Changes in ctDNA levels as an early indicator of outcomes in advanced NSCLC treated with TKI: Initial findings from a retrospective aggregate analysis of 8 clinical trials

Hillary Stires, PhD,[1] Nevine Zariffa, MMath,[2] Megan Eisele, MS,[3] Emily Goren, PhD,[3] Carin R. Espenschied, MS,[4] Minakshi Guha, PhD,[5] Jiannan Guo,[6] Dilafruz Juraeva, PhD,[7] Jean-Francois Martini, PhD,[8] Brittany McKelvey, PhD,[1] Katherine K. Nishimura, MPH, PhD,[3] Gary A. Pestano, PhD,[9] Sorena Rahmanian, PhD,[10] Adam Rosenthal, MS,[3] Mark Stewart, PhD,[1] Anna Szpurka, PhD,[11] Diana Vega, PhD,[12] Antje Hoering, PhD,[3] Jeff Allen, PhD,[1] and the ctMoniTR Step 2 Working Group

1. Friends of Cancer Research 2. NMD Group, LLC. 3. Cancer Research And Biostatistics (CRAB) 4. Guardant Health 5. Takeda Development Center Americas, Inc. 6. Resolution Bioscience, a part of Agilent 7. the healthcare business of Merck KGaA, Darmstadt, Germany 8. Pfizer Inc. 9. Biodesix Inc. 10. Illumina 11. Loxo@Lilly, Eli Lilly and Company 12. AstraZeneca
Corresponding author is Hillary Stires (hstires@focr.org)

FRIENDS of CANCER RESEARCH

@cancerresrch

Abstract # 3030

Background

ctMoniTR Project Overview

To determine whether changes in circulating tumor DNA (ctDNA) levels reflect treatment outcome, Friends of Cancer Research created the ctDNA to Monitor Treatment Response (ctMoniTR) Project with collaborators from industry, government, academia, and advocacy (project members).

- **ctMoniTR Step 1** analyzed 5 clinical trials and showed an association between decreases in ctDNA levels and improved outcomes in patients with advanced non-small cell lung cancer (aNSCLC) treated with an anti-PD-(L)1.[A]
- **ctMoniTR Step 2** expands on this work into additional tumor types and treatment modalities.

Step 2 Module 1 – aNSCLC treated with TKI

DATASET
Retrospective aggregate analysis of 8 unique clinical trials of patients with aNSCLC treated with a tyrosine kinase inhibitor (TKI; i.e., anti-EGFR, ALK, RET, or MET; n=1590) broken into three research objectives

TRAINING/VALIDATION
We randomly divided the dataset into training (2/3 of the data) and validation (1/3 of the data) datasets stratified by clinical trial cohort (i.e., arm), age, tumor stage, and prior lines of therapy, then ran initial analyses on the training dataset (presented herein).

RESEARCH OBJECTIVE 1
Do early changes in ctDNA levels associate with long-term clinical outcomes?

RESEARCH OBJECTIVE 2
Do "early" changes in ctDNA complement 1st RECIST to assess treatment efficacy? Best overall response?

RESEARCH OBJECTIVE 3
Does combining ctDNA with radiographic response data (i.e., RECIST) improve associations with outcomes?

Step 2 Module 2

aNSCLC with anti-PD(L)1 and/ or chemotherapy

Step 2 Module 3

Solid tumors with anti-PD(L)1 or TKI

Step 2 Cross Module Analysis

Combine data from all modules (TBD)

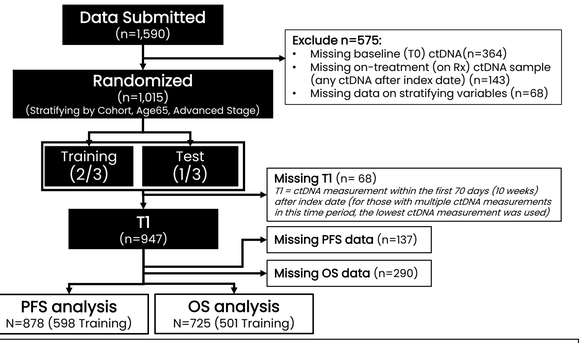
Methods

Approach

Align on statistical analysis plan *Project Members* → Upload patient-level data to secure portal *Trial sponsors* → Review data and share private dataset report *CRAB/ Trial Sponsors* → Perform analyses *CRAB* ⇄ Discuss findings and next steps *Project Members* → Align on key findings and disseminate results *Project Members*

Differences in OS (Overall Survival) and PFS (Progression-Free Survival) by ctDNA category were evaluated using Kaplan Meier plots with log-rank p-values. Univariate and multivariable Cox proportional hazards models were stratified by cohort and landmarked at 70 days (10 weeks) after enrollment and patients with an event during the 70-day landmark were excluded from the analysis. Demographic and clinical confounders were also included in the models.

CONSORT Diagram & Patient/Assay Characteristics



Trait	Description	Cohort 1 n=183	2 n=180	3 n=176	4 n=100	5 n=98	6 n=95	7 n=89	8 n=36	9 n=29	10 n=24	11 n=5	*p*	Overall, n/N (%) n=1015
Age	Age ≥ 65 years at enrollment , No. (%)	86 (47%)	85 (47%)	78 (44%)	38 (38%)	28 (29%)	25 (26%)	24 (27%)	10 (28%)	12 (41.4%)	6 (25%)	4 (80%)	<0.001	396/1015 (39.0%)
Sex	Female, No. (%)	109 (60%)	111 (62%)	109 (62%)	55 (55%)	52 (53%)	61 (64%)	53 (60%)	14 (39%)	23 (79%)	12 (50%)	1 (20%)	0.0349	600/1013 (59.2%)
Race	White, No. (%)	49 (27%)	54 (30%)	55 (32%)	46 (46%)	42 (43%)	47 (50%)	51 (57%)	16 (44%)	14 (48%)	19 (79%)	2 (40%)	<0.001	395/1011 (39.1%)
Smoking Status	Ever smoked, No. (%)	73 (40%)	64 (36%)	62 (35%)	47 (47%)	35 (36%)	33 (35%)	39 (44%)	16 (44%)	10 (35%)	8 (33%)	3 (60%)	0.5818	390/1014 (38.5%)
ECOG	ECOG performance status ≥ 1, No. (%)	115 (63%)	102 (57%)	107 (61%)	51 (51%)	65 (66%)	57 (60%)	57 (64%)	18 (50%)	25 (86%)	18 (75%)	1 (20%)	0.0131	616/1015 (60.7%)
Stage*	Advanced stage (stage IV), No. (%)	169 (92%)	165 (92%)	144 (82%)	99 (99%)	97 (99%)	93 (98%)	86 (97%)	35 (97%)	29 (100%)	24 (100%)	5 (100%)	<0.001	946/1015 (93.2%)
Prior Therapy	Prior lines of systemic therapy ≥ 1, No. (%)	16 (9%)	21 (12%)	176 (100%)	11 (11%)	30 (31%)	5 (5%)	29 (33%)	36 (100%)	29 (100%)	22 (92%)	3 (60%)	<0.001	378/1015 (37.2%)
Histology	Adenocarcinoma, No. (%)	180 (98%)	177 (98%)	174 (99%)	94 (94%)	90 (92%)	91 (96%)	88 (99%)	35 (97%)	27 (93%)	23 (96%)	4 (80%)	0.0095	983/1015 (96.8%)
ctDNA Samples	Median ctDNA measurements (Range)	5 (1-24)	5 (1-28)	6 (2-19)	3 (2-3)	2 (2-4)	3 (2-3)	2 (2-3)	2 (2-3)	2 (2-3)	4 (2-5)	4 (3-4)		
Assay		ddPCR	ddPCR	ddPCR	NGS	NGS	NGS	NGS	NGS	NGS	NGS	NGS		
LOD		2 copies	2 copies	2 copies	0.1-0.3%	0.1%	0.1-0.3%	0.1%	0.1%	0.5%	0.1-0.3%	0.1-0.3%		
ND	Nondetected ctDNA at T0, No. (%)	48 (26%)	52 (29%)	47 (27%)	26 (26%)	53 (54%)	49 (52%)	42 (47%)	15 (42%)	0 (0%)	1 (4%)	0 (0%)	0.0036	257/1015 (25.3%)
VAF ≤0.5*	ctDNA Samples per cohort, No. (%)	72 (39%)	83 (46%)	63 (36%)	50 (50%)	53 (54%)	49 (52%)	42 (47%)	15 (42%)	0 (0%)	8 (33%)	3 (60%)	<0.001	438/1015 (43.2%)

*Stage at enrollment for all except cohort 1-3, which used stage at diagnosis; ^0.5 chosen because it was the max LOD across cohorts
P value from Fishers' exact test comparing cohorts

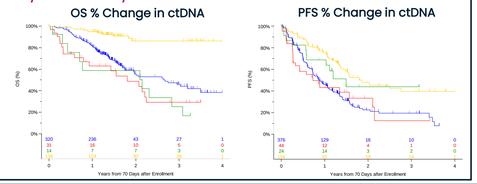
Results

Creating ctDNA Categories and Assessing Associations with Long-term Outcomes

Initially, we created categories of ctDNA based on a change in ctDNA using descriptive ctDNA trends.[A] This led to 4 categories focused on a percent change in ctDNA levels:

- "Decrease" = >50% decrease in ctDNA from T0 to T1 (and those with detected (D) ctDNA levels at T0 and non-detected (ND) ctDNA levels at T1)
- "Increase" = >20% increase from T0 to T1 (and those with ND ctDNA levels at T0 and D ctDNA levels at T1)
- "Intermediate" = 50% decrease to 20% increase from T0 to T1
- "ND/ND" = ND ctDNA levels at T0 and T1

Categorizing the samples by percent change did not demonstrate separation of Kaplan-Meier Curves.

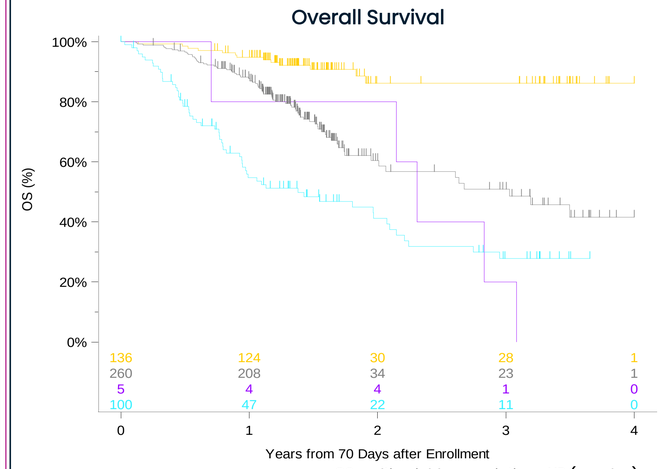


There is no clear cut-point in the data to separate out the samples with D at T0 and T1.
We ran an Optimal Cut-point analysis using a running log-rank test to select a cut-point which maximized the difference in OS based on % change in ctDNA (data not shown).

We created new categories based on whether ctDNA was detected or not at T0 and T1.[B]

- "ND/ND" = ND levels of ctDNA at T0 and T1
- "D/ND" = D at T0 and ND at T1
- "ND/D" = ND at T0 and D at T1
- "D/D" = D at T0 and T1

Overall Survival

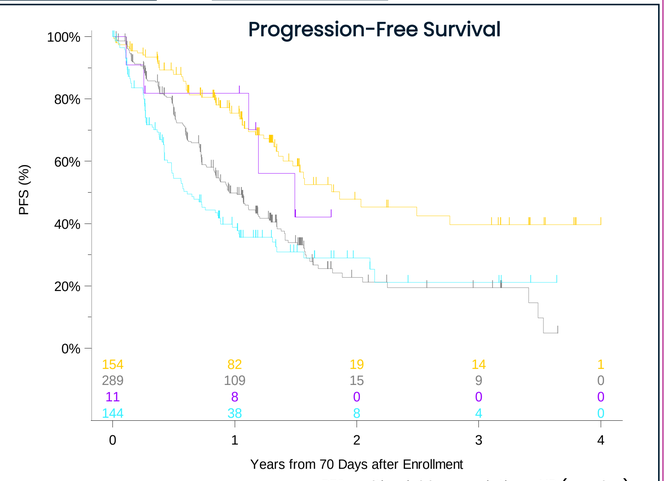


OS multivariable associations, HR (p-value)

	Events / N	Median Years (Range)	1-Yr Estimate % (IQR)
ND/ND	13/136	NR	95% (91, 99)
D/ND	78/260	3 (2, .)	88% (84, 92)
ND/D	5/5	2.3 (0.7, .)	80% (45, 100)
D/D	58/100	1.4 (0.9, 2.1)	55% (44, 65)

Comparator	Reference: ND/ND	D/ND	ND/D	D/D
ND/ND				
D/ND	3.03 (0.002)			
ND/D	9.63 (<0.001)	3.28 (0.026)		
D/D	6.88 (<0.001)	2.27 (<0.001)	0.69 (0.512)	

Progression-Free Survival



PFS multivariable associations, HR (p-value)

	Events / N	Median Years (Range)	1-Yr Estimate % (IQR)
ND/ND	56/154	1.9 (1.5, .)	75% (68, 83)
D/ND	171/289	1 (0.8, 1.2)	50% (44, 56)
ND/D	5/11	1.5 (1.1, .)	82% (59, 100)
D/D	87/144	0.6 (0.5, 0.9)	39% (30, 47)

Comparator	Reference: ND/ND	D/ND	ND/D	D/D
ND/ND				
D/ND	2.07 (<0.001)			
ND/D	3.11 (0.023)	1.50 (0.408)		
D/D	3.24 (<0.001)	1.56 (0.005)	1.04 (0.936)	

Non-detected ctDNA at T1 (D/ND) was associated with improved OS and PFS over patients with detected levels of ctDNA (D/D) in patients with aNSCLC treated with TKI. The figure demonstrates Kaplan-Meier plots for OS or PFS and the 4 ctDNA categories, landmarked at 70 days from treatment initiation (the sampling window for the first on-treatment ctDNA sample). Multivariable Cox regression models identified statistically significant associations between OS and age and performance status, and between PFS and performance status and tumor stage. Multivariable associations for ctDNA categories are included in the table below each Kaplan Meier plot.

Conclusions

- In a retrospective aggregate analysis of 8 clinical trials in aNSCLC treated with TKI, non-detected ctDNA on treatment (D/ND) associates with better OS compared with patients with detected levels of ctDNA on treatment (D/D).
- ctDNA samples collected within 10 weeks following initial treatment can be used to assess response to treatment and are an indicator of long-term benefit.

Next Steps

Remaining findings analyzing changes in ctDNA with long-term outcomes (Research Objective 1), will be presented at a *Friends'* hosted meeting in Washington, DC on July 11:

- Additional analyses will assess the association between ctDNA categories and the first RECIST measurement and best overall response (Research Objectives 2 and 3).
- We will perform and present validation studies.

We will then move on to Module 2 and 3.

Key Definitions

- **TKI** – Tyrosine Kinase Inhibitor (anti-EGFR, ALK, RET, or MET)
- **Index Date** – Date of randomization / date of treatment initiation
- **Baseline (T0) ctDNA** – ctDNA measurement no more than 14 days (2 weeks) prior to index date, and must not be after index date
- **T1 ctDNA** – ctDNA measurement within the first 70 days (10 weeks) after index date (for those with multiple ctDNA measurements in this time period, the lowest ctDNA measurement was used)
- **Max VAF** – Percent change in maximum variant allele frequency (VAF) between T0 and T1 using tumor-derived variants provided by sponsors for each unique patient sample
- **ND** – Not detected – the ctDNA measurement of the sample was determined to be ND (limit of detection was defined by the sponsor)
- **D** – Detected – the ctDNA measurement of the sample was determined to be D

ctDNA Categories

- **ND/ND** – Patients who had non-detected levels of ctDNA at T0 and T1
- **ND/D** – Patients who had non-detected levels of ctDNA at T0 and detected ctDNA at T1
- **D/ND** – Patients who had detected levels of ctDNA at T0 and non-detected ctDNA at T1
- **D/D** – Patients who had detected levels of ctDNA at T0 and T1

A) Vega DM, et al. Changes in Circulating Tumor DNA Reflect Clinical Benefit Across Multiple Studies of Patients With Non-Small-Cell Lung Cancer Treated With Immune Checkpoint Inhibitors. JCO Precis Oncol 2022;6:e2100372. B) Mack PC, et al. Circulating tumor DNA (ctDNA) kinetics predict progression-free and overall survival in EGFR TKI-treated patients with EGFR-mutant NSCLC (SWOG S1403). Clin Cancer Res 2022; 28(17): 3752–3760.