The role of ctDNA in drug development across solid tumors

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natera™ | Signatera™
Residual disease test (MRD)

Agenda

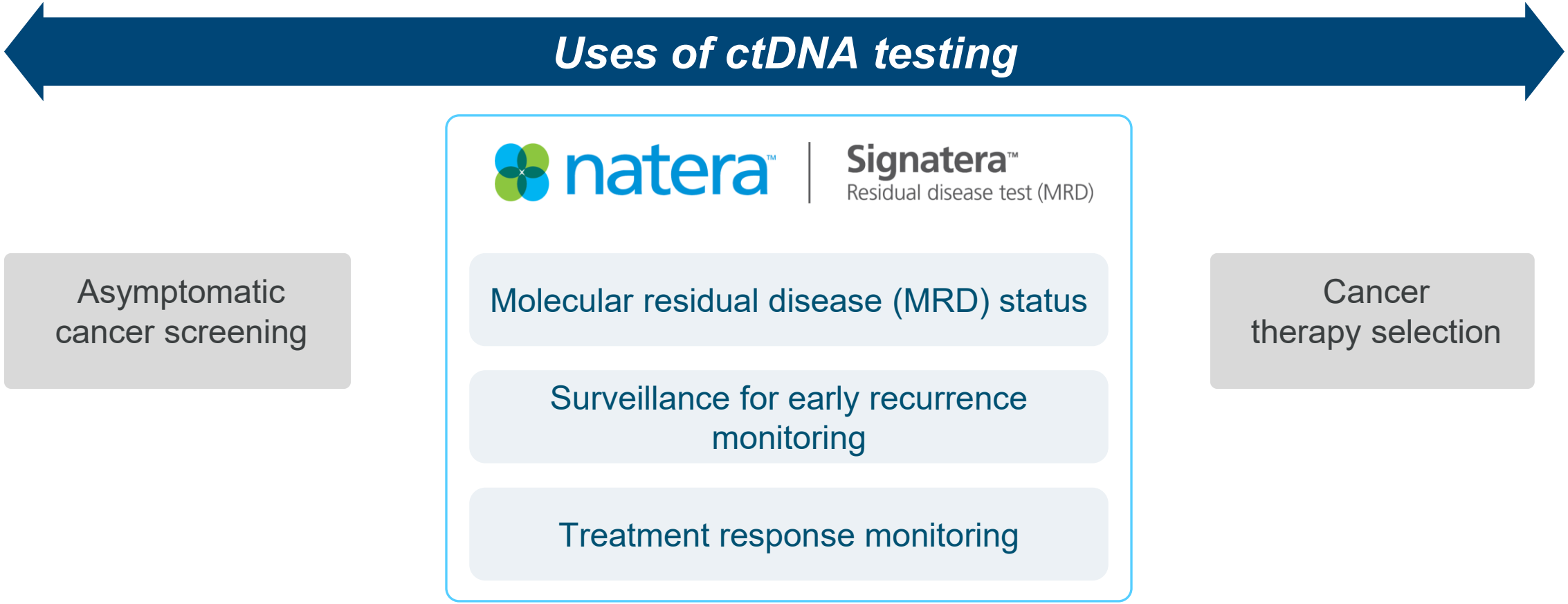
MRD testing technologies

Clinical data

Applications in clinical development

Closing thoughts

ctDNA MRD Testing is focused on residual disease detection and applications in cancer monitoring



Signatera FDA designation and Medicare coverage

Breakthrough Device

3 breakthrough device designations by the FDA

May 2019, March 2021

- As a lab-developed test (LDT), Signatera does not need FDA approval for clinical use
- Breakthrough helps clear regulatory pathway to support biopharma studies

Final coverage for colorectal cancer (CRC)

September 2020

- Finalized a local coverage determination (LCD) to provide Medicare benefits for serial use of Signatera in patients with stage II or III CRC

Draft immunotherapy coverage

September 2020

- Draft LCD proposes coverage of Signatera for immunotherapy response monitoring in all clinically validated solid tumors

Why personalized and tumor-informed?

Technique	Approach	Limit of detection	Advantages/Limitations
Candidate gene analysis qPCR, dPCR, ddPCR	Single-locus or multiplex assays	0.01% to 1%	• Can only query small number of specific variants or mutations concurrently • Only able to monitor known mutations
Tumor-informed NGS CAPP-seq, PCM assay, Signatera™, RaDaR®	Personalized, multiplex PCR targets	<0.01% to 1.0%	• Highly sensitive and specific to detect small traces of ctDNA • Quantitative measurement, ideal for monitoring MRD over time • Requires WES of tumor tissue to design personalized assay
Tumor-naïve NGS SAFE-SeqS, TEC-seq, Guardant360®, FoundationOne®Liquid	Panel-based, targeted sequencing	0.01% to >0.1%	• Does not require a priori knowledge of the molecular alteration • Not designed for monitoring MRD in solid tumors

Sources: 1. Siravegna G, Marsoni S, Siena S. *et al.* Integrating liquid biopsies into the management of cancer. *Nat Rev Clin Oncol 2017;*14:531–548 (2017). https://doi.org/10.1038/nrclinonc.2017. 2. Chin RI, Chen K. Usmani A, *et al.* Detection of Solid Tumor Molecular Residual Disease (MRD) Using Circulating Tumor DNA (ctDNA). *Mol Diagn Ther* 2019;23:311–331. https://doi.org/10.1007/s40291-019-00390-5. 3. Abbosh C, et al. Phylogenetic tracking and minimal residual disease detection using ctDNA in early-stage NSCLC: A lung TRACERx study. AACR Annual Meeting 2020 Virtual Meeting I, CT023. 4. Marsico G, et al. Analytical development of the RaDaR assay, a highly sensitive and specific assay for the monitoring of minimal residual disease. AACR Annual Meeting 2020 Virtual Meeting, Poster no. 3097.

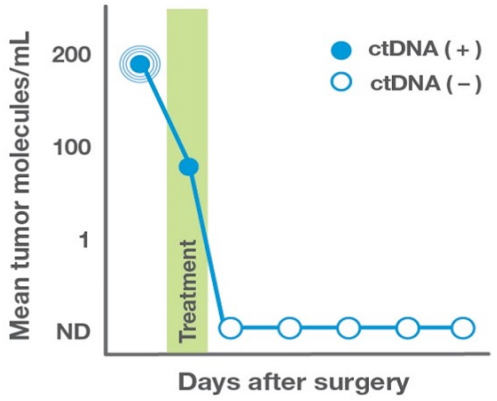
Signatera™ residual disease test (MRD)

The personalized and tumor-informed approach

Sequence tumor tissue to identify unique signature of tumor mutations

Custom design and manufacture personalized mPCR assay for each patient, targeting top clonal mutations found in tumor

Use personalized assay to test patient's blood for presence of circulating tumor DNA (ctDNA)



What is needed in an AV study| High sensitivity, high specificity

- Sensitivity of at least 0.01% VAF
- LOD confirmation with clinical samples
- Specificity needs to be > 99%
- Well designed AV studies
- Consistent performance across multiple patient samples

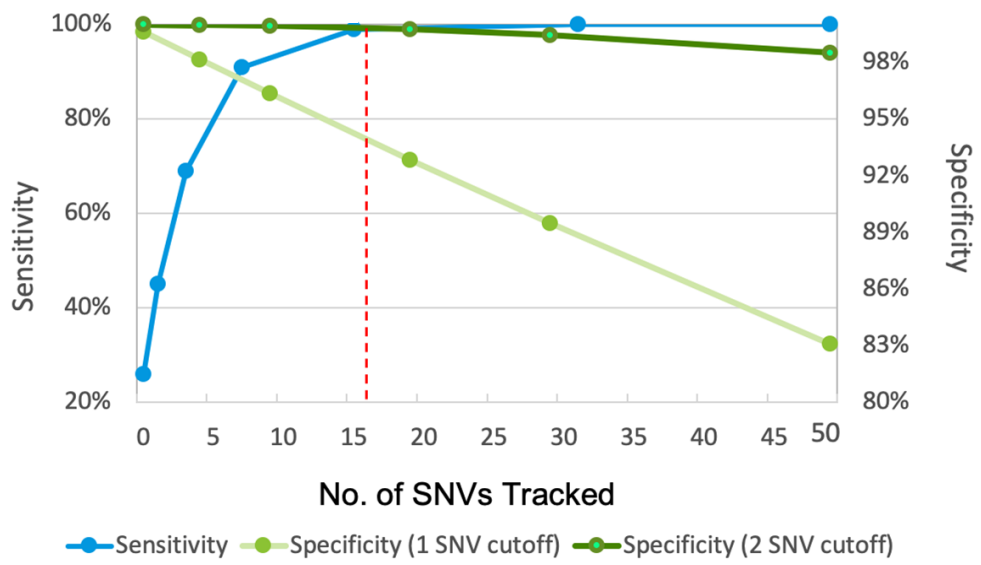
Proportion of positive results (PPR) for estimating analytical sensitivity

Intended MTM per mL plasma	Intended VAF	Empirical PPR pos/[pos+neg]
0.15	0.0075%	72/76 (94.7%)
0.2	0.0100%	75/76 (98.7%)
0.3	0.0150%	76/76 (100%)
0.4	0.0200%	76/76 (100%)

Source: Natera internal data from Signatera analytical validation

Optimizing sensitivity and specificity: a delicate balance

Expected sample sensitivity and specificity by variants tracked



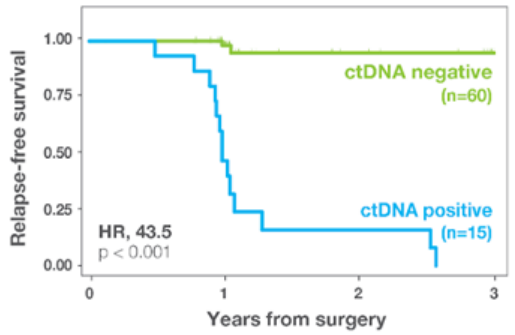
- Adding more targets above 16:
  - Negative impact to specificity
  - Unclear impact to sensitivity
  - Higher failure rate in certain histologies
- 16 ensures stable algorithm and workflow across histologies
  - Variable targets per patient complicates development, validation and commercialization

1. Abbosh C, Birkbak NJ, Wilson GA, et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature. 2017;545(7655):446-451. 2. Natera internal analysis.

High performance and consistent results across multiple tumor types

JAMA ONCOLOGY

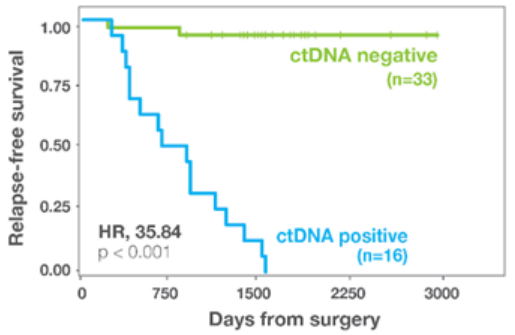
Colon[1]



88% sensitivity to relapse
Average lead time 8.7 mos

CLINICAL CANCER RESEARCH

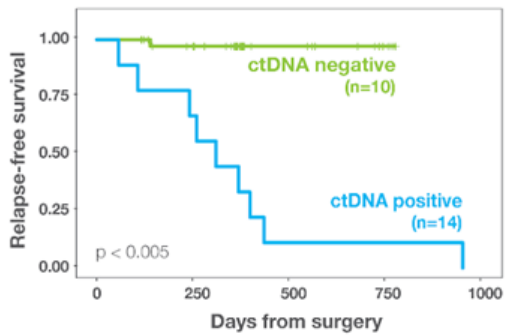
Breast[2]



89% sensitivity to relapse
Average lead time 9.5 mos

NATURE

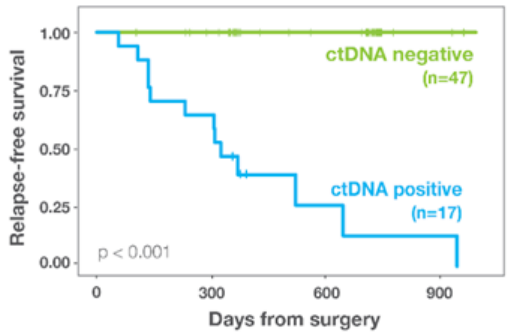
Lung[3]



92% sensitivity to relapse
Average lead time 4.0 mos

JOURNAL OF CLINICAL ONCOLOGY

Bladder[4]



100% sensitivity to relapse
Average lead time 2.8 mos

Positive Signatera result, without further treatment, has predicted relapse with overall PPV > 98%.[1-4]

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. 2. 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. 3. Abbosh C, Birkbak NJ, Wilson GA, et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. *Nature*. 2017;545(7655):446-451. 4. Christensen E, Birkenkamp-Demtroder 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.

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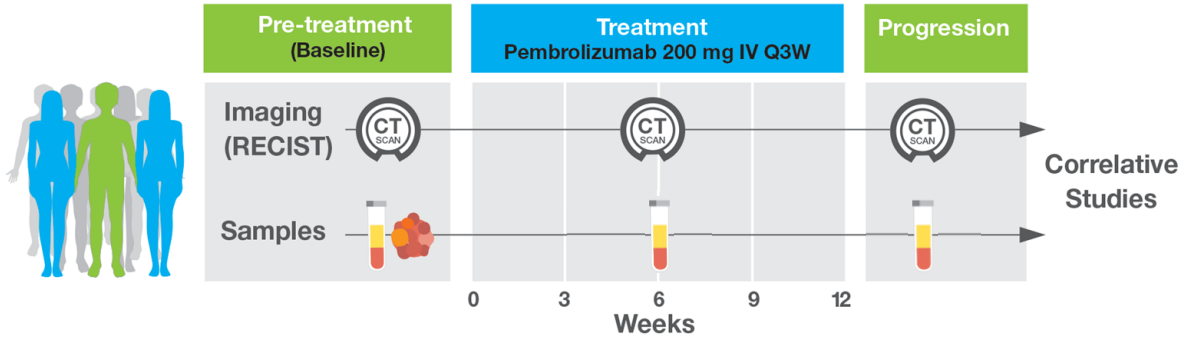
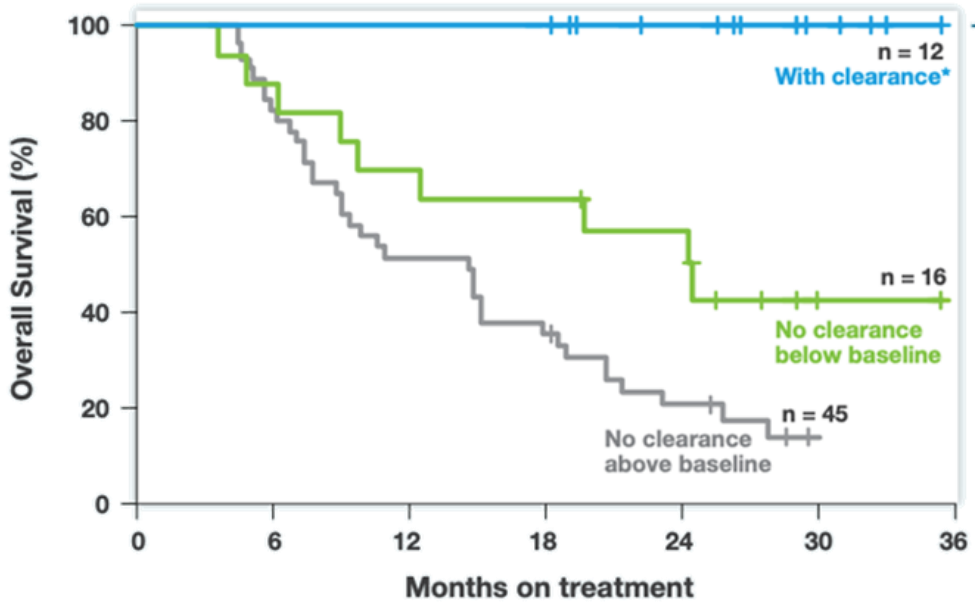
Decrease from baseline or clearance of ctDNA is predictive of outcomes

INSPIRE Trial



94 patients with various solid tumors in the Phase II INSPIRE trial treated with single agent pembrolizumab (200 mg IV q3W).

98% **of patients had detectable baseline ctDNA**



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

1. Bratman SV, Yang SMC, 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.

IMvigor010 data demonstrated the predictive power of Signatera ctDNA for treatment benefit

809 patients in IMvigor010 ITT population

- Atezolizumab (n=406)
- Observation (n=403)

190 with insufficient tumour, matched normal or plasma sample

4 failed tumour library prep

31 did not have C1D1 data

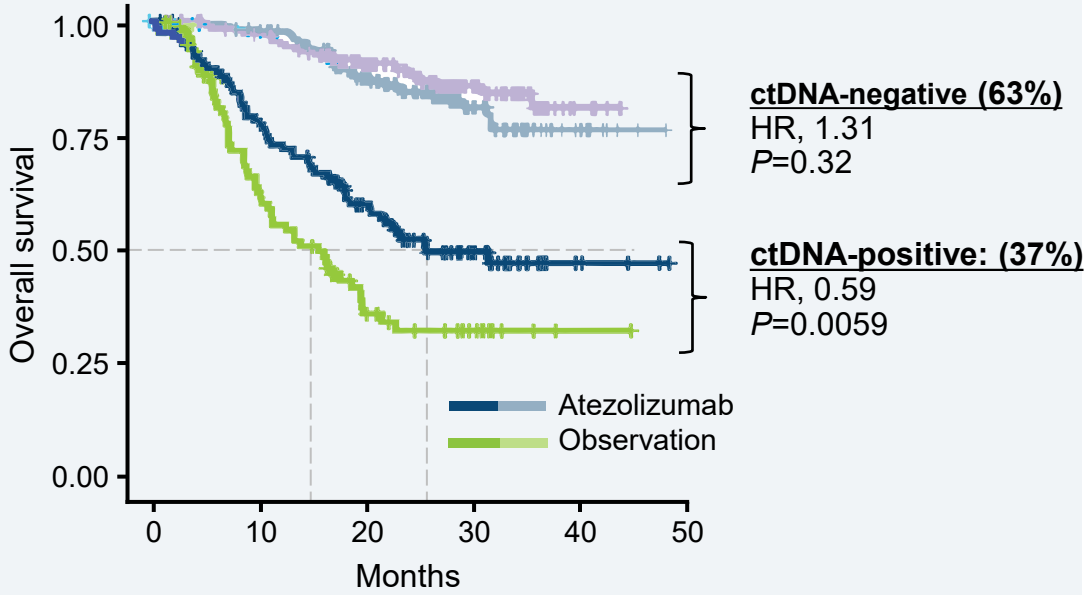
3 failed quality control checks

581 ctDNA-evaluable patients (72% of ITT population)

Observation (n=281)	Atezolizumab (n=300)
ctDNA(+): 98 (35%)	ctDNA(+): 116 (39%)
ctDNA(–): 183 (65%)	ctDNA(–): 184 (61%)

Key results

41% increase in OS benefit for Signatera ctDNA-positive patients treated with atezolizumab, while no treatment benefit was observed in the ctDNA-negative population

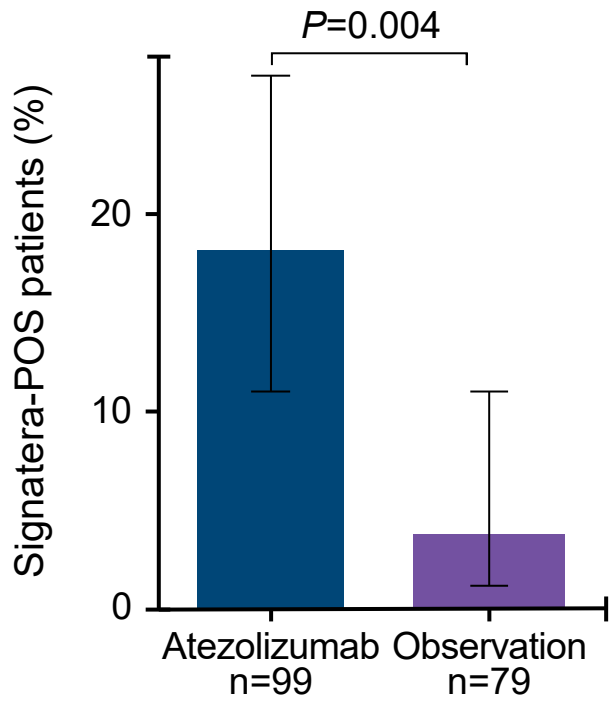


MIBC = muscle invasive bladder cancer

1. Powles T, Assaf ZJ, Davarpanah N, et al. Clinical outcomes in ctDNA-positive urothelial carcinoma patients treated with adjuvant immunotherapy. *Nature.* 2021. *(Accepted)*

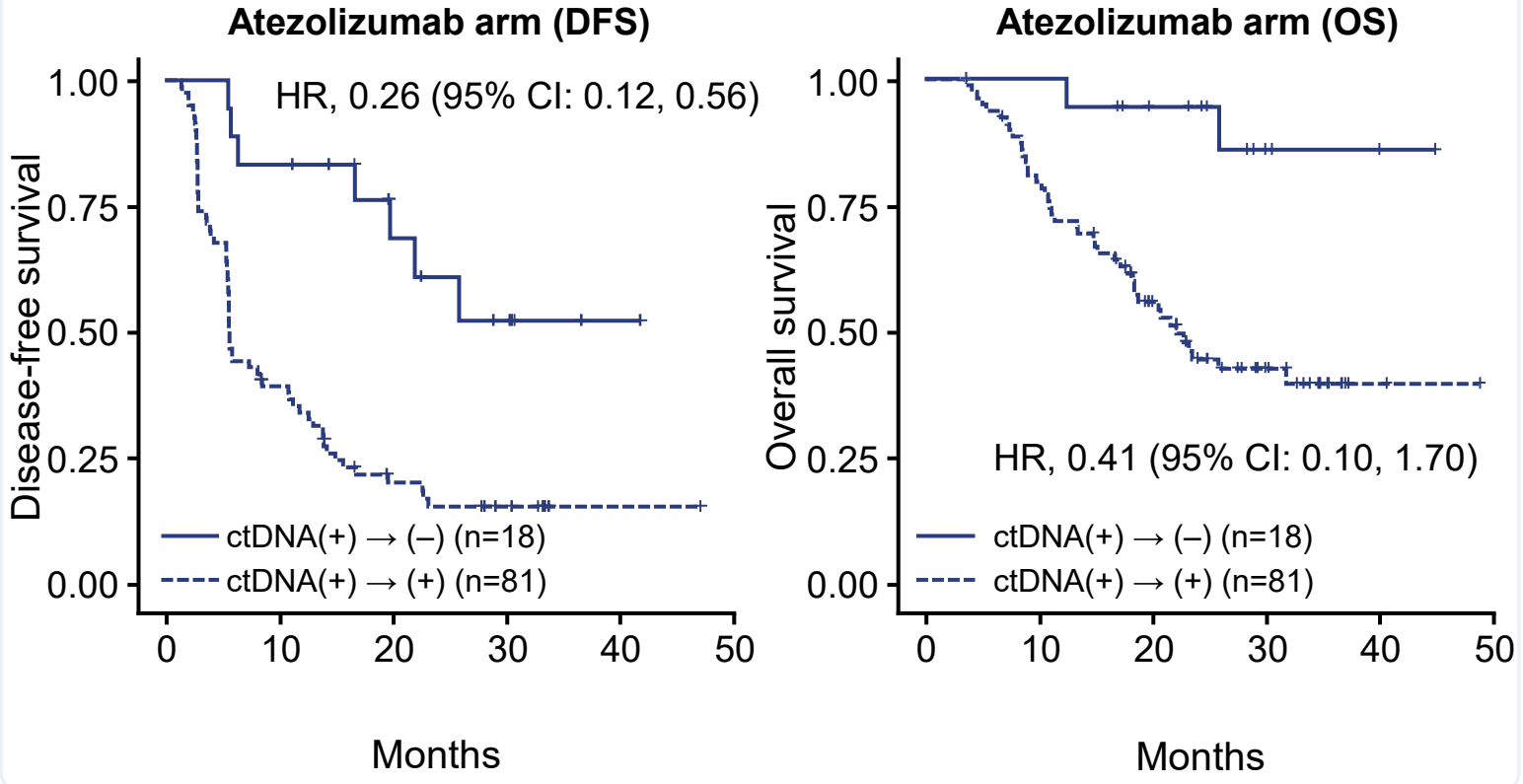
ctDNA clearance was associated with improved outcomes in the atezolizumab arm

ctDNA clearance at C3D1 occurs at higher rate in treatment arm vs. obs



	Atezolizumab n=99	Observation n=79
POS ➔ NEG	**18 (18.8%)**	3 (3.8%)

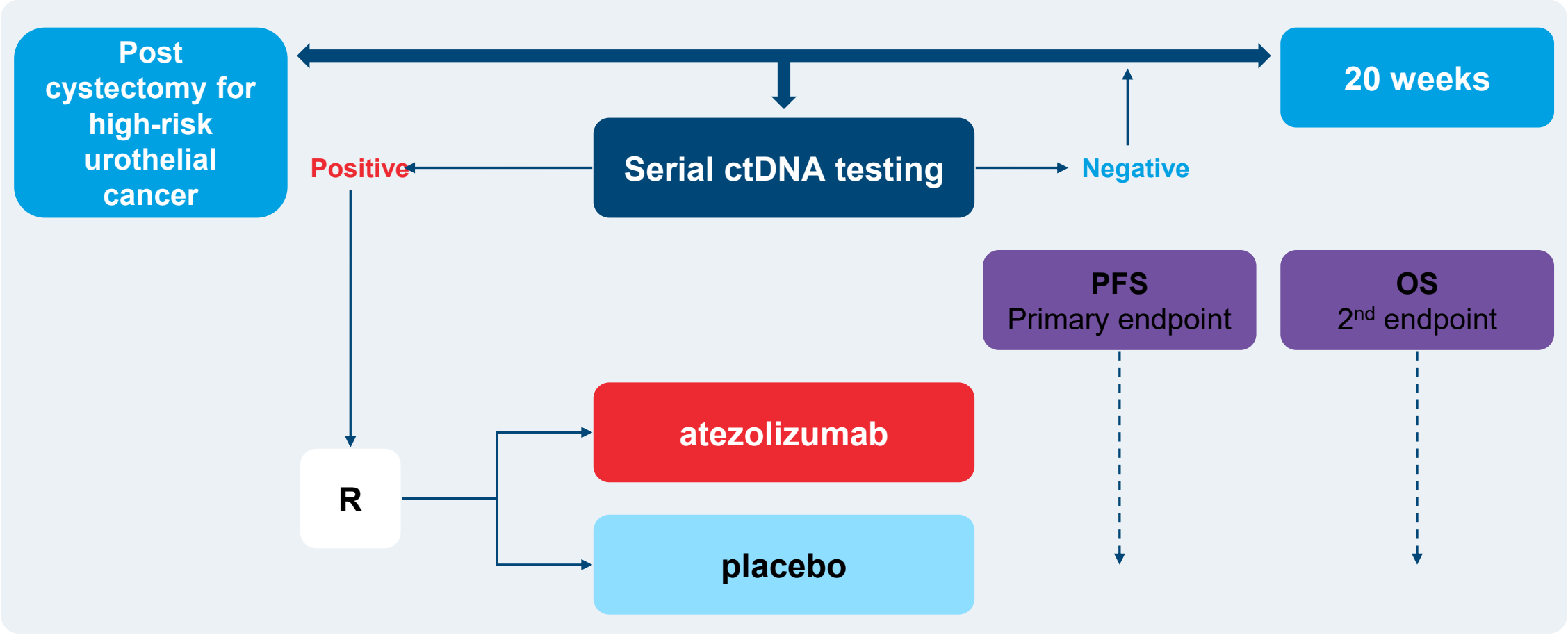
ctDNA clearance associated with improved DFS and OS outcomes in the treatment arm



Assessed using Fisher exact test.

1. Powles T, Assaf ZJ, Davarpanah N, et al. Clinical outcomes in ctDNA-positive urothelial carcinoma patients treated with adjuvant immunotherapy. *Nature.* 2021. *(Accepted)*

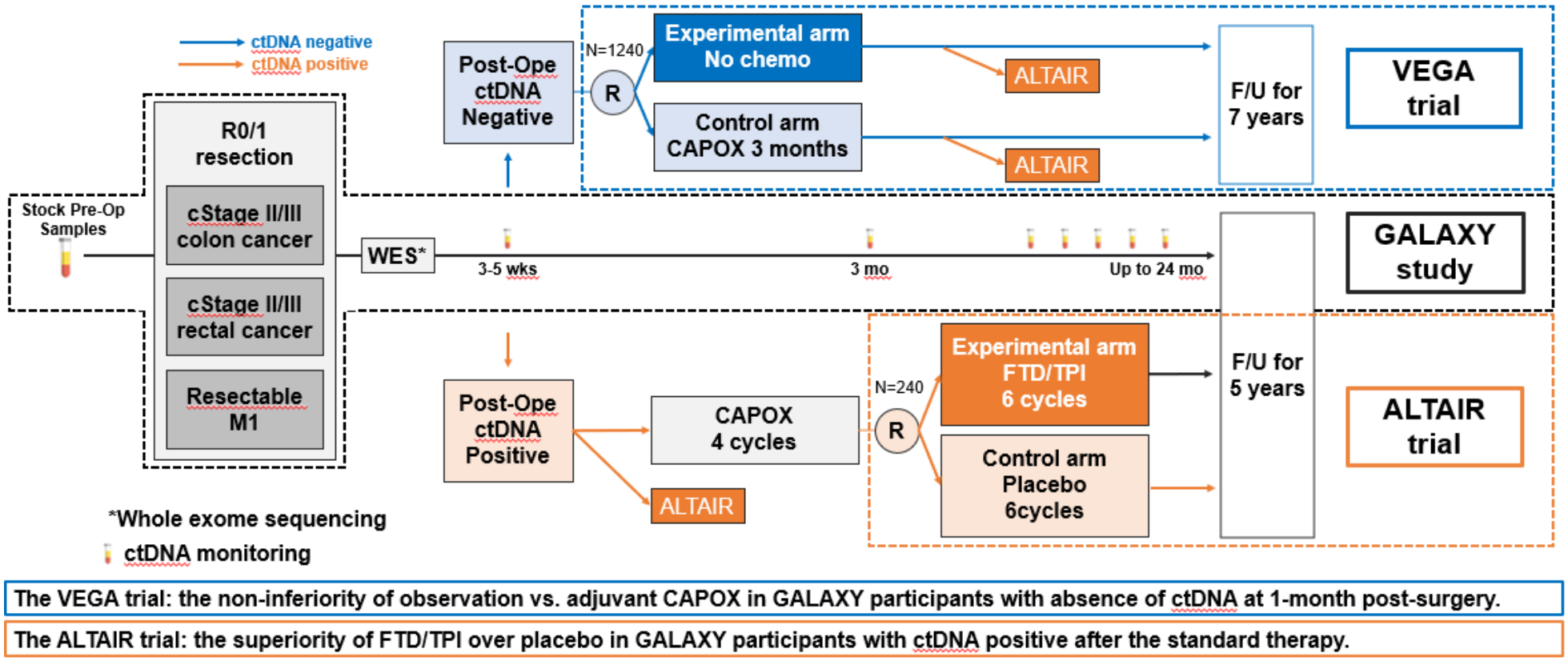
IMvigor011 | Adjuvant atezolizumab vs placebo in MIBC patients who are ctDNA positive following cystectomy



1. NCT04660344. Clinicaltrials.gov. Accessed April 27, 2021.

CIRCULATE Japan| Escalation and de-escalation in Stage II/III CRC

CIRCULATE Japan study schema



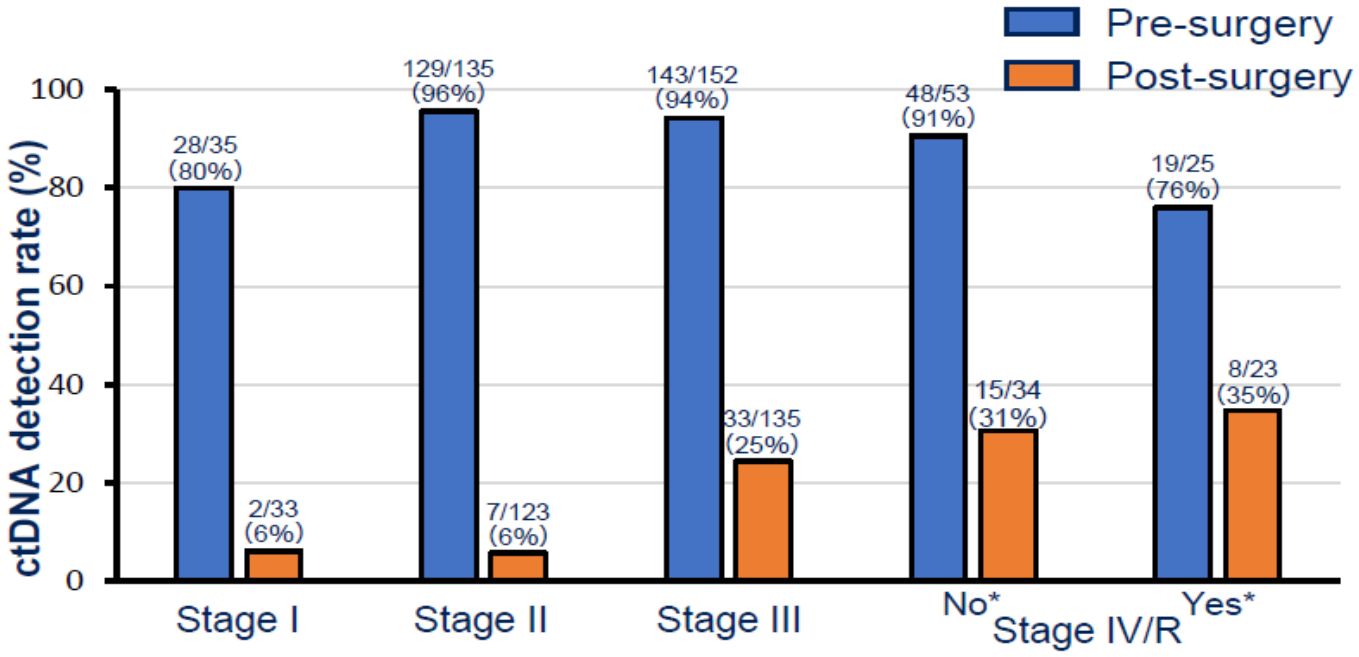
The VEGA trial: the non-inferiority of observation vs. adjuvant CAPOX in GALAXY participants with absence of ctDNA at 1-month post-surgery.

The ALTAIR trial: the superiority of FTD/TPI over placebo in GALAXY participants with ctDNA positive after the standard therapy.

1. Yukami H, Mishima S, Kotani D, et al. Trial in progress: Prospective Observational Study Monitoring Circulating Tumor DNA in Resectable Colorectal Cancer Patients Undergoing Radical Surgery: GALAXY Study in CIRCULATE-Japan. Poster (P-120) presented at: ESMO: GI – World Congress on Gastrointestinal Cancer. July 1-4, 2020; Virtual Meeting.

Yukami et al
ASCO 2021

Signatera ctDNA detection rates pre- and post-surgery



pStage	Pre-surgery	Post-surgery
	Median ctDNA level (MTM/ml)	Median ctDNA level (MTM/ml)
pStage I	0.73	0.92
pStage II	3.66	0.72
pStage III	4.54	0.46
pStage IV/R	27.07	1.81

*History of chemotherapy prior to surgery within 6 months

Across multiple studies in CRC, Signatera has shown to have pre-surgical detection 89-94%[1-2]

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. 2. CIRCULATE data on file.

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Prospective trials to assess association of MRD-positivity with treatment response

Trial/ NCT#	Stage / Tumor type	Description	Phase
MERMAID-1 *NCT04385368*	Stage II/III NSCLC	Durvalumab + chemo vs chemo for MRD+ after resection	3
MERMAID-2 *NCT04642469*	Stage II/III NSCLC	Durvalumab vs placebo for MRD+ after resection and possible neoadj/adjuv treatment	3
CATHAYA *NCT04611776*	Stage IB-IIIB NSCLC	Atezolizumab + chemo vs placebo + chemo for MRD+ after resection	2
IMvigor011 *NCT04660344*	Muscle-invasive bladder cancer	Atezolizumab vs placebo for MRD+ after resection	3
ISPY-2 *NCT01042379*	Neoadjuvant breast cancer	ctDNA dyanamics pre- and post-neoadjuvant chemotherapy prior to resection	2
c-TRAK-TN *NCT03145961*	High risk, early stage TNBC	Pembrolizumab vs placebo for MRD+ after resection and possible neoadj/adjuv tx	2
LEADER *NCT03285412*	Stage I-III breast cancer (ER+, HER2-)	Ribociclib + endocrine therapy vs endocrine therapy for MRD+ patients after possible neoadj/adjuv tx	2
DARE *NCT04567420*	Stage II-III breast cancer (ER+, HER2-)	Palbociclib + fulvestrant vs SoC endocrine tx for MRD+ patients treated with adjuvant aromatase inhibitor/tamoxifen	2
CIRCULATE-Japan *UMIN000039205*	Stage II-III CRC	Treatment escalation with experimental therapies in MRD+ patients after surgery, and treatment de-escalation (no chemo) in MRD- patients after surgery	3
MGH 18-397 *NCT03803553*	Stage III CRC	Nivolumab, Encorafenib/Binimetinib/Cetuximab, FOLFIRI, or active surveillance as appropriate for MRD+	3
COBRA *NCT04068103*	Stage IIA colon cancer	FOLFOX6 or CAPOX vs surveillance for resected MRD+	2/3
TAPISTRY *NCT04589845*	TMB-high, advanced solid tumors	TKIs vs atezolizumab vs ipatasertib vs trastuzumab vs idasanutlin vs GDC-0077	2
NCT04510285	HER2+ esophagogastric tumors	Trastuzumab + placebo vs trastuzumab + pembrolizumab in MRD+	2

Sources: 1. Clinicaltrials.gov. Accessed April 27, 2021; 2. Yukami H, Mishima S, Kotani D, et al. Trial in progress: Prospective Observational Study Monitoring Circulating Tumor DNA in Resectable Colorectal Cancer Patients Undergoing Radical Surgery: GALAXY Study in CIRCULATE-Japan. Poster (P-120) presented at: ESMO: GI – World Congress on Gastrointestinal Cancer. July 1-4, 2020; Virtual Meeting.

Using ctDNA to bring life-saving therapeutics to patients

- Clinical studies and data shows strong correlation for quantification of ctDNA levels and clinical outcomes
- ctDNA can serve as a biomarker in various manners including prognostic, predictive, monitoring, and treatment response
- ctDNA status has the potential to be used as:
  - A surrogate endpoint of treatment efficacy to accelerate clinical trial results
  - A stratification method to identify the subset of patients who may still benefit from therapies studied in trials that failed to meet their primary endpoint

Open questions| Guidance needed from FDA / PMDA

- Pathway for treatment-related ctDNA dynamics to become a surrogate endpoint for drug approvals
- Pathway for updating label based on MRD data using retrospective banked samples
  - Complementing other surrogate endpoints with ctDNA, or work through regulatory paradigms like accelerated approvals?

Thank you!

Questions

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