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A COMPARATIVE STUDY ON CANCER DETECTION ACROSS DIFFERENT STAGES OF CARCINOMA DEVELOPMENT USING MOLECULAR BIOLOGY TECHNIQUES

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Abstract
Carcinoma, a malignancy arising from epithelial cells, is the most common cancer globally and is linked to significant morbidity and mortality, especially due to late diagnosis and disease advancement. The goal of this study is to compare the usefulness of molecular biology approaches including polymerase chain reaction (PCR), next-generation sequencing (NGS), and biomarker analysis in finding cancer at different stages of development. The study concentrates on evaluating stage-specific diagnostic accuracy, expression patterns of molecular markers, and the sensitivity and specificity of different diagnostic methodologies. The results show that PCR-based methods, notably droplet digital PCR, are very good at finding known genetic alterations and circulating tumor DNA in the early stages of cancer. NGS offers extensive genomic profiling and is essential for detecting new mutations, tumor heterogeneity, and drug resistance, rendering it particularly successful in intermediate and later stages. Biomarker analysis, which looks at proteins, microRNAs, and epigenetic markers, is a way to screen for diseases, keep an eye on them, and figure out how likely they are to get better without having to do any intrusive tests. The combination of various molecular diagnostic technologies greatly improves the accuracy of detection and helps with planning individualized treatment. Nevertheless, significant obstacles to wider clinical adoption persist, including prohibitive costs, the necessity for extensive technical proficiency, and the unpredictability in biomarker expression. The study's conclusion is that a multi-modal diagnostic technique that combines PCR, NGS, and biomarker analysis is a promising way to improve early detection, treatment decisions, and survival rates in people with carcinoma.

Keywords: *Carcinoma Detection, Molecular Biology Techniques, Polymerase Chain Reaction (PCR), Next-Generation Sequencing (NGS), Biomarker Analysis, Circulating Tumor DNA, Molecular Diagnostics, Personalized Cancer Therapy*

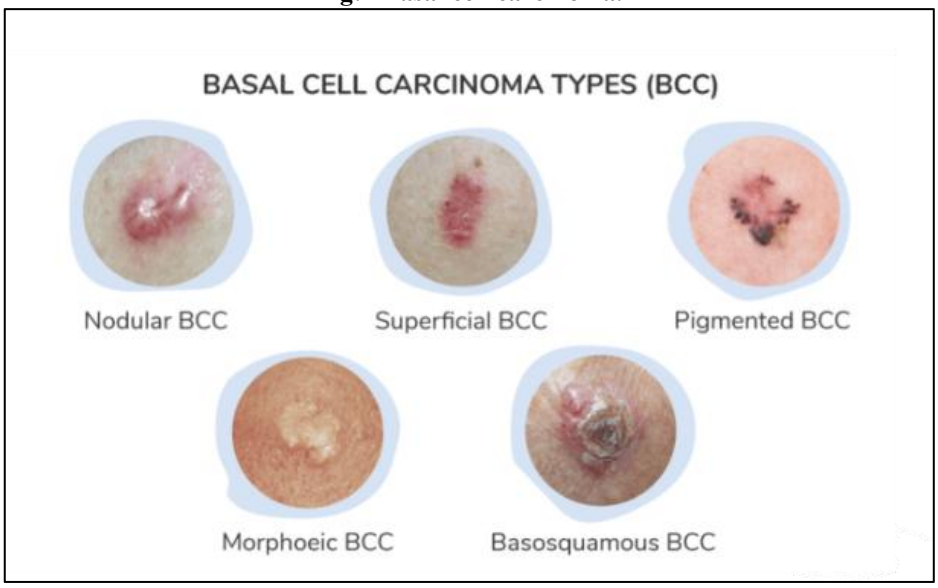
Introduction

Carcinoma is a comprehensive term that refers to many tumors originating from epithelial cells in the skin, internal organs, and bodily cavities. These cells function to protect, absorb chemicals, secrete fluids, and filter waste. Upon the occurrence of aberrant genetic mutations in epithelial cells, they may proliferate uncontrolled, resulting in malignant tumors termed carcinomas.

Carcinoma is the most common cancer, accounting for around 80-90 percent of all diagnosed cases worldwide. Carcinoma's great incidence and ability to affect multiple organs have rendered it a major worldwide health concern. The disease has elevated morbidity and fatality rates, primarily due to late detection, rapid progression, and the capacity of malignant cells to metastasize to distant organs, rendering it very lethal. **(Brown et al., 2023), (Benz, 2017)**

Carcinoma arises from the transformation of epithelial cells caused by genetic mutations, environmental exposures, or lifestyle risk factors. These reengineered cells forfeit the standard mechanisms that regulate their growth and division, leading to unregulated proliferation. As the tumor proliferates, carcinoma cells may invade adjacent organs and disseminate throughout the body via the bloodstream or lymphatic system, a process known as metastasis. Unlike sarcomas, which originate from connective tissues such as bone, muscle, or cartilage, carcinomas arise directly from epithelial tissue and have distinct morphological and molecular characteristics. Carcinomas can be categorized into distinct types and behavioral classifications based on their organ of origin and the specific epithelial cells involved in their development. Carcinomas in early stages may remain localized; but, in mature stages, tissue invasion and metastasis generally occur, worsening the prognosis. **(Benz, 2017), (Sinha, 2018), (Carbone, 2020)**

Fig:1 Basal cell carcinoma.



Source: https://miiskin.com/skin-cancer/basal-cell-carcinoma/

The prevalence of carcinomas is evidenced by the greatest incidence of cancer globally, indicating widespread population distribution. Lung cancer, with 2.5 million new cases in 2022, was the most prevalent cancer, predominantly including non-small cell lung cancer, including adenocarcinoma and squamous cell carcinoma. The subsequent most common malignancy was breast carcinoma, with roughly 2.3 million new cases, predominantly ductal or lobular carcinoma. Colorectal carcinoma accounted for around 1.9 million new occurrences, predominantly comprising adenocarcinoma.

Approximately 20 million new cancer cases were diagnosed globally in 2022, with carcinomas representing the majority in over 185 countries. In industrialized and high-income regions, higher rates are attributed to lifestyle and screening practices, whereas the incidence of carcinoma is escalating swiftly in low- and middle-income countries. The increase is largely ascribed to population aging, urbanization, environmental pollution, and heightened exposure to risk factors such as tobacco use, unhealthy diets, and sedentary lifestyles. **(Xia et al., 2022), (Sharma & Khubchandani, 2024), (Cao et al., 2024), (Zhu et al., 2024)**

Table: 1

Carcinoma Type	Est. 2022 New Cases (millions)	Top Regions	References
Lung	2.5	Eastern Asia, Europe	(Sharma & Khubchandani, 2024) ("Global Cancer Burden

			Growing, amidst Mounting Need for Services," 2024)
Breast	2.3	Western countries, global	("Global Cancer Burden Growing, amidst Mounting Need for Services," 2024), (Liao, 2025)
Colorectal	1.9	High HDI nations	("Global Cancer Burden Growing, amidst Mounting Need for Services," 2024)
Esophageal (mostly squamous)	0.6	Eastern Asia, Africa	(Morgan et al., 2022)

Main types

Adenocarcinoma: Adenocarcinoma originates from glandular epithelial cells that produce chemicals. It is a carcinoma that is quite prevalent and can arise in the lungs, breast, prostate, pancreas, and colon. Adenocarcinomas generally originate in glandular or gland-like tissues and may vary in terms of differentiation and aggressiveness. Adenocarcinoma is predominantly linked to lifestyle and environmental factors, including smoking, obesity, and dietary habits. Certain adenocarcinoma variants exhibit improved responsiveness to early detection and targeted therapy, albeit prognosis is contingent upon the stage and affected organ. **(McCart Reed et al., 2021), (Liu et al., 2018)**

Squamous Cell Carcinoma: Squamous cell carcinoma originates in the flat, thin squamous epithelial cells that comprise the skin and the epithelial lining of internal organs, including the lungs, esophagus, cervix, and head and neck. It is closely associated with prolonged sun exposure, tobacco usage, alcohol intake, and viral infections such as human papillomavirus (HPV). It can disseminate to the parenchymal tissues and proliferate, however it may require time to manifest if left untreated. The clinical presentation varies based on the tumor's location, with treatment typically involving surgery, radiation therapy, or chemotherapy. **(Di Stasio et al., 2022), (Zamani & Rezaei, 2024), (Morgan et al., 2022)**

Other Subtype: Numerous more subtypes of cancer are classified by cellular origin and anatomical region. Basal cell carcinoma, the most prevalent form of skin cancer, often originates from basal epidermal cells and is mostly due to prolonged exposure to UV light. Transitional cell carcinoma (urothelial carcinoma) is a kind of bladder cancer primarily affecting the bladder lining, frequently linked to cigarette smoking and chemical exposure. Large cell neuroendocrine carcinoma is a rare and severe variant of lung cancer characterized by rapid proliferation and lack of curability. Carcinoma is a complex and diverse illness characterized by the specific biology, diagnostic criteria, and treatment outcomes of each subtype. **(Liu et al., 2018), (Suster & Suster, 2025)**

Objectives

- To evaluate and compare the effectiveness of various molecular biology techniques (such as PCR, next-generation sequencing, and biomarker analysis) in detecting carcinoma at different stages of development.
- To analyze the expression patterns of specific molecular markers associated with early, intermediate, and advanced stages of carcinoma to determine their diagnostic and prognostic significance.
- To assess the sensitivity and specificity of selected molecular diagnostic tools in identifying stage-wise progression of carcinoma for improving early detection and treatment planning.

Research Methodology

The current study utilized a secondary research methodology to gather and analyze pertinent data regarding cancer diagnosis at various phases of carcinoma development by molecular biology techniques. We got secondary data from a lot of different real and trustworthy sources that are available online. These include peer-reviewed research articles, scientific journals, government health reports, international cancer databases, conference papers, and publications from well-known groups like the World Health Organization (WHO), the National Cancer Institute (NCI), and other academic repositories. We employed a lot of online scholarly resources like Google Scholar, PubMed, ResearchGate, and ScienceDirect to find recent and trustworthy literature. The sources were chosen because they were relevant to molecular diagnostic procedures such Polymerase Chain Reaction (PCR), Next-Generation Sequencing (NGS), and biomarker analysis, as well as to distinct phases of cancer progression. We carefully looked over, compared, and put together the data we collected to find trends, similarities, and variations in how well these approaches worked for diagnosing, how sensitive and specific they were, and how useful they were in the clinic. Studies released in the last few years were given more weight to make sure the information was up-to-date and backed by science. Furthermore, statistical data, experimental outcomes, and clinical observations from several studies were aggregated to enhance the research's dependability and validity. The secondary data strategy allowed for a thorough comprehension of current knowledge, minimized time and resource limitations, and permitted a comparative assessment of molecular detection technologies. To keep academic integrity and avoid plagiarism, the right ways to cite and reference were used. This

strategy made sure that the study was founded on solid scientific data and gave a wide range of analytical views on ways to find cancer.

Effectiveness at Different Stages of Carcinoma Development

Biomarker Analysis: Initial Stages (I-II: Localized, Minimal Tumor Burden)

In the initial stages of illness, the amount of tumor-associated material, such as circulating tumor DNA (ctDNA), is markedly low—often constituting less than 1 percent of the total cell-free DNA. Consequently, precise measures are required to avoid overlooking a tumor. Individuals at elevated risk should have non-invasive blood testing for screening purposes.

PCR: PCR is essential in detecting established hotspot mutations, such as KRAS in colorectal cancer and TP53 in ovarian cancer, as well as in discovering methylation markers. Droplet digital PCR (ddPCR) may detect ctDNA concentrations as low as 0.1-1, making it valuable for the early identification of pancreatic or colorectal cancers. ddPCR demonstrates a sensitivity ranging from 35% to 78% when utilized in conjunction with protein markers such as CA19-9. Quantitative PCR (qPCR) exhibits lower sensitivity (overall sensitivity around ~0.32-66 in HPV-related malignancies), although it is more rapid and cost-effective for large-scale screening. qPCR exhibits good specificity (about 90-94 percent) but is deficient in detecting novel or mixed variants. PCR can identify early methylation changes associated with breast cancer in liquid biopsies, with an area under the curve of 0.889, a sensitivity of 100%, and a specificity of 75%. **(Ma et al., 2024), (Naegele et al., 2024)**

NGS: Next-generation sequencing (NGS) offers extensive profiling capabilities. It identifies many alterations, including new mutations and epigenetic modifications, such as methylation DNA markers in liver cancer. NGS exhibits a sensitivity of 95% and an AUC of 0.96. Next-generation sequencing (NGS) exhibits greater sensitivity in low-tumor-burden instances, with an overall sensitivity of approximately ~0.91 to 0.93 in HPV-related malignancies, in contrast to polymerase chain reaction (PCR). The extensive coverage offers a competitive advantage in heterogeneous malignancies, including lung and breast cancer. The compromise is that NGS necessitates supplementary DNA and, in certain instances, enrichment processes for ultra-low fractions. It is optimal for early screening panels of multi-cancer; nevertheless, the turnaround time is prolonged (days) and more costly compared to PCR. **(Ma et al., 2024), (Naegele et al., 2024), (Passaro et al., 2024)**

Biomarker analysis: Biomarker analysis encompasses non-genetic markers. Examples include protein assays such as PSA in prostate cancer via ELISA and microRNA in blood panels for lung cancer. Genetic analysis typically use PCR/NGS to examine ctDNA or exosomes. These panels have varying sensitivities; miRNA panels demonstrate a sensitivity range of 94-97% in Wilms tumor. They may also be advantageous in the initial liquid biopsy diagnosis of uroepithelial carcinoma (e.g., in conjunction with methylation and mutation markers), with a sensitivity of 96% and specificity of 88%. Tissue immunohistochemistry is less invasive yet can be beneficial in confirming early lesions. Biomarker tests are often broad, however they are influenced by the specific target and the style of the test. **(Ma et al., 2024), (Das et al., 2024)**

NGS is more suitable for first detection because to its breadth, while PCR is chosen for rapid targeted testing. Biomarker panels are a synthesis of multiple markers integrated into a single test.

Intermediate Stages (III: Regional Dissemination)

During the intermediate phases, the tumor mass expands, hence elevating detection rates. Nonetheless, the tumor exhibits increased diversity, necessitating vigilant monitoring of its progression.

PCR: PCR is reliable for identifying known mutations, particularly those crucial for monitoring responses in colorectal cancer. ddPCR exhibits high sensitivity and demonstrates a concordance rate of approximately ~87-93% with tissue samples. It is effective for serial monitoring; nevertheless, it is ineffective in scenarios involving the emergence of novel mutations. **(Su et al., 2022), (Ma et al., 2024)**

NSG: Next-Generation Sequencing (NGS) is highly successful in tracking clonal evolution and resistance mutations, particularly in lung cancer. It exhibits a high sensitivity of ~91% in stages II to IV. NGS demonstrates enhanced overall genotyping capabilities, identifying a greater number of mutations than PCR. Next-generation sequencing can predict disease development earlier in liquid samples compared to imaging techniques. **(Ma et al., 2024)**

Biomarker analysis: Dynamic monitoring can be efficacious through biomarker analysis. The progression-free survival correlates with the concentration of ctDNA assessed via PCR-integrated assays. Protein markers, such as CA15-3 in breast cancer, assist in assessing the cancer's stage; elevated levels indicate further dissemination.**(Das et al., 2024), (Zafar et al., 2025)**

Both methods operate effectively, with NGS offering more comprehensive prognostic information.

Subsequent Initiation (IV: Metastatic)

In advanced stages, the tumor burden is sufficiently substantial for detection, exhibiting heterogeneity and therapeutic resistance that confound analysis.

PCR: PCR remains relevant for recognized targets, such as EGFR in lung cancer, demonstrating a concordance rate of 87-93% with NGS. The sensitivity of ddPCR in metastatic HPV tumors ranges from ~0.77 to 0.89. PCR is an economical method for monitoring resistance; however it does not capture all mutations. **(Su et al., 2022), (Naegele et al., 2024), (Bestvina et al., 2024)**

NGS: Most effective, finding 100% of advanced lung cancer cases and new variations for customized treatment. Long-term lower expenses because of superior results (for example, longer progression-free survival compared to PCR in metastatic non-small cell lung cancer). Perfect for liquid biopsies of metastases that can't be reached. **(Ma et al., 2024), (Bestvina et al., 2024), (Bestvina et al., 2024)**

Biomarker analysis: Prognosis necessitates the utilization of biomarkers. Elevated ctDNA levels are associated with worse outcomes. The integration of biomarker data and next-generation sequencing (NGS) can yield multi-omics, shown by the prediction of immunotherapy response using tumor mutational burden (TMB) assessed via NGS. This approach exhibits a sensitivity of 0.89 when utilized in conjunction with PD-L1 testing. The assessment of microsatellite instability in advanced gastrointestinal malignancies is highly specific. **(Shi et al., 2023), (Ma et al., 2024)**

Table: 2 Comparison Table.

Aspect	PCR	NGS	Biomarker Analysis
Sensitivity	High for targeted (ddPCR: 0.77–0.89; qPCR: 0.32–0.66); best for low-burden early stages	Highest overall (0.91–0.93); broad for heterogeneous tumors across stages	Variable (80–97%); high when multi-marker (e.g., 96% in early uroepithelial)
Specificity	High (90–94%); low false positives for known targets	High (similar to PCR); excellent for novel variants	High (88–94%); depends on assay (e.g., IHC: tumor-specific)
Advantages	Fast (hours), low-cost, simple; ideal for serial monitoring and early targeted detection	Comprehensive, detects unknowns; better for prognosis and therapy guidance	Versatile (proteins/miRNAs); non-invasive; integrates with PCR/NGS for multi-omics
Limitations	Limited to known targets; misses heterogeneity	Complex, costly, longer time (days); bioinformatics needed	Assay-dependent; may require validation for novel biomarkers
Suitability by Stage	Early: Good for low ctDNA; Intermediate/Advanced: Effective but limited scope	Early: Strong with enrichment; Intermediate/Advanced: Optimal for complexity	Early: Screening via liquids; Advanced: Prognostic via levels
Cost & Time	Low cost, quick turnaround	Higher cost, slower	Moderate; varies by method
Examples in Carcinoma	KRAS in colorectal (early detection)	Multi-gene panels in lung (advanced monitoring)	CA19-9 + ctDNA in pancreatic (all stages)

NGS is usually the best way to find cancer at all stages, especially late carcinoma. PCR, on the other hand, is a good way to find cancer at early stages. Biomarker analysis enhances both by providing complementary data, often boosting sensitivity when combined (e.g., PCR + NGS). The choice depends on the clinical situation, and liquid biopsies make it possible to act sooner. **(Passaro et al., 2024), (Ma et al., 2024), (Meridianbioscience, 2026)**

Molecular Markers in Carcinoma Progression

Tumor Suppressor Gene/Protein TP53.

Expression Patterns: During the early stages, TP53 is typically wild-type and is normally expressed or even lowly. Mutations start piling up -TP53 mutations have been observed in about 50% of cases of NSCLC and bladder carcinoma. The intermediate phase is characterized by an increase in TP53 overexpression which is demonstrated by IHC and it is involved in the disruption of cell cycle. Mutant TP53 expression prevails in progressive disease, over 70% Nederlandse slokwoudier en NSCLC metastases, and is highly correlated with genomic instability and resistance to treatment.

Diagnostic Significance: Mutations detectable in ctDNA for early bladder/NSCLC screening; IHC on tissue confirms malignancy (sensitivity ~80% in advanced).

Prognostic Significance: TP53 overexpression/mutation is associated with poorer overall survival (OS) and progression-free survival (PFS), and hazard ratio of 1.5-2.0 in NSCLC. It also has an independent predictive value of recurrence with bladder and ovarian carcinoma. (Mehta et al., 2010)

EGFR (Epidermal Growth Factor Receptor, Oncogene/Protein)

Patterns of Expression: The early disease is characterized by normal or low levels of EGFR. Moderate overexpression is observed at intermediate stages, which is frequently caused by the amplification of genes in 10-20% of NSCLC cases. High overexpression or activating mutations (e.g., exon 19/21 in NSCLC, bladder) are the characteristic feature of advanced disease that promotes proliferation and metastasis. **(Mehta et al., 2010)**

Diagnostic Significance: EGFR mutations are able to be identified in liquid biopsies and offer early cancer diagnoses to NSCLC with 70-90% sensitivities. In bladder and rectal cancers, staging is done with the help of IHC and FISH.

Prognostic Significance: EGFR overexpression is associated with high-grade or advanced disease and a lower OS; patients can have their survival of rectal cancer reduced by 20-30%. EGFR status predicts response to tyrosine-kinase inhibitors, but this is bad prognosis in cases where resistance-conferring mutations develop. **(Mehta et al., 2010)**

HER2/neu (Human Epidermal Growth Factor Receptor 2, Oncogene/Protein)

Patterns of Expression: HER2 is minimally amplified in early breast and bladder cancers. Approximately 15-20% of breast tumors in intermediate stages amplify. HER2 overexpression or amplification appears to be highest in advanced disease and 25-30% of metastatic breast cancer has these alterations and is highly correlated with aggressive behavior. **(Mehta et al., 2010)**

Diagnostic Significance: FISH and IHC determine the status of HER2 in breast cancer subtyping. HER2 levels in serums are used to track the development of the disease in gastric and bladder cancers.

Prognostic Significance: Overexpression of HER2 is associated with increased risk of developing metastasis and worsening OS (1.8-hazard ratio in breast cancer). Nonetheless, the patients characterized by HER2-positive disease are frequently treated with improved outcomes in case of targeted HER2 therapy. **(Mehta et al., 2010)**

KRAS (Oncogene)

Patterns of Expression: KRAS mutations are early onset, occurring in 30 -40% of colorectal cancer and NSCLC. The mutation rate is maintained at intermediate levels and during advanced disease it grows clonally, which adds to the heterogeneity of tumours. **(Mehta et al., 2010)**

Diagnostic Significance: PCR-based detection in ctDNA for early CRC screening; high specificity for malignancy.

Prognostic Significance: KRAS mutations are predictive of shorter OS in NSCLC and colorectal cancer and in advanced disease, PFS declines. They also confer resistance to EGFR-targeted therapy. **(Mehta et al., 2010)**

VEGF (Vascular Endothelial Growth Factor, Angiogenesis Promoter)

Patterns of expressions: Low levels of VEGF are observed in early tumors, owing to low levels of hypoxia. There is an intermediate phase where the tumor is upregulated and its size increases. Enhanced disease especially in renal cell carcinoma (RCC) and metastatic colorectal cancer expresses a high rate of VEGF to help in neovascularization. **(Mehta et al., 2010)**

Diagnostic Significance: VEGF concentrations in serum are useful in the detection of advanced RCC, whereas IHC assesses tumor angiogenic activity.

Prognostic Significance: High VEGF is associated with poorer OS of clear cell RCC (HR 2.0) and increased risk of metastasis. **(Mehta et al., 2010)**

ER/PR (Estrogen/Progesterone Receptors, Hormone Receptors in Breast Carcinoma)

Patterns in Expression: Early breast cancers turn out to be often ER-positive (70-80%). The process of expression loss involves intermediate phases with the dedifferentiation of tumors. Triple negative subtypes and/or advanced disease can frequently exhibit low or no ER/PR expression.

Diagnostic Significance: IHC for subtyping and early detection in breast; guides endocrine therapy eligibility. **(Mehta et al., 2010)**

Prognostic Significance: Positive status predicts better OS (e.g., 5-year survival >80% vs. 60% in negative); loss indicates aggressive progression. **(Mehta et al., 2010)**

RAD52 (DNA Repair Gene)

Patterns of expression: RAD52 is normally expressed in early bladder cancer or it expresses high. The level of RNA decreases in intermediate, muscle-invasive disease. Later stages have an additional loss that impairs the ability to repair DNA.

Diagnostic Significance: RAD52 RNA may be assayed in tissue or urine to identify bladder cancer at an early stage, and is a part of DNA-damage-response gene panels.

Prognostic Significance: The reduced expression of RAD52 is associated with a reduced disease-free survival especially in those individuals who receive cisplatin treatment. Analysis of TCGA supports negative outcomes. **(Tappia & Ramjiawan, 2023)**

MAL (Myelin and Lymphocyte Protein, Gene in Ovarian Carcinoma)

Patterns of Expression: MAL is lowly expressed in early-stage ovarian cancer, as well as long-term survivors. It increases to intermediate levels in the intermediate disease, and in the advanced stages is three times higher in short-term survivors than long-term survivors.

Diagnostic Significance: The levels of malignancy in ovarian cancer are distinguished by microarray or qRT-PCR tests.

Prognostic Significance: High expression predicts short survival (<3 years) in stage III/IV; part of gene signatures for OS prediction (90% accuracy in models). **(Berchuck et al., 2005)**

miR-99b-5p (microRNA in Prostate Carcinoma)

Patterns of Expression: High in early prostate; downregulated in intermediate; substantially low in advanced, encouraging aggressiveness.

Diagnostic significance: Serum and urine tests for early aggressive prostate screening.

Prognostic Significance: Downregulation associated with unfavorable overall survival through the AR-mTOR axis; a possible biomarker for therapeutic resistance. **(Tappia & Ramjiawan, 2023)**

Sensitivity and Specificity in Molecular Diagnostic Tools for Carcinoma Detection

Molecular diagnostic methods such as PCR (including variants like qPCR and ddPCR), NGS, and biomarker analysis are essential for determining the course of carcinoma through its stages: early (I-II: localized, low burden), intermediate (III: regional dissemination), and advanced (IV: metastatic). Sensitivity (true positive rate) tells you how well you can find cancer at a certain stage, while specificity (true negative rate) tells you how well you can rule out instances that aren't cancer.

These numbers are important for finding cancer early, which increases survival rates (for example, the 5-year survival rate is over 90% for early-stage cancers and less than 20% for late cancers in many types of cancer). They are also

important for planning therapy, such as choosing targeted medicines or keeping an eye on how the disease is becoming worse.

According to data from meta-analyses and studies, sensitivity often rises with stage because there is a bigger tumor burden (for example, more ctDNA release), while specificity stays high (often >90%) to reduce false positives. Tumor heterogeneity, low analyte levels at early stages, and differences across types of carcinoma (such lung, breast, and colorectal) are all problems. In liquid biopsies, tools are often utilized to find things without cutting into the body.

Using more than one method at a time (like multi-marker panels) improves performance. We assess chosen techniques below, concentrating on ctDNA, mutations, and protein/epigenetic indicators, informed by recent research.

PCR

PCR makes certain nucleic acids bigger so that mutations (such KRAS or TP53) or methylation can be found more easily. ddPCR gives you exact numbers and is very sensitive to uncommon variations. It is great at finding known targets early on, but it could overlook new changes.

- **Early Stages:** Sensitivity ranges from 53.8% to 92% (for example, ddPCR for multi-cancer is 53.8% to 100%, and qPCR for cfDNA is about 92% for EGFR in NSCLC). Specificity: 80–96%. Less sensitive since ctDNA is hard to find, but good for screening (for example, the PanSeer test can find cancer four years before diagnosis with about 88% overall post-diagnostic sensitivity, which means it can be useful early on). **(Neefs et al., 2025), (Chen et al., 2020), (Gao et al., 2022), (Zafar et al., 2025)**
- **Intermediate Stages:** Sensitivity 78–98% (for example, ddPCR for lung adenocarcinoma: 96.5% at 93% spec). Specificity: 90 to 97%. Better because of a moderate tumor load.
- **Advanced Stages:** Sensitivity 92–100% (for example, ddPCR for NSCLC: ~100%). Specificity: 94–99%. High burden makes it easier to find things, which helps in tracking progression and resistance. **(Guo et al., 2022), (Rubinstein et al., 2024)**

NGS

NGS allows for high-throughput genomic profiling of a wide range of things, such as mutations, CNVs, and fusions. It works best for tumors that are different from each other and finding new variants, but it needs bioinformatics and takes longer.

- **Early Stages:** Sensitivity ranges from 16.8% to 65.6%, including 16.8% for stage I multi-cancer, 59–71% for stage I-II in colorectal/breast/ovarian/lung using TEC-Seq, and 65.6% in prostate urine/plasma. Specificity: 95–99.67%. Lower sensitivity for stage I (e.g., 27–62%) restricts standalone early screening, although enrichment enhances it. **(Klein et al., 2021), (Jiang et al., 2025), (Lin et al., 2021), (Silva et al., 2024), (Rubinstein et al., 2024)**
- **Intermediate Stages**: Sensitivity 60–96.92% (for example, 60–87% for stage III and 96.92% for SNVs at 0.5% AF). Specificity: 99–99.67%. Better at finding clonal evolution.
- **Advanced Stages:** Sensitivity ranges from 67.6% to 100% (for example, 100% for stage II-IV NSCLC and 67.6% for advanced prostate plasma). 95–99% specificity. Does a great job of guiding therapy (for example, TKI for EGFR). **(Lescuyer et al., 2025), (Jiang et al., 2025), (Lin et al., 2021)**

Analysis of Biomarkers

This includes tests for proteins (such ELISA for CA19-9 and PSA), miRNA, and multi-omics panels. Usually combines PCR/NGS for better accuracy; panels make it easier to find things early on.

- **Early Stages:** Sensitivity 43–94.64% (for example, CancerSEEK: 43% stage I; CTC profiling for breast: 94.64%; multi-biomarker for NSCLC: 91.2% stage I-II). Specificity: 93.1% to 97%. Variable; low for single markers (e.g., CA19-9: low sens/spec), but panels boost (e.g., AFP + cfDNA: 94.4% spec). **(Crook et al., 2022), (Shao et al., 2023), (Tenchov et al., 2024), (Zhou et al., 2024), (Thomas et al., 2025)**
- **Intermediate Stages:** Sensitivity 60–95% (for example, CancerSEEK: 78% stage II; MIP sensors: high for multi-markers). Specificity: 90% to 99%. Helpful for staging and making predictions.
- **Advanced Stages:** Sensitivity 75–100% (e.g., >75% for multiple tumors; 100% for stage IV in some panels). Specificity: 95–99%. Strong for keeping an eye on response and metastases. **(Shao et al., 2023), (Büyüktiryaki, 2025), (Zhao et al., 2025), (Tappia & Ramjiawan, 2023)**

Implications for Early Detection and Treatment Planning

Early Detection: NGS and biomarker panels look well for screening for more than one type of cancer (for example, MCED tests have a sensitivity of 27–95% at >95% specificity), but PCR is faster and cheaper for finding early signs. Combining technologies (such NGS and biomarkers) gets more than 90% of early NSCLC cases right, which lowers the number of false positives.

Planning treatment: Metrics for each stage help make decisions. For example, low early sens means more imaging or biopsies, whereas high advanced sens means more monitoring for resistance (such EGFR by NGS/PCR). These tools make it possible to make individualized strategies, which can improve results by 20–50% in cases where they work, but they need to be tested in different groups of people. **(Kisiel et al., 2022), (Kim et al., 2023), (Zafar et al., 2025), (Crosby et al., 2022), (Chen et al., 2020)**

Comparative analysis

The comparative analysis combines the results of molecular diagnostic approaches at different phases of cancer development. This section brings together the results of stage-wise evaluations to find the best way to diagnose and show how PCR, NGS, and biomarker analysis can work together.

Table: 3 Stage-Wise Comparative Superiority of Molecular Diagnostic Techniques in Carcinoma Detection.

Carcinoma Stage	Tumor Characteristics	Most Effective Technique	Supporting Techniques	Reason for Superiority	Clinical Application
Stage I – II (Early / Localized)	Low tumor burden, minimal ctDNA release, early molecular alterations	PCR (especially ddPCR)	Biomarker Panels, NGS (with enrichment)	Extremely high sensitivity for detecting rare known mutations and methylation markers; rapid and cost-effective	Early screening, high-risk population monitoring, mutation-specific detection
Stage III (Intermediate / Regional Spread)	Increased tumor burden, genetic heterogeneity begins, clonal evolution occurs	NGS	PCR for mutation monitoring, Biomarker analysis	Comprehensive genomic profiling detects emerging mutations and tumor heterogeneity	Disease progression monitoring, therapy selection, resistance mutation identification
Stage IV (Advanced / Metastatic)	High tumor burden, extensive heterogeneity, treatment resistance mutations	NGS + Biomarker Analysis (Multi-omics Approach)	PCR for targeted resistance tracking	Detects novel mutations, immunotherapy markers, tumor mutational burden, and prognostic biomarker levels	Personalized therapy planning, immunotherapy selection, prognosis evaluation

Table: 4 Overall Comparative Ranking of Techniques.

Technique	Strength Area	Weakness Area	Overall, Role
PCR	High sensitivity for known mutations, cost-effective, rapid results	Cannot detect unknown mutations, limited genomic scope	Best for early detection and mutation monitoring
NGS	Broad mutation detection, detects heterogeneity, supports personalized medicine	Expensive, technically complex, longer turnaround time	Best for intermediate and advanced carcinoma analysis
Biomarker Analysis	Non-invasive screening, prognostic evaluation, supports multi-omics	Variable accuracy if single marker used	Best as complementary diagnostic and prognostic tool

Table:5 Comparative Effectiveness of Molecular Techniques Across Carcinoma Stages

Carcinoma Stage	PCR Effectiveness	NGS Effectiveness	Biomarker Analysis Effectiveness	Overall Diagnostic Strategy
Early Stage (Stage I–II: Localized Tumor)	Very high sensitivity for detecting known mutations and low ctDNA levels; rapid and cost-effective	Moderate effectiveness; capable of detecting multiple mutations but may require enrichment and higher sample quantity	High when multi-marker panels are used; useful for non-invasive screening and early risk detection	PCR is primary diagnostic tool supported by biomarker panels and selective NGS screening

Intermediate Stage (Stage III: Regional Spread)	High accuracy for monitoring known mutations and tracking disease progression	Very high effectiveness due to ability to detect tumor heterogeneity, clonal evolution, and emerging resistance mutations	Moderate to high effectiveness; useful for monitoring tumor burden and prognostic evaluation	NGS becomes dominant technique, supported by PCR for mutation tracking and biomarker monitoring
Advanced Stage (Stage IV: Metastatic Cancer)	Effective for targeted resistance mutation monitoring but limited in detecting novel alterations	Highest effectiveness; provides comprehensive genomic profiling, therapy selection guidance, and mutation discovery	Very high effectiveness for prognosis, immunotherapy response prediction, and disease monitoring	Combined NGS and biomarker analysis provide most accurate diagnostic and therapeutic decision support, with PCR as complementary monitoring tool

Discussion

The comparative assessment of molecular biology methodologies indicates that cancer detection has markedly enhanced due to the progression of PCR, next-generation sequencing (NGS), and biomarker-driven strategies. The efficiency of each approach varies according to the stage of cancer development. In the initial stages of carcinoma, characterized by limited tumor burden and circulating tumor DNA (ctDNA) levels, extremely sensitive techniques such as droplet digital PCR and multi-marker biomarker panels offer dependable screening and early detection. NGS improves early detection even further by finding several genomic and epigenetic changes at the same time.

However, its high cost and technical difficulty make it hard to use in all cases. As carcinoma moves into intermediate stages, the tumors become more diverse, which means that full molecular profiling is needed. NGS is especially good at finding clonal evolution and resistance mutations, although PCR is still good for keeping an eye on specific mutations and tracking disease development.

Biomarker analysis enhances these methodologies by facilitating non-invasive assessment of protein, microRNA, and epigenetic markers linked to tumor proliferation and metastasis. In advanced metastatic phases, molecular diagnostics are crucial for therapeutic decision-making and prognosis assessment. NGS gives a lot of genomic information that is needed for customized medicine, such as choosing targeted medication and immunotherapy. Biomarkers like ctDNA levels and tumor mutational burden help us guess how well a treatment will work and how the disease will progress.

Combining different molecular diagnostic technologies makes them more sensitive and specific at all stages, which means that treatment may start sooner and disease can be monitored better. But broad use is limited by problems including high costs, the need for advanced lab equipment, and the fact that biomarker expression varies between various forms of carcinoma. So, it looks like the best way to improve the detection and management of carcinoma is to combine low-cost PCR-based screening with thorough NGS and biomarker analysis.

Conclusion

The study emphasizes the essential function of molecular biology approaches in enhancing the identification, monitoring, and prognosis of cancer at various developmental stages. PCR-based approaches are useful for early screening and illness monitoring because they can quickly, cheaply, and very specifically find known mutations. NGS provides extensive genomic profiling, facilitating the detection of new mutations, tumor heterogeneity, and therapy-resistant variations, which is crucial for customized treatment planning. Biomarker analysis is an additional method that uses protein, microRNA, and epigenetic markers to help with non-invasive detection and prognostic evaluation. Using these molecular approaches together makes diagnoses more accurate, helps treatments work better, and promotes early intervention efforts. Even if there are problems with cost and technological complexity, molecular diagnostics and multi-omics techniques are projected to keep becoming better, making them easier to use and more reliable. In general, using molecular biology approaches together is a promising way to improve the identification of cancer and the survival rates of patients.

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