



CASE REPORT

**LIQUID BIOPSY APPROACHES TO CAPTURE TUMOUR
EVOLUTION AND CLINICAL OUTCOMES DURING
CANCER IMMUNOTHERAPY**

Liquid Biopsy Approaches to Capture Tumour Evolution and Clinical Outcomes During Cancer Immunotherapy

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Abstract

A new biomarker called ctDNA is present in blood samples every time there's a tumor or metastasis. By measuring the ctDNA from circulation, we can identify mutations caused by treatment of the primary tumors via chemotherapy or immunotherapy (immunotherapeutic ligands). With this information, we can determine how the malignant cells respond to the immunotherapy treatment compared to their counterparts which do not express these mutations. Thus, we can determine how to improve or develop new therapies. Furthermore, we can use ctDNA as a rapid and accurate method to measure the degree of change and progress of a tumor from one treatment to the next. However, there remains a lack of unique information from a single ctDNA measurement that identifies targeted anti-tumour immune response mechanisms. Therefore, it is necessary to utilise more complex assays that model tumour-immune interaction to study the kinetics associated with effective antitumor immune responses and the tumor cell reversal process. To date, ctDNA testing has proven to be beneficial in clinical settings where ctDNA has been used for certain genomic biomarkers (e.g., tumor mutation burden, microsatellite instability) to aid in predicting patient outcomes when treated with immune checkpoint inhibitors and allow for real-time assessments (measurement) of tumor burden changes. Therefore, the development of ctDNA assays for use in clinical practice is becoming increasingly significant in selecting patients for cancer immunotherapy and determining the success of immunotherapies.

Introduction

Immunotherapy has proven itself to be a milestone in the treatment of various solid tumours using the body's own immunity to combat malignant diseases.[1,]

Development of immune checkpoint inhibitors (ICIs) such as anti-PD-1, anti-PD-L1, and anti-CTLA-4 treatments has brought about significant progress in increasing the longevity of patients suffering from various types of cancer. Yet only certain individuals receive benefits from immunotherapies, and there is an urgent need to develop reliable

biomarkers that will help select the appropriate therapy and evaluate its efficacy in individual cases. Tissue biopsy was considered the most effective method for identifying biomarkers; yet, this technique does not allow tracking changes in tumours over time, is highly invasive and biased. Liquid biopsy, especially ctDNA analysis, provides an innovative opportunity to track tumour activity in a non-invasive way. Repeated sampling allows for evaluating a patient's condition throughout the course of cancer and determining the level of tumour load and treatment response.



Insert Figure 1 here: Approaches for the Detection and Analysis of ctDNA in Immuno-Oncology. **Adopted from:** Sivapalan, L., Murray, J. C., Canzoniero, J. V., Landon, B., Jackson, J., Scott, S., Lam, V., Levy, B. P., Sausen, M., & Anagnostou, V. (2023). Liquid biopsy approaches to capture tumor evolution and clinical outcomes during cancer immunotherapy. *Journal for Immunotherapy of Cancer*, 11(1), e005924. <https://doi.org/10.1136/jitc-2022-005924>

Biology and Detection of Circulating Tumour DNA

CtDNA is the portion of cell-free DNA that comes from tumours through apoptotic, necrotic, and secretory mechanisms. The quantity of ctDNA released in the blood circulation often reflects the size and development stage of tumours, therefore

making it an effective biomarker for the detection of cancer. Recent technological advancements in molecular diagnostics have made it possible to detect ctDNA at high sensitivity and specificity. Some of the existing techniques of ctDNA detection

include droplet digital PCR (ddPCR), targeted NGS, and whole-genome sequencing. Using such technologies allows the detection of various genetic mutations including point mutations, CNVs, rearrangements, methylations, and fragmentations. In addition, tumour-informed and tumour-agnostic methods can be used for ctDNA analysis, [2], depending on whether the tumour-specific

Tracking Tumour Evolution During Immunotherapy

Evolution of tumours can be described because of genetic instability and selection due to treatments. In immunotherapy, there will always be ongoing evolution of cancer cells because they continuously adapt to the attacks from the immune system, thereby altering their clonal structure. ctDNA longitudinal analysis gives a chance to track such evolutionary changes. Several studies found that the [3]

ctDNA-Based Biomarkers for Patient Selection

One of the most significant uses of liquid biopsy in immuno-oncology involves predicting biomarkers, which help in selecting a population suitable for immunotherapy. In that regard, blood tumor mutational burden (bTMB) is an excellent biomarker since a mutation leads to neoantigen production, which can induce immunity reactions against tumors. As opposed to TMB, bTMB

measures mutations from several metastatic sites and hence reflects tumor heterogeneity. The POPLAR, OAK, and MYSTIC clinical trials have shown

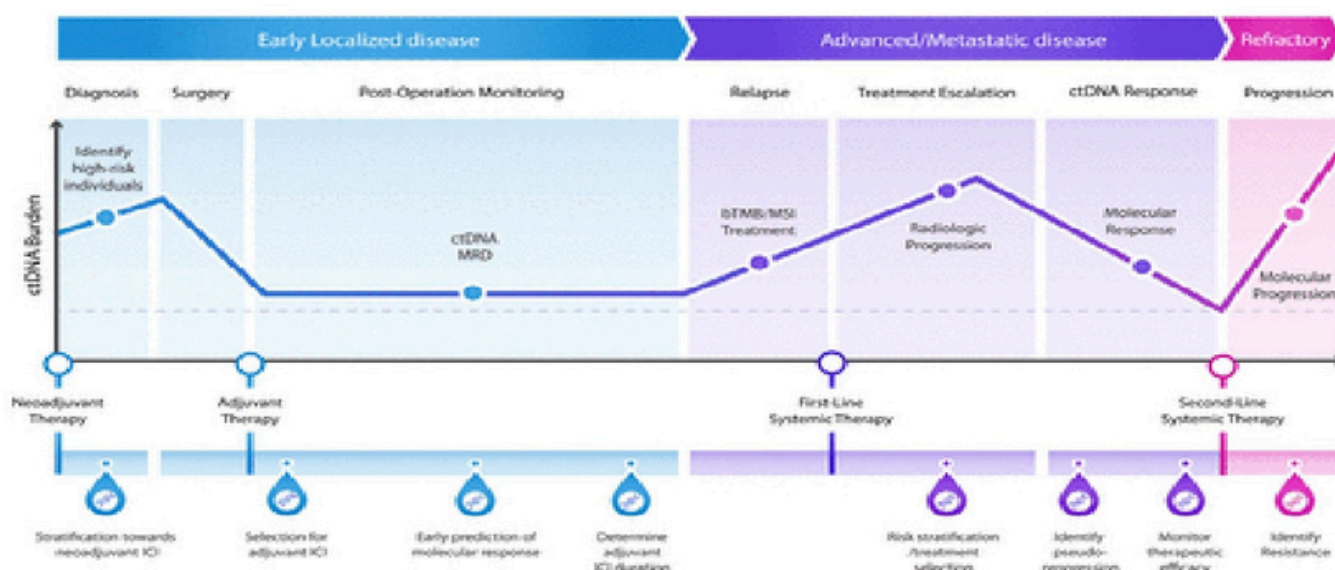
mutations are known or not. While both approaches provide comprehensive molecular data, tumour-informed methods rely on already discovered genetic mutations in a patient's tumour to achieve higher sensitivity. The possibility of repeated sampling of ctDNA through blood tests makes liquid biopsy revolutionary in precision oncology.

Analysis of ctDNA can show how tumour clonality changes over time and allow detecting new resistance mutations. At the same time, this type of analysis can also explain why some therapies fail. Additionally, information about neoantigen evolution and immune evasion of cancer cells can be useful because those factors determine the effectiveness of immunotherapies. With the integration of ctDNA profiling results and the information obtained with TCR sequencing, one can achieve a more detailed picture of interactions between the tumor cells and the immune system.

correlation between bTMB and positive patient outcomes after immune checkpoint inhibition. Nonetheless, the use of bTMB for prediction purposes faces several challenges, including differences in testing techniques and thresholds.

Another biomarker whose relevance cannot be overlooked is blood-based microsatellite instability (MSI). The phenomenon occurs when there are defects within the DNA mismatch repair pathway, which can enhance response rates to immunotherapy. With the advancements in sequencing technologies, blood-based detection of MSI is now possible using ctDNA tests. Recent research shows that blood-based detection of MSI is highly correlated with conventional assessment techniques. Consequently, the method can be applied

in predicting patients' eligibility for immunotherapy and can be used when there are insufficient tissue specimens.



Insert Figure 2 here: Potential Applications of ctDNA to Guide Clinical Decision-Making During Immunotherapy . **Adopted from:** Sivapalan, L., Murray, J. C., Canzoniero, J. V., Landon, B., Jackson, J., Scott, S., Lam, V., Levy, B. P., Sausen, M., & Anagnostou, V. (2023). Liquid biopsy approaches to capture tumor evolution and clinical outcomes during cancer immunotherapy. *Journal for Immunotherapy of Cancer*, 11(1), e005924. <https://doi.org/10.1136/jitc-2022-005924>

Minimal Residual Disease Detection and Adjuvant Immunotherapy

MRD stands for the few residual cancer cells in the patient's body following curative treatment which leads to disease recurrence. One of the clinically relevant applications of ctDNA testing is the detection of MRD. Detection of ctDNA post-surgery has always been associated with a high risk of recurrence. The use of ctDNA test for MRD in immunotherapy has provided means through which patients who need additional therapy can be identified while sparing others from toxicity. Various clinical trials such as CheckMate-816, have indicated a strong correlation between ctDNA clearance in the immunotherapy setting and pathologic complete response.[4]

On the other hand, the clinical trial IMvigor010 showed that those patients who had ctDNA after surgery during adjuvant atezolizumab therapy gained some advantage as opposed to those patients whose ctDNA was not detectable. Such trials have confirmed that ctDNA is an essential biomarker for risk stratification as well as therapy decision-making. This is evident from the results of the DYNAMIC trial, which have demonstrated the avoidance of unnecessary treatment based on ctDNA tests. In line with the continued gathering of evidence, ctDNA-based MRD testing will become an integral part of personalised medicine in the future.

Monitoring Treatment Response and Disease Progression

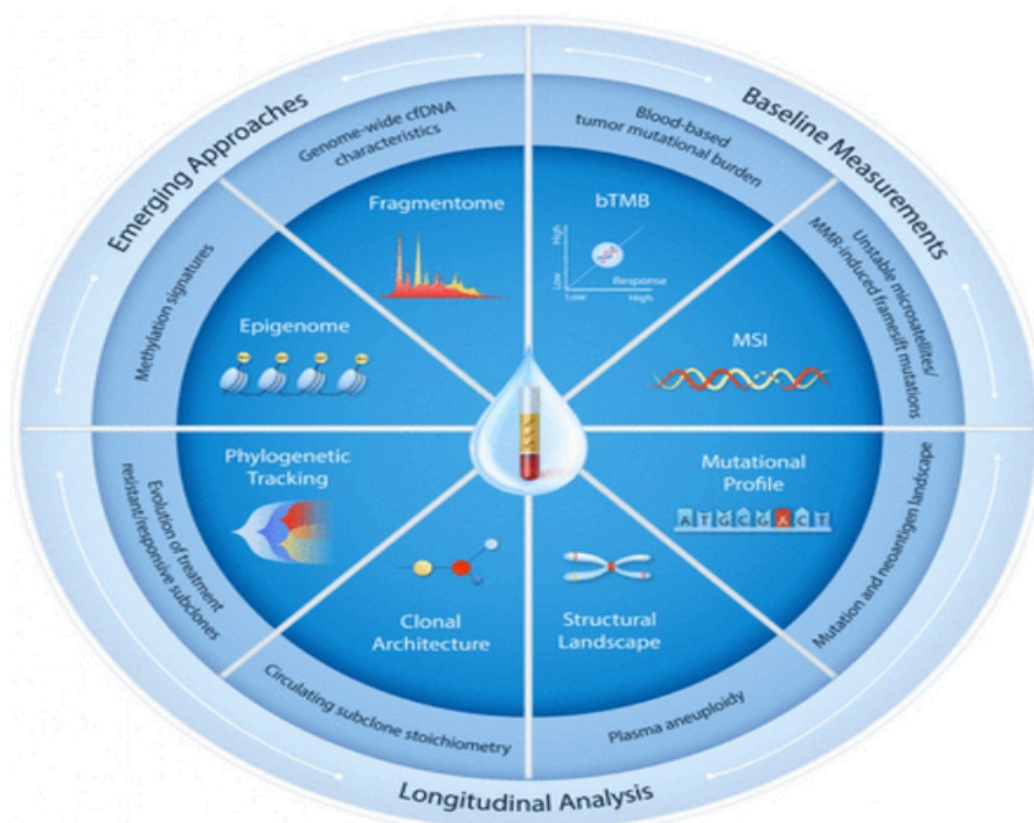
The evaluation of the treatment's response is still problematic in cancer immunotherapy. Radiography cannot always distinguish between actual progressive disease and pseudo-progression – a condition when tumors first become bigger because of immune cells' infiltration and then start shrinking. ctDNA testing appears to be much more sensitive and dynamic way of determining the effectiveness of the therapy.

ctDNA-Guided Clinical Trials and Future Perspectives

Increasingly relevant ctDNA has given rise to many interventional trials involving the use of a liquid biopsy in informing therapy. Current ongoing studies include MERMAID-1, MERMAID-2, TOMBOLA, PERSEVERE, and ATLAS, which are exploring whether a ctDNA-based intervention can benefit patients. Such trials involve MRD-based adjuvant therapy, treatment escalation by assessing molecular response, and immunotherapy that adapts depending on ctDNA status.

There is no doubt that the reduction of the ctDNA levels observed during the therapy is a predictor of good results, while the maintenance or growth of these molecules relates to disease progression and treatment resistance. What is crucial, ctDNA dynamics sometimes precede the disease's progression observed radiographically by a few months. It allows adjusting treatment early. The absence of ctDNA is one of the best indicators of the positive effect and long-term survival. In addition, ctDNA helps distinguish between pseudo-progression and actual progression and prevents stopping effective treatment.

In addition to genetic mutations, future advancements in liquid biopsy technology are likely to consider other parameters. For instance, new technologies can include DNA methylation, fragmentation patterns, chromatin accessibility, and other epigenetic characteristics. Incorporating such data together with the existing immune biomarkers along with artificial intelligence can greatly advance the value of liquid biopsies in predicting disease behaviour and thus informing precision therapy.



Insert Figure 3 here: Leveraging the Hallmarks of cfDNA to Expand Current and Future Uses in Immuno-Oncology Adopted From: Sivapalan, L., Murray, J. C., Canzoniero, J. V., Landon, B., Jackson, J., Scott, S., Lam, V., Levy, B. P., Sausen, M., & Anagnostou, V. (2023). Liquid biopsy approaches to capture tumor evolution and clinical outcomes during cancer immunotherapy. *Journal for Immunotherapy of Cancer*, 11(1), e005924.

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Conclusion

The liquid biopsy approach has shown promise and proven itself to be a valuable modality in the field of cancer immunotherapy. By using the ctDNA testing technique, medical professionals can learn more about tumour progression through a relatively non-invasive procedure. Liquid biopsy has also shown promising results when used in assessing

therapeutic response and detecting minimal residual disease. Despite the issues that exist, ongoing research and clinical trials prove the value of using this approach in oncology medicine. The application of this technique is bound to become increasingly important for enhancing patient outcomes from therapy and ultimately prolonging life expectancy

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