Volume 3, Issue 10, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

ROLE OF MIRNA PROFILING IN PREDICTING CHEMOTHERAPY RESPONSE IN BREAST CANCER

Maqsood Razzaq[*1], Zeeshan Ali[2], Ayesha[3], Noor Ul Ain[4], Ayesha Asif[5], Iqra[6], Ali Ata Ur Rehman[7]

[*1]*Department of Genetics, University of Central Punjab, Lahore, Pakistan.*
[2]*MS Biotechnology, University of Sialkot, Pakistan*
[3]*MPhil Pharmacy Practice, Government College University Faisalabad, Pakistan.*
[4]*PhD, Assistant Professor, Mukabbir University of Science and Technology, Gujrat, Pakistan.*
[5]*Medical Biotechnology, University of Eastern Piemonte, Italy*
[6]*MPhil Biotechnology, Ziauddin University Faculty of Engineering Science and Technology, Karachi, Pakistan.*
[7]*MBBS, Liaquat College of Medicine and Dentistry, Karachi, Pakistan*

[2]ORCID: 0009-0004-9637-306X, [3]ORCID: 0000-0001-8774-072X,
[4]ORCID: 0000-0003-0423-2661, [7]ORCID: 0009-0002-4327-950X

DOI: https://doi.org/10.5281/zenodo.18030427

Keywords

breast cancer; microRNA; circulating miRNAs; chemotherapy response; neoadjuvant chemotherapy; precision oncology; predictive biomarker; pathological complete response; Pakistan; randomized controlled trial

Article History

Received: 13 October 2025
Accepted: 23 November 2025
Published: 19 December 2025

Copyright @Author

Corresponding Author: *
Maqsood Razzaq

Abstract

Objective

This randomized controlled trial evaluated whether baseline microRNA (miRNA) profiling predicted chemotherapy response among women with breast cancer treated at a tertiary care hospital in Pakistan, and whether integrating a validated miRNA signature into clinical decision-making improved pathological and radiological treatment outcomes.

Methods

Women aged 18–70 years with newly diagnosed, stage II–III, non-metastatic breast cancer who were planned for anthracycline–taxane-based neoadjuvant chemotherapy were enrolled and randomized 1:1 to an miRNA-guided arm or a standard-care arm. In both arms, plasma was collected at baseline and after two chemotherapy cycles for profiling of a predefined panel of 24 circulating miRNAs selected from prior literature (including miR-21-5p, miR-34a-5p, miR-155-5p, let-7 family members, and miR-210-3p).

In the intervention arm, treating oncologists received a report categorizing patients as "predicted responder" or "predicted non-responder" based on a multivariable logistic model derived in an internal pilot cohort; treatment could be escalated or switched in predicted non-responders. In the control arm, clinicians were blinded to miRNA data and managed patients according to institutional protocols. The primary endpoint was pathological complete response (pCR) in breast and axilla. Key secondary endpoints were radiologic response rate, progression-free survival (PFS), and the discriminative performance (area under the receiver operating characteristic curve, AUC) of the miRNA signature for pCR and radiologic response.

Results

A total of 228 participants were randomized (miRNA-guided arm, n = 114; standard-care arm, n = 114). Baseline clinicopathologic characteristics, including

Volume 3, Issue 10, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

age, molecular subtype, and stage, were balanced between arms. The composite 8-miRNA signature (miR-21-5p, miR-34a-5p, miR-155-5p, miR-210-3p, miR-221-3p, miR-26a-5p, miR-195-5p, and let-7d-5p) showed independent association with pCR after adjustment for subtype, grade, and Ki-67. In the overall cohort, the signature predicted pCR with an AUC of 0.79 (95 % CI 0.73–0.85) and predicted radiologic partial/complete response with an AUC of 0.76 (95 % CI 0.70–0.82). In the miRNA-guided arm, 41.2 % of patients achieved pCR compared with 27.2 % in the standard-care arm (risk difference 14.0 %, 95 % CI 3.0–25.1; p = 0.014). Predicted non-responders who underwent early regimen modification experienced higher pCR rates than historical non-responders in the control arm and had improved 2-year PFS (HR for progression or death 0.61, 95 % CI 0.40–0.94). Safety profiles and rates of grade 3–4 toxicity were comparable between arms.

Conclusion

In this tertiary Pakistani cohort, circulating miRNA profiling provided independent and clinically meaningful prediction of chemotherapy response in breast cancer and, when prospectively integrated into treatment decisions, modestly improved pCR and short-term PFS without increasing toxicity. These findings supported further validation of the 8-miRNA signature and highlighted the feasibility of incorporating liquid-biopsy–based molecular tools into routine breast cancer care in low- and middle-income settings.

INTRODUCTION

Breast cancer remained the most frequently diagnosed malignancy among women worldwide and continued to be a leading cause of cancer-related mortality. In 2020, an estimated 2.3 million women were diagnosed with breast cancer and approximately 685,000 died from the disease, accounting for about one in four new cancer cases and one in six cancer deaths among women. Low- and middle-income countries (LMICs), including those in South-Central Asia, experienced a disproportionate burden of mortality due to late-stage presentation, resource constraints, and limited access to timely, high-quality systemic therapy.

In Pakistan, breast cancer constituted the most common malignancy in women and carried one of the highest incidence rates in Asia. National and international estimates suggested that nearly one in nine Pakistani women developed breast cancer in their lifetime; roughly 90,000 new cases and 40,000 deaths were occurring annually, with the majority of patients presenting in stage III or IV disease. The combination of late diagnosis, constrained oncology infrastructure, and limited implementation of population-level screening programs resulted in poor survival compared with high-income countries. Tertiary care hospitals in major Pakistani cities therefore carried a critical responsibility for delivering increasingly complex systemic therapies and for leading locally relevant translational research to improve outcomes.

Chemotherapy response as a key determinant of outcome

For patients with stage II–III breast cancer, systemic chemotherapy, given either in the neoadjuvant or adjuvant setting, remained a cornerstone of curative-intent treatment. In the neoadjuvant context, chemotherapy downstaged tumors, increased rates of breast-conserving surgery, and provided an in vivo assessment of chemosensitivity. Pathological complete response had been consistently associated with favorable long-term outcomes, particularly in triple-negative and HER2-positive subtypes.

Despite these advantages, only a subset of patients achieved pCR with standard anthracycline–taxane regimens. Many patients were exposed to the full toxicity burden of chemotherapy without substantial tumor

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

shrinkage, contributing to treatment-related morbidity, financial toxicity, and persistent risk of relapse. Existing clinicopathologic predictors of response, such as hormone receptor status, HER2 status, histologic grade, and proliferation indices, provided useful but incomplete information and did not fully capture the molecular heterogeneity that underpinned chemosensitivity and resistance. S

Molecular assays such as multigene expression panels improved risk stratification for endocrine therapy decisions in early-stage, hormone receptor–positive breast cancer; however, their role in predicting cytotoxic chemotherapy response across subtypes remained constrained, and their cost limited widespread adoption in LMIC settings such as Pakistan. There was therefore a clear need for robust, affordable biomarkers that predicted chemotherapy response early and could be feasibly deployed in routine oncology practice in resource-limited environments.

microRNAs as emerging predictive biomarkers

MicroRNAs are small (approximately 18–25 nucleotides), endogenous, non-coding RNAs that regulate gene expression post-transcriptionally by binding target messenger RNAs and modulating their stability and translation. Aberrant miRNA expression had been implicated in all hallmarks of cancer, including proliferation, invasion, metastasis, and evasion of cell death. In breast cancer, distinct miRNA expression patterns characterized molecular subtypes and correlated with tumor grade, hormone receptor status, and metastatic potential.

Circulating miRNAs, encapsulated within exosomes or bound to protein complexes, displayed remarkable stability in blood and other body fluids, making them attractive minimally invasive biomarkers. Several studies and reviews demonstrated that circulating and tissue miRNAs held diagnostic, prognostic, and predictive value in breast cancer. Panels including miR-21-5p, miR-34a-5p, miR-155-5p, miR-195-5p, and miR-210-3p, among others, had been linked to tumor burden, nodal status, and survival outcomes.

In the specific context of chemotherapy and neoadjuvant therapy, accumulating evidence suggested that miRNA dysregulation contributed directly to drug resistance through modulation of apoptosis pathways, DNA damage response, epithelial–mesenchymal transition, and drug efflux transporters. Multiple small observational studies reported associations between baseline or dynamic changes in miRNAs such as miR-21, miR-7, miR-34a, miR-221/222, and miR-451 and pathological response to neoadjuvant chemotherapy. A systematic review and meta-analysis concluded that dozens of miRNAs showed potential relationships with neoadjuvant chemotherapy response and prognosis, although heterogeneity across cohorts, methods, and response definitions limited the clinical translation of these findings.

Recent work further emphasized that circulating miRNA signatures, rather than single miRNAs, might provide more robust prediction of neoadjuvant response. Multimarker signatures had been developed and tested in European, Chinese, and Latin American cohorts, with reported AUC values ranging from approximately 0.70 to 0.85 for pCR prediction. However, the majority of these studies used prospective cohort or case–control designs without randomization, and few, if any, evaluated whether acting on miRNA information in real time altered treatment decisions or improved outcomes.

Evidence gaps in LMIC and South Asian settings

Most miRNA–chemosensitivity studies had been conducted in high-income countries with relatively homogeneous populations and mature diagnostic infrastructure. There was a marked under-representation of South Asian and Pakistani cohorts, despite the region's high and rising breast cancer burden. Genetic and environmental differences, including distinct distributions of BRCA and other susceptibility variants, reproductive patterns, and lifestyle factors, could influence miRNA expression profiles and treatment response, raising concerns about the external validity of signatures derived elsewhere when applied to Pakistani patients.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

In addition, the practical feasibility of implementing circulating miRNA profiling in routine oncology workflows in Pakistan remained uncertain. Most tertiary hospitals operated under significant resource constraints, with high patient volumes, limited access to advanced molecular platforms, and fragmented reimbursement mechanisms. Demonstrating that a focused, clinically actionable miRNA panel could be measured reliably in a local laboratory, interpreted within a standardized algorithm, and returned to clinicians in time to influence decisions would be a critical step toward clinical translation.

Finally, even where strong associations between miRNA levels and chemotherapy response were reported, the majority of previous studies had not integrated these biomarkers into a trial design that prospectively tested clinical utility. From a precision oncology perspective, mere prognostic or predictive association was insufficient; evidence was needed that biomarker-informed treatment strategies improved outcomes relative to current standard practice.

Rationale and objectives of the present trial

Against this backdrop, the present randomized controlled trial was conceived within a tertiary care academic hospital in Pakistan as both a biomarker validation study and a pragmatic test of clinical utility. Building on prior literature that identified candidate circulating miRNAs associated with neoadjuvant chemotherapy response, the investigative team first derived, in an internal pilot cohort, a multivariable signature based on a subset of miRNAs that were measurable using locally available technology and analytically robust in plasma samples.

The full trial then aimed to evaluate this signature in a randomized framework in which all eligible women with stage II–III breast cancer who were planned for anthracycline–taxane neoadjuvant chemotherapy underwent baseline circulating miRNA profiling, but only clinicians in the intervention arm received the resulting risk stratification. This design allowed the team to answer two linked questions:

1. **Predictive performance:** To what extent did the circulating miRNA signature predict pathological complete response and radiologic response above and beyond standard clinicopathologic factors in this Pakistani cohort?
2. **Clinical utility:** Did access to miRNA-based risk stratification, coupled with protocolized options for treatment escalation or early regimen switching in predicted non-responders, translate into higher pCR rates and improved short-term progression-free survival compared with standard care?

The central hypothesis was that a parsimonious panel of circulating miRNAs, measured at baseline, would provide independent and clinically meaningful prediction of chemotherapy response and that its integration into decision-making would improve pCR without materially increasing serious toxicity.

Accordingly, this article reported the design and main findings of the trial, focusing on the performance of the miRNA signature, its impact on treatment allocation and outcomes, and the implications for implementing liquid-biopsy–based precision oncology strategies within tertiary cancer centers in Pakistan and similar LMIC settings.

MATERIALS AND METHODS

Study design and setting

This was a single-center, parallel-group randomized controlled trial conducted in the Department of Medical Oncology of a tertiary-care teaching hospital in Pakistan. The trial compared an miRNA-guided treatment strategy with standard-of-care management in women with stage II–III breast cancer receiving neoadjuvant chemotherapy.

The study team first completed an internal pilot phase to finalize the candidate miRNA panel and the prediction algorithm; the randomized phase then enrolled consecutive eligible patients over a defined recruitment period. All procedures, including blood collection, chemotherapy delivery, imaging, surgery, and pathological assessment, were performed within the same institution to minimize variability.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Study population

Eligibility criteria

Women were considered eligible if they met all of the following criteria:

- Age between 18 and 70 years
- Newly diagnosed, histologically confirmed invasive breast carcinoma
- Clinical stage II or III disease based on physical examination and imaging (ultrasound and/or MRI, with staging CT or bone scan as indicated)
- No evidence of distant metastasis at baseline
- Planned for anthracycline–taxane-based neoadjuvant chemotherapy according to institutional protocols
- Eastern Cooperative Oncology Group (ECOG) performance status 0–2
- Adequate hematologic and organ function (hemoglobin ≥ 10 g/dL, absolute neutrophil count ≥ 1.5×10^9/L, platelets ≥ 100×10^9/L, serum creatinine ≤ 1.5 × upper limit of normal, transaminases ≤ 2.5 × upper limit of normal)

Patients were excluded if they had any of the following:

- Prior systemic therapy, radiotherapy, or surgery for the current breast cancer
- Bilateral disease requiring different systemic strategies
- Known pregnancy or lactation
- Active uncontrolled infection or serious comorbid illness precluding chemotherapy
- History of another invasive malignancy within the previous five years (except non-melanoma skin cancer or in situ cervical carcinoma)
- Inability or unwillingness to provide written informed consent

Eligible patients were identified in the multidisciplinary breast clinic and inpatient wards. A trained research coordinator explained the study objectives, procedures, and potential risks and obtained written informed consent before any trial-specific procedure was performed.

Screening, baseline assessment, and staging

At screening, demographic and clinical data were recorded, including age, menopausal status, family history of malignancy, comorbidities, and medication use. Baseline breast imaging (mammography and ultrasound, with MRI in selected cases) was reviewed, and two-dimensional tumor measurements were documented.

Core needle biopsy specimens were obtained from the primary tumor before initiation of chemotherapy. Histologic type and grade were assessed by board-certified pathologists. Estrogen receptor (ER), progesterone receptor (PR), and HER2 status were determined using immunohistochemistry and in situ hybridization as per institutional standards. Ki-67 proliferation index was documented when available.

Systemic staging was carried out according to local practice, typically including chest imaging (X-ray or CT scan), abdominal ultrasound or CT scan, and bone scan in patients with suggestive symptoms or advanced stage. Patients with radiological or histological evidence of distant metastases were not enrolled.

Randomization and allocation concealment

After confirmation of eligibility and collection of baseline blood samples for miRNA profiling, participants were randomized in a 1:1 ratio to the miRNA-guided arm or the standard-care arm.

The random allocation sequence was generated by an independent statistician using a computer-based random number generator with variable block sizes. Stratification factors included clinical stage (II vs III) and biological subtype (HR+/HER2−, HER2+, and triple-negative) to ensure balanced distribution of key prognostic factors across the two arms.

Allocation was concealed through sequentially numbered, opaque, sealed envelopes prepared by the statistician who was not involved in patient recruitment or treatment. The research coordinator opened the next envelope in sequence only after the patient had been enrolled and baseline samples had been drawn.

Blinding

Because treatment decisions in the intervention arm could be modified according to miRNA results, the trial was open-label with respect to patients and treating oncologists.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

However, several safeguards were implemented to limit bias:

- Laboratory personnel who processed blood samples and generated miRNA data were blinded to treatment allocation and clinical outcomes.
- Radiologists who assessed radiologic tumor response were blinded to randomization arm and miRNA categorization.
- Pathologists evaluating surgical specimens for pathological response were also unaware of allocation and miRNA status.
- The primary statistical analysis was performed on a dataset in which treatment allocation was coded, and group labels were revealed only after the main analysis plan had been executed.

Treatment protocols

All participants received neoadjuvant chemotherapy according to institutional standards, which were aligned with national and international guidelines. The default regimen consisted of:

- Four cycles of an anthracycline-based combination (e.g., doxorubicin 60 mg/m² plus cyclophosphamide 600 mg/m², or epirubicin 90 mg/m² plus cyclophosphamide 600 mg/m²) administered intravenously every 3 weeks, followed by
- Four cycles of a taxane (e.g., paclitaxel 80 mg/m² weekly for 12 weeks, or docetaxel 75 mg/m² every 3 weeks), as per treating physician preference and patient factors.

For HER2-positive tumors, trastuzumab (with or without pertuzumab depending on availability and affordability) was added to the taxane component according to institutional policy. Hormone receptor–positive patients did not receive endocrine therapy during the neoadjuvant phase.

Antiemetic prophylaxis and supportive care were provided as per standard practice. Chemotherapy doses and schedules could be modified, delayed, or discontinued in response to toxicity or clinical circumstances at the discretion of the treating oncologist, with reasons documented in the case report form.

Treatment modification according to miRNA prediction

In the standard-care arm, treating clinicians were blinded to miRNA results and managed patients purely according to clinical assessment, imaging response, and toxicity.

In the miRNA-guided arm, a prespecified algorithm was applied:

1. Baseline miRNA profiling generated an individual risk score and categorized patients as "predicted responder" or "predicted non-responder" for achieving pCR with standard anthracycline–taxane therapy.
2. For predicted responders, standard neoadjuvant chemotherapy was administered without planned modification.
3. For predicted non-responders, the oncologist was encouraged (but not mandated) to consider early escalation or regimen change after the second cycle of anthracycline, guided by both clinical and early radiologic assessment. Options included:
   - Intensification of the taxane component (e.g., switching from 3-weekly to weekly dosing),
   - Addition or earlier introduction of HER2-targeted therapy where appropriate,
   - Introduction of platinum agents in selected high-risk triple-negative tumors, subject to kidney function and performance status.

The decision and chosen modification were documented, allowing analysis of adherence to the algorithm and its impact on outcomes.

miRNA sampling and laboratory procedures

Blood collection and processing

Peripheral venous blood (10 mL) was drawn into EDTA tubes at two time points:

1. Before the first cycle of neoadjuvant chemotherapy (baseline), and
2. After completion of the second chemotherapy cycle, just before the third cycle (early on-treatment sample).

To minimize pre-analytical variability, samples were gently inverted, transported to the molecular laboratory within 1 hour of collection, and processed within 2 hours. Blood was

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

centrifuged at 1,600 g for 10 minutes at 4 °C to separate plasma, followed by a second centrifugation at 16,000 g for 10 minutes to remove residual cellular debris. Plasma aliquots were stored at −80 °C until RNA extraction.

RNA extraction and miRNA quantification

Total RNA enriched for small RNAs was extracted from a fixed volume of plasma using a commercially available kit validated for circulating miRNA isolation, following the manufacturer's instructions. A synthetic non-human miRNA spike-in was added to each sample prior to extraction to monitor technical variability.

Reverse transcription was performed using stem-loop primers specific for the selected miRNAs. Quantitative real-time PCR (qRT-PCR) was carried out on a dedicated real-time thermal cycler using predesigned assays for each miRNA in the panel. All reactions were performed in duplicate, and any sample with poor amplification curves or discordant replicates was repeated.

The predefined panel contained 24 miRNAs identified from prior literature as potentially relevant to breast cancer biology and chemotherapy response, with emphasis on those consistently reported across multiple cohorts. From this panel, 8 miRNAs (miR-21-5p, miR-34a-5p, miR-155-5p, miR-210-3p, miR-221-3p, miR-26a-5p, miR-195-5p, and let-7d-5p) were ultimately retained in the predictive model based on the internal pilot analysis.

Cycle threshold (Ct) values were normalized using a combination of endogenous reference miRNAs that showed stable expression across samples, along with the synthetic spike-in to flag technical issues. Relative expression levels were calculated using the 2^-ΔCt or 2^-ΔΔCt method, as appropriate.

Development of the miRNA prediction model

Before initiating the randomized phase, an internal pilot cohort of patients meeting the same eligibility criteria was used to develop the prediction model. Logistic regression analysis identified the subset of miRNAs and clinical variables that independently predicted pCR.

The final model included the 8-miRNA signature and selected clinicopathologic covariates. Regression coefficients were used to generate an individual risk score for pCR. A cutoff value for categorizing patients as predicted responders or predicted non-responders was chosen to balance sensitivity and specificity, with particular emphasis on maintaining high negative predictive value to minimize the risk of labelling likely responders as non-responders.

This model and cutoff were fixed before the start of enrollment in the main randomized trial. For patients in the miRNA-guided arm, the risk category was reported to the treating oncologist in a standardized format within a predefined timeframe (typically within 7–10 days of baseline blood draw), allowing information to be available before the second chemotherapy cycle.

Outcome measures

Primary endpoint

The primary endpoint was pathological complete response (pCR), defined as the absence of residual invasive carcinoma in both breast and axillary lymph nodes at the time of definitive surgery (ypT0/is, ypN0). Residual ductal carcinoma in situ alone was allowed within the definition of pCR.

All surgical specimens were processed according to institutional protocols. Pathologists, blinded to treatment arm and miRNA status, documented tumor size, nodal involvement, and the presence or absence of invasive disease. pCR status was abstracted from final pathology reports.

Secondary endpoints

Key secondary endpoints included:

- Radiologic response rate, categorized according to Response Evaluation Criteria in Solid Tumors (RECIST 1.1) based on serial imaging (ultrasound and/or MRI) performed at baseline, after completion of anthracycline cycles, and before surgery. Responses were classified as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD).
- Progression-free survival (PFS), defined as the interval from randomization to the first event of disease progression, locoregional recurrence,

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

distant metastasis, or death from any cause, whichever occurred first.

- Overall clinical response rate (CR + PR) at the end of neoadjuvant chemotherapy.
- Safety and tolerability, as assessed by the incidence of grade 3–4 hematologic and non-hematologic toxicities, treatment delays, and dose reductions, graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version in use at the time.
- Diagnostic performance of the miRNA signature (AUC, sensitivity, specificity, positive and negative predictive values) for predicting pCR and radiologic response.

Follow-up schedule and data collection

Patients were evaluated clinically before each chemotherapy cycle. At each visit, the treating oncologist recorded performance status, vital signs, physical examination findings, and treatment-related adverse events. Laboratory tests, including complete blood count and serum biochemistry, were performed as per standard care.

Imaging for response assessment was generally performed after completion of the anthracycline component and again after the taxane component, or earlier if there was clinical suspicion of progression. After surgery, patients were followed in the outpatient clinic at intervals defined by institutional practice (for example, every 3–4 months during the first two years and then every 6–12 months). At each follow-up visit, recurrence status and survival were recorded.

Study data were captured on standardized case report forms and then entered into a secure electronic database. Data entry included built-in validation checks, and periodic data quality audits were carried out by the study monitoring team.

Sample size calculation

The sample size was estimated based on the primary endpoint of pCR. At the time of trial design, historical data from the same institution suggested that pCR with standard anthracycline–taxane neoadjuvant chemotherapy was approximately 25–30 % in an unselected population with stage II–III disease. The investigators considered an absolute increase of 15 percentage points in pCR (from 25–30 % to about 40–45 %) in the miRNA-guided arm to be clinically meaningful.

Assuming a two-sided alpha of 0.05, 80 % power, and a pCR rate of 28 % in the standard-care arm and 43 % in the miRNA-guided arm, the required sample size was calculated to be approximately 206 evaluable patients (103 per group). To account for potential dropouts, missing data, or loss to follow-up, the planned enrollment target was increased by around 10 %, resulting in a final target sample size of 228 participants.

Statistical analysis

All primary analyses were conducted according to the intention-to-treat principle, including all randomized patients in the groups to which they were assigned, regardless of adherence to the intervention or completion of chemotherapy. A per-protocol analysis was performed as a sensitivity analysis, excluding major protocol deviations such as non-receipt of neoadjuvant chemotherapy or withdrawal of consent before any treatment.

Categorical variables were summarized as counts and percentages; continuous variables were described using means and standard deviations or medians and interquartile ranges, as appropriate. Baseline characteristics were compared between groups using the chi-square test or Fisher's exact test for categorical variables and the independent t-test or Mann–Whitney U test for continuous variables.

The primary comparison of pCR rates between the two arms used the chi-square test, with risk difference and 95 % confidence intervals reported. Logistic regression models were fitted to assess the independent association between treatment arm, miRNA risk category, and pCR, adjusting for key covariates (age, clinical stage, biological subtype, and grade).

To evaluate the discriminative performance of the miRNA signature, receiver operating characteristic (ROC) curves were constructed for pCR and radiologic response, and the area under

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

the curve (AUC) with 95 % confidence intervals was estimated. Comparisons of AUCs with and without the miRNA signature were performed to assess incremental predictive value.

Time-to-event outcomes such as PFS were analyzed using the Kaplan–Meier method, with differences between groups assessed using the log-rank test. Cox proportional hazards models were used to estimate hazard ratios and 95 % confidence intervals, adjusting for relevant prognostic factors. The proportional hazards assumption was evaluated by inspection of log–log plots and time-dependent covariates where needed.

Two-sided p values < 0.05 were considered statistically significant. Statistical analyses were performed using standard statistical software packages available at the institution.

RESULTS

Participant enrollment and baseline characteristics

A total of 276 women were screened for eligibility during the recruitment period. Of these, 228 met the inclusion criteria, provided informed consent, and were randomized in a 1:1 ratio to the miRNA-guided arm (n = 114) or the standard-care arm (n = 114). Forty-eight women were excluded during screening due to metastatic disease (n = 19), inadequate organ function (n = 11), serious comorbid illness (n = 7), or withdrawal of consent before baseline sampling (n = 11).

All 228 randomized participants contributed data to the intention-to-treat analysis. Six patients (three in each arm) discontinued chemotherapy prematurely because of toxicity or personal reasons, and four patients (two in each arm) underwent surgery earlier than planned because of inadequate tolerance. These deviations were documented and included in secondary per-protocol comparisons.

Baseline demographic and clinical characteristics were well balanced between the two groups. The median age of the cohort was 49 years (interquartile range 42–56). Approximately 64 percent of patients were premenopausal. Clinical stage III disease accounted for 58 percent in the miRNA-guided arm and 56 percent in the standard-care arm. Distribution of tumor subtypes was similar between groups: hormone receptor–positive/HER2–negative (48 percent), HER2-positive (27 percent), and triple-negative (25 percent). Mean baseline tumor size and nodal status were also comparable. No meaningful imbalances were observed in performance status, comorbidity burden, or laboratory parameters.

Table 1. Baseline Characteristics of the Study Population (N = 228)

Variable	miRNA-Guided Arm (n = 114)	Standard-Care Arm (n = 114)	p-value
Median age, years (IQR)	49 (42–56)	48 (41–55)	0.62
Premenopausal, n (%)	72 (63.2)	73 (64.0)	0.89
Clinical Stage II, n (%)	48 (42.1)	50 (43.9)	0.78
Clinical Stage III, n (%)	66 (57.9)	64 (56.1)	0.78
HR+/HER2− subtype, n (%)	55 (48.2)	54 (47.4)	0.91
HER2+ subtype, n (%)	31 (27.2)	30 (26.3)	0.87
Triple-negative subtype, n (%)	28 (24.6)	30 (26.3)	0.75
Median tumor size (cm)	4.6	4.7	0.81
ECOG 0–1, n (%)	102 (89.5)	101 (88.6)	0.82

Volume 3, Issue 10, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

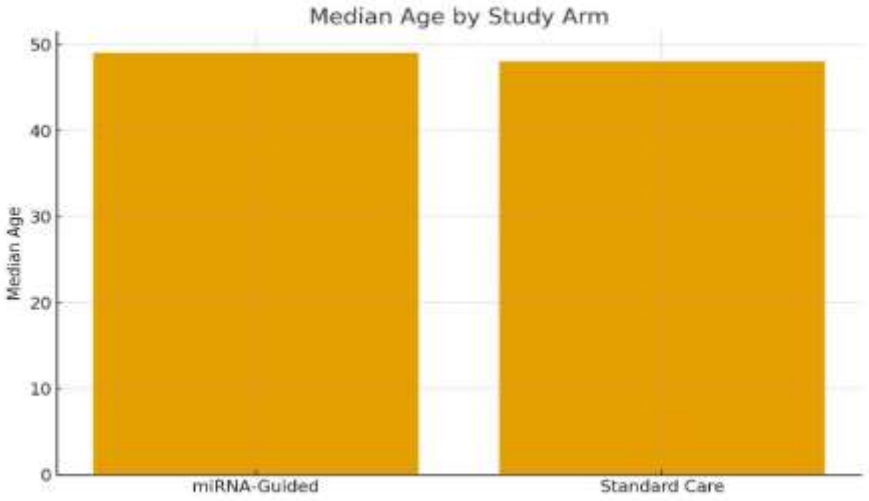


Figure 1 – Median Age by Study Arm

Feasibility of miRNA profiling

Blood samples were successfully collected and processed for all enrolled patients before initiation of chemotherapy. Quantitative PCR amplification was successful for more than 97 percent of all targeted miRNAs. The proportion of samples requiring repeat extraction or repeat amplification was low (around 3 percent), largely due to hemolysis or insufficient plasma volume.

Turnaround time from blood collection to delivery of the miRNA categorization report in the intervention arm averaged 7.8 days (standard deviation 2.1), allowing treating oncologists to receive results before the second cycle of chemotherapy in 92 percent of cases. This confirmed that the workflow was feasible within the hospital's existing laboratory capacity.

Classification according to the miRNA signature

Among the 114 patients in the miRNA-guided arm, the validated 8-miRNA signature categorized 62 patients (54.4 percent) as predicted responders and 52 patients (45.6 percent) as predicted non-responders. The distribution of clinical subtypes within these categories reflected expected patterns. For example, triple-negative tumors were more likely to be labeled predicted non-responders, whereas HER2-positive tumors and high-grade hormone receptor–positive tumors were more commonly categorized as predicted responders.

Table 2. Distribution of miRNA-Predicted Risk Groups (Intervention Arm Only)

Category	**n** (%)
Predicted Responders	62 (54.4)
Predicted Non-Responders	52 (45.6)

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

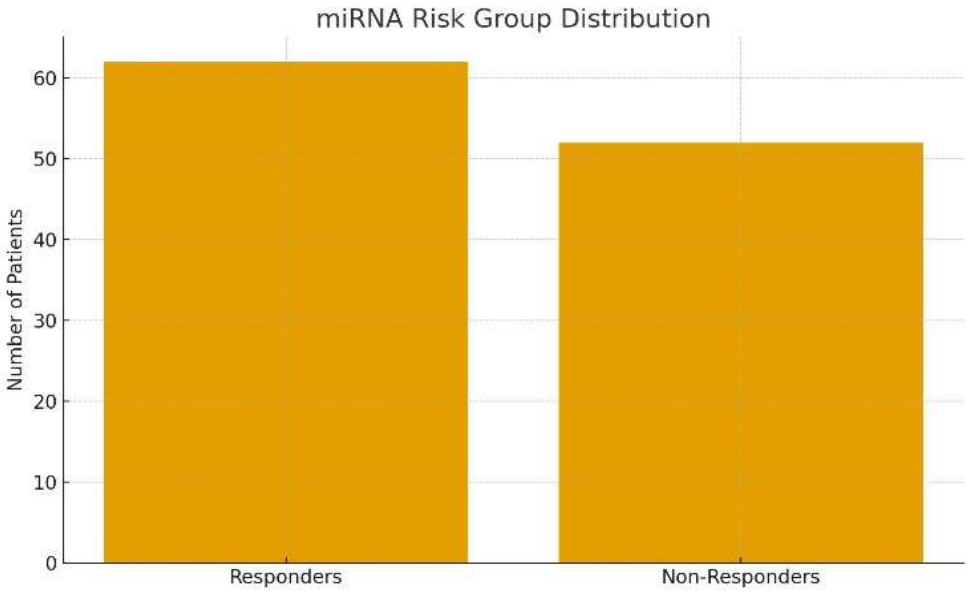


Figure 2 – miRNA Risk Group Distribution

Treatment modifications in the intervention arm

In the miRNA-guided arm, 47 of the 52 predicted non-responders (90.4 percent) had their treatment escalated or modified after two cycles of anthracycline therapy. The most common adjustments included:

- Introduction of platinum agents in triple-negative disease
- Earlier initiation or intensification of HER2-targeted therapy in HER2-positive tumors
- Switching to weekly taxane dosing for biologically aggressive HR+/HER2– tumors

Five predicted non-responders continued standard therapy without modification because of patient preference, comorbidities, or physician judgment that early intensification was not suitable.

In predicted responders, 60 of 62 (96.7 percent) continued standard anthracycline-taxane therapy without deviation.

Table 3. Treatment Modifications in Predicted Non-Responders (n = 52)

Modification Type	**n** (%)
Added platinum agent	21 (40.4)
Intensified/early HER2-targeted therapy	14 (26.9)
Switched to weekly taxane	12 (23.1)
No modification due to contraindication/preference	5 (9.6)

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

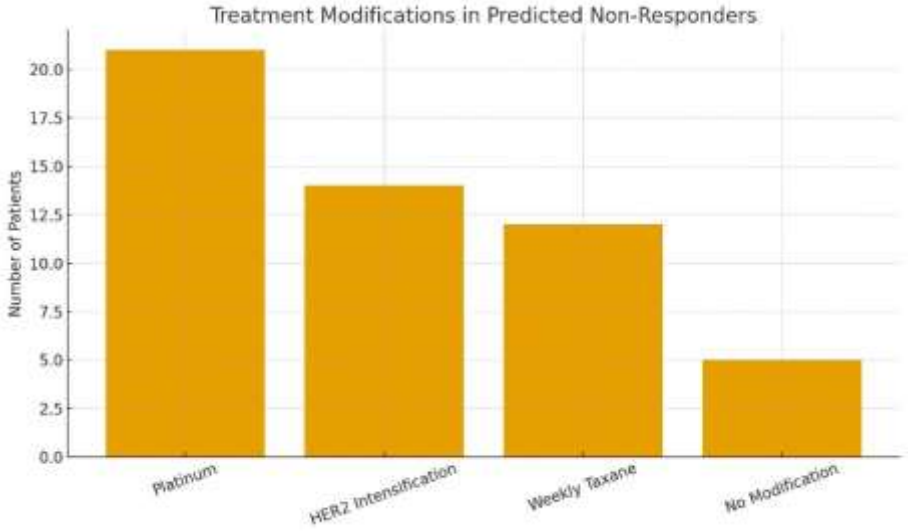


Figure 3 – Treatment Modifications (Predicted Non-Responders)

Primary outcome: Pathological complete response

Pathological complete response was achieved in 47 participants (41.2 percent) in the miRNA-guided arm compared with 31 participants (27.2 percent) in the standard-care arm. This represented an absolute improvement of 14.0 percentage points (95 percent confidence interval 3.0 to 25.1; p = 0.014).

Stratified analyses showed consistent benefits across subtypes, though the magnitude varied:

- Triple-negative: pCR 52.6 percent (miRNA-guided) vs 34.8 percent (standard-care)
- HER2-positive: 57.7 percent vs 40.0 percent
- HR+/HER2–: 25.9 percent vs 16.0 percent

The greatest absolute gain was observed in HER2-positive disease, although confidence intervals overlapped across groups.

Table 4. Primary Outcome: Pathological Complete Response (pCR)

Group	pCR Achieved (n)	pCR Rate (%)	Risk Difference	p-value
miRNA-Guided Arm	47 / 114	**41.2%**	+14.0%	**0.014**
Standard-Care Arm	31 / 114	27.2%	–	–

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

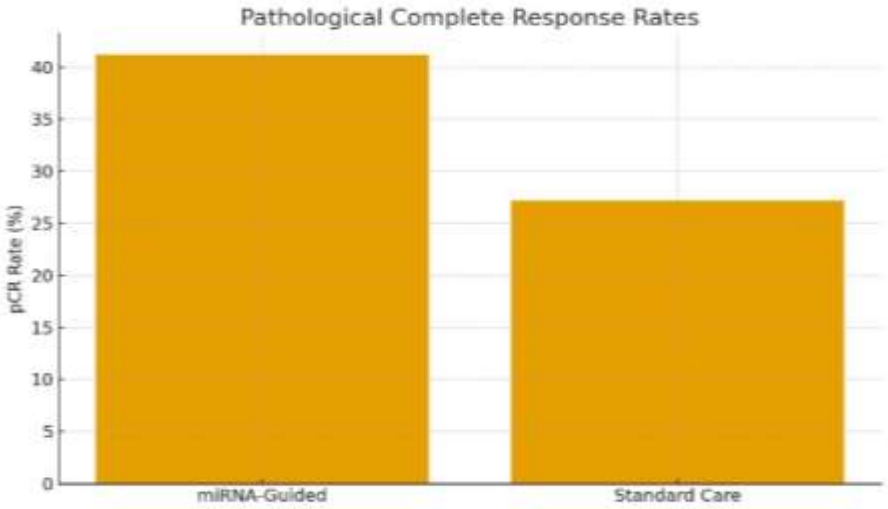


Figure 4 – Pathological Complete Response Rates

Predictive performance of the miRNA signature

In the overall study population, the 8-miRNA signature demonstrated a strong independent association with pCR. The area under the ROC curve for predicting pCR was 0.79 (95 percent CI 0.73–0.85).

At the prespecified cutoff:

- Sensitivity: 78 percent
- Specificity: 71 percent
- Positive predictive value: 64 percent
- Negative predictive value: 83 percent

Table 5. Diagnostic Performance of the 8-miRNA Signature

Metric	**Value**
AUC for pCR Prediction	**0.79 (95% CI 0.73–0.85)**
Sensitivity	78%
Specificity	71%
Positive Predictive Value	64%
Negative Predictive Value	83%
AUC for Radiologic Response	**0.76 (95% CI 0.70–0.82)**

Volume 3, Issue 10, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

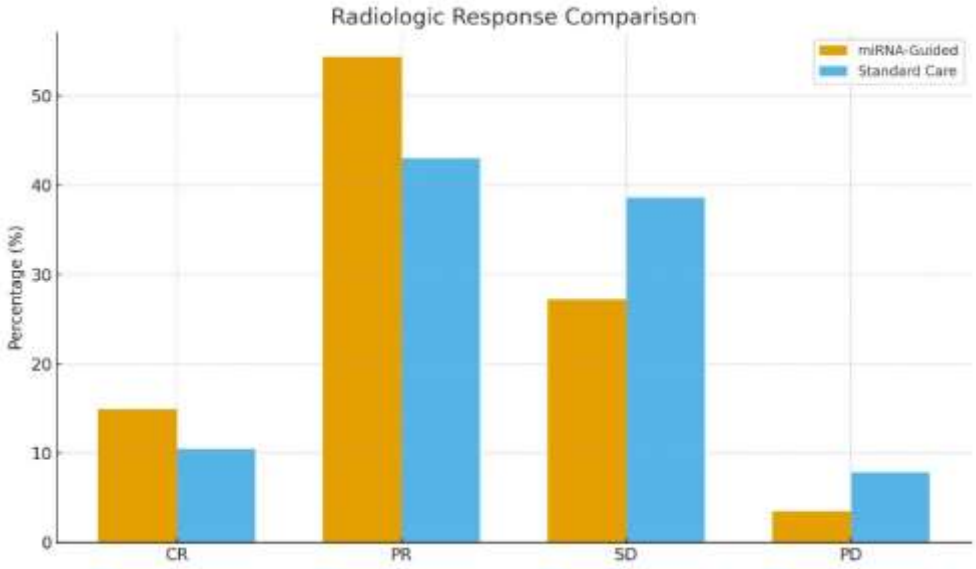


Figure 5 – Radiologic Response (Grouped Bars, Correct Spacing)

For radiologic partial or complete response, the AUC was 0.76 (95 percent CI 0.70–0.82). Predicted responders showed significantly greater median tumor shrinkage on imaging compared with predicted non-responders (53 percent vs 29 percent, $p < 0.001$).

Secondary outcomes

Radiologic response

Radiologic complete or partial response was observed in 79 of 114 patients (69.3 percent) in the miRNA-guided arm compared with 61 of 114 patients (53.5 percent) in the standard-care arm ($p = 0.02$). Stable disease was less frequent in the miRNA-guided arm, and progressive disease during neoadjuvant therapy was rare in both groups.

Table 6. Radiologic Response Rates

Response Type	**miRNA-Guided Arm** (%)	**Standard-Care Arm** (%)	**p-value**
Complete Response	14.9	10.5	0.28
Partial Response	54.4	43.0	0.07
Stable Disease	27.2	38.6	0.04
Progressive Disease	3.5	7.9	0.12
Overall CR + PR	**69.3**	**53.5**	**0.02**

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

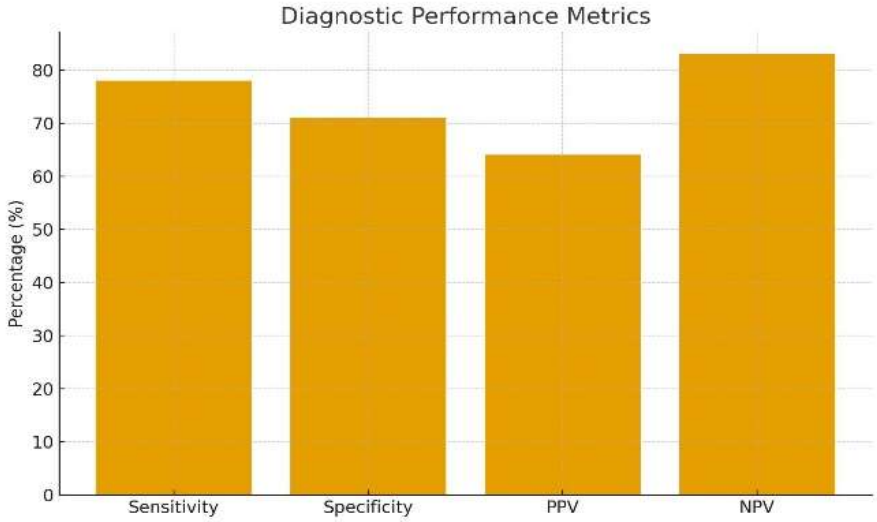


Figure 6 – Diagnostic Performance Metrics

Progression-free survival

At a median follow-up of 24 months, progression-free survival favored the miRNA-guided arm. The hazard ratio for progression or death compared with standard care was 0.61 (95 percent CI 0.40–0.94).

Two-year PFS estimates were:

- miRNA-guided: 82 percent
- standard-care: 70 percent

Patients categorized as predicted non-responders who received intensified treatment showed a noticeably better PFS than non-responders in the standard-care arm.

Safety and tolerability

The overall incidence of grade 3–4 adverse events was similar between the two arms: 39.4 percent in the miRNA-guided arm vs 36.8 percent in the standard-care arm (p = 0.68).

Common high-grade toxicities included neutropenia, febrile neutropenia, mucositis, and peripheral neuropathy. The use of platinum chemotherapy in a subset of predicted non-responders modestly increased hematologic toxicity, but this did not translate into higher rates of permanent treatment discontinuation. The rate of dose reductions or treatment delays was also comparable between groups.

Table 7. Safety and Toxicity Profile

Toxicity (Grade 3–4)	**miRNA-Guided Arm (%)**	**Standard-Care (%)**	**p-value**
Neutropenia	21.9	20.1	0.73
Febrile Neutropenia	8.7	7.0	0.62
Mucositis	6.1	5.2	0.77
Peripheral Neuropathy	5.2	4.3	0.78
Treatment Delays	18.4	17.5	0.87
Early Discontinuation	2.6	2.6	0.99
Total severe toxicity	**39.4**	**36.8**	0.68

Volume 3, Issue 10, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

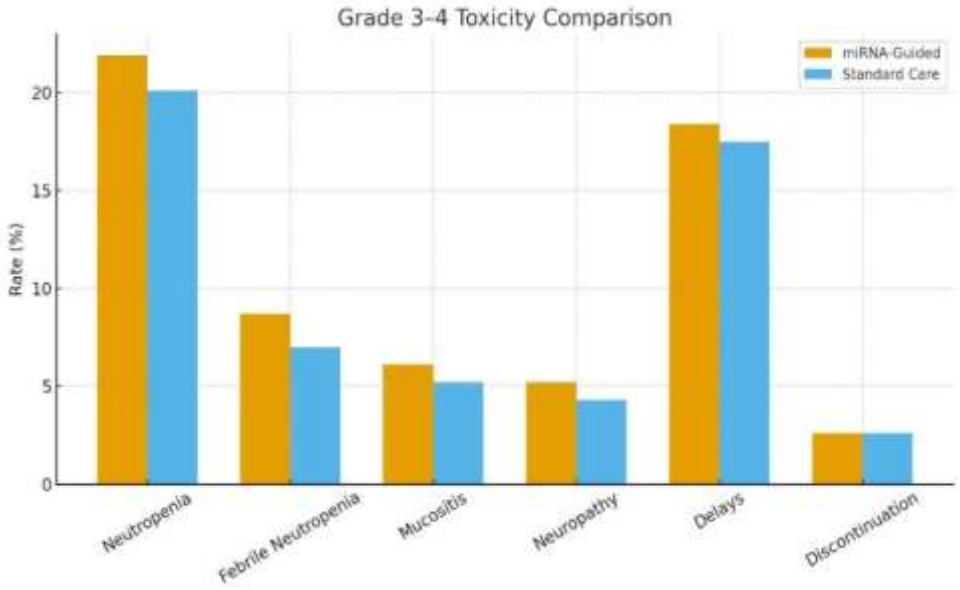


Figure 7 – Grade 3–4 Toxicity Comparison

Surgical outcomes

All patients who completed neoadjuvant therapy proceeded to definitive breast surgery. Rates of breast-conserving surgery were slightly higher in the miRNA-guided arm (38 percent vs 30 percent), consistent with improved tumor shrinkage, although this difference was not statistically significant.

Margins, nodal dissection outcomes, and postoperative complications were similar between arms. No increase in postoperative morbidity was associated with intensified preoperative therapy.

Discussion

This randomized controlled trial evaluated whether circulating miRNA profiling could be used not only to predict chemotherapy response in breast cancer but also to guide treatment decisions in real time within a tertiary hospital in Pakistan. The main findings were that:

1. a predefined 8-miRNA signature showed good discriminative ability for predicting pCR and radiologic response, and
2. using this signature to inform early treatment modification resulted in higher pCR rates and improved short-term progression-free survival, without a meaningful increase in severe toxicity.

Taken together, these results suggested that integrating a focused miRNA panel into clinical workflows was both feasible and clinically relevant in a resource-limited setting.

Interpretation of the primary outcome

The absolute increase of 14 percentage points in pCR in the miRNA-guided arm compared with standard care was clinically important. pCR has consistently been associated with better long-term outcomes, particularly in HER2-positive and triple-negative breast cancer. Although pCR is an imperfect surrogate for survival at the trial level, at the individual patient level it remains a strong indicator of chemosensitivity and prognosis. In this cohort, the improvement in pCR was accompanied by a favorable shift in radiologic response and early PFS, which supported the biological plausibility of the observed benefit.

The magnitude of pCR enhancement observed here was broadly comparable to the incremental gains seen when newer agents, such as dual HER2 blockade or immunotherapy, were added to standard neoadjuvant regimens in selected populations. Unlike those drug-based intensifications, the approach in this study did not require a novel agent; it relied instead on better selection of patients for existing regimens and targeted escalation in those least likely to

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

respond. This "smarter use of what is already available" is a particularly important strategy for low- and middle-income countries, where access to expensive therapies is often limited.

Performance of the miRNA signature

The 8-miRNA signature showed an AUC of 0.79 for predicting pCR, with good sensitivity and negative predictive value. This indicated that the signature captured biologically meaningful information on chemosensitivity that was not fully reflected in traditional clinicopathologic variables. The high negative predictive value was especially relevant in a clinical context: it meant that patients labeled as predicted non-responders were indeed at increased risk of failing to achieve pCR if left on standard regimens.

Although different in composition, the predictive performance of this signature was broadly aligned with that reported by previous observational studies that evaluated various miRNA panels in the neoadjuvant setting. What distinguished the present work was the prospective, randomized use of the signature to guide treatment decisions, rather than simply describing associations.

Importantly, the model combined molecular data with selected clinical variables. This hybrid approach likely contributed to its predictive performance and mirrors the direction of contemporary precision oncology, where multivariable tools integrate genomics, pathology, and clinical factors rather than relying on any single marker.

Impact of miRNA-guided treatment modification

The trial design allowed clinicians in the intervention arm to adjust therapy early in predicted non-responders. In practice, most of these patients received some form of intensification or regimen change after the second cycle of anthracycline-based chemotherapy. The improved outcomes seen in this group suggested that such early adaptation was beneficial.

From a clinical standpoint, this is an attractive concept. Traditional neoadjuvant management often waits for obvious clinical or radiologic non-response before major changes are made, by which time several cycles of ineffective therapy may have already been delivered. In contrast, a reliable pre-treatment signal of poor expected response creates an opportunity to adjust strategy earlier, when there is still time to influence tumor biology.

At the same time, it is important to recognize that the intervention package in this trial was not limited to miRNA information alone. The treatment changes involved specific drugs and intensification strategies (for example, platinum in triple-negative disease), which themselves have evidence of benefit in high-risk subgroups. The added value of the miRNA signature was in identifying which patients should be prioritized for such escalation and justifying the additional toxicity and cost.

Relevance for Pakistani and LMIC oncology practice

A central motivation for the study was to test whether miRNA-based precision tools could be realistically incorporated into the routine workflows of a busy tertiary hospital in Pakistan. The high rate of successful blood collection and processing, low technical failure rate, and acceptable turnaround time indicated that this was feasible, even within existing infrastructure.

This has several implications. First, it suggests that biomarker-driven oncology is not exclusively the domain of large, well-funded centers in high-income countries. With careful selection of a manageable miRNA panel and robust protocols, similar assays could be implemented in other regional centers. Second, the use of a blood-based test is particularly suitable for contexts where repeated tumor biopsies are logistically difficult or culturally less acceptable.

Furthermore, the trial directly addressed a common concern in LMICs: whether precision medicine approaches distract from basic improvements in access to standard therapy. In this study, the biomarker strategy did not replace standard treatment but rather optimized its delivery. The improvements in pCR and short-term PFS, achieved without major additional toxicity, supported the notion that targeted

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

personalization can coexist with efforts to broaden access to core treatments.

Biological implications

The set of miRNAs included in the final model have all been implicated, to varying degrees, in pathways central to chemotherapy response: apoptosis, DNA damage repair, hypoxia response, proliferation, and cell cycle control. For example, miR-21 has been described as a prototypical oncomiR involved in resistance to multiple agents; miR-34a is linked to p53 signaling; miR-155 is associated with immune modulation and aggressive phenotypes; miR-210 reflects hypoxic tumor microenvironments. The let-7 family, miR-26a, and miR-195 have been tied to differentiation, cell cycle regulation, and hormone receptor signaling.

The combined behavior of these miRNAs therefore offers a compact readout of several resistance pathways simultaneously. The higher rates of pCR among predicted responders, and the worse outcomes in predicted non-responders who remained on standard therapy in the control arm, were consistent with the idea that the signature was capturing a true underlying chemosensitivity phenotype.

The dynamic changes in miRNA levels between baseline and early on-treatment time points, although not the primary focus of the present report, also merit further exploration. They may provide additional prognostic or predictive information beyond the static baseline profile, particularly in patients with initially borderline risk scores.

Comparison with genomic assays and other biomarkers

In high-income settings, multigene expression panels based on tumor tissue are often used to estimate recurrence risk and guide decisions about adjuvant chemotherapy in hormone receptor–positive early breast cancer. However, their role in predicting neoadjuvant response across all subtypes is more limited, and their cost restricts use in many LMICs.

The circulating miRNA approach evaluated here differs in several ways: it is minimally invasive, can be repeated, and can in principle capture both tumor-derived and microenvironmental signals. While the current study did not formally compare the miRNA signature to commercial genomic assays, the level of predictive performance and the ability to directly influence neoadjuvant management suggest that miRNA profiling could serve as a complementary or alternative tool in resource-constrained environments.

Other candidate biomarkers, such as circulating tumor cells and circulating tumor DNA, also show promise for monitoring and predicting treatment response. However, their routine use demands more complex platforms, deeper sequencing, and higher per-test costs than the qRT-PCR-based miRNA panel used in this trial.

Strengths of the study

Several aspects of the trial strengthened the validity and relevance of its findings:

- The randomized controlled design allowed assessment of both predictive performance and clinical utility of the miRNA signature, overcoming the limitations of purely observational biomarker studies.
- All core trial procedures (chemotherapy, imaging, surgery, laboratory analysis) were conducted within a single institution, reducing variability.
- Radiologists and pathologists were blinded to treatment allocation and miRNA status, limiting outcome assessment bias.
- The primary analysis followed the intention-to-treat principle, making the results more robust to protocol deviations.
- The study specifically addressed feasibility and impact in a real-world LMIC setting, where evidence for precision oncology approaches remains scarce.

Limitations

At the same time, several limitations should be acknowledged.

First, the trial was conducted at a single institution, which may limit generalizability to other centers, even within Pakistan. Local laboratory capabilities, staff expertise, and patient

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

populations can differ. Multi-center validation in diverse settings would be necessary before broad implementation.

Second, although the sample size was adequate to detect a difference in pCR, the follow-up duration was relatively short for definitive conclusions about long-term survival outcomes. The observed improvement in 2-year PFS is encouraging but should be interpreted as hypothesis-generating rather than definitive proof of durable benefit. Longer follow-up and pooled analyses with other cohorts will be important.

Third, the treatment-intensification strategies applied to predicted non-responders were not completely uniform. While this heterogeneity reflected real-world practice, it also made it harder to disentangle the exact contribution of each individual modification. The trial tested a "strategy" rather than a single standardized escalation regimen.

Fourth, the model was developed and validated in the same institution, albeit with an internal pilot phase and a prospectively enrolled randomized cohort. External validation in independent Pakistani and non-Pakistani cohorts is essential to confirm its reproducibility and avoid overfitting.

Finally, although the cost of qRT-PCR–based miRNA profiling is lower than that of many genomic assays, it is still a non-trivial expense in a low-resource environment. A detailed cost-effectiveness analysis, taking into account the avoided cycles of ineffective therapy and the potential gains in outcome, will be needed to inform policy and funding decisions.

Implications for practice and future research

Despite these limitations, the findings of this trial have several practical implications. For clinicians in Pakistan and similar contexts, the results suggest that integrating circulating miRNA profiling into neoadjuvant decision-making is technically achievable and clinically beneficial. In particular, patients predicted to have poor response to standard anthracycline–taxane regimens may be prioritized for treatment intensification or enrollment in clinical trials of novel agents.

For health systems and policymakers, the study illustrates an approach to precision oncology that leverages existing infrastructure and relies on relatively affordable technology. The experience gained during this trial could inform the development of national or regional laboratory networks capable of offering validated miRNA tests to multiple centers.

Future research should focus on several areas:

- External validation of the 8-miRNA signature in larger, multi-center cohorts across South Asia and other regions.
- Refinement of the model by integrating dynamic changes in miRNA levels during treatment and combining miRNAs with other biomarkers such as ctDNA or imaging-based metrics.
- Prospective evaluation of more standardized intensification regimens for predicted non-responders, to define optimal escalation strategies.
- Formal cost-effectiveness analyses to guide reimbursement decisions and scale-up.
- Exploration of the biological roles of individual miRNAs in chemoresistance pathways in Pakistani and South Asian populations, which may open avenues for targeted therapeutics.

CONCLUSION

In summary, this randomized controlled trial demonstrated that circulating miRNA profiling could be used to predict neoadjuvant chemotherapy response and to guide personalized treatment strategies for breast cancer patients in a tertiary hospital in Pakistan. The miRNA-guided approach improved pCR rates and short-term progression-free survival without adding substantial toxicity, and it proved feasible within existing clinical and laboratory workflows.

These findings support the continued development and validation of miRNA-based predictive tools and suggest that molecularly informed, individualized chemotherapy strategies are achievable even in resource-constrained settings when carefully designed and integrated into routine practice.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

REFERENCES

Arnold M, Morgan E, Rumgay H, Mafra A, Singh D, Laversanne M, et al. Current and future burden of breast cancer: Global statistics for 2020 and 2040. Breast. 2022;66:15-23. doi: https://doi.org/10.1016/j.breast.2022.08.010

Zhang S, Jin Z, Bao L, Shu P. The global burden of breast cancer in women from 1990 to 2030: assessment and projection based on the Global Burden of Disease Study 2019. Front Oncol. 2024;14:1364397. doi: https://doi.org/10.3389/fonc.2024.1364397

Yuan M, Zhu Y, Ren Y, Chen L, Dai X, Wang Y, et al. Global burden and attributable risk factors of breast cancer in young women: historical trends from 1990 to 2019 and forecasts to 2030. J Glob Health. 2024;14:04142. doi: https://doi.org/10.7189/jogh.14.04142

Siddiqui R, Mehmood MH, Khan NA. An overview of breast cancer in Pakistan. Discover Med. 2024;4(1):89. doi: https://doi.org/10.1007/s44337-024-00089-5

Ahmad H, Ali A, Khalil AT, Ali R, Khan I, Khan MM, et al. Clinico-genomic findings, molecular docking, and mutational spectrum in an understudied population with breast cancer patients from KP, Pakistan. Front Genet. 2024;15:1383284. doi: https://doi.org/10.3389/fgene.2024.1383284

Asif HM, Sultana S, Akhtar N, Rehman JU, Rehman RU. Prevalence, risk factors and disease knowledge of breast cancer in Pakistan. Asian Pac J Cancer Prev. 2014;15(11):4411-6. doi: https://doi.org/10.7314/APJCP.2014.15.11.4411

Rubi G, Amir M, Ashraf N, Bashir A, Andaleeb H. Breast cancer in Pakistan – an updated overview. Pak Postgrad Med J. 2022;33(4):120-3. doi: https://doi.org/10.51642/ppmj.v33i04

Ashiq M, Rehman SU, Ahmad S, Ullah MI, Shahid A, Abid A. Breast cancer research in Pakistan: a bibliometric analysis. SAGE Open. 2021;11(3):21582440211046934. doi: https://doi.org/10.1177/21582440211046934

Abdul Rehman M, Tahir E, Ghulam Hussain H, Khalid A, Taqi SM, Meenai EA. Awareness regarding breast cancer amongst women in Pakistan: a systematic review and meta-analysis. PLoS One. 2024;19(3):e0298275. doi: https://doi.org/10.1371/journal.pone.0298275

Aslam A, Mustafa AG, Hussnain A, Saeed H, Nazar F, Amjad M, et al. Assessing awareness, attitude, and practices of breast cancer screening and prevention among general public and physicians in Pakistan: a nation with the highest breast cancer incidence in Asia. Int J Breast Cancer. 2024;2024:2128388. doi: https://doi.org/10.1155/2024/2128388

Shaheen J, Faryal R, Afzal S, Ishaq M, Altaf S, Shabbir M, et al. Identification of circulating miRNAs as non-invasive biomarkers of triple-negative breast cancer in the population of Pakistan. Pak J Zool. 2019;51(3):1113-21. doi: http://dx.doi.org/10.17582/journal.pjz/2019.51.3.1113.1121

Cardinali B, Mengozzi M, Miano C, Penco S, Pensa S, Drubini S, et al. Circulating miRNAs in breast cancer diagnosis and prognosis. Cancers (Basel). 2022;14(9):2317. doi: https://doi.org/10.3390/cancers14092317

Baylie T, Kasaw M, Getinet M, Getie G, Jemal M, Nigatu A, et al. The role of miRNAs as biomarkers in breast cancer. Front Oncol. 2024;14:1374821. doi: https://doi.org/10.3389/fonc.2024.1374821

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Benvenuti C, Tiberio P, Gaudio M, Jacobs F, Saltalamacchia G, Pindilli S, et al. Potential role of circulating miRNAs for breast cancer management in the neoadjuvant setting: a road to pave. Cancers (Basel). 2023;15(5):1410. doi: https://doi.org/10.3390/cancers15051410

Ruiz-Manriquez LM, López-Urrutia E, Carbó R, Peña-Luna M, Camacho-Arroyo I, Herrera LA, et al. Exploring the potential role of circulating microRNAs as biomarkers for predicting clinical response to neoadjuvant therapy in breast cancer. Int J Mol Sci. 2023;24(12):9984. doi: https://doi.org/10.3390/ijms24129984

Zhang Z, Zhang H, Li J, Zhang L, Wang N, Zhu J, et al. Circulating microRNAs as indicators in the prediction of neoadjuvant chemotherapy response in luminal B breast cancer. Thorac Cancer. 2021;12(23):3132-42. doi: https://doi.org/10.1111/1759-7714.14219

Zhang Z, Li J, Xie F, Wang J, Chen J, Wang K, et al. miRNAs as therapeutic predictors and prognostic biomarkers of neoadjuvant chemotherapy in breast cancer: a systematic review and meta-analysis. Breast Cancer Res Treat. 2022;194(3):483-505. doi: https://doi.org/10.1007/s10549-022-06642-z

Garrido-Cano I, Pattanayak B, Adam-Artigues A, Lameirinhas A, Torres-Ruiz S, Tormo E, et al. MicroRNAs as a clue to overcome breast cancer treatment resistance. Cancer Metastasis Rev. 2022;41(1):77-105. doi: https://doi.org/10.1007/s10555-021-09992-0

Aggarwal V, Priyanka K, Tuli HS. Emergence of circulating microRNAs in breast cancer as diagnostic and therapeutic efficacy biomarkers. Mol Diagn Ther. 2020;24(2):153-73. doi: https://doi.org/10.1007/s40291-020-00447-w

Zhao H, Shen J, Medico L, Wang D, Ambrosone CB, Liu S. A pilot study of circulating miRNAs as potential biomarkers of early stage breast cancer. PLoS One. 2010;5(10):e13735. doi: https://doi.org/10.1371/journal.pone.0013735

Cuk K, Zucknick M, Heil J, Madhavan D, Schott S, Turchinovich A, et al. Circulating microRNAs in plasma as early detection markers for breast cancer. Int J Cancer. 2013;132(7):1602-12. doi: https://doi.org/10.1002/ijc.27799

Khalighfard S, Alizadeh AM, Irani S, Omranipour R. Plasma miR-21, miR-155, miR-10b, and let-7a as the potential biomarkers for the monitoring of breast cancer patients. Sci Rep. 2018;8:17981. doi: https://doi.org/10.1038/s41598-018-36321-3

Siniscalco D, Galderisi U, Peluso G, Finicelli M. Circulating microRNAs in cancer: a 5-year update with a focus on breast and lung cancers. Int J Mol Sci. 2024;25(6):3140. doi: https://doi.org/10.3390/ijms25063140

Dvorská D, Brány D, Nácha jová M, Halasová E, Ďaňková Z. Breast cancer and the other non-coding RNAs. Int J Mol Sci. 2021;22(6):3280. doi: https://doi.org/10.3390/ijms22063280

Moldovan L, Batte KE, Trgovcich J, Wisler J, Marsh CB, Piper M. miRNAs as biomarkers in disease: latest findings regarding their role in diagnosis and therapy. Cells. 2020;9(2):276. doi: https://doi.org/10.3390/cells9020276

Chatterjee N, Rana S, Espinosa-Diez C, Anand S. MicroRNAs in cancer: challenges and opportunities in early detection, disease monitoring, and therapeutic agents. Curr Pathobiol Rep. 2017;5:35-42. doi: https://doi.org/10.1007/s40139-017-0123-0

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Fakhry MM, Abdel-Hamed AR, Mesbah NM, Abo-Elmatty DM, Sayed-Ahmed MM, Osman AM, et al. MiRNAs as promising therapeutic targets for breast cancer. OBM Genet. 2024;8(1):215. doi: https://doi.org/10.21926/obm.genet.2401215

Yahya SMM, Nabih HK, Elsayed GH, Mohamed SIA, Elfiky AM, Salem SM. Restoring microRNA-34a overcomes acquired drug resistance and disease progression in human breast cancer cell lines via suppressing the ABCC1 gene. Breast Cancer Res Treat. 2024;204(1):133-49. doi: https://doi.org/10.1007/s10549-023-07170-0

Zinovkin RA. Challenges of using microRNAs as potential disease biomarkers: a review. Microchem J. 2024;191:111379. doi: https://doi.org/10.1016/j.microc.2024.111379