including KRAS, BRAF, ESR1 and BRCA1. High MSI was detected in two patients, with one of them having no actionable alterations. Taken together, actionable changes that could be amended by either genomically-driven targeted therapies or immunotherapy were seen in 47/114 patients with detected somatic variants (41%). Blood TMB (at tumor fractions of ≥1%) was observed at a median score of 19.3 Mut/Mb (range: 11.2-66) in 35/142 patients and most frequently associated with lung, breast and prostate cancer; 15 of these patients had no actionable variants.
Conclusions: This study supports the use of PanTracer LBx as a tool for guiding patients with advanced malignancies to appropriate, guideline-recommended therapies for improved disease outcomes.

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**62**

**The Journal of Liquid Biopsy 9 (2025) 100383**

## Genomic Profiles of Early-Stage Non-Small Cell Lung Cancer Patients, and Association with Pre-Treatment Blood Circulating Tumour DNA Detection and Levels

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**Introduction:** Tumour-informed blood circulating tumour DNA (ctDNA) assays are used for sensitive, non-invasive detection of disease in cancer patients. The influence of tumour characteristics, including driver gene mutation status, on ctDNA detection and quantity, remain little explored.
**Methods:** We compared whole exome sequencing (WES)-identified tumour mutations to pre-treatment (pre-Tx) ctDNA detection using personalised panels (RaDaR 1.0) applied to 165 patients with stage 0-III NSCLC, recruited to two retrospective clinical studies; LEMA (n=87) and LUCID (n=78). Pre-Tx samples were prioritised to limit confounding effects of treatment. Analyses focussed on the association of mutation status of select tumour driver genes (TP53, KRAS, EGFR) with pre-Tx ctDNA detection and estimated Variant Allele Fraction (eVAF), total cfDNA (copies/mL), and survival. Biological and clinical characteristics such as tumour stage, smoking status, histology and treatment were also explored.
**Results:** Findings indicate mutation-specific differences in ctDNA detection across EGFR-, KRAS- and TP53-mutated tumours, and tumours with no mutations of these genes (WT) (p=0.0026). Pre-Tx ctDNA was detected in 73%, 35% and 0% of patients with tumour mutations in TP53, KRAS, and EGFR, respectively, and in 51% of WT patients. Patients with TP53-mutated tumours had higher ctDNA detection and eVAF upon multivariable analysis and were associated with higher total cfDNA levels than WT ($p<0.05$). In survival analyses, low-level pre-Tx ctDNA detection (≤0.01% eVAF), was associated with worse overall survival (OS) in univariate analysis, but this association was non-significant in adjusted Cox model. Stage III had a markedly higher hazard than stage 0/I (HR = 10.0, $p<0.001$), and patients with TP53-mutated tumours had worse OS than WT (HR = 4.24, p=0.020). A formal ctDNA-TP53 interaction was non-significant, likely due to limited numbers. Additional correlates of ctDNA detection and mutation status included smoking history, tumour mutational burden, and tumour size.
Conclusions: Pre-Tx ctDNA detection and burden were genotype-dependent in this cohort of early-stage NSCLC patients, with TP53 mutations linked to higher ctDNA detection and eVAF, and total cfDNA levels. These data highlight the potential importance of underlying tumour biology on ctDNA, and their clinical utility in patients with differing genomic profiles.

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**63**

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## Clinical Utility of Soluble Immune Mediators and cfDNA in Endometrial Cancer

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**Introduction:** Endometrial cancer is the most common gynecological malignancy in developed countries. Although most cases are diagnosed early, advanced-stage disease and aggressive subtypes display poor outcomes. Consequently, liquid biopsy, through the analysis of circulating cell-free DNA (cfDNA) and soluble immune mediators, emerges as a minimally invasive approach with significant diagnostic and prognostic potential.
Objective: To determine the clinical utility of liquid biopsy in endometrial cancer by comprehensively characterizing soluble immune mediators and performing a comparative analysis of genomic alterations between primary tumor tissue and matched plasma samples.
Material and **Methods:** Serum soluble immune mediators were analyzed in 66 patients using multiplex immunoassay (MILLIPLEX® Immuno-Oncology Panel 2). Parametric and non-parametric tests were employed for statistical analysis. Receiving Operating Curve (ROC) method was used to determine prognostic power of analytes. For survival analysis, Cox regression and Kaplan Meier with log-rank test were performed. Diagnostic FFPE tissue and plasma samples (baseline and follow-up) from 13 patients were analyzed using the Oncomine™ Gynecological panel.
**Results:** Of 66 patients, 46 patients had early-stage (I–II) and 20 advanced-stage disease (III–IV). In early-stage cohort, sGalectin-1 levels were higher in those >70 kg (p=0.036), while smokers had lower sCD276 and sGalectin-3 concentrations (p=0.033). Relapse was associated with reduced baseline sMICA (p=0.006; cutoff of 16.16 pg/mL, sensitivity 100%, specificity 97.5%, AUC 0.979). In survival analysis, low sMICA levels correlated with increased recurrence risk (p=0.024), whereas post-menopausal status was associated with better RFS and OS ($p<0.05$). In advanced stages, more aggressive tumors had higher sGalectin-3 levels (p=0.030), and absence of estrogen receptors were related to worse PFS (p=0.013), and elevated Galectin-3 to worse OS (p=0.015). Regarding NGS analyses, concordance was observed in six cases between FFPE tissue and baseline plasma. Due to DNA degradation in FFPE, complex BRCA1/2 mutations were undetectable in tissue but identified in plasma.
Conclusions: Serum immune mediators, particularly sMICA and sGalectin-3, emerge as potential prognostic biomarkers in endometrial cancer. Validation in larger cohorts with longer follow-up is required.
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**64**

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## Lung Cancer Detection via Nasal Swab cfDNA and mFAST-SeqS Analysis - A Proof-of-Concept Study

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**Introduction:** Accurate and timely diagnosis is critical in lung cancer, with early detection being the key factor to improve patient outcomes. The nasal cavity is

an easily accessible source of airway-derived biomolecules and may carry tumor-derived biomarkers from the lower respiratory tract, due to the anatomical connection to the lower airways [1]. Circulating tumor DNA (ctDNA), a fraction of cell-free DNA shed by tumor cells, can be detected in plasma but often with limited sensitivity [2]. We therefore hypothesized that nasal cfDNA may provide a more proximal source of ctDNA. To test this, we evaluated whether nasal swabs analyzed by modified Fast Aneuploidy Screening Test-Sequencing System (mFAST-SeqS), a genome-wide aneuploidy assay, can detect tumor-derived signal as a complementary diagnostic approach in lung cancer.

**Methods:** We prospectively enrolled 10 female, never-smoker, treatment-naïve patients with pathologically confirmed non–small cell lung cancer (NSCLC) at Hospital Universitario Central de Asturias (HUCA) and three self-reported healthy controls. mFAST-SeqS was performed on nasal cfDNA by amplifying, sequencing, and mapping LINE-1 elements across chromosome arms. Sample-level aneuploidy z-scores were computed against a pool of healthy controls. As in prior plasma studies, $z > 5$ defined ctDNA-presence [3].

**Results:** Among samples collected from patients with lung cancer z-scores ranged 4.67–38.28 (median 5.80, IQR 5.17–6.83). Using the predefined threshold, 8/10 patients were ctDNA-positive (80%, 95% CI 44–97%). Two were borderline but below threshold (4.84, 4.67). By comparison, the three healthy controls showed uniformly low z-scores (2.07, 1.96, 1.49; median 1.96), and none met the ctDNA-positivity threshold. Consequently, every positive sample scored above every control, yielding complete separation between groups and supporting the biological validity of nasal cfDNA as a source of tumor-derived material.

Conclusions: Despite the limited sample size, this study provides the first proof-of-concept that nasal swabs can capture tumor-derived genomic biomarkers, setting the stage for their use as a non-invasive, lung-proximal, and scalable liquid-biopsy modality. These findings provide a rationale for larger, controlled trials to define the clinical integration of nasal swabs in the diagnostic pathway of early lung cancer detection.

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[2] https://doi.org/10.3390/arm93030017
[3] https://doi.org/10.1002/1878-0261.13196

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## 66

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### Performance Comparison of RaDaR 1.0 and RaDaR ST Assays for Circulating Tumor DNA Detection Across Solid Tumor Types

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**Introduction:** Circulating tumor DNA (ctDNA) represents a promising tool for detection of minimal residual disease (MRD) and treatment monitoring in patients with cancer. Assays designed to detect ctDNA must demonstrate reproducibility across technical updates. This study evaluated concordance between two versions of a tumor-informed MRD assay: RaDaR 1.0, a clinically validated and approved version, and the updated RaDaR ST assay. This presentation will explore the analytical and technical performance and potential clinical utility of RaDaR ST across cancer types.

**Methods:** A total of 175 plasma samples from patients, representing 15 types of solid tumors, were tested using RaDaR 1.0 and RaDaR ST. Both assays represent tumor-informed workflows, previously described, with the updated assay employing modified chemistry. Assay limit of detection (LoD) is 0.001% for both assays. Sample testing was covered under IRB-approved protocols. Samples taken at the same day and time were used to avoid temporal variability in ctDNA. Concordance between the assays (matching 'ctDNA detected' or 'ctDNA not detected' calls) was assessed, as well as ctDNA estimated variant allele fraction (eVAF). Discordant results and technical considerations are explored.

**Results:** Roughly equal quantities of DNA were used as input to each assay (median input = 7,900 copies, range 1,075-20,000, variance = 10.25%). Of 166 matched samples, concordant ctDNA detection was observed in 161 (96.99%). In concordant 'ctDNA detected' samples, eVAF was highly correlated between the two assays ($R^2 = 0.969$). The five discordant cases saw eVAF measurements near or below the assay LoD (0.001–0.003% eVAF). In some instances, RaDaR ST may have detected 'true positive' results not detected by RaDaR 1.0 (indicated by matched clinical data consistent with recurrence).

**Conclusion:** This bridging study demonstrated high concordance between RaDaR 1.0 and RaDaR ST, supporting the transition to RaDaR ST for research and clinical use. These data affirm the reliability of RaDaR ST for ctDNA detection across diverse tumor types and clinical scenarios, including those previously validated by RaDaR 1.0.

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## 67

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### Plasma and Pleural Effusion as reflective mirrors of the Immune Landscape in two histotypes of Pleural Mesothelioma

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**Introduction:** Pleural mesothelioma (PM) is a rare, asbestos-related cancer with poor prognosis. Immune checkpoint inhibitors have demonstrated clinical activity in PM, particularly in non-epithelioid (NE) subtypes, although only a subset of patients benefits from this therapeutic strategy. Video-Assisted Thoracic Surgery (VATS), a surgical procedure remains the diagnostic gold standard, highlighting the need for reliable, non-invasive biomarkers. In this context, pleural effusion (PE) and peripheral blood might act as alternative sources for biomarker identification and disease monitoring. Here, we performed a pilot study of a panel of cytokines and soluble immune checkpoint molecules (sICs) in both plasma and PE from a cohort of PM patients.

**Methods:** Twenty patients who underwent VATS for a suspected PM within a prospective multicenter study, were included in this pilot study. For all patients, both tumor and plasma samples were collected at diagnosis, whereas in 12/20 PE specimens were also obtained with curative intent. Tumor microenvironment (TME) characterization of both tumor- and PE-derived cells was performed using flow cytometry with two multiplex panels targeting lymphoid and myeloid populations. Furthermore, levels of 4 sICs (PD-L1/CTLA-4/TIM-3/LAG-3) and 7 cytokines (IFNγ/CXCL10/IL-2/IL-10/IL-6/MIG/TNF-α) were evaluated in plasma and PE samples by ELLA platform (Bio-Techne).

**Results:** Twelve patients had epithelioid and eight NE. In plasma, circulating markers showed a trend toward higher levels in the NE subtype, particularly for LAG-3. In contrast, PE analysis revealed significantly higher IL-10 levels in the epithelioid histotype. Overall, marker concentrations were generally higher in PE compared with paired plasma samples, especially for IL-6. TIM-3, IFNγ, IL-10, MIG, and TNF-α significantly higher in PE versus paired plasma in epithelioid PM; conversely, TIM-3 and IL-6 were significantly higher in PE compared with matched plasma in NE histology.

**Conclusion:** This pilot study shows that plasma and PE may reflect the immune status of PM, with PE providing a stronger representation, probably due to its direct contact with tumor cells. Ongoing characterization of tumor- and PE-derived cells could provide complementary information on the PM immune landscape, supporting the identification of prognostic markers and potential therapeutic targets.

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