MADRID 2023 ESMO congress

CANCER RESEARCH UK CLINICAL ACADEMIC TRAINING PROGRAMME

THE FRANCIS CRICK INSTITUTE

TRACERx

Personalis®

An ultra-sensitive and specific ctDNA assay provides novel pre-operative disease stratification in early stage lung cancer

TRACERx: NCT01888601

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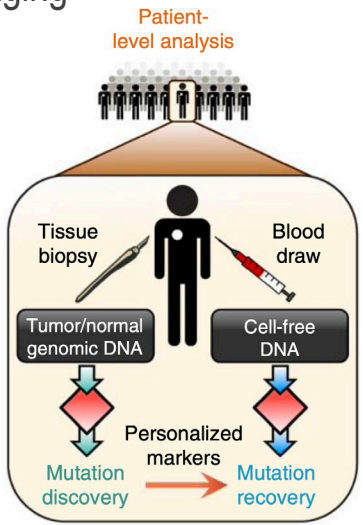
DECLARATION OF INTERESTS

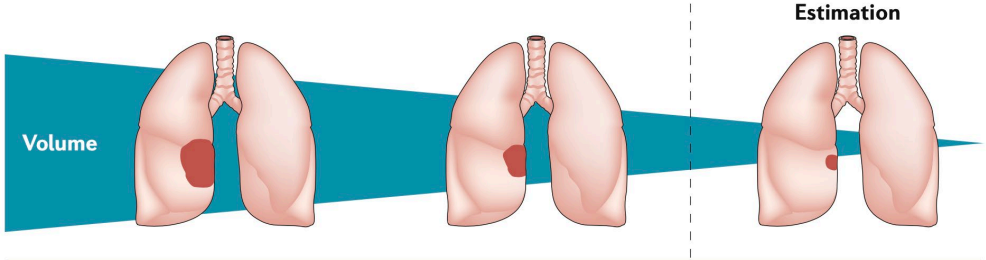
Dr James Black:

Data and analysis presented here was supported in part by funding from Personalis Inc.

Circulating tumour DNA

- Often comprises tiny fraction of cell-free DNA
- Stratifies patients for outcome pre and post-operatively
- Minimal residual disease can predict relapse prior to imaging





MAF	1.4% (0.62–3.1%)	0.1% (0.06–0.18%)	0.008% (0.002–0.03%)
Nodule diameter	5.8 cm	2.6 cm	1.2 cm
Nodule volume	100 cm^3	10 cm^3	1 cm^3
T stage	T3	T1c	T1b

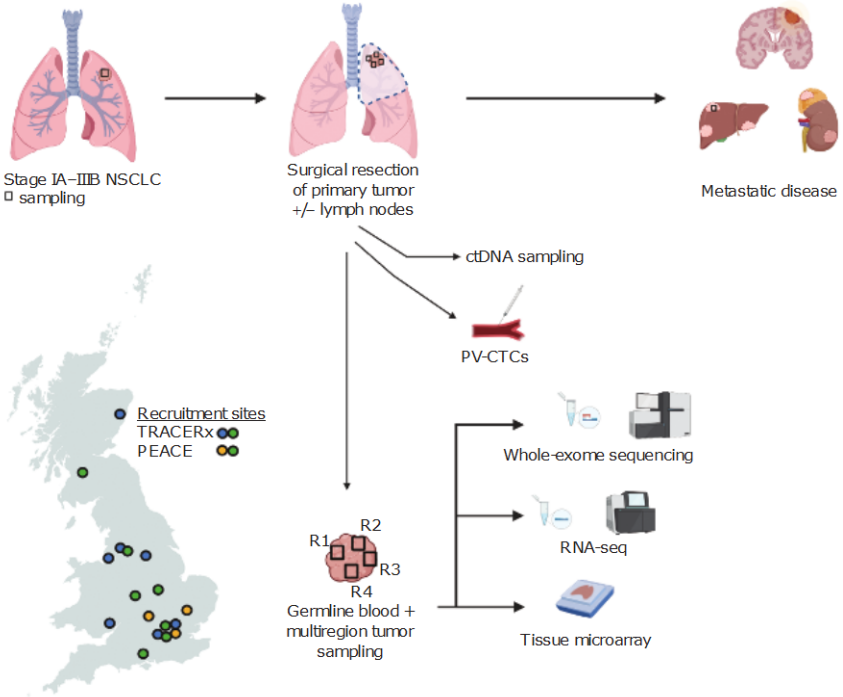
ctDNA detection: requirements

- High sensitivity
- High specificity
- Low input amounts
- Applicable across representative sample of tumours

Newman et al., *Nat Med.* 2014

Abbosh et al., 2018, *Nat Rev Clin Onc.* 2018

ctDNA in TRACERx lung



How much clinical performance is lost due to assay sensitivity?

First generation tumour-informed ctDNA assays

Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution

Christopher Abbosh, Nicolai J. Birkbak, Gareth A. Wilson, Mariam Jamal-Hanjani, Tudor Constantin, Raheleh Salari, John Le Quesne, David A. Moore, Selvaraju Veeriah, Rachel Rosenthal, Teresa Marafioti, Eser Kirkizlar, Thomas B. K. Watkins, Nicholas McGranahan, Sophia Ward, Luke Martinson, Joan Riley, Francesco Fraioli, Maise Al Bakir, Eva Grönroos, Francisco Zambrana, Raymondo Endozo, Wenya Linda Bi, Fiona M. Fennessy, The TRACERx consortium, The PEACE consortium & ... Charles Swanton

+ Show authors

Nature **545**, 446–451 (2017) | Cite this article

16 variant personalised panel: LOD95*
~0.01% VAF (100 PPM)

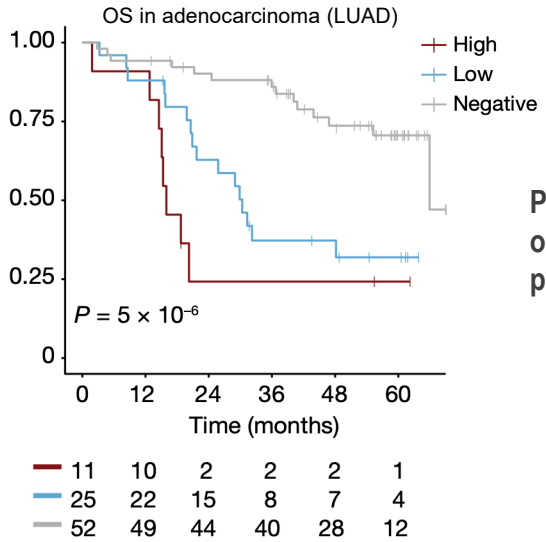
Tracking early lung cancer metastatic dissemination in TRACERx using ctDNA

Christopher Abbosh, Alexander M. Frankell, Thomas Harrison, Judit Kisistok, Aaron Garnett, Laura Johnson, Selvaraju Veeriah, Mike Moreau, Adrian Chesh, Tafadzwa L. Chaunzwa, Jakob Weiss, Morgan R. Schroeder, Sophia Ward, Kristiana Grigoriadis, Aamir Shahpurwalla, Kevin Litchfield, Clare Puttick, Dhruva Biswas, Takahiro Karasaki, James R. M. Black, Carlos Martínez-Ruiz, Maise Al Bakir, Oriol Pich, Thomas B. K. Watkins, TRACERx Consortium, ... Charles Swanton

+ Show authors

Nature **616**, 553–562 (2023) | Cite this article

50 variant personalised panel: LOD95*
~0.008% VAF (~80 PPM)



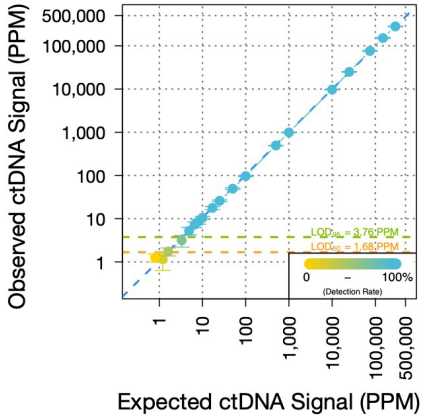
Presence of ctDNA within pre-operative plasma sample predicts poor OS in adenocarcinomas

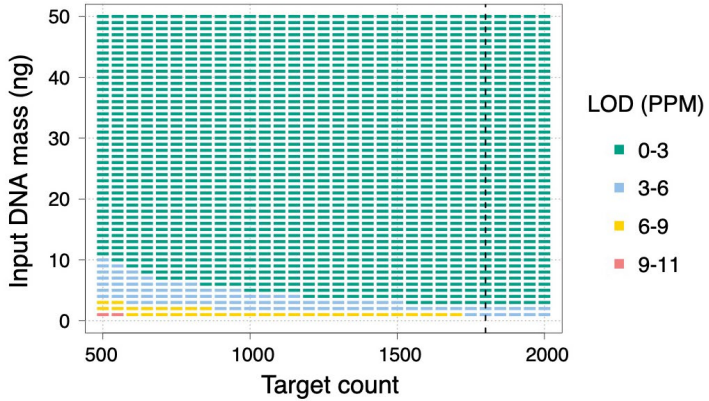
Abbosh et al., 2017, *Nature*
Sethi et al., 2018, *AACR*
Bailey, Black et al., 2021, *Cancer Discov.*
Zhou et al., 2023, *Mol Diagn Ther.*
Abbosh et al., 2023, *Nature*

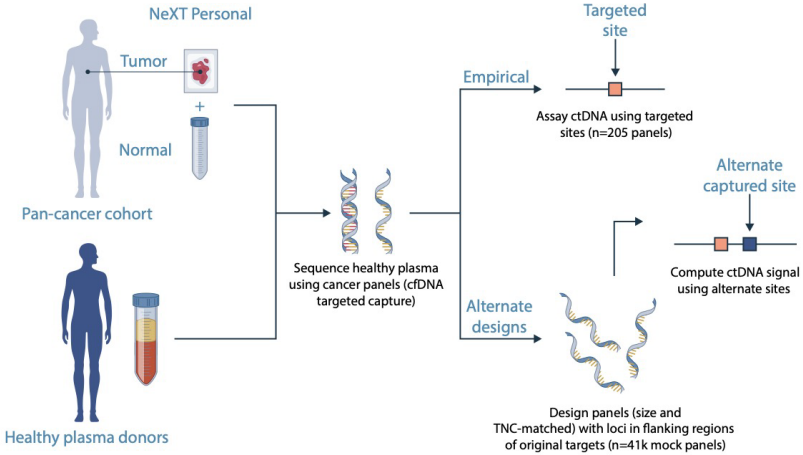
*LOD95: estimated ctDNA fraction which would be detected in 95% of replicates

NeXT Personal: ultra-sensitive ctDNA detection

- Tracks ~1,800 mutations per patient
- LOD95* of 3.76 PPM (0.00037% ctDNA fraction)
- Simulations suggest LOD < 10 PPM (0.001% ctDNA fraction) with 1ng DNA input
- Estimated specificity 99.98%







Number of Patient-Specific Panels	True Negative ctDNA Detections	False Positive ctDNA Detections	Specificity (95% CI)
205	205	0	100% (98.22-100%)

Number of Simulated Mock Panels	True Negative ctDNA Detections	False Positive ctDNA Detections	*in silico* Specificity (95% CI)
40,600	40,593	7	99.98% (99.96-99.99%)

*LOD95: estimated ctDNA fraction which would be detected in 95% of replicates

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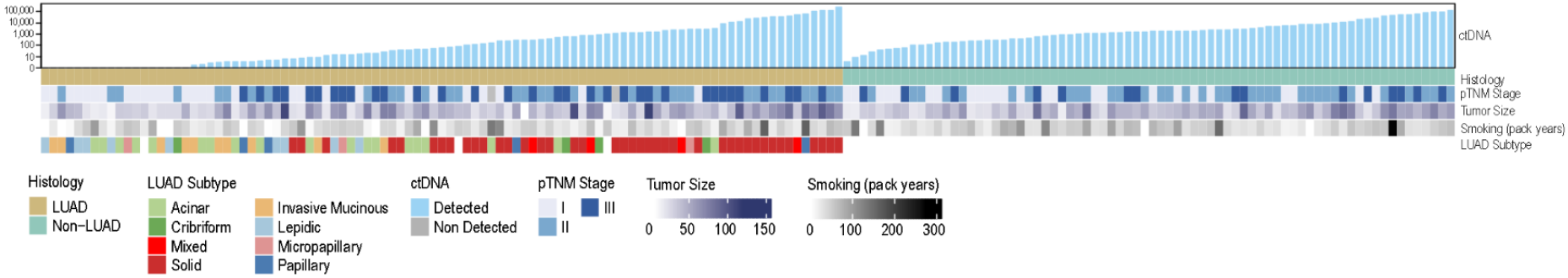
Cohort overview

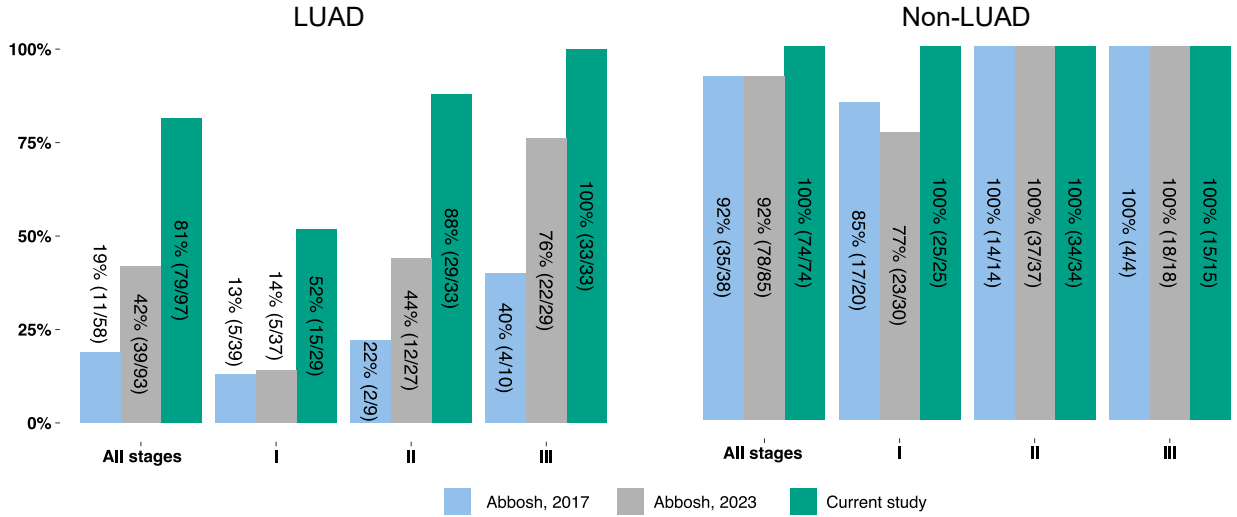
- Early-stage NSCLC patients enrolled in TRACERx
- Surgery with curative intent
- 48% of patients received adjuvant treatment; nil neo-adjuvant treatment
- Analysis of blood plasma collected at pre-operative timepoint
- Median follow-up of 5 years

		LUAD	Non-LUAD
n	-	97	74
Age	Mean (SD)	67.3 (8.8)	70.1 (8.1)
Sex	Female	41 (42.3)	28 (37.8)
	Male	56 (57.7)	46 (62.2)
Stage	IA	11 (11.3)	6 (8.1)
	IB	18 (18.6)	19 (25.7)
	IIA	15 (15.5)	21 (28.4)
	IIB	19 (19.6)	13 (17.6)
	IIIA	34 (35.1)	15 (20.3)
Smoking status	Current Smoker	15 (15.5)	10 (13.5)
	Ex-Smoker	42 (43.3)	45 (60.8)
	Never Smoked	6 (6.2)	2 (2.7)
	Recent Ex-Smoker	34 (35.1)	17 (23.0)
Adjuvant treatment	Adjuvant	47 (48.5)	35 (47.3)
	No adjuvant	50 (51.5)	39 (52.7)
LUAD subtype	Acinar	19 (19.6)	
	Cribriform	4 (4.1)	
	Invasive Mucinous	10 (10.3)	
	Lepidic	9 (9.3)	
	Micropapillary	3 (3.1)	
	Mixed	4 (4.1)	
	Missing	3 (3.1)	
	Papillary	5 (5.2)	
	Solid	40 (41.2)	
Histology	Adenocarcinoma	97 (100.0)	
	Other		18 (24.3)
	Squamous cell carcinoma		56 (75.7)
Followup (OS)	Median (# of events)	1830 (39)	1850 (35)
Followup (RFS)	Median (# of events)	1821 (51)	1843 (38)

Pre-operative detection of ctDNA using NeXT Personal

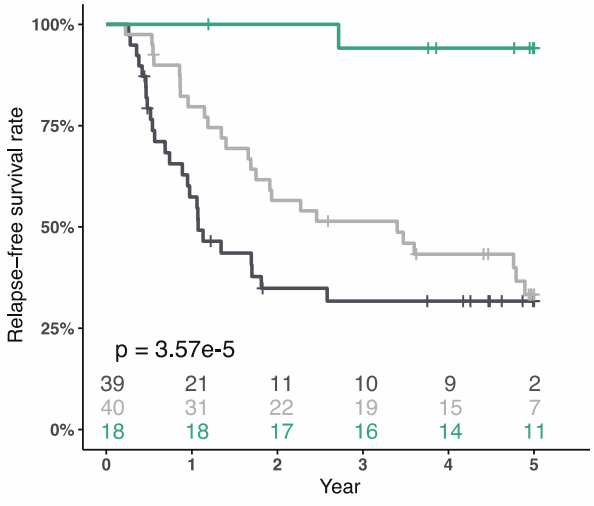


- ctDNA detected from 1.7 to 253,826 PPM
- Pre-operative ctDNA detected in **81%** of LUAD and **100%** of non-LUAD patients
- Includes **52%** of pTNM stage I LUAD patients

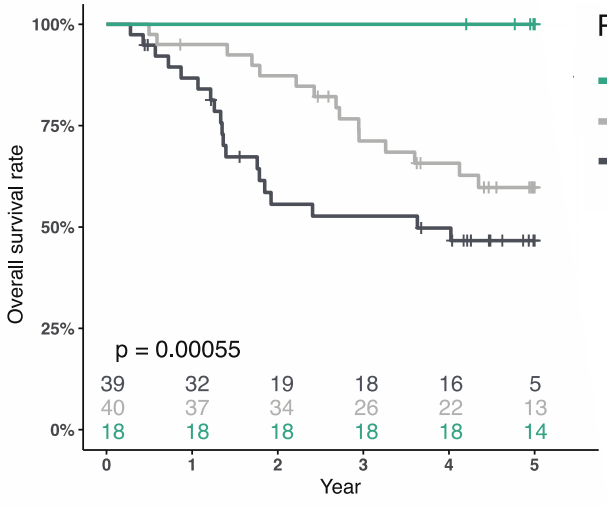


Patient stratification with pre-operative ctDNA: adenocarcinomas

ctDNA status independently predicts RFS and OS



ctDNA low: HR=13.7, 95% CI:1.8-105.8
ctDNA high: HR=20.8, 95% CI: 2.5-172.74



ctDNA low: HR=9.1 95% CI:1.1-72.5
ctDNA high: HR=23.6, 95% CI:2.7-206.0

Pre–operative ctDNA
- Not detected
- Low
- High

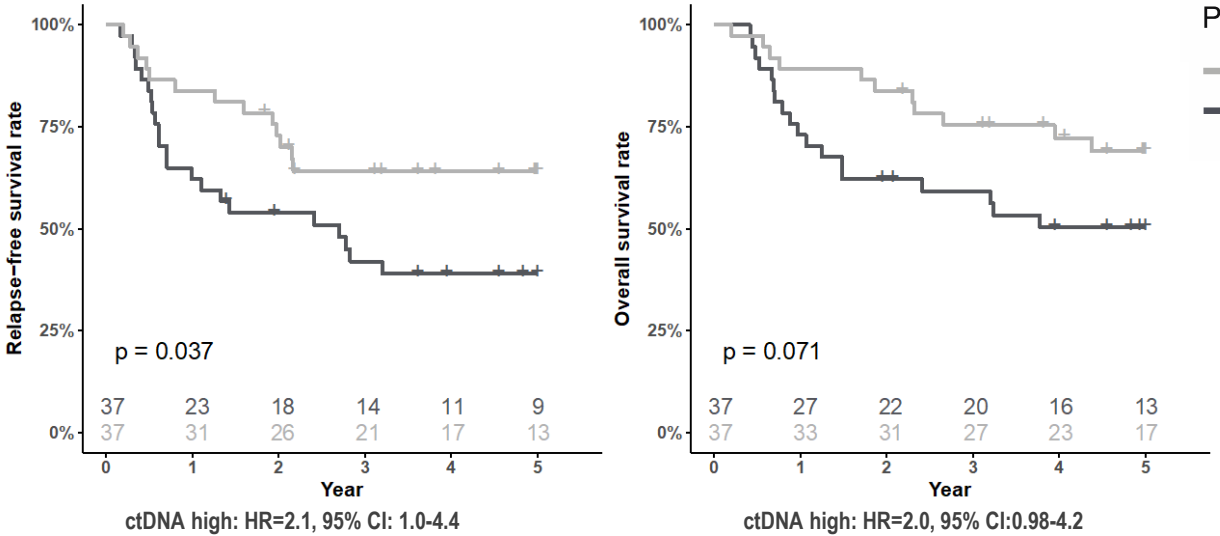
	5-year RFS	5-year OS
ctDNA negative	94%	100%
Below-median ctDNA	33.3%	59.8%
Above-median ctDNA	31.7%	46.6%

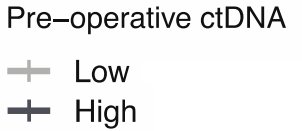
HRs adjusted in multivariable Cox regression model containing ctDNA level (not detected, low, high), treatment status (adjuvant treatment, no adjuvant treatment), smoking status (per 10 pack years), pTNM stage (I, II, III) and age (per 10 years).

Low: below-median ctDNA-positive tumours; High: above-median ctDNA-positive tumours.

Patient stratification with pre-operative ctDNA: non-adenocarcinomas

ctDNA status independently predicts RFS





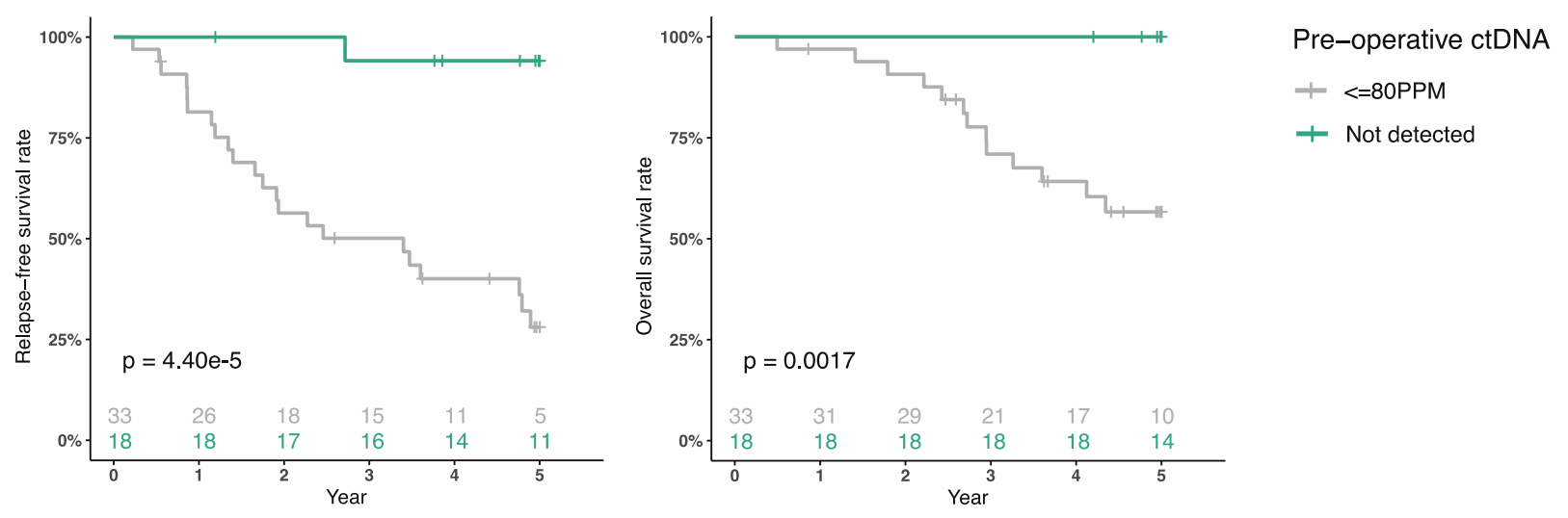
	5-year RFS	5-year OS
ctDNA negative	NA	NA
Below-median ctDNA	64.1%	69.0%
Above-median ctDNA	38.9%	50.3%

HRs adjusted in multivariable Cox regression model containing ctDNA level (low, high), histology (squamous cell, non-squamous cell), treatment status (adjuvant treatment, no adjuvant treatment), smoking status (per 10 pack years), pTNM stage (I, II, III) and age (per 10 years).

Low: below-median ctDNA-positive tumours; High: above-median ctDNA-positive tumours.

Adenocarcinoma with ultra-low pre-operative ctDNA levels

Patients below 95LOD of assay used in Abbosh et al. 2023 (80 PPM ctDNA) stratified for RFS and OS by NeXT Personal



NeXT Personal identifies ultra low-risk group of adenocarcinomas

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Abbosh et al., 2023, *Nature*

Zhou et al., 2023, *Mol Diagn Ther.*

Future plans in TRACERx

- Planned analysis of ~450 TRACERx patients ongoing
- Approximately 4200 plasma samples being analysed
- 350 tumour-specific subclonal mutations tracked in each patient
- Future expanded analyses will focus on clinical performance, clonal evolution through treatment, acquisition of treatment resistance and factors governing ctDNA shedding

	Lead time (days)
All tumours	173
LUAD	145
Non-LUAD	213
Landmark positive	331

PPM: parts per million ctDNA. Landmark positive: ctDNA detection within first 120 days of follow up. After adj. treatment: following completion of final treatment with curative intent. Minimum follow-up to be considered relapse-free: 3 years. Median follow-up: 5 years (range 89-2927 days). Landmark lead times were calculated for landmark positive patients, longitudinal lead time includes all patients. Median time to relapse LUAD: 391 days (range 82-1782); Non-LUAD: 443 days (range 59-1747).

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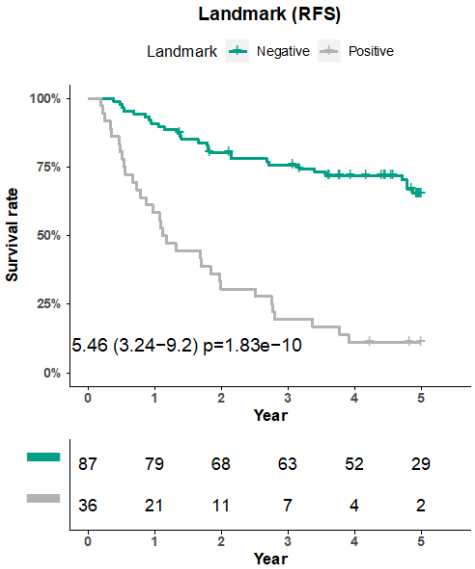
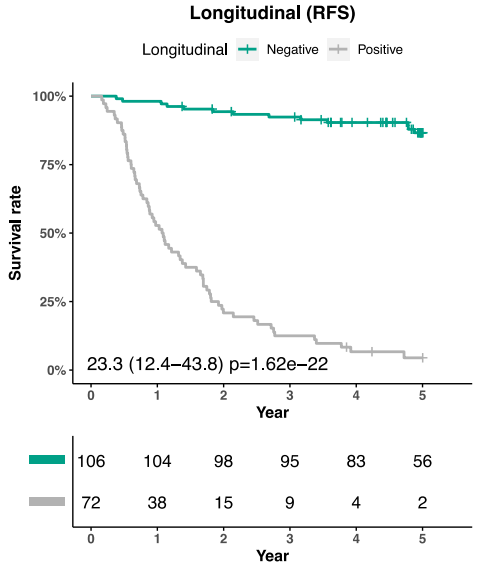
Preliminary data from post-operative timepoints

	PPV	NPV	Sensitivity	Specificity	ctDNA PPM (IQR)
Landmark (n=123)	89%	69%	54%	94%	239 (21-1547)
Landmark (n=74, LUAD only)	90%	58%	46%	94%	159 (21-1166)
Landmark (n=49, Non-LUAD only)	87%	85%	72%	94%	358 (25-1830)
Landmark (from end of curative treatment, n=110)	94%	73%	58%	97%	152 (14-1332)
Longitudinal (n=178)	94%	89%	85%	96%	

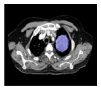




NeXT Personal Landmark Performance

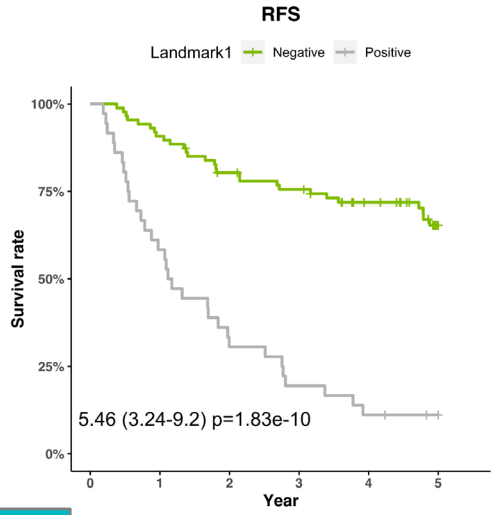
Surgery
+/- Adjuvant

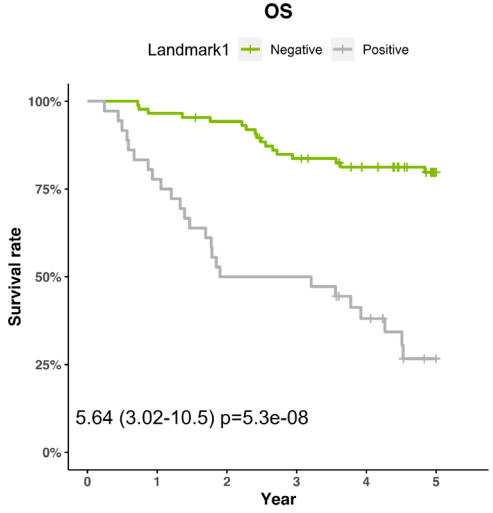
Recurrence on Imaging



331 days median lead time to recurrence *(NeXT Personal)*

228 days *(Abbosh '23)*





- Best overall landmark performance to date in TRACERx cohort
- Landmark in NSCLC is challenging because of ultra-low ctDNA levels after surgery +/- adjuvant
- In the first 120 days after surgery or end of treatment, able to identify 54% to 58% of patients that went on to recur within 5 years
- Exceptional lead time over imaging detection in landmark positive patients, with an ~11 month median lead time
- NeXT Personal median lead time is ~3 months earlier[1] compared to prior TRACERx studies
- Predictive of 5-year RFS and OS

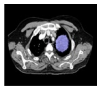
Non-ESMO Slide

1 For median lead time evaluation across Abbosh '23 and this study, while the patients were drawn from the TRACERx cohort, the specific patients analyzed may be different, which may lead to potential differences in: duration of follow-up, frequency and timing of blood draws, frequency and timing of imaging, etc. Median lead time to recurrence was not reported in Abbosh '17.
Source: ESMO 2023 TRACERx proferred talk

NeXT Personal Longitudinal Performance

Surgery
+/- Adjuvant

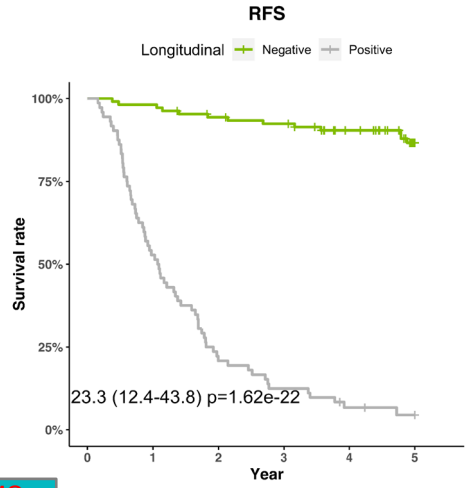
Recurrence on Imaging

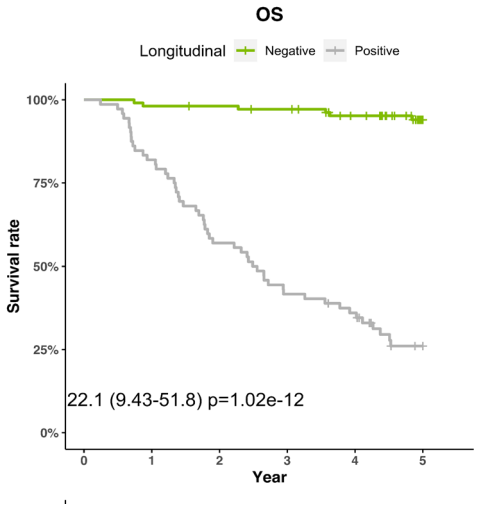


173 days median lead time to recurrence *(NeXT Personal)*

119 days *(Abbosh '23)*

70 days *(Abbosh '17)*





- Exceptional lead time over imaging detection, with a median ~6 month lead time
- NeXT Personal median lead time is ~2-3 months earlier[1] compared to prior TRACERx studies
- Strong PPV (94%), NPV (89%), sensitivity (85%) and specificity (96%) as expected
- Predictive of 5-year RFS and OS
- Median follow up time was 5 years

1 For median lead time evaluation across Abbosh '17, Abbosh '23, and this study, while the patients were drawn from the TRACERx cohort, the specific patients analyzed may be different, which may lead to potential differences in: duration of follow-up, frequency and timing of blood draws, frequency and timing of imaging, etc.

Source: ESMO 2023 TRACERx proferred talk

Non-ESMO Slide

Summary

- Ultra-sensitive ctDNA detection identifies pre-operative ctDNA in 81% of patients with LUAD, including 52% of Stage I's
- High ctDNA level predicts poor OS and RFS in LUAD and RFS in non-LUADs
- **Added sensitivity is clinically meaningful:** ultra-sensitive detection below 80 PPM (95LOD of approach taken in Abbosh et al., 2023) stratifies ctDNA-positive LUAD patients for worse OS and RFS
- Full study will explore impact of added technical sensitivity on clinical performance at landmark timepoint
- Preliminary data highlights promise of ultra-sensitive approach for post-operative disease stratification

Abbosh et al., 2023, *Nature*

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