IJHSR
ijhighschoolresearch.org
REVIEW ARTICLE

Understanding Cancer Through Biomarkers: Integrating Diagnostic Testing with Literature Analysis

Avni Goswami

Lancers International School, Gurugram, India; avnigoswami08@gmail.com

ABSTRACT: This study investigates the diagnostic and prognostic significance of key cancer biomarkers—KRAS, BCR-ABL, PIVKA-II, and HPV—through literature review and laboratory-based molecular diagnostics. Real-time PCR and immunoassays were employed to analyze clinical samples for genetic, proteomic, and viral biomarkers. The findings revealed that KRAS mutations were identified in codon 12, while BCR-ABL fusion transcripts were detected in multiple blood samples, confirming leukemia diagnosis. Elevated PIVKA-II levels indicated hepatocellular carcinoma, and HPV 16 & 18 strains were identified in cervical samples. These results highlight the importance of molecular diagnostics in early detection and the planning of treatment. The study also highlights challenges in biomarker variability and sample size, and emphasizes the future potential of synthetic biomarkers and AI-based diagnostics in cancer care.

KEYWORDS: Translational Medical Sciences, Disease Detection and Diagnosis, Biomarkers, Cancer RT PCR, BCR-ABL, HPV, KRAS Mutation.

Introduction

Biomarkers in cancer refer to measurable indicators of biological or pathological processes, including specific molecules in bodily fluids, tissues, or cells that provide valuable insights into cancer progression, type, or presence.[1] Biomarkers can be proteins, genes, metabolites, or even lipids that are differentially expressed in cancerous tissues as compared to normal tissue. Cancer biomarkers can be broadly classified as proteomic, genetic, and epigenetic. Genetic biomarkers are alterations in the DNA sequence that may drive the development of cancer. KRAS and EGFR are common examples of genetic biomarkers.[2] Additionally, HPV (human papillomavirus) detection serves as an epigenetic and viral biomarker in cervical and oropharyngeal cancers. High-risk HPV strains, particularly HPV-16 and HPV-18, are implicated in the pathogenesis of these cancers.[3] Proteomic biomarkers define a change in protein levels, which reflects alterations in cell signaling, metabolic pathways, or immune response.[4] PIVKA-II is an abnormal form of prothrombin used in hepatocellular carcinoma (HCC),[5] and is an example of a protein biomarker that will be examined in the literature review **(Table 1)**.

Protein biomarkers include overexpressed proteins such as HER2 in breast cancer and mutated proteins like BCR-ABL in chronic myeloid leukemia (CML), which is critical for treatment with tyrosine kinase inhibitors.[6]

Biomarkers guide cancer management by classifying tumors based on molecular signatures that often predict therapeutic outcomes. For instance, HER2 overexpression in breast cancer identifies patients likely to respond to targeted therapies like Trastuzumab, improving prognosis. Predictive biomarkers also help determine a tumor's likelihood of responding to specific treatments. In the context of targeted therapies, biomarkers like EGFR in non-small cell lung cancer (NSCLC) play a crucial role in identifying patients who will benefit from tyrosine kinase inhibitors (TKIs), such as erlotinib or gefitinib.[7]

One of the primary challenges in oncology is accurately distinguishing between various cancer types, as tumors with similar histological features may have distinct molecular profiles and clinical behaviors. Genetic biomarkers have become indispensable in overcoming this challenge, particularly in the case of histological overlap or ambiguous clinical representation. For instance, mutations in KRAS, a gene encoding a GTPase involved in cell signaling,[8] are common in colorectal cancer and pancreatic cancer, where they not only provide prognostic value but also detect resistance to therapies such as anti-EGFR monoclonal antibodies like cetuximab.[9] Similarly, EGFR mutations, most notably exon 19 deletions, and L858R point mutations are frequent in adenocarcinoma of the lungs, particularly in non-smokers and Asian populations,[10] and help differentiate between cancer subtypes while guiding the use of EGFR inhibitors for targeted treatment.[11] For instance, BRCA1/2 mutations: these tumor suppressor genes predispose individuals to hereditary breast and ovarian cancer, where mutations correlate with high-grade tumors and guide the use of PARP inhibitors such as Olaparib, significantly improving patient outcomes in BRCA-mutated cancers, thus proving that biomarkers are central to the precision medicine approach in oncology.[12]

DOI: 10.36838/v8i1.14

Table 1: List of Biomarkers associated with different Cancers. This table summarizes relevant biomarkers identified in various cancer types, including the gene name, associated cancer(s), specific mutation(s), diagnostic application, brief explanation of biomarker relevance-prognostic value, predictive utility, or clinical applicability.

Name of the gene	Cancer type/types	Mutation	Diagnosis	Explanation	References
KRAS	Colorectal, Pancreatic, and lung cancer	G12A, G12V, G12C	Sanger sequencing	KRAS mutations are oncogenic drivers that promote uncontrolled cell growth; they are common in solid tumors and influence treatment decisions.	Shackelford *et al.* 2012[13] Huang *et al.* 2021[14]
PIVKA-II	Liver, Pancreatic cancer	-	Biochemical analysis of the levels of PIVKA-II (Protein Induced by Vitamin K Absence-II)	PIVKA-II is an abnormal prothrombin protein elevated in vitamin K deficiency and hepatocellular carcinoma, used as a tumor marker for liver cancer.	Zhu *et al.* 2024[15]
HPV Human papillomavirus	Cervical, Anal, Vulvar, Vaginal, Penile, and Oropharyngeal Cancer	HPV-16, HPV-18	HPV test on samples taken from the cervical cells.	Persistent infection with high-risk HPV types leads to genomic instability and is the primary cause of cervical and other anogenital cancers.	Tsakogiannis *et al.* 2022[16]
BCR-ABL	Blood (Chronic Myeloid Leukemia, Acute Lymphoblastic Leukemia)	T215I, E255K/V, Y253F/H, F3171/L, V299L.	Flow cytometry	BCR-ABL fusion gene results from the Philadelphia chromosome translocation and is a key driver in CML; resistance mutations impact therapy response.	Löf *et al.* 2017[17] Hochhaus *et al.* 2011[18]

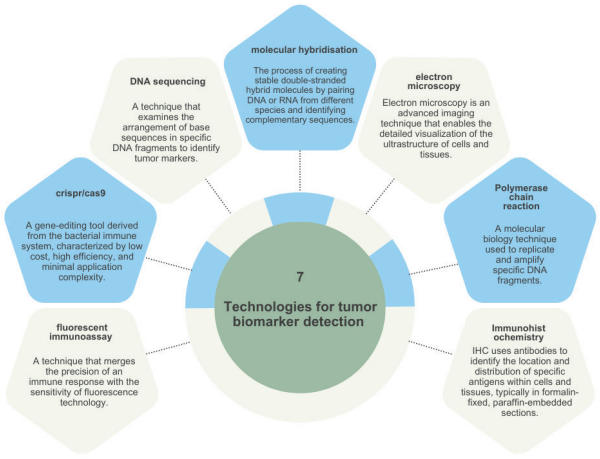


Figure 1: Schematic representation of different diagnostic techniques used to detect tumor biomarkers.

To investigate the diagnostic and prognostic potential of specific cancer biomarkers, I compiled and analyzed studies mainly published between 2010 and 2024 by querying databases like PubMed, Google Scholar, Kaggle, SpringerLink, and PMC for peer-reviewed articles, datasets, and review papers focused primarily on genetic, proteomic, and epigenetic biomarkers across various cancer types. The studies selected were mainly from randomized controlled trials (RCTs), systematic reviews, and meta-analyses to ensure high-quality evidence, with preference given to studies that addressed well-established biomarkers in breast, colorectal, lung, and ovarian cancer. Studies from 2015 to 2024 were mainly chosen to reflect the latest developments in cancer biomarker research.

The present study aims to investigate specific biomarkers' diagnostic and prognostic potential by comparing the findings in healthy versus cancer patients. The research will help examine how these biomarkers can help differentiate cancer types, predict treatment, and provide insights into disease progression. By synthesizing current evidence and analyzing data from a range of studies, this work will contribute to the ongoing work to refine cancer diagnostics and personalized therapeutic strategies. Additionally, while previous literature has extensively described the roles of individual biomarkers in cancer diagnosis or prognosis, this study aims to provide a comparative analysis of four key biomarkers through both literature review and molecular diagnostics, to analyze their combined diagnostic and prognostic potential across different cancer types.

Methodology

KRAS Mutation Diagnosis:

For KRAS mutation analysis, genomic DNA was extracted from fresh, frozen, or formalin-fixed paraffin-embedded (FFPE) tumor tissues using the QIAamp® FFPE DNA Tissue Kit. DNA purity was verified using spectrophotometric analysis, ensuring an A260/A280 ratio between 1.7 and 1.9. Each PCR reaction used 150–200 ng of purified genomic DNA. The DNA extraction process began by removing excess paraffin from the tissue block using a scalpel. Up to eight sections, each 5–10 μm thick, were cut from the block. If the outermost section was exposed to air, the first 2–3 sections were discarded to minimize contamination. The remaining sections were transferred to a 1.5- or 2-ml microcentrifuge tube, to which 1 ml of xylene was added. The tube was vortexed vigorously for 10 seconds, followed by centrifugation at full speed for 2 minutes at room temperature (15–20 °C). The supernatant was then carefully removed without disturbing the pellet. Subsequent steps were carried out according to the QIAamp® DNA FFPE Tissue Kit protocol to complete the extraction.

The detection of KRAS mutations was based on allele-specific amplification using the Amplification Refractory Mutation System (ARMS) in real-time PCR. This method employed specific primers to selectively amplify mutated DNA sequences, while fluorescent probes (HEX and FAM) were used to differentiate between mutant and wild-type alleles. During amplification, Taq polymerase cleaved the probes, releasing a fluorescent signal proportional to the number of DNA copies. The data was analyzed using the BIORAD CFX Maestro software.

For the PCR setup, the reaction mix included a master multiplex mix composed of a reaction buffer, $MgCl_2$, stabilizers, hot-start DNA polymerase, and dNTPs (dATP, dCTP, dGTP, dTTP). Additionally, specific primer-probe mixes (PPM) were prepared for each KRAS mutation along with an internal control primer-probe mix. Internal controls serve to monitor the efficiency of amplification and detect the presence of potential inhibitors, ensuring assay integrity. Each DNA sample underwent 12 different PCR reactions, each targeting a distinct KRAS mutation.

PCR reaction mixes per assay:

Component	Volume per reaction (µl)
Multiplex master mix (including reaction buffer, $MgCl_2$, stabilizers, DNA polymerase, and dNTPs)	10
Primer probe mix (PPM) specific to the target mutation	2.5
Internal control primer probe mix	2.5
Total volume (excluding DNA sample)	15
DNA sample (150-200 ng)	Up to 5

Mutation Detection by Real-Time PCR:

Thermal cycling for the real-time PCR assay was performed under the following conditions: an initial denaturation step at 94˚C for 10 minutes, followed by 40 amplification cycles consisting of denaturation at 94˚C for 15 seconds and a combined annealing and fluorescence acquisition step at 60˚C for 60 seconds. Each reaction included appropriate controls—sterile water as the negative control and KRAS-positive control DNA provided by the manufacturer as the positive control. Mutation detection was carried out by analyzing cycle threshold (Ct) values. The Δ Ct value, calculated as the difference between the mutation-specific Ct and the reference Ct, was compared to predefined thresholds (as shown in **Table 2**). Samples with Δ Ct values below the cut-off were classified as mutation-positive.

Table 2: Key KRAS mutations along with their codons, exons, and Δ Ct thresholds for detection using TRUPCR® KRAS Mutation Kit.

KRAS Mutation	Codon	Exon	Mutation Type	Amino Acid Change	Δ Ct Cut-off (≤)	Clinical Relevance
G12S	12	2	Missense	Gly → Ser	7.0	Common in colorectal and lung cancers
G12D	12	2	Missense	Gly → Asp	4.5	Predicts resistance to anti-EGFR therapies
G12R	12	2	Missense	Gly → Arg	8.5	Rare; seen in pancreatic cancer
G13D	13	2	Missense	Gly → Asp	5.5	May retain some EGFR-inhibitor sensitivity
G12C	12	2	Missense	Gly → Cys	3.5	Targeted by sotorasib (AMG 510), adagrasib
G12V	12	2	Missense	Gly → Val	6.5	Common in NSCLC
G12A	12	2	Missense	Gly → Ala	7.5	Occurs in pancreatic and colorectal cancer
A59x	59	2	Other (e.g., stop)	Ala → X (Stop)	4.0	Rare; associated with aggressive phenotype
Q61x	61	2	Other	Gln → X (e.g., His, Leu)	4.5	Found in various malignancies
K117x	117	3	Other	Lys → X	5.5	Less frequent, linked with therapy resistance
A146x	146	3	Other	Ala → X	8.0	Detected in colorectal and hematologic cancers

BCR-ABL:

RNA Extraction and Reverse Transcription for BCR-ABL1 Detection:

Peripheral blood or bone marrow samples were collected in EDTA tubes and stored at 2–8˚C. Total RNA was extracted using either the 3B SpeedTools RNA Blood Kit, Qiagen® RNeasy Mini Kit, or QIAmp® RNA Blood Mini Kit, following the manufacturer's protocols. RNA purity was confirmed using spectrophotometry (A260/A280 ratio of 1.7–2.0), and 1 µg of RNA per sample was reverse-transcribed using the TRUPCR® BCR-ABL1 Kit.

qPCR for BCR-ABL1 Transcript Detection:

Quantitative PCR was performed using the TRUPCR® BCR-ABL1 Kit, which targets major, minor, and micro BCR-ABL1 fusion transcripts and ABL1 as a reference. Fluorescent probes (FAM, HEX) enabled detection via Taq polymerase-mediated probe hydrolysis, with signal intensity proportional to RNA copy number. Data were analyzed using BIORAD CFX Maestro software. Each reaction included a master mix (buffer, $MgCl_2$, dNTPs, stabilizers, hot-start polymerase), transcript-specific primer-probe mixes, nuclease-free water, and 5 µl cDNA. Four reactions per sample were run. Thermal cycling involved initial denaturation at 94˚C for 10 min, followed by 45 cycles of 94˚C for 15s and 60˚C for 60s (fluorescence acquisition). Negative (sterile water), positive (BCR-ABL1 standard), and internal (ABL1) controls were included.

DNA Extraction for HPV Detection:

DNA was extracted from cervical swabs, urine, or FFPE tissue using the TRUPCR® Tissue DNA Extraction Kit. DNA quality (A260/A280 ratio 1.7–2.0) was verified spectrophotometrically. A volume of 10 µl of DNA per sample was used for amplification.

qPCR for HPV Genotyping:

HPV detection was performed using the TRUPCR® HPV HR with 16 and 18 Differentiation Kit. Primers targeted the E6/E7 regions of HPV 16, 18, and 12 other high-risk genotypes. Fluorescent probes (FAM, HEX, Texas Red, Cy5) differentiated between genotypes and internal controls. Taq polymerase cleaved the probes during amplification, generating genotype-specific signals. Data were analyzed with BIORAD CFX Maestro. Each reaction included a multiplex master mix (buffer, $MgCl_2$, dNTPs, polymerase, ROX), primer-probe mix, and appropriate controls. Samples were tested in a single-tube multiplex format. Thermal cycling included 94˚C for 10 min (initial denaturation), followed by 38 cycles of 94˚C for 15s, 62˚C for 30s (annealing), 72˚C for 15s (extension), and a final fluorescence step at 60˚C for 30s. Controls included sterile water (negative), standard HPV DNA (positive), and an internal human gene control.

Fluorescent channel selection:

Table 3: Reporter dyes used for the detection of different HPV genotypes for TRUPCR® HPV HR with 16 & 18 differentiation kit (Single tube) reaction.

Target	Reporter/ detection channel	Interpretation / Purpose
HPV HR (14 genotypes) "HPV HR" refers to pooled detection of the 14 high-risk types (e.g., 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68).	FAM/green	Detects the presence of any of the 14 high-risk HPV genotypes
HPV 16	Texas Red/Rox	Differentiates specifically the HPV 16 genotype
HPV 18	HEX/Vic	Differentiates specifically the HPV 18 genotype
Internal control	Cy5/Red	Confirms DNA extraction and PCR reaction validity

DOI: 10.36838/v8i1.14

PIVKA II-Protein Induced by Vitamin K Absence or Antagonist-II:

Sample preparation:

Human serum or plasma samples were collected using standard sampling conditions or tubes containing a separating gel. Acceptable anticoagulants included lithium heparin, K_2-EDTA, and K_3-EDTA. The Cobas e 801 analytical unit used 24 µl of the sample. The sample was automatically prediluted 1:5 with Diluent Universal, and 12 µl of the prediluted sample was used in the assay.

Assay procedure:

First incubation: The pre-diluted sample was incubated with a biotinylated monoclonal PIVKA-II-specific antibody and a monoclonal PIVKA-II-specific antibody labeled with a ruthenium complex, forming a sandwich complex.

Second incubation: Streptavidin-coated microparticles were added, allowing the complex to bind to the solid phase via a biotin-streptavidin interaction.

Measurement: The reaction mixture was aspirated into the measuring cell, where microparticles were magnetically captured onto the electrode surface. Unbound substances were removed, and the electrochemiluminescent signal was measured; the total assay duration was 18 minutes.

Analytical Specifications and Detection Protocol:

The assay had a measuring range of 3.5–12,000 ng/mL, with a limit of detection (LoD) of ≤3.5 ng/mL. Repeatability, expressed as the coefficient of variation (CV), ranged from 1.0% to 1.8%. The thermal cycling protocol included an initial incubation for 18 minutes at room temperature, followed by electrochemiluminescent signal measurement for detection. Control reactions included sterile water as a negative control, a PIVKA-II standard dilution series as the positive control, and an internal control to ensure consistency across multiple calibrations.

Results

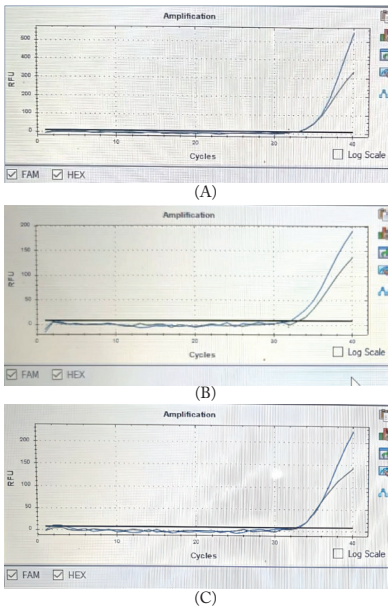
This study analyzed four key cancer biomarkers—KRAS, BCR-ABL, PIVKA-II, and HPV—across multiple clinical samples using real-time PCR and immunoassays. KRAS mutation analysis of sample M023-A0347 revealed three oncogenic mutations—G12C, G12V, and G12A—indicating a KRAS-positive profile commonly associated with colorectal, lung, and pancreatic cancers. BCR-ABL testing across 10 hematological samples showed fusion transcripts in 8 cases, supporting a diagnosis of chronic myeloid leukemia (CML) or Philadelphia-positive acute lymphoblastic leukemia (ALL). In the HPV analysis, 3 out of 10 cervical samples tested positive for high-risk genotypes (HPV 16 and/or 18), suggesting viral oncogenic involvement in a subset of the population. PIVKA-II levels, assessed in liver cancer risk cases, were elevated in 7 of 10 samples, indicating a potential diagnosis of hepatocellular carcinoma (HCC). Collectively, these findings validate the clinical utility of molecular diagnostics in cancer detection, while also highlighting biomarker-specific patterns that inform diagnosis, prognosis, and potential therapeutic strategies.

KRAS sample analysis:

Detection of KRAS mutations in Sample M023-A0347 using the TRUPCR® KRAS Kit across 11 assays. The table summarizes Ct values for both mutant (FAM) and control (HEX) channels, Δ Ct calculations, and interpretation against reference thresholds. The analysis revealed that mutations G12C, G12V, and G12A were detected as positive, indicating the presence of clinically relevant KRAS mutations in this sample.

Table 4: KRAS mutation analysis of Sample M023-A0347 using the TRUPCR® KRAS Kit. Δ Ct values were compared against reference thresholds across 11 assays. The mutations G12C, G12V, and G12A were detected, indicating a KRAS-positive result.

KRAS Mutation	Ct Fam (sample)	Ct HEX (control)	Δ Ct calculated	Δ Ct Reference	Result
G12C - PPM	31.65	32.95	1.27	≤ 3.5	G12C Positive
612S - PPM	-	32.41	32.41	≤ 7.0	Wild type or below LOD
G12R - PPM	-	33.31	33.31	≤ 8.5	Wild type or below LOD
612V - PPM	31.79	32.27	0.48	≤ 6.5	G12V Positive
G12D - PPM	-	33.61	33.61	≤ 4.5	Wild type or below LOD
G12A - PPM	32.15	33.01	0.86	≤ 7.5	G12A Positive
G13D - PPM	-	33.13	33.13	≤ 5.5	Wild type or below LOD
A59X - PPM	-	32.67	32.16	≤ 4.0	Wild type or below LOD
Q61X - PPM	-	32.80	32.80	≤ 4.5	Wild type or below LOD
K117X - PPM	-	32.63	32.63	≤ 5.5	Wild type or below LOD
A146X - PPM	-	32.62	32.62	≤ 8.0	Wild type or below LOD
Reference ppm	30.71	33.42	2.71		No reference was provided for direct comparison.



(A) (B) (C)

Figure 2: Graph showing RT PCR results for the identification of KRAS mutation in the sample. **A.** Dual-target amplification showing positive detection of KRAS G12C mutation (FAM, green) alongside internal control (HEX, blue). **B.** Positive detection of KRAS G12V. **C.** Positive detection of KRAS G12A. Distinct amplification curves and Δ Ct values confirm the presence of these mutations, indicating a KRAS-mutant profile in the sample.

BCR-ABL1 sample analysis:

Table 5: Summary of RT-PCR results for BCR-ABL1 detection using ABL1 as the reference gene. Out of 10 samples analyzed, 8 tested positive and 2 tested negative, indicating the presence of BCR-ABL1 fusion transcripts in the majority of the cases.

S.No.	Sample ID	Result
1	M02400715	positive
2	M02400718	Negative
3	M02400720	Positive
4	M02470021	Positive
5	M02400725	Positive
6	M02400726	Positive
7	M02500001	Positive
8	M02500002	Positive
9	M02500005	Negative
10	M02500009	Positive

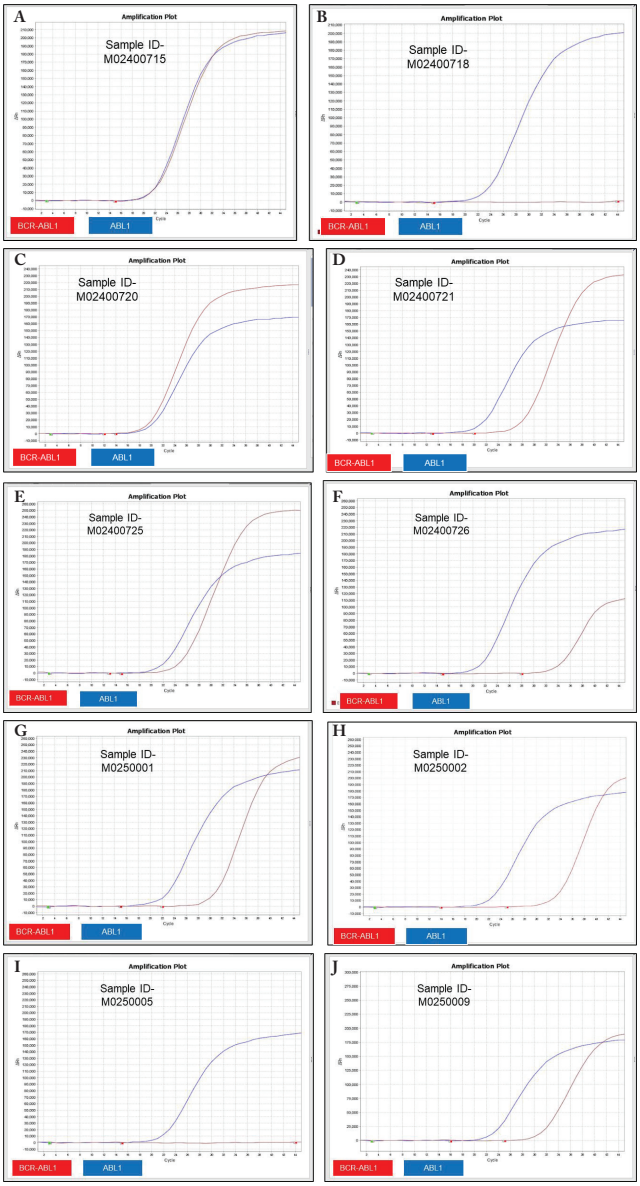


Figure 3: RT-PCR amplification curves for BCR-ABL1 detection across multiple samples. Positive samples (A, C–H, J) display dual amplification curves for BCR-ABL1 (red) and the internal control ABL1 (blue), while negative samples (B, I) show only the ABL1 control curve. These results align with the sample analysis summarized in Table 5.

HPV sample analysis:

Table 6: RT-PCR analysis of HPV genotypes in clinical samples. Out of 10 samples tested, 3 were positive for high-risk HPV genotypes, while 7 showed no detectable HPV DNA, indicating the presence of HPV infection in a subset of the tested population.

S.No.	Sample ID	Result
1	MO24-00061	Negative
2	MO24-00062	Negative
3	MO24-582	Negative
4	MO24-583	Negative
5	MO24-590	Positive
6	MO25-015	Negative
7	MO25-056	Positive
8	MO25-084	Negative
9	MO25-092	Positive
10	MO25-103	Negative

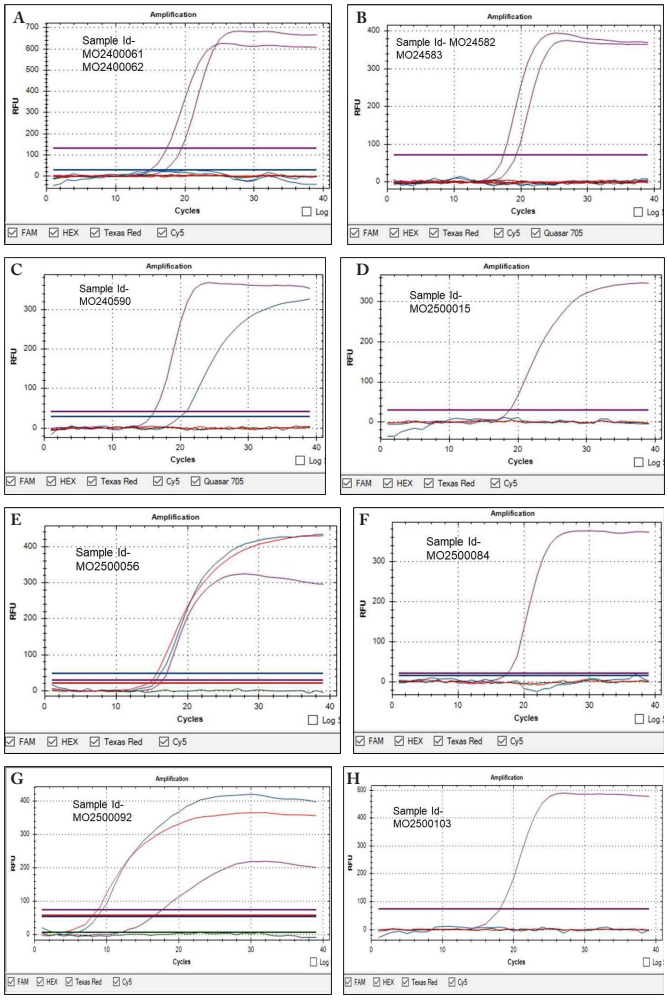


Figure 4: Graphs showing RT PCR results for the identification of HPV genotypes (HPV 16 and 18) in a sample. All samples showed Cy5/Red line (internal control). Graph C is positive for HPV 18 (HEX) (blue line). Graphs E and G (Texas red and HEX blue) are positive for both HPV 16 and HPV 18.

DOI: 10.36838/v8i1.14

PIVKA II sample analysis::

Table 7: PIVKA-II levels in samples with risk classification based on the reference range (<28.4 ng/mL). Values above this indicate high risk, while those below are normal.

Sample ID	Result	Risk	Bio Ref Range
IMM2403116	22.13	Normal	<28.4
IMM2403112	23.27	Normal	<28.4
IMM2403116	35.77	High	<28.4
IMM2403117	19.78	Normal	<28.4
IMM2403118	126.7	High	<28.4
IMM2403122	33.91	High	<28.4
IMM2500005	45.64	High	<28.4
IMM2500006	243.9	High	<28.4
IMM2500011	30.65	High	<28.4
IMM2500031	55.70	High	<28.4

Clinical Significance of the Results:

The clinical interpretation of the results obtained in this study is summarized **(Table 8)** below.

Table 8: Clinical Interpretation of Biomarker Results.

Biomarker	Detected Mutation / Result	Associated Cancer(s)	Clinical Implication
KRAS	G12C, G12V, G12A mutations	Colorectal, Lung, Pancreatic	Confirms oncogenic mutations; indicates likely resistance to anti-EGFR therapies (e.g., cetuximab); supports the need for alternative targeted treatments.
BCR-ABL	Fusion gene detected in multiple samples	Chronic Myeloid Leukemia (CML), Philadelphia-positive ALL	Confirms diagnosis; guides use of tyrosine kinase inhibitors (TKIs) such as imatinib; essential for monitoring treatment response and disease progression.
PIVKA-II	Elevated in multiple samples (e.g., 35.77 ng/mL)	Hepatocellular Carcinoma (HCC)	Suggests liver malignancy; aids early diagnosis and monitoring of tumor burden or recurrence; valuable in surveillance of high-risk patients.
HPV	High-risk types 16 and 18 were detected	Cervical, Oropharyngeal	Confirming oncogenic viral infection, associated with elevated cancer risk, guides screening, prevention (e.g., HPV vaccination), and early treatment strategies.

Discussion

Introduction to Biomarkers in Oncology:

This literature review explores the diagnostic and prognostic significance of key biomarkers–KRAS, BCR-ABL, PIVKA-II, and HPV–in various cancers **(Table 1)**. This study analyzes existing mutations and evaluates their effectiveness in early detection, disease monitoring, and treatment stratification. Understanding the clinical utility of these biomarkers is improving patient care, as accurate diagnosis and prognosis can lead to more targeted and effective therapeutic strategies, ultimately enhancing survival and quality of life.

KRAS in Solid Tumors:

The KRAS gene encodes a GTPase integral to the RAS/MAPK[14] signaling pathway, which regulates cellular proliferation, differentiation, and survival. Mutations in KRAS, particularly at codons 12, 13, and 61, result in constitutive activation of the RAS protein, leading to uncontrolled cell division and tumorigenesis.[14] These mutations are present in various malignancies, including pancreatic (approximately 90%), colorectal (30-50%), and non-small cell lung cancers (15-30%).[14] The presence of KRAS mutations **(Table 4; Figure 2)** serves as a diagnostic marker, differentiating malignant from benign lesions, and has prognostic implications, often correlating with resistance to particular therapies and poorer clinical outcomes.

BCR-ABL and Hematologic Malignancies:

BCR-ABL is a fusion oncogene resulting from the t(9;22)(q34;q11) chromosomal translocation, known as the Philadelphia chromosome.[18] This translocation juxtaposes the breakpoint cluster region (BCR) gene on chromosome 22 with the Abelson murine leukemia viral oncogene homolog 1 (ABL1) gene on chromosome 9, producing a constitutively active tyrosine kinase. The BCR-ABL fusion protein drives leukemogenesis by activating multiple signaling pathways that enable proliferation and inhibit apoptosis. This fusion gene is a hallmark of chronic myeloid leukemia (CML) **(Table 5; Figure 3)** and is also present in a subset of Acute Lymphoblastic Leukemia (ALL) cases.[19] Detection of BCR-ABL is diagnostic for this leukemia and guides targeted therapies with tyrosine kinase inhibitors, such as imatinib,[18] which have improved patient outcomes.

PIVKA-II as a marker for Hepatocellular Carcinoma:

PIVKA-II, or des-γ-carboxy prothrombin, is an abnormal form of the blood-clotting protein prothrombin. It is produced in the absence of vitamin K or under the influence of vitamin K antagonists. Elevated PIVKA-II levels **(Table 7)** are strongly associated with hepatocellular carcinoma (HCC), as malignant hepatocytes show impaired prothrombin carboxylation. This biomarker helps distinguish malignant hepatic tumors from benign liver conditions and is especially valuable for early HCC detection in high-risk populations by identifying tumor-specific proteins in the blood.[20]

HPV in Virus-Associated Cancers:

Human Papillomavirus (HPV), a double-stranded DNA virus from the Papillomaviridae family, includes high-risk subtypes like HPV 16 and 18, which are major drivers of cervical and oropharyngeal cancers.[21] These subtypes promote oncogenesis via E6 and E7 oncoproteins that inactivate tumor suppressors p53 and pRB, enabling uncontrolled proliferation.[22] HPV DNA **(Table 6; Figure 4)** and E6/E7 mRNA tests support early detection and risk stratification. Additionally, HPV integration into host DNA acts as a prognostic marker, influencing tumor behavior and therapeutic response.[23]

Prognostic Value Across Biomarkers:

Prognostic implications of biomarkers **(Table 8)** differ across cancers. KRAS mutations are linked to aggressive tumor phenotypes and poor survival, notably in colorectal and lung cancers.[14] These mutations cause constitutive activation of proliferative pathways, enhancing invasiveness and resistance to apoptosis. KRAS-mutant tumors also display increased metastatic potential, signifying a worse prognosis. Similarly, BCR-ABL transcript levels are a recognized prognostic factor

in hematologic malignancies such as chronic myeloid leukemia (CML). Elevated baseline levels or inadequate molecular response to tyrosine kinase inhibitors (TKIs) indicate a higher risk of disease progression to blast crisis.[24] Continuous monitoring of BCR-ABL helps assess relapse risk and optimize therapy. In HCC, PIVKA-II also serves a prognostic role.

Elevated levels correlate with larger tumors, vascular invasion, and reduced survival.[25] Post-surgical PIVKA-II levels predict recurrence, identifying patients who may need adjuvant therapy or intensive follow-up.

Biomarkers in Treatment Monitoring and Resistance:

Biomarkers are also critical for assessing treatment efficacy and resistance. For example, KRAS mutations predict resistance to EGFR-targeted therapies in colorectal cancer.[14] Patients with KRAS mutations do not benefit from anti-EGFR agents like cetuximab and panitumumab, underscoring the need for genotyping before treatment to avoid ineffective regimens and unnecessary costs. In CML, BCR-ABL transcript quantification informs treatment response. A major molecular response (MMR), defined as a ≥3-log reduction in BCR-ABL transcripts, correlates with prolonged progression-free survival.[24] Failure to achieve MMR suggests primary resistance, warranting dose adjustment or switching to second-generation TKIs such as dasatinib or nilotinib. Some patients may develop additional ABL mutations, like T315I, necessitating third-generation TKIs like ponatinib. In HCC, persistently high PIVKA-II levels post-treatment may indicate minimal residual disease or early recurrence.[26] Successful resection or targeted therapy typically reduces levels, while sustained elevation suggests incomplete tumor clearance or resistant disease, highlighting PIVKA-II's role in treatment monitoring.

Validation of Molecular Diagnostics in the study:

This study explored molecular diagnostics using RT-PCR for detecting BCR-ABL, HPV, and KRAS mutations, alongside PIVKA-II screening for HCC **(Figure 1)**. Although limited in sample size, findings support RT-PCR as a sensitive and specific technique for oncologic diagnostics.[27] PIVKA-II results aligned with previous studies validating its diagnostic role in HCC.5 Future research with larger cohorts can confirm these methods' clinical utility.

Challenges in Biomarker-Based Diagnostics:

Despite progress, early tumor detection via biomarkers faces challenges. Low biomarker abundance in early-stage cancers impairs detection, and non-specific expression in benign conditions can cause false positives. Furthermore, the sensitivity of current techniques may not suffice for detecting low-level ctDNA, leading to false negatives.[28] Tumor heterogeneity and dynamic biomarker expression add complexity, demanding more robust assays.

Future Directions:

Recent innovations address these limitations.[29] Paper-based microfluidic devices offer rapid, low-cost biomarker detection, even outside clinical labs. Such platforms have been developed for KRAS mutation screening, facilitating early cancer diagnosis.[30] Additionally, inter-patient variability, including genetic differences, tumor microenvironment conditions, and immunity status, contributes to differential biomarker expression, which in turn affects detection sensitivity and reliability.

AI integration further enhances diagnostic accuracy. AI algorithms can identify patterns in imaging and molecular data, improving early detection.[31] AI-assisted imaging, for example, has advanced breast cancer screening outcomes.[32] Additionally, AI aids in interpreting liquid biopsies, increasing sensitivity and specificity in cancer detection.[33,34] Interestingly, some of the clinical samples tested negative for biomarkers despite being suspected cases. For instance, two samples expected to show BCR-ABL transcripts (Table 5) and seven samples for HPV (Table 6) were negative. These outcomes could be due to several factors: (1) low disease burden resulting in biomarker levels below detection thresholds, especially if the disease is in the early or latent stages; (2) technical limitations such as RNA degradation or suboptimal sample storage; and (3) In some cases, the disease may behave differently in different people (biological heterogeneity), and might not involve the specific biomarker we tested for. This means a person could still have the disease, but test negative because their version of the illness is caused by a different mechanism (e.g., non-HPV cervical cancers or atypical BCR-ABL-negative leukemia). Additionally, host immune response and virus clearance might explain HPV-negative results in previously exposed individuals.

Traditional biomarkers like ctDNA, proteins, and metabolites have improved cancer diagnostics but often face challenges such as low abundance, variability, and limited early-stage detection. To overcome these, synthetic biomarkers—engineered biological molecules[29,36] introduced into the body to amplify disease signals—are emerging as promising tools. These rationally designed molecules or nano sensors interact with tumor-specific enzymes or microenvironmental changes, producing detectable signals.[29] They improve sensitivity and specificity by amplifying weak biological signals.

Recent innovations include nano sensors that release synthetic biomarkers when triggered by tumor enzymes,[35] enabling early tumor detection. An MIT study[36] showcased a paper-based test using synthetic biomarkers for accurate, non-invasive cancer screening. Beyond detection, synthetic biomarkers show prognostic value. By dynamically responding to tumor progression, they help monitor treatment and recurrence, supporting personalized treatment plans and better outcomes.[37]

■ Limitations

This literature review offers insights into the diagnostic and prognostic value of biomarkers, but some limitations must be noted. A primary constraint was the inability to perform independent lab analyses due to the biological nature of the samples. As a minor, and following Good Laboratory Practices and lab regulations, I was restricted from handling most clinical specimens. Consequently, the sample size was small, and

DOI: 10.36838/v8i1.14

access to positive cases was limited, impacting the validation of certain biomarker trends.

Another limitation was biomarker heterogeneity across cancers. Although KRAS, BCR-ABL, and PIVKA-II are established biomarkers, their expression and clinical relevance can vary among patients. Tumor evolution, genetic mutations, and technical issues in detection also complicate standardization for early diagnosis and prognosis.

Conclusion

This review and lab investigation emphasized the role of KRAS, PIVKA-II, HPV, and BCR-ABL in distinguishing cancerous from healthy tissues. These biomarkers enhance early detection and guide clinical decisions. They also offer prognostic insights into disease progression, treatment response, and survival, aiding in therapy selection and risk stratification. Continued research on synthetic and novel biomarkers is crucial to improving diagnostic precision and advancing personalized oncology care.

Ethical Statement

This study was conducted under the supervision of qualified professionals in a certified diagnostic laboratory. The student did not handle any clinical specimens directly. All sample processing followed institutional biosafety and ethical guidelines, and no personal or identifiable data were used.

Acknowledgment

I sincerely thank my mentor, Dr. Ankita Dave (PhD. Molecular Biology, CSMCRI, India), for her invaluable guidance and support throughout this research. I am also grateful to Shaurya Bansal and my mother for their helpful insights and encouragement.

References

1. National Institutes of Health (US). *Molecular Testing for Chronic Myeloid Leukemia*; Co-published by National Institutes of Health (US): Bethesda, MD, **2016**. https://www.ncbi.nlm.nih.gov/books/NBK402289/.
2. Das, S.; Dey, M. K.; Devireddy, R.; Gartia, M. R. Biomarkers in Cancer Detection, Diagnosis, and Prognosis. *Sensors* **2023**, *24* (1), 37. https://doi.org/10.3390/s24010037.
3. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. *Human Papillomaviruses*; International Agency for Research on Cancer: Lyon, France, 2007; IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 90. https://www.ncbi.nlm.nih.gov/books/NBK321770/.
4. Safaei, A.; Rezaei-Tavirani, M.; Sobhi, S.; Akbari, M. E. Breast Cancer Biomarker Discovery: Proteomics and Genomics Approaches. *Iran J. Cancer Prev*. **2013**, *6* (Suppl), 45–53.
5. Seo, S. I.; Kim, H. S.; Kim, W. J.; Shin, W. G.; Kim, D. J.; Kim, K. H.; Jang, M. K.; Lee, J. H.; Kim, J. S.; Kim, H. Y.; Kim, D. J.; Lee, M. S.; Park, C. K. Diagnostic Value of PIVKA-II and Alpha-Fetoprotein in Hepatitis B Virus-Associated Hepatocellular Carcinoma. *World J. Gastroenterol*. **2015**, *21* (13), 3928–3935. https://doi.org/10.3748/wjg.v21.i13.3928.
6. Na, I. K.; le Coutre, P. Emerging Role of Tyrosine Kinases as Druggable Targets in Cancer. *Biomark. Insights* **2015**, *10* (Suppl 3), 29–31. https://doi.org/10.4137/BMI.S22432.
7. Sharma, S.; Bell, D.; Settleman, J.; *et al*. Epidermal Growth Factor Receptor Mutations in Lung Cancer. *Nat. Rev. Cancer* **2007**, *7*, 169–181. https://doi.org/10.1038/nrc2088.
8. Kriegshäuser, G.; Auner, V.; Zeillinger, R. New and Potential Clinical Applications of KRAS as a Cancer Biomarker. *Expert Opin. Med. Diagn*. **2010**, *4* (5), 383–395. https://doi.org/10.1517/17530059.2010.510512.
9. van Krieken, J. H. J. M.; Jung, A.; Kirchner, T.; *et al*. KRAS Mutation Testing for Predicting Response to Anti-EGFR Therapy for Colorectal Carcinoma: Proposal for a European Quality Assurance Program. *Virchows Arch*. **2008**, *453*, 417–431. https://doi.org/10.1007/s00428-008-0665-y.
10. Shi, Y.; Au, J. S. K.; Thongprasert, S.; Srinivasan, S.; Tsai, C. M.; Khoa, M. T.; Yang, P. C. A Prospective, Molecular Epidemiology Study of EGFR Mutations in Asian Patients with Advanced Non-Small-Cell Lung Cancer of Adenocarcinoma Histology (PIONEER). *J. Thorac. Oncol*. **2014**, *9* (2), 154–162.
11. Cagle, P. T.; Chirieac, L. R. Advances in Treatment of Lung Cancer with Targeted Therapy. *Arch. Pathol. Lab. Med.* **2012**, *136* (5), 504–509. https://doi.org/10.5858/arpa.2011-0618-RA.
12. Tung, N. M.; Garber, J. E. BRCA1/2 Testing: Therapeutic Implications for Breast Cancer Management. *Br. J. Cancer* **2018**, *119* (2), 141–152. https://doi.org/10.1038/s41416-018-0127-5
13. Shackelford, R. E.; Vora, M.; Mayhall, K. R. KRAS Testing: A Tool for the Implementation of Personalized Medicine. *Genes Cancer* **2012**, *3* (7-8), 459–466. https://doi.org/10.1177/1947601912460547.
14. Huang, L.; Guo, Z.; Wang, F.; *et al*. KRAS Mutation: From Undruggable to Druggable in Cancer. *Sig. Transduct. Target. Ther.* **2021**, *6*, 386. https://doi.org/10.1038/s41392-021-00780-4.
15. Zhu, W.; Wang, W.; Zheng, W.; *et al*. Diagnostic Performance of PIVKA-II in Identifying Recurrent Hepatocellular Carcinoma Following Curative Resection: A Retrospective Cohort Study. *Sci. Rep*. **2024**, *14*, 8416. https://doi.org/10.1038/s41598-024-59174-5.
16. Tsakogiannis, D.; Nikolaidis, M.; Zagouri, F.; Zografos, E.; Kottaridi, C.; Kyriakopoulou, Z.; Tzioga, L.; Markoulatos, P.; Amoutzias, G. D.; Bletsa, G. Mutation Profile of HPV16 L1 and L2 Genes in Different Geographic Areas. *Viruses* **2022**, *15* (1), 141. https://doi.org/10.3390/v15010141.
17. Löf, L.; Arngården, L.; Olsson-Strömberg, U.; *et al*. Flow Cytometric Measurement of Blood Cells with BCR-ABL1 Fusion Protein in Chronic Myeloid Leukemia. *Sci. Rep*. **2017**, *7*, 623. https://doi.org/10.1038/s41598-017-00755-y.
18. Hochhaus, A.; Kreil, S.; Corbin, A. S.; La Rosée, P.; Müller, M. C.; Lahaye, T.; Hanfstein, B.; Schoch, C.; Cross, N. C. P.; Berger, U.; Hehlmann, R.; Goldman, J. M.; Daley, G. Q. Impact of BCR-ABL Mutations on Patients with Chronic Myeloid Leukemia. *Cell Cycle* **2011**, *10* (2), 250–260. https://doi.org/10.4161/cc.10.2.14537.
19. El-Tanani, M.; Nsairat, H.; Matalka, I. I.; Lee, Y. F.; Rizzo, M.; Aljabali, A. A.; Mishra, V.; Mishra, Y.; Hromić-Jahjefendić, A.; Tambuwala, M. M. The Impact of the BCR-ABL Oncogene in the Pathology and Treatment of Chronic Myeloid Leukemia. *Pathol. Res. Pract*. **2024**, *254*, 155161. https://doi.org/10.1016/j.prp.2024.155161.
20. Svobodova, S.; Karlikova, M.; Topolcan, O.; Pecen, L.; Pestova, M.; Kott, O.; Treska, V.; Slouka, D.; Kucera, R. PIVKA-II as a Potential New Biomarker for Hepatocellular Carcinoma – A Pilot Study. *In Vivo (Athens, Greece)* **2018**, *32* (6), 1551–1554. https://doi.org/10.21873/invivo.11413.
21. Soheili, M.; Keyvani, H.; Soheili, M.; Nasseri, S. Human Papillomavirus: A Review Study of Epidemiology, Carcinogenesis,

Diagnostic Methods, and Treatment of All HPV-Related Cancers. *Med. J. Islam. Repub. Iran* **2021**, *35*, 65. https://doi.org/10.47176/mjiri.35.65.
22. Yim, E. K.; Park, J. S. The Role of HPV E6 and E7 Oncoproteins in HPV-Associated Cervical Carcinogenesis. *Cancer Res. Treat.* **2005**, *37* (6), 319–324. https://doi.org/10.4143/crt.2005.37.6.319.
23. Ye, R.; Wang, A.; Bu, B.; Luo, P.; Deng, W.; Zhang, X.; Yin, S. Viral Oncogenes, Viruses, and Cancer: A Third-Generation Sequencing Perspective on Viral Integration into the Human Genome. *Front. Oncol.* **2023**, *13*, 1333812. https://doi.org/10.3389/fonc.2023.1333812.
24. Press, R. D.; Kamel-Reid, S.; Ang, D. BCR-ABL1 RT-qPCR for Monitoring the Molecular Response to Tyrosine Kinase Inhibitors in Chronic Myeloid Leukemia. *J. Mol. Diagn.* **2013**, *15* (5), 565–576. https://doi.org/10.1016/j.jmoldx.2013.04.007.
25. Yang, Y.; Li, G.; Lu, Z.; Liu, Y.; Kong, J.; Liu, J. Progression of Prothrombin Induced by Vitamin K Absence-II in Hepatocellular Carcinoma. *Front. Oncol.* **2021**, *11*, 726213. https://doi.org/10.3389/fonc.2021.726213.
26. Feng, H.; Li, B.; Li, Z.; *et al*. PIVKA-II Serves as a Potential Biomarker That Complements AFP for the Diagnosis of Hepatocellular Carcinoma. *BMC Cancer* **2021**, *21*, 401. https://doi.org/10.1186/s12885-021-08138-3.
27. Bernard, P.S.; Wittwer, C.T. Real-Time PCR Technology for Cancer Diagnostics. *Clin. Chem.* **2002**, *48* (8), 1178–1185.
28. Wen, X.; Pu, H.; Liu, Q.; Guo, Z.; Luo, D. Circulating Tumor DNA—A Novel Biomarker of Tumor Progression and Its Favorable Detection Techniques. *Cancers* **2022**, *14* (24), 6025. https://doi.org/10.3390/cancers14246025.
29. Zhou, Y.; Tao, L.; Qiu, J.; *et al*. Tumor Biomarkers for Diagnosis, Prognosis, and Targeted Therapy. *Sig. Transduct. Target Ther.* **2024**, *9*, 132. https://doi.org/10.1038/s41392-024-01823-2.
30. Kwong, G. A.; Ghosh, S.; Gamboa, L.; *et al*. Synthetic Biomarkers: A Twenty-First Century Path to Early Cancer Detection. *Nat. Rev. Cancer* **2021**, *21*, 655–668. https://doi.org/10.1038/s41568-021-00389-3.
31. Erkocyigit, B. A.; Ozufuklar, O.; Yardim, A.; Guler Celik, E.; Timur, S. Biomarker Detection in Early Diagnosis of Cancer: Recent Achievements in Point-of-Care Devices Based on Paper Microfluidics. *Biosensors* **2023**, *13*, 387. https://doi.org/10.3390/bios13030387.
32. Alshuhri, M. S.; Al-Musawi, S. G.; Al-Alwany, A. A.; Uinarni, H.; Rasulova, I.; Rodrigues, P.; Alkhafaji, A. T.; Alshanberi, A. M.; Alawadi, A. H.; Abbas, A. H. Artificial Intelligence in Cancer Diagnosis: Opportunities and Challenges. *Pathol. Res. Pract.* **2024**, *253*, 154996. https://doi.org/10.1016/j.prp.2023.154996.
33. Chen, Y.; Shao, X.; Shi, K.; Rominger, A.; Caobelli, F. AI in Breast Cancer Imaging: An Update and Future Trends. *Semin. Nucl. Med.* **2025**, *55* (3), 358–370. https://doi.org/10.1053/j.semnuclmed.2025.01.008.
34. Foser, S.; Maiese, K.; Digumarthy, S. R.; Puig-Butille, J. A.; Rebhan, C. Looking to the Future of Early Detection in Cancer: Liquid Biopsies, Imaging, and Artificial Intelligence. *Clin. Chem.* **2024**, *70* (1), 27–32. https://doi.org/10.1093/clinchem/hvad196.
35. Khazaei, M.; Hosseini, M. S.; Haghighi, A. M.; Misaghi, M. Nanosensors and Their Applications in Early Diagnosis of Cancer. *Sens. Bio-Sens. Res.* **2023**, *41*, 100569. https://doi.org/10.1016/j.sbsr.2023.100569.
36. Hao, L.; Zhao, R. T.; Welch, N. L.; *et al*. CRISPR-Cas-Amplified Urinary Biomarkers for Multiplexed and Portable Cancer Diagnostics. *Nat. Nanotechnol.* **2023**, *18*, 798–807. https://doi.org/10.1038/s41565-023-01372-9.
37. Zhou, Y.; Tao, L.; Qiu, J.; *et al*. Tumor Biomarkers for Diagnosis, Prognosis, and Targeted Therapy. *Sig. Transduct. Target Ther.* **2024**, *9*, 132. https://doi.org/10.1038/s41392-024-01823-2.

Author

Avni Goswami is an aspiring oncologist from India, currently studying in the International Baccalaureate (IB) program with a strong focus on biology and chemistry. Her goal is to study medicine in the UK, where she hopes to build a solid foundation for her future career in Medicine.

DOI: 10.36838/v8i1.14