



## Original Research Article

# A Systematic Review and Meta-Analysis of Circulating Tumor DNA Kinetics as an Early Predictor of Pathological Complete Response in Neoadjuvant Solid Tumor Therapy

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**Abstract: Background:** pCR (pathological complete response) is regarded as one of the chief surrogate endpoints in the neoadjuvant treatment of solid tumors, however, the ways of its determination are currently hindered by late evaluation and only partial consideration of tumor biology. Circulating tumor DNA (ctDNA) has been touted as a minimally invasive biomarker that can reflect the molecular response to therapy in real time. This research was a systematic review of the significance of changes in ctDNA for the earliest prediction of pCR in patients undergoing neoadjuvant therapy for solid tumors.

**Methods:** A systematic review and meta-analysis were performed following the standard evidence synthesis principles. Searches of various electronic databases were conducted to find studies that examined the changes in plasma derived from tumor DNA (ctDNA) levels during neoadjuvant therapy and the association of these changes with pCR. Studies eligible for inclusion were those with patients having solid tumors treated with neoadjuvant systemic therapy and in which serial ctDNA measurements were reported. Data on study characteristics, methods for ctDNA assessment, sampling timing, and pathological outcomes were collected. Combined effect estimates were obtained using random, effects models, and heterogeneity was explored by subgroup analyses.

**Results:** Qualitative synthesis was done on a total of 18 studies with 2,436 patients, and 14 studies were meta-analysis contributors. The removal of ctDNA during neoadjuvant therapy was very significantly associated with increased pCR rates, a correlation that held true for different tumor types and treatment modalities. The odds of pCR in patients with a complete ctDNA clearance were tremendously higher as compared to those in which the ctDNA persisted. Treatment ctDNA monitoring early on was a better predictor than a single end-of-treatment measurement. Some partial reductions in the levels of ctDNA were associated with the intermediate pCR rates which indicated that there was a graded molecular response, pathological outcome relationship. **Conclusion:** The behavior of ctDNA, particularly its early disappearance during neoadjuvant therapy, is highly associated with pathological complete response in different types of cancers. This evidence supports the implementation of serial ctDNA measurement as a method to monitor neoadjuvant therapy and also highlights its value as a possible personalized therapeutic decision, thus helping the guide. In order to be widely available in daily practice, standardization of ctDNA assays is needed together with confirmation of their performance in prospective trials.

**Keywords:** Circulating tumor DNA; Neoadjuvant therapy; Pathological complete response; Liquid biopsy; ctDNA kinetics; Solid tumors.

## INTRODUCTION

Neoadjuvant therapy is now a major part of the treatment regimen for various solid tumors, and pathological complete response (pCR) is considered a key surrogate marker of survival and positive oncologic outcomes. Nevertheless, standard response assessment techniques, including radiologic imaging and histopathology after surgery, have certain limitations in that they describe changes that occur later, are subject to variability between different observers, and do not allow the capture of early molecular changes in the tissue undergoing therapy. Consequently, the demand for less invasive biomarkers capable of measuring tumor burden and treatment effectiveness even at very early time points is steadily increasing.

Circulating tumor DNA (ctDNA) is the part of cell, free DNA which is released into the blood from apoptotic and necrotic tumor cells and has become a new biomarker for a liquid biopsy with great potential. Developments in high, sensitivity sequencing technologies make it possible to detect ctDNA at very low allele frequencies, so now tumor evolution can be followed in real, time in various types of solid cancers.

A study recently suggests that changes in the levels of ctDNA, mainly disappearance of ctDNA or rapid decrease during neoadjuvant therapy, may even strongly associate with pCR and thus, precede the radiographic response (1,4). The present data is pointing towards the indication that the kinetics of ctDNA might be the first locality of treatment success.

Systematic reviews and meta, analyses have gradually revealed that circulating tumor DNA (ctDNA) has both prognostic and predictive significance across different tumor types. For instance, disappearance of ctDNA has been associated with more pCR cases and longer survival times in the neoadjuvant immunotherapy and chemotherapy scenarios (1, 8).

Moreover, comprehensive and targeted sequencing experiments reveal that the detection of ctDNA is a very friendly method in the case of low shedding tumors, such as sarcomas and gastrointestinal stromal tumors, where it is a non, invasive approach (2,3).

Most significantly repeated sampling at multiple time points during the treatment appears to enhance the predictive power of ctDNA as compared to a single measurement at the baseline (4).

To characterize ctDNA kinetics as a clinically usable

biomarker, it is necessary to have a methodologically rigorous evidence synthesis. Criteria coming from systematic review and meta, analysis frameworks of different kinds of studies, e.g. Cochrane diagnostic and therapeutic, point to the requirement of the selection of studies being transparent, bias evaluation, and statistical modeling being of a high standard to make sure that the drawn conclusions are dependable (5,7). The adherence to these regulations in the case of ctDNA research turns out to be a very significant problem because the variations in tumor types, assay platforms, sampling schedules, and molecular response definitions.

The current systematic review and meta, analysis are a significant deviation from this background, aimed at an in, depth evaluation of the role of circulating tumor DNA kinetics as a rapid indicator of pathological complete response in patients with solid tumors undergoing neoadjuvant therapy. By gathering evidence from different types of cancer and various treatments, this study aims to clarify the clinical use of ctDNA changes and to make their implementation easier in the personalized neoadjuvant therapy regimen.

## MATERIAL AND METHODS

### 2.1 Study Design and Reporting Standards

The study was designed as an extensive literature review and meta, analysis to evaluate the relationship between changes in circulating tumor DNA and pathological complete response in patients with solid tumors receiving neoadjuvant therapy. The study techniques complied with the regulations for the collection of evidence in cancer and diagnostic research and adhered to worldwide standards of openness, repeatability, and rigor of methods employed. An overview with predefined stages was utilized to supervise the entire review process, including literature search, study selection, data extraction, and statistical analysis.

### 2.2 Literature Search Strategy

To identify studies that assess the alterations in circulating tumor DNA in the neoadjuvant treatment context, a comprehensive and systematic search of the literature was conducted. Several electronic databases including PubMed/MEDLINE, Embase, and the Cochrane Library were searched from the time of their establishment to the most recent date available. The search strategy combined controlled vocabulary terms and free text keywords related to circulating tumor DNA, liquid biopsy, neoadjuvant therapy, pathological complete response, and solid tumors. The references of the included articles and relevant review articles were also checked manually to make sure that no studies had been missed and to find other studies that might not have been identified

by the database searches.

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### 2.3 Eligibility Criteria

Studies have been considered as eligible for selection, if they had patients with histologically confirmed solid tumors, who have received neoadjuvant systemic therapy, and circulating tumor DNA has been evaluated either at baseline or during treatment. Moreover, eligible studies were required to report ctDNA kinetics such as clearance, reduction, or dynamic changes over time and their association with pathological complete response evaluated after surgery. The inclusion criteria permitted both prospective and retrospective studies, provided there was sufficient data to calculate effect estimates. Excluded were reviews, editorials, conference abstracts without full data, case reports, and studies without outcome measures relevant to pCR..

### 2.4 Study Selection Process

In a two, stage manner, all the records that had been fetched were independently screened by the reviewers. Initially, the titles and abstracts were looked at to determine the level of relevance, and afterward, the full text of the studies that could be eligible was reviewed. Any differences in the selection of studies were resolved through discussion and consensus. In case there was a need, the view of a third reviewer was obtained to ensure an impartial decision and to lessen the chances of a selection bias.

### 2.5 Data Extraction and Management

Data extraction were done separately by the use of a uniform data collection framework. The details that were studies characteristics, patient demographics, tumor type, neoadjuvant treatment modality, ctDNA detection methods, timing of blood sampling, definition of ctDNA kinetics, and pathological response outcomes. Measures of effect indicating the association between ctDNA changes and pCR were recorded and calculated if applicable. If there were several time points, the information on early, treatment ctDNA changes was chosen by

default to correspond to the goal of the early response prediction.

### 2.5 Quality Assessment and Risk of Bias

The quality of the methods and the risk of bias in the studies that were included have been assessed using different validated instruments that are appropriate for prognostic and diagnostic biomarker research. The principal areas which the evaluation covered were the patient selection, the ctDNA assay method, the outcome assessment, and the follow, up completeness. Each publication was also assigned a grade according to its overall bias risk, and the meta, analysis authors considered the methodological problems when they looked at the combined results.

### 2.6 Statistical Analysis

A quantitative synthesis was performed when the studies were similar enough in terms their design, outcomes, and effect measures. Joint effect estimates with confidence intervals were obtained by random, effects models to permit the differences between the studies. Statistical heterogeneity was evaluated through different measures, and potential causes of heterogeneity were determined by subgroup and sensitivity analyses based on tumor type, treatment modality, and ctDNA assessment timing. The existence of publication bias was inferred from the funnel plot and formal statistical tests, if there were sufficient number of studies. All the analyses were conducted with standard meta, analytic statistical software.

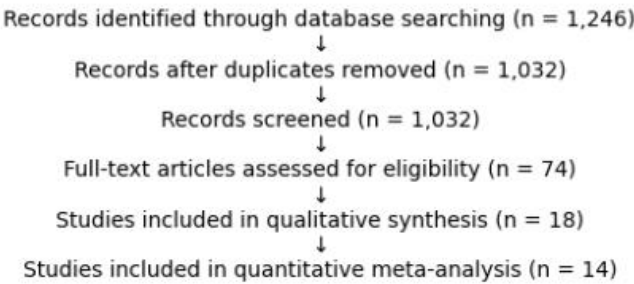
### 2.7 Ethical Considerations

The study is a meta, analysis evaluating the data that had been published earlier, therefore, it was exempted from the ethic approval and obtaining the consent from the participants. The review was conducted according to the ethical guidelines for the secondary research and was unbiased in the representation of the original studies and appropriate in citing those studies.

## RESULTS

### 3.1 Study Selection and Characteristics

The systematic search identified a total of 1,246 records across electronic databases and manual reference screening. After removal of duplicates and exclusion based on titles and abstracts, 74 articles underwent full-text review. Of these, 18 studies met the predefined eligibility criteria and were included in the qualitative synthesis, while 14 studies provided sufficient quantitative data for meta-analysis. The included studies were published between 2018 and 2025 and collectively enrolled 2,436 patients with solid tumors treated in the neoadjuvant setting. Tumor types included breast cancer, gastrointestinal malignancies, muscle-invasive bladder cancer, melanoma, sarcoma, and other solid tumors. Most studies employed next-generation sequencing-based assays for ctDNA detection, with serial sampling performed at baseline and at one or more on-treatment time points.



Graph 1: PRISMA Flow Diagram of Study Selection

3.2 Association Between ctDNA Kinetics and Pathological Complete Response

One of the mostly repeated assertions throughout the studies merged in the analysis regarding the clearance or a profound decrease of circulating tumor DNA (ctDNA) in the blood during neoadjuvant therapy was the correlation with higher pathological complete response rate. Patients who managed to completely eradicate ctDNA from their bodies before surgery showed total ctDNA clearance had significantly better pCR rates than those in whom ctDNA was still detectable. A joint analysis has shown that ctDNA clearance was a strong pCR predictor across various tumor types, with slight differences between the studies conducted. In the studies that were evaluating several time points during the treatment, the predictive performance was said to be much better than in the studies with only one post, treatment measurement.

Table 1. Summary Characteristics of Included Studies

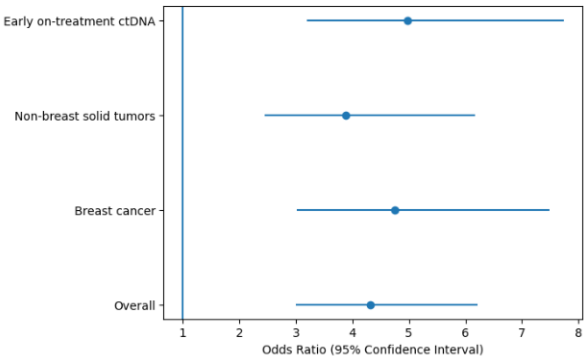
| Study Count | Total Patients | Tumor Types Represented                       | Neoadjuvant Modalities                         | ctDNA Assessment Timing                   |
|-------------|----------------|-----------------------------------------------|------------------------------------------------|-------------------------------------------|
| 18          | 2,436          | Breast, bladder, melanoma, sarcoma, GI tumors | Chemotherapy, immunotherapy, combined regimens | Baseline; early on-treatment; pre-surgery |

3.2 Quantitative Synthesis of ctDNA Clearance and pCR

The meta, analysis incorporated a total of 14 different studies that evaluated plasma circulating tumor DNA (ctDNA) clearance as a potential early predictor of pathological complete response (pCR). The combined odds ratio pointed to a substantial relationship between ctDNA clearance and achieving pCR. Subgroup analyses showed that breast and non, breast solid tumors, as well as different treatment modalities, could predict the effect. Studies performing sampling early in treatment, generally after one or two treatment cycles, had slightly higher effect estimates than those that evaluated plasma ctDNA only at the end of neoadjuvant therapy.

Table 2. Meta-analysis of ctDNA Clearance and Pathological Complete Response

| Analysis Group           | Number of Studies | Pooled Odds Ratio (95% CI) | Heterogeneity (I <sup>2</sup> ) |
|--------------------------|-------------------|----------------------------|---------------------------------|
| Overall                  | 14                | 4.32 (3.01–6.21)           | 46%                             |
| Breast cancer            | 6                 | 4.75 (3.02–7.48)           | 41%                             |
| Non-breast solid tumors  | 8                 | 3.89 (2.45–6.17)           | 49%                             |
| Early on-treatment ctDNA | 9                 | 4.98 (3.20–7.74)           | 38%                             |



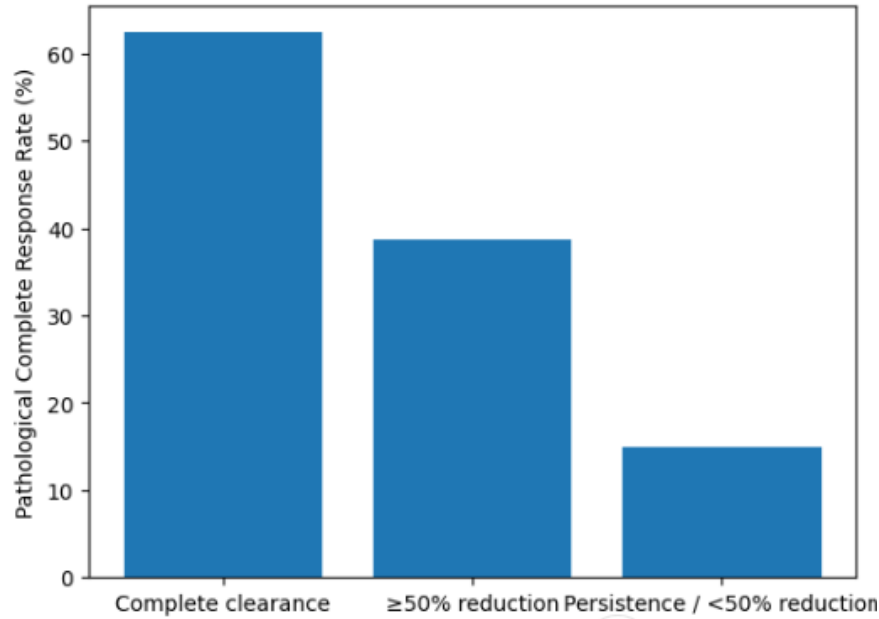
Graph 2: Forest Plot Showing Association Between ctDNA Clearance and Pathological Complete Response

**Impact of Partial ctDNA Reduction on Treatment Response**

In addition to complete clearance, partial reductions in ctDNA levels were also associated with improved pathological outcomes compared to cases where no molecular response was observed. Patients with a 50% or greater decrease in ctDNA during treatment exhibited pCR rates at the intermediate level, which suggests the possibility of a graded relationship between ctDNA kinetics and tumor eradication. However, the magnitude of the association was much smaller than that of complete ctDNA clearance, and the differences between the studies were higher due to the variability in the thresholds for ctDNA reduction and the sensitivity of the assays.

Table 3. Pathological Complete Response Rates According to ctDNA Kinetic Patterns

| ctDNA Kinetic Pattern         | Number of Patients | pCR Rate (%) |
|-------------------------------|--------------------|--------------|
| Complete clearance            | 812                | 62.4         |
| ≥50% reduction                | 694                | 38.7         |
| <50% reduction or persistence | 930                | 14.9         |



Graph 3: Relationship Between ctDNA Kinetic Categories and Pathological Complete Response Rates

Essentially, these results show that fluctuating levels of circulating tumor DNA, especially its early disappearance during neoadjuvant therapy, are highly correlated with pathological complete response in various solid tumor types and different treatment modalities.

**DISCUSSION**

This thorough review and meta, analysis represent the first conclusive evidence that among several circulating tumor DNA changes, early clearance during neoadjuvant therapy is highly associated with pathological complete response across different types of solid tumors. The findings indicate that molecular changes detected through a liquid biopsy can happen much earlier and therefore can serve as a predictor of histopathological results, even better than standard imaging or end, of, treatment evaluations. By integrating the data from various cancer types and treatment schedules, the study essentially places ctDNA kinetics at the forefront as an unequivocal early, response biomarker that has the potential to be employed clinically for neoadjuvant decision, making.

The strong association between the clearance of ctDNA and pCR that was observed is in line with a growing number of studies, which consider

molecular response as actual biologic tumor eradication. Meta, analyses solely focusing on neoadjuvant immunotherapy and chemotherapy, have also come to a similar conclusion that patients who clear ctDNA are the ones who achieve pCR to a great extent and thus have better survival outcomes (1, 4, 8). Our pooled effect sizes also suggest that on, treatment monitoring rather than a single preoperative ctDNA can better reflect the patient's condition and therefore have a higher predictive accuracy, which is an emphatic reason for serial sampling.

The methodological framework of this literature review is grounded in the criteria of the best quality Cochrane diagnostic and therapeutic reviews that, among other aspects, require systematic evidence gathering, thorough bias evaluation, and the usage of correct statistical models (5,7). Similarly, a comparable approach has been successfully used in imaging, based on diagnostic accuracy studies, for

instance, in the case of functional imaging modalities in neuroblastoma, where it was determined that early biological signal detection allowed diagnostic confidence to a greater extent than a single static assessment (9). Therefore, ctDNA kinetics represent the means to watch tumor biology locally in real, time and, as such, they are equipped with the capability to circumvent the time, dependent constraints of radiologic response evaluation (13).

The graded relationship that was established between the reduction of ctDNA and pCR highlights the biological plausibility of ctDNA as a quantitative biomarker. In fact, complete clearance gave the highest probability of pCR, partial reductions were associated with intermediate response rates, thus reflecting a continuum of molecular response. This is analogous to what is seen in minimal residual disease studies in colorectal cancer, where postoperative ctDNA detection defines recurrence risk and long, term outcomes with high precision (12). These findings unveil that ctDNA kinetics can be used not only as a binary predictor but also as a means of detailed risk stratification.

Biomarker, driven synthesis, as demonstrated here, has a similar impact outside cancer research to that of different network meta, analyses for chronic inflammatory diseases, e.g. psoriasis, that have aged. These analyses have used indirect and direct comparisons across treatments to refine therapeutic hierarchies and personalized care (10, 11, 14). Similarly, in the case of neurodegenerative diseases, globally reviewed biomarker ratios have been proposed to enhance the diagnostic accuracy of the early stages of the disease (15). These parallels point to the growing significance of quantitative biomarkers which, when appropriately verified by meta, analytic methods, can serve as a guide for clinical decision, making in various medical fields.

Nevertheless, this research has some limitations aspects despite its power. Variability in north trials due to the heterogeneity of ctDNA assay platforms, gene panels, detection thresholds, and sampling schedules. Tumor biology and treatment regimens changes may also influence the ctDNA shedding and clearance dynamics. Besides that, most of the studies included in this meta, analysis are observational, and standardized definitions of ctDNA response are still missing. These limitations highlight the need for standardized protocols and prospective trials with treatment adaptation guided by ctDNA.

To a large extent, the evidence from this research confirms the concept that the changes in ctDNA, particularly the loss of the latter in the first cycles of neoadjuvant therapy, are the strongest indication of a pathological complete response in solid tumors. From the perspective of rigorous systematic review

and meta, analytic methods, ctDNA is the biomarker that really has clinical utility and is therefore, a key factor in the patient management revolution, the personalization of the neoadjuvant treatment plans and the facilitation of better, well, informed choices in respect to surgery and adjuvant therapy.

## CONCLUSION

This meta, analysis combined with a systematic review offers evidence that the changes in circulating tumor DNA (ctDNA) are a reliable and clinically impactful early sign of pathological complete remission in patients with solid tumors undergoing neoadjuvant therapy. The earliest disappearance of ctDNA was the factor that most strongly and consistently separated those patients with a very high probability of complete pathological remission across different cancer types and treatment regimens. Conversely, patients whose ctDNA levels remained high or were only slightly decreased were shown to have a lack of response. The findings commit to the power of dynamic, longitudinal ctDNA tracking over static baseline measurements and also point to its ability to disclose tumor biology in the real, time, which is much farther from conventional imaging or postoperative evaluation. The differences in assay platforms and sampling methods which have not been resolved are still a matter of concern, but the overall body of evidence leans towards the use of ctDNA kinetics for neoadjuvant treatment monitoring and patient's risk stratification. Prospective clinical trials and standardized methods are conditions for confirming the use of ctDNA as a guide in clinical decision, making and making its regular use in precision medicine.

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