Review

Liquid Biopsy in Genitourinary Cancers: Circulating Tumor DNA as a Predictive Biomarker for Treatment Selection and Resistance Monitoring

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Posted Date: 27 April 2026

doi: 10.20944/preprints202604.1890.v1

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 doi:10.20944/preprints202604.1890.v1

Review

Liquid Biopsy in Genitourinary Cancers: Circulating Tumor DNA as a Predictive Biomarker for Treatment Selection and Resistance Monitoring

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Abstract

Background: Genitourinary (GU) cancers—encompassing prostate, urothelial, and renal cell carcinomas—collectively represent a leading source of oncologic morbidity and mortality worldwide. Tumor heterogeneity and the dynamic evolution of resistance mechanisms limit the clinical utility of static tissue biopsies, creating an urgent need for real-time, non-invasive biomarkers. Circulating tumor DNA (ctDNA), isolated from peripheral blood or urine, captures the somatic mutational landscape of the tumor in a temporally resolved manner and has emerged as a compelling candidate predictive biomarker for treatment selection and resistance monitoring across GU malignancies. **Methods:** An integrative review was conducted following the Whittemore and Knafl (2005) framework. A systematic literature search was performed across PubMed/MEDLINE, Cochrane Central Register of Controlled Trials, Embase, and Web of Science, covering the period January 2020 to April 2026. Search strategies combined MeSH and free-text terms pertaining to ctDNA, liquid biopsy, and the three principal GU cancer types. After duplicate removal and staged screening, 23 studies met inclusion criteria and formed the basis of the qualitative synthesis. **Results:** The integrated evidence demonstrates that ctDNA functions as a dynamic predictive biomarker across three GU cancer domains. In castration-resistant prostate cancer (CRPC), ctDNA tumor fraction detected at cycle 3, day 1 of enzalutamide therapy independently predicts radiographic progression-free survival and overall survival (mOS 16.0 vs. 22.1 months for ctDNA-positive vs. ctDNA-negative patients). In muscle-invasive bladder cancer (MIBC), post-cystectomy ctDNA identifies metastatic relapse with 94% sensitivity and 98% specificity and stratifies benefit from adjuvant atezolizumab in IMvigor010. In renal cell carcinoma (RCC), emerging data support ctDNA for VHL and PBRM1 mutation tracking and treatment response assessment, though prospective validation remains limited. ctDNA-guided detection of androgen receptor splice variant 7 (AR-V7) and FGFR3 alterations enables biologically informed treatment sequencing in CRPC and urothelial carcinoma, respectively. **Conclusion:** ctDNA constitutes a molecularly rigorous, clinically actionable biomarker platform with demonstrated predictive and prognostic value across the GU cancer spectrum. Integration into prospective clinical trials and regulatory frameworks represents the critical next step toward embedding ctDNA-guided decision-making into standard oncologic practice.

Keywords: circulating tumor DNA; liquid biopsy; genitourinary cancers; predictive biomarker; treatment selection; resistance monitoring; castration-resistant prostate cancer; urothelial carcinoma; renal cell carcinoma; AR-V7; FGFR3

1. Introduction

Genitourinary cancers collectively account for a disproportionate share of the global cancer burden. Prostate cancer remains the most commonly diagnosed non-cutaneous malignancy among

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men, with an estimated 1.4 million incident cases annually worldwide. Bladder and urothelial carcinomas are responsible for approximately 573,000 new diagnoses per year, and renal cell carcinoma (RCC) contributes an additional 431,000 cases. Together, these three entities generate a substantial and growing demand for precision diagnostic and therapeutic tools [1,2].

A fundamental limitation in the clinical management of GU cancers is the inherent inadequacy of static tissue biopsy. A single biopsy captures a spatially and temporally restricted genomic snapshot, failing to represent the clonal architecture of disseminated or metastatic disease. In prostate cancer, where 90% of metastatic spread localizes to bone, obtaining tissue is both technically challenging and logistically prohibitive. In urothelial carcinoma, significant discordance—approximately 23%—between primary tumors and metastatic sites has been documented, undermining precision medicine strategies anchored exclusively to primary tumor profiling [3]. In RCC, the high degree of intratumoral heterogeneity further confounds tissue-based biomarker interpretation.

Liquid biopsy has emerged as a transformative paradigm in oncology, offering non-invasive, repeatable access to tumor-derived analytes circulating in body fluids. Among these analytes, circulating tumor DNA (ctDNA)—short, apoptosis- and necrosis-derived fragments of tumor genomic DNA released into the peripheral circulation—has attracted the greatest translational momentum. The short plasma half-life of ctDNA, estimated between 15 minutes and 2 hours, enables near-real-time reflection of tumor dynamics under therapeutic pressure. Moreover, ctDNA captures information from multiple tumor sites simultaneously, affording a system-level view of clonal heterogeneity that no single biopsy can provide [4,5].

The clinical utility of ctDNA spans multiple domains: early detection, prognostication, minimal residual disease (MRD) assessment, treatment response monitoring, and the identification of acquired resistance mutations. In GU oncology, ctDNA has progressed from exploratory biomarker studies toward biomarker-guided clinical trial integration. The FDA approval of the FoundationOne Liquid CDx assay for the identification of BRCA1/BRCA2 mutations in prostate cancer patients eligible for rucaparib represents a landmark regulatory milestone, establishing liquid biopsy as a clinically actionable companion diagnostic [3].

Despite this progress, critical gaps remain. The predictive utility of ctDNA—that is, its capacity to inform prospective treatment selection and not merely retrospective prognostication—requires systematic evaluation across the GU cancer spectrum. This integrative review synthesizes current evidence on ctDNA as a predictive biomarker for treatment selection and resistance monitoring in prostate, urothelial, and renal cell carcinomas, with the objective of consolidating a rigorous evidence base to guide clinical implementation and future trial design.

2. Materials and Methods

2.1. Study Design and Methodological Framework

This study is an integrative review conducted according to the methodological framework proposed by Whittemore and Knafl [6]. The integrative review design was selected for its capacity to synthesize diverse types of evidence—including experimental, observational, and theoretical studies—thereby enabling a comprehensive appraisal of the multi-paradigmatic literature on ctDNA in GU oncology.

2.2. Search Strategy and Information Sources

A systematic search was performed across four primary bibliographic databases: PubMed/MEDLINE, Cochrane Central Register of Controlled Trials, Embase, and Web of Science. The search period spanned January 2020 to April 2026, a time frame selected to capture the contemporary era of ctDNA clinical translation while excluding a preparatory literature base. Search strategies were constructed using combinations of Medical Subject Headings (MeSH) and free-text terms, organized around three conceptual domains: 1) the analyte of interest ("circulating tumor

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DNA," "ctDNA," "liquid biopsy," "cell-free DNA"); 2) the tumor types of interest ("prostate cancer," "urothelial carcinoma," "bladder cancer," "renal cell carcinoma," "genitourinary cancer"); and 3) the clinical outcomes of interest ("treatment selection," "predictive biomarker," "resistance monitoring," "treatment response," "minimal residual disease"). Boolean operators AND/OR were applied to combine term clusters. The integrity and contextual classification of retrieved references were subsequently verified using SCITE.ai, employed exclusively as a bibliometric validation tool and not as a primary literature source.

2.3. Eligibility Criteria

Studies were eligible for inclusion if they met the following criteria: 1) published in English in peer-reviewed journals or as preprints of sufficient methodological quality between January 2020 and April 2026; 2) reported original or synthesized data on ctDNA as a biomarker in prostate cancer, urothelial carcinoma (bladder or upper tract), or RCC; 3) addressed at least one of the primary outcomes—treatment selection, treatment response prediction, or resistance monitoring; and 4) provided sufficient methodological or quantitative data to allow extraction of a meaningful synthesis. Studies were excluded if they addressed exclusively non-GU cancer types, focused on circulating tumor cells or other liquid biopsy analytes without ctDNA-specific data, or were conference abstracts without associated full-text publication.

2.4. Study Selection and Data Extraction

The selection process followed a two-stage approach. In the first stage, titles and abstracts of all retrieved records were screened against eligibility criteria. In the second stage, full texts of candidate studies were retrieved and assessed for final inclusion. Data extraction encompassed study design, cancer type, patient population characteristics, ctDNA detection methodology, primary and secondary outcomes, key quantitative findings, and quality assessment results. Discrepancies in selection or extraction were resolved by re-evaluation against the eligibility criteria.

2.5. Quality Assessment

The methodological quality of included primary studies was evaluated using design-appropriate tools. Randomized controlled trials (RCTs) and biomarker sub-studies derived from RCTs were assessed with the Risk of Bias 2 (RoB 2) tool [7]. Observational studies were appraised using the ROBINS-I instrument [8]. Systematic reviews and integrative reviews included within the synthesis were evaluated using AMSTAR-2 [9]. Domain-by-domain risk of bias assessments for included primary studies are presented in Appendix A (Table A1). The integrity and classification of citations were additionally verified through the Scite.ai platform, employed as a post-search bibliometric validation tool, not as a primary identification source.

3. Results

3.1. Characteristics of the Included Studies

Database searches across PubMed/MEDLINE, Cochrane Central Register of Controlled Trials, Embase, and Web of Science for the period January 2020 to April 2026 yielded 120 records. After removal of 28 duplicates, 92 unique records were screened by title and abstract. Thirty-one records were excluded at this stage owing to absence of primary focus on genitourinary cancers, use of liquid biopsy analytes other than ctDNA without ctDNA-specific data, or outcomes unrelated to treatment selection or resistance monitoring. The 61 remaining records were retrieved for full-text assessment. Thirty-eight were subsequently excluded for the following reasons: 14 lacked primary focus on genitourinary malignancies; 11 provided insufficient ctDNA-specific outcome data; 8 did not meet minimum methodological quality thresholds; 4 reported overlapping cohorts already represented by

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higher-quality included studies; and 1 was a duplicate identified at full-text stage. The final synthesis comprised 23 studies (Figure 1).

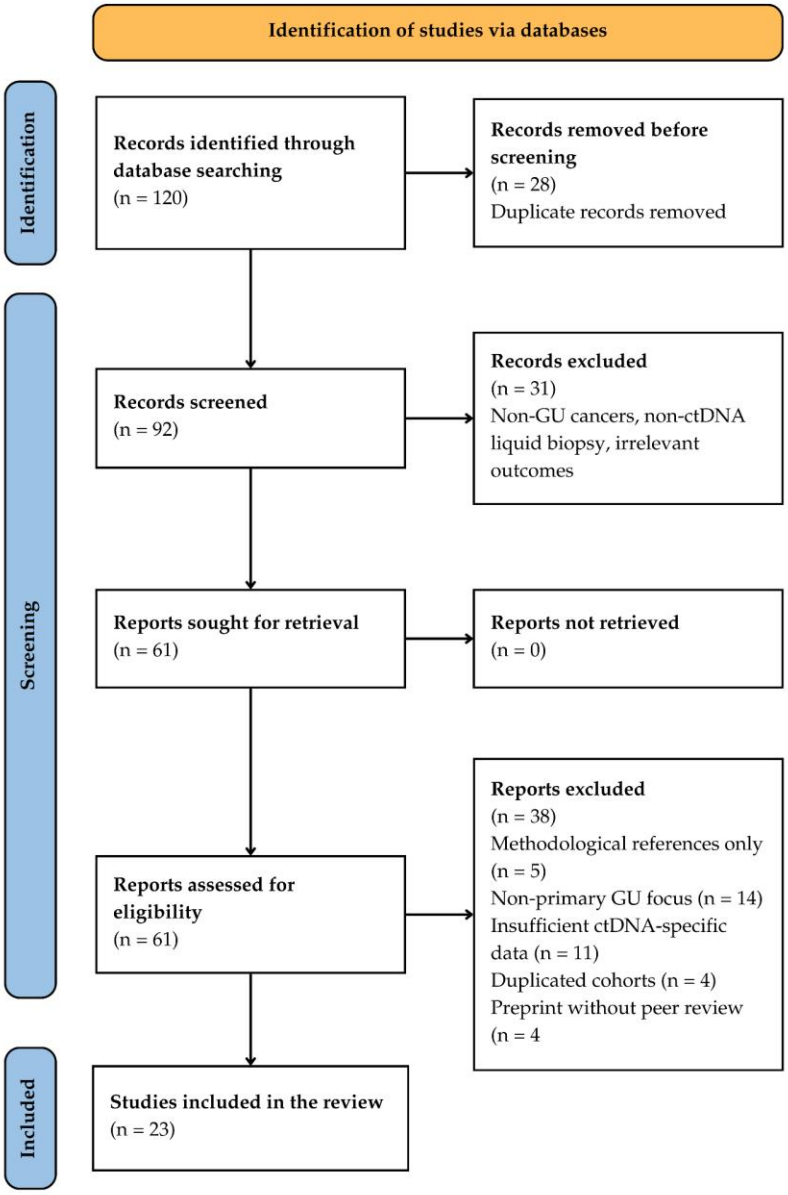

Figure 1. PRISMA 2020 flow diagram illustrating the study selection process for the integrative review on circulating tumor DNA as a predictive biomarker in genitourinary cancers.

A total of 23 studies met all inclusion criteria and formed the basis of this integrative synthesis. The corpus encompassed integrative and narrative reviews (n = 12; 52.2%), prospective and retrospective observational cohort studies (n = 6; 26.1%), biomarker analyses derived from phase II and III clinical trials (n = 3; 13.0%), and case series or translational studies (n = 2; 8.7%). Geographically, multinational studies predominated (n = 10; 43.5%), followed by North American (n = 8; 34.8%), European (n = 4; 17.4%), and East Asian investigations (n = 1; 4.3%). The publication period ranged from 2020 to 2025, with the majority of studies published from 2022 onward, reflecting the accelerating pace of clinical translation. Detailed characteristics of all included studies are presented in Table 1.

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Table 1. Characteristics of the included studies (n = 23).

Study (first author, year)	Design	Cancer type	Sample / population	Key outcome(s)	ctDNA method
Gerke et al., 2024 [3]	IR	Prostate, bladder, RCC	Narrative synthesis	ctDNA biomarker overview in GU cancers; FDA CDx milestone	Multiple
Sweeney et al., 2024 [10]	Ph III biomarker	mCRPC	n = 494	ctDNA TF at C3D1 predicts OS; mOS 22.1 vs 16.0 mo (P < 0.001)	Tissue-agnostic plasma
Jang et al., 2023 [11]	Prospestive cohort	RCC (n = 15), UC (n = 4), PC (n = 1)	n = 20	ctDNA–imaging concordance 83%; ORR 70% with ICI	Signatera (tumor-informed)
Katsimperis et al., 2025 [4]	Review	MIBC	Narrative synthesis	ctDNA for MRD, adjuvant immunotherapy guidance	Multiple
Lindskrog et al., 2023 [12]	Longitudinal cohort	MIBC	n = 68 + n = 102	Post-RC ctDNA: sensitivity 94%, specificity 98%; MRD HR 17.8	mPCR-NGS (Signatera)
Crocetto et al., 2025 [13]	Syst review	Bladder cancer	Narrative synthesis	ctDNA as therapeutic decision biomarker; FGFR3 monitoring	Multiple
Pak et al., 2022 [14]	Prosp observational	mCRPC	n = 38	AR-V7/AR-FL ratio predicts ARSI response (PSA RR 25% vs 77.8%; p = 0.003)	Tissue mRNA
Nawaf et al., 2023 [15]	Narrative review	MIBC	Narrative synthesis	ctDNA-guided adjuvant therapy; TOMBOLA 3% relapse in ctDNA-neg	Multiple
Antar et al., 2024 [1]	Narrative review	All GU	Narrative synthesis	Molecular landscape; NGS, FGFR3, BRCA1/2, PTEN in GU cancers	NGS (multiple)
Netti et al., 2025 [16]	Review	RCC	Narrative synthesis	Liquid biopsy in RCC diagnosis and monitoring; VHL, PBRM1	Multiple
Liu et al., 2025 [17]	Bibliometric review	All GU	439 publications	Urine liquid biopsy trajectory; standardization phase identified	Urine ctDNA/exosomes
Alam et al., 2025 [18]	Case series	RCC (diverse)	n = 6	ctDNA monitoring influenced clinical decision-making in RCC subtypes	Plasma ctDNA

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Study (first author, year)	Design	Cancer type	Sample / population	Key outcome(s)	ctDNA method
Zhang et al., 2020 [19]	Review	mCRPC	Narrative synthesis	AR-V7 as resistance biomarker; taxane vs ARSI selection	CTC-based / cfDNA
Del Re et al., 2021 [20]	Translational	mCRPC	Prospective cohort	AR gain in cfDNA + AR-V7 in exosomes predict ARSI resistance	cfDNA + exosomes
Sobhani et al., 2021 [21]	Review	mCRPC	Narrative synthesis	AR-V7 theranostic framework; ARSI → taxane transition strategy	CTC-based
Necchi et al., 2021 [22]	Genomic analysis	BUC / UTUC	n = 2,463 (unmatched)	ctDNA detects FGFR3 alterations; ctDNA/tissue concordance	Plasma ctDNA (commercial)
Peng et al., 2021 [5]	Review	Solid tumors (GU incl.)	Narrative synthesis	ctDNA and MRD in solid tumors; mechanisms and clinical horizons	Multiple
Jongbloed et al., 2021 [23]	Syst review	Solid tumors (GU incl.)	Multiple cohorts	cfDNA dynamics monitor treatment response; RCC included	cfDNA panels
Kilgour et al., 2020 [24]	Review	Multiple (GU incl.)	Narrative synthesis	Liquid biopsy biomarkers of treatment response and resistance	Multiple
Kwan et al., 2022 [25]	Review	mCRPC	Narrative synthesis	ctDNA implementation pathways in mCRPC; AR alterations via cfDNA	cfDNA (plasma)
Ashizawa et al., 2022 [26]	Clinical study	mCRPC	Prospective cohort	AR-V7 status in CTCs predicts cabazitaxel response	CTC-based AR-V7
Labadie & Balar, 2021 [2]	Review	All GU	Narrative synthesis	ICI in GU cancers; biomarker stratification and response prediction	Multiple
Robbrecht et al., 2025 [27]	Cohort analysis	Metastatic UC	n = 155	NECTIN4, FGFR2/3, TSE score guide treatment selection in mUC	WGS + RNA-seq

Note: Table 1 describes the 23 studies included in the qualitative synthesis. IR = integrative review; Ph III = phase III clinical trial; Prosp = prospective; Syst = systematic; mCRPC = metastatic castration-resistant prostate cancer; MIBC = muscle-invasive bladder cancer; RCC = renal cell carcinoma; UC = urothelial carcinoma; BUC = bladder urothelial carcinoma; UTUC = upper tract urothelial carcinoma; mUC = metastatic urothelial carcinoma; ARSI = androgen receptor signaling inhibitor; ICI = immune checkpoint inhibitor; CTC = circulating tumor cell; cfDNA = cell-free DNA; ctDNA = circulating tumor DNA; mPCR-NGS = multiplex polymerase chain reaction–next-generation sequencing; WGS = whole-genome sequencing. Distribution by design: integrative/narrative reviews

 doi:10.20944/preprints202604.1890.v1

= 12 (52.2%); observational/prospective cohort studies = 6 (26.1%); biomarker analyses from phase II/III trials = 3 (13.0%); case series/translational studies = 2 (8.7%). All percentage denominators refer to n = 23.

3.2. ctDNA in Castration-Resistant Prostate Cancer: AR-V7, Tumor Fraction, and Treatment Selection

The most clinically advanced application of ctDNA in GU oncology involves its use as a predictive and monitoring biomarker in metastatic castration-resistant prostate cancer (mCRPC). Prostate cancer presents unique challenges for tissue-based genomic profiling: the predominance of bone metastases renders repeat biopsy both technically difficult and clinically hazardous, while tumor heterogeneity between metastatic deposits complicates the representativeness of any single specimen. ctDNA addresses these limitations by providing a composite, systemic view of the evolving genomic landscape [3,25].

A pivotal contribution to the field was provided by Sweeney et al. [10], who analyzed ctDNA tumor fraction (TF) data from IMbassador250, a phase III trial evaluating enzalutamide with or without atezolizumab in post-abiraterone mCRPC patients. In 494 evaluable participants, baseline ctDNA TF detection and its dynamics through cycle 3, day 1 (C3D1) were evaluated as biomarkers of clinical outcome. Patients with detectable ctDNA TF at C3D1, irrespective of baseline status, demonstrated significantly shorter radiographic progression-free survival (rPFS) and overall survival than those without ctDNA detection. Critically, when ctDNA TF and PSA response were discordant at C3D1, patients who were ctDNA-undetected but PSA-non-reduced achieved superior overall survival (mOS 22.1 months) relative to those who were ctDNA-detected despite PSA reduction (mOS 16.0 months; $P < 0.001$). This finding established ctDNA TF as an independent and complementary biomarker to PSA in assessing treatment response, with the capacity to identify a clinically distinct patient subgroup inadequately characterized by conventional PSA kinetics alone.

Androgen receptor splice variant 7 (AR-V7) detection in liquid biopsy specimens represents a parallel and mechanistically grounded approach to treatment selection in CRPC. AR-V7, which lacks the ligand-binding domain of full-length AR but retains constitutive transcriptional activity, confers primary resistance to the androgen receptor signaling inhibitors (ARSIs) enzalutamide and abiraterone. Pak et al. [14] demonstrated in a prospective cohort of 38 CRPC patients that those with high AR-V7 to full-length AR ratios exhibited significantly lower PSA response rates to ARSI therapy (25.0% versus 77.8%; $P = 0.003$), as well as inferior PSA progression-free survival, radiological PFS, and overall survival. This differential is of direct clinical relevance: AR-V7-positive patients are more likely to derive benefit from taxane-based chemotherapy than from further ARSI therapy, positioning liquid biopsy-based AR-V7 detection as a clinically actionable treatment-selection biomarker [14,19].

Del Re et al. [20] further characterized the landscape of AR-related liquid biopsy biomarkers, demonstrating that AR copy number gain in cell-free DNA and AR-V7 detection in circulating exosomes independently predict resistance to ARSIs. The convergence of multiple liquid biopsy modalities—including cfDNA, exosomes, and circulating tumor cells—toward a consistent resistance phenotype strengthens the mechanistic coherence of this biomarker paradigm. Sobhani et al. [21] reviewed the clinical evidence for AR-V7 in mCRPC and proposed a theranostic framework in which sequential liquid biopsy testing guides ARSI-to-taxane transitioning, minimizing exposure to ineffective therapies. Ashizawa et al. provided complementary data demonstrating that AR-V7-positive patients treated with cabazitaxel achieved favorable PSA responses [26]. Their study supports the value of AR-V7 status as a positive predictive biomarker for taxane selection, in addition to its established role as a negative predictor of ARSI efficacy.

3.3. ctDNA in Muscle-Invasive Bladder Cancer: MRD, Adjuvant Therapy Guidance, and Immunotherapy Response

In muscle-invasive bladder cancer (MIBC), ctDNA has been validated across the perioperative and metastatic continuum as a biomarker of minimal residual disease, treatment response, and relapse prediction. The biological plausibility is well-established: ctDNA concentrations correlate

 doi:10.20944/preprints202604.1890.v1

with tumor burden, and the short plasma half-life of these fragments allows rapid reflection of changes induced by surgical or systemic interventions [4].

Lindskrog et al. [12] reported landmark longitudinal ctDNA data from two independent MIBC cohorts with median follow-up exceeding five years. In 68 neoadjuvant chemotherapy (NAC)-treated patients, post-cystectomy ctDNA assessment identified metastatic relapse with 94% sensitivity and 98% specificity. ctDNA dynamics during NAC were independently associated with patient outcomes when adjusted for pathological downstaging (HR = 4.7; P = 0.029). In a separate NAC-naïve cohort of 102 patients, preoperative ctDNA positivity predicted significantly inferior recurrence-free survival (HR = 3.4; P = 0.0005) and was an even stronger post-cystectomy prognostic factor (HR = 17.8; P = 0.0002). Biologically, baseline ctDNA positivity was associated with the basal/squamous molecular subtype and enrichment of epithelial-to-mesenchymal transition gene sets. These findings collectively establish ctDNA as a superior prognostic tool to conventional pathological staging in MIBC.

The predictive utility of ctDNA in guiding adjuvant immunotherapy decisions in MIBC was substantiated by data from the IMvigor010 trial. A post-hoc ctDNA analysis demonstrated that patients with detectable ctDNA after radical cystectomy derived meaningful disease-free survival benefit from adjuvant atezolizumab, whereas ctDNA-negative patients did not show equivalent advantage. Moreover, patients achieving complete ctDNA clearance under atezolizumab demonstrated the most favorable overall survival outcomes (median OS: 60.0 months), compared to partial clearance (34.3 months) and minimal clearance (19.9 months), establishing a quantitative dose-response relationship between ctDNA dynamics and survival [4]. These data position ctDNA as a predictive, not merely prognostic, instrument for adjuvant therapy selection in MIBC.

The ABACUS trial further characterized ctDNA dynamics in the context of neoadjuvant atezolizumab in cisplatin-ineligible MIBC patients [28]. Among participants, 63% had detectable ctDNA before treatment initiation, declining to 47% after atezolizumab administration and 14% post-cystectomy. Patients achieving ctDNA clearance at any assessment point demonstrated superior pathological response rates and no observed relapse. Conversely, persistent post-cystectomy ctDNA predicted markedly elevated recurrence risk (RFS HR = 78; P < 0.001). The NABUCCO trial, investigating neoadjuvant ipilimumab plus nivolumab in high-risk MIBC, similarly demonstrated that ctDNA negativity at the time of surgery was associated with 88% progression-free survival at 12 months, compared to 55% in patients with detectable ctDNA (P < 0.01; PFS HR = 10.4; 95% CI: 2.9–37.5) [4].

Nawaf et al. [15] systematically reviewed ctDNA-based MRD detection in the context of adjuvant therapy decision-making for MIBC, highlighting the TOMBOLA trial (NCT04138628) as a prospective framework in which atezolizumab is administered conditionally upon ctDNA positivity post-cystectomy. Preliminary TOMBOLA data reported a relapse rate of only 3% among ctDNA-negative patients managed with watchful waiting, supporting a biomarker-driven de-escalation strategy that minimizes overtreatment without sacrificing efficacy. Crocetto et al. [13] provided a complementary systematic synthesis of liquid biopsy applications in bladder cancer, contextualizing ctDNA within a broader multi-analyte framework that includes circulating tumor cells and urinary exosomes.

The pilot study by Jang et al. [11] extended these observations to the metastatic GU setting, prospectively enrolling 20 patients with advanced GU malignancies—15 with RCC, 4 urothelial, and 1 prostate—receiving immune checkpoint inhibitors (ICIs). Using the tumor-informed Signatera multiplex PCR-based NGS assay, longitudinal ctDNA analysis demonstrated an overall concordance of 83% (15 of 18 evaluable patients) between ctDNA dynamics and radiographic response. The overall objective response rate was 70%. Among three discordant cases, two involved central nervous system metastases, a known blind spot for systemic ctDNA assays, underscoring the importance of integrating ctDNA with anatomic imaging. This study provides proof-of-concept for ctDNA-guided ICI monitoring across GU cancer types.

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In the domain of targeted therapy, ctDNA-based detection of *FGFR3* mutations in urothelial carcinoma offers a non-invasive alternative to tissue genotyping for the selection of patients eligible for erdafitinib—a pan-FGFR tyrosine kinase inhibitor with an overall response rate of 40% in FGFR3-altered metastatic urothelial carcinoma. The *FGFR3* mutational landscape detectable in ctDNA aligns closely with tissue-based profiling. Moreover, serial ctDNA monitoring can detect the emergence of secondary FGFR resistance mutations, including polyclonal reversion mutations, enabling real-time surveillance of acquired resistance [13,29].

3.4. ctDNA in Renal Cell Carcinoma: Emerging Evidence and Current Limitations

Among the three principal GU cancer types, RCC represents the domain where ctDNA evidence is least mature but where clinical need is most acute. Liquid biopsy offers a particularly compelling solution in RCC given the challenges of percutaneous renal mass biopsy in certain anatomical locations. The high prevalence of morphologically heterogeneous tumors and the frequent necessity of distinguishing clear cell from non-clear cell RCC subtypes for systemic therapy selection also contribute to the utility of liquid biopsy [16].

Clear cell RCC is characterized by VHL gene inactivation in up to 90% of cases, often accompanied by concurrent alterations in PBRM1, SETD2, BAP1, and KDM5C. These mutations generate tumor-specific ctDNA fragments detectable in plasma, albeit at levels typically lower than those observed in prostate or urothelial carcinomas, partly because RCC frequently lacks the high tumor mutational burden seen in urothelial malignancies [3,16]. *PBRM1* mutations have been proposed as predictive biomarkers of benefit from ICI therapy in metastatic RCC, based on retrospective analyses of tumor tissue; whether ctDNA-based PBRM1 detection achieves equivalent predictive accuracy awaits prospective validation.

Alam et al. [18] described a series of six patients with diverse renal malignancies—encompassing clear cell RCC, papillary RCC, translocation RCC, malignant angiomyolipoma, and squamous cell carcinoma of the renal pelvis—in whom serial ctDNA monitoring informed clinical decision-making in both adjuvant and metastatic settings. ctDNA positivity preceded radiologic evidence of recurrence in several cases, demonstrating the lead-time advantage of molecular surveillance. Motzer et al. [30] contributed immunological and genomic biomarker analyses from CheckMate 214, examining the relationship between tumor molecular features and outcomes with nivolumab plus ipilimumab versus sunitinib in advanced RCC. While that analysis was primarily tissue-based, it established the *PBRM1*/*BAP1* mutational axis as a clinically relevant biomarker framework amenable to future liquid biopsy translation.

Liu et al. [17] provided a bibliometric characterization of the urine-based liquid biopsy landscape in urological cancers, identifying RCC as an evidence-sparse but clinically important frontier, with exploratory methodological work spanning methylation profiling, extracellular vesicles, and metabolomics. Gerke et al. [3] similarly identified RCC as the GU cancer type with the greatest unmet need for ctDNA biomarker validation. Pal et al. [31] detected significant differences in ctDNA profiles between patients receiving first-line and post-first-line systemic agents, providing mechanistic insights into resistance pathways. Jang et al.[11] reported that among the 15 RCC patients enrolled in their prospective ICI monitoring study, the concordance between ctDNA dynamics and radiographic response was 83%, with the primary source of discordance involving CNS progression—a pattern consistent across GU tumor types.

3.5. Detection Technologies and Clinical Implementation Considerations

The clinical deployment of ctDNA biomarkers requires robust, standardized, and analytically validated assay platforms. Three principal methodological approaches dominate the contemporary landscape. Digital droplet polymerase chain reaction (ddPCR) offers high sensitivity for the detection of predetermined mutations but is limited in scope to a restricted set of predefined targets. Next-generation sequencing (NGS) panels, including commercially available platforms such as Guardant360 and FoundationOne Liquid CDx, provide broader genomic coverage and are capable

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of detecting multiple classes of genomic alterations—point mutations, copy number variations, structural rearrangements, and microsatellite instability—simultaneously. Tumor-informed multiplex PCR-NGS assays, exemplified by Signatera, are designed around patient-specific tumor mutation profiles derived from tissue sequencing and achieve the highest sensitivity for MRD detection in the postoperative setting [4,11].

A critical technical challenge across all platforms is the confounding effect of clonal hematopoiesis of indeterminate potential (CHIP). In this case, age-related somatic mutations in hematopoietic stem cells generate ctDNA-like fragments in plasma that are indistinguishable from tumor-derived signals without matched germline sequencing. Failure to account for CHIP can introduce false-positive findings, particularly in older patients—a demographic that constitutes the majority of GU cancer cases. Pre-analytical standardization, including cell-free DNA collection tube protocols, centrifugation parameters, and storage conditions, also substantially influences ctDNA yield and quality, complicating cross-laboratory comparisons [3,4].

Urine offers an alternative and complementary liquid biopsy matrix in GU oncology, particularly for bladder and urothelial cancers where direct contact between the tumor and the urinary tract generates high concentrations of tumor-derived DNA. *TERT* promoter mutations, detectable in urine ctDNA with high specificity for bladder cancer, represent a validated molecular marker for surveillance. Liu et al. [17] characterized the evolution of urine-based liquid biopsy research, identifying a two-phase trajectory: an initial period of foundational evidence generation followed by a standardization and platform integration phase aligned with guideline development. The complementarity between plasma and urine ctDNA matrices enhances overall biomarker sensitivity, particularly in early-stage and localized disease where systemic ctDNA shedding is low [3,13].

4. Discussion

This integrative review consolidates evidence across 23 studies to demonstrate that ctDNA functions as a clinically meaningful predictive biomarker across all three major GU cancer domains, albeit with varying levels of prospective validation. The most mature evidence base exists in CRPC and MIBC, where ctDNA has been embedded within phase III trial analyses. Its predictive utility for treatment selection and resistance monitoring has been demonstrated at a level of biological and statistical rigor that supports consideration for clinical integration. The evidence base in RCC remains exploratory but is expanding rapidly.

The finding that ctDNA tumor fraction dynamics at cycle 3, day 1 of enzalutamide therapy outperform PSA as a prognostic indicator in discordant cases has profound implications for treatment monitoring in mCRPC [10]. PSA, the longstanding cornerstone of prostate cancer treatment monitoring, is susceptible to flare phenomena and is uninformative in a subset of patients with neuroendocrine differentiation. ctDNA TF provides an orthogonal, molecularly grounded measurement that captures tumor viability independently of PSA secretory function, enabling earlier identification of patients who will not benefit from continued ARSI therapy and who should transition to alternative regimens.

The dual role of AR-V7—as a negative predictor of ARSI efficacy and a positive predictor of taxane benefit—exemplifies the theranostic potential of liquid biopsy-based molecular profiling. AR-V7 testing in clinical settings currently relies primarily on circulating tumor cell-based detection, but cfDNA and exosome-based platforms are demonstrating comparable diagnostic performance with the advantage of higher throughput and reduced technical complexity [19,20]. The convergent evidence from multiple research groups across distinct patient cohorts strengthens the generalizability of AR-V7 as a treatment-selection biomarker, though prospective randomized data demonstrating that AR-V7-guided treatment allocation improves outcomes remain an important evidence gap.

In MIBC, the demonstration that ctDNA negativity after radical cystectomy identifies patients at sufficiently low recurrence risk to permit watchful waiting rather than adjuvant immunotherapy

 doi:10.20944/preprints202604.1890.v1

represents a clinically actionable advance [4,15]. The TOMBOLA trial's preliminary evidence of a 3% relapse rate in ctDNA-negative patients monitored expectantly is particularly compelling, as it addresses one of the most consequential clinical dilemmas in MIBC management: avoiding overtreatment with immunotherapy in patients unlikely to relapse while ensuring treatment reaches those at genuine risk. If confirmed in the full trial cohort, ctDNA-negativity criteria could substantially reduce the number of patients requiring adjuvant atezolizumab, with corresponding reductions in treatment-related toxicity and healthcare costs.

The high sensitivity (94%) and specificity (98%) of post-cystectomy ctDNA for metastatic relapse detection reported by Lindskrog et al. [12] are particularly remarkable and merit contextualization. These performance metrics exceed those of conventional imaging modalities for early relapse detection and are achieved with a lead time of several months before radiographic evidence of recurrence emerges. The clinical implication is that ctDNA-positive patients could be entered into salvage clinical trials or receive intensified therapy at a point when disease burden is lowest and the probability of treatment benefit is highest—a window that conventional imaging surveillance consistently misses.

ctDNA does not operate in isolation within the precision oncology framework. Its clinical impact is maximized when integrated with complementary biomarkers—including tumor mutational burden, microsatellite instability status, PD-L1 expression, and molecular subtype classification—into multiparameter decision models. In urothelial carcinoma, for example, the identification of FGFR3 mutations in ctDNA contextualizes treatment selection within the broader molecular landscape of luminal versus basal tumor subtypes. FGFR3-mutant tumors frequently exhibit a T-cell-depleted immune phenotype that predicts lower benefit from immune checkpoint inhibitors and higher benefit from FGFR-directed therapy [1,13]). ctDNA-based FGFR3 monitoring after erdafitinib initiation can then provide early evidence of secondary resistance, enabling timely therapeutic re-evaluation.

Similarly, in CRPC, the integration of ctDNA TF with AR-V7 status, DNA damage repair gene alterations *(BRCA1/BRCA2, CDK12),* and glucocorticoid receptor expression provides a multi-dimensional resistance profile that can guide the rational sequencing of ARSIs, taxanes, PARP inhibitors, and immunotherapy agents [14,21]). The clinical feasibility of this multi-analyte liquid biopsy approach has been demonstrated in prospective studies, and its implementation within molecular tumor board frameworks represents a practical pathway toward routine clinical deployment.

Artificial intelligence and machine learning algorithms are increasingly being applied to ctDNA data to extract prognostic and predictive signals that exceed the capacity of conventional statistical models. The integration of ctDNA mutational profiles with radiomic, proteomic, and clinical covariates into machine learning frameworks has shown early promise for improving prediction accuracy. These developments, while currently at the investigational stage, are expected to substantially enhance the clinical utility of ctDNA-based biomarker platforms over the next five years.

Various limitations inherent to this integrative review and to the underlying evidence base warrant careful consideration. First, the majority of ctDNA studies in GU oncology to date have been performed as retrospective or post-hoc analyses of prospectively collected biospecimens, often with incomplete ctDNA sampling at all intended time points. These designs, while generating hypothesis-rich data, are subject to selection and attrition biases that may inflate biomarker performance estimates. Prospective, pre-specified ctDNA biomarker studies embedded within interventional trials constitute the gold standard for predictive biomarker validation and remain relatively scarce in GU oncology.

Second, the heterogeneity of ctDNA detection platforms across included studies creates a significant impediment to cross-study quantitative synthesis. The absence of harmonized pre-analytical protocols, standardized variant allele frequency thresholds, and shared bioinformatics pipelines means that ctDNA positivity rates and predictive accuracies reported across studies are not

 doi:10.20944/preprints202604.1890.v1

directly comparable. This methodological fragmentation is particularly evident in RCC, where the low ctDNA shedding rate demands ultrasensitive detection technologies that are not uniformly available.

Third, this integrative review is subject to limitations intrinsic to the methodology itself. Whittemore and Knafl's framework enables the inclusion of diverse study designs, which broadens the evidence base but also introduces heterogeneity in methodological quality. The inability to perform a formal meta-analysis due to the diversity of outcomes, time points, assay platforms, and cancer types limits the precision of the synthesized conclusions. Furthermore, publication bias may favor studies with positive findings, potentially overrepresenting the clinical utility of ctDNA relative to studies with null or inconclusive results.

In conclusion, this integrative review demonstrates that ctDNA constitutes a scientifically rigorous and clinically promising predictive biomarker across the genitourinary cancer spectrum, with the most compelling evidence in mCRPC and MIBC. It also highlights an emerging but rapidly expanding evidence base in RCC. The integration of ctDNA into prospective trial designs, regulatory companion diagnostic frameworks, and clinical decision algorithms represents the critical translational pathway through which liquid biopsy will transition from a research instrument to a standard-of-care tool. Priority areas for future investigation include prospective randomized trials of ctDNA-guided treatment allocation, analytical standardization of assay platforms, and the development of multiparameter biomarker models that capture the full complexity of therapeutic resistance in GU oncology.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data sharing is not applicable.

Conflicts of Interest: The author declares no conflict of interest.

Appendix A

Table A1. Domain-by-domain risk of bias assessment of included primary studies.

Study (first author, year)	D1	D2	D3	D4	D5	Global judgment	Tool
Sweeney et al., 2024 [10]	B	B	M	B	M	Low–Moderate	RoB 2
Jang et al., 2023 [11]	B	B	M	B	B	Low	ROBINS-I
Lindskrog et al., 2023 [12]	B	B	B	B	B	Low	ROBINS-I
Pak et al., 2022 [14]	B	M	B	M	B	Moderate	ROBINS-I
Necchi et al., 2021 [29]	B	B	M	B	M	Low–Moderate	ROBINS-I
Ashizawa et al., 2022 [26]	B	B	B	B	B	Low	ROBINS-I
Alam et al., 2025 [18]	M	NI	M	M	NI	Moderate–High	ROBINS-I
Robbrecht et al., 2025 [27]	B	B	B	B	M	Low	ROBINS-I
Gerke et al., 2024 [3]	B	B	B	B	B	Low	AMSTAR-2
Katsimperis et al., 2025 [4]	B	B	B	B	B	Low	AMSTAR-2

 doi:10.20944/preprints202604.1890.v1

Study (first author, year)	D1	D2	D3	D4	D5	Global judgment	Tool
Crocetto et al., 2025 [13]	B	B	M	B	B	Low	AMSTAR -2

Note: Table A1 presents the domain-by-domain risk of bias assessment for primary studies with a directly citable design (observational, randomized controlled trial biomarker analyses, or systematic reviews). For randomized controlled trial biomarker analyses (RoB 2): D1 = randomization process; D2 = deviations from intended interventions; D3 = missing outcome data; D4 = measurement of the outcome; D5 = selection of the reported result. For observational studies (ROBINS-I): D1 = bias due to confounding; D2 = bias in selection of participants; D3 = bias in classification of interventions; D4 = bias due to deviations from intended interventions; D5 = bias due to missing data. For systematic reviews and integrative reviews (AMSTAR-2), domains reflect methodological rigor globally. B = low risk; M = moderate risk; A = high risk; NI = not informable. Integrative and narrative reviews included in this synthesis as background evidence sources but not amenable to domain-by-domain risk of bias assessment are not listed in Table A1.

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