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Review

The Multi-Omic Transformation of Breast Cancer Diagnostics: A Comprehensive Review of the Transition from Immunohistochemistry to Liquid Biopsy and Next-Generation Sequencing

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Abstract

Background: Breast cancer (BC) diagnostics are undergoing a transformative shift from traditional morphological assessment to a complex multi-omic characterization. While Immunohistochemistry (IHC) remains the clinical bedrock for subtyping, its inherent inability to account for intra-tumoral heterogeneity and the dynamic nature of clonal evolution has necessitated the integration of high-throughput genomic and transcriptomic tools. **Main body:** This review examines the technological evolution from "gold standard" protein-based assays to precision oncology enabled by Next-Generation Sequencing (NGS) and liquid biopsy. We provide a rigorous analysis of multi-gene expression signatures (Oncotype DX, MammaPrint, Prosigna, EndoPredict) and their impact on clinical decision-making. Furthermore, we explore the clinical utility of circulating tumor DNA (ctDNA) for molecular residual disease (MRD) detection and the identification of acquired resistance mechanisms involving ESR1, PIK3CA, AKT1, and PTEN mutations. We further address the complex bioinformatics challenges, including variant interpretation, the implementation of Unique Molecular Identifiers (UMIs), and the integration of artificial intelligence in analyzing massive multi-omic datasets. **Conclusion:** The integration of IHC with longitudinal genomic profiling is essential for the future of breast cancer management. This multi-modal approach ensures that therapeutic strategies evolve alongside the tumor's molecular landscape. Future clinical trials must focus on MRD-guided interventions to validate the clinical benefit of early genomic detection.

Keywords: breast cancer; next-generation sequencing; liquid biopsy; immunohistochemistry; precision medicine; ctDNA; multi-omics

1. Introduction

1.1. The Evolving Landscape of Oncology

Breast cancer (BC) remains the most prevalent malignancy worldwide, accounting for one in four cancer cases among women and serving as a leading cause of oncological mortality [1]. Historically, the treatment paradigm was defined by clinical staging (TNM) and a limited set of immunohistochemical (IHC) markers: the Estrogen Receptor (ER), Progesterone Receptor (PR), and Human Epidermal Growth Factor Receptor 2 (HER2) [2,3].

However, we now recognize that classic IHC subtypes mask a vast landscape of underlying genetic diversity [22]. The transition to a multi-omic diagnostic framework marks a shift from reactive to proactive oncology. By integrating NGS, transcriptomics, and non-invasive liquid biopsy techniques, clinicians can now track the "molecular life cycle" of a tumor.

1.2. Aim of the review

This systematic review aims to elucidate the relationship between advancing genomic technologies and clinical outcomes in breast cancer, specifically focusing on the transition from static protein-based assays to dynamic liquid biopsy and the role of NGS in identifying therapeutic resistance.

2. Search strategy

A comprehensive literature search was conducted across PubMed, Scopus, Google Scholar, and clinicaltrials.gov for studies published between 2000 and 2025. Search terms included "Breast Cancer," "Next-Generation Sequencing," "Liquid Biopsy," "ctDNA," "Oncotype DX," and "Molecular Residual Disease." Priority was given to Level 1 clinical trials (TAILORx, MINDACT, SOLAR-1) and international guidelines (ASCO/CAP) to ensure the highest quality of evidence synthesis.

3. Results and Discussion

3.1. The Foundation and Fragility of the IHC Standard

For over three decades, IHC has been the indispensable tool of the surgical pathologist. Its primary strength lies in the preservation of spatial architecture, allowing for the correlation of biomarker expression with histological features such as lymphovascular invasion, stromal involvement, and tumor-infiltrating lymphocytes (TILs).

3.1.1. Technical Nuances and the Challenge of Standardization

Despite its dominance, the technical limitations of IHC are increasingly apparent. Inter-observer variability remains a primary concern; for markers like Ki-67 or low-level HER2 expression ("HER2-low"), studies have shown significant discrepancies even among senior pathologists [4]. Pre-analytical variables, including tissue fixation time and the choice of antibody clones (e.g., SP197 vs. 4B5), can significantly alter results, directly impacting patient eligibility for targeted therapies [13]. A core needle biopsy captures only a small fraction of the total tumor volume; in multifocal tumors, a single IHC report may miss high-grade or drug-resistant clones located elsewhere in the breast [22].

3.1.2. The HER2 Spectrum: From Binary to Continuum

A paradigm shift is occurring in the classification of HER2. Traditionally binary (positive or negative), the emergence of the "HER2-low" category (IHC 1+ or IHC 2+/FISH negative) has gained clinical significance following the DESTINY-Breast04 trial [13]. This trial proved that HER2-targeted Antibody-Drug Conjugates (ADCs) like Trastuzumab Deruxtecan (T-DXd) are highly effective in patients previously classified as HER2-negative. Similarly, the Trop-2 targeted ADC Sacituzumab Govitecan has shown efficacy in HR+/HER2-negative metastatic settings [18], further blurring traditional IHC boundaries [19].

3.2. The Transcriptomic Shift: Multi-Gene Expression Assays

Beyond IHC, mRNA-based expression profiling provides deeper insights into tumor proliferation [4]. The major commercially available multi-gene assays are summarized in Table 1.

Table 1. Comparison of multi-gene expression assays used in breast cancer.

Assay	Gene Count	Patient Population	Key Trial	Evidence Level	Reference
Oncotype DX	21	HR+/HER2- early BC	TAILORx	Level I	[10]
MammaPrint	70	Stage I-II BC	MINDACT	Level I	[11]
Prosigna	50	Postmenopausal HR+	TransATAC	Level II	[4]

3.2.1. Oncotype DX and the TAILORx Trial

The 21-gene recurrence score (Oncotype DX) has revolutionized the management of HR-positive, HER2-negative early-stage breast cancer. The assay analyzes genes involved in proliferation, estrogen signaling, and HER2 signaling. The landmark TAILORx trial, involving 10,273 women, provided Level 1 evidence that patients over age 50 with a mid-range score (11-25) can safely forgo adjuvant chemotherapy. This has fundamentally changed the standard of care, sparing thousands of women from unnecessary toxicity [10].

3.2.2. MammaPrint and the MINDACT Trial

The 70-gene MammaPrint assay provides a binary classification of "High Risk" vs "Low Risk." The MINDACT trial proved that even if a patient is considered "Clinically High Risk" (due to tumor size), a "Genomic Low Risk" result indicates a high survival rate without chemotherapy. This study highlighted the superiority of genomic risk over clinical risk in approximately 46% of high-risk cases [11].

3.2.3. Prosigna (PAM50) and EndoPredict: Precision in Late Recurrence

Beyond risk of recurrence, the Prosigna assay provides the "intrinsic subtype" (Luminal A, Luminal B, HER2-enriched, or Basal-like). This is critical for determining the risk of late recurrence (5-10 years post-diagnosis). EndoPredict has similarly shown high efficacy in identifying patients who may benefit from extended endocrine therapy beyond 5 years, a critical clinical question for HR+ patients [4].

3.3. Next-Generation Sequencing (NGS): Mapping the BC Genome

While transcriptomics looks at gene expression, NGS looks at the DNA itself. This allows for the identification of the specific "driver" mutations that cause a normal cell to become malignant [21].

3.3.1. Comparative Analysis: Illumina vs. Ion Torrent Platforms

In medical genetics, the choice of platform is critical.

- **Illumina (Sequencing by Synthesis):** Dominates the market due to its extremely high accuracy and throughput. It is the gold standard for WES and large targeted panels.
- **Ion Torrent (Semiconductor Sequencing):** Offers faster turnaround times and requires less starting DNA, making it attractive for small biopsies where tissue is limited. However, its error rate in homopolymer regions (repeated bases like AAAAAA) is higher than Illumina.

3.3.2. Targeted Panels vs. Whole Exome Sequencing (WES)

- **Targeted Panels:** These sequence 300-500 genes known to be relevant in cancer (e.g., FoundationOne). They provide high "depth" of coverage (often >500x), allowing for the detection of mutations present in only 1-2% of cells (Variant Allele Frequency - VAF).
- **Whole Exome Sequencing (WES):** WES sequences all ~20,000 protein-coding genes. In medical genetics, WES is essential for identifying Tumor Mutational Burden (TMB) and Microsatellite Instability (MSI), which are predictive markers for response to immunotherapy with Pembrolizumab [6].

3.3.3. Actionable Mutations and Resistance Mechanisms

The most significant impact of NGS is in the identification of actionable mutations that drive therapeutic decisions:

- **PIK3CA Mutations:** Found in ~40% of HR+ breast cancers, these mutations activate the PI3K/AKT/mTOR pathway, leading to cell proliferation and resistance to endocrine therapy. The

SOLAR-1 trial demonstrated that Alpelisib significantly improves progression-free survival in these patients [12].

- **ESR1 Mutations** (Y537S, D538G): These mutations typically develop under the selective pressure of Aromatase Inhibitors (AI). They cause the Estrogen Receptor to remain "always on" even without estrogen. Detecting these via NGS informs a switch to Selective Estrogen Receptor Degraders (SERDs) like Elacestrant [7].
- **BRCA1/2 Mutations:** These alterations in DNA repair pathways predict response to PARP Inhibitors, particularly in triple-negative or germline-associated cases [21].

Clinically actionable genomic alterations and associated therapies are summarized in Table 2.

Table 2. Actionable genomic alterations in breast cancer and associated targeted therapies.

Mutation	Pathway	Targeted Therapy	Clinical Utility	Reference
PIK3CA	PI3K/AKT/mTOR	Alpelisib	Advanced HR+ BC	[12]
ESR1	Estrogen Receptor	Elacestrant	Endocrine Resistance	[7]
BRCA1/2	DNA Repair	PARP Inhibitors	Triple Negative/Germline	[21]

3.4. Liquid Biopsy: The Non-Invasive Diagnostic Revolution

The most significant hurdle in genomic monitoring is the invasive nature of repeated tissue biopsies. Liquid biopsy, which analyzes circulating tumor DNA (ctDNA) shed into the blood, offers a non-invasive, "real-time" view of the cancer. Despite its promise, ctDNA sensitivity remains limited in low-tumor-burden and early-stage disease, necessitating careful clinical interpretation [5,20]. A schematic overview of the liquid biopsy workflow is shown in Figure 1.

Figure 1. Schematic overview of the liquid biopsy workflow in breast cancer diagnostics. Peripheral blood is collected, plasma is isolated, circulating tumor DNA (ctDNA) is extracted and labeled with unique molecular identifiers (UMIs), followed by next-generation sequencing (NGS), bioinformatic analysis, and clinical decision-making.

3.4.1. Molecular Residual Disease (MRD) and the "Lead Time"

After surgery, ctDNA can detect "Molecular Residual Disease" (MRD) months before a tumor is large enough to be seen on a CT scan. This "lead time" (often 6-10 months) provides a critical window for early intervention. Assays like Signatera use patient-specific "fingerprints" to achieve detection limits as low as 0.01% VAF [8].

3.4.2. Clonal Evolution and the "Dynamic Biopsy"

Tumors are dynamic entities. A liquid biopsy performed every 3-6 months allows clinicians to watch the "clonal evolution" of the cancer [22]. If a new resistance mutation appears in the blood, the treatment can be adjusted immediately. This is far more effective than the traditional "treat until progression" model, where changes are only made after a tumor has already caused clinical symptoms or radiological changes [9].

3.5. The Bioinformatics Engine: From Raw Data to Clinical Report

The transition to multi-omics creates a massive "data deluge." A single NGS run generates gigabytes of raw data (FASTQ files).

- **The Pipeline:** Raw data must be aligned to the human reference genome (GRCh38). Sophisticated algorithms like BWA-MEM or Bowtie2 are used for alignment, followed by variant calling with tools like GATK or MuTect2.
- **Unique Molecular Identifiers (UMIs):** In liquid biopsy, where the signal is very weak, UMIs are used to label each DNA fragment before sequencing. This allows the computer to distinguish

between a real mutation and a sequencing error, pushing the sensitivity to unprecedented levels [9].

- Artificial Intelligence (AI) and Machine Learning: AI models are being trained to integrate digital pathology slides (H&E) with NGS data. These "multi-modal" AI tools can predict survival outcomes better than any single test. AI also assists in solving the "Variant of Uncertain Significance" (VUS) problem by simulating the structural impact of a mutation on the protein's fold.

3.6. Socio-Economic Barriers and the Ethical Landscape

The global implementation of multi-omics faces significant barriers:

- The Genomic Divide: The high cost of NGS (often >\$3,000 per test) creates a gap between high-income and low-income healthcare systems. This creates a moral imperative to reduce costs and standardize protocols globally.
- Incidental Findings: Sequencing can reveal germline mutations (e.g., *BRCA1/2* or *TP53*) that have implications for the patient's family [21]. This requires robust genetic counseling services and clear institutional policies on "the right to know."

4. Conclusions and Future Directions

The transformation of breast cancer diagnostics from a single IHC-based assessment to a multi-omics, longitudinal pipeline is the hallmark of modern precision medicine. While IHC remains essential for initial screening and morphological context, it is no longer sufficient for the management of complex, evolving disease.

Future Directions:

1. **Interventional Genomics:** Clinical trials must now prioritize treating patients based on MRD status rather than waiting for radiological recurrence.
2. **Multi-modal AI Integration:** The future lies in combining "spatial transcriptomics" with serial liquid biopsy to create "digital twins" of patient tumors.
3. **Equitable Precision Medicine:** Standardizing bioinformatics and reducing sequencing costs is vital to bridge the "genomic divide" and ensure global access.

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Abbreviations

ADC	Antibody-Drug Conjugate
BC	Breast Cancer
ctDNA	Circulating tumor DNA
IHC	Immunohistochemistry
MRD	Molecular Residual Disease
NGS	Next-Generation Sequencing
TMB	Tumor Mutational Burden

UMI	Unique Molecular Identifier
VAF	Variant Allele Frequency
VUS	Variant of Uncertain Significance
WES	Whole Exome Sequencing

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