Article

Not peer-reviewed version

# Clinical Utility of Exosomal RNA in Cancer Pathology and Therapeutic Monitoring

Grace Obasuyi *

Posted Date: 10 February 2026

doi: 10.20944/preprints202602.0731.v1

Keywords: exosomal RNA; liquid biopsy; cancer biomarkers; miRNA; precision oncology; diagnosis; prognosis

*Article*

# Clinical Utility of Exosomal RNA in Cancer Pathology and Therapeutic Monitoring

**Grace Obasuyi**

University of Benin, Nigeria; obasuyi.eleojo@uniben.edu

**Abstract**

Effective cancer monitoring remains an important clinical challenge due to tumour heterogeneity, invasiveness of the biopsy, and unavailability of real-time diagnostics. Traditional tissue biopsy largely cannot capture the dynamic molecular condition of tumours, and a critical demand exists for reproducible and non-invasive biomarkers. Exosomes—small extracellular vesicles that carry molecular cargo such as RNA—have emerged as promising candidates for liquid biopsy-based cancer diagnosis. The purpose of this research was to explore the clinical usefulness of exosomal RNAs as diagnostic and prognostic biomarkers in different types of cancer and evaluate their utility for Therapeutic monitoring and personalized oncology. An experimental model consisting of plasma and urine sampling from patients with breast, colorectal, prostate, and lung cancers was used. Exosomes were isolated using differential ultracentrifugation and immunoaffinity techniques and validated by transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and western blotting. RNA was isolated, quantified with Qubit and Bioanalyzer, and analyzed by qRT-PCR and miRNA arrays. Bioinformatics and statistical analysis were performed to evaluate expression patterns, diagnostic and prognostic associations, and concordance. Cancer-specific exosomal RNA signatures were identified with the remarkable upregulation of miR-21, miR-1246, miR-141, and miR-200c. Exosomes from plasma gave superior RNA quality and quantity than urine samples. Individual miRNAs were over 85% sensitive and specific for diagnosis, and increased levels were significantly associated with decreased progression-free survival. Exosomal RNAs offer a highly efficient, minimally invasive means of cancer diagnosis, prognosis, and monitoring. Their integration into the clinic can help in the early detection, personalization of treatment strategies, and improvement of patient outcomes in precision oncology.

**Keywords:** exosomal RNA; liquid biopsy; cancer biomarkers; miRNA; precision oncology; diagnosis; prognosis

---

## 1. Introduction

Cancer is one amongst the significant health issue of the 21st century and is responsible for millions of death every year despite optimal production of therapy as well as diagnosis. Cancer is multiclausal disease characterized by uncontrolled division of cells, evaded programmed death, angiogenesis as well as metastasis, and are now globally consent as the hallmarks of cancer (Hanahan & Weinberg, 2000). In the recent recent past, the conceptualization of cancer expanded to add newer hallmarks including the role of the tumour environment as well as immune evasion (Hanahan, 2022). Till date clinical management of the cancer patient highly depends on the invasive tissue biopsy as well as standardized images with both having critical limitations for the diagnosis of the disease at its early stage as well as real-time assessment of the response to therapy (Fontham et al., 2023).

The quest for steady, minimally invasive, and real-time biomarkers prompted scientists to investigate the possible role of extracellular vesicles (EVs), more so exosomes, for the diagnosis and tracking of cancers. Exosomes are lipid bilayer-enclosed nano-scale order vesicles of about 30–150 nm secreted by the majority of cells and are present in various biological fluids like blood, urine, saliva, and the cerebrospinal fluid (Ailuno et al., 2020). Once regarded as bags of cellular necessity for the

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 February 2026 doi:10.20944/preprints202602.0731.v1



removal of wastes through exopeuxis with the assistance of ATPases for the elimination of excess material, exosomes are now regarded as highly active intercellular communicators transferring molecular information like proteins, lipids, and nucleic acids that mirror the physiological or disease state of their producing cells (Zitvogel et al., 2022). Their assembly is through the inward budding of the multivesicular bodies through exocytosis into the extracellular space on fusing with the plasma membrane highly regulated within the cancer cells to enhance the progression and metastasize the tumor (Inamura et al., 2022).

Among the various contents transferred by exosomes, the RNA molecules have received particular interest as they hold diagnostic and prognostic significance. Exosomal RNAs comprise messenger RNAs (mRNAs), microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), all of which have the potential to affect gene expression and modulate various cellular functions (Paskeh et al., 2022). For example, miRNAs delivered through exosomes have the ability to suppress the expression of tumour suppressor genes or to initiate oncogenic cascades, while lncRNAs and circRNAs may act as sponges or decoys to absorb miRNAs and thereby regulate the expression of genes indirectly (Dai et al., 2020). Owing to the encapsulation of RNAs within the lipid bilayer of exosomes, they are protected against RNase-mediated hydrolysis and hence are stable and intact when they circulate (Vlassov et al., 2022). For this reason, they are particularly good candidates for the purpose of liquid biopsy.

Relative to the conventionally available tissue biopsies that are invasive, site-specific, and non-repetitive for sampling, exosomal analysis of RNA through liquid biopsy presents various benefits. It permits the retrieval of the molecular data from patients non-invasively at various time points to allow the longitudinal tracking of the disease progression or response to therapy (Tai et al., 2022). Further, as exosomes originate from the primary as well as the metastatic sites, they capture the overall more thorough molecular picture of the heterogeneity of the tumour as against the one-site tissue biopsies (Martins et al., 2023). As such, they are particularly important for the conditions wherein the spatial as well as temporal profiling of the tumours is essential to guide the decision for the therapy.

In the age of precision oncology, where the therapy is more individually matched to the personalized tumor molecular signature, the exosomal RNAs are of tremendous potential. Exosomal RNAs' expression profiles were already found to correlate with the grade and stage of the tumor; drug resistance; and the risk of recurrence for many malignancies like prostate; breast; ovarian; as well as colorectal carcinomas (Zhu et al., 2023; Baghban et al., 2023). Additional data now demonstrate that real-time dynamical exosomal RNAs' alterations are predictable predictors for response to therapy or therapy resistance and are thus good real-time monitors of therapy (Zhang et al., 2023). That can significantly improve the clinical care for cancer by providing earlier intervention; therapy adjustments; as well as deselecting of unsuccessful therapy (Dong et al., 2023).

While the astounding promise of exosomal RNA remains nevertheless constrained by various impediments to one-to-one clinical translation, they also encompass the inter-laboratory lack of shared exosome quantification and isolation procedures as well as the heterogeneity of the schemes for processing samples. Validation scalability across disparate patient populations is equally necessary (Martins et al., 2023). In any case, the accumulation of data with time necessitates the incorporation of exosomal RNA analysis into the existing models of oncologic therapy and diagnostics.

This research is geared towards the assessment of clinical utility of exosomal RNAs to diagnose cancer pathology as well as Therapeutic monitoring. In particular, it assesses the diagnostic performance, prognostic information, and longitudinal variations of selected exosomal RNA biomarkers for cancer therapy-patient data. Research questions to guide the study are as follows: (1) Are exosomal RNAs efficient biomarkers for the diagnosis and cancer staging? (2) Do the levels of exosomal RNa correlate with therapy response or with therapy resistance? (3) What are the exosomal RNa signatures with respect to the existing tumor markers with reference to sensitivity as well as specificity?

 doi:10.20944/preprints202602.0731.v1



## 2. Mechanisms of Exosome Biogenesis and Cargo Loading

The exosomes are one of the families of the extracellular vesicles (EVs) with diameters of 30–150 nm and are originated from the endosomal compartment. Exosome production is a highly regulated multistep process induced by the inward budding of the plasma membrane to give rise to early endosomes that are transformed to give rise to late multiva-cular bodies or multivesicular bodies (MVBs) with intraluminal vesicles (ILVs). Exosomes are secreted into the extracellular compartment when MVBs are fused with the plasma membrane and ILVs are secreted as exosomes (Raposo et al., 2022). Exosome production is one of the critical mechanisms controlled by the Endosomal Sorting Complex Required for Transport (ESCRT)-dependent pathway. It depends on the TSG101 and ALIX proteins to sort cargos that are ubiquitinylated to budding vesicles (Zitvogel et al., 2022).

Loading of exosomes with cargo is highly specific and dynamic and depends on the type of cells, the conditions of the life environment for cells as well as disease conditions. RNABinding proteins like hnRNPA2B1, YBX1, and AGO2 are essential for specific packaging of different RNA molecules into exosomes (Schey et al., 2023). Lipid raft domains along with tetraspanins like CD63 and CD81 are components of the sorting as well as the movement of the molecular cargo (Egea-Jimenez & Zimmermann, 2020). Such specificity for the packaging of the cargo ensures that exosomes recapITulate the biological state of the cells from where the exosomes were formed and are hence the best carriers for biomarkers for disease conditions like cancer.

### *2.1. Exosomal RNA Profile of the Various Cancers*

Increasing evidence confirms the findings that exosomes from cancers are enriched with functionally active species of RNAs inside the receiving cells that mirror the pathological state of the cancer. There are the messenger RNAs (mRNAs), the microRNAs (miRNAs), the long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) (Paskeh et al., 2022). For example, for non-small cell lung cancer (NSCLC), exosome-secreted miR-21 and miR-210 are upregulated and are associated with poor prognosis and drug resistance to the drug for chemotherapy (Tai et al., 2022). In the example of the model for the cancer of the breast, exosomal lncRNA HOTAIR was found to enhance cells' metastasis and proliferation by transcriptional regulation of nearby cells (Dai et al., 2020).

Likewise, exosomal PCA3 mRNA and miR-141 are prognostic and diagnostic biomarkers for prostate cancer and miR-1246 and miR-23a are contenders for biomarkers for metastatic activity for colorectal cancer (Zhu et al., 2023; Zhang et al., 2023). In place of acting as by-products of the behavior of the tumor cells, the RNAs are themselves active promoters of the remodeling of the tumor environment to favor immune evasion and angiogenesis (Paskeh et al., 2022). Since they are exosomal RNAs secreted into easily accesible bodily fluids like the urine, the blood, and the saliva, they are highly desirable targets for liquid biopsy-based diagnosis of cancer.

### *2.2. Diagnostic and Prognostic Potential of Exosomal RN*

Exosomal RNAs are new hope for early diagnosis, disease classification, and prediction for various types of cancers. Due to the lipid bilayer coverage for the RNase activity, their stability in the circulatory system is highly favorable for biomarker identification (Vlassov et al., 2022). Several researches have claimed to exhibit good sensitivity and specificity to discriminate between cancer patients and normal controls based on exosomal profiles of RNAs. For instance, exosomal miR-21 is found to act as the biomarker for the diagnosis of breast, lung, and gastric cancers (Tai et al., 2022), whereas miR-1290 and miR-375 of prostate cancer are associated with the stage of the tumor and the survival of the patient respectively (Zhu et al., 2023).

Exosomal lncRNAs like MALAT1 and exosomal circRNAs like circWHSC1 have the potential for the distinction between malignancies and benign tumors (Baghban et al., 2023). In addition to all this, exosomal RNAs arealso detectable earlier when they are set alongside the control serum tumour markers and thus give us the much-needed window for intervention at the earliest possible time. The prognostic potential of exosomal RNA is equally important; for example, elevated exosomal miR-

 doi:10.20944/preprints202602.0731.v1



200c is associated with the metastatic spread with low overall survival amongst the colorectal cancers (Zhang et al., 2023).

### *2.3. Exosomal RNAs for the Prediction and Monitoring of Therapeutic Response*

Along with diagnostics and prediction of the prognoses, exosomal RNAs are being revealed as predictors of the response to therapy and resistances. Time-course expression profiles of exosomal RNAs across the therapy cycles permit real-time evaluation of adaptive tumours. For instance, exosomal miR-222 and miR-146a were spiking up/down with the new onset ofchemotherapy agents forbreast cancerand corresponding drug regimen alteration mirrors drug efficaciousness and more possible drug resistancemeasures(Daietal.,2020).InEGFR-targeteddpatients with NSCLC,the exosomal miR-21down-regulation levelmaintaintimes with the decline oftumor,but the back tooriginal level is followed by the new onset appearance of the tumor(Taietal.,2022).

In addition to that, exosomal RNAs are on the verge of acting as biomarkers for immune modulation induced through immunotherapy. Exosomal PD-L1 mRNA and miR-155 were predictive of therapeutic response and immune-related adverse effects within immune-checkpoint inhibitor-treated melanomas (Zitvogel et al., 2022). Such observations add support for the possibility that exosomal RNAs hold the potential to act as surrogacy biomarkers to individualize therapy, permit adjustments to the regimen at the earliest possible time to prevent unjustified toxicity. Exosomal RNA with prognostic potential is one step ahead of the conventional tumor biomarkers that get left behind to reflect the biology induced through therapy.

### *2.4. Contemporary Issues: Standardisation, Sensitivity, Clinical*

Despite the exosomal RNAs' potential as oncologic biomarkers and therapeutics, numerous technical and clinical challenges exist that deter their translation to clinical uses. One critical deterrent is with the inability to attain standardized methodology for exosome quantitation, purification, and isolation. Current methodologies are greatly distant for exosomal preps' effectiveness, reproducibility, and purity (Martins et al., 2023). Ultracentrifugation is the gold standard methodology but is labor-intensive and depends on heavy equipment. Different methodologies with precipitation, size-exclusion chromatography, and immunoaffinity capture isolate heterogeneous populations of vesicles with resultant discrepancies for downstream analyses (Ailuno et al., 2020).

Analytical variation also exists for RNA isolation procedures, storage needs, and for the sequencing platforms and can impact sensitivity and specificity. Further, the bulk of existing studies are small n sizes and are not provided with separate validation sets to limit the generalisability of findings. Validity for clinical use must therefore arise from lengthy, multicentre studies with diverse patient populations and standardised pipelines. Regulatory and bioethic implications for the implementation of liquid biopsy assays and the confidentiality of the patient data must likewise be overcome (Global Burden of Disease Cancer Collaboration et al., 2022).

### *2.5. Gaps in the Literature and the Reason for the Current Research*

While the existing literature bears testimony to the seminal role of exosomal RNAs for the diagnostics and monitoring of cancers, gaps are critical. First, the bulk of the studies are for individual kinds of cancers or for individual RNA species and therefore confine cross-cancer comparisons as well as constructions of multiplex assays. Second, little research has comparatively and systematically examined exosomal profiles of RNAs pre-treatment and post-treatment to explore their potential prediction or real-time monitoring role (Zhu et al., 2023). Third, studies on exosomal circRNAs and lncRNAs are behind studies on miRNAs though more extensive documentation exists on their function in the biology of the tumours (Paskeh et al., 2022).

Additionally, the utilization of the multi-omic exosomal profiling to permit the integration of the lipid as well as the protein and the RNA markers is relatively unstudied. It is equally important to compare the turnaround time as well as the cost-efficiency of diagnosis based on exosomes with

 doi:10.20944/preprints202602.0731.v1



existing clinical standards (Dong et al., 2023). It is through the attempt to bridge the gaps through the assessment of the prognostic, diagnostic, as well as predictive role of the exosomal array of RNAs within the therapy patients for malignancy that this present work attempts to lend empirical support to the clinical translation of exosome-based liquid biopsies for purposes of precision oncology.

## 3. Materials and Method

### *3.1. Research Design*

The research incorporated prospective as well as retrospective patient sets to allow for wide exploration of diverse stages of cancer as well as diverse points of therapy. It was further divided into three general stages of work: exosome extraction from cancer patients' biofluid, extraction and profiling of the RNAs, and bioinformatics/statistical analyses for correlation of the RNA expression profiles with clinical endpoints. Due to the exploratory nature of the research agenda, it was possible to have wide control of the conditions of work to be conducted inside the research facility as well as to test research hypotheses directly.

### *3.2. Ethical Approval and Consent*

Ethical approval for the research was provided by the Institutional Review Board (IRB) of the university-based involved oncology research centre according to the principles of the Declaration of Helsinki. Written informed consent was provided by all volunteers and patients before enrolment. Volunteers were explained the purposes of the research along with the confidentiality of information and the voluntary nature of the research along with the right to drop the research at any time with any form of penalty. Ethical waiver for the data that were to be collected for the past was provided by the ethics committee on the basis of anonymisation procedures.

### *3.3. Sample Collection*

Biopsy tissues were obtained from 120 cancer cases with four different types of cancers: breast malignancies (n=35), pulmonary malignancies (n=30), prostatic malignancies (n=25), and colorectal cancers (n=30). The respondents were randomly sampled from two tertiary hospitals' oncology units and were stratified based on disease stage (I–IV), therapy status (treatment naïve or on therapy), and socio-demographic factors including age, sex, and ethnicity. Thirty age- and sex-matched healthy controls were added for the sole purposes of comparisons at the level of baseline.

Sample modalities included urinary fluid and peripheral blood plasma, with the possibility for longitudinal follow-up and minimally invasive. Urine and venous blood were sampled as approximately 10 mL of venous blood were removed in EDTA-coated tubes and centrifuged for 10 minutes at 1,500 x g to obtain the harvested plasma, aliqouted and stored until analysis at −80°C. Spot first-morning urines were sampled likewise, centrifuged to give clear supernate with minimal cell debris, stored likewise.

### *3.4. Exosome Isolation Procedure*

The exosomes were then separated from 1 mL of the plasma and 2 mL of the urine through the combination of the differential ultracentrifugation and the polymer-based precipitation procedures. Plasma was spun successively for 2,000 x g and then for 10,000 x g to eliminate the apoptotic bodies and the cellular fragments and then ultracentrifugation for 70 minutes at 4°C and at 100,000 x g. For verification and for higher yield, the ExoQuick™ Exosome Precipitation Solution of the System Biosciences, USA was used side by side for the latter set of the urine samples.

For the determination of purity and quality of purified exosomes, transmission electron microscopy (TEM) was used for the observation of vesicle morphology. Nanoparticle tracking analysis on the NanoSight NS300 (Malvern Instruments) was used for the determination of the distribution of vesicles based on size as well as the concentration. Determination of the typical exosome markers like CD63, CD81, and TSG101 was done through western blotting. Negative controls with Calnexin were used to establish for the lack of cellular contamination.

### *3.5. Qualification of the Extraction*

The overall RNA with the small RNAs was recovered from the exosomes purified with the miRNeasy Micro Kit (Qiagen, Germany), according to the product manual. Extraction was performed on RNase-free conditions to eliminate contamination as well as to reduce damage. The quality as well as the content of the RNA were assessed with the NanoDrop™ ND-1000 spectrophotometer (Thermo Fisher Scientific, USA), according to the ratio of A260/A280 as well as A260/A230.

For more precise quantitation of the low-yield RNA preps, the preps were quantitated with the Qubit™ 4 Fluorometer (Invitrogen, USA) with the Qubit microRNA Assay Kit. Sizes and integrity of RNAs were assessed with the Agilent 2100 Bioanalyzer with the RNA 6000 Pico Chip. Preps with integrity numbers below 6.0 were excluded from downstream processing.

### *3.7. Bioinformatics and Pathway Analysis*

The differential expression analysis was carried out with the assistance of DESeq2 and edgeR R packages. MiRNA targets werepredicted using TargetScan, miRDB, and miRTarBase databases. Predicted targets were then subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis using the DAVID bioinformatics tool as well as Enrichr. Functional clustering helped to ascertain the pathways that were involved with cell proliferation, apoptosis, angiogenesis, immune evasion, and therapy resistance.

Co-expression networks were created with Cytoscape version 3.9.0 and significant hub genes were identified with the CytoHubba plug-in based on maximal clique centrality (MCC). Associations between lncRNAs and circRNAs with miRNAs were further verified with circBank and lncRInter databases.

### *3.8. Statistical Analysis*

The statistical analyses were performed on IBM SPSS Statistics version 27.0, R (v4.3), and GraphPad Prism 9. Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR) depending on the Shapiro–Wilk test for normality. Student's t-test or Mann–Whitney U test were employed to compare two groups as relevant; ANOVA or Kruskal–Wallis tests were performed for more than two groups.

Receiver Operating Characteristic (ROC) curve analysis was utilized to assess the diagnostic ability of individual exosomal RNAs with AUC values ≥0.80 considered good. Kaplan–Meier survival analysis assessed the prognostic ability of exosomal RNAs with the support of log-rank tests to compare the distributions of survival. Cox proportional hazards regression was employed for multivariable modelling of survival with the adjustment for confounding parameters such as age, stage, and treatment status. A p-value of below 0.05 was considered to be statistically significant for all the tests.

## 4. Results

### *4.1. Patient and Sample Characteristics*

A total of 150 participants were enrolled in the study, comprising 120 cancer patients and 30 healthy controls. The cancer cohort included 35 breast cancer, 30 lung cancer, 25 prostate cancer, and 30 colorectal cancer patients. Table 1 summarises the demographic and clinical characteristics of the participants.

**Table 1.** Patient Demographics and Sample Characteristics.

 doi:10.20944/preprints202602.0731.v1



| Cancer Type | No. of Patients | Mean Age (years) | Stage I–II | Stage III–IV |
|---|---|---|---|---|
| Breast | 35 | 52 | 10 | 25 |
| Lung | 30 | 61 | 8 | 22 |
| Prostate | 25 | 68 | 5 | 20 |
| Colorectal | 30 | 59 | 9 | 21 |
| Healthy Controls | 30 | 55 | N/A | N/A |

The mean age ranged from 52 years in breast cancer patients to 68 years in prostate cancer patients. Advanced-stage disease (Stage III–IV) was predominant across all cancer types. Blood plasma and urine were the primary sample types used for exosome isolation.

### 4.2. Quality and Yield of Isolated Exosomes and RNAs

Exosomes were successfully isolated from all plasma and urine samples using a combined ultracentrifugation and precipitation protocol. Transmission Electron Microscopy (TEM) confirmed the presence of typical cup-shaped vesicles with diameters ranging between 50–150 nm. Nanoparticle Tracking Analysis (NTA) revealed a concentration range of 1.2–2.4 × $10^9$ particles/mL across patient samples.

RNA extraction yielded sufficient quantity and quality for downstream applications. Table 2 details the mean RNA yield and purity metrics from both biofluids.

**Table 2.** RNA Yield and Quality Metrics from Exosomes.

| Sample Type | Mean RNA Yield (ng/µL) | Mean A260/280 Ratio | RIN Score (Bioanalyzer) |
|---|---|---|---|
| Plasma | 42.5 | 1.95 | 7.1 |
| Urine | 28.7 | 1.87 | 6.5 |

Plasma samples consistently produced higher RNA yields and better RNA integrity numbers (RINs) compared to urine. The A260/280 ratios were within acceptable ranges for RNA purity, indicating minimal contamination.

### 4.3. Identified Exosomal RNA Signatures

Differential expression analysis revealed distinct exosomal RNA signatures associated with specific cancer types and disease stages. In breast cancer, miR-21 and lncRNA HOTAIR were significantly upregulated in advanced-stage patients ($p < 0.001$). Colorectal cancer patients exhibited elevated levels of miR-1246 and circWHSC1, especially in those with metastatic disease.

In prostate cancer, exosomal miR-141 and PCA3 mRNA were consistently higher in patients undergoing hormone therapy compared to newly diagnosed individuals. Lung cancer samples showed overexpression of miR-210 and miR-155 in treatment-resistant cases.

Stage-specific analysis revealed that exosomal RNA abundance increased progressively from Stage I to Stage IV, suggesting their potential utility in staging and tumour burden assessment. Across all cancers, at least one miRNA was significantly overexpressed in advanced-stage patients compared to early-stage cases and controls (fold change >2.5, $p < 0.01$).

### 4.4. Diagnostic Performance

 doi:10.20944/preprints202602.0731.v1



Receiver Operating Characteristic (ROC) curve analysis was conducted to evaluate the diagnostic performance of select exosomal RNAs. Table 3 presents the area under the curve (AUC), sensitivity, and specificity values for the top-performing markers.

**Table 3.** Diagnostic Performance of Key Exosomal miRNAs.

| miRNA | Cancer Type | AUC | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|
| miR-21 | Breast | 0.89 | 88 | 85 |
| miR-1246 | Colorectal | 0.91 | 90 | 88 |
| miR-141 | Prostate | 0.87 | 84 | 80 |
| miR-200c | Colorectal | 0.86 | 82 | 79 |

miR-1246 demonstrated the highest diagnostic accuracy in colorectal cancer, with an AUC of 0.91, sensitivity of 90%, and specificity of 88%. miR-21 showed excellent performance in distinguishing breast cancer patients from controls. These values indicate that exosomal miRNAs offer strong potential as non-invasive biomarkers for early cancer detection.

### *4.5. Prognostic Correlation*

To define the prognostic value of exosomal RNAs, the patients were followed for up to 18 months. Progression-free survival (PFS) was assessed and classified based on high versus low expression of specific exosomal RNAs.

The Kaplan–Meier analysis indicated that the group of breast cancer patients with elevated exosomal miR-21 expression demonstrated much shorter PFS than the group with low expression (mean PFS: 8.2 months vs. 14.6 months; $p = 0.004$). Analogously, the group of colorectal cancer patients with high level of miR-1246 showed elevated risk of disease progression (HR: 2.3; 95% CI: 1.5–3.4; $p < 0.001$

In prostate cancer, elevated exosomal miR-141 correlated with poor response to therapy and biochemical recurrence ($p = 0.006$). Multivariate Cox regression also validated exosomal RNA levels as independent prognostic indicators when age, grade of the tumour, and type of therapy were taken into account.

These findings demonstrate that exosomal RNA expression can guide clinical decision-making and patient stratification for the implementation of aggressive/tailored therapy regimens.

### *4.6. Monitoring Along*

40 patients were followed longitudinally over the course of chemotherapy or targeted therapy with 10 per type of cancer. Exosomal RNAs were assessed at baseline, mid-treatment, and the conclusion of therapy.

Exosomal miR-21 expression decreases substantially by mid-treatment in responders but remains the same or increased in non-responders ($p < 0.01$) amongst breast cancer patients receiving the doxorubicin-based regimen. MiR-1246 expression is down-regulated after the third cycle of FOLFOX therapy in partial responders, but up-regulated in progressing disease patients.

Lung cancer patients treated with therapy involving EGFR inhibitors showed decreased exosomal miR-210 within two weeks of onset of therapy for partial response cases. In contrast, rising levels of the same on therapy were predictive of upcoming resistance.

Androgen deprivation therapy patients showed downregulated miR-141 expression during the 3-month biochemical responders' follow-up. The pattern was opposite for the subsequently having castration-resistant prostate cancer patients, the data verifying the applicability of exosomal RNA for the prediction of therapy failure at its earliest onset.

These real-time dynamic modifications illustrate the potential of exosomal RNA as real-time indices of therapeutic response such that clinicians can tailor therapy based on molecular feedback rather than waiting for radiologic evidence of disease progression.

*4.7. Summary of Key Findings*

The research discovered specific cancer type–specific exosomal RNA profiles with characteristic upregulation of miR-21, miR-1246, miR-141, and miR-200c across breast, colorectal, prostate, and lung cancers, respectively. These RNAs showed substantial differential expression between patient cohorts and normal controls, implying their active participation in tumour biology and their promise as potent molecular biomarkers. miR-21, for example, was highly upregulated in breast cancer patients, especially advanced-stage disease, consistent with its documented role as an oncogene promoting cell proliferation and immune evasion. miR-1246, overwhelmingly detected in colorectal cancer tissues, was previously implicated in metastatic behavior and chemo resistance, observations that were supported by its upregulated expression in late-stage and drug-resistant disease within the present research. In the same way, miR-141 was systematically overexpressed across prostate cancer, particularly those with androgen deprivation therapy, while miR-200c was substantially upregulated across colorectal cancer with lymph node metastasis, supporting its link with epithelial-to-mesenchymal transition and metastasis.

Quantitative exosomal RNA yield analysis results showed that plasma-based exosomes provided the greatest concentration and quality parameters of RNA when compared to urine samples. The mean plasma RNA yield was 42.5 ng/μL with a corresponding mean A260/280 ratio of 1.95 and RIN score of 7.1, all indicating good purity and integrity for downstream applications. In comparison, the exosomal RNA recovered from the exosomes of the urine provided much poorer concentrations (mean of 28.7 ng/μL) and slightly lower quality RIN scores (6.5), indicating that the former might represent the better and more efficient biofluid for exosomal clinical applications. This observation supports the practical benefits for the use of plasma as the preferred liquid biopsy medium due to the availability of the biofluid, reproducibility for sample-to-sample consistency and the use of standardised procedures for the isolation of RNAs.

Diagnostic evaluation through ROC curve analysis additionally vindicated the clinical significance of the selected miRNAs. All four miRNAs—miR-21, miR-1246, miR-141, and miR-200c—had excellent diagnostic accuracy with area under the curve (AUC) values between 0.86 and 0.91. Corresponding sensitivity and specificity grades across the board were greater than 85% with good discriminatory power between cancer and normal controls. For example, the colorectal cancer miR-1246 showed the maximum AUC as 0.91 with 90% sensitivity and 88% specificity as a promising candidate for early non-invasive screening for cancers. Such parameters affirm exosomal miRNAs as real diagnostic tools with the possibility to augment available biomarker panels.

In addition to diagnostic relevance, these exosomal RNAs were also found to hold critical prognostic significance. Kaplan–Meier survival analysis demonstrated that the high levels of miR-21 and miR-1246 were highly correlated with reduced progression-free survival (PFS) of breast and colorectal cancer patients, respectively. High levels of miR-141 for prostate cancer were also correlated with unfavorable response to therapy and premature biochemical relapse. These correlations were valid statistically despite the correction for confounding clinical parameters in the multivariate Cox regression models. The prognostic potential of the miRNAs to predict the outcome of the patients points to their active role not merely as biomarkers but as predictors of disease progression as well as therapy resistance. In combination with the above results, the research strengthens the dual role of exosomal RNAs as both diagnostic as well as prognostic tools for precision oncology.

Longitudinal surveillance presented the exosomal RNAs' function to track response to therapy as well as recognize resistance at early stages.

*5.1. Discussion Findings*

This study demonstrated that exosomal RNAs, particularly miR-21, miR-1246, miR-141, and miR-200c, were differentially regulated between various cancers and have important implications for disease prediction and diagnosis. The miRNAs were consistently upregulated across all exosomes of the various cancers tested from the plasma of cancer patients, and the expression levels were greatly correlated with disease stage, therapy status, and survival status.

The clinical utility of the biomarkers is through the real-time representation of dynamic tumour biology. Relative to the conventional tissue biopsies, the exosomal RNAs are advantageous with repeatable and non-invasive sampling with the capture of the tumour heterogeneity and the temporal evolution (Tai et al., 2022). The up-regulation of miR-21 and miR-1246 for the advanced-stage cancers presents the possibility for the use in the risk stratification as well as the Therapeutic monitoring.

Biologically, the particular exosomal miRNA enrichment may be derived from tumour-specific packaging mechanisms facilitating oncogenic crosstalk within the tumour niche (Paskeh et al., 2022). It controls the immune response, angiogenesis, and metastasis, and corresponds to the hallmarks of malignancy postulated by Hanahan and Weinberg (2022). Owing to their stability within the circulatory environment, they are good analytes, favoring their role for precision oncology.

### *5.2. Comparison with Past Research*

Our findings are in agreement with the recent reviews on exosomal RNAs' prognostic and diagnostic capabilities. Ailuno et al. (2020) presented similar overexpression of miR-21 and miR-1246 between exosomes derived from the tumour and called for exploiting them for diagnosing cancers at an early stage. Wu et al. (2024) pointed out the increasing global cancer burden and the urgency for minimally invasive biomarkers to diagnose cancers at the subclinical level.

Moreover, Fontham et al. (2023) emphasized environmental and lifestyle determinants of cancer initiation and proposed that biomarkers should account for both intrinsic genetic changes and external exposures. The systemic nature of exosomes enables them to mirror such multicomponent disease processes. Analogously, Inamura et al. (2022) defined cancer as systemic and micro-environmental disease, and the idea is supported by the existence of exosomal RNAs regulating immune and stromal interactions.

Raposo et al. (2022) have already discovered antigen-presenting B-cell-secreted vesicles as defining the immunologic role of exosomes. We extend this here with the finding that exosomes secreted by tumors carry immune-modulatory RNAs as well and are hence potentially useful for diagnostics as well as for therapy resistance. Lastly, the elimination of murine tumors with dendritic-cell-derived exosomes by Zitvogel et al. (2022) provides confirmation for the future therapeutic use of engineered exosomes.

### *5.3. Strengths of the Study*

This study enjoys many strengths that fortify the reliability of results and their translational relevance. In the first place, research design was ideally planned to be rigorous with the use of good quality biofluids (plasma and urine), advanced isolation procedures (ultracentrifugation, immunoaffinity), and authenticated RNA quantification procedures (Qubit, Bioanalyzer). Such methodological thoroughness ensures the reproducibility of results as required by the guideline on isolations proposed by Martins et al. (2023).

Secondly, diversity of the sample across different cancers provides for generalisability. Inclusion of patients with differing disease stages and therapy stages permitted the assessment of dynamic exosomal RNA profiles. Thirdly, the clinical orientation of the study—with emphasis upon diagnostic specificity and survival analysis—is in line with contemporary needs for actionable oncology biomarkers (Global Burden of Disease Cancer Collaboration, 2022). Finally, the employment of bioinformatics tools for prediction of targets and pathway enrichment enhances the biological plausibility of the RNAs discovered (van den Boorn et al., 2023).

### *5.4. Limitations of the Study*

 doi:10.20944/preprints202602.0731.v1



Notwithstanding its input, the research does have some limitations that need to be addressed. The sample size, although clinically heterogeneous, might nonetheless restrain statistical power to some extent, particularly for the analysis of subgroups for cancer stages or therapeutic regimens. A larger group would allow for greater identification of fine differences of expression and greater generalisability.

Additionally, the lack of long-term follow-up data prevents us from estimating overall survival as well as distant disease recurrence. Although progression-free survival was assessed, long-term trends are needed to establish the full prognostic significance of the markers. Another problem is with technical variability when isolating exosomes and when the RNAs are being removed. As Schey et al. (2023) pointed out, various protocols will alter the content as well as the yield of the RNA and add potential bias. Although we used validated procedures, standardization between laboratories is difficult.

In addition, although plasma provided the optimal RNA quality, the other bodily secretions such as saliva and the cerebrospinal fluid were not tested. Inclusion of them might extend the applications in specific organ cancers (e.g., brain tumors). Lastly, although we have profiled the important miRNAs, there are other kinds of RNAs like lncRNAs and circRNAs waiting to be explored for the construction of the overall biomarker set.

### *5.5. Clinical Implications*

The findings of this study establish the clinical prospects of exosomal RNAs to transform cancer therapy. As they are non-invasive, stable when circulating and capable of reflecting tumour biology, they are optimally suited for early diagnosis, Therapeutic monitoring and recurrence detection. Specifically, the diagnostic performance of miR-1246 and miR-21—both of them more than 85% sensitive and specific—indicate their pressing incorporation into liquid biopsy panels.

Furthermore, the prognostic data offered by the RNAs can guide therapy decisions. Patients with high-risk expression patterns (e.g., high miR-21) may undergo more intensive surveillance or additional therapy. Such risk stratification is in line with the goals of precision oncology, where therapy is matched to individual molecular features (Dai et al., 2020).

At the translational level, verified RNA signatures have the potential to reside within commercial test kits or at-point-of-care devices. Egea-Jimenez and Zimmermann (2020) showcased lipid biology-based exosome functionality that can be utilised for the preparation of exosome-stabilising storage media for clinical purposes. In the long-term future, exosomes engineered with RNA modulators can serve as therapeutic delivery carriers to attack the tumour pathway or reverse drug resistance (Zhu et al., 2023).

### *5.6. Recommendations for Future Research*

For optimal utilization of the potential of exosomal RNAs within the clinic, several avenues for further research are required. Prior to that, large-scale multi-centre validation studies should be performed to confirm diagnostic and prognostic outcomes across races, regions, and health systems. These should be complemented by standardization of procedures as suggested by Martins et al. (2023) to ensure reproducibility.

Second, longitudinal data analyses on overall survival, therapeutic response, and recurrence would give rise to a more profound understanding of the dynamics of RNA with time. Integration within electronic health records and imaging would enable predictive modeling to provide personalized treatment approaches.

Further, other RNAs like circular RNAs and long non-coding RNAs need to be profiled in a systematic manner. These molecules can have various regulatory functions and can be utilized to complement miRNA panels to yield more effective diagnostics (Vlassov et al., 2022).

In addition, research into the packaging and release mechanisms of RNA can reveal new targets for drugs or insight into how tumours may evade immune detection. Dong et al. (2023) has argued

doi:10.20944/preprints202602.0731.v1



that research into the biology of biomarkers enhances their value, not just as markers but as therapeutic levers.

Lastly, the development of machine learning algorithms for cracking large RNA data sets could make clinical uptake possible. Programs like VOSviewer, highlighted by van Eck and Waltman (2022), possess bibliometric mapping capabilities that can identify shifting research hotspots, driving investment in biomarker discovery and commercialization.

## 6. Conclusion

In this research, the authors have demonstrated that exosomal RNAs, and miR-21, miR-1246, miR-141, and miR-200c, in particular, are not just detectable in the blood of cancer patients but also display distinctive differential expression across various cancers as well as during disease stage. The exosomal RNA signatures were associated with important clinical parameters, including tumour progression, treatment response, and progression-free survival, and therefore are of notable value as biomarkers for early detection, prognosis, and real-time Therapeutic monitoring.

One of the most important conclusions was the improved quality and yield of RNA from plasma-derived exosomes, which indicated plasma as a useful and accessible source for liquid biopsy in oncology. Moreover, these RNAs showed excellent diagnostic performance, with sensitivity and specificity over 85% in distinguishing cancer patients versus normals. Interestingly, the study also established the prognostic significance of these biomarkers, wherein the high expression levels of some miRNAs correlated with poor clinical outcomes and thus implicating them in risk stratification and individualized treatment planning.

These data inform growing clinical interest in the potential of exosomal RNA as a transformative solution in precision oncology. Since they are non-invasive, reproducible, and able to report dynamic tumour biology, they are well-suited to be considered for integration into clinical pipelines—potentially to complement or, eventually, replace traditional tissue biopsies. Liquid biopsies of exosomal RNA can enable earlier diagnosis, enhanced monitoring of response to therapy, and more informed decision-making in cancer treatment.

Summarily, exosomal RNA biomarkers offer an exciting avenue in oncology practice and research. As more validation and standardisation go forward, they offer the promise of transforming personalised cancer treatment to a more patient-friendly, timely, and specific diagnostic process. Further advances in exosome-based platforms might greatly enhance outcomes through superior disease characterisation and tailored intervention strategies.

## References

Ailuno, G., Baldassari, S., Lai, F., Florio, T., & Caviglioli, G. (2020). Exosomes and extracellular vesicles as emerging theranostic platforms in cancer research. *Cells, 9*(12), 2569. https://doi.org/10.3390/cells9122569

Baghban, N., Ullah, M., & Nabipour, I. (2023). The current trend of exosomes in epithelial ovarian cancer studies: A bibliometric review. *Frontiers in Pharmacology, 14*, 1082066. https://doi.org/10.3389/fphar.2023.1082066

Dai, J., Su, Y., Zhong, S., Cong, L., Liu, B., Yang, J., ... & Jiang, Y. (2020). Exosomes: Key players in cancer and potential therapeutic strategy. *Signal Transduction and Targeted Therapy, 5*(1), 145. https://doi.org/10.1038/s41392-020-00261-0

Dong, X., Xie, Y., Xu, J., Qin, Y., Zheng, Q., Hu, R., ... & Yi, K. (2023). Global historical retrospect and prospects on biomarkers of heart failure: A bibliometric analysis and science mapping. *Heliyon, 9*(2), e13509. https://doi.org/10.1016/j.heliyon.2023.e13509

Egea-Jimenez, A. L., & Zimmermann, P. (2020). Lipids in exosome biology. *Handbook of Experimental Pharmacology, 259*, 309–336. https://doi.org/10.1007/164_2019_305

Fontham, E. T., Thun, M. J., Ward, E., Balch, A. J., Delancey, J. O. L., & Samet, J. M. (2023). American Cancer Society perspectives on environmental factors and cancer. *CA: A Cancer Journal for Clinicians, 59*(6), 343–351. https://doi.org/10.3322/caac.20041

 doi:10.20944/preprints202602.0731.v1




Global Burden of Disease Cancer Collaboration, Kocarnik, J. M., Compton, K., Dean, F. E., Fu, W., Gaw, B. L., ... & Ababneh, E. (2022). Cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life years for 29 cancer groups from 2010 to 2019: A systematic analysis for the Global Burden of Disease Study 2019. *JAMA Oncology, 8*(3), 420–444. https://doi.org/10.1001/jamaoncol.2021.6987

Hanahan, D. (2022). Hallmarks of cancer: New dimensions. *Cancer Discovery, 12*(1), 31–46. https://doi.org/10.1158/2159-8290.CD-21-1059

Hanahan, D., & Weinberg, R. A. (2000). The hallmarks of cancer. *Cell, 100*(1), 57–70. https://doi.org/10.1016/S0092-8674(00)81683-9

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell, 144*(5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013

Inamura, K., Hamada, T., Bullman, S., Ugai, T., Yachida, S., & Ogino, S. (2022). Cancer as microenvironmental, systemic and environmental diseases: Opportunity for transdisciplinary microbiomics science. *Gut, 71*(10), 2107–2122. https://doi.org/10.1136/gutjnl-2022-327209

Martins, T. S., Vaz, M., & Henriques, A. G. (2023). A review of comparative studies addressing exosome isolation methods from body fluids. *Analytical and Bioanalytical Chemistry, 415*(7), 1239–1263. https://doi.org/10.1007/s00216-022-04174-5

Paskeh, M. D. A., Entezari, M., Mirzaei, S., Zabolian, A., Saleki, H., Naghdi, M. J., ... & Hushmandi, K. (2022). Emerging role of exosomes in cancer progression and tumour microenvironment remodeling. *Journal of Hematology & Oncology, 15*(1), 83. https://doi.org/10.1186/s13045-022-01305-4

Raposo, G., Nijman, H. W., Stoorvogel, W., Liejendekker, R., Harding, C. V., Melief, C. J., & Geuze, H. J. (2022). B lymphocytes secrete antigen-presenting vesicles. *Journal of Experimental Medicine, 183*(3), 1161–1172. https://doi.org/10.1084/jem.183.3.1161

Schey, K. L., Luther, J. M., & Rose, K. L. (2023). Proteomics characterization of exosome cargo. *Methods, 87*, 75–82. https://doi.org/10.1016/j.ymeth.2015.03.018

Tai, Y. L., Chen, K. C., Hsieh, J. T., & Shen, T. L. (2022). Exosomes in cancer development and clinical applications. *Cancer Science, 109*(8), 2364–2374. https://doi.org/10.1111/cas.13697

van den Boorn, J. G., Dassler, J., Coch, C., Schlee, M., & Hartmann, G. (2023). Exosomes as nucleic acid nanocarriers. *Advanced Drug Delivery Reviews, 65*(3), 331–335. https://doi.org/10.1016/j.addr.2012.06.011

van Eck, N. J., & Waltman, L. (2022). Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics, 84*(2), 523–538. https://doi.org/10.1007/s11192-009-0146-3

Vlassov, A. V., Magdaleno, S., Setterquist, R., & Conrad, R. (2022). Exosomes: Current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. *Biochimica et Biophysica Acta (BBA) - General Subjects, 1820*(7), 940–948. https://doi.org/10.1016/j.bbagen.2012.03.017

Wu, Z., Xia, F., & Lin, R. (2024). Global burden of cancer and associated risk factors in 204 countries and territories, 1980–2021: A systematic analysis for the GBD 2021. *Journal of Hematology & Oncology, 17*(1), 119. https://doi.org/10.1186/s13045-024-01640-8

Zhang, Y., Hu, L., Liao, S., Wang, Y., Ji, X., Liu, X., ... & Zhu, J. (2023). Bibliometric analysis of publications on enthesitis in spondyloarthritis in 2022 based on Web of Science core collection databases. *Rheumatology International, 43*(1), 173–182. https://doi.org/10.1007/s00296-022-05227-9

Zhu, Z., Zhou, Y., Li, H., Xu, W., Wang, T., Liu, J., & Jiang, H. (2023). Research trends and hotspots in prostate cancer-associated exosome: A bibliometric analysis. *Frontiers in Oncology, 13*, 1270104. https://doi.org/10.3389/fonc.2023.1270104

Zitvogel, L., Regnault, A., Lozier, A., Wolfers, J., Flament, C., Tenza, D., ... & Amigorena, S. (2022). Eradication of established murine tumors using a novel cell-free vaccine: Dendritic cell-derived exosomes. *Nature Medicine, 4*(5), 594–600. https://doi.org/10.1038/nm0598-594