2020)[26] conducted the USON9735 trial in 2020 and reported that four cycles of TC were superior to AC in BC patients, especially those with hormone receptor-positive tumors (71.2%) and Grade III tumors (75%), with a 95.5% OS rate. This interim study also supports this observation, DFS AC-T regimen exhibited a slight advantage over concomitant anthracycline-taxane (TAC) and anthracycline alone (FEC). Mean DFS times were: FEC 37 months, TAC 38 months, and AC-T 39 months with nonsignificant ($P = 0.534$) association. OS was significantly associated with higher tumor grade ($P = 0.008$) and poor chemotherapy response ($P = 0.001$) indicating its prognostic value in predicting survival outcomes. Remarkably, the FEC regimen exhibited the worst OS, whereas patients undergoing either sequential (AC-T) or concomitant (TAC) anthracycline and taxane therapies demonstrated similar OS results. The mean OS was 40 months (95% CI: 34.645–45.691) for FEC, 43.5 months (95% CI: 40.578–46.520) for TAC, and 43.6 months (95% CI: 39.522–44.942) for AC-T. This suggests that while FEC showed the least favorable prognosis, there was no significant distinction in OS between patients receiving anthracycline and taxane therapies in either sequential or concomitant administration. Concluding that the survival distributions were not equal among the different treatment regimen groups. Mackey *et al*., in BCIRG-005 randomized trial studied the differential outcome of AC-T versus TAC as a sequential to concurrent combination in node positive and nonmetastatic BC patients. The result of this trial, revealed that, in terms of OS, TAC (OS 78.0%) arm was not superior over AC-T (OS 79.9%) arm ($P = 0.506$).[27] In a similar study by van Rossum *et al*. in 2020, the comparison between adjuvant, dose-dense anthracycline-taxane (ddAC) and TAC among high risk BC where no significant difference was observed on RFS and OS after 6 cycles with 7 years' median follow-up.[28] DFS analysis in this study showed, suggests that the achievement of disease-free status was notably shorter for NRs compared to the overall population across different treatment regimens. AC-T regimen exhibited a slight advantage over the others. Although a significant relationship was found between treatment response and DFS ($P < 0.0001$), patients who did not respond to chemotherapy (NRs group) experienced a median DFS only at 6 ± 2.449 months (95% CI: 1.199–10.801).

QoL stands as a crucial endpoint in oncology research. Several anthropometric factors such as body mass index, skin fold thickness, and mid-upper arm circumference had significant impact on QoL of BC patient.[29] Evaluating this factor not

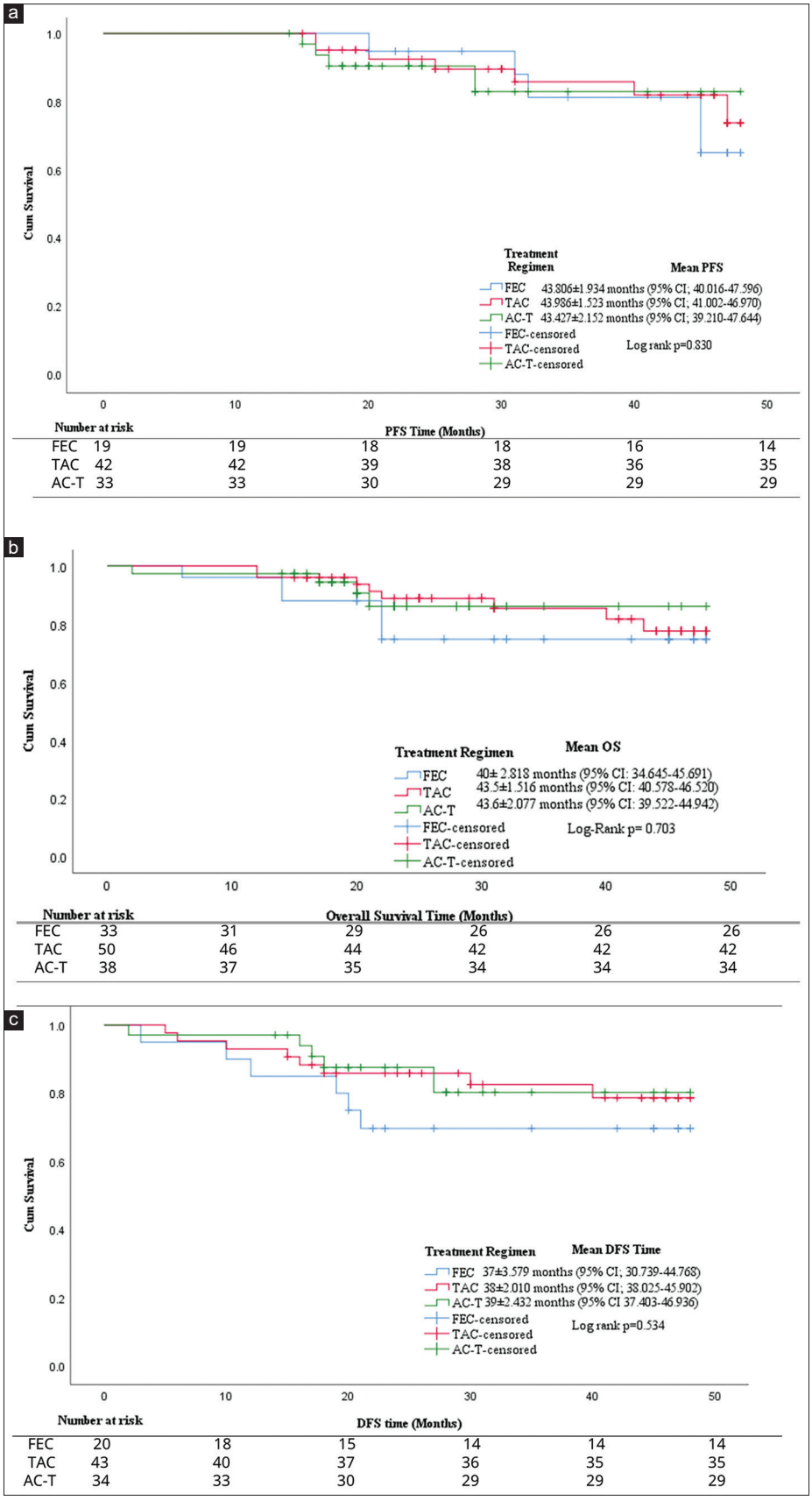

Figure 4: Kaplan–Meier analysis of different treatment regimen (FEC/TAC/AC-T) (a) Progression-Free Survival (PFS) (b) Overall Survival (OS) (c) Disease-Free Survival (DFS). FEC: 5-fluorouracil epirubicin–cyclophosphamide, TAC Docetaxel + Doxorubicin + Cyclophosphamide, AC-T- Doxorubicin + Cyclophosphamide followed by Taxane, CI: Confidence interval

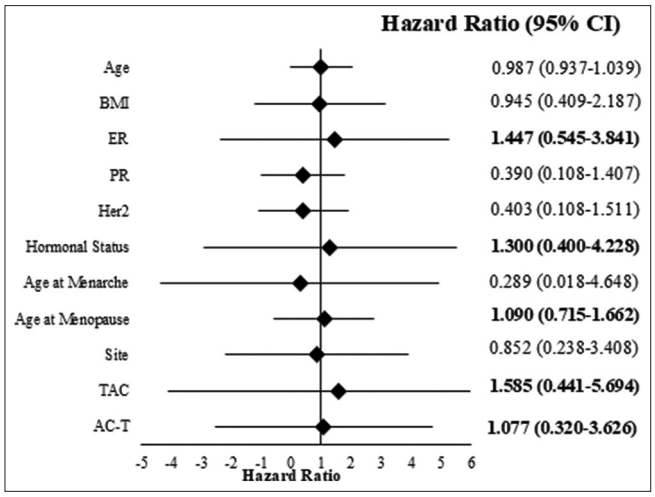

Figure 5: Hazard ratio of several baseline factors. BMI: Body mass index, CI: Confidence interval, ER: Estrogen receptor, PgR: Progesterone Receptor, TAC: Docetaxel + Doxorubicin + Cyclophosphamide, AC-T-Doxorubicin + Cyclophosphamide followed by Taxane

only enhances treatment protocols but also plays a pivotal role in advancing survival rates. Therefore, this study delved into examining how chemotherapy influenced the QoL. Hatam *et al*. reported that TAC group had significantly lower functional status in various domains (physical, role, emotional, cognitive, and social functioning) and global health status compared to the FAC group.[30] However, our observations were different as we noticed QoL declined notably during chemotherapy treatment (6th month), likely due to chemo-toxicity, but improved after chemotherapy completion (12th month), possibly due to enhanced increased family/social support spiritual faith, and reduced symptom distress.

Chemotherapy-induced toxicity played a vital role in shaping patients' QoL outcomes as well as clinical efficacy. Hatam *et al*. (2011)[30] reported, chemotherapy-induced toxicity (such as amenorrhea, anaemia, nail discoloration, febrile neutropenia, hyperpigmentation, neurologic toxicity, and edema), the TAC arm exhibited higher toxicity compared to the FAC arm. Batra *et al*. in 2020 (USON9735 trial), reported that four cycles of TC were superior to AC in BC patients. The majority of these patients had hormone receptor-positive (71.2%), and three-fourths had grade III tumours, with a remarkable OS rate of 95.5%.[26] Ferreira *et al*. (2018) studied a prospective Phase II trial of neoadjuvant treatment of Stage IIB/III TNBC with cyclophosphamide, doxorubicin, and cisplatin; where they reported very few grade ≥3 toxicity such as nausea (16.3%), vomiting (14.0%), and neutropenia (9.3%).[31] In our study, we detected Grade 2–3 toxicity in a limited patient subset, potentially linked to the administration of standard premedication. This may be attributed to the preventive measures provided to the patients, which likely contributed to the enhanced well-being among the survival group.

Potential limitations of the study included the inherent biases associated with the small sample size and the single-center nature of the study. Efforts were made to minimize these limitations through standardized data collection procedures, rigorous statistical analyses, and appropriate adjustment for the confounding variables. Due to less follow-up time, OS may not be matured enough. The findings of this research underscore the need for molecular investigations to gain deeper insights into treatment outcomes. Moreover, future research could also focused on these results by conducting a dedicated cost-effectiveness analysis, particularly addressing the economic considerations for low-to-middle-income countries.

Conclusions

Prognosis of the Indian BC patients treated with anthracycline and taxane either sequentially or concomitantly was better in terms of survival and efficacy with that of only anthracycline receiving patients. Notably, the AC-T regimen does not improve DFS and OS; however, the observed trend toward DFS and OS was improved, suggesting its potential as a preferred option in specific patient cohorts. However, only anthracycline was least favorable among all. This, outcome among Indian heterogeneous population was very similar with western world. DFS was shorter among NR patients, although this interim 4-year study on the clinical outcome in BC patients facilitate evidence-based decision-making to optimize long-term survival with better QoL as the primary end-point of treatment.

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Conflicts of interest

There are no conflicts of interest.

References

1. Ferlay J, Ervik M, Lam F, Laversanne M, Colombet M, Mery L, *et al*. Global Cancer Observatory: Cancer Today. International Agency for Research on Cancer; 2024. Available from: https://gco.iarc.who.int/today. [Last accessed on 2024 Apr 27].
2. Winters S, Martin C, Murphy D, Shokar NK. Breast cancer epidemiology, prevention, and screening. Prog Mol Biol Transl Sci 2017;151:1-32.
3. Nath A, Mitra S, Mistry T, Pal R, Nasare VD. Molecular targets and therapeutics in chemoresistance of triple-negative breast cancer. Med Oncol 2021;39:14.
4. Mistry T, Nath A, Pal R, Ghosh S, Mahata S, Kumar Sahoo P, *et al.* Emerging futuristic targeted therapeutics: A comprising study towards a new era for the management of TNBC. Am J Clin Oncol 2024;47:132-48.
5. Leclerc AF, Jerusalem G, Devos M, Crielaard JM, Maquet D. Multidisciplinary management of breast cancer. Arch Public Health

2016;74:50.
6. Deepa KV, Gadgil A, Löfgren J, Mehare S, Bhandarkar P, Roy N. Is quality of life after mastectomy comparable to that after breast conservation surgery? A 5-year follow up study from Mumbai, India. Qual Life Res 2020;29:683-92.
7. Bonneterre J, Roché H, Kerbrat P, Brémond A, Fumoleau P, Namer M, *et al.* Epirubicin increases long-term survival in adjuvant chemotherapy of patients with poor-prognosis, node-positive, early breast cancer: 10-year follow-up results of the French Adjuvant Study Group 05 randomized trial. J Clin Oncol 2005;23:2686-93.
8. von Minckwitz G, Loibl S. Evolution of adjuvant chemotherapy for breast cancer. Lancet 2015;385:1812-4.
9. Henderson IC, Berry DA, Demetri GD, Cirrincione CT, Goldstein LJ, Martino S, *et al.* Improved outcomes from adding sequential paclitaxel but not from escalating doxorubicin dose in an adjuvant chemotherapy regimen for patients with node-positive primary breast cancer. J Clin Oncol 2003;21:976-83.
10. Martín M, Lluch A, Seguí MA, Ruiz A, Ramos M, Adrover E, *et al.* Toxicity and health-related quality of life in breast cancer patients receiving adjuvant docetaxel, doxorubicin, cyclophosphamide (TAC) or 5-fluorouracil, doxorubicin and cyclophosphamide (FAC): Impact of adding primary prophylactic granulocyte-colony stimulating factor to the TAC regimen. Ann Oncol 2006;17:1205-12.
11. Mehrotra R, Yadav K. Breast cancer in India: Present scenario and the challenges ahead. World J Clin Oncol 2022;13:209-18. doi: 10.5306/wjco.v13.i3.209.
12. Viral P, Pavithran K, Beena K, Shaji A, Vijaykumar DK. Ten-year survival outcome of breast cancer patients in India. J Carcinog 2021;20:1.
13. Bhattacharyya GS, Doval DC, Desai CJ, Chaturvedi H, Sharma S, Somashekhar SP. Overview of breast cancer and implications of overtreatment of early-stage breast cancer: An Indian perspective. JCO Glob Oncol 2020;6:789-98.
14. Coombes RC, Bliss JM, Wils J, Morvan F, Espié M, Amadori D, *et al.* Adjuvant cyclophosphamide, methotrexate, and fluorouracil versus fluorouracil, epirubicin, and cyclophosphamide chemotherapy in premenopausal women with axillary node-positive operable breast cancer: Results of a randomized trial. The International Collaborative Cancer Group. J Clin Oncol 1996;14:35-45.
15. Mamounas EP, Bryant J, Lembersky B, Fehrenbacher L, Sedlacek SM, Fisher B, *et al.* Paclitaxel after doxorubicin plus cyclophosphamide as adjuvant chemotherapy for node-positive breast cancer: Results from NSABP B-28. J Clin Oncol 2005;23:3686-96.
16. Provincial Health Services Authority. BC Cancer; 2022. Available from: https://www.bccancer.bc.ca/. [Last accessed on 2023 Jan 02].
17. Davies C, Pan H, Godwin J, Gray R, Arriagada R, Raina V, *et al.* Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. Lancet 2013;381:805-16.
18. Cardoso F, Paluch-Shimon S, Senkus E, Curigliano G, Aapro MS, André F, *et al.* 5th ESO-ESMO international consensus guidelines for advanced breast cancer (ABC 5). Ann Oncol 2020;31:1623-49.
19. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, *et al.* New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228-47.
20. Dueck AC, Mendoza TR, Mitchell SA, Reeve BB, Castro KM, Rogak LJ, *et al.* Validity and reliability of the US National Cancer Institute's patient-reported outcomes version of the common terminology criteria for adverse events (PRO-CTCAE). JAMA Oncol 2015;1:1051-9.
21. Brady MJ, Cella DF, Mo F, Bonomi AE, Tulsky DS, Lloyd SR, *et al.* Reliability and validity of the functional assessment of cancer therapy-breast quality-of-life instrument. J Clin Oncol 1997;15:974-86.
22. Anderson BO, Ilbawi AM, Fidarova E, Weiderpass E, Stevens L, Abdel-Wahab M, *et al.* The global breast cancer initiative: A strategic collaboration to strengthen health care for non-communicable diseases. Lancet Oncol 2021;22:578-81.
23. Matikas A, Möbus V, Greil R, Andersson A, Steger GG, Untch M, *et al.* Tailored dose-dense versus standard adjuvant chemotherapy for high-risk early breast cancer: End-of-study results of the randomized PANTHER trial. J Clin Oncol 2024;42:3077-82.
24. Amer MH. Genetic factors and breast cancer laterality. Cancer Manag Res 2014;6:191-203.
25. Swain S, Jeong J, Geyer C, Costantino J, Pajon E, Fehrenbacher L, *et al*. NSABP B-30: Definitive analysis of patient outcome from a randomized trial evaluating different schedules and combinations of adjuvant therapy containing doxorubicin, docetaxel and cyclophosphamide in women with operable, node-positive breast cancer. Cancer Res 2009;69 Suppl 2:75. Available from: https://aacrjournals.org/cancerres/article/69/2_Supplement/75/552634/NSABP-B-30-definitive-analysis-of-patient-outcome. [Last accessed on 2023 Apr 24].
26. Batra A, Hannouf MB, Alsafar N, Lupichuk S. Four cycles of docetaxel and cyclophosphamide as adjuvant chemotherapy in node negative breast cancer: A real-world study. Breast 2020;54:1-7.
27. Mackey JR, Pieńkowski T, Crown J, Sadeghi S, Martin M, Chan A, *et al.* Long-term outcomes after adjuvant treatment of sequential versus combination docetaxel with doxorubicin and cyclophosphamide in node-positive breast cancer: BCIRG-005 randomized trial. Ann Oncol 2016;27:1041-7.
28. van Rossum AG, Kok M, van Werkhoven E, Opdam M, Mandjes IA, van Leeuwen-Stok AE, *et al.* Adjuvant dose-dense doxorubicin-cyclophosphamide versus docetaxel-doxorubicin-cyclophosphamide for high-risk breast cancer: First results of the randomised MATADOR trial (BOOG 2004-04). Eur J Cancer 2018;102:40-8.
29. Mistry T, Pal R, Ghosh S, Choudhury T, Mandal S, Nath P, *et al.* Impact of low BMI and nutritional status on quality of life and disease outcome in breast cancer patients: Insights from a tertiary cancer center in India. Nutr Cancer 2024;76:596-607.
30. Hatam N, Ahmadloo N, Ahmad Kia Daliri A, Bastani P, Askarian M. Quality of life and toxicity in breast cancer patients using adjuvant TAC (docetaxel, doxorubicin, cyclophosphamide), in comparison with FAC (doxorubicin, cyclophosphamide, 5-fluorouracil). Arch Gynecol Obstet 2011;284:215-20.
31. Ferreira AR, Metzger-Filho O, Sarmento RM, Bines J. Neoadjuvant treatment of stage IIB/III triple negative breast cancer with cyclophosphamide, doxorubicin, and cisplatin (CAP regimen): A single arm, single center phase II study (GBECAM 2008/02). Front Oncol 2018;7:329.

Emerging Futuristic Targeted Therapeutics

A Comprising Study Towards a New Era for the Management of TNBC

Tanuma Mistry, MSc,† Arijit Nath, MTech,‡ Ranita Pal, MSc,* Sushmita Ghosh, MSc,* Sutapa Mahata, PhD,* Pranab Kumar Sahoo, MPharm,* Sinjini Sarkar, PhD,* Trisha Choudhury, MSc,* Partha Nath, MBBS,§ Neyaz Alam, MS,‖ and Vilas D. Nasare, PhD**

Abstract: Triple-negative breast cancer is characterized by high lethality attributed to factors such as chemoresistance, transcriptomic, and genomic heterogeneity, leading to a poor prognosis and limiting available targeted treatment options. While the identification of molecular targets remains pivotal for therapy involving chemo drugs, the current challenge lies in the poor response rates, low survival rates, and frequent relapses. Despite various clinical investigations exploring molecular targeted therapies in conjunction with conventional chemo treatment, the outcomes have been less than optimal. The critical need for more effective therapies underscores the urgency to discover potent novel treatments, including molecular and immune targets, as well as emerging strategies. This review provides a comprehensive analysis of conventional treatment approaches and explores emerging molecular and immune-targeted therapeutics, elucidating their mechanisms to address the existing obstacles for a more effective management of triple-negative breast cancer.

Key Words: triple-negative breast cancer (TNBC), chemoresistance, genomic heterogeneity, poor prognosis, molecular targets, potent novel treatments

(*Am J Clin Oncol* 2024;47:132–148)

Breast cancer (BC) is the most common ailment affecting women worldwide and cumulates to about 11.7% of all feasible cancer cases summing up to about 2.3 million new cases as recorded in 2020.[1] Among all subtypes, triple-negative breast cancer (TNBC) is the most aggressive with heightened chemoresistance and lowered prognosis. TNBC is characterized by immunohistochemically negative expression of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2/neu).[2] High recurrence rates, metastatic nature, and complex molecular machinery with superior chemoresistance make TNBC very hard to target, challenging treatment options that reduce therapeutic prognosis. Standard treatments including neoadjuvant chemotherapy–like taxane and anthracycline-based procedures along with post–neoadjuvant chemotherapy are only effective to some extent.[3–5] Frequency of the response rates declines to worsen the prognosis and therapeutic approach, thereby affirming the constant ineffectivity of present therapies due to heightened chemoresistance.[5] Various clinical investigations reveal a wide range of potential new approaches, targets, and therapies in TNBC to render therapeutic failure. Several approaches have surfaced as a conventional strategy including poly (ADP-ribose) polymerase (PARP) inhibition, receptor tyrosine kinase (RTK), AKT pathway targeting, etc. to reduce the previous therapeutic failure and chemoresistance. However, fluent paucity in TNBC therapeutics forces the need for the identification of novel molecular targets. Therefore, different studies are ongoing to focus on a few newly emerging therapies by targeting reactive oxygen species (ROS), RNA, and immune molecules along with their mode of delivery (clustered regularly interspaced short palindromic repeats [CRISPR] targeting) to shortcomings of the previous therapeutic failure and bring plausible rendition to TNBC therapy. This review highlights the novel targets and therapeutic approaches involving them to overcome obstacles posed for developing a target-specific treatment strategy that will enhance the overall TNBC patient survival.

From the Departments of *Pathology and Cancer Screening; §Medical Oncology; ‖Surgical Oncology, Chittaranjan National Cancer Institute; †Department of Life Science and Biotechnology, Jadavpur University, Kolkata, West Bengal; and ‡Kalinga Institute of Industrial Technology (KIIT), Deemed to be University, School of Biotechnology, Bhubaneswar, Odisha, India.

T.M., A.N., and R.P. share equal authorship.

The authors declare no conflicts of interest.

Correspondence: Vilas D. Nasare, PhD, Department of Pathology and Cancer Screening, Chittaranjan National Cancer Institute, 37, S.P. Mukherjee Road, Kolkata 700026, West Bengal, India. E-mail: vilas.dr@gmail.com.

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CONVENTIONAL MOLECULAR TARGETS

TNBC forms a subtype of BC that is heterogenous and presents with complex molecular machinery that expresses superior chemoresistance making TNBC very hard to target and challenging treatment options that reduce therapeutic options.[6] Conventional chemotherapy (anthracycline and taxane in mono and combination and dose-dense therapy) forms the basic effective mainstay of all therapeutic approach, which provides somewhat sensitive outcomes in few cases, though high prevalent chemoresistance, fluctuated pathologic complete response rates with lower survival rates, and hindered outcomes are common in most cases.[5] The only possible way to bring suitable therapy in this condition is to target the TNBC resistance pathways and mechanisms that hinder suitable therapies. Exploiting such signal cascades and possible resistance build-up mechanisms through target identification and target-based therapies have shown results and hence several clinical trials are being conducted to make their possible modes of therapy in TNBC.[7] One such emerging therapeutic is targeting RTK receptors that include epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor, and

fibroblast growth factor receptor.[8] RTKs modulate important factors like TNBC proliferation, survival metastases, and angiogenesis, and several inhibitors such as cetuximab against EGFR, bevacizumab against vascular endothelial growth factor, and PD173074 against fibroblast growth factor receptor have proven effective and progressing towards clinical trials.[9–11] Another proven emerging therapy is PARP inhibitors that target PARP1-based single-stranded DNA repair that leads to double-stranded DNA breakage. The HR pathway through functional BRCA1/2 repairs such defects.[12] PARP inhibitors in mono and combination with chemo drugs has proven effective in TNBC (both BRCA-mutated and wild-type) through united loss of HR, PARP-mediated repair, and several inhibitors like olaparib, talazoparib, and rucaparib are efficacious and under clinical trials.[13-15] In addition, targeting non-RTKs as PI3K-AKT-mTOR (PAM) pathway, SRC oncoprotein, and MEK (a component of mitogen-activated protein specific kinase signaling pathway) have proven effective against TNBC. Targeting TNBC through epigenetic modes and additionally through immune-based targets have provided results and are under clinical trial.[7] Table 1 shows all the emergent and approved molecular targets and their related therapies.

Such mechanisms though efficacious to some point face resistance and have provided unexpectedly low results in the long term. The lack of predictive biomarkers and inefficient patient group selection poses a hindrance to these therapies.[7] In addition, monotherapies including such therapies are not completely effective, though in combination they are efficacious but present with low therapeutic value. Moreover, the presence of an active cancer stem cell (CSC) niche, tumor microenvironment (TME) modalities, and the presence of several other cytokines, chemokines, leptin, and fatty acid also pose a therapeutic blockade in this scenario.[49,50] Last, the mode of delivery of several drugs, inhibitors, and therapeutic molecules served as a shortcoming that led to low therapeutic efficacy and chemoresistance enhancement.[5] Chemotherapy along with radiation and excision-based approaches (presently applicable) are only tumor site-specific giving rise to the present dominant chemoresistance and targeted-therapeutic failure. The paucity of TNBC therapeutics forces the need for the identification of novel molecular targets along with the devising of additional innovative therapies that will target tumorigenesis and bring plausible rendition to TNBC therapy.[5,51-54] Therefore, a few novel approaches have been listed in the following sections that surpass a few of the previous hindrances allowing possible therapy. Some of these novel approaches have undergone several clinical trials, while some are in a preclinical state. Further investigation is required to make them clinically acceptable but are not established for proper clinical application (Fig. 1).

EMERGING THERAPIES

CRISPR-based Targeting

CRISPR and their associated Cas proteins (CRISPR/Cas) seem to be the best-suited cancer therapy due to their specificity, efficiency, and definite results against tumorigenic gene knockout.[55] Apart from gene knockout, CRISPR/Cas can be used as an early detection technique using polymerase chain reaction and circulating tumor DNA as a target, whereby particular genes containing nontarget DNA are removed via Cas9 and cpf1 action and recognized at different PAM sequences breaking TNBC heterogeneity.[56,57] A suitable amount of research shows that protein upregulation and downregulation a enter the causal factors behind TNBC progression and chemoresistance, whereby protein upregulation can be easily targeted with CRISPR-mediated knockdown for a better therapeutic approach.[58] Upregulated protein classes may constitute the EGFR family, heat shock proteins, transcription/translation controlling proteins, and metabolic regulators.[59] CRISPR-Cas gene editing becomes specifically important in this scenario, as it is useful for target-specific delivery, accurate precision, and downregulation of suitable target proteins.

CRISPR-Cas knockout of UBR5 E3-ubiquitin ligase (an important regulator of metastatic growth and proliferation) showed reduced growth and proliferation in TNBC.[60,61] In addition, targets like transforming growth factor β (TGFβ) (induces epithelial-mesenchymal transition and mesenchymal-epithelial transition [EMT/MET] and superior proliferation) or TOP2A (builds resistance to anthracyclines), and PARP3 (proliferator of TNBC) can be aimed by CRISPR-Cas-mediated knockdown that can regulate growth and proliferation in TNBC.[58] Furthermore, mTORC2 (the main driver in TNBC development) can be targeted to suppress TNBC. mTORC2 regulated by RICTOR overexpress AKT and activates RAC1 through the Rac-GEF Tiam 1 that affects the proliferation, metastases, and chemotaxis in TNBC.[58] mTORC2 also forms a basic link between glucose metabolization and EGFR-tyrosine kinase inhibitor resistance.[5] RICTOR resists apoptosis via AKT activation (s473 phosphorylation) and its deletion has led to lapatinib sensitivity and apoptosis in HER2-improved BC, thereby asserting CRISPR-mediated gene editing as a therapeutic.[58,62-64] Table 2 shows other important CRISPR targets in TNBC therapeutics.

CRISPR-Cas-mediated target knockout can be used against chemoresistance with success. Drug resistance in TNBC can be attributed to several proteins. P-glycoprotein class multidrug resistance protein 1, CDC25A (regulator of AKT and ATR), and oncogene moesin (enhances metastases and drug resistance) can be targeted by this system.[58,68,69] In addition, downregulation of MALAT1 in several investigations has reduced cancer progression and in the BT-549 TNBC model, CRISPR/Cas9-mediated promoter targeting enhanced paclitaxel and doxorubicin sensitivity[70] CRISPR/Cas-mediated knockout of specific proteins has also yielded success in TNBC therapeutics as observed in several cell lines. TGFβ-mediated growth through smad2/3 is affluent in TNBC and TGFβ-Smad3-TMEPAI axis poses a serious threat that can be targeted with CRISPR/Cas9 system as a possible therapeutic.[71] Similarly, ceramide kinase is a novel target in TNBC progression[51] and CRISPR/Cas-mediated ceramide kinase inhibition will lead to its suppression. Other drivers like FOXM1 or tuftelin (TUFT1) can also be targeted with this system to provide a plausible therapy against TNBC, though further suitable CRISPR technique development is required[51] CRISPR-based gene editing, though highly efficacious, faces problems in its delivery to the target cell and consecutive gene. Guo et al[67] encapsulated CRISPR-gRNA conjugated plasmids in a deformable tumor-targeting nanolipogel constituting fat molecules and hydrogels that could penetrate the TME efficiently tumor-targeting nanolipoge composes of both zwitterionic and anionic lipids collectively termed "non-catanionic" and encapsulates CRISPR plasmids without any electrostatic forces that negated cationic toxicity and enhanced specificity through antibody–mediated targeting. Apart from the nanodelivery of the CRISPR-Cas system, phages can also be used in the delivery of CRISPR-gRNA-fused plasmids specifically to the tumor cells in the case of lung adenocarcinoma investigated by Zhou et al[72] and can form a similar novel tactic in TNBC cases. In conclusion, the CRISPR system provides a unique way of specific targeting of any genes inducing knockout

TABLE 1. Conventional Molecular Targets of Triple-negative Breast Cancer

Sl. no	Targeted molecule	Drug	Mode of mechanism	Monotherapy/ combinational therapy	Status of clinical trials	Phase	Route of drug administration	References
1	Akt	Uprosertib (GSK2141795)	Akt inhibitor	Combination with trametinib	NCT01964924 Completed	Phase II	Orally	16
		Ipatasertib (GDC-0068)		Combination with paclitaxel	NCT02162719 Completed	Phase II	Orally	17
		Capivasertib (AZD5363)		Combination with paclitaxel	NCT02423603 Active, not recruiting	Phase II	Orally	18
2	PI3K	Copanlisib (Aliqopa)	PI3K inhibitor	Combination with eribulin mesylate	NCT04345913 (Recruiting)	Phase I/II	Intravenous infusion	19
		Alpelisib (Piqray)		Combination with fulvestrant	NCT05038735 (Recruiting)	Phase III	Orally	20
3	mTOR	Everolimus (Afinitor)	mTOR inhibitor	Combination with carboplatin	NCT02531932 (Recruiting)	Phase II	Orally	21
		Gedatolisib (PF-05212384)		Combination with either cisplatin or docetaxel or with other antitumor agents	NCT01920061 Completed	Phase I	Intravenous infusion	22
4	Androgen	Enzalutamide (Xtandi)	Nonsteroidal androgen receptor inhibitor (androgen antagonist)	Enzalutamide in combination with taselisib	NCT02457910 (Active, not recruiting)	Phase I/II	Orally	23
		Bicalutamide (Casodex®)		Monotherapy	NCT03055312 (Terminated)	Phase III	Orally	24
5	CYP17	Seviteronel (VT-464/ INO-464)	It is a nonsteroidal antiandrogen, an inhibitor of the enzyme CYP17A1	Monotherapy	NCT02580448 (Completed)	Phase I/II	Orally	25
6	PD-1	Pembrolizumab (Keytruda®)	Pembrolizumab is a PD-1 receptor blocker. It prevents the binding and activation of PD-L1 and PD-L2, which causes the activation of T-cell-mediated immune responses against tumor cells	Combination with chemotherapy	NCT03036488 Approved by FDA on July 26, 2021 Pembrolizumab is a highly selective humanized monoclonal IgG4 antibody	Phase III	Intravenous infusion	26,27
		Camrelizumab	An engineered anti-programmed death-ligand 1 (PD-1) antibody	Combination with chemotherapy	NCT04613674 (Recruiting)	Phase III	Intravenous infusion	28
7	PD-L1	Atezolizumab (MPDL3280A)	Atezolizumab is a humanized monoclonal antibody immune checkpoint inhibitor that selectively binds to PD-L1 to stop the interaction between PD-1 and B7	Combination with paclitaxel	NCT02425891 (Completed)	Phase III	Intravenous infusion	29
8	PARP	Talazoparib (Talzenna)	PARP inhibitor	Monotherapy/combination with chemotherapy	NCT01945775 (Completed) Approved by FDA on October 16, 2018	Phase III	Orally	30
		Olaparib (Lynparza®)	PARP inhibitor	Combination with chemotherapy	NCT02000622 (Active)	Phase III	Orally	14
		Rucaparib (Rubraca®)	PARP inhibitor	Combination with chemotherapy cisplatin	NCT01074970 (Completed)	Phase II	Orally	31
		Niraparib (Zejula®)	PARP inhibitor	Combination with chemotherapy	NCT01905592 (Completed; ended due to serious adverse events)	Phase III	Orally	32
		Veliparib	PARP inhibitor	In combination with the chemotherapeutic drug paclitaxel, carboplatin	NCT02163694 (Active, not recruiting)	Phase III	Orally	33

9	EGFR	Cetuximab (Erbitux)	Cetuximab is a monoclonal antibody developed to inhibit the binding of growth factors and the subsequent activation of epidermal growth factor receptor (EGFR)	Monotherapy/combination with chemotherapy	NCT00463788 (Completed)	Phase II	Intravenously infusion	34
10	VEGF	Bevacizumab (Avastin)	Bevacizumab selectively binds with circulating VEGF, thus inhibiting this molecule from binding with its cell surface receptors	Combination with chemotherapy	NCT03577743 (Completed)	Phase II	Intravenous infusion.	35
11	WNT	LGK974 (WNT974)	Upon oral administration, WNT974 binds to and inhibits PORCN in the endoplasmic reticulum (ER), which blocks post-translational acylation of Wnt ligands and inhibits their secretion	Monotherapy or in combination with PDR001	NCT01351103 (Recruiting)	Phase I	Orally	36
12	Trop2	Sacituzumab govitecan (Trodelvy)	Sacituzumab govitecan is an antibody-drug conjugate targeting Trop-2 expressing cells and selectively delivering SN-38, an active metabolite of irinotecan	Monotherapy	NCT02574455 (Completed) Approved by FDA on April 7, 2021	Phase III	Intravenous infusion	37
13	VEGF-TKIs	Cabozantinib (Cabometyx®)	Cabozantinib inhibits the activity vascular endothelial growth factor receptor (VEGFR) thereby leading to reduced tumor angiogenesis, motility and invasiveness	Cabozantinib in combination with atezolizumab	NCT03170960 (Recruiting)	Phase II	Orally	38
14	MEK	Trametinib	MEK inhibitor	Combination with GSK2141795	NCT01964924 (Completed)	Phase II	Orally	39
15	cMET	Cabozantinib (XL184)	cMET inhibitor	Monotherapy	NCT01738438 (Completed)	Phase II	Orally	40
16	HSP90	Ganetespib	HSP90 inhibitor	Monotherapy	NCT01677455 (Completed)	Phase II	Intravenous infusion	41
		Onalespib	HSP90 inhibitor	Combination with paclitaxel	NCT02474173 (Active, not recruiting)	Phase I	Intravenous infusion	42
17	JAK/STAT	Ruxolitinib	JAK inhibitor	Combination with chemotherapy	NCT02876302 (Active, not recruiting)	Phase II	Orally	43
		TTI-101	STAT-3 inhibitor	Monotherapy	NCT03195699 (Recruiting)	Phase I	Orally	44
18	TGFβ	Galunisertib	TGFβ antagonist	Combination with paclitaxel	NCT02672475 (Recruiting)	Phase I	Orally	45
19	Notch	PF-03084014	Notch inhibitor	Combination with paclitaxel	NCT01876251 (Terminated)	Phase I	Orally	46
20	Hedgehog	Vismodegib	Hedgehog inhibitor	In combination with neoadjuvant chemotherapy	NCT02694224 (Unknown)	Phase II	Orally	47
21	Aurora	ENMD-2076	Aurora inhibitor	Monotherapy	NCT01639248 (Completed)	Phase II	Orally	48

FDA indicates Food and Drug Administration; TGF, transforming growth factor.

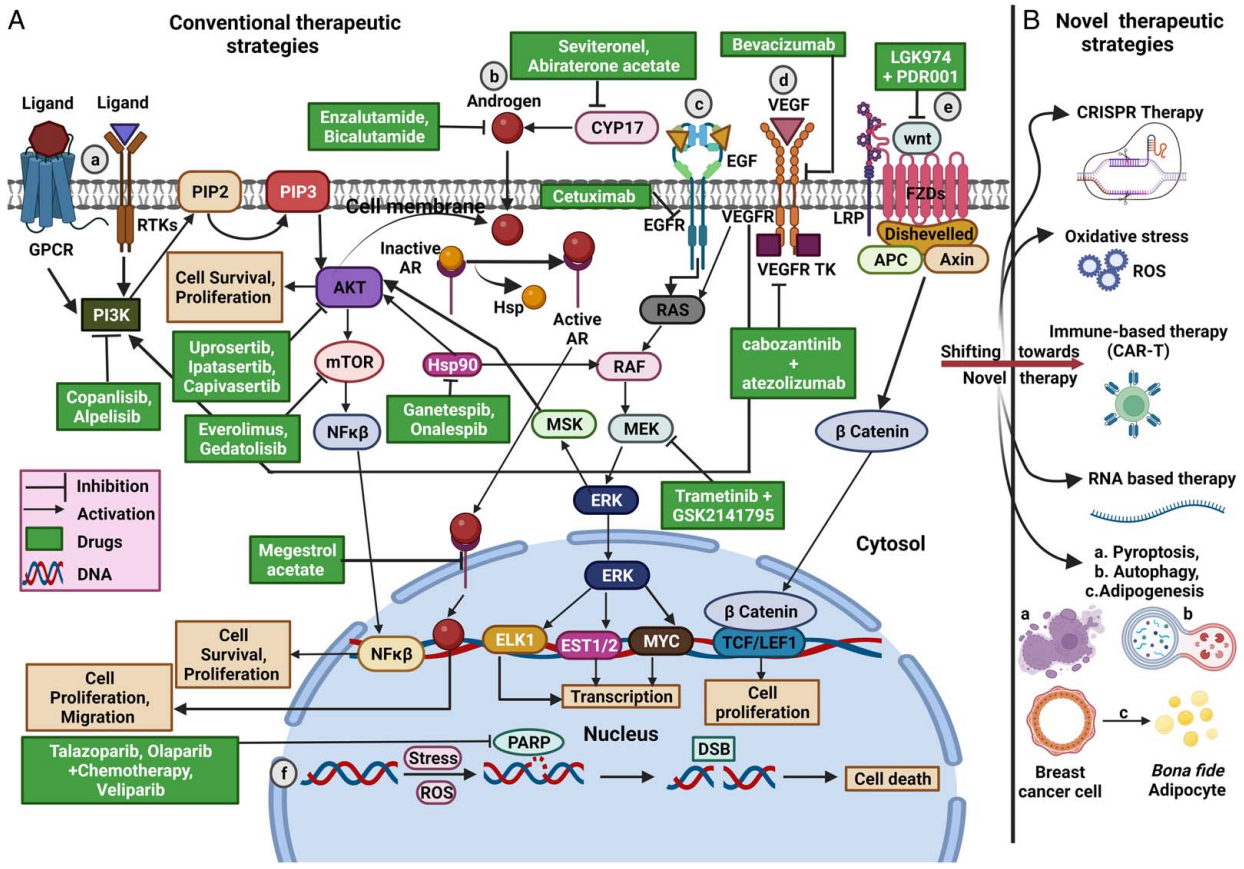

FIGURE 1. Conventional molecular targets of triple-negative breast cancer shifting towards novel therapeutic strategies. A. Conventional molecular targets include, a, Cross-talk between PI3K/Akt/mTOR and G-protein coupled receptors (GPCR) pathway regulated by extracellular molecules (ligands). b, Androgens mediating cell proliferation and migration. c, Mitogen-activated protein specific kinase (MAPK) pathway. d, Vascular endothelial growth factor (VEGF)-mediated angiogenesis. e, WNT/β-catenin pathway regulating tumor proliferation. f, Poly-adenosine diphosphate-ribose polymerase (PARP) inhibitory pathway leads to cell death. B. Novel therapeutic strategies includes CRISPR, ROS, CAR-T, RNA therapy, Pyroptosis, Autophagy. full color online

efficiently. CRISPR-Cas system is affordable, takes preferably less time, and shows off-target effects of gene knockout. Thus, it can be conjugated with nano molecules that will enhance the specificity and efficacy forming a strong suppression system to oppose the ever-increasing TNBC chemoresistance and aggressiveness.

Oxidative Stress Targeting

The oxidative condition in TNBC is diverse and depends on the hypoxic status of the TME, the presence of oxidative stress inducers (nitric oxide, hydrogen peroxide), and several organelles that release and maintain the ROS level and modulates the overall tumorigenic properties.[73] Constant hypoxia induces high hypoxia-related genes and hypoxia-inducible factors that mediates transcription of a large number of genes and protein that upregulates tumor vascularization, extracellular matrix remodeling, and the overall chemoresistance pattern.[74-76] Unbalanced mitochondrial activity in the TME in hypoxic conditions further triggers mitochondrial reactive oxygen species (mtROS) that promote malignancy; with oncogenic signal (NF-κβ, NRF2, Wnt, and EGFR) upregulation accentuated with hydrogen peroxide presence.[77] Other organelles like tumorigenic lysosome, endoplasmic reticulum (ER), and macrophages present in the TME also have roles in ROS upregulation,[78-80] which drives its tumorigenic properties and chemoresistance. Therefore, hypoxic or organelle-produced ROS influences tumor progression and overall chemoresistant properties; targeting such oxidative imbalances will form a suitable mode of therapeutic in TNBC therapy.

Oxidative targeting-based therapies have become popular, varying from inhibition of organelles that produce ROS to silencing several genes which upregulate oxidative stress. In addition, antioxidative defense reduces excess oxidative stress and inhibits ROS-induced molecular progression. Yang et al[81] reported that cystine transporters can be used as a potential biomarker to detect oxidative stress using ^{18}F-5-fluoro aminosuberic acid radiotracer in vivo (xenograft model) and in vitro of BC models. Therefore, the estimation of oxidative stress in TME becomes important for further targeted TNBC therapy and several modes of therapies are under investigation. Techniques like photodynamic therapies are becoming a major component to target mitochondria and their associated ROS. BODIPY-Ir(III) conjugate acts as a light-dependent ROS inducer–based mitochondrial destruction system that was highly effective in TNBC cases, where it congregated inside mitochondrial walls and induced apoptosis after irradiation.[82] In addition, branched-chain ketoacid dehydrogenase kinase (BCKDK) from BCAA catabolic pathway can activate the RAS/RAF/MEK/ERK pathway and modulate mitochondrial activity with tumor proliferation.[58] BCKDK silencing is a powerful technique against oxidative

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TABLE 2. CRISPR Molecular Targets With Their Effects When Silenced in TNBC

Sl. no.	CRISPR targets	Effect in TNBC after silencing	References
1	Cripto-1	TGF signaling pathway receptor that mutates in TNBC and aids in Notch receptor maturation	58,65
2	ctDNA	Particular genes containing nontarget DNA can be removed via Cas9 and cpf1 action and further recognize at different PAM sequences and detection using PCR	58
3	UBR5 E3-ubiquitin ligase	CRISPR-Cas9 knockdown reduced growth and proliferation in TNBC	58
4	TGFβ	Inhibits EMT/MET and superior proliferation	58
5	PARP3	Knockdown by CRISPR-Cas9 prevents migration and proliferation	58
6	RICTOR/mTORC2	CRISPR-Cas9 knockdown inhibits mTORC2 which is regulated by RICTOR and is found to overexpress AKT and activate RAC1 through the Rac-GEF Tiam 1, which further affects the proliferation, metastases, chemotaxis, glucose metabolism and EGFR-TKI	58
7	Multidrug resistance protein 1 (MDR1)	CRISPR-Cas9 targeting prevents regulation of AKT thereby suppressing the growth and proliferation of TNBC	66
8	CDC25A	CRISPR-Cas9 targeting prevents regulation of ATR thereby suppressing growth and metastasis of TNBC	58
9	Moesin	The oncogene is silenced to suppress metastases and drug resistance	58
10	MALAT1	CRISPR/Cas9-mediated promoter targeting enhanced paclitaxel and doxorubicin sensitivity, and reduced cancer progression	58
11	Ceramide kinase (CERK)	CRISPR/Cas9 knockdown may suppress cancer proliferation	51
12	FOXM1	Can be a plausible target	51
13	Tuftelin (TUFT1)	Maybe a possible target	51
14	Lipocalin 2 (Lcn2)	Novel CRISPR nanotherapeutic to effectively suppress TNBC tumor growth	67
15	Programmed cell death 1 (PD-1)	To target cell death machinery to prevent the progression of cancer	58
16	CD19	CarT techniques to prevent growth, proliferation, and metastasis	58
17	TP63	Cas9 prevents migration and proliferation	58
18	TP73	Cas9 prevents growth and proliferation	58
19	TOP2A	Knockdown using CRISPR-Cas9 increases sensitivity to anthracyclines	58
20	Proteasome deprivation of major cellular proteins	The target for cancer therapy/drug resistance progression	58

CRISPR indicates clustered regularly interspaced short palindromic repeats; PCR, polymerase chain reaction; TGF, transforming growth factor; TNBC, triple-negative breast cancer.

stress management and has resulted in lowered mitochondrial ETS and protein expression, ATP production, and downregulated mitochondrial genes that dysregulated its function and upregulated apoptotic pathways.[58] Moreover, BCKDK silencing upregulated sestrin2/Hi95 (SESN2 gene), an oxidative stress recovery protein remodeled BCAA flux and reinstated doxorubicin cytotoxicity.[58] Furthermore, mitochondrial OXPHOS inhibitors like novel IACS-10759 (in combination with palbociclib and cabozantinib separately,[83] IM156 (phase I),[84] and application of Palmitoyl-CoA (Pal-CoA),[85] a mitochondrial permeability transition pore inducer are effective in inhibiting mitochondrial function and induce apoptosis, thereby reducing mtROS. Inhibition of other ROS-inducing-organelles like lysosome, ER, and tumor-associated macrophages are an option for reducing oxidative stress in the TNBC TME. Several microtubule poisons like colchicine, peloruside A, epothilones, and taxanes can act as lysosome inhibitors,[86,87] while colony-stimulating factor 1 receptor forms an important target in tumor-associated macrophages and can be inhibited by PLX397 (colony-stimulating factor 1 receptor antagonist).[88] The hypoxic environment prevalent in the TME also incites XBP1 activation, with further supplementation from ER membrane–based UPR sensor IRE1α kinase.[89,90] XBP1 further accentuates hypoxia-inducible factor 1α and modulates transcription factor MYC and angiogenesis factor VEFGA.[89-91] IRE1α kinase inhibition therefore can act to inhibit ER-mediated stress reverting ER-structure-distension as well as targeting XBP1 that links with hypoxia and overall oxidative stress. Last, overall oxidative stress can be managed with antioxidative defense though antioxidant-based procedures are controversial as they are effective in selective cases. Synthetic antioxidants N-acetyl cysteine along with glutamine and glycine has provided plausible outcomes in TNBC cell lines.[77,92] There are several other antioxidants like curcumin, epigallocatechin gallate, selenium, and hydroxytyrosol along with several synthetic antioxidants that have completed clinical trials and can be used in therapy.[93] Figure 2 represents oxidative-based targeting in TNBC. Therefore, oxidative molecules damage DNA and alter several signaling pathways acting as a morphogen that aids in progression, metastasis, and chemoresistance upregulation and targeting ROS-induced oxidative stress will pose as a brilliant therapeutic against such.

RNA-based Targeting

RNA molecules regulate several gene expressions and functions that are linked with tumor progression, metastasis, immune escape, and drug resistance profile. RNA therapeutics includes RNA and RNA-targeted molecules as a drug to expand the range of treatment therapy in cancer which depends on the specificity of cell proteins and their regulation in cell signaling pathways that induce tumor cell proliferation, motility, and survival.[94] RNA therapeutics are becoming extremely popular that aim at several proteins and their related pathways modulating the "druggable" targets that optimize collective therapy.[95] Different classes of RNA have emerged as therapeutics, including small interference RNA (siRNA), microRNA (miRNA), messenger RNA (mRNA), circular RNA, and piwi RNA.[96,97] Moreover, RNA therapy categories depend on the mechanisms like inhibition of mRNA translation, the agents of RNA interference, the catalytic activity of RNA molecules, and RNAs binding proteins with other molecular ligands[98] (Fig. 3). Multiple mechanisms are responsible for cellular defense mechanisms against pharmacological RNA that induce an immunogenic

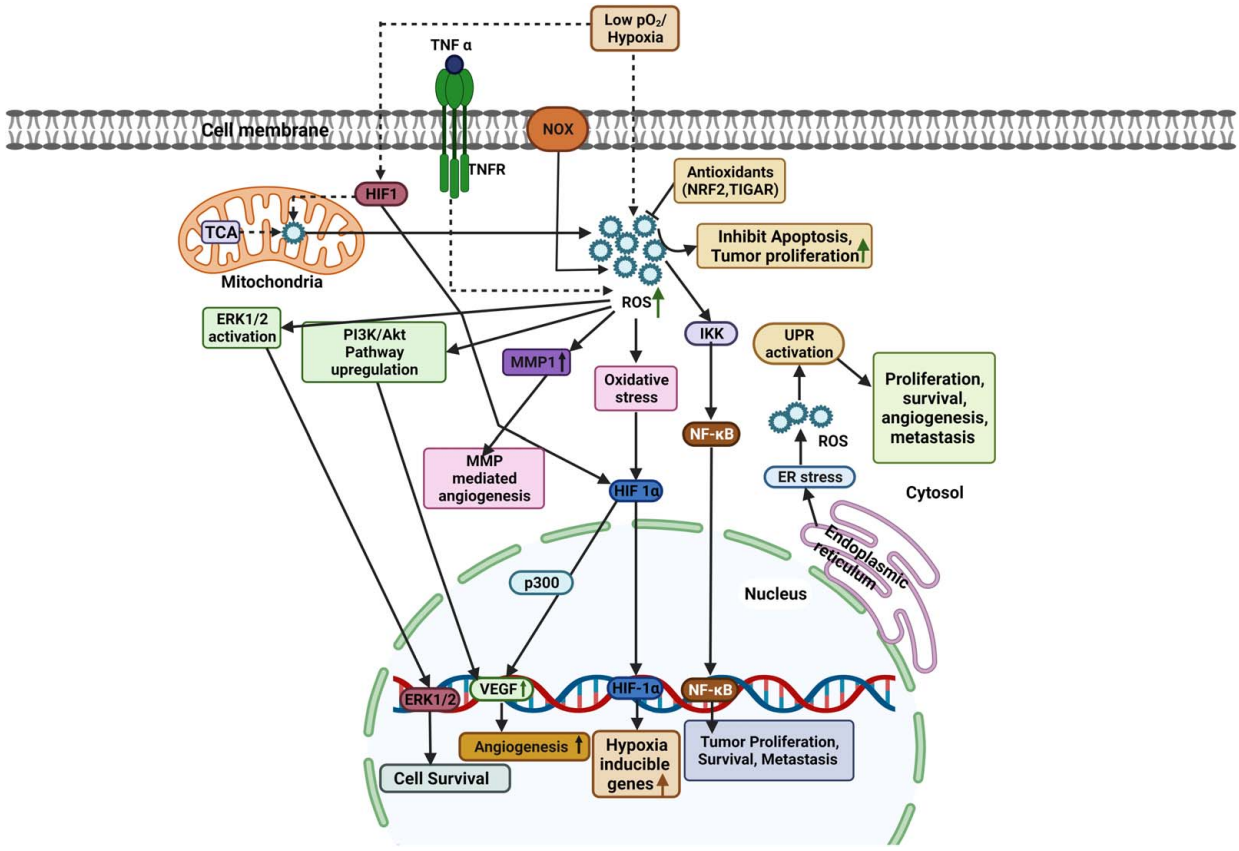

FIGURE 2. Reactive oxygen species (ROS) induced oxidative stress regulating tumor proliferation in triple-negative breast cancer. Tumorigenic hypoxia induces oxidative stress and generates ROS, hypoxia-inducible factors (HIFs) matrix metalloproteinases (MMPs). ROS-HIF-MMP signaling elevates transcription of numerous genes and proteins that mediates tumor vascularization, chemoresistance, triple-negative breast cancer proliferation and angiogenesis via PI3K/Akt, MAPK pathway, NF-κβ, VEGF pathway. Endoplasmic reticulum (ER) stress upregulated unfolded protein response (UPR) along with unbalanced mitochondrial activity in hypoxic condition which combinedly induces cellular ROS generation which promote malignancy. full color online

response or release cytokine. Furthermore, this mode of action relies on the measures and structures of RNA molecules.[99] RNA-based therapeutics prevalent against TNBC mainly involve RNA interference (RNAi) techniques that use gene-silencing methods to target RNA-modulated pathways (siRNA and miRNA) and RNA monotherapy, which delivers RNA-conjugated nanoparticles to target cells.[100] siRNA conjugation with nanoparticles silences the target mRNA, while miRNA-based therapeutics can be approached primarily as an inhibitor against endogenous miRNAs (miRNAendo). Second, they are targeted and degraded as a replacement strategy where artificial miRNAs are engaged to copy miRNAendo-based mRNA degradation.[101] This technique utilizes both inhibition and augmentation functions that enhance the overall gene-silencing efficacy. Targeted RNAi can be used to silence all types of dysfunctional and deregulated mRNA in TNBC that helps in progression, and drug-resistant properties and further can be supplemented through synthetic RNA production specific to each mRNA-protein product.[100] Targeted RNAi is even successful in inhibiting TNBC CSCs and several RNAi-based therapeutics against CSC markers (CD24, CD44, CD133, and ABCG2) are under investigation.[102] In addition, RNA-binding-protein (RBP) inhibition is also a plausible method of RNA-based therapeutics. Furthermore, anti-miRNA-based therapies are becoming popular, and quite recently, Yin et al, in 2019, used a heat/chemical stabilized 3-way junction motif as an RNA aptamer scaffold targeting CD133 (a TNBC CSC marker) and delivered locked nucleic acid sequence targeting miRNA21 that inhibited its action, thereby downregulating TNBC migration with PTEN and PDCD4 (tumor suppressor) upregulation.[102] Although RNA molecules are very promising for the effective treatment of TNBC application is extremely constrained due to their stability, poor cell penetration ability, and cost of production.[103] Despite having various challenges, RNA targeting might be a quite modern therapeutic for the successful treatment of TNBC proliferation, chemoresistance, and aggressiveness.

IMMUNE-BASED TARGETING

Bispecific T-cell Redirecting Engagers (BiTEs)

BiTEs are bispecific antibodies that target 2 different antigens by redirecting immune cells to cancer cells for the delivery of drugs and blocking 2 pathways simultaneously for the tumor.[104] BiTEs are made up of 2 single-chain variable fragments (scFv) with unique specificity. In these, one scFv generally targets CD3 of the T-cell receptor and the other one binds to targeted tumor antigen. These molecules can cross-link with the cancer cells and cytotoxic cells without major histocompatibility complex restriction and act as a bridge to activate T cells.[105] The basic requirement for successful BiTEs therapy is the proper identification of tumor-associated antigens that are

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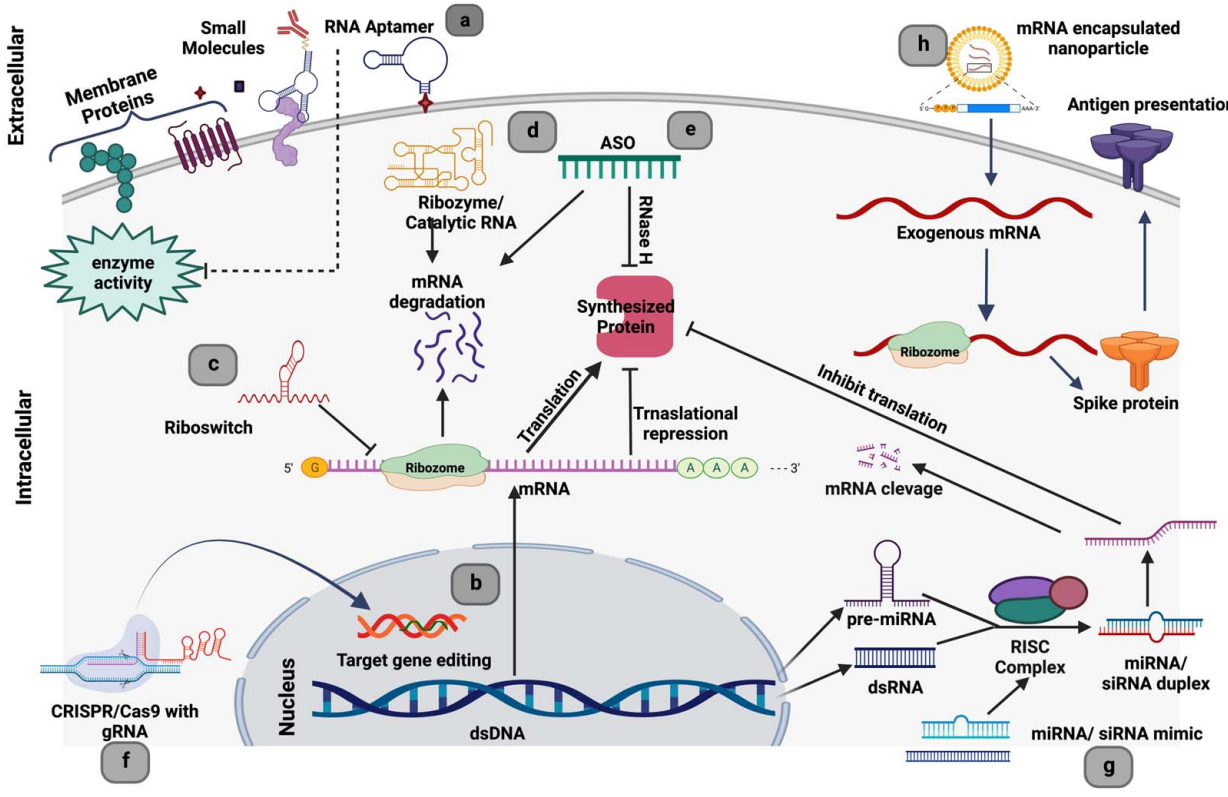

FIGURE 3. RNA molecules and their probable pathways as potential therapeutic targets in triple-negative breast cancer. a, Aptamers bind the intra and extracellular proteins and small molecules to block enzymatic activity via protein-protein interaction, can be a potential targeted drug delivery. b, Genomic transcription of ncRNAs (noncoding RNAs) and mRNAs (messenger RNAs), few translates into protein directly targeted by RNA-based drugs. c, Riboswitch inhibits the mRNA translation (if the ligand is absent). d, Ribozymes hybridize and degrade the target mRNA thus, inhibiting its expression. e, ASOs (antisense oligonucleotides) target intracellular mRNAs through complementary base-pairing responsible for gene. f, gRNA (guide RNA)-mediated CRISPR-Cas9 used to alter or knockout the targeted gene expression. g, miRNA (microRNA)/siRNA (short mimics) inhibit the translation of target mRNAs. h, Exogenous mRNAs are introduced into cells as vaccine/therapeutics to elicit an immune response. CRISPR/Cas indicates clustered regularly interspaced short palindromic repeats and their associated Cas proteins. full color online

expressed on the surface of tumor cells. The BiTE antibody transmitting T cells to kill tumor cells has shown favorable clinical outcomes both in hematological malignancies as well as solid tumors like TNBC.[106] However, many cases show resistance to this therapy. Promising preclinical data on BiTE therapy for TNBC is presently available and many pharmaceutical companies are funding the further development of bispecific antibody treatment for TNBC.[107] Current research has suggested that the loss of antigen along with immunosuppressive factors, precisely inhibition of immune checkpoint molecules, are the main cause of treatment failure. Thus, new approaches for improved BiTE constructs to develop novel BiTEs therapeutics with multiple targets are currently under investigation through a series of preclinical and clinical trials. Thus, a combination of BiTE antibody therapies and other therapeutic approaches may lead to an exciting era of immunotherapy.

Cancer Vaccines (CVs)

CVs involve immunotherapy that upregulates immune cells against cancer-specific antigens that are normally absent in nontumorigenic cells and target them specifically. In this manner, a person can be immunized against cancer-specific antigens and suppress cancer progression, destroy any cancerous cells after chemotherapy and suppress tumorigenic growth using his immunity. CV is mainly efficacious against BC and poses as a suitable approach in TNBC, though further immunogenic studies are required to make it a permanent solution.[108] Table 3 lists all the possible vaccine-based therapies against TNBC.

Immune Checkpoint Inhibitor (ICI)

The complete activation of T cells depends on 2 signals, one is a derivative of cross-talk between major histocompatibility complex present on the surface of APC and T-cell receptor, whereas, the second one is an antigen-independent molecule. The T-cell activation is completely regulated by the costimulatory known as immune checkpoints.[114] These checkpoints aim to reduce the damage to normal tissue to prevent unwanted autoimmunity. Cancer cells have the potential to generate ligands that are capable to bind with coinhibitory receptor molecules. Thus, the blockades of ICIs are capable to raise antitumor response.[115] The emergence of ICI become a therapeutic landscape for some types of cancers. ICI therapy does not directly destroy the cancer cells but induces the power to enhance the endogenous antitumor activity within the immune system of patients.[116] It is one of the most studied strategies of immunotherapy for the treatment of various cancer. Immune inhibitors block the communication of inhibitory signals through the activation of cytotoxic T lymphocytes which induce the antitumor effects.[117] Some ICIs

TABLE 3. List of Possible Vaccine-based Therapies in Triple-negative Breast Cancer

Sl. no.	Type of vaccine	Target	Mode of delivery	References
1	Dendritic cells (DC)	Runt-associated transcription factor 2 (Runx2)	Runx2 lentivirus transfection system	109
2	Anti-idiotypic antibody vaccine	B7-1 (CD80)/IL-12	TMV-based vaccine immunotherapy in combination with anti-CTLA-4 mAb treatment	110
3	Personalized peptide vaccination (PPV)	Proinflammatory cytokine interferon-γ (IFNγ)/HLA-I/HLA-II	Peptides (typically 8-12 amino acids in length) are loaded onto nascent HLA-I molecules based on their suitability to bind to the individual's HLA allotypes and transported to the cell surface for $CD8^+$ T-cell recognition/ HLA-II pathway relies on lysosomal degradation of proteins by professional antigen-presenting cells (APCs), with peptide loading occurring in the late endosome (with peptides 13-17 amino acids in length) and transport of the HLA-II-peptide complexes to the cell surface for recognition by $CD4^+$ T cells	111
4	Antigen-presenting cell (APC) and DC-based tumor vaccination	Stem and central memory $CD8^+$ T cells, denoted by their expression of CD62L, CCR7, and β-catenin	Adoptive T-cell transfer (ACT)	112
5	Chimeric antigen receptor (CAR) T-cell therapy (CAR T)	B-cell maturation antigen/CD20/CD22/CD30/CD33/CD38/ CD123/CD138	Adoptive T-cell transfer (ACT)	113

including anti–cytotoxic T-lymphocyte–associated antigen-4 (CTLA-4), anti–programmed cell death protein 1 (PD-1), and programmed cell death ligand 1 (PD-L1) have demonstrated their clinical importance in a certain type of tumors. In 2011, anti-CTLA-4 monoclonal antibody (mAb) (ipilimumab) was approved to treat melanoma, and thereafter, anti-PD-1 mAbs (pembrolizumab, nivolumab, cemiplimab, etc.), as well as an anti-PD-L-1 antibody (atezolizumab, avelumab, durvalumab, etc.), are in use to treat locally advanced and/or metastatic cancers.[118,119] However, the overall response rate of the CTLA-4, PD-1, and PD-L1 mAbs is low as most of the patients develop acquired or primary resistance.[120] Due to this unsatisfactory outcome of the present immune checkpoint therapy, extensive studies are in progress to find out other novel ICIs targets. The next-generation targeted checkpoints are lymphocyte activation gene-3 (LAG-3), T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), T-cell immunoglobulin and ITIM domain (TIGIT), V domain immunoglobulin suppressor of T-cell activation (VISTA), B7 homolog 3 protein (B7-H3), B-cell and T-cell lymphocyte attenuator, and inducible T-cell costimulatory may be the promising targets to treat solid tumors as well as TNBC. In TNBC, there was no substantial advancement had been seen for clinical management but now ICIs yield promising results for the treatment of early-stage and advanced BC. However, alone these novel strategies are not efficient enough to treat BC patients, but in combination with existing therapies provide a potential effect.[121] Although, some of the explored immune checkpoint modulators like anti-LAG-3, and anti-TIM-3 have been chosen as novel therapeutic targets and several clinical trials have been registered in ClinialTrial.gov also the number is increasing exponentially, however, no drugs entered into clinical practice to date.[122] Moreover, still some riddles need to be solved like studying the outcome of the ligands of TIGIT, VISTA, and B7-H3 for their therapeutic potential and more attempts to be taken to design combinational immunotherapy that targets various pathways to achieve the best possible effect to inhibit tumor growth.[123]

Chimeric Antigen Receptor T Cells (CAR T)

Although targeted therapy and immunotherapy have a great impact on the treatment of hematological and solid tumor malignancies, a large portion of patients develops resistance after receiving the therapy. Adoptive cell therapy (ACT) may provide added treatment options to those patients as ACT works based on an autologous or allogeneic transfer of host cell vial viral, nonviral, and plasmid vector to eliminate tumor cells.[124] CAR T-cell therapy is an emerging form of adoptive antitumor treatment that can attack specific cancer cells by targeting their surface antigen. Genetically modified T-cell express a CAR known as CAR T cells. CAR is a receptor protein that has been altered in T cells to target a specific protein.[125] CAR T cells of both $CD4^+$ and $CD8^+$ can be employed to redirect the recognition of the target cell. However, it depends on the efficient, safe, and stable condition of gene transfer.[126] CAR T-cell therapy has shown an extensive clinical response in hematological malignancies, for example, lymphoma, leukemia, and multiple myeloma. Now, the effectiveness of CAR T-cell therapy for solid tumors is in consideration to treat other malignancies.[127] Various clinical trials are under process to treat different types of cancers. The major remarkable success of CAR T-cell therapy was the Food and Drug Administration (FDA) approval of Kymriah and Yescarta; 2 second-generation CAR T-cell products targeting CD19.[128] After this success, the quality of research on CAR T cells immunotherapy focusing on solid tumors and TNBC has increased exponentially over the last few years. The antitumor activity associated with targets of tumor antigens and CAR T cells has been demonstrated in POC

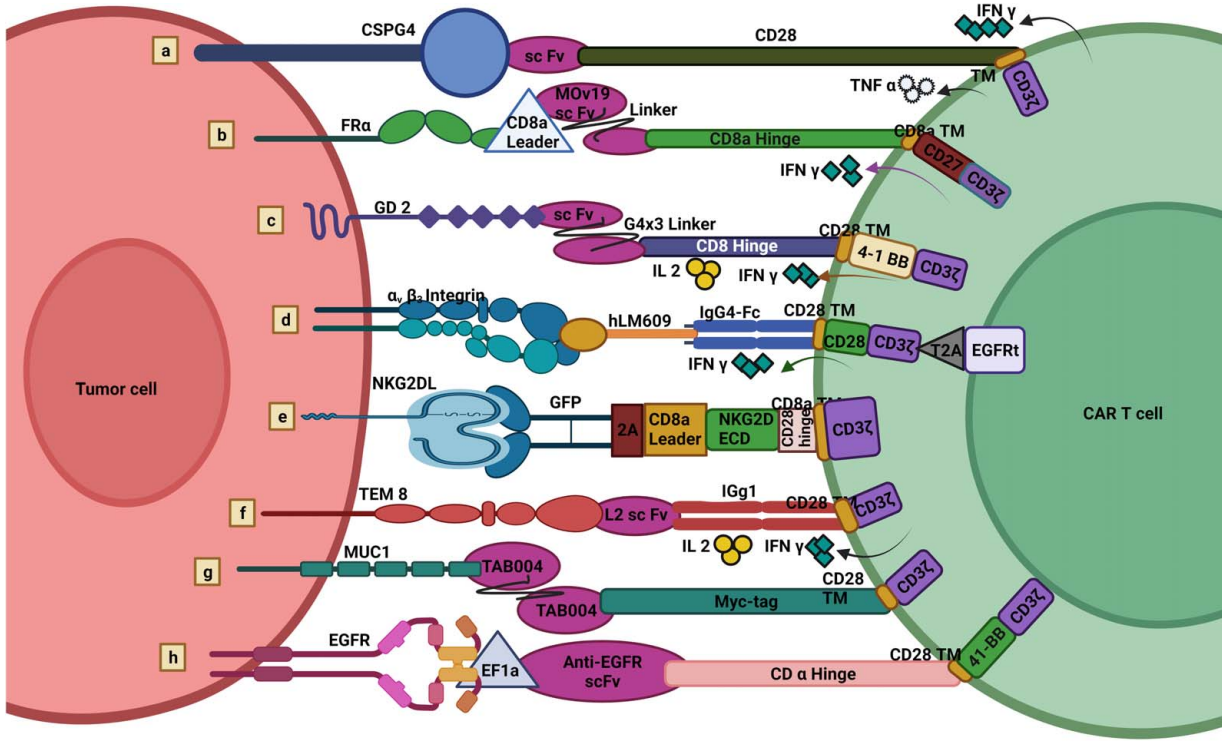

FIGURE 4. CAR T-cell–mediated therapeutic approach. CAR T-cell–mediated therapeutic approach. Second-generation and third-generation CAR engineered T cell interacting with surface tumor antigens. a, Chondroitin sulfate proteoglycan 4 (CSPG4). b, folate receptor alpha (FRα). c, GD2. d, Integrin αvβ3. e, natural killer group 2, member D ligand (NKG2DL). f, TEM8. g, MUC1. h, EGFR to induce cytokines-mediated tumor cell. CAR indicates chimeric antigen receptor; EGFR, epidermal growth factor receptor; IFN γ, interferon-γ; TNBC, triple-negative breast cancer; TNF, tumor necrosis factor. full color online

(Proof of concept) studies.[129] These antigens are classified as tumor-specific, tumor-associated, and cancer germline. Developing effective CAR therapy for TNBC selection of appropriate cell surface antigen is the preliminary step that may contribute to the pathophysiology of cancer[130] (Fig. 4). The novel and promising TNBC CAR T cells targets include AXL, EGFR, mesothelin, Mucin 1 (MUC1), c-Met, chondroitin sulfate proteoglycan 4 (CSPG4), Fc-gamma receptors (FCγR), receptor tyrosine kinase–like orphan receptor 1 (ROR1), folate receptor alpha (FRα), GD2, intracellular adhesion molecule 1 (ICAM1), integrin αvβ3, natural killer group 2, member D (NKG2D), stage-specific embryonic antigen-4, tumor endothelial marker 8, and trophoblast cell-surface antigen 2 (Table 4). Despite having many positive aspects, CAR T-cell therapy is associated with many obstacles including lack of targeted antigen specificity and its heterogeneity, immunosuppressive TME, improper expression of immune checkpoint molecules due to inefficient intratumoral trafficking, and poor persistence. Thus, CAR T therapy is one of the most sought-after therapeutic approaches since it is one of the safest and most effective forms of ACT. It not only specifically targets the TSA, but it can also recognize the polymorphs, and the absence of cell surface proteins present in normal cells. This increases the chance of detection and diagnosis of cancer since it may account for unique antigens resistant to other techniques.

FUTURISTIC APPROACHES

Pyroptosis

Cellular death via inflammatory pathway-mediated programmed cell death, also known as pyroptosis is becoming popular and a recent panel of researchers claims that pyroptosis can act as a therapeutic in TNBC.[146] Chronic pyroptosis acts as a tumor inducer, while acute pyroptosis leads to necrosis and necrosis-mediated cell death (necroptosis), therefore, pyroptosis mediation can act as both tumor mediator and tumor suppressor, though the focus has been drawn on acute pyroptosis.[147,148] Moreover, short-sparred pyroptosis can be used as a potent therapeutic against cancer growth and suppress chemoresistance, which becomes an arsenal in TNBC therapy. Several molecules and drugs have been reported to induce acute pyroptosis, which is becoming a therapeutic pillar in this scenario. TNBC targeting via pyroptosis proceeds mainly through oxidative stress induction, though several other pathway modulations are also involved. Cadmium exposure to TNBC cells (MDA-MB-231) produced cell cytotoxicity, reduced cell viability, and arrested cell cycle at the S phase through cyclin 1A1/1D1 and CDK2 modulation along with enhanced LDL production and pyroptosis induction (enhanced Bax and gasdermin E-mediated GSDME-NT cleavage) that reduced cancer growth and proliferation.[149] In another scenario, tetra arsenic hexoxide was used as a therapeutic agent against TNBC, where it induced pyroptosis via reduced phosphorylation of mitochondrial STAT3 that enhanced mtROS, mediated mtROS-caspase-3-gasdermin-E pathway, and exhibited all pyroptotic symptoms.[150] Other instances state that omega-3 docosahexaenoic acid, *Spatholobus suberectus* Dunn percolation extract (SSP) also have antitumor properties and can induce pyroptosis in TNBC.[151,152] Investigation in age-old anticancer drugs like cisplatin[153] has revealed that cisplatin too can induce pyroptotic cell death, thereby curbing the chemoresistant nature of TNBC and reducing its proliferation. Other drugs like

TABLE 4. CAR T Targets and Their Preclinical Outputs in TNBC

Sl no.	Targets of CAR T cell	CAR	CAR T cell	Preclinical output in TNBC	References
1	AXL	Single-chain variable fragment (scFv) against AXL	AXL-CAR T cell	In TNBC cells, AXL-CAR T cells show in vitro cytotoxicity thus, reducing tumor cell growth found in a xenograft mouse model of TNBC Genetically engineered IL7R with AXL-CAR T cells show enhanced anti-TNBC activity in vitro but show increased survival in a xenograft model of TNBC	131 132
2	EGFR	Anti-EGFR single-chain variable fragment (scFv1/2)	EGFR-specific CAR T cell	EGFR-CAR T cells initiate TNBC cell lysis in vitro which inhibits the growth of TNBC tumors in vivo	133
3	Mesothelin	Meso-binding single chain variable domain (SS1-scFV)	Meso CAR T cell	Disruption of PD-1 gene locus by CRISPR/Cas9 ribonucleoprotein-mediated editing to enhance the antitumor activity of Mesothelin targeted CAR T cells both in vitro and in vivo	134
4	tMUC1	scFv motif derived from TAB004	MUC28z CAR T cell	tMUC1-CAR T cells promote cytotoxicity in TNBC cells both in vitro and reduce TNBC tumor growth in-vivo	135
5	c-Met	c-Met scFv	mRNA-transfected c-Met-CAR T cell	mRNA-mediated c-Met-CAR T cells lysis of TNBC cells showed significant tumor shrinkage in a TNBC xenograft mouse model	136
6	CSPG4	Murine scFv fragments: 225.28S, TP41.2, 149.53 and G71.1 (specific for CSPG4)	CSPG4-specific CAR T cell	CSPG4-CAR T cells elicit cytolytic activity against TNBC cells in vitro	137
7	ROR1	2A2 and R12 scFv	ROR1-CAR T cell	ROR1-CAR T cells infiltrate TNBC cells and elicit antitumor activity within in vitro 3D cultures	138
8	FRα	MOv19 scFv	FRα-specific CAR T cell	FRα-CAR T cells induce the killing of TNBC cells in vitro and a TNBC xenograft mouse model, which promotes tumor regression	139
9	GD2	scFv derived from the monoclonal antibody (mAb) ch14.18 (also known as dinutuximab beta), ie, anti-GD2 CARs	GD2-CAR T cell	GD2-CAR T cells initiate cytotoxicity of TNBC cells in vitro but prevent metastasis to lungs in a xenograft mouse model of TNBC	140
10	ICAM-1	ICAM-1 scFv	ICAM-1-CAR T cell	ICAM-1-CAR T cells facilitate in vitro killing of TNBC cells	141
11	Integrin $\alpha_v\beta_3$	hLM609	Integrin $\alpha_v\beta_3$-CAR T cell	Engineered Integrin $\alpha_v\beta_3$-CAR T cells with an EGFR showed inhibition of safety switch for growth of TNBC cell in vitro	142
12	NGD2DL	NGD2D	NKG2D-CAR T cell	NKG2D-CAR T cells demonstrate prolonged persistence of TNBC cells in vivo and demonstrate tumor regression in a mouse model of TNBC	143
13	TEM8	L2 scFv	TEM8-CAR T cell	In a TNBC xenograft mouse model, TEM8-CAR T cells demonstrate antitumor activity TEM8-CAR T cells arbitrate on-tumor and off-target toxicity in an in vivo model of TNBC	144 145

CAR indicates chimeric antigen receptor; CRISPR, clustered regularly interspaced short palindromic repeats; EGFR, epidermal growth factor receptor; ICAM1, intracellular adhesion molecule 1; NKG2D, natural killer group 2, member D; PD-1, programmed cell death protein 1; ROR1, receptor tyrosine kinase–like orphan receptor 1; TNBC, triple-negative breast cancer.

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triclabendazole can induce pyroptosis via a novel caspase-3-mediated pathway in BC,[154] but its relevance in TNBC needs further investigations. Pyroptosis mediation is a relatively an old topic but its application as a therapeutic in cancer and especially TNBC is novel and has proven to be effective in multiple scenarios. Pyroptosis-mediated cellular death has deep implications in most cancers, including TNBC and predominantly it can be used to target and suppress cancer, taking GSDMD as a prognostic marker. Until now, it is not clear how inflammasomes modulate tumor progression, and a large number of the complex in vivo molecular studies and clinical trials are required to make pyroptosis viable therapeutics of the future.

Autophagy

Autophagy can be described as the degradation of the old proteins and damaged/old cells or organelles by the phagosome-phagolysosome formation in normal cells while during tumorigenesis autophagy has dual roles.[155] Normally, it facilitates tumor suppression via cell death but it can also aid in cancer progression generally in highly progressed cancers, thereby categorizing autophagy-targeted cancer treatment in the controversial zone. Even though, several investigations have shown that it can be used as a viable therapeutic option and therefore pose as dependable therapy of the future. Cancer cells mainly progress by autophagy deficiency induction and recent advances reveal several novel bio-molecular targets and techniques through which autophagic deficiency can be inhibited resulting in successful cancer therapy via autophagy induction in all BC and TNBC subsequently.[155] Investigators like Wang et al[156] have shown that autophagy can be used as a potential therapeutic in TNBC and identified eukaryotic elongation factor 2 kinase as a paclitaxel resistance modulator of autophagy. Novel autophagy biomarkers like LC3, ULK1 (regulates autophagosome formation), and VPS34 (diverse cellular processes including autophagy) are some of the targets where target-specific inhibitors can be used as a potential TNBC therapeutic targeting autophagy.[157] Recently, another novel compound CD661 has shown efficacy against autophagy via lysosome deacidification.[158] Furthermore, combinatorial therapy including EGFR inhibition in a gefitinib-dependent manner induced autophagy along with ROCK inhibition led to autophagic vacuole accumulation that hampered autophagosome clearance resulting from cytoskeletal modulation that led to apoptosis.[159] Last, Tenascin-C another marker in this scenario can be inhibited with other immunogenic factors that reduce TNBC progression.[160] Autophagy acts as a cytoprotective survival pathway targeting TNBC through autophagy induction is an interesting technique for averting long-term tissue damage that induces tumorigenesis. Therefore, induction or complete restoration of autophagy can stop TNBC induction and spread, although dysfunctional autophagic progression needs to be diverted. Autophagy modulation and induction is a therapy of potential that needs further investigation and is currently under heavy scrutiny.

Adipogenesis

All cancers in general and TNBC specifically express EMT/MET that provide the enhanced potential for metastatic progression and upregulated drug resistance.[161–163] During EMT/MET transitions, TN cells exhibit upregulated cellular plasticity and stem cell–like properties, which can be used as a therapeutic approach in this scenario.[163–165] Ishay-Ronen et al report that cellular plasticity and stem-like property expression mediated by EMT in BC cells can be used to generate *bona fide* adipocytes (adipogenesis), through transdifferentiation of mesenchymal, malign tumor cells.[166] It is well known that *bona fide* adipocytes are generally nonplastic and show almost nil differentiative capabilities including growth arrest reducing further tumorigenic mutations, thereby becoming an effective mode of BC therapeutics. Preadipocytes 3T3-L1 fibroblasts, when treated with insulin with doses of dexamethasone along with rosiglitazone in presence of peroxisome proliferator-activated receptor γ (PPARγ), preadipocytic 3T3-L1, upregulated and expressed PPARγ and C/EBPα, which are considered to be transcription factors regulating adipogenesis.[163,166-169] With the successive expression of PPARγ and C/EBPα, they showed morphological changes and progressed with lipid droplet accumulation with the presence of perilipin (adipocyte-specific protein) into *bona fide* adipocytes[163,166] The same was tested in BC cells derived from 2 models: (i) MTflECad from MMTV-*Neu* transgenic mouse carrying E-cadherin (Floxed) that went into irreversible EMT when E-cadherin was expressed via Cre recombinase system; (ii) Py2T cells from MMTV-PyMT transgenic mouse underwent EMT/MET with TGFβ addition/removal, respectively.[166,170] When these systems expressing EMT/MET conditions, were treated with bone morphogenetic protein 2, they showed transitions to adipocytes morphologically and were most effective in combination with bone morphogenetic protein 2 and rosiglitazone.[163,171] The adipocytic state was maintained even after the removal of all the differentiation factors, which becomes an attractive target.[163] Hence, the same when introduced in TNBC therapeutic can pose as a beautiful end-of-line therapy, in light of high drug resistance and failure in TNBC therapeutics. Recently, Ishay-Ronen et al[166] used a patient-derived xenograft model (x-3078) established as a nontreated primary TNBC clump to test the long-term adipogenesis effect in a clinical situation based on an experimental set-up. Combinational treatment with MEK inhibitor trametinib and rosiglitazone yielded reduced primary TNBC clump size. Trametinib inhibited ERK phosphorylation and induced adipogenesis differentiation with perilipin enhancement, while rosiglitazone aided in combination.[166] Hence, adipogenesis in all BC is possible and provides a sustained option in TNBC setups for futuristic approaches. Furthermore, several model-based investigations are required to understand adipogenesis, and look into its negative sides that will determine its effective role as a therapeutic in TNBC patients.

CONCLUSIONS

TNBC remains the most complex disease when compared with other subtypes due to negative expression of estrogen receptor, progesterone receptor, and HER2/neu immunohistochemically that builds high invasiveness with early recurrence rate, and poor prognosis level. Conventional molecular targeting-based therapy remains widely suited for TNBC therapy, though the high resistance patterns and unexpectedly low therapeutic results from such therapies are persistent and pose a problem. Interesting successful investigations have resulted in new therapeutics, which are more precise and target-specific molecular mechanisms that characterize TNBC. Therapeutic techniques based on CRISPR-Cas gene editing, immunotherapies, and approaches targeting the tumor oxidative stress, cell death mechanisms through pyroptosis and autophagy, and complete tumor cell differentiation through adipogenesis are novel and pose as formidable therapeutic that could overcome the TNBC burden. Effective clinical trials, model optimization, and in vivo testing models will present an important turnover for these techniques. These insights will be helpful in future research in combating TNBC progression and chemoresistance.

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REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*. 2021;71: 209–249.
2. Yin L, Duan JJ, Bian XW, et al. Triple-negative breast cancer molecular subtyping and treatment progress. *Breast Cancer Res*. 2020;22:1–13.
3. Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. *N Engl J Med*. 2010;363:1938–1948.
4. Kim C, Gao R, Sei E, et al. Chemoresistance Evolution in Triple-Negative Breast Cancer Delineated by Single-Cell Sequencing. *Cell*. 2018;173:879–893e13.
5. Nedeljković M, Damjanović A. Mechanisms of Chemotherapy Resistance in Triple-Negative Breast Cancer-How We Can Rise to the Challenge. *Cells*. 2019;8.
6. Andreopoulou E, Schweber SJ, Sparano JA, et al. Therapies for triple negative breast cancer. *Expert Opin Pharmacother*. 2015; 16:983–998.
7. Lee A, Djamgoz MBA. Triple negative breast cancer: Emerging therapeutic modalities and novel combination therapies. *Cancer Treat Rev*. 2018;62:110–122.
8. Butti R, Das S, Gunasekaran VP, et al. Receptor tyrosine kinases (RTKs) in breast cancer: Signaling, therapeutic implications and challenges. *Mol Cancer*. 2018;17:1–18.
9. Shamseddine AI, Farhat FS. Platinum-based compounds for the treatment of metastatic breast cancer. *Chemotherapy*. 2012;57: 468–487.
10. Mohamed RF, Bakri HAM, Abdelfattah ON, et al. Does bevacizumab carry a hope for metastatic triple-negative breast cancer in the era of immunotherapy. *Anticancer Drugs*. 2022;33: E604–E609.
11. Turner N, Lambros MB, Horlings HM, et al. Integrative molecular profiling of triple negative breast cancers identifies amplicon drivers and potential therapeutic targets. *Oncogene*. 2010;29: 2013–2023.
12. Han Y, Yu X, Li S, et al. New Perspectives for Resistance to PARP Inhibitors in Triple-Negative Breast Cancer. *Front Oncol*. 2020:10.
13. Ettl J, Quek RGW, Lee KH, et al. Quality of life with talazoparib versus physician's choice of chemotherapy in patients with advanced breast cancer and germline BRCA1/2 mutation: Patient-reported outcomes from the EMBRACA phase III trial. *Ann Oncol*. 2018;29:1939–1947.
14. Robson M, Im SA, Senkus E, et al. Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. *N Engl J Med*. 2017;377:523–533.
15. Miller K, Tong Y, Jones DR, et al. Cisplatin with or without rucaparib after preoperative chemotherapy in patients with triple negative breast cancer: Final efficacy results of Hoosier Oncology Group BRE09-146. *J Clin Oncol*. 2015;33(15_suppl):1082–1082.
16. Trametinib and Akt Inhibitor GSK2141795 in Treating Patients With Metastatic Triple-Negative Breast Cancer. clinicaltrials.gov. Published 2013. Accessed March 6, 2023 https://www.clinicaltrials.gov/ct2/show/results/NCT01964924?view=results
17. Kim SB, Maslyar DJ, Dent R, et al. Ipatasertib plus paclitaxel versus placebo plus paclitaxel as first-line therapy for metastatic triple-negative breast cancer (LOTUS): a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Oncol*. 2017;18:1360–1372.
18. Schmid P, Abraham J, Chan S, et al. Capivasertib plus paclitaxel versus placebo plus paclitaxel as first-line therapy for metastatic triple-negative breast cancer: The PAKT trial. *Journal of Clinical Oncology*. 2020;38:423–433.
19. NCT04345913. Testing the Addition of Copanlisib to Eribulin for the Treatment of Advanced-Stage Triple Negative Breast Cancer. https://clinicaltrials.gov/show/NCT04345913. Published online 2020. Accessed March 3, 2023. https://clinicaltrials.gov/ct2/show/NCT04345913
20. EUCTR2021-001966-39-DE. A clinical study to assess the efficacy of alpelisib plus fulvestrant in participants with HR-positive, HER2-negative, advanced breast cancer after treatment with a CDK4/6 inhibitor and an aromatase inhibitor. https://trialsearch.who.int/Trial2.aspx?TrialID=EUCTR2021-001966-39-DE Published online 2021. Accessed March 19, 2023 https://clinicaltrials.gov/ct2/show/NCT05038735
21. NCT02531932. Comparison of Single-Agent Carboplatin vs the Combination of Carboplatin and Everolimus for the Treatment of Advanced Triple-Negative Breast Cancer. *clinicaltrials.gov*. Published online 2015:2015-2017. Accessed March 19, 2023. https://clinicaltrials.gov/ct2/show/NCT02531932
22. NCT01920061. A Study Of PF-05212384 In Combination With Other Anti-Tumor Agents and in Combination With Cisplatin in Patients With Triple Negative Breast Cancer in an Expansion Arm (TNBC). clinicaltrials.gov. Published 2021. Accessed March 19, 2023. https://clinicaltrials.gov/ct2/show/NCT01920061
23. Abramson V Taselisib and Enzalutamide in treating patients with Androgen Receptor Positive Triple-Negative Breast Cancer - NCT02457910. https://clinicaltrials.gov/ct2/show/NCT02457910 Published 2022. Accessed March 19, 2023. https://clinicaltrials.gov/ct2/show/NCT02457910
24. NCT03055312. Bicalutamide in Treatment of Androgen Receptor (AR) Positive Metastatic Triple Negative Breast Cancer. Published online 2017. Accessed March 3, 2023. https://clinicaltrials.gov/show/nct03055312; https://clinicaltrials.gov/ct2/show/NCT03055312
25. NCT02580448. CYP17 Lyase and Androgen Receptor Inhibitor Treatment With Seviteronel Trial (INO-VT-464-006; NCT02580448) - Full Text View - ClinicalTrials.gov. Published 2015. Accessed March 3, 2023. https://clinicaltrials.gov/ct2/show/NCT02580448
26. Pérez-García J, Soberino J, Racca F, et al. Atezolizumab in the treatment of metastatic triple-negative breast cancer. *Expert Opin Biol Ther*. 2020;20:981–989. doi:10.1080/14712598.2020.1769063
27. Schmid P, Cortes J, Pusztai L, et al. Pembrolizumab for Early Triple-Negative Breast Cancer. *N Engl J Med*. 2020;382:810–821.
28. Mizuki M, Fenski R, Halfter H, et al. Flt3 mutations from patients with acute myeloid leukemia induce transformation of 32D cells mediated by the Ras and STAT5 pathways. *Blood*. 2000;96: 3907–3914.
29. Wang H, Ma H, Sové RJ, et al. Quantitative systems pharmacology model predictions for efficacy of atezolizumab and nab-paclitaxel in triple-negative breast cancer. *J Immunother Cancer*. 2021;9:e002100.
30. Blum JL, Douglas Laird A, Litton JK, et al. Determinants of Response to Talazoparib in Patients with HER2-Negative, Germline BRCA1/2-Mutated Breast Cancer. *Clin Cancer Res*. 2022;28:1383–1390.
31. NCT01074970. PARP Inhibition for Triple Negative Breast Cancer (ER-/PR-/HER2-)With BRCA1/2 Mutations. clinicaltrials.gov. Published 2010. Accessed March 19, 2023. https://clinicaltrials.gov/ct2/show/NCT01074970
32. Tesaro Inc. A. A Phase III Trial of Niraparib Versus Physician's Choice in Her2 Negative, Germline BRCA Mutation-positive Breast Cancer Patients. Clinicaltrials.Gov. Published 2013. Accessed March 19, 2023. https://clinicaltrials.gov/ct2/show/NCT01905592
33. Ayoub JP, Wildiers H, Friedlander M, et al. Safety and efficacy of veliparib plus carboplatin/paclitaxel in patients with HER2-negative metastatic or locally advanced breast cancer: subgroup analyses by germline BRCA1/ 2 mutations and hormone receptor status from the phase-3 BROCADE3 trial. *Ther Adv Med Oncol*. 2021:13.
34. Maennling AE, Tur MK, Niebert M, et al. Molecular targeting therapy against egfr family in breast cancer: Progress and future potentials. *Cancers (Basel)*. 2019:11.
35. Brufsky A, Valero V, Tiangco B, et al. Second-line bevacizumab-containing therapy in patients with triple-negative breast cancer:

Subgroup analysis of the RIBBON-2 trial. *Breast Cancer Res Treat*. 2012;133:1067–1075.

36. Rodon J, Argilés G, Connolly RM, et al. Phase 1 study of single-agent WNT974, a first-in-class Porcupine inhibitor, in patients with advanced solid tumours. *Br J Cancer*. 2021;125:28–37.
37. Bardia A, Tolaney SM, Punie K, et al. Biomarker analyses in the phase III ASCENT study of sacituzumab govitecan versus chemotherapy in patients with metastatic triple-negative breast cancer. *Ann Oncol*. 2021;32:1148–1156.
38. Pal SK, McGregor B, Suárez C, et al. Cabozantinib in Combination With Atezolizumab for Advanced Renal Cell Carcinoma: Results From the COSMIC-021 Study. *J Clin Oncol*. 2021;39:3725–3736.
39. NCT01964924. Trametinib and Hydroxychloroquine in Treating Patients With Pancreatic Cancer - Full Text View - ClinicalTrials.gov. https://www.clinicaltrials.gov/ct2/show/NCT01964924 Published 2013. Accessed March 19, 2023. https://www.clinicaltrials.gov/ct2/show/NCT01964924
40. Weber ZT, Collier KA, Tallman D, et al. Modeling clonal structure over narrow time frames via circulating tumor DNA in metastatic breast cancer. *Genome Med*. 2021;13:1–17.
41. NCT01677455. An Open-Label Multicenter Phase 2 Window of Opportunity Study Evaluating Ganetespib in Women With Breast Cancer. Synta Pharmaceuticals Corp. Published 2012. Accessed March 19, 2023. https://clinicaltrials.gov/ct2/show/NCT01677455
42. NCT02474173. Galunisertib and Paclitaxel in Treating Patients With Metastatic Androgen Receptor Negative (AR-) Triple Negative Breast Cancer - Full Text View - ClinicalTrials.gov. Published 2022. Accessed March 19, 2023. https://clinicaltrials.gov/ct2/show/NCT02474173
43. NCT02876302. Study Of Ruxolitinib (INCB018424) With Preoperative Chemotherapy For Triple Negative Inflammatory Breast Cancer - Full Text View - ClinicalTrials.gov. Published 2022. Accessed March 6, 2023. https://clinicaltrials.gov/ct2/show/NCT02876302
44. NCT03195699. Oral STAT3 Inhibitor, TTI-101, in Patients With Advanced Cancers - Full Text View - ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT03195699 Published 2017. Accessed March 6, 2023. https://clinicaltrials.gov/ct2/show/NCT03195699
45. NCT02672475. Galunisertib and Paclitaxel in Treating Patients With Metastatic Androgen Receptor Negative (AR-) Triple Negative Breast Cancer - Full Text View - ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT02474173 Published 2022. Accessed March 19, 2023. https://clinicaltrials.gov/ct2/show/NCT02474173
46. NCT01876251. A Study Evaluating The PF-03084014 In Combination With Docetaxel In Patients With Advanced Breast Cancer - Full Text View - ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT01876251 Published 2013. Accessed March 6, 2023. https://clinicaltrials.gov/ct2/show/NCT01876251
47. NCT02694224, Clinica Universidad de Navarra U de N. Addition of Vismodegib to Neoadjuvant Chemotherapy in Triple Negative Breast Cancer Patients - Full Text View - ClinicalTrials.gov. Published 2018. Accessed March 6, 2023. https://clinicaltrials.gov/ct2/show/NCT02694224
48. NCT01639248. Phase 2 Study - Aurora + Angiogenic Kinase Inhibitor ENMD-2076 in Previously Treated Locally Advanced + Metastatic TNBC - Full Text View - ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT01639248 Published 2012. Accessed March 19, 2023. https://clinicaltrials.gov/ct2/show/NCT01639248
49. Zheng Q, Banaszak L, Fracci S, et al. Leptin receptor maintains cancer stem-like properties in triple negative breast cancer cells. *Endocr Relat Cancer*. 2013;20:797–808.
50. Balaban S, Shearer RF, Lee LS, et al. Adipocyte lipolysis links obesity to breast cancer growth: adipocyte-derived fatty acids drive breast cancer cell proliferation and migration. *Cancer Metab*. 2017;5. doi:10.1186/s40170-016-0163-7
51. Nath A, Mitra S, Mistry T, et al. Molecular targets and therapeutics in chemoresistance of triple-negative breast cancer. *Med Oncol*. 2022;39:14.
52. Yu T, Di G. Role of tumor microenvironment in triple-negative breast cancer and its prognostic significance. *Chinese J Cancer Res*. 2017;29:237–252.
53. Zheng H, Siddharth S, Parida S, et al. Tumor microenvironment: Key players in triple negative breast cancer immunomodulation. *Cancers (Basel)*. 2021:13. doi:10.3390/cancers13133357
54. Mahata S, Sahoo PK, Pal R, et al. PIM1/STAT3 axis: a potential co-targeted therapeutic approach in triple-negative breast cancer. *Med Oncol*. 2022;39:1–11.*2022 397*.
55. Haranath C, Jonnala R, Bhargav E, et al. Clustered Regularly Interspaced Short Palindromic Repeats and its Associated Protein-9: Drug Delivery and Therapeutic Applications. *J Young Pharm*. 2022;14:268–272.
56. Lee S, Rauch J, Kolch W. Targeting MAPK signaling in cancer: Mechanisms of drug resistance and sensitivity. *Int J Mol Sci*. 2020;21. doi:10.3390/ijms21031102
57. Lee SH, Yu J, Hwang GH, et al. CUT-PCR: CRISPR-mediated, ultrasensitive detection of target DNA using PCR. *Oncogene*. 2017;36:6823–6829.
58. Deepak Singh D, Han I, Choi EH, et al. CRISPR/Cas9 based genome editing for targeted transcriptional control in triple-negative breast cancer. *Comput Struct Biotechnol J*. 2021;19:2384–2397.
59. Shao F, Sun H, Deng CX. Potential therapeutic targets of triple-negative breast cancer based on its intrinsic subtype. *Oncotarget*. 2017;8:73329–73344.
60. Liao L, Song M, Li X, et al. E3 ubiquitin ligase UBR5 drives the growth and metastasis of triple-negative breast cancer. *Cancer Res*. 2017;77:2090–2101.
61. Yang H, Jaeger ML, Walker A, et al. Break breast cancer addiction by CRISPR/Cas9 genome editing. *J Cancer*. 2018;9:219–231.
62. Breuleux M, Klopfenstein M, Stephan C, et al. Increased AKT S473 phosphorylation after mTORC1 inhibition is rictor dependent and does not predict tumor cell response to PI3K/mTOR inhibition. *Mol Cancer Ther*. 2009;8:742–753.
63. Ruicci KM, Plantinga P, Pinto N, et al. Disruption of the RICTOR/mTORC2 complex enhances the response of head and neck squamous cell carcinoma cells to PI3K inhibition. *Mol Oncol*. 2019;13:2160–2177.
64. White MK, Khalili K. CRISPR/Cas9 and cancer targets: future possibilities and present challenges. *Oncotarget*. 2016;7:12305.
65. Castro NP, Fedorova-Abrams ND, Merchant AS, et al. Cripto-1 as a novel therapeutic target for triple negative breast cancer. *Oncotarget*. 2015;6:11910–11929.
66. Bossennec M, Di Roio A, Caux C, et al. MDR1 in immunity: friend or foe. *Oncoimmunology*. 2018;7. doi:10.1080/2162402X.2018.1499388
67. Guo P, Yang J, Huang J, et al. Therapeutic genome editing of triple-negative breast tumors using a noncationic and deformable nanolipogel. *Proc Natl Acad Sci USA*. 2019;116:18295–18303.
68. Hsu PY, Hsu HK, Hsiao TH, et al. Spatiotemporal control of estrogen-responsive transcription in ERα-positive breast cancer cells. *Oncogene*. 2016;35:2379.
69. Wagner JM, Kaufmann SH. Prospects for the Use of ATR Inhibitors to Treat Cancer. *Pharmaceuticals*. 2010;3:1311.
70. Shaath H, Vishnubalaji R, Elango R, et al. Single-cell long noncoding RNA (lncRNA) transcriptome implicates MALAT1 in triple-negative breast cancer (TNBC) resistance to neoadjuvant chemotherapy. *Cell Death Discov*. 2021;7:1–14.
71. Singha PK, Pandeswara S, Geng H, et al. Increased smad3 and reduced smad2 levels mediate the functional switch of tgf-β from growth suppressor to growth and metastasis promoter through tmepai/pmepa1 in triple negative breast cancer. *Genes and Cancer*. 2019;10:134–149.
72. Zhou JY, Suwan K, Hajitou A. Initial steps for the development of a phage-mediated gene replacement therapy using CRISPR-Cas9 technology. *J Clin Med*. 2020;9.
73. Gadi V, Gupta D, Shetty S. Emerging potentials of nano-therapeutics in breast cancer microenvironment targeting. *Open-Nano*. 2022:8. doi:10.1016/J.ONANO.2022.100101
74. Semenza GL. The hypoxic tumor microenvironment: A driving force for breast cancer progression. *Biochim Biophys Acta - Mol Cell Res*. 2016;1863:382–391.
75. Sun X, Luo H, Han C, et al. Identification of a Hypoxia-Related Molecular Classification and Hypoxic Tumor Microenvironment

Signature for Predicting the Prognosis of Patients with Triple-Negative Breast Cancer. *Front Oncol*. 2021:11.
76. Jing X, Yang F, Shao C, et al. Role of hypoxia in cancer therapy by regulating the tumor microenvironment. *Mol Cancer*. 2019;18.
77. Sarmiento-Salinas FL, Delgado-Magallón A, Montes-Alvarado JB, et al. Breast cancer subtypes present a differential production of reactive oxygen species (ROS) and susceptibility to antioxidant treatment. *Front Oncol*. 2019;9(JUN10.3389/fonc.2019.00480
78. Nohl H, Gille L. Lysosomal ROS formation. *Redox Rep*. 2005;10: 199–205.
79. Yoboue ED, Sitia R, Simmen T. Redox crosstalk at endoplasmic reticulum (ER) membrane contact sites (MCS) uses toxic waste to deliver messages. *Cell Death Dis*. 2018;9. doi:10.1038/s41419-017-0033-4
80. Forman HJ, Torres M. Reactive oxygen species and cell signaling: Respiratory burst in macrophage signaling. *American Journal of Respiratory and Critical Care Medicine*. 2002;166. In: American Thoracic Society.
81. Yang H, Miao Q, Jenni S, , et al Oxidative stress imaging in triple negative breast cancer using a cystine transporter tracer [18F]5-fluoro aminosuberic acid (FASu) | Request PDF. Soc Nuclear Med. Published 2014. Accessed March 11, 2023. https://www.researchgate.net/publication/291457269_Oxidative_stress_imaging_in_triple_negative_breast_cancer_using_a_cystine_transporter_tracer_18F5-fluoro_aminosuberic_acid_FASu
82. Qiao L, Liu J, Kuang S, et al. A mitochondrion-targeted BODIPY-Ir(III) conjugate as a photoinduced ROS generator for the oxidative destruction of triple-negative breast cancer cells. *Dalt Trans*. 2021;50:14332–14341.
83. Evans KW, Yuca E, Scott SS, et al. Oxidative phosphorylation is a metabolic vulnerability in chemotherapy-resistant triple-negative breast cancer. *Cancer Res*. 2021;81:5572–5581.
84. Rha SY, Beom SH, Shin YG, et al. Phase I study of IM156, a novel potent biguanide oxidative phosphorylation (OXPHOS) inhibitor, in patients with advanced solid tumors. *J Clin Oncol*. 2020;38 (15_suppl):3590–3590.
85. Furuno T, Kanno T, Arita K, et al. Roles of long chain fatty acids and carnitine in mitochondrial membrane permeability transition. *Biochem Pharmacol*. 2001;62:1037–1046.
86. Škubník J, Jurášek M, Ruml T, et al. Mitotic poisons in research and medicine. *Molecules*. 2020;25. doi:10.3390/molecules25204632
87. Seglen PO. Inhibitors of lysosomal function. *Methods Enzymol*. 1983;96(C):737–764.
88. Cassetta L, Kitamura T. Targeting tumor-associated macrophages as a potential strategy to enhance the response to immune checkpoint inhibitors. *Front Cell Dev Biol*. 2018;6(APR):38. doi:10.3389/fcell.2018.00038
89. Harnoss JM, Le Thomas A, Reichelt M, et al. IRE1α disruption in triple-negative breast cancer cooperates with antiangiogenic therapy by reversing ER stress adaptation and remodeling the tumor microenvironment. *Cancer Res*. 2020;80:2368–2379. doi:10.1158/0008-5472.CAN-19-3108
90. Chen X, Iliopoulos D, Zhang Q, et al. XBP1 promotes triple-negative breast cancer by controlling the HIF1α pathway. *Nature*. 2014;508:103–107. doi:10.1038/nature13119
91. Zhao N, Cao J, Xu L, et al. Pharmacological targeting of MYC-regulated IRE1/XBP1 pathway suppresses MYC-driven breast cancer. *J Clin Invest*. 2018;128:1283–1299. doi:10.1172/JCI95873
92. Kwon Y. Possible beneficial effects of N-acetylcysteine for treatment of triple-negative breast cancer. *Antioxidants*. 2021;10: 1–22. doi:10.3390/antiox10020169
93. Griñan-Lison C, Blaya-Cánovas JL, López-Tejada A, et al. Antioxidants for the Treatment of Breast Cancer: Are We There Yet. *Antioxidants (Basel, Switzerland)*. 2021;10:1–44.
94. Costales MG, Childs-Disney JL, Haniff HS, et al. How We Think about Targeting RNA with Small Molecules. *J Med Chem*. 2020; 63:8880–8900. doi:10.1021/acs.jmedchem.9b01927
95. Yu AM, Jian C, Yu AH, et al. RNA therapy: Are we using the right molecules. *Pharmacol Ther*. 2019;196:91–104.
96. Burnett JC, Rossi JJ. RNA-based therapeutics: Current progress and future prospects. *Chem Biol*. 2012;19:60–71.
97. Crooke ST, Baker BF, Xia S, et al. Integrated Assessment of the Clinical Performance of GalNAc 3 -Conjugated 2′-O-Methoxyethyl Chimeric Antisense Oligonucleotides: I. Human Volunteer Experience. *Nucleic Acid Ther*. 2019;29:16–32.
98. Melnikova I. RNA-based therapies. *Nat Rev Drug Discov*. 2007;6: 863–864.
99. Santos R, Ursu O, Gaulton A, et al. A comprehensive map of molecular drug targets. *Nat Rev Drug Discov*. 2016;16:19–34.
100. Haque S, Cook K, Sahay G, et al. RNA-Based Therapeutics: Current Developments in Targeted Molecular Therapy of Triple-Negative Breast Cancer. *Pharmaceutics*. 2021;13.
101. Lam JKW, Chow MYT, Zhang Y, et al. siRNA versus miRNA as therapeutics for gene silencing. *Mol Ther - Nucleic Acids*. 2015;4:e252.
102. Yin H, Xiong G, Guo S, et al. Delivery of Anti-miRNA for Triple-Negative Breast Cancer Therapy Using RNA Nanoparticles Targeting Stem Cell Marker CD133. *Mol Ther*. 2019;27:1252–1261.
103. Granot Y, Peer D. Delivering the right message: Challenges and opportunities in lipid nanoparticles-mediated modified mRNA therapeutics—An innate immune system standpoint. *Semin Immunol*. 2017;34:68–77.
104. Suurs FV, Lub-de Hooge MN, de Vries EGE, et al. A review of bispecific antibodies and antibody constructs in oncology and clinical challenges. *Pharmacol Ther*. 2019;201:103–119.
105. Klinger M, Benjamin J, Kischel R, et al. Harnessing T cells to fight cancer with BiTE® antibody constructs - past developments and future directions. *Immunol Rev*. 2016;270:193–208.
106. Yu L, Wang J. T cell-redirecting bispecific antibodies in cancer immunotherapy: recent advances. *J Cancer Res Clin Oncol*. 2019; 145:941–956.
107. Deshaies RJ. Multispecific drugs herald a new era of biopharmaceutical innovation. *Nature*. 2020;580:329–338.
108. Cao Y, Chen C, Tao Y, et al. Immunotherapy for triple-negative breast cancer. *Pharmaceutics*. 2021;13:2003.
109. Tang M, Liu Y, Zhang QC, et al. Antitumor efficacy of the runx2-dendritic cell vaccine in triple-negative breast cancer in vitro. *Oncol Lett*. 2018;16:2813–2822.
110. Pack CD, Bommireddy R, Munoz LE, et al. Tumor membrane-based vaccine immunotherapy in combination with anti-CTLA-4 antibody confers protection against immune checkpoint resistant murine triple-negative breast cancer. *Hum Vaccines Immunother*. 2020;16:3184–3193.
111. Goncalves G, Mullan KA, Duscharla D, et al. IFNγ Modulates the Immunopeptidome of Triple Negative Breast Cancer Cells by Enhancing and Diversifying Antigen Processing and Presentation. *Front Immunol*. 2021:12.
112. Neal LR, Bailey SR, Wyatt MM, et al. The Basics of Artificial Antigen Presenting Cells in T Cell-Based Cancer Immunotherapies. *J Immunol Res Ther*. 2017;2:68–79; Accessed March 10, 2023 https://pubmed.ncbi.nlm.nih.gov/28825053/
113. Dees S, Ganesan R, Singh S, et al. Emerging CAR-T cell therapy for the treatment of triple-negative breast cancer. *Mol Cancer Ther*. 2020;19:2409–2421.
114. La-Beck NM, Jean GW, Huynh C, et al. Immune Checkpoint Inhibitors: New Insights and Current Place in Cancer Therapy. *Pharmacotherapy*. 2015;35:963–976.
115. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12:252–264.
116. Marin-Acevedo JA, Dholaria B, Soyano AE, et al. Next generation of immune checkpoint therapy in cancer: New developments and challenges. *J Hematol Oncol*. 2018;11.
117. Sadreddini S, Baradaran B, Aghebati-Maleki A, et al. Immune checkpoint blockade opens a new way to cancer immunotherapy. *J Cell Physiol*. 2019;234:8541–8549.
118. Qin S, Xu L, Yi M, et al. Novel immune checkpoint targets: Moving beyond PD-1 and CTLA-4. *Mol Cancer*. 2019;18:1–14.
119. Vaddepally RK, Kharel P, Pandey R, et al. Review of Indications of FDA-Approved Immune Checkpoint Inhibitors per NCCN Guidelines with the Level of Evidence. *Cancers (Basel)*. 2020;12. doi:10.3390/CANCERS12030738
120. Shergold AL, Millar R, Nibbs RJB. Understanding and overcoming the resistance of cancer to PD-1/PD-L1 blockade. *Pharmacol Res*. 2019:145. doi:10.1016/j.phrs.2019.104258

121. Adams S, Diamond JR, Hamilton E, et al. Atezolizumab Plus nab-Paclitaxel in the Treatment of Metastatic Triple-Negative Breast Cancer with 2-Year Survival Follow-up: A Phase 1b Clinical Trial. *JAMA Oncol*. 2019;5:334–342.
122. Anderson AC, Joller N, Kuchroo VK. Lag-3, Tim-3, and TIGIT: Co-inhibitory Receptors with Specialized Functions in Immune Regulation. *Immunity*. 2016;44:989–1004.
123. Cao G, Xiao Z, Yin Z. Normalization cancer immunotherapy: Blocking siglec-15. *Signal Transduct Target Ther*. 2019;4. doi:10.1038/s41392-019-0045-x
124. Luke JJ, Flaherty KT, Ribas A, et al. Targeted agents and immunotherapies: Optimizing outcomes in melanoma. *Nat Rev Clin Oncol*. 2017;14:463–482.
125. June CH, O'Connor RS, Kawalekar OU, et al. CAR T cell immunotherapy for human cancer. *Science*, 80-2018;359: 1361–1365.
126. Chmielewski M, Hombach AA, Abken H. Antigen-specific T-cell activation independently of the MHC: Chimeric antigen receptor-redirected T cells. *Front Immunol*. 2013;4. 10.3389/fimmu.2013.00371
127. Chen N, Li X, Chintala NK, et al. Driving CARs on the uneven road of antigen heterogeneity in solid tumors. *Curr Opin Immunol*. 2018;51:103–110. doi:10.1016/j.coi.2018.03.002
128. Yang X, xiang Wang G, feng Zhou J. CAR T Cell Therapy for Hematological Malignancies. *Curr Med Sci*. 2019;39:874–882.
129. Abreu TR, Fonseca NA, Gonçalves N, et al. Current challenges and emerging opportunities of CAR-T cell therapies. *J Control Release*. 2020;319:246–261.
130. Li Z, Qiu Y, Lu W, et al. Immunotherapeutic interventions of Triple Negative Breast Cancer. *J Transl Med*. 2018;16.
131. Wei J, Sun H, Zhang A, et al. A novel AXL chimeric antigen receptor endows T cells with anti-tumor effects against triple negative breast cancers. *Cell Immunol*. 2018;331:49–58.
132. Zhao Z, Li Y, Liu W, et al. Engineered IL-7 Receptor Enhances the Therapeutic Effect of AXL-CAR-T Cells on Triple-Negative Breast Cancer. *Biomed Res Int*. 2020:2020. doi:10.1155/2020/4795171
133. Liu Y, Zhou Y, Huang KH, et al. EGFR-specific CAR-T cells trigger cell lysis in EGFR-positive TNBC. *Aging (Albany NY)*. 2019;11:11054–11072.
134. Hu W, Zi Z, Jin Y, et al. CRISPR/Cas9-mediated PD-1 disruption enhances human mesothelin-targeted CAR T cell effector functions. *Cancer Immunol Immunother*. 2019;68:365–377.
135. Zhou R, Yazdanifar M, Das Roy L, et al. CAR T cells targeting the tumor MUC1 glycoprotein reduce triple-negative breast cancer growth. *Front Immunol*. 2019;10(MAY).10.3389/fimmu.2019.01149
136. Tchou J, Zhao Y, Levine BL, et al. Safety and efficacy of intratumoral injections of chimeric antigen receptor (CAR) T cells in metastatic breast cancer. *Cancer Immunol Res*. 2017;5:1152–1161.
137. Beard RE, Zheng Z, Lagisetty KH, et al. Multiple chimeric antigen receptors successfully target chondroitin sulfate proteoglycan 4 in several different cancer histologies and cancer stem cells. *J Immunother Cancer*. 2014;2. doi:10.1186/2051-1426-2-25
138. Wallstabe L, Göttlich C, Nelke LC, et al. ROR1-CAR T cells are effective against lung and breast cancer in advanced microphysiologic 3D tumor models. *JCI Insight*. 2019;4. doi:10.1172/jci.insight.126345
139. Song DG, Ye Q, Poussin M, et al. Effective adoptive immunotherapy of triple-negative breast cancer by folate receptor-alpha redirected CAR T cells is influenced by surface antigen expression level. *J Hematol Oncol*. 2016;9. doi:10.1186/s13045-016-0285-y
140. Seitz CM, Schroeder S, Knopf P, et al. GD2-targeted chimeric antigen receptor T cells prevent metastasis formation by elimination of breast cancer stem-like cells. *Oncoimmunology*. 2020;9. doi:10.1080/2162402X.2019.1683345
141. Yang Y, Vedvyas Y, McCloskey JE, et al. Abstract 2322: ICAM-1 targeting CAR T cell therapy for triple negative breast cancer. *Cancer Res*. 2019;79(13_Supplement):2322–2322.
142. Wallstabe L, Mades A, Frenz S, et al. CAR T cells targeting α v β 3 integrin are effective against advanced cancer in preclinical models. *Adv Cell Gene Ther*. 2018;1:e11.
143. Han Y, Xie W, Song DG, et al. Control of triple-negative breast cancer using ex vivo self-enriched, costimulated NKG2D CAR T cells. *J Hematol Oncol*. 2018;11. doi:10.1186/S13045-018-0635-Z
144. Byrd TT, Fousek K, Pignata A, et al. TEM8/ANTXR1-Specific CAR T cells as a targeted therapy for triple-negative breast cancer. *Cancer Res*. 2018;78:489–500.
145. Petrovic K, Robinson J, Whitworth K, et al. TEM8/ANTXR1-specific CAR T cells mediate toxicity in vivo. *PLoS One*. 2019; 14. doi:10.1371/journal.pone.0224015
146. Fang Y, Tian S, Pan Y, et al. Pyroptosis: A new frontier in cancer. *Biomed Pharmacother*. 2020:121. doi:10.1016/J.BIOPHA.2019.109595
147. Hou J, Zhao R, Xia W, et al. PD-L1-mediated gasdermin C expression switches apoptosis to pyroptosis in cancer cells and facilitates tumour necrosis. *Nat Cell Biol*. 2020;22:1264–1275.
148. Wang L, Qin X, Liang J, et al. Induction of Pyroptosis: A Promising Strategy for Cancer Treatment. *Front Oncol*. 2021:11. doi:10.3389/FONC.2021.635774
149. Tang J, Bei M, Zhu J, et al. Acute cadmium exposure induces GSDME-mediated pyroptosis in triple-negative breast cancer cells through ROS generation and NLRP3 inflammasome pathway activation. *Environ Toxicol Pharmacol*. 2021:87. doi:10.1016/J.ETAP.2021.103686
150. An H, Heo JS, Kim P, et al. Tetraarsenic hexoxide enhances generation of mitochondrial ROS to promote pyroptosis by inducing the activation of caspase-3/GSDME in triple-negative breast cancer cells. *Cell Death Dis*. 2021;12. doi:10.1038/S41419-021-03454-9
151. Pizato N, Luzete BC, Kiffer LFMV, et al. Author Correction: Omega-3 docosahexaenoic acid induces pyroptosis cell death in triple-negative breast cancer cells. *Sci Rep*. 2018;8. doi:10.1038/S41598-018-27850-Y
152. Zhang F, Liu Q, Ganesan K, et al. The Antitriple Negative Breast cancer Efficacy of Spatholobus suberectus Dunn on ROS-Induced Noncanonical Inflammasome Pyroptotic Pathway. *Oxid Med Cell Longev*. 2021:2021. doi:10.1155/2021/5187569
153. Yan H, Luo B, Wu X, et al. Cisplatin induces pyroptosis via activation of meg3/ nlrp3/caspase-1/gsdmd pathway in triple-negative breast cancer. *Int J Biol Sci*. 2021;17:2606–2621.
154. Yan L, Liu Y, Ma XF, et al. Triclabendazole Induces Pyroptosis by Activating Caspase-3 to Cleave GSDME in Breast Cancer Cells. *Front Pharmacol*. 2021;12:670081.
155. Bellio C, Villanueva J. Hitting the brakes on autophagy for overcoming acquired resistance in triple negative breast cancer. *Ann Transl Med*. 2020;8:848–848.
156. Wang RX, Xu XE, Huang L, et al. eEF2 kinase mediated autophagy as a potential therapeutic target for paclitaxel-resistant triple-negative breast cancer. *Ann Transl Med*. 2019;7:783–783.
157. Dolgin E. Anticancer autophagy inhibitors attract "resurgent" interest. *Nat Rev Drug Discov*. 2019;18:408–410.
158. Rebecca VW, Nicastri MC, Fennelly C, et al. PPT1 promotes tumor growth and is the molecular target of chloroquine derivatives in cancer. *Cancer Discov*. 2019;9:220–229.
159. Rontogianni S, Iskit S, Van Doorn S, et al. Combined EGFR and ROCK Inhibition in Triple-negative Breast Cancer Leads to Cell Death Via Impaired Autophagic Flux. *Mol Cell Proteomics*. 2020;19:261–277.
160. Li ZL, Zhang HL, Huang Y, et al. Autophagy deficiency promotes triple-negative breast cancer resistance to T cell-mediated cytotoxicity by blocking tenascin-C degradation. *Nat Commun*. 2020;11. doi:10.1038/s41467-020-17395-y
161. Lawson DA, Bhakta NR, Kessenbrock K, et al. Single-cell analysis reveals a stem-cell program in human metastatic breast cancer cells. *Nat*. 2015;526:131–135.
162. Saxena M, Stephens MA, Pathak H, et al. Transcription factors that mediate epithelial-mesenchymal transition lead to multidrug resistance by upregulating ABC transporters. *Cell Death Dis*. 2011;2. doi:10.1038/cddis.2011.61
163. Ishay-Ronen D, Christofori G. Targeting Cancer Cell Metastasis by Converting Cancer Cells into Fat. *Cancer Res*. 2019;79: 5471–5475.
164. Gupta PB, Pastushenko I, Skibinski A, et al. Phenotypic Plasticity: Driver of Cancer Initiation, Progression, and Therapy Resistance. *Cell Stem Cell*. 2019;24:65–78.

165. Nieto MA. Epithelial plasticity: a common theme in embryonic and cancer cells. *Science*. 2013;342. doi:10.1126/SCIENCE.1234850
166. Ishay-Ronen D, Diepenbruck M, Kalathur RKR, et al. Gain Fat—Lose Metastasis: Converting Invasive Breast Cancer Cells into Adipocytes Inhibits Cancer Metastasis. *Cancer Cell*. 2019;35:17–32.e6.
167. Brasaemle DL. Thematic review series: adipocyte biology. The perilipin family of structural lipid droplet proteins: stabilization of lipid droplets and control of lipolysis. *J Lipid Res*. 2007;48: 2547–2559.
168. Rosen ED, Hsu CH, Wang X, et al. C/EBPα induces adipogenesis through PPARγ: a unified pathway. *Genes Dev*. 2002;16:22.
169. Lefterova MI, Zhang Y, Steger DJ, et al. PPARgamma and C/EBP factors orchestrate adipocyte biology via adjacent binding on a genome-wide scale. *Genes Dev*. 2008;22: 2941–2952.
170. Waldmeier L, Meyer-Schaller N, Diepenbruck M, et al. Py2T Murine Breast Cancer Cells, a Versatile Model of TGFβ-Induced EMT In Vitro and In Vivo. *PLoS One*. 2012;7.
171. Gubelmann C, Schwalie PC, Raghav SK, et al. Identification of the transcription factor ZEB1 as a central component of the adipogenic gene regulatory network. *Elife*. 2014;3(August2014): 1–30.

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between the HER2- *versus* the Basal- and Luminal-Type tumors. Statistically significant differentially expressed genes were identified and further selected based on coefficient of variation (CV) for the most stable genes. Functional annotation and pathway analysis was performed using Gene Ontology and Reactome Pathway Analysis followed by comparison of the new signature with previously reported molecular subtyping signatures using Principal Component Analysis (PCA).

Results: Our approach resulted in an expanded 29-gene HER2-Type signature. The 29 genes had an exceptionally low CV of ~5% indicating that they have a high stability. Among the 29 genes, 7 are from the HER2 amplicon and known to be upregulated in pathologically confirmed HER2+ tumors. Pathways associated with these genes include PI3 K and AKT signaling, which have a key role in cancer development.

Similarly to the BP 80-gene assay, the expanded BP HER2 signature could also better identify the molecularly HER2-Type versus non-HER2-Type tumors, based on the higher percentage of variance captured by PCA, than previously reported molecular subtyping signatures, while having an excellent concordance (97%) with the original 80-gene BP.

Conclusions: The expanded 29-gene BluePrint HER2-Type signature represents even more biological diversity within the HER2-Type tumors, thereby capturing the modern definition of HER2+ tumors. Indeed, the 29 genes include known HER2 amplicon genes and other genes involved in several oncogenic signaling pathways. Importantly, it is yet to be determined whether improved recognition of the HER2-Type increases treatment response prediction with HER2-targeted therapies, which is part of future research.

Conflict of interest:
Other Substantive Relationships: All authors are non-commercial employees of Agendia, the company that markets the 80-gene molecular subtyping assay, known as BluePrint.

240 (PB-064) Poster

A novel biomarker to predict DNA-Repair-inhibitor response in stage I-III high risk breast cancer patients

A. Barcaru[1], M.M. Kuilman[1], D. Wolf[2], C. Yau[3], E.B.M. Choy[1], W.M. Audeh[4], L. Brown-Swigart[2], G.L. Hirst[3], F.W. Symmans[5], M.C. Liu[6], R. Nanda[7], L.J. Esserman[3], L.J. van 't Veer[1], A.M. Glas[1], L. Mittempergher[8]. [1]*I-SPY 2 Investigators, Agendia N. V., Research and Development, Amsterdam, Netherlands;* [2]*University of California San Francisco, Department of Laboratory Medicine, San Francisco, USA;* [3]*University of California San Francisco, Department of Surgery, San Francisco, USA;* [4]*Agendia Inc., Medical Affairs, Irvine, USA;* [5]*University of Texas MD Anderson Cancer Center, Department of Pathology, Houston, USA;* [6]*Mayo Clinic, Department of Surgery, Rochester, USA;* [7]*University of Chicago, Department of Medicine, Chicago, USA;* [8]*Agendia N.V., Research and Development, Amsterdam, Netherlands*

Background: The combinations of PARP inhibitor (PARPi) and platinum-based drugs are gaining more interest as first line therapy for early-stage breast cancer. The I-SPY2 trial (NCT01042379) qualified different DNA-Damage-Repair (DDR) deficiency biomarkers that predict response to DNA damage agents. Here we aimed to translate the I-SPY2 research findings to a robust clinical grade platform signature to predict sensitivity to PARPi and platinum-based chemotherapy.

Material and methods: For this study, 72 fresh frozen pre-treatment biopsies from patients enrolled in the ISPY2 Veliparib+Carboplatin (VC) arm, were analyzed with whole transcriptome microarray following standard diagnostics at Agendia. All 72 patients had a High Risk MammaPrint® 70-gene profile. Pathological complete response (pCR) was defined as no residual invasive cancer in breast or nodes at the time of surgery. From the total set, 27 patients had pCR (5 HR(hormonal receptor)+HER2- 22 Triple Negative (TN)) and 45 had residual disease (RD) (28 HR+HER2- 17 TN). Biomarker development was based on the identification of significantly differentially expressed genes between pCR and RD groups, while balancing the HR status as well as prior knowledge on the biological relevance of the genes (i.e. genes relevant to DDR were prioritized). A leave-one-out cross-validation was employed due to a limited sample size. The significance criteria were based on the absolute value of the effect size (|ES|>0.5). Signature performance was evaluated on the RNAseq data from the carboplatin arm (n = 122) of the BrighTNess trial (NCT01525966), using 8-fold cross validation with support vector classifier.

Results: A set of 60 genes was selected after passing the significance criteria. Large majority of the signature genes (>70%) are related to DDR pathways among which homologous recombination repair, non-homologous end joining repair, Fanconi anemia and other conserved DDR genes. The performance of the biomarker on the development set was 94% accuracy, 96% sensitivity and 93% specificity across all patients. Sensitivity and specificity in the TN group were 95% and 94%, and in HR+HER2- 100% and 93%, respectively. Independent performance assessment using the BrighTNess data set yielded an average of 67% accuracy (with standard deviation σ = 11%), 67% sensitivity (σ = 10%) and 65% specificity (σ = 11%). Relatively large standard deviation pointed to heterogeneity within this cohort.

Conclusion: In the ISPY2 VC arm, RePrint predicts pCR with high accuracy, sensitivity and specificity. The performance on the BrighTNess dataset indicates the potential DRD predictive value of RePrint on RNAseq data. The signature includes genes from various DDR pathways indicating that it may detect patients with DDR deficiency that could be candidate for DNA damage response therapy.

Conflict of interest:
Other Substantive Relationships: Barcaru A, Kuilman M.M., Choy E.B.M., Audeh M.W., van 't Veer L.J., Glas A.M. and Mittempergher L. are non-commercial employees of Agendia.

241 (PB-065) Poster

Single nucleotide polymorphisms of ABCB1 (rs1128503) and ABCC2 (rs145008610) genes and its clinical impact in ER & PR positive breast cancer patients in a tertiary care hospital of India

T. Mistry[1], S. Ghosh[1], P. Sahoo[1], S. Mahata[1], R. Pal[1], S. Sarkar[1], T. Choudhury[1], N. Alam[2], S. Mandal[3], V.D. Nasare[1]. [1]*Chittaranjan National Cancer Institute, Pathology and Cancer Screening, Kolkata, India;* [2]*Chittaranjan National Cancer Institute, Surgical Oncology, Kolkata, India;* [3]*Chittaranjan National Cancer Institute, Epidemiology and biostatistics, Kolkata, India*

Background: Inter-individual differences in drug response are frequent clinical challenge due to genetic variation. ATP-binding cassette (ABC) transporters are crucial determinants of drug disposition and have been studied extensively in response to chemotherapeutic regimen and tamoxifen treatment. But no major findings were established till date in Indian scenario that correlates the effect of drug in ABC polymorphism.

In our study we aim to investigate the impact of ABCB1 (rs1128503) and ABCC2 (rs145008610) gene polymorphisms with reference to the clinical characteristics and adverse drug reactions in hormone receptor positive Breast Cancer (BC) patients who received tamoxifen adjuvant therapy.

Materials and Methods: In this monocentric, observational study, 121 patients were recruited with histologically proven hormone receptor positive BC from surgical OPD of Chittaranjan National Cancer Institute, Kolkata. Tamoxifen therapy (20 mg orally daily till 3 years) was given to the recruited patients after first-line treatment with surgery followed by adjuvant/ neo adjuvant chemotherapy with different regimens administered according to NCCN guidelines. The dose was determined as per patient's BSA value. 5 ml peripheral blood was withdrawn during the treatment to isolate genomic DNA and polymorphism analysis of ABCB1 (167964T>C) and ABCC2 (58626T>C) gene was performed using PCR-RFLP method. PET-CT/ CECT/MRI reports were clinically correlated with genomic data to assess the drug response and adverse drug effect among the attendees.

Results: Majority of the BC patients (n = 121) are diagnosed in stage II (52.9%), 41–60 (57.9%) age group are more prone to develop breast cancer. Infiltrating Ductal Carcinoma (83.5%) found to be the most common pathological subtype, maximally with grade II tumor (58%); tumor size range between >2cm-≤5 cm were most prevalent. In this study, significantly different in response categories among treatment group and significantly unequal survival outcome were seen between responder, non-responder and partial responder (long rank p = 0.225). Median overall survival was achieved within 48 months. Overall response rate was 94.2%. ABCB1 and ABCC2 gene polymorphism is non-significant with clinical parameters. Furthermore, no statistical significance (p > 0.05) was found with adverse events of Chemotherapeutic regimens and tamoxifen adjuvant therapy in contrast to ABCB1 and ABCC2 gene polymorphism.

Conclusion: Our study interprets that, ABCB1 (rs1128503) & ABCC2 (145008610) gene polymorphism may not be a predictor of treatment outcome of patients with respect to hormone positive breast cancer patients. Moreover, transporter genes may not significantly associate with adverse drug reaction thus no effect in overall survival. However, small sample size of our study restricts the statistical power.

No conflict of interest.

patients received all planned cycles of chemotherapy before surgery. Overall pathological complete response (pCR) was seen in 27.3% of patients (47/175). pCR rates were significantly higher in the TNBC cohort compared to HR+ cohort (38.2% vs 15.6 %, P - 0.001). TNBC variant and higher histologic grade (Grade III) were associated with significantly higher pCR rates. At a median follow-up of 30.8 months, 23.2% of patients (40/172) developed relapse (26.9% in TNBC, 19.2 % in HR+). Patients who achieved pCR had significantly lower chances of recurrence compared to those with non-pCR to chemotherapy (12.7% vs 27.2%, P- 0.045).

Conclusions: Triple-negative breast cancer subtype and higher histologic grade are associated with significantly higher pathological complete response to NACT. Achieving complete pathological response is associated with lower risk of breast cancer recurrence, and it is an important surrogate marker for recurrence-free and overall survival.

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45P Demographic determinants of pathological complete response after neoadjuvant chemotherapy in breast cancer

A. Dharanikota

Surgical Oncology, Basavatarakam Indo American Cancer Hospital & Research Institute, Hyderabad, India

Background: Pathologic complete response (pCR) is often utilized as a surrogate marker for overall survival in breast cancer. Significant differences in pCR rates are reported in many studies depending on the biological tumor profile and molecular classification. Based on our observations we hypothesize that few breast cancer patients are less likely to achieve pCR after neoadjuvant chemotherapy (NACT). The role of demographic variables in predicting pCR is still not clear. The aim of this study was to evaluate various demographic factors which could impact pCR rates.

Methods: A prospective analysis of 1246 patients with breast carcinoma who had undergone neoadjuvant chemotherapy (NACT) followed by surgery was done from June 2020 to December 2022. Demographic, surgical, and pathological data were collected on completion of therapy. Categorical variables were analyzed using $\chi 2$ or Fisher's exact test and continuous data were analyzed using t-tests. Multiple linear regressions were used to study interactions between various demographic factors and pCR. Statistical analysis was done using SPSS v25.

Results: A total of 1324 patients were offered NACT, of which 1246 (94.1%) who underwent resection post-NACT were included in the analysis. Overall, 275 (22.1%) patients had pCR. 39 (14.2%) in ER+/HER2- group, 131 (47.6%) in HER2+ group; and 105 (38.2%) in ER-/HER2- group had pCR. Univariate analysis showed significant association between age $<$50 years, low body-mass index, and ability to achieve pCR. However, women with obesity had higher odds of residual disease (OR $=$ 0.191 [0.029-1.157]; p=0.076). The results were consistent even after controlling for confounding variables such as grade, receptor status, and clinical T and N stages.

Conclusions: Younger age can predict a pCR and is an independent prognostic factor for locoregional recurrence in locally advanced breast cancer patients after NACT. Obesity is a risk factor for failure to achieve PCR in women undergoing NACT. The contrary was observed in non-obese patients as they had higher odds of achieving PCR. Studies with larger groups are needed to validate this observation. Further studies evaluating the role of BMI in drug resistance would be valuable.

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46P Predicting toxicity following cancer chemotherapy by detecting transporter gene ABCB1 (C1236T, G2677T/A, C3435CT) polymorphism in breast cancer patients receiving chemotherapy with anthracycline and taxane either sequentially or concomitantly

T. Mistry[1], R. Pal[1], S. Ghosh[1], P. Nath[2], N. Alam[3], V.D. Nasare[1]

[1]Pathology and Cancer Screening, Chittaranjan National Cancer Institute, Kolkata, India; [2]Medical Oncology, Chittaranjan National Cancer Institute, Kolkata, India; [3]Surgical Oncology, Chittaranjan National Cancer Institute, Kolkata, India

Background: ABCB1 gene reported to affect chemo-induced toxicity through drug disposition and efflux mechanisms. This study aims to evaluate the influence of ABCB1 (C1236T, G2677T/A, C3435T) polymorphisms on, chemotoxicity, and survival outcomes in BC patients treated with anthracycline and taxane either sequentially or concomitantly.

Methods: 100 patients were included who received sequential or concomitant anthracycline and taxane as per clinician discretion and underwent modified radical mastectomy. Chemotoxicity grade was analyzed by CTCAE v.4. All patients received standard premedication with $5HT_3$, NK1 receptor antagonist, dexamethasone, H2 blocker/ PPI. SNP of ABCB1 (C1236T, G2677T/A, C3435T) gene was detected by PCR-RFLP and sequencing.

Results: Patients mostly reported at Stage III (52.89%) with infiltrating ductal carcinoma subtype (90.9%) who received sequential (50%) & concomitant (50%) chemotherapy. Grade $\geq$2 chemo toxicity like neutropenic fever, significantly associated with C1236T SNP with odd ratio (OR) for TT genotypes was 2.00 (95% CI: 0.125-31.975, p=0.000), while in CT genotypes, it stood at 1.100 (95% CI: 0.063-15.988; p $=$ 0.035). Meanwhile, vomiting was significantly associated with C3435T for TT genotypes, with an OR of 3.031 (95% CI: 0.031-7.994, p=0.05). TT genotypes (C1236T) were significantly associated with nausea (OR: 2.667; 95% CI: 1.043-6.815; p $=$ 0.04). Alopecia was observed 88% patients. However, ABCB1 showed no significant (p $=$ 0.416, Log-rank) correlation for overall survival. Although, no significant associations were found for G2677T/A in terms of toxicity and survival.

Conclusions: The homozygous mutant TT genotypes (C1236T and C3435T) and heterozygous CT genotypes (C1236T) showed significant association to chemo-induced toxicity (hematological toxicity, nausea, vomiting, alopecia) in patients underwent anthracycline and taxane. Hence, these SNPs could serve as predictive markers to mitigate chemotherapy's adverse effects and optimize treatment to reduce the grade of toxicity and improves patients quality of life.

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47P Sequencing of chemotherapy and surgery among older triple-negative and HER2-positive breast cancer patients with comorbidities

A. Dharanikota

Surgical Oncology, Basavatarakam Indo American Cancer Hospital & Research Institute, Hyderabad, India

Background: Studies have shown disparities in management of older patients with breast cancer, resulting in undertreatment. Older patients with co-morbidities pose a challenge for cancer care providers who must balance the risk of death due to toxicity from treatment versus the benefit of standard multimodality treatment. The aim of the study was to evaluate whether the sequencing of chemotherapy and surgery impact the ability to deliver both modalities of treatment.

Methods: A retrospective analysis of prospective data was evaluated between 2018 and 2022. We included patients $>$60 years of age with chronic comorbidities, with a clinical stage T1c-3 and N0-3, HER2-positive or triple-negative invasive breast cancer treated with chemotherapy alone, surgery alone, or both surgery and chemotherapy. Kaplan-Meier curves were plotted to compare the survival outcomes. Statistical analysis was done using SPSS v25.

Results: A total of 821 patients met the inclusion criteria, of whom 85.9% (N=705) underwent surgery as the initial treatment. Among patients who received chemotherapy first (N=116), 73.3% (N=85) were able to complete subsequent surgery. Factors associated with completion surgery after chemotherapy were younger age and clinical node-negative status. Among patients treated with surgery first, only 36.3% (N=256) received adjuvant chemotherapy. Among patients who received both modalities of treatment (N=341), women with more advanced stage tumors and those diagnosed in recent years were more likely to receive neoadjuvant chemotherapy. With a median follow-up of 19.3 months, cN0 patients who underwent both surgery and chemotherapy had significantly better overall survival compared to patients who received single modality of treatment.

Conclusions: In older, triple-negative or HER2-positive breast cancer patients with comorbidities, receipt of chemotherapy and surgery was associated with improved survival. Neoadjuvant chemotherapy group were twice as likely to receive both modalities of treatment than those undergoing surgery first. A multidisciplinary approach to evaluate geriatric patients with comorbidities is essential to deliver appropriate treatment and improve outcomes in this vulnerable population.

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Gene

Low BMI and ABCC2 Genotype Associated with Poor Clinical Response among Sequential Anthracycline-Taxane Chemotherapy Receiving Breast Cancer Patients: A Tertiary Care Hospital-Based Study

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Corresponding Author:	Vilas D. Nasare, Ph.D. Chittaranjan National Cancer Institute Kolkata, West Bengal INDIA
First Author:	Tanuma Mistry, M.Sc
Order of Authors:	Tanuma Mistry, M.Sc
	Sanghamitra Sengupta, PhD
	Ram Suresh Kumar, PhD
	Nisha Thakur, PhD
	Partha Nath, MBBS
	Neyaz Alam, MBBS, MS
	Vilas D. Nasare, Ph.D.
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Abstract:	Sequential anthracycline-taxane chemotherapy remains the standard treatment for BC. However, significant inter-individual variability in treatment response and toxicity persists. Notably, there was a significant gap in research addressing the challenges faced by patients with low BMI, whose transporter gene variations may influence therapeutic outcomes. This study investigates combine effect of low BMI and possible pharmacogenetic influence of ABC gene polymorphisms in treatment responses of BC patients. Nutritional parameter (BMI) was analysed prior to commencement of chemotherapy. Clinical response was evaluated by radiological imaging and categorised as per RECISTv.1 criteria. SNPs(C1236T, C3435T, C58626A) located in ABCB1 and ABCC2 gene were selected. 148 samples were analysed using PCR-RFLP. Appropriate statistical methods were employed to perform the association analysis. ABCC2(58626AA) was significantly associated with treatment non-responsiveness in all genetic models namely dominant(OR:2.954;[1.442-6.051];p=0.003), recessive(5.723[2.48-13.20];p<0.0001), codominant(2 21.219;p<0.0001). The proportion of ORR and NRs were significantly different between low(<18.5) and high(≥18.5) BMI classes (16.097[7.12-36.35]; p<0.0001). Furthermore, when treatment response was combined with BMI groups, significant associations were observed for C58626A SNP across all genetic models for low BMI group: dominant (3.324[1.012-10.406]; p=0.041), recessive (7.250[1.533-34.278];p=0.012) and codominant (28.657;p=0.013). Both PFS(35.31 months;p=0.005) and OS(39.75 months;p=0.032) were lowered among 58626AA genotype while the hazard risk of this genotype was further increased in low BMI patients(HR:1.936). 3435CT genotypes in ABCB1 gene showed 87% reduction in risk of death (HR 0.13;p=0.025). Low BMI independently and jointly with 58626AA genotype of ABCC2 gene was responsible for poor chemotherapy response and survival outcome among AC-T regimen receiving BC patients. Together, this study underscores the importance of genetic counselling and nutritional assessment for favourable treatment outcomes.