natera™ | Signatera™
Residual disease test (MRD)

Know
cancer's next move

Treat
with confidence

Signatera
Personalized, tumor-informed molecular residual disease (MRD) detection

13011 McCallen Pass, Building A Suite 100 | Austin, TX 78753 | natera.com

Signatera has been developed and its performance characteristics determined by the CLIA-certified laboratory performing the test. The test has not been cleared or approved by the US Food and Drug Administration (FDA). CAP accredited, ISO 13485 certified, and CLIA certified. © 2022 Natera, Inc. All Rights Reserved. NAT-9300000

Signatera™ provides insight when current measures may be delaying answers to critical questions

Is there cancer left in the body?

Is additional treatment beneficial?

Is the treatment working?

Reliable results from a single test, deeper insights with serial sampling

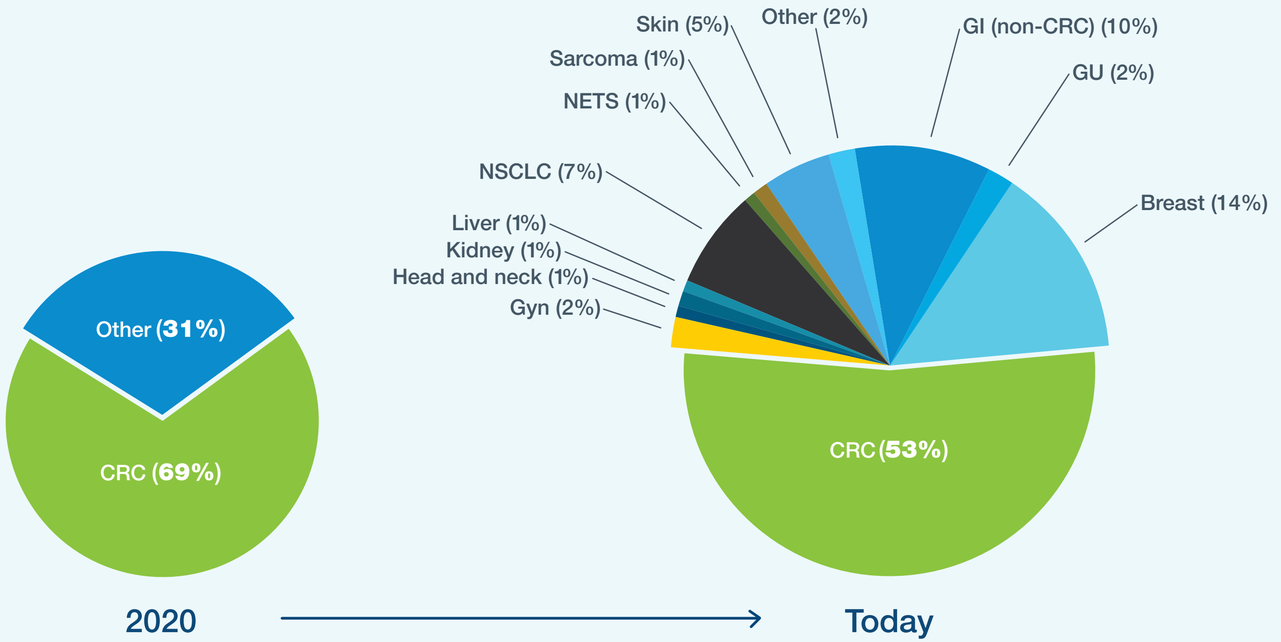
	From a single test	With serial sampling
+	**Residual disease present** 97% of MRD-positive patients with early-stage CRC will relapse without further treatment[1,2]	**Actionable kinetics** Know if disease burden is increasing or shrinking with trackable MTM values[3]
−	**No evidence of residual disease** Only 12% of MRD-negative patients with early-stage CRC will relapse after surgery[1]	**Reduced recurrence risk** Only 3% of patients with serial ctDNA negative results relapsed[1]

CRC=colorectal cancer; ctDNA=circulating tumor DNA; MRD=molecular residual disease; MTM=mean number of tumor molecules.

References: 1. Reinert T, Henriksen TV, Christensen E, et al. Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer. *JAMA Oncol*. 2019;5(8):1124-1131. doi:10.1001/jamaoncol.2019.0528 **2.** Natera. Data on file. **3.** Henriksen TV, Tarazona N, Frydendahl A, et al. Circulating tumor DNA in stage III colorectal cancer, beyond minimal residual disease detection, towards assessment of adjuvant therapy efficacy and clinical behavior of recurrences. *Clin Cancer Res*. Published online October 8, 2021. doi:10.1158/1078-0432.CCR-21-2404

Personalized approach, pan-cancer applicability

Broad clinical use[1]



Signatera is a tumor-informed approach, clinically validated across multiple tumor types/settings[1]

Clinically validated

- >25K Signatera™ tests conducted in the United States since launch[1]*
- An additional 3000+ patient cases published or presented at major congresses[1]

Established Medicare coverage

- Medicare coverage for Stage II-III CRC, Stage IV oligometastatic CRC, and pan-cancer IO monitoring[1]

Breakthrough designation from the FDA

*CLIA samples processed from 2H'19-1H'21.

CLIA=Clinical Laboratory Improvement Amendments; CRC=colorectal cancer; GI=gastrointestinal; GU=genitourinary; Gyn=gynecological; IO=immuno-oncology; NETS=neuroendocrine tumors; NSCLC=non-small cell lung cancer.

Reference: 1. Natera. Data on file.

Signatera™ delivers deeper knowledge across the treatment journey

	Signatera clinical applications	Why tumor-informed MRD?
1	**Neoadjuvant response monitoring**	Tailor neoadjuvant treatment or surgical strategies to patient's specific needs (e.g., rectal cancer TNT)
2	**Postsurgical MRD assessment**	Identify patients who may or may not benefit from adjuvant therapy
3	**Recurrence monitoring**	Triage indeterminate nodules; rule in/rule out disease recurrence
4	**Assess treatment effectiveness**	Monitor ctDNA kinetics (increase or decrease in ctDNA levels) to quickly identify if there is any response to treatment

Add the Altera™ tumor genomic profiling test when you order Signatera to get clinically relevant biomarkers and MRD monitoring with no additional sample. Altera utilizes whole-exome and whole-transcriptome sequencing. **Add Empower™ hereditary cancer test** to inform treatment options following a cancer diagnosis.

ctDNA=circulating tumor DNA; MRD=molecular residual disease; TNT=total neoadjuvant treatment.

Signatera™ is a simple solution

1. Simple to order

Neoadjuvant → Adjuvant → Surveillance → Advanced/ metastatic

- Order Signatera at any time; only one tissue sample is needed
- Common initial time points are at diagnosis or before treatment
- Order Altera™ along with Signatera for genomic profiling to aid in treatment selection

2. Simple for MRD monitoring

Recurring order program
(cadence can be based on surgery date or customized to patient's needs)

3. Simple sampling

Resection or diagnostic biopsy obtained from pathology *(initial test only)*

+

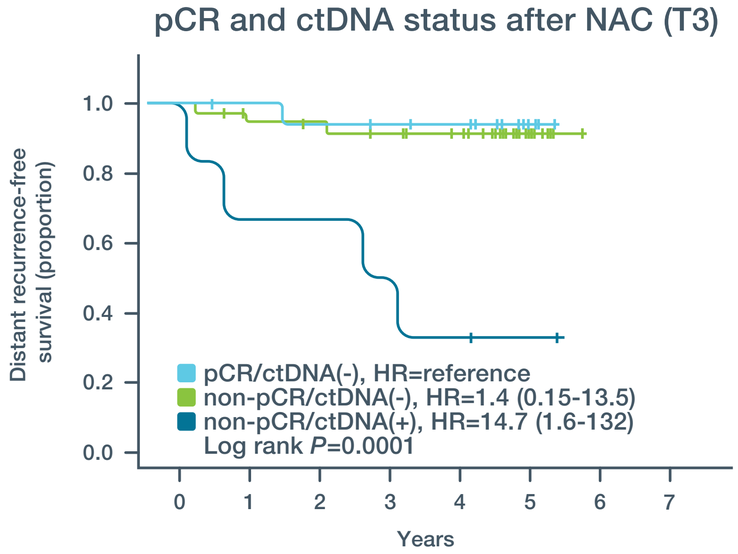
Initial and follow-up tests use a single blood draw from the clinic or patient's home using mobile phlebotomy

MRD=molecular residual disease.

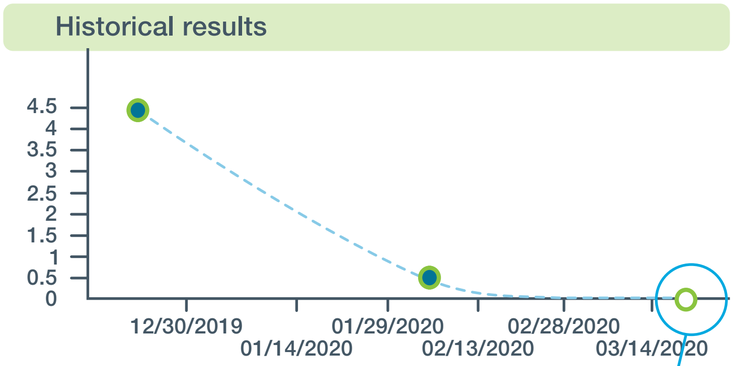
Signatera™ is proven across treatment phases and tumor types

1. Neoadjuvant response monitoring

Tailor neoadjuvant treatment or surgical strategies based on MRD status (e.g., rectal cancer TNT)



Breast cancer patients achieving ctDNA clearance but not pCR demonstrated similar risk of recurrence as those who achieved pCR.[1]



Date	MTM/mL
Dec 20, 2019	4.44
Feb 06, 2020	0.48
Mar 19, 2020	0.00

Case example: Rectal cancer patient who achieved ctDNA clearance during TNT elected for nonsurgical management[2]

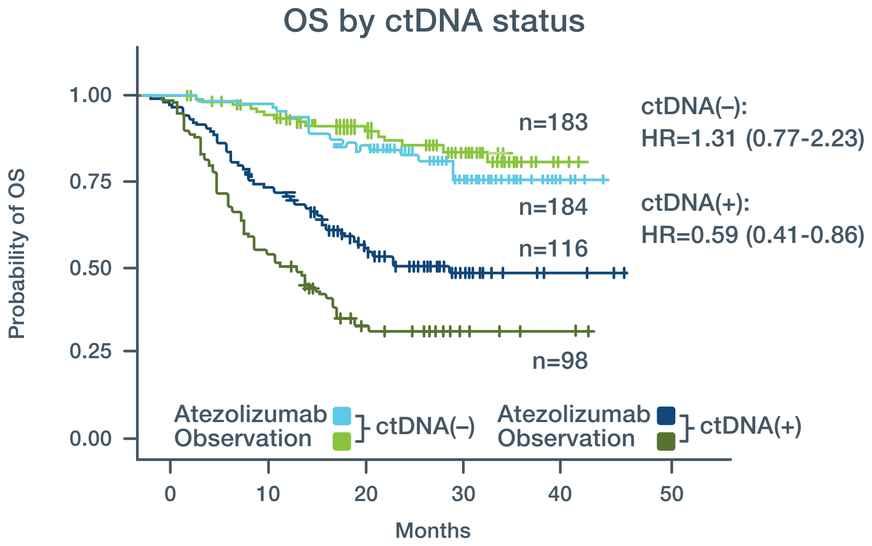
ctDNA=circulating tumor DNA; MRD=molecular residual disease; MTM=mean number of tumor molecules; NAC=neoadjuvant chemotherapy; pCR=pathologic complete response; TNT=total neoadjuvant treatment.

References: 1. Magbanua MJM, Swigart LB, Wu H-T, et al. Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. *Ann Oncol*. 2021;32(2):229-239. doi:10.1016/j.annonc.2020.11.007 **2.** Natera. Data on file.

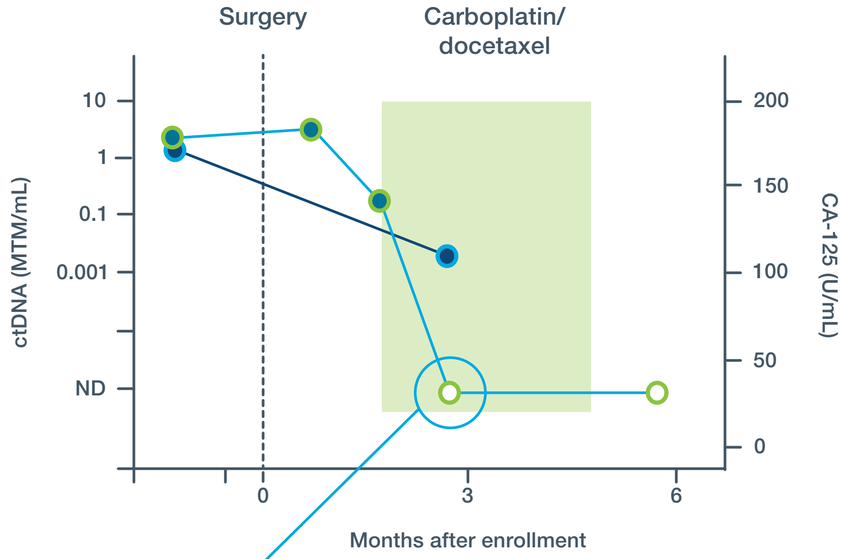
Signatera™ is proven across treatment phases and tumor types

2. Postsurgical MRD assessment

Evaluate the need for adjuvant therapy by identifying risk of postsurgical relapse



MIBC patients who were MRD-positive postsurgery derived treatment benefit with adjuvant therapy (OS HR=0.59). MRD-negative patients saw no improvement despite adjuvant treatment (OS HR=1.31).[1]



Case example: Ovarian cancer patient, who was MRD-positive after surgery, cleared ctDNA with carboplatin/docetaxel[2]

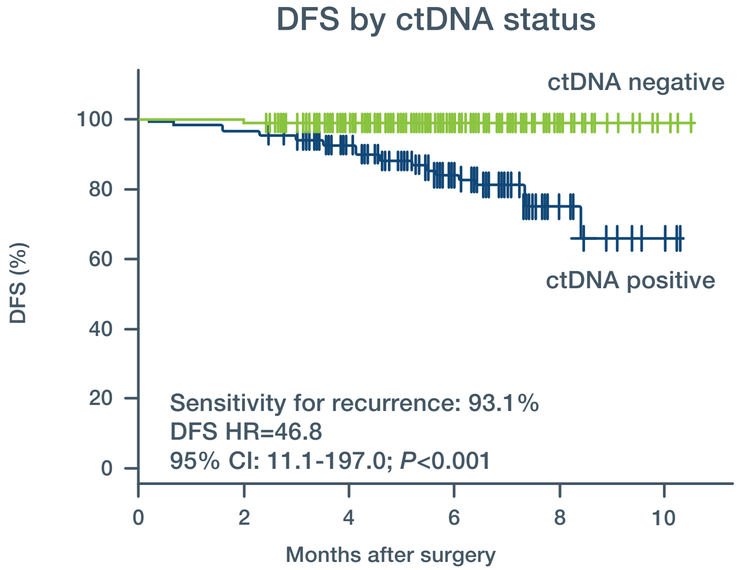
ctDNA=circulating tumor DNA; MIBC=muscle-invasive bladder cancer; MRD=molecular residual disease; MTM=mean number of tumor molecules; OS=overall survival.

References: 1. Powles T, Assaf ZJ, Davarpanah N, et al. ctDNA guiding adjuvant immunotherapy in urothelial carcinoma. *Nature*. 2021;595(7867):432-437. doi:10.1038/s41586-021-03642-9 **2.** Chapman JS, Pierson WE, Smith-McCune K, et al. Circulating tumor DNA predicts disease recurrence in ovarian cancer patients. Presented at: American Association of Cancer Research; April 9-14, 2021; Virtual.

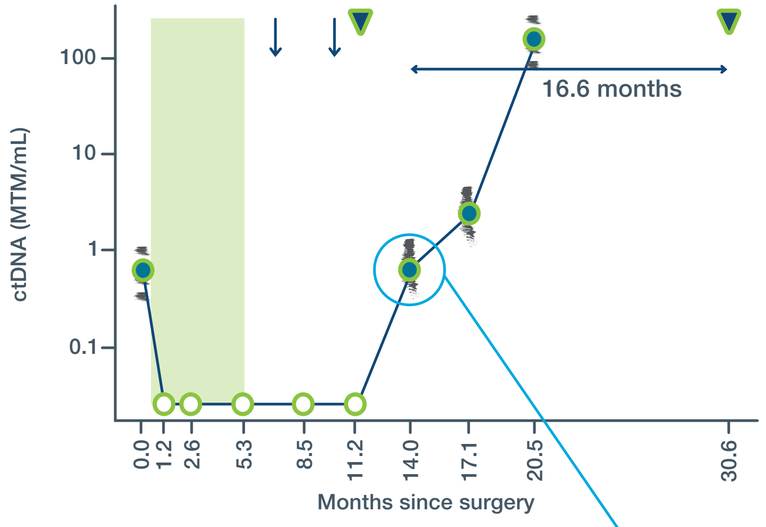
Signatera™ is proven across treatment phases and tumor types

3. Recurrence monitoring

Triage indeterminate nodules; detect disease recurrence early while the tumor may still be resectable



Signatera demonstrated 93% relapse sensitivity in a longitudinal analysis of more than 800 patients with colorectal cancer.[1]



Case example: Colon cancer patient with ctDNA detected 16.6 months ahead of radiographic recurrence[2]

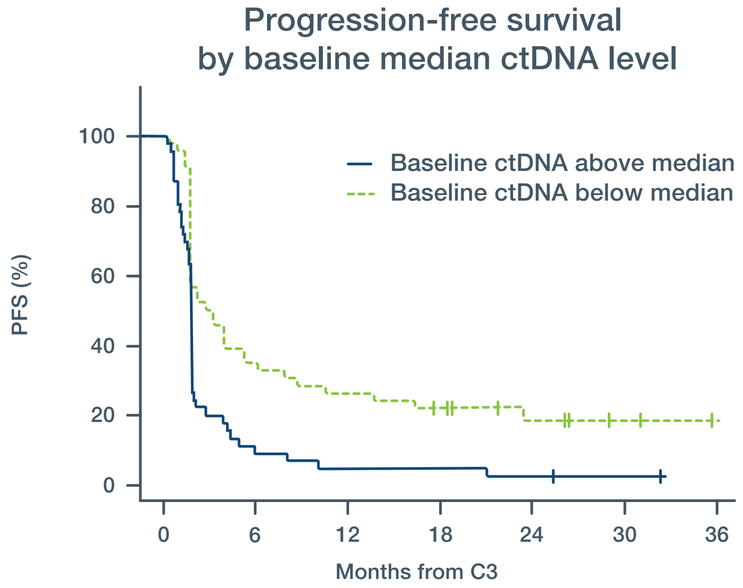
ctDNA=circulating tumor DNA; DFS=disease-free survival; MTM=mean number of tumor molecules.

References: 1. Shirasu H, Taniguchi H, Watanabe J, et al. Monitoring molecular residual disease by circulating tumor DNA in resectable colorectal cancer: molecular subgroup analyses of a prospective observational study GALAXY in CIRCULATE-Japan. Presented at: ESMO World Congress on Gastrointestinal Cancer; June 30-July 3, 2021; Lugano, Switzerland; Virtual. **2.** Reinert T, Henriksen TV, Christensen E, et al. Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer. *JAMA Oncol*. 2019;5(8):1124-1131. doi:10.1001/jamaoncol.2019.0528

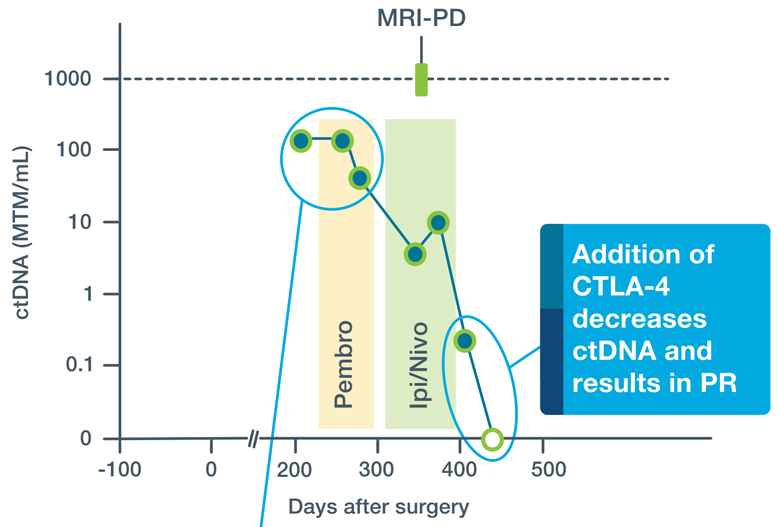
Signatera™ is proven across treatment phases and tumor types

4. Assess treatment effectiveness

Identify patients who may not be responding to therapy, as well as exceptional responders who clear ctDNA



Signatera assessment of ctDNA kinetics at 6 weeks in conjunction with imaging was 100% predictive of treatment nonresponse to immunotherapy.[1]



Case example: CRC patient with elevated ctDNA, despite pembrolizumab monotherapy, experiences radiographic recurrence[2]

CRC=colorectal cancer; ctDNA=circulating tumor DNA; MRI-PD=magnetic resonance imaging proton density; MTM=mean number of tumor molecules; PFS=progression-free survival; PR=partial response.

References: 1. Bratman SV, Yang SYC, Iafolla MAJ, et al. Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. *Nat Cancer*. 2020;1:873-881. doi:10.1038/s43018-020-0096-5 **2.** Kasi P, Krainock M, Budde G, et al. Circulating tumor DNA (ctDNA) serial analysis during progression on PD-1 blockade and later CTLA-4 rescue in patients with mismatch repair-deficient metastatic colorectal cancer. Presented at: Society for Immunotherapy of Cancer 35th Annual Meeting; November 9-14, 2020; Virtual.

Knowledge at every step to support optimal patient care

Is your cancer MRD test

- Validated in >3000 patients
- Pan-cancer
- Able to track ctDNA kinetics
- Personalized
- Tumor informed
- Breakthrough designated by FDA
- **Signatera™**

ctDNA=circulating tumor DNA; MRD=molecular residual disease.

Bladder cancer

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Neoadjuvant

Adjuvant therapy

Postsurgical MRD assessment

MIBC: Postcystectomy MRD assessment can help risk-stratify patients and identify those with evidence of residual disease who may benefit from adjuvant treatment,[1] including the recently approved nivolumab immunotherapy.[2]

Surveillance

Recurrence monitoring

MIBC: ctDNA can predict disease recurrence 3 months before traditional imaging methods, enabling treatment before visceral crisis.[3]

Metastatic

Assess treatment response

Monitor ctDNA kinetics serially to evaluate response to SA immunotherapy (i.e., pembrolizumab, atezolizumab, nivolumab) and thus, inform treatment duration. ctDNA clearance is associated with improved prognosis.[4]

ctDNA=circulating tumor DNA; MIBC=muscle-invasive bladder cancer; MRD=molecular residual disease; SA=single agent.

References: 1. Powles T, Assaf ZJ, Davarpanah N, et al. ctDNA guiding adjuvant immunotherapy in urothelial carcinoma. *Nature*. 2021;595(7867):432-437. doi:10.1038/s41586-021-03642-9 **2.** FDA approves nivolumab for adjuvant treatment of urothelial carcinoma. News release. Food and Drug Administration; August 20, 2021. Accessed November 2, 2021. https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approvesnivolumab-adjuvant-treatment-urothelial-carcinoma **3.** Christensen E, Birkenkamp-Demtröder K, Sethi H, et al. Early detection of metastatic relapse and monitoring of therapeutic efficacy by ultra-deep sequencing of plasma cell-free DNA in patients with urothelial bladder carcinoma. *J Clin Oncol*. 2019;37(18):1547-1557. doi:10.1200/JCO.18.02052 **4.** Bratman SV, Yang SYC, Iafolla MAJ, et al. Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. *Nat Cancer*. 2020;1:873-881. doi:10.1038/s43018-020-0096-5

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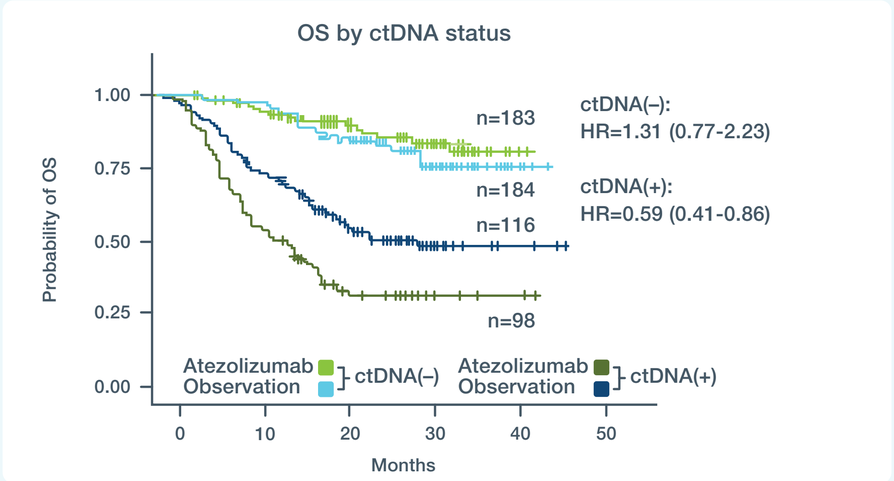
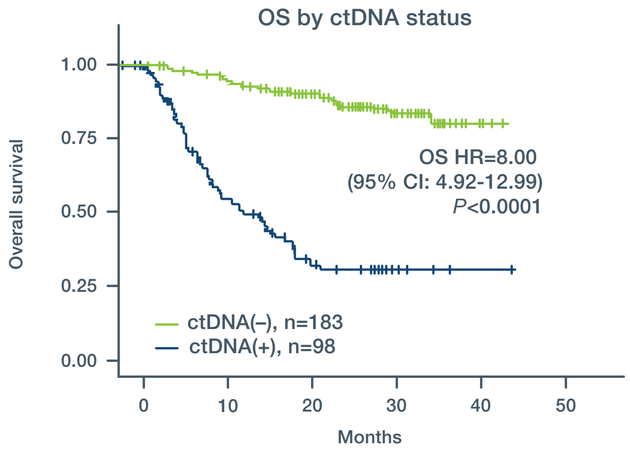
IMvigor010 study background: Phase III, randomized clinical trial of atezolizumab vs observation in high-risk adjuvant MIBC[1]





Predictive:

- ctDNA(+) after cystectomy may be predictive of treatment benefit with atezolizumab (OS HR=0.59)
- ctDNA(–) patients had similar OS on atezolizumab vs observation

Prognostic:

- IMvigor010 confirmed the prognostic value of ctDNA status

Signatera ctDNA analysis can help identify MIBC patients whose disease is likely to recur after cystectomy if not given further treatment

Add the Altera™ tumor genomic profiling test when you order Signatera to get clinically relevant biomarkers (e.g., *FGFR*, *ERCC2*) and MRD monitoring with no additional sample. Altera utilizes whole-exome and whole-transcriptome sequencing.
Add Empower™ hereditary cancer test to inform treatment options following a cancer diagnosis.

ctDNA=circulating tumor DNA; *ERCC2*=excision repair cross-complementation group 2; *FGFR*=fibroblast growth factor receptor; MIBC=muscle-invasive bladder cancer; OS=overall survival.

Reference: 1. Powles T, Assaf ZJ, Davarpanah N, et al. ctDNA guiding adjuvant immunotherapy in urothelial carcinoma. *Nature*. 2021;595(7867):432-437. doi:10.1038/s41586-021-03642-9

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Metastatic bladder cancer case review*

Is the tumor truly progressing?

- **Age:** 71
- **Original diagnosis:** pT3N0M0 muscle-invasive bladder cancer
- **Treatment course:** Preexisting renal insufficiency precluded the use of neoadjuvant cisplatin-based chemotherapy. Patient underwent radical cystectomy
- **Monitoring:** Two postoperative ctDNA tests were negative, then ctDNA begins to rise as the patient displays clinical symptoms. CT shows no evidence of recurrence at this time

*Adapted from a real-world Signatera patient case.
CT=computed tomography; ctDNA=circulating tumor DNA.
Case features modified to protect patient confidentiality. No treatment recommendations are made or should be implied.

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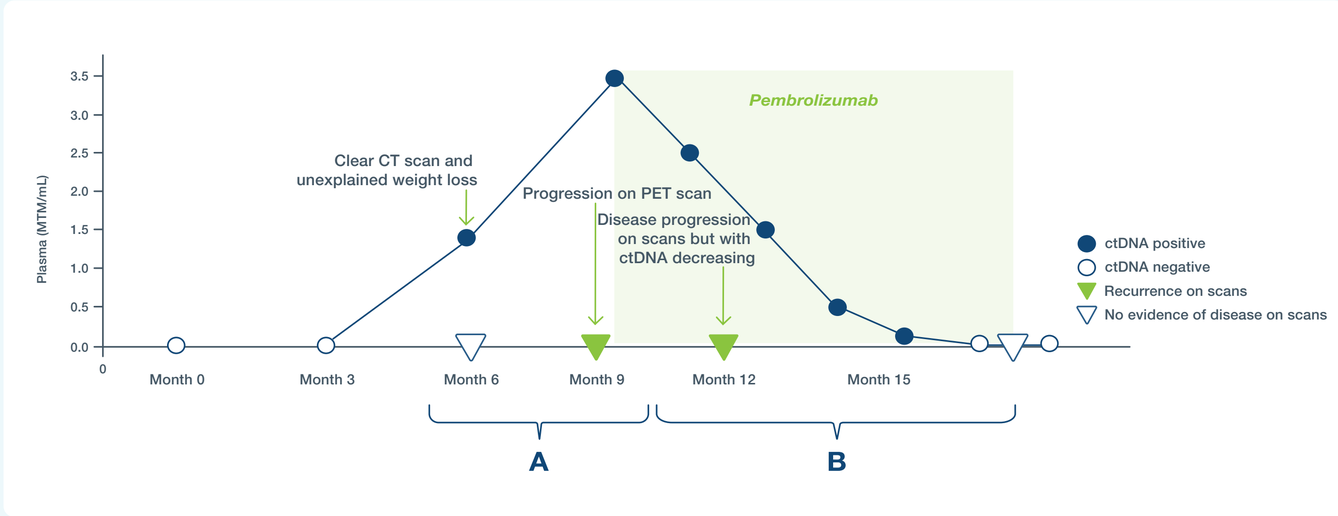
Signatera can identify relapse early; ctDNA kinetics can inform treatment response assessment to immunotherapy in conjunction with imaging[1-3]

Evidence of disease progression



A
- CT scan did not find evidence of disease
- Signatera identified disease progression 3 months before progression was identified by PET scan

B
- After starting pembrolizumab, CT scan initially identified progressive disease, but ctDNA levels were declining
- Pembrolizumab treatment was continued
- Patient cleared ctDNA and scans subsequently cleared

CT=computed tomography; ctDNA=circulating tumor DNA; MTM=mean number of tumor molecules; PET=positron emission tomography.

References: 1. Powles T, Assaf ZJ, Davarpanah N, et al. ctDNA guiding adjuvant immunotherapy in urothelial carcinoma. *Nature*. 2021;595(7867):432-437. doi:10.1038/s41586-021-03642-9 **2.** Christensen E, Birkenkamp-Demtröder K, Sethi H, et al. Early detection of metastatic relapse and monitoring of therapeutic efficacy by ultra-deep sequencing of plasma cell-free DNA in patients with urothelial bladder carcinoma. *J Clin Oncol*. 2019;37(18):1547-1557. doi:10.1200/JCO.18.02052 **3.** Bratman SV, Yang SYC, Iafolla MAJ, et al. Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. *Nat Cancer*. 2020;1:873-881. doi:10.1038/s43018-020-0096-5

Breast cancer

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Neoadjuvant

Response monitoring

High-risk patients
Assess the risk of recurrence by monitoring ctDNA dynamics during or post completion of NAC.

Adjuvant

Surveillance

Recurrence monitoring

High-risk patients
- Monitor and detect recurrence with serial ctDNA measurements
- Use ctDNA to determine recurrence in questionable clinical scenarios (e.g., equivocal scans, concerning symptoms, abnormal blood test)
- Refer high-risk ctDNA-positive patients to ongoing clinical trials when appropriate
- Encourage adherence to long-term therapy such as endocrine therapy, anti-HER2 therapy, capecitabine, olaparib, or immunotherapy

Metastatic

Assess treatment effectiveness

TNBC
Monitor ctDNA serially to help evaluate response to immunotherapy (e.g., pembrolizumab + chemotherapy in PD-L1 high patients).

ctDNA=circulating tumor DNA; HER2=human epidermal growth factor receptor 2; NAC=neoadjuvant chemotherapy; PD-L1=programmed death-ligand 1; TNBC=triple-negative breast cancer.

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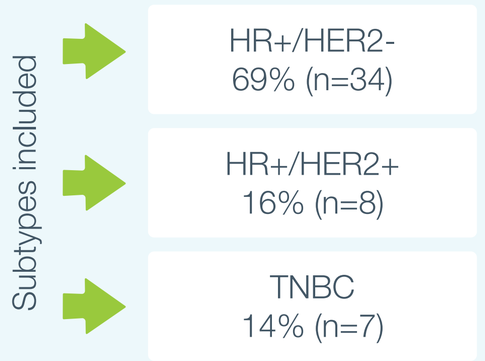
EBLIS study cohort, a retrospective analysis: Is there a more sensitive technology that can detect preclinical metastasis?

EBLIS objectives

Primary: Determine lead interval between ctDNA detection and clinical metastatic disease.
Secondary: Determine whether ctDNA in plasma can detect recurrent disease earlier than traditional methods.

Trial design[1]

- Cohort of 49 Stage I-III breast cancer patients with a >50% risk of mortality at 10 years without prior therapy
- Patients received Signatera testing every 6 months for up to 4 years following completion of surgery and adjuvant therapy
- Additional monitoring tests, including CT imaging, LFT, and CA 15-3, were also performed



CT=computed tomography; ctDNA=circulating tumor DNA; HER2=human epidermal growth factor receptor 2; HR=hormone receptor; LFT=liver function test; TNBC=triple-negative breast cancer.

Reference: 1. Coombes RC, Page K, Salari R, et al. Personalized detection of circulating tumor DNA antedates breast cancer metastatic recurrence. *Clin Cancer Res*. 2019;25(14):4255-4263. doi:10.1158/1078-0432.CCR-18-3663

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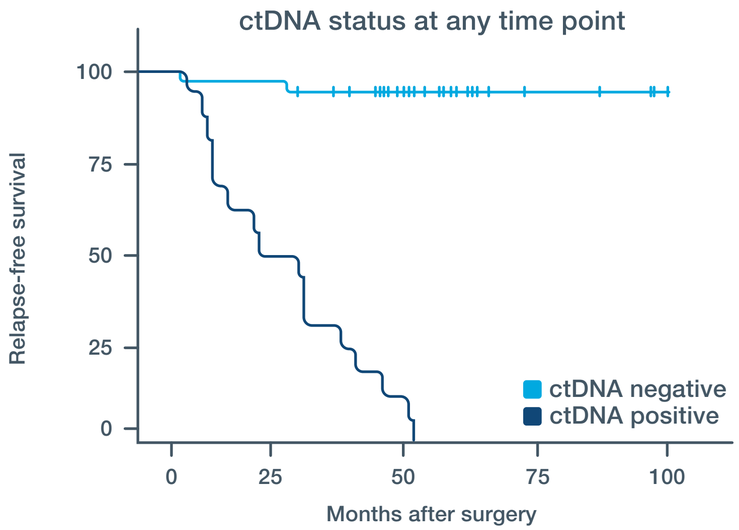
Signatera can detect recurrence up to 2 years earlier[1]



- 89% sensitivity: Signatera detected plasma ctDNA in 16 out of 18 patients prior to clinical or radiographic relapse
- 100% specificity: None of the 31 nonrelapsing patients were ctDNA positive during the course of the study
  - ctDNA detected relapses up to 2 years ahead of traditional clinical indicators:
    - 8.9 months earlier than radiologic relapse
    - 200 days before CA 15-3 detection on average

Add the Altera™ tumor genomic profiling test when you order Signatera to get clinically relevant biomarkers (e.g., *BRCA1/2*, *NTRK* fusions, MSI, TMB) and MRD monitoring with no additional sample. Altera utilizes whole-exome and whole-transcriptome sequencing.
Add Empower™ hereditary cancer test to inform treatment options following a cancer diagnosis.

BRCA=breast cancer gene; ctDNA=circulating tumor DNA; MRD=molecular residual disease; MSI=microsatellite instability; *NTRK*=neurotrophic tropomyosin receptor kinase; TMB=tumor mutational burden.

Reference: 1. Coombes RC, Page K, Salari R, et al. Personalized detection of circulating tumor DNA antedates breast cancer metastatic recurrence. *Clin Cancer Res*. 2019;25(14):4255-4263. doi:10.1158/1078-0432.CCR-18-3663

Triple-negative breast cancer (TNBC) case review*

Are you missing early-stage recurrence during surveillance?

- **Histological subtype:** TNBC
- **Age:** 57
- **Initial diagnosis:** Stage IA, grade 2, cT1N0M0 in right breast
- **Neoadjuvant therapy:** Fluorouracil (5-FU), epirubicin, cyclophosphamide + docetaxel
- **Surgery:** Lumpectomy
- **Pathology results:** 9-mm invasive residual disease ypT1ypN0 tumor
- **Adjuvant therapy:** Radiation therapy

*Adapted from a real-world Signatera patient case.
Case features modified to protect patient confidentiality. No treatment recommendations are made or should be implied.

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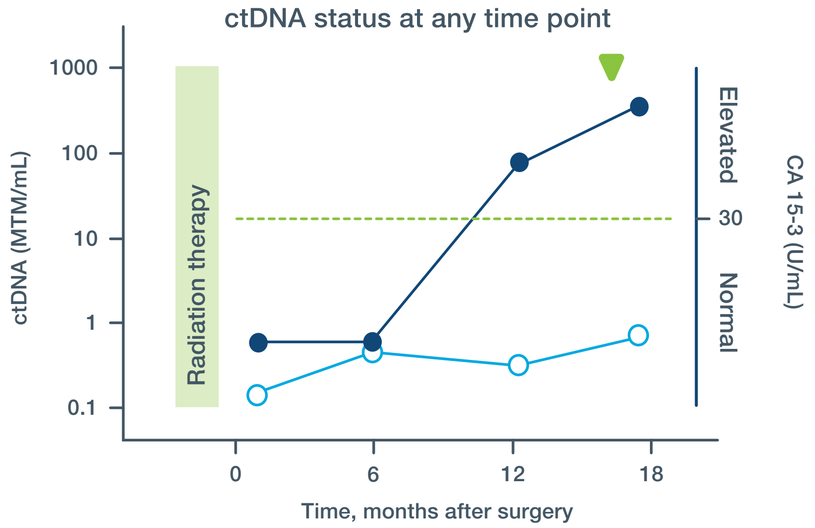
Assessment of ctDNA detects relapse months before that of CA 15-3



- **Molecular surveillance:** ctDNA levels spiked 12 months after radiation therapy while CA 15-3 levels remained at baseline
- **Clinical confirmation of relapse:** Cancer recurrence found in clavicle and axillary lymph nodes

To find clinical trials actively enrolling patients in the surveillance setting, search “ctDNA” on clinicaltrials.gov

ctDNA=circulating tumor DNA; MTM=mean number of tumor molecules.

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Clinical trials actively enrolling in the surveillance setting

- **DARE (NCT04567420): DNA-guided second-line adjuvant therapy for high-residual risk, Stage II-III, HR+ HER2- breast cancer[1]**
- **LEADER (NCT03285412): ribociclib, a CDK 4/6 inhibitor, with adjuvant endocrine therapy for ER+ breast cancer[2]**
- **ZEST (NCT04915755): efficacy and safety comparison of niraparib vs placebo for participants with either HER2- breast cancer susceptibility gene mutation (*BRCA*mut) or TNBC with molecular disease[3]**

BRCA=breast cancer gene; CDK=cyclin-dependent kinase; ER=estrogen receptor; HER2=human epidermal growth factor receptor 2; HR=hormone receptor; TNBC=triple-negative breast cancer.

References: 1. DNA-guided second line adjuvant therapy for high residual risk, stage II-III, hormone receptor positive, HER2 negative breast cancer (DARE). ClinicalTrials.gov identifier: NCT04567420. Updated November 5, 2021. Accessed November 12, 2021. https://clinicaltrials.gov/ct2/show/NCT04567420?term=NCT04567420&draw=2&rank=1 **2.** CDK 4/6 inhibitor, ribociclib, with adjuvant endocrine therapy for ER-positive breast cancer. ClinicalTrials.gov identifier: NCT03285412. Updated October 27, 2021. Accessed November 12, 2021. https://clinicaltrials.gov/ct2/show/NCT03285412?term=NCT03285412&draw=2&rank=1 **3.** Efficacy and safety comparison of niraparib to placebo in participants with either human epidermal growth factor 2 negative (HER2-) breast cancer susceptibility gene mutation (BRCAmut) or triple-negative breast cancer (TNBC) with molecular disease. ClinicalTrials.gov identifier: NCT04915755. Updated October 21, 2021. Accessed November 12, 2021. https://clinicaltrials.gov/ct2/show/study/NCT04915755?term=NCT04915755&draw=2&rank=1

Colorectal cancer (CRC)

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Neoadjuvant	Adjuvant therapy	Surveillance	Metastatic
Response monitoring	**Postsurgical MRD assessment**	**Recurrence monitoring**	**Assess treatment response**
May be considered for select “watch and wait” patients to support a nonsurgical approach.	Use Signatera after surgery to evaluate the need for adjuvant chemotherapy and potentially avoid unnecessary treatment.	Use Signatera along with CEA testing to detect recurrence earlier while the tumor may still be resectable.	Signatera enables real-time monitoring of ctDNA kinetics for early and sensitive evaluation of treatment response.

Signatera accurately identifies patients at high risk of recurrence[1]

97% **of patients with a positive Signatera result will relapse without additional treatment**

Only 3% of patients who test negative serially will relapse

CEA=carcinoembryonic antigen; ctDNA=circulating tumor DNA; MRD=molecular residual disease.

Reference: 1. Reinert T, Henriksen TV, Christensen E, et al. Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer. *JAMA Oncol*. 2019;5(8):1124-1131. doi:10.1001/jamaoncol.2019.0528

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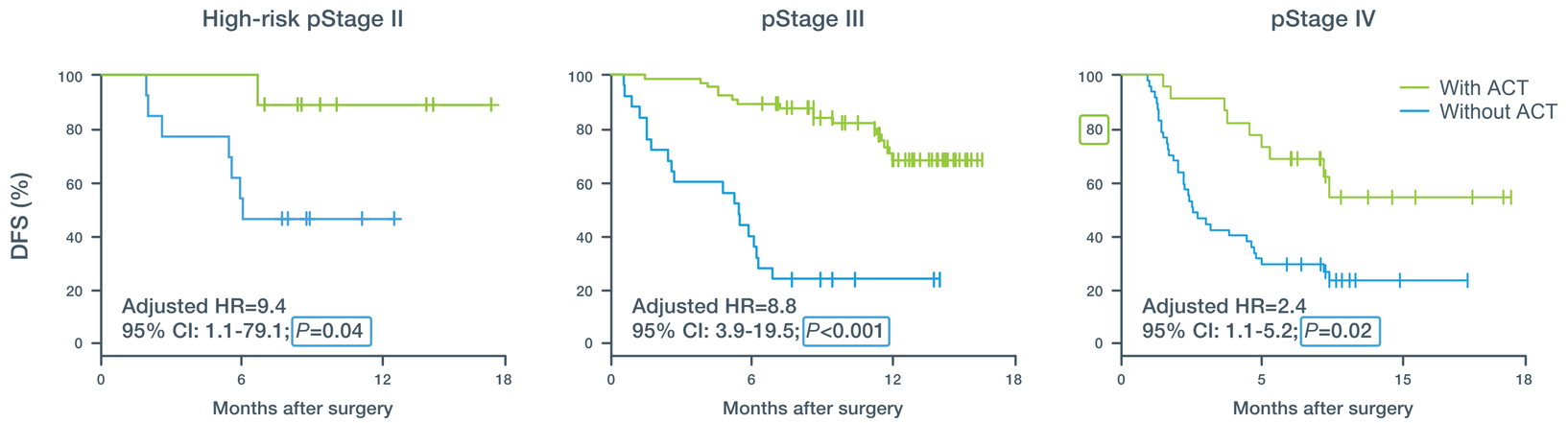
MRD-positive patients benefit significantly from adjuvant chemotherapy[1]



Add the Altera™ tumor genomic profiling test when you order Signatera to get clinically relevant biomarkers (e.g., *EGFR*, *KRAS*, *NRAS*, *BRAF*, MET, MSI, TMB) and MRD monitoring with no additional sample. Altera utilizes whole-exome and whole-transcriptome sequencing.
Add Empower™ hereditary cancer test to inform treatment options following a cancer diagnosis.

ACT=adjuvant chemotherapy; *BRAF*=B-Raf oncogene; DFS=disease-free survival; *EGFR*=epidermal growth factor receptor; *KRAS*=Kirsten rat sarcoma (RAS) viral oncogene homolog; MET=mesenchymal–epithelial transition; MRD=molecular residual disease; MSI=microsatellite instability; *NRAS*=neuroblastoma RAS viral oncogene homolog; pStage=pathologic stage; TMB=tumor mutational burden.

Reference: 1. Kotaka M, Shirasu H, Watanabe J, et al. Association of circulating tumor DNA dynamics with clinical outcomes in the adjuvant setting for patients with colorectal cancer from an observational GALAXY study in CIRCULATE-Japan. Presented at: 2022 ASCO Gastrointestinal Cancers Symposium; January 20-22, 2022; San Francisco, CA.

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Stage II colon cancer review*

For patients with high-risk clinicopathologic risk features, how would you monitor your patients for early recurrence?

- **Molecular profile:** MSS/MSI-L, *BRAF* WT, *KRAS* p.G12V
- **Age:** 69-year-old male with T3N0Mx R-sided colon adenocarcinoma
- **Pathology:** PNI+, negative margins, 35LN-
- **Adjuvant treatment:** XELOX

*Adapted from a real-world Signatera patient case.

BRAF=B-Raf oncogene; CNS=central nervous system; *KRAS*=Kirsten rat sarcoma (RAS) viral oncogene homolog; MSI-L=microsatellite instability-low; MSS=microsatellite stability; PNI=perineural invasion; WT=wild type; XELOX=capecitabine oxaliplatin regimen.

Case features modified to protect patient confidentiality. No treatment recommendations are made or should be implied.

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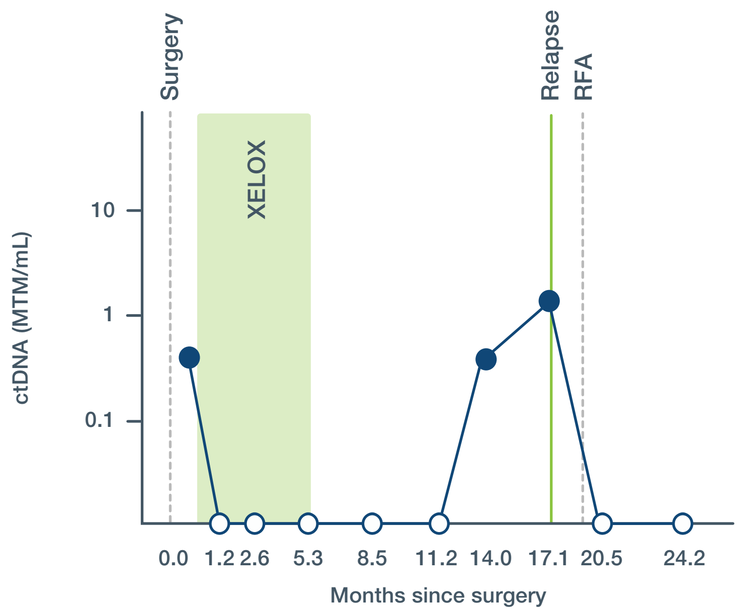
Signatera enables ultrasensitive and specific detection of MRD to allow for early intervention[1]

Historical results



- Signatera™ test became positive 14 months after surgery
- As a result, physician increased frequency of CT scans and considered escalation to PET
- 3 months later, a 2-cm hypodense lesion was found in the liver by CT scan
- Patient underwent RFA with curative intent and remains disease free at 2-year follow-up

CT=computed tomography; ctDNA=circulating tumor DNA; MRD=molecular residual disease; MTM=mean number of tumor molecules; PET=positron emission tomography; RFA=radiofrequency ablation; XELOX=capecitabine oxaliplatin regimen.

Reference: 1. Reinert T, Henriksen TV, Christensen E, et al. Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer. *JAMA Oncol*. 2019;5(8):1124-1131. doi:10.1001/jamaoncol.2019.0528

Gynecological cancers

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Neoadjuvant → **First-line therapy** → **Surveillance/ maintenance** → **Second-line therapy**

MRD assessment for maintenance

Ovarian: Use to risk-stratify patients to inform maintenance therapy with PARPi (e.g., non-*BRCA*m, HRD+ patients)

Recurrence monitoring

- **Ovarian:** Use alongside CA-125 when results are indeterminate or when CA-125 is not expressed (~20%)
- **Cervical:** No biomarkers—imaging difficult to interpret after full pelvic radiation
- **Endometrial:** No biomarkers—guidelines do not recommend imaging without clinical symptoms

Assess treatment effectiveness

- **Ovarian:** Use serially in MSI-H patients to monitor response to pembrolizumab when CA-125 is tough to interpret[1]
- **Cervical:** Use serially in PD-L1 high patients to monitor response to single-agent pembrolizumab[1]
- **Endometrial:** Monitor for exceptional response to pembrolizumab + lenvatinib in non-MSI-H patients or with dostarlimab in dMMR patients

BRCA=breast cancer gene; dMMR=deficient MisMatch repair; HRD=homologous recombination deficiency; MRD=molecular residual disease; MSI-H=microsatellite instability-high; PARPi=poly(adenosine diphosphate-ribose) polymerase inhibitor; PD-1=programmed death-ligand 1.

Reference: 1. Bratman SV, Yang SYC, Iafolla MAJ, et al. Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. *Nat Cancer*. 2020;1:873-881. doi:10.1038/s43018-020-0096-5

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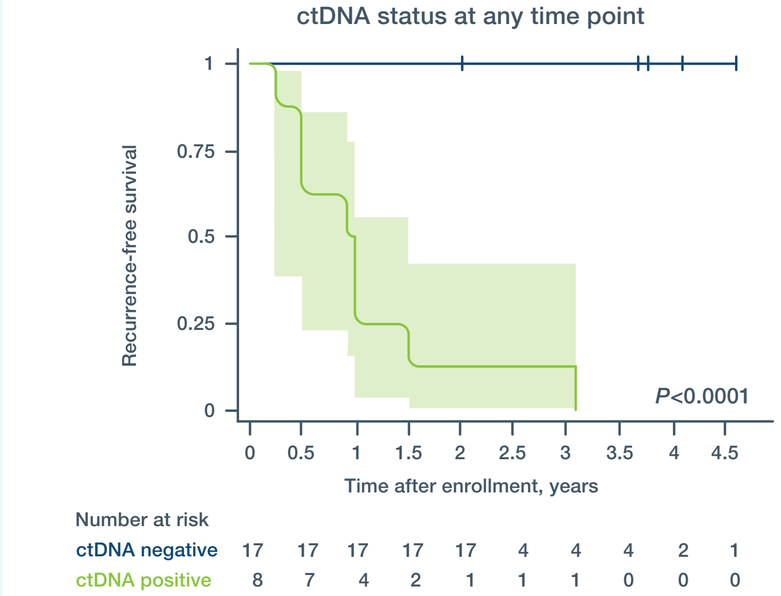
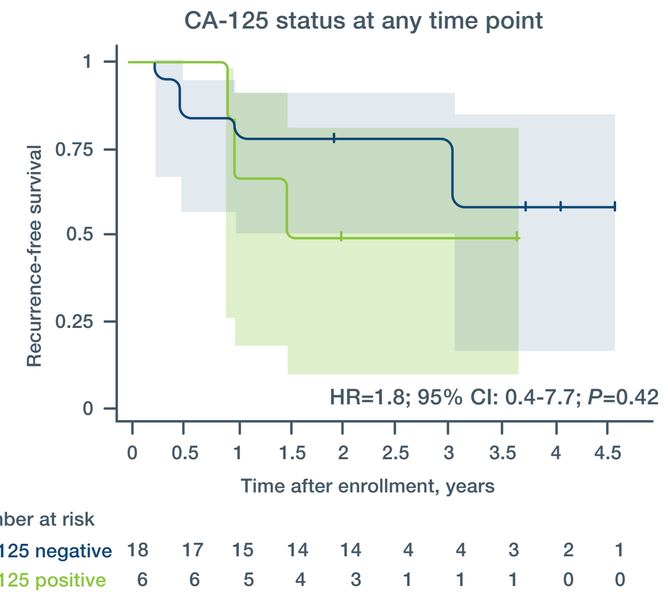
In ovarian cancer, longitudinal Signatera MRD assessment was the strongest predictor of disease recurrence[1]





- **In the longitudinal setting**, all MRD-positive patients experienced relapse (8/8), while all MRD-negative patients (17/17) remained recurrence free, yielding a sensitivity and specificity of 100%
- **Signatera detected recurrence** an average of 10 months earlier vs <1 month earlier for the CA-125 test

Add the Altera™ tumor genomic profiling test when you order Signatera to get clinically relevant biomarkers (e.g., *BRCA1/2*, *NTRK* fusions, MSI, TMB) and MRD monitoring with no additional sample. Altera utilizes whole-exome and whole-transcriptome sequencing.
Add Empower™ hereditary cancer test to inform treatment options following a cancer diagnosis.

BRCA=breast cancer gene; ctDNA=circulating tumor DNA; MRD=molecular residual disease; MSI=microsatellite instability; *NTRK*=neurotrophic tropomyosin receptor kinase; TMB=tumor mutational burden.

Reference: 1. Chapman JS, Pierson WE, Smith-McCune K, et al. Circulating tumor DNA predicts disease recurrence in ovarian cancer patients. Presented at: American Association of Cancer Research; April 9-14, 2021; Virtual.

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Ovarian cancer case review*

Is the tumor truly progressing?

- **Molecular profile:** *BRCA2+*
- **Age:** 67
- **Diagnosis:** Stage IIIC (T3N1Mx) HGSOC
- **Sites of metastasis:** Brain, aortocaval lymph node
- **Treatment history:** Received upfront neoadjuvant paclitaxel/cisplatin followed by an interval cytoreductive procedure. Received carboplatin/docetaxel in the adjuvant setting. Subsequently underwent a craniotomy for recurrence and olaparib was initiated.*

*Adapted from a real-world Signatera patient case.
BRCA=breast cancer gene; HGSOC=high-grade serous ovarian cancer.
Case features modified to protect patient confidentiality. No treatment recommendations are made or should be implied.

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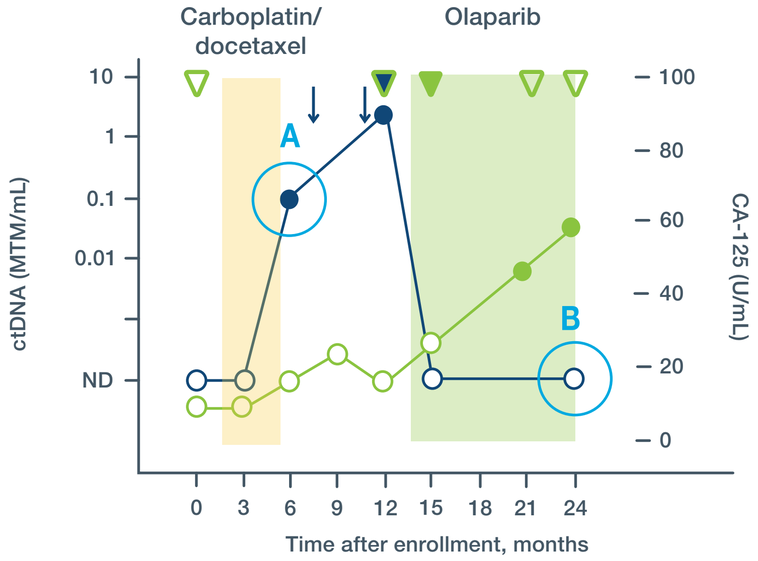
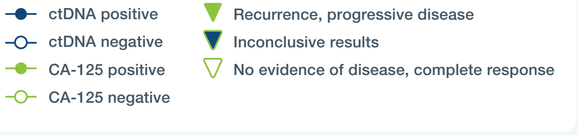
Signatera shows superior performance over CA-125 to assess risk of radiologic recurrence, with greater lead time[1]





A Signatera identified recurrence after completion of neoadjuvant and first-line therapy, approximately 6 months ahead of radiographic findings; CA-125 remained undetectable during that time

B Signatera showed response to PARP inhibition (olaparib) prior to imaging, while CA-125 continued to rise

ctDNA kinetics can be used to monitor treatment effectiveness in the maintenance setting with IO therapy[2]

ctDNA=circulating tumor DNA; IO=immuno-oncology; MTM=mean number of tumor molecules; PARP=poly(adenosine diphosphate-ribose) polymerase.

References: 1. Chapman JS, Pierson WE, Smith-McCune K, et al. Circulating tumor DNA predicts disease recurrence in ovarian cancer patients. Presented at: American Association of Cancer Research; April 9-14, 2021; Virtual.
2. Bratman SV, Yang SYC, Iafolla MAJ, et al. Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. *Nat Cancer*. 2020;1:873-881. doi:10.1038/s43018-020-0096-5

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Neoadjuvant

Adjuvant

Postsurgical MRD assessment

Use MRD status to identify patients who may benefit from adjuvant treatment with IO PD-1 (nivolumab), IO CTLA-4 (ipilimumab), or *BRAF*/MEK inhibitors (*BRAF* V600) vs observation.

Surveillance

Recurrence monitoring

Monitor and detect recurrence earlier with serial ctDNA measurements.

Metastatic

Assess treatment effectiveness

- Monitor ctDNA kinetics to help determine whether ipilimumab should be added to single-agent nivolumab
- Confirm pseudoprogression
- Identify exceptional responders and assess whether a treatment break is appropriate

BRAF=B-Raf oncogene; ctDNA=circulating tumor DNA; CTLA-4=cytotoxic T-lymphocyte associated antigen 4; IO=immuno-oncology; MEK=mitogen-activated extracellular signal-regulated kinase; MRD=molecular residual disease; PD-1=programmed cell death protein 1.

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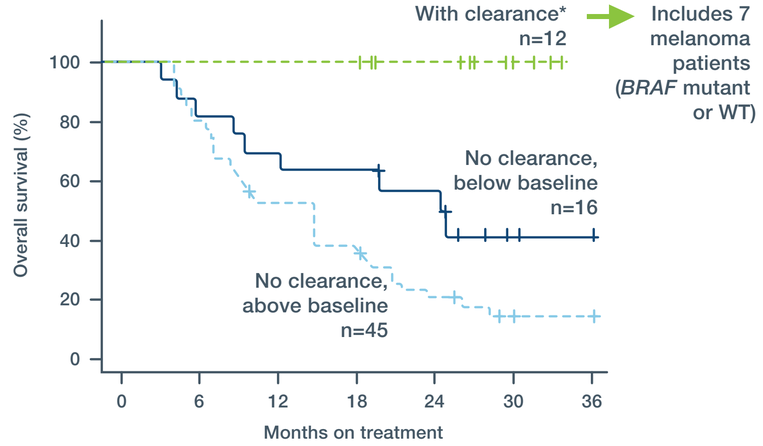
Combined with standard imaging, Signatera predicts benefits of immunotherapy as early as week 6 with ctDNA kinetics[1]

98% **of patients (92/94) had detectable baseline ctDNA**

None of the patients with an increase in both ctDNA level and tumor size at week 6 achieved OR at any time during the study



*Median follow-up beyond first clearance of 25.4 months (range 10.8-29.5).

Clearance of ctDNA at any time point results in superior clinical outcomes
OS was 100% in patients who achieved ctDNA clearance at least once during treatment (median follow-up of 25.4 months beyond first clearance)

Add the Altera™ tumor genomic profiling test when you order Signatera to get clinically relevant biomarkers (e.g., *BRAF*, *NRAS*, *KIT*, *NTRK* fusions, MSI, TMB) and MRD monitoring with no additional sample. Altera utilizes whole-exome and whole-transcriptome sequencing.
Add Empower™ hereditary cancer test to inform treatment options following a cancer diagnosis.

BRAF=B-Raf oncogene; ctDNA=circulating tumor DNA; *KIT*=receptor tyrosine kinase; MRD=molecular residual disease; MSI=microsatellite instability; *NRAS*=neuroblastoma rat sarcoma (RAS) viral oncogene homolog; *NTRK*=neurotrophic tropomyosin receptor kinase; OR=objective response; OS=overall survival; TMB=tumor mutational burden; WT=wild type.

Reference: 1. Bratman SV, Yang SYC, Iafolla MAJ, et al. Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. *Nat Cancer*. 2020;1:873-881. doi:10.1038/s43018-020-0096-5

Melanoma case review*

When should a change in therapy be considered?

- **Molecular profile:** *BRAF* WT
- **Age:** 69
- **Site of metastasis:** Lung
- **First-line treatment:** Nivolumab
- **Treatment history:** Patient had no CNS metastasis and had good performance status. Nivolumab monotherapy was chosen after weighing tolerability vs potential clinical benefit

*Adapted from a real-world Signatera patient case.
BRAF=B-Raf oncogene; CNS=central nervous system; WT=wild type.
Case features modified to protect patient confidentiality. No treatment recommendations are made or should be implied.

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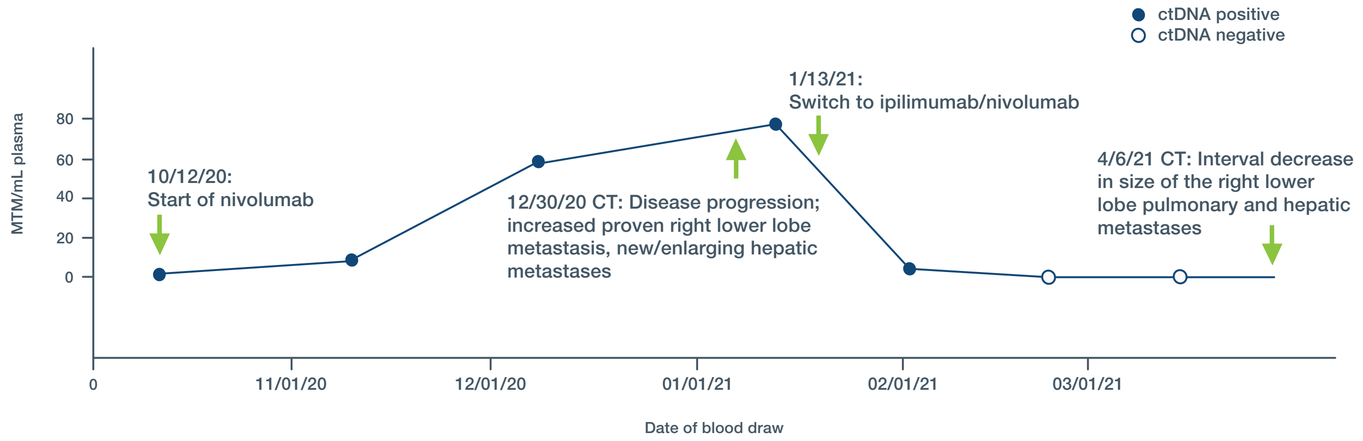
Signatera ctDNA analysis can help clarify when the addition of ipilimumab may improve tumor response in patients treated with single-agent nivolumab[1]

Historical results



- **Progression on nivolumab:** Growth of lung metastases and appearance of new hepatic metastasis was observed at week 10 of nivolumab treatment
- **Switch to PD-1+ CTLA-4 doublet:** Signatera ctDNA analysis confirmed progression by imaging. Ipilimumab was added to nivolumab monotherapy
- **Partial response and clearance of ctDNA:** After switching to the immunotherapy doublet, Signatera ctDNA cleared; later confirmed by decrease in size of metastases on CT scans

CT=computed tomography; ctDNA=circulating tumor DNA; CTLA-4=cytotoxic T-lymphocyte-associated antigen 4; MTM=mean number of tumor molecules; PD-1=programmed cell death protein 1.

Non-small cell lung cancer (NSCLC)

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Neoadjuvant

Response monitoring

Postsurgical MRD assessment

IO will likely become a part of neoadjuvant and adjuvant treatment in the future. Use MRD status and ctDNA kinetics to help inform treatment decisions, such as when and for which patients to add IO to neoadjuvant or adjuvant chemotherapy.

Surveillance

Recurrence monitoring

No biomarkers—detect recurrence earlier with serial measurements.

Advanced/metastatic

Assess treatment effectiveness

- **Stage III (unresectable):** After definitive CRT, identify patients who may benefit from durvalumab consolidation, or determine optimal length of durvalumab therapy
- **Stage IV:** Monitor ctDNA kinetics to decide whether platinum/pemetrexed should be added to single-agent pembrolizumab in high PD-L1 expression
- Confirm pseudoprogression
- Identify exceptional responders and assess whether a treatment break is appropriate

CRT=conformal radiation therapy; ctDNA=circulating tumor DNA; IO=immuno-oncology; MRD=molecular residual disease; PD-L1=programmed death-ligand 1.

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Combined with standard imaging, Signatera predicts benefits of immunotherapy as early as week 6 with ctDNA kinetics[1]

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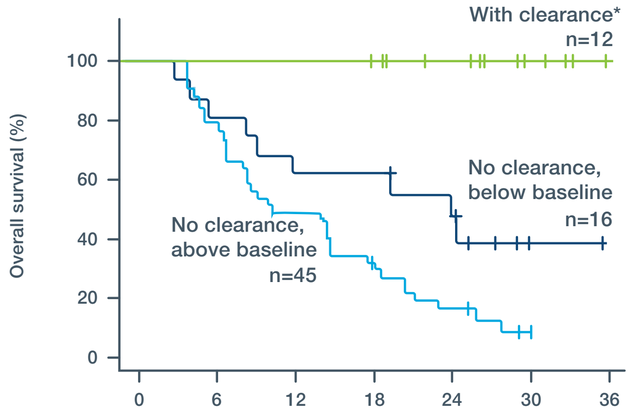
None of the patients with an increase in both ctDNA level and tumor size at week 6 achieved OR at any time during the study



*Median follow-up beyond first clearance of 25.4 months (range 10.8-29.5).

Clearance of ctDNA at any time point correlates with favorable clinical outcomes[1]
OS was 100% in patients who achieved ctDNA clearance at least once during treatment (median follow-up of 25.4 months beyond first clearance)

Add the Altera™ tumor genomic profiling test when you order Signatera to get clinically relevant biomarkers (e.g., *EGFR*, *KRAS*, *ALK*, *ROS1*, MET, RET, *NTRK*, MSI, TMB) and MRD monitoring with no additional sample. Altera utilizes whole-exome and whole-transcriptome sequencing.
Add Empower™ hereditary cancer test to inform treatment options following a cancer diagnosis.

ALK=anaplastic lymphoma kinase; ctDNA=circulating tumor DNA; *EGFR*=epidermal growth factor receptor; *KRAS*=Kirsten rat sarcoma (RAS) viral oncogene homolog; MET=mesenchymal–epithelial transition; MRD=molecular residual disease; MSI=microsatellite instability; *NTRK*=neurotrophic tropomyosin receptor kinase; OR=objective response; OS=overall survival; RET=rearranged during transfection; *ROS1*=c-ros oncogene 1; TMB=tumor mutational burden.

Reference: 1. Bratman SV, Yang SYC, Iafolla MAJ, et al. Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. *Nat Cancer*. 2020;1:873-881. doi:10.1038/s43018-020-0096-5

NSCLC case review*

For patients with high PD-L1 starting on single-agent pembrolizumab, when would you decide if a platinum/pemetrexed doublet should be added to increase tumor response?

- **Molecular profile:** No driver mutations
- **PD-L1 expression:** 60%
- **Age:** 68
- **Site of metastasis:** Adrenal glands; lymph nodes in the neck and mediastinum
- **First-line treatment:** Pembrolizumab
- **Treatment history:** Patient received pembrolizumab, first-line, for 10 months before an 8-month loss to follow-up and discontinuation

*Adapted from a real-world Signatera patient case.
NSCLC=non-small cell lung cancer; PD-L1=programmed death-ligand 1.
Case features modified to protect patient confidentiality. No treatment recommendations are made or should be implied.

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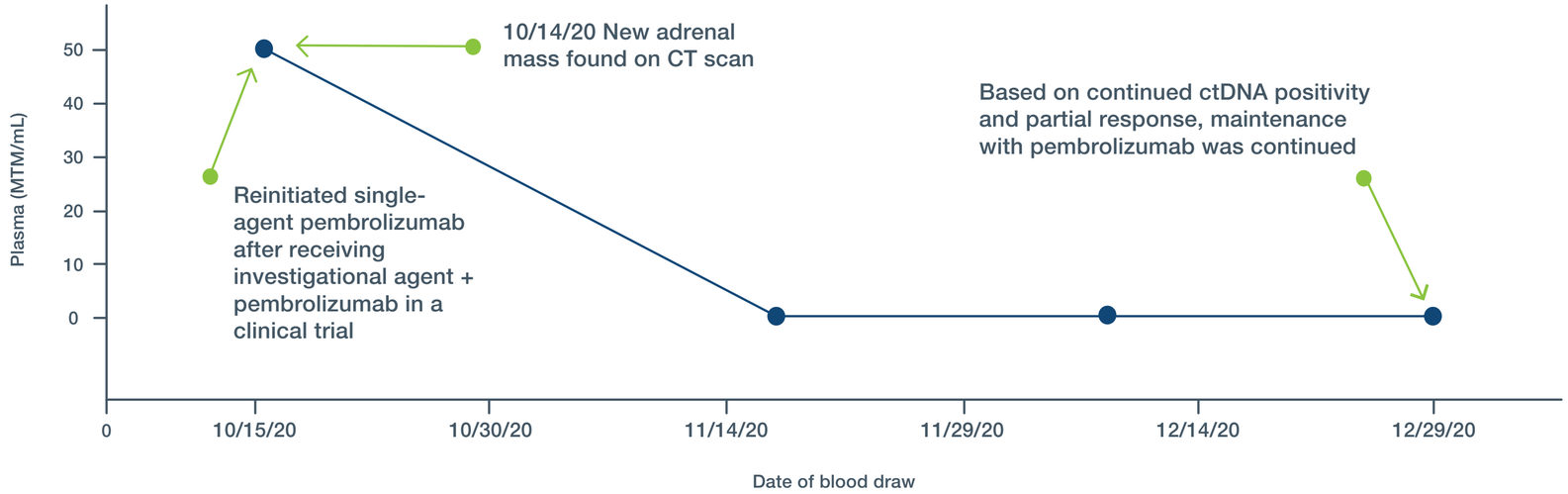
Signatera enables real-time monitoring of ctDNA kinetics for early and sensitive evaluation of treatment response to immunotherapy[1]

Historical results



- **Reinitiating pembrolizumab treatment:** A new adrenal mass was detected on CT scan and Signatera™ ctDNA level was 48.81 MTM/mL
- **One month after reinitiation:** Signatera ctDNA remained positive but declined sharply to 0.20 MTM/mL
- **Serial Signatera testing:** Signatera ctDNA results were consistently positive at low levels of MTM/mL, correlating with partial response on repeat imaging
- **Clinical decision:** Based on the continued ctDNA positivity and partial response, the decision was made to continue pembrolizumab as maintenance

CT=computed tomography; ctDNA=circulating tumor DNA; MTM=mean number of tumor molecules.

Reference: 1. Bratman SV, Yang SYC, Iafolla MAJ, et al. Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. *Nat Cancer*. 2020;1:873-881.