ACE BRIEF FOR NEW AND EMERGING HEALTH TECHNOLOGIES

The Signatera Test for Patients Previously Diagnosed with Cancer

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Summary of Key Points

- In the context of solid tumours, molecular residual disease (MRD) refers to the small number of isolated or circulating tumour cells in patients following a curative treatment which may lead to further regional or distant recurrence.
- Currently, surveillance for cancer recurrence includes physical, clinical and radiological examinations, which is limited by its detection accuracy and is resource intensive.
- The Signatera test (Natera, Inc.) is a personalised and tumour-informed liquid biopsy assay that identifies circulating tumour DNA (ctDNA) for the detection of MRD to monitor treatment response and early disease recurrence.
- Based on the local disease burden and available evidence, this brief focused on patients with breast and colorectal cancer (CRC).
- There was no major safety concern expected related to the test.
- In terms of accuracy, ctDNA showed moderate to high sensitivity in detecting CRC relapse (42% to 91.4%) across multiple studies while limited data showed good sensitivity in detecting breast cancer relapse (89%). A high overall specificity (93% to 100%) was reported. There is some evidence showing that the test may lead to earlier detection of CRC or breast cancer recurrence by a mean or median interval of up to 8.9 months.
- However, ctDNA was found to have a lower sensitivity compared with guideline-recommended CRC surveillance (imaging with carcinoembryonic antigen level; 53.3% *vs.* 73.3%) and similar lead time of relapse detection (median, 14.3 *vs.* 15 months).
- The evidence further demonstrated association of ctDNA with disease recurrence.
    - In patients with resected CRC before adjuvant chemotherapy, ctDNA positivity was associated with increased risk of disease recurrence and poorer survival outcomes.
    - In patients with CRC or breast cancer, longitudinal ctDNA surveillance showed that inadequate ctDNA clearance upon chemotherapy treatment correlated with disease relapse while ctDNA positivity after definitive treatment was significantly associated with higher rate of relapse and 15 to 50.8-fold increased risk of disease recurrence.
- The clinical utility of ctDNA outcomes in terms of patient management and treatment outcomes remains unclear. Further intervention trials would be required to show that MRD detection is an actionable finding, with ongoing studies expected to be completed largely beyond the next two to three years. Potential healthcare system benefits include resource use optimisation based on risk stratification. However, the results were limited by the modest sample size and weak evidence base for breast cancer.
- No studies reported on cost-effectiveness of the Signatera test.
- The Signatera test was estimated to cost US$1,750 to profile five plasma samples.
- Key implementation considerations include assay turnaround time, staff training, infrastructure concerns and collaboration between primary and tertiary care providers.
- Various ongoing developments of MRD tests for solid tumours were identified.

I. Background

Cancer is a group of diseases characterised by uncontrolled cell growth, which may lead to the formation of malignant tumours.[1] Following curative treatment for the primary tumour, patients may harbour a small number of isolated or circulating tumour cells known as minimal residual disease in the context of haematological tumours.[2] In solid tumours, minimal residual disease is also referred to as molecular residual disease (MRD) as it can be identified with rapid advancements in diagnostic technologies.[2] The presence of MRD may indicate ineffective treatment or the occurrence of treatment resistance, which may lead to further regional or distant recurrence. In addition, these residual tumour cells do not show clinical signs of cancer and cannot be detected with conventional diagnostic tools.[2] As such, postoperative MRD detection may provide substantial clinical value in oncology practice.

In Singapore, cancer accounted for one of the leading causes of disability adjusted life years (DALYs), responsible for 13.3% of the total DALYs in 2017.[3] From 2015 to 2019, there were 78,204 patients diagnosed with cancer with an age-standardised incidence rate of 235 per 100,000 population.[4] Of which, colorectal and breast cancer were the most commonly diagnosed cancers with leading cancer mortality in males and females respectively.[4] Despite curative treatments, patient may experience relapse. The recurrence rate following curative treatments depends on the cancer type and stage, with a rate of 30% in patients with breast cancer[5] and 30% to 50% in patients with colorectal cancer (CRC).[6]

Current surveillance strategies to detect disease recurrence, including routine postoperative physical, haematological and radiological examinations, have its limitations.[7] These include varying patterns of recurrence and tumour heterogeneity that can affect detection accuracy, while being onerous to patients and requiring intensive medical resources.[7] Together, these indicates a clinical unmet need for a simpler, less resource intensive, accurate and early diagnosis of disease recurrence in cancer survivors.

II. Technology

Signatera (Natera, Inc.) is a personalised, tumour-informed, liquid biopsy assay that identify circulating tumour DNA (ctDNA) for the detection of MRD, monitoring of treatment response and early disease recurrence. It involves a whole exome sequencing of the primary tumour tissue and matched normal blood of individual patients with next-generation sequencing (NGS) technology (Figure 1). Based on the sequencing results, the top 16 somatic single nucleotide and indel variants are selected to design a bespoke assay specific to each patient's tumour mutation signature. Specifically, multiplex polymerase chain reaction (PCR) primers are designed for each variant to create a 16-plex personalised assay. At predefined timepoints during longitudinal surveillance of patients, whole blood is collected followed by plasma isolation, ctDNA extraction and amplification with the patient specific 16-plex PCR. NGS will be conducted and analysed to detect for the presence or absence of ctDNA, which can be used to inform on cancer recurrence.

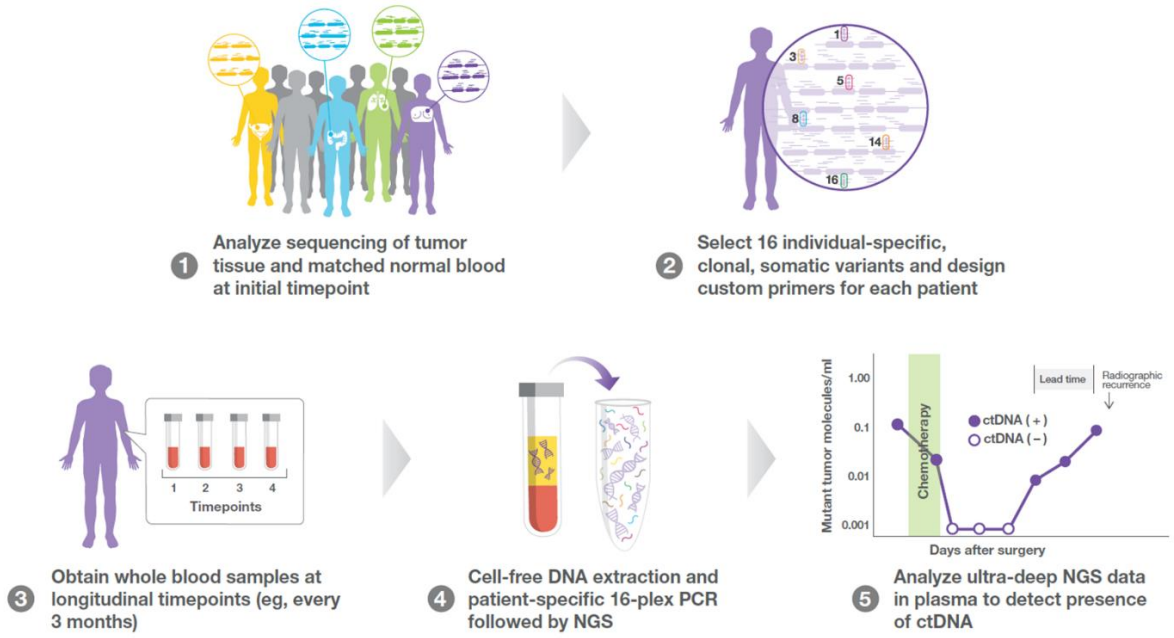

Figure 1: Workflow of the Signatera assay. Image adapted from Signatera's whitepaper, available from https://www.natera.com/resource-library/oncology/seeing-beyond-the-limit-detect-residual-disease-and-assess-treatment-response

Using ctDNA as a biomarker, the Signatera test can detect for MRD at the molecular level and predict disease recurrence. The non-invasive and dynamic nature of ctDNA may also provide a real-time indicator of treatment effectiveness, while guiding and monitoring treatment response.[8] However, the test may be limited by potential subjectivity of tumour variant selection in the custom gene panel, changes in tumour makeup over time, challenges in obtaining a tumour biopsy for initial sequencing in certain cancer types, as well as tumour heterogeneity where the initial site-specific tissue sample obtained may not be representative of all genetic alterations present in the tumour.[9]

III. Regulatory and Subsidy Status

The Signatera test was granted a total of three Breakthrough Device Designations by the US Food and Drug Administration (FDA) as a companion diagnostic to different cancer therapies, including one designation for early-stage breast cancer.[10] It is available as a laboratory developed test (LDT) in the United States and is covered by Medicare for patients with stage II or III CRC or those on immunotherapy. The test also received the CE mark in August 2020.

IV. Stage of Development in Singapore

The Signatera test has not been approved by the Health Sciences Authority and was locally investigated in a clinical study conducted at the National Cancer Centre Singapore.[11] It is also locally available through a private provider.

☐	Yet to emerge	☐	Established
☒	Investigational / Experimental (subject of clinical trials or deviate	☐	Established *but* modification in indication or technique

from standard practice and not routinely used)

☐ Nearly established

☐ Established *but* should consider for reassessment (due to perceived no/low value)

V. Treatment Pathway

The surveillance for recurrent or secondary cancer is an integral part of survivorship care, with multiple modalities used including medical history, physical examinations, tumour biomarkers, endoscopic visualisation and radiographic imaging.[12] Table 1 summarised the high-level recommendations for cancer surveillance in patients with standard risk of relapse following curative treatments for the common cancers in the US as reported by the American Society of Clinical Oncology (ASCO) Post.[12,13]

Table 1: High-level recommendations on tests for cancer recurrence in patients at standard risk of relapse

Tumour site	Tests for cancer recurrence
Breast	Annual mammography
Prostate	Prostate-specific antigen, digital rectal exam
Lung	Chest CT
Colon/rectum	Annual chest/abdominal/pelvic CT for 3 to 5 years; CEA every 3 to 6 months up to 5 years; colonoscopy every 5 years; rectosigmoidoscopy every 6 to 12 months for 3 to 5 years
Bladder	Chest/abdominal/pelvic CT, urine cytology, liver function test, creatine clearance up to 2 years
Thyroid	Biomarkers, ultrasound (subtypes)
Melanoma	Chest X-ray/CT, brain MRI with or without PET/CT for 3 years in patients with stage IIB to IV disease
Abbreviations: CEA, carcinoembryonic antigen; CT, computed tomography; MRI, magnetic resonance imaging; PET, positron emission tomography. Table adapted from The American Society of Clinical Oncology (ASCO) Post.[13]	

The introduction of the Signatera test may disrupt existing clinical pathways, leading to a switch in current surveillance strategies to a blood-based test to detect ctDNA biomarkers for MRD. At the same time, it is also possible that ctDNA testing may complement the current tests used for cancer surveillance.

VI. Summary of Evidence

Based on the local disease burden and number of available evidence identified from PubMed and Embase, this brief focused on the use of the Signatera test for patients with CRC and breast cancer.

The assessment was conducted based on the Population, Intervention, Comparison and Outcome (PICO) criteria presented in Table 2, where a total of six studies[14-19] were included. Although MRD was generally defined as residual disease following curative treatment, one included study[18] investigated the role of the Signatera test prior to surgical resection. In addition, two studies[20,21] based on the INSPIRE trial across multiple cancer types, including head and neck, breast, ovarian, melanoma and mixed solid tumours, served as supporting evidence. The evidence base was listed in Table A1 (Appendix A) while the study design and characteristics of the included studies were summarised in Table A2 (Appendix A).

Table 2: Summary of PICO criteria

Population	Patients with breast or colorectal cancer
Intervention	Signatera
Comparison	Standard-of-care assessment for cancer recurrence, if available
Outcome	Safety, clinical and cost effectiveness

Safety

The studies included did not report on safety of the Signatera test. As the test involves phlebotomy which is routinely performed in clinical practice, there were no major safety concerns expected. In addition, the use of the Signatera test may lead to the avoidance of radiation exposure from radiological surveillance of cancer recurrence.

Effectiveness

Predictive accuracy

Five studies[14-17,19] reported on the predictive accuracy of the Signatera test in detecting cancer recurrence in patients with resected CRC or breast cancer. For patients with CRC, multiple studies[15-17,19] reported a moderate to high sensitivity of relapse detection across single timepoint assessment and longitudinal ctDNA surveillance (range, 42% to 91.4%) while a high sensitivity of 89% was reported in one study[14] (n=49) for patients with breast cancer (Table 3). In both CRC and breast cancer, high specificity of ctDNA in detecting relapse was reported (range, 93.3% to 100%).[14,15,17,19]

In comparison with carcinoembryonic antigen (CEA), which is a prognostic biomarker of CRC relapse, ctDNA demonstrated better predictive accuracy (Table 3).[15,17,19] However, the sensitivity of ctDNA in predicting CRC relapse appeared to be worse than the combination of imaging and CEA according to the National Comprehensive Cancer Network (NCCN) guidelines (53.3% *vs.* 73.3%; Table 3).[15] Moreover, due to the limited evidence, the high sensitivity of ctDNA in predicting breast cancer relapse requires further validation.

Table 3: Predictive accuracy of ctDNA and other modalities on cancer recurrence

Study	Cancer type	N	Biomarker or imaging modalities to predict disease recurrence					
			ctDNA		CEA		Imaging + CEA*	
			Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Single timepoint assessment (postoperative)								
Loupakis et al. (2021)[17]	Colorectal	112	72%	93.3%	—	—	—	—
		55†	84.6%	—	46%	—	—	—
Henriksen et al. (2022)[16]		140	42%	—	—	—	—	—
Longitudinal surveillance								
Loupakis et al. (2021)[17]	Colorectal	50	91.4%	93.3%	—	—	—	—
Reinert et al. (2019)[19]		75	88%	98%	69%	64%	—	—
Henriksen et al. (2022)[16]		114	88%	—	—	—	—	—
Fakih et al. (2022)[15]		48	53.3%	100%	20%	90.9%	73.3%	87.9%

Coombes et al. (2019)[14]	Breast	49	89%	100%	—	—	—	—

* NCCN guideline recommendation for identifying CRC relapse. † Subset of patients with both ctDNA and CEA results available postoperatively.

Note: Recurrence was based on radiological or clinical relapse.

Abbreviations: CEA, carcinoembryonic antigen; ctDNA, circulating tumour DNA.

Association of ctDNA with disease recurrence

Across six studies[14-19], ctDNA positivity was found to be associated with increased risk of disease recurrence in patients with CRC and breast cancer. However, the translation of these findings into clinical utility in terms of patient management and treatment outcomes requires further investigation.

Predicting risk of recurrence before adjuvant therapy

In patients with resected CRC, three studies[16,17,19] demonstrated the role of the Signatera test in predicting the risk of disease recurrence based on postoperative ctDNA levels before adjuvant chemotherapy (ACT). Patients who were ctDNA-positive had a higher recurrence rate of 70% to 80% in contrast to 11.9% to 18% in ctDNA-negative patients (Table B1 in Appendix B).[16,19] Further, the presence of ctDNA was associated with a 16-fold and 7-fold increased risk of death and disease recurrence, respectively, compared with ctDNA-negative patients (Table 4).[16,19] This corroborated the hazard ratio (HR) for recurrence-free survival (RFS) in CRC reported in a meta-analysis of various MRD assays, including Signatera (HR, 7.9; 95% CI, 4.49 to 13.91).[22] Of note, postoperative ctDNA but not CEA, was found to be significantly correlated with disease progression (Table B2 in Appendix B).[17] To further add, pooled data across multiple cancer types substantiated these findings, where lower ctDNA levels before treatment with pembrolizumab was generally associated with better overall survival (OS) and progression-free survival (PFS; Figure B1 in Appendix B).[20] Of note, subcohort analysis of patients with breast cancer showed no association of ctDNA levels with improved survival outcomes before administration of pembrolizumab, although this remained inconclusive due to the small sample size (n=18, see cohort B in Figure B1 in Appendix B).[20]

Table 4: Association of postoperative ctDNA levels prior to adjuvant chemotherapy with clinical outcomes

Study	Cancer type	N	Timepoint	Comparison	Endpoint	Outcome	p-value
Reinert et al. (2019)[19]	Colorectal	94	Post-operative before ACT	ctDNA-positive *vs.* negative	RFS, HR (95% CI)	7.2 (2.7 to 19.0)	<0.001
Loupakis et al. (2021)[17]		112			DFS, HR (95% CI)	5.8 (3.5 to 9.7)	<0.001
					OS, HR (95% CI)	16.0 (3.9 to 68.0)	<0.001
Henriksen et al. (2022)[16]		140			RFS, HR (95% CI)	7.0 (3.7 to 13.5)	<0.001

Abbreviations: ACT, adjuvant chemotherapy; ctDNA, circulating tumour DNA, DFS, disease-free survival; HR, hazard ratio; OS, overall survival; PFS, progression-free survival; RFS, recurrence-free survival.

Together, these findings indicate the potential ability of ctDNA to predict and stratify patient's risk of recurrence and survival outcomes before treatment initiation. Further investigation would be required to support its potential clinical utility in sparing low-risk ctDNA-negative

patients from adjuvant therapy and its toxic side effects while intensifying treatment regimens for high-risk ctDNA-positive patients.

Longitudinal ctDNA monitoring to assess treatment effectiveness

In addition, longitudinal ctDNA assessment could serve as a surrogate measure of treatment efficacy, where clearance of ctDNA could suggest treatment response. Briefly, across patients with high risk early stage breast cancer and resected CRC upon treatment with neoadjuvant chemotherapy (NAC) or ACT respectively, ctDNA clearance was correlated with reduced or no disease relapse while patients with ctDNA experienced relapse or had an increased risk of relapse (see Figures B2 to B4 in Appendix B).[16,18,19] Likewise, pooled data across multiple cancer types during treatment with pembrolizumab showed the ability of ctDNA to assess treatment effectiveness, where ctDNA kinetics was associated with survival outcomes and complemented changes in tumour burden and lesion measurement to stratify patients based on their treatment response (see Figures B5 to B9 in Appendix B).[20,21] Notably, ctDNA kinetics was not associated with improved survival outcomes in the subcohort of patients with breast cancer, although the results were equivocal due to the limited sample size (see cohort B in Figure B5 in Appendix B).[20]

Overall, these findings point to the potential value of ctDNA in assessing treatment response at the molecular level, where incomplete MRD elimination could lead to disease recurrence. The test may be potentially useful to identify non-responders who may benefit from early switch to more effective therapies while potentially de-escalating treatment in early responders to avoid side effects, although further evidence is required.

Longitudinal ctDNA monitoring to detect risk of relapse

There are some evidence showing that, following treatment, longitudinal ctDNA surveillance may detect MRD to identify risk of relapse earlier than standard surveillance techniques. Across colorectal and breast cancers after definitive treatment, the Signatera test anticipated disease relapse earlier than clinical or radiological diagnoses by an average or median interval of up to 8.9 months (Table 5).[14,16,19] Notably, findings by Fakih et al. (2022)[15] reported no significant difference between ctDNA and imaging±CEA in the lead time for detecting CRC relapse. This was postulated to be due to inadequate radiographic surveillance in the study by Reinert et al. (2019)[19] which may have led to a bias towards the superiority of ctDNA over imaging.[15]

Table 5: Lead time of longitudinal ctDNA surveillance over standard methods after definitive treatment

Study	Cancer type	Comparison	Lead time, months	p-value
Reinert et al. (2019)[19]	Colorectal	ctDNA *vs.* CT scan	Mean, 8.7 (range, 0.8 to 16.5)	<0.001
Henriksen et al. (2022)[16]			Median, 6 (IQR, 2 to 9)	NR
Fakih et al. (2022)[15]		ctDNA *vs.* imaging*	Median, 0.7	0.45
		ctDNA *vs.* imaging* + CEA	Median, 0.7	0.79
Coombes et al. (2019)[14]	Breast	ctDNA *vs.* SOC assessments†	Median, 8.9 (range, 0.5 to 24)	NR

* Imaging-detected recurrence includes metastatic lesion detected by CT scan or MRI. † SOC assessment for breast cancer refers to clinical and biochemical measurements, including CA 15-3.

Abbreviations: CEA, carcinoembryonic antigen; CT: computed tomography; ctDNA, circulating tumour DNA; IQR, interquartile range; MRI, magnetic resonance imaging; NR, not reported; SOC, standard-of-care.

Although the ability of ctDNA to detect CRC recurrence earlier than standard surveillance methods remains ambiguous, substantial increase in tumour burden in terms of detectable plasma ctDNA was reported between the time of a positive ctDNA finding to CRC relapse.[16,19] Studies in patients with CRC found a five-fold increase in ctDNA variant allele frequency as well as a ctDNA growth rate of 25% to 143% per month, indicating rapid proliferation of MRD in the lead up to disease relapse.[16,19] Indeed, longitudinal ctDNA assessment following definitive treatment found that ctDNA-positive patients with CRC had a significantly higher rate of recurrence compared to ctDNA-negative patients (range, 93.3% to 96% *vs.* 3% to 3.3%; Table B3 in Appendix B).[16,19] The presence of ctDNA in patients with CRC or breast cancer was also significantly associated with 15 to 50.8-fold increased risk of disease recurrence compared with ctDNA-negative patients (Table 6).[14,16,17,19]

Table 6: Association of longitudinal post-treatment ctDNA status with clinical outcomes

Study	Cancer type	N	Comparison	Endpoint	Outcome	p-value
Reinert et al. (2019)[19]	Colorectal	75	ctDNA-positive *vs.* negative	RFS, HR (95% CI)	43.5 (9.8 to 193.5)	<0.001
Henriksen et al. (2022)[16]		114			50.8 (14.9 to 172)	<0.001
Loupakis et al. (2021)[17]		50		DFS, HR (95% CI)	15.0 (4.3 to 49)	<0.001
Coombes et al. (2019)[14]	Breast	49		RFS, HR (95% CI)	35.8 (8.0 to 161.3)	<0.001

Abbreviations: ctDNA, circulating tumour DNA; DFS, disease-free survival; HR, hazard ratio; RFS, recurrence-free survival.

In sum, post-treatment longitudinal ctDNA surveillance may stratify and identify patients with higher risk of relapse, potentially providing clinicians with advanced notice and a potential therapeutic window to reduce MRD before disease recurrence.

Healthcare system benefits

Besides patient's benefit, the Signatera test may bring potential healthcare system benefits. Patients determined with a negative ctDNA status who are at a lower risk of disease recurrence could be placed on less frequent radiological surveillance, while such resources can be redirected to higher risk ctDNA-positive patients.[16] This may potentially allow better long-term allocation of imaging resources based on risk stratification, optimising healthcare resource utilisation.[16]

Cost effectiveness

No studies were identified on the cost effectiveness of the Signatera test.

Ongoing trials

Several ongoing clinical trials were identified from the ScanMedicine database (NIHR Innovation Observatory) as well as Natera's website (Table 7). Multiple studies are underway to further validate the clinical utility of the Signatera test, including the large-scale CIRCULATE-JAPAN, CIRCULATE-US and BESPOKE studies. In addition, evidence supporting the use of the Signatera test as a companion diagnostic for early-stage breast cancer as part of

the FDA breakthrough designation indication is investigated in the ZEST trial that is estimated to be completed in 2029.

Table 7: Ongoing clinical trials

Study (Trial ID)	Estimated enrollment	Brief description	Estimated study completion date
Colorectal cancer			
BESPOKE CRC (NCT04264702)	2,000	A prospective case-control study to examine the impact of Signatera on adjuvant treatment decisions and determine the rate of recurrence in patients with stage I-IV CRC.	January 2025
CIRCULATE-JAPAN (GALAXY, VEGA and ALTAIR)	2,500	CIRCULATE-Japan is composed of one observational study and two randomized phase III trials. This project aims to detect MRD and measure treatment responsiveness in resectable CRC using ctDNA testing. Ultimately, CIRCULATE-Japan aims to use ctDNA to guide the administration of more precise adjuvant therapy treatment regimens in patients.	December 2023 for the ALTAIR RCT
CIRCULATE-US (NCT05174169)	1,912	A Phase II/III RCT to evaluate appropriate chemotherapy to recommend to patients based on the presence or absences of circulating tumor DNA (ctDNA) after surgery for colon cancer.	March 2030
Rapid 1 Trial (NCT04786600)	78	A randomized, phase II RCT to investigate the use of the Signatera assay versus the standard scan-based approach to guide treatment in patients with metastatic colorectal cancer.	May 2025
Study of ctDNA Guided Change in Tx for Refractory Minimal Residual Disease in Colon Adenocarcinomas (NCT04920032)	22	A phase II, prospective, two-arm, randomized, open-label clinical trial determining the efficacy of adjuvant trifluridine and tipiracil (TAS-102) in combination with irinotecan in patients with ctDNA positive colon adenocarcinoma. The Signatera MRD ctDNA assay will be used to measure ctDNA positivity.	June 2024
KISIMA-01 (NCT04046445)	96	KISIMA-01 will assess the safety, tolerability, and preliminary efficacy of ATP128 in combination with a PD1 blockade in defined patient populations with stage IV colorectal cancer. The Signatera test will be used as a biomarker to evaluate treatment response.	December 2023
Breast cancer			
ZEST (NCT04915755)	800	A phase III trial that uses the Signatera test to identify early-stage breast cancer patients eligible for investigational treatment with GSK's PARP inhibitor niraparib.	August 2029
CIPHER (NCT05333874)	30	To examine the impact of ctDNA on treatment decision making in patients with early-stage breast cancer after neoadjuvant therapy and surgery.	August 2026
DARE (NCT04567420)	100	A randomized, phase II trial of ctDNA-guided second line adjuvant therapy for high residual risk, stage II-III, estrogen receptor positive, HER-2 negative breast cancer.	December 2026
LEADER (NCT03285412)	120	LEADER is Phase II randomized clinical trial of Ribociclib for the treatment of ER-positive breast cancer. The Signatera test will be used to determine patient enrollment eligibility based on presence of ctDNA via longitudinal monitoring and to evaluate response based on ctDNA clearance as the primary endpoint.	October 2026
Across multiple cancer types			
BESPOKE IO (NCT04761783)	1,539	A prospective case-control study to examine the impact of Signatera on clinical decision-making regarding continuation, discontinuation, escalation, or de-escalation of immunotherapy in	May 2025

		patients with advanced solid tumours, including melanoma, NSCLC and CRC.	
Abbreviations: CRC, colorectal cancer; ctDNA, circulating tumour DNA; MRD, molecular residual disease; NSCLC, non-small cell lung cancer.			

Summary

The Signatera test was found to be safe with no major safety concerns, while potentially providing advantages in avoiding radiation exposure from radiological surveillance. In terms of accuracy, ctDNA showed a moderate to high sensitivity for detecting CRC relapse (range, 42% to 91.4%) while limited data showed good sensitivity in detecting breast cancer relapse (89%). The test showed a high overall specificity across both cancer types (range, 93.3% to 100%). There is some evidence showing that the test may lead to earlier detection of CRC or breast cancer relapse by an average or median interval of up to 8.9 months. However, when compared to current NCCN guidelines for CRC surveillance (imaging with CEA), ctDNA showed poorer sensitivity (53.3% *vs.* 73.3%) and no significant difference in lead time of relapse detection (median, 14.3 *vs.* 15 months).

Furthermore, the test showed that ctDNA positivity before ACT was associated with increased risk of disease recurrence and poorer survival outcomes in patients with CRC. In patients with CRC or breast cancer, longitudinal ctDNA surveillance showed that inadequate ctDNA clearance upon chemotherapy treatment correlated with disease relapse while ctDNA positivity after definitive treatment was significantly associated with a higher rate of relapse and 15 to 50.8-fold increased risk of disease recurrence. However, the clinical utility in terms of its role in guiding and refining treatment decisions based on risk stratification and subsequent patient outcomes remains unclear. The Signatera test may bring potential healthcare system benefits by optimising use of imaging resources based on risk stratification. Also, the cost-effectiveness of the test is currently uncertain.

Nevertheless, the are some limitations that should be noted, including the modest sample size of the studies and the weak evidence base for breast cancer. In addition, further intervention trials may be required to show that MRD detection is an actionable finding.[23] These could be addressed by ongoing studies, including the randomised trials in CIRCULATE-JAPAN, CIRCULATE-US and the large-scale BESPOKE studies.

VII. Estimated Costs

The cost of a targeted ctDNA profiling assay, similar to the Signatera test, was estimated to be US$1,750 per patient for the sequencing of a single tumour region, design of a personalised assay panel and profiling of five plasma samples.[24] As a reference, the annual cost of CEA tests performed every three months for patients with CRC locally amounted to less than [redacted] (Personal communication: Colorectal surgeon from National University Hospital, 13 May 2022).

VIII. Implementation Considerations

There are several considerations in implementing the Signatera test into existing clinical workflows, including turnaround time, staff training, infrastructure concerns and collaboration between primary and tertiary care providers. The introduction of a MRD assay

that is personalised to each patient's unique mutation signature profile would require the sequencing of the excised tumour tissue, development of a bespoke assay and testing of the patient's plasma sample to determine for MRD. In order for patients to receive prompt treatment, the process should be performed in a time-sensitive manner to inform on clinical decisions.[25] It has been suggested that a consortium-led approach with clinical, academic and industry inputs would be required for the clinical implementation of such test.[25] Furthermore, in contrast to the conventional 'plug-and-play' assays, the personalised nature of the test may require additional training for existing laboratory staff. To this end, the set-up of a central laboratory to consolidate expertise and resources may improve the turnaround time while ensuring quality control in designing and performing the assay. However, this may impose additional infrastructure concerns.

In addition, institutional buy-in may be required to facilitate its adoption as the assay may disrupt deeply entrenched cancer surveillance practices. Moreover, the accessibility and ease of administering the blood based Signatera test may potentially shift surveillance testing to the community care setting while patients continue to be managed by specialists. This may require a greater level of partnership between primary and tertiary care providers.

IX. Concurrent Developments

Similar to the Signatera test, there are multiple tumour-informed assays in ongoing development to detect for MRD to guide treatment decision and monitor for cancer recurrence (Table 8).

Table 8: Ongoing technologies in development

Technology (Manufacturer)	Brief description	Status
Guardant Reveal (Guardant Health, Inc.)	The test detects plasma ctDNA in post-operative patients to identify those with MRD who may benefit from adjuvant therapy. The first indication is early-stage CRC.	Commercially available in the United States
Personalized Cancer Monitoring platform (Invitae Corporation)	A pan-cancer, tumor-informed, liquid biopsy assay to detect MRD and monitor for cancer recurrence.	
RaDaR (Invitae Corporation)	RaDaR is a multi-tumour, personalized blood test able to detect residual disease and recurrence with exceptional sensitivity.	CE marked; FDA BDD
Exact Sciences' MRD solution (Exact Sciences Corporation)	The MRD test that Exact Sciences is developing is intended for patients diagnosed with solid tumour malignancies to detect ctDNA before, during, and after cancer treatment.	Undergoing clinical trial
Genetron's MRD assay (Genetron Health)	Genetron Health is collaborating with AstraZeneca to develop and validate a personalized, solid tumor MRD assays for cancer monitoring and recurrence.	In developmental stage
Abbreviations: BDD, Breakthrough Device Designation; CRC, colorectal cancer; ctDNA, circulating tumour DNA; LDT, laboratory developed test; FDA, US Food and Drug Administration; MRD, molecular residual disease.		

X. Additional Information

Ethical concerns may arise with use of the Signatera test, which can potentially provide patients with early notice of a likely future cancer relapse before the site of recurrence is localised. This may further exacerbate the fear of cancer recurrence, which is a prominent clinical issue that causes distress and reduces patient's quality of life. This is of particular

concern in patients who have completed adjuvant therapy, who may be harmed with such news without any treatment options except for repeated imaging and waiting for the cancer to relapse.[23]

In addition, five studies[14,16-19] reported conflict of interest where some authors were employed by Natera, received support from or hold ownership interest in Natera.

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Appendix

Appendix A: Studies identified and study design

Table A1: List of included studies

Cancer type	Number of studies included
Colorectal cancer	4
Breast cancer	2

Note:

1. Inclusion criteria
   a. Studies that fulfil the PICO criteria listed in Table 2.
2. Exclusion criteria
   a. Studies only available in the abstract form.
   b. Case reports or case series of n<5.

Table A2: Design and characteristics of included studies

Study	Cancer type	Study design	N	Population
Reinert et al. (2019)[19]	Colorectal	Prospective cohort	130	Patients with stages I to III CRC who were treated with curative intent
Loupakis et al. (2021)[17]		Prospective cohort	112	Patients with metastatic CRC who underwent resection of metastases with curative intent
Henriksen et al. (2022)[16]		Prospective cohort	168	Patients with stage III CRC who were scheduled for curative intent treatment, with no metastatic disease evidence on CT of chest, abdomen and pelvis before surgery.
Fakih et al. (2022)[15]		Retrospective cohort	48	Patients with curatively resected stage I to IV CRC
Coombes et al. (2019)[14]	Breast	Prospective cohort	49	Patients with breast cancer following surgery and adjuvant therapy
Magbanua et al. (2021)[18]		Retrospective study	84	Patients enrolled in the I-SPY 2 trial who have ≥2.5cm stage II/III breast cancer, and received standard NAC combined with MK-2206 (AKT inhibitor) or standard NAC alone

Abbreviations: CRC, colorectal cancer; CT, computed tomography; NAC, neoadjuvant chemotherapy.

Appendix B: Supplementary Tables and Figures

Table B1: Rate of disease recurrence in ctDNA positive *vs.* negative patients before treatment initiation

Study	Cancer type	Rate of recurrence, n/N (%)	
		ctDNA-positive	ctDNA-negative
Reinert et al. (2019)[19]	Colorectal	7/10 (70%)	10/84 (11.9%)
Henriksen et al. (2022)[16]		16/20 (80%)	22/120 (18%)

Abbreviation: ctDNA, circulating tumour DNA.

Table B2: Comparison of post-operative ctDNA and CEA level in predicting disease-free survival in patients with colorectal cancer

Study	N	Biomarker	Endpoint	Outcome	p-value
Loupakis et al. (2021)[17]	55	ctDNA	DFS, HR (95% CI)	6.4 (3.0 to 13.0)	<0.001
		CEA		1.5 (0.83 to 2.1)	0.18
Abbreviation: CEA, carcinoembryonic antigen; ctDNA, circulating tumour DNA; DFS, disease-free survival; HR, hazard ratio.					

Table B3: Rate of disease recurrence in ctDNA positive *vs.* negative patients during longitudinal monitoring after the end of definitive treatment

Study	Cancer type	Rate of recurrence, n/N (%)		p-value
		ctDNA-positive	ctDNA-negative	
Reinert et al. (2019)[19]	Colorectal	14/15 (93.3%)	2/60 (3.3%)	<0.001
Henriksen et al. (2022)[16]		21/22 (96%)	3/92 (3%)	<0.001
Abbreviation: ctDNA, circulating tumour DNA.				

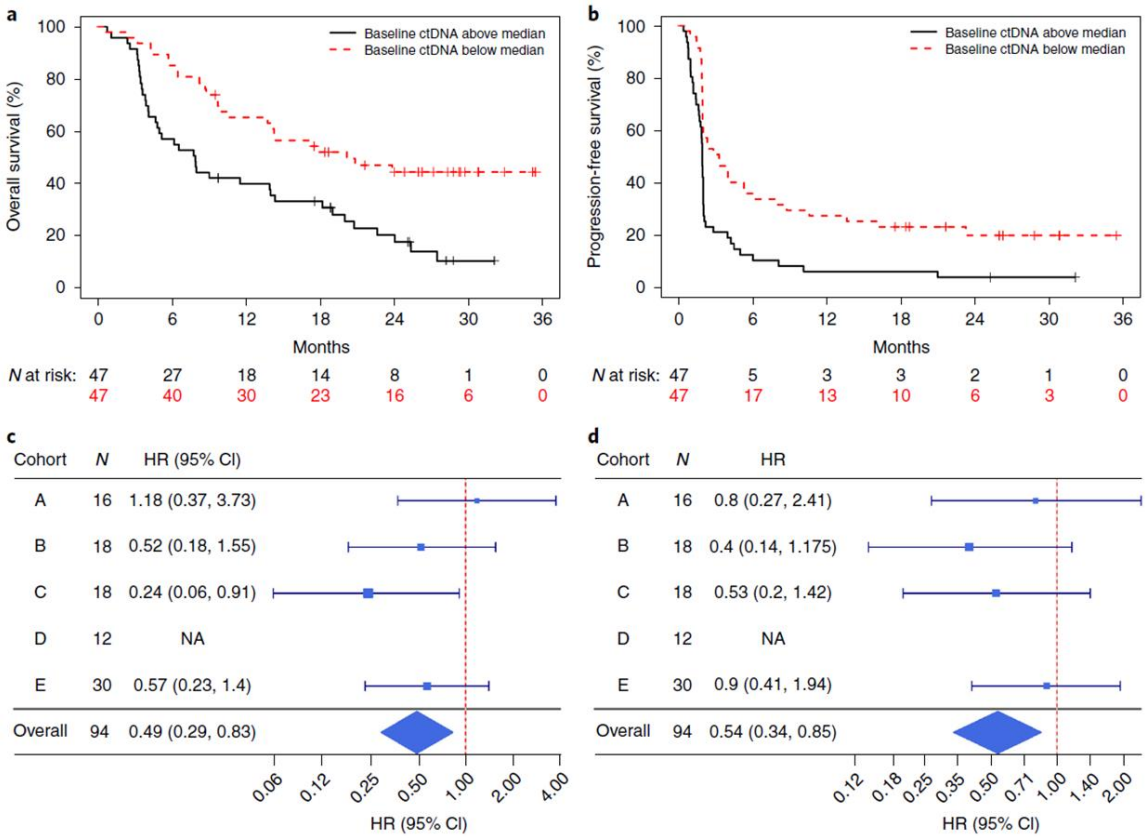

Figure B1: Baseline ctDNA level before treatment with pembrolizumab is associated with overall survival (OS) and progression-free survival (PFS) in patients across multiple cancer types. (a, b) Kaplan-Meier curves of **(a)** OS and **(b)** PFS. **(c, d)** Forest plot indicating the association of baseline ctDNA levels with **(c)** OS and **(d)** PFS in the five subcohorts (A: squamous cell cancer of head and neck; B: triple negative breast cancer; C: high-grade serous ovarian cancer; D: malignant melanoma; E: mixed solid tumours). Image adapted from Bratman et al. (2020)[20].

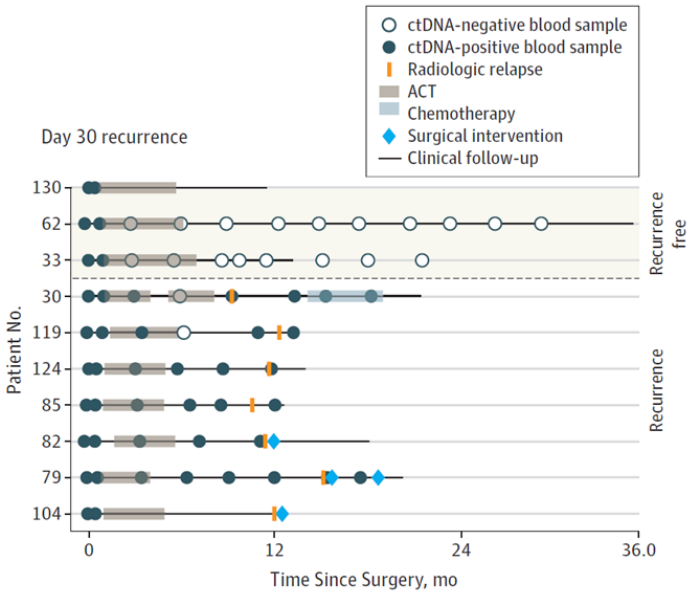

Figure B2: Longitudinal ctDNA monitoring to assess treatment effectiveness in patients with resected CRC. In 10 patients with CRC who were ctDNA positive before treatment with ACT, 8 patients had longitudinal ctDNA monitoring. 4 out of the 8 patients remained ctDNA-positive throughout treatment and experienced disease recurrence. The remaining 4 patients had ctDNA clearance, of which 2 patients with persistent clearance remained recurrence free while the other 2 patients who regained ctDNA positivity relapsed. Image adapted from Reinert et al. (2019)[19].

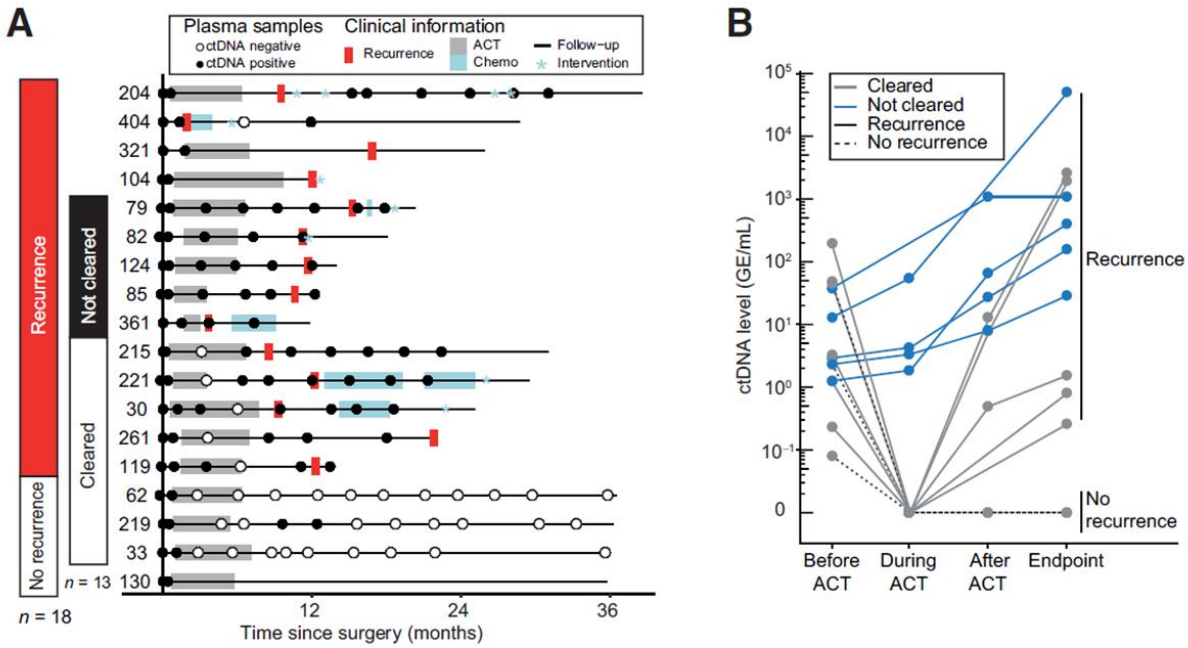

Figure B3: Longitudinal ctDNA monitoring to assess treatment effectiveness in patients with resected CRC. 13 patients with CRC were monitored before, during and after ACT treatment. Of the 13 patients, 3 had persistent ctDNA clearance after ACT and did not relapse. The remaining 10 patients who had a transient clearance or did not clear their plasma ctDNA experienced relapse. Image adapted from Henriksen et al. (2022)[16].

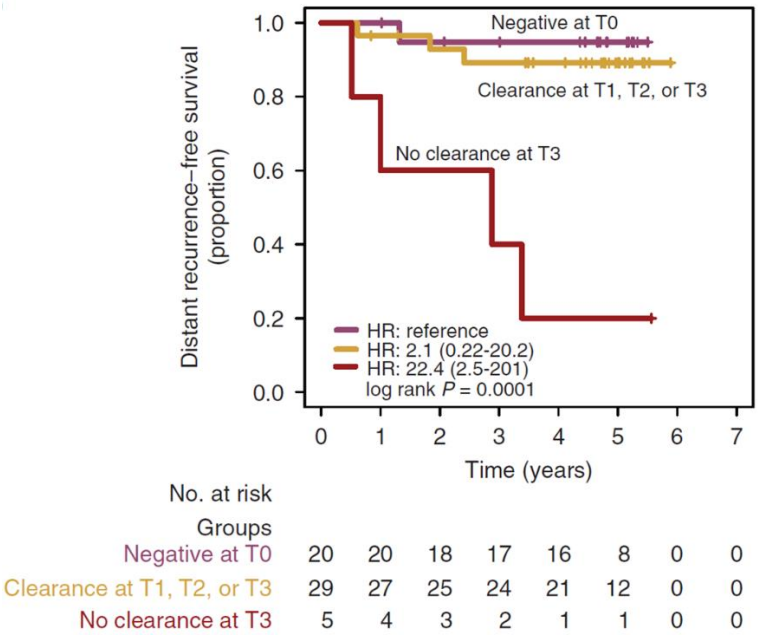

Figure B4: Distant recurrence-free survival in patients with breast cancer on treatment with neoadjuvant chemotherapy. Patients with early (stage II/III) breast cancer were stratified into pretreatment (T0), 3 weeks after initiation of paclitaxel (T1), between paclitaxel and anthracycline regimens (T2) or prior to surgery (T3) groups. Patients who cleared ctDNA at T1, T2 or T3 had similar risk of metastatic recurrence compared to those who are ctDNA-negative at T0 (n=20; HR, 2.1; 95% CI, 0.22 to 20.2). On the other hand, patients who did not had ctDNA clearance at T3 had a significantly higher risk of metastatic recurrence (n=5; HR, 22.4; 95% CI, 2.5 to 201; p<0.001). Image adapted from Magbanua et al. (2021)[18].

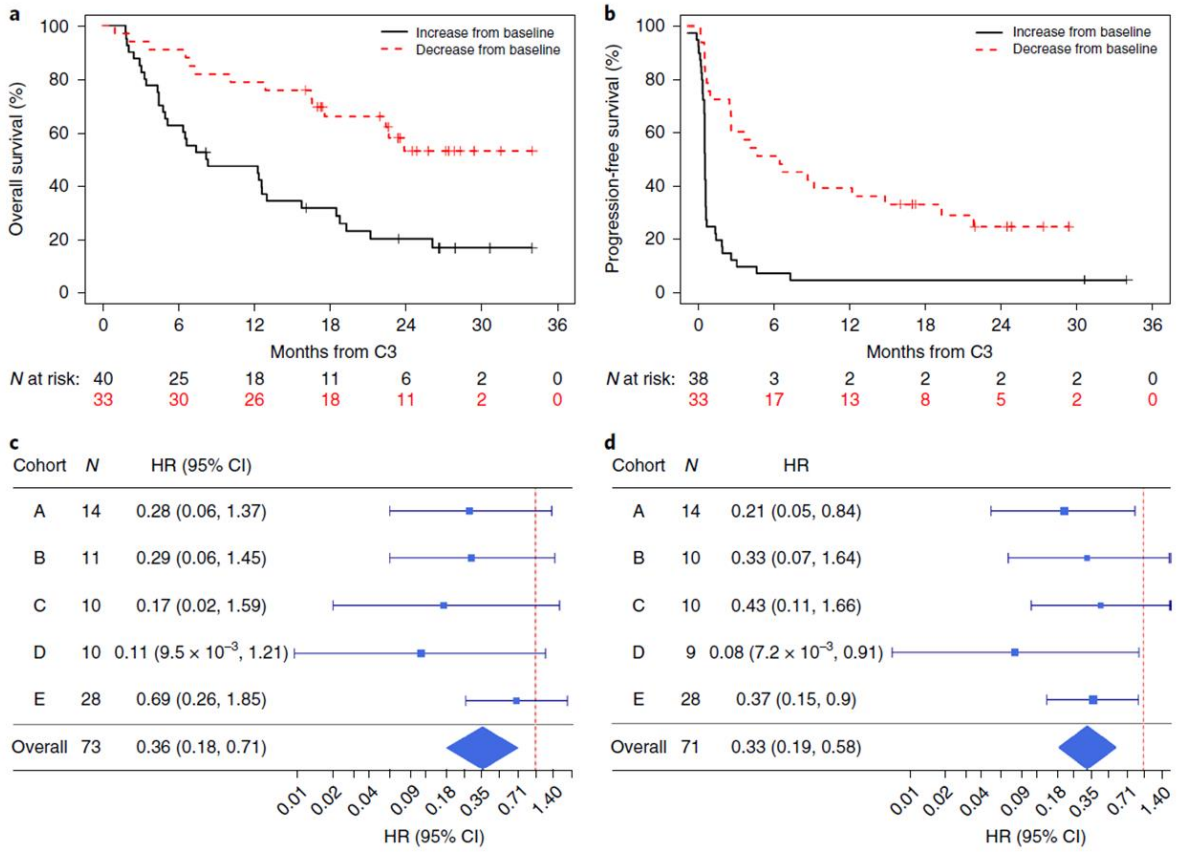

Figure B5: Changes in ctDNA level from baseline to after second cycle of pembrolizumab treatment in patients across multiple cancer types is associated with overall survival (OS) and progression-free survival (PFS). (a, b) Kaplan-Meier curves of **(a)** OS and **(b)** PFS in patients stratified with increase or decrease ctDNA values following second cycle of pembrolizumab treatment from baseline. **(c, d)** Forest plot indicating the association of baseline ctDNA levels with **(c)** OS and **(d)** PFS in the five subcohorts (A: squamous cell cancer of head and neck; B: triple negative breast cancer; C: high-grade serous ovarian cancer; D: malignant melanoma; E: mixed solid tumours). Image adapted from Bratman et al. (2020)[20].

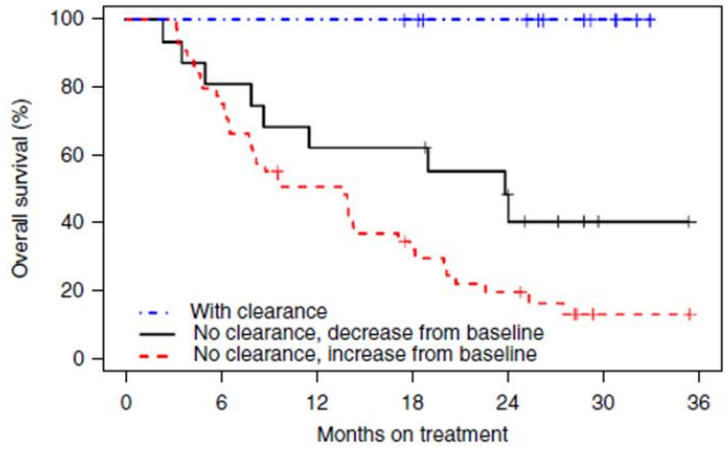

Figure B6: Kaplan-Meier curve of overall survival among patients across multiple cancer types with at least two ctDNA measurements during treatment with immune checkpoint inhibitor (pembrolizumab). Across multiple cancer types, patients with rise in ctDNA levels above baseline were associated with poor survival (median OS, 13.7 months), while those with detectable ctDNA levels that remained below baseline had a marginally longer survival (median OS, 23.8 months). For patients who had ctDNA clearance, there was a 100% OS at a median follow-up of 25.4 months. Image adapted from Bratman et al. (2020)[20].

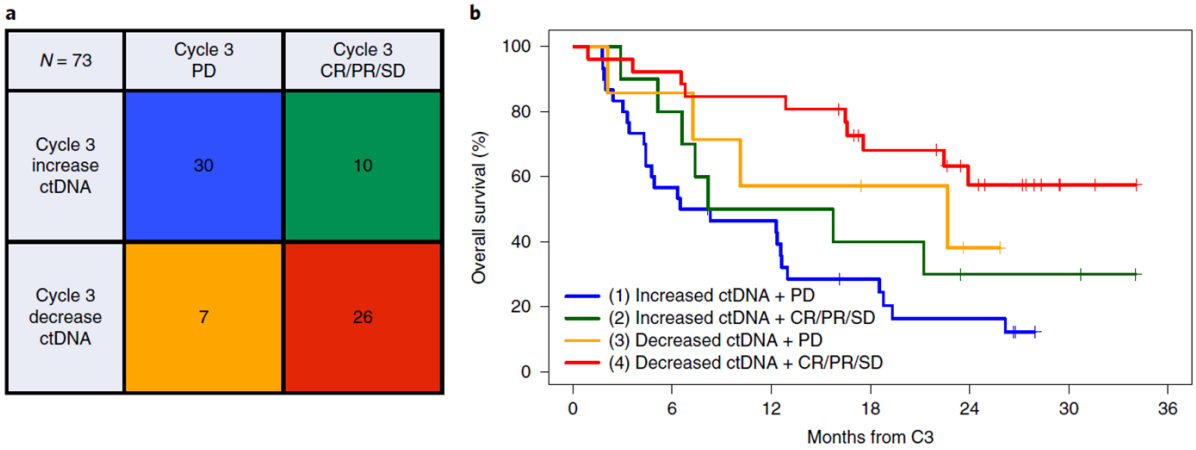

Figure B7: Stratification of patients across multiple cancer types to different risk groups based on ctDNA kinetics and early clinical response. (a) The table indicates the RECIST classification of patients at the beginning of cycle 3 of immune checkpoint inhibitor (pembrolizumab) and the ctDNA kinetics results (that is, increase or decrease from baseline at the beginning of the cycle 3). **(b)** Kaplan-Meier curve of overall survival among patients with at least two ctDNA measurements (n=73) stratified based on cycle 3 RECIST and ctDNA kinetics at the beginning of the third cycle of pembrolizumab. Image adapted from Bratman et al. (2020)[20].

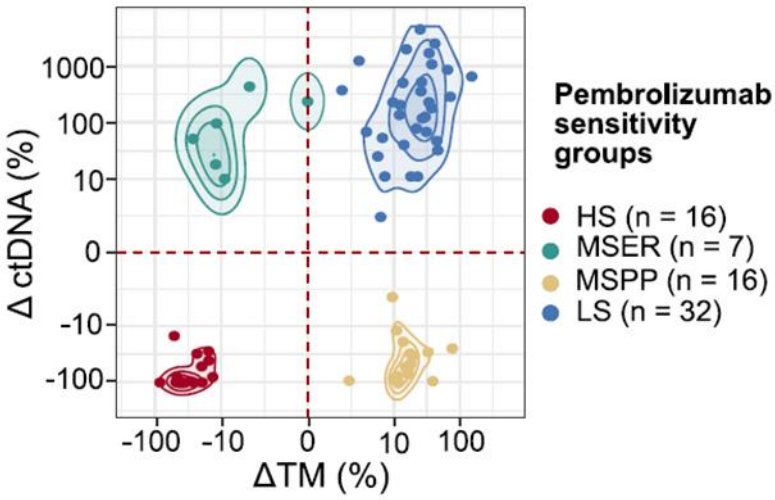

Figure B8: Stratification of patients across multiple cancer types based on ctDNA kinetics (ΔctDNA) and change in tumour lesion measurement (ΔTM). Contours around each group indicate the density within each group with the median shown as the center of the contour. Abbreviations: HS, high sensitivity; LS, low sensitivity; MSER, mixed sensitivity with emerging resistance; MSPP, mixed sensitivity with pseudo-progression. Image adapted from Yang et al. (2021)[21].

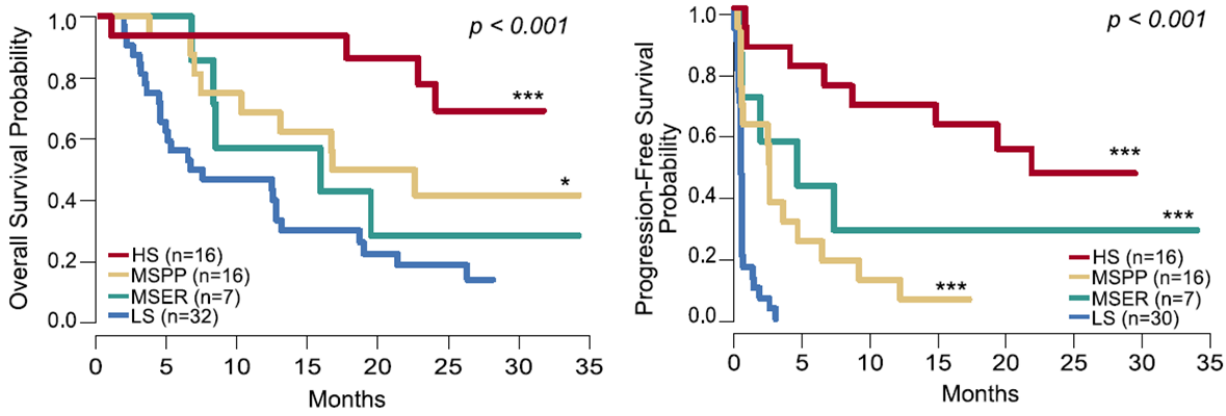

Figure B9: Kaplan-Meier plot of overall survival (left) and progression-free survival (right) from treatment cycle 3 in patients across multiple cancer types grouped by pembrolizumab sensitivity. Abbreviations: HS, high sensitivity; LS, low sensitivity; MSER, mixed sensitivity with emerging resistance; MSPP, mixed sensitivity with pseudo-progression. Image adapted from Yang et al. (2021)[21].