

A ctDNA-Based Longitudinal Classification System Stratifies Risk in NSCLC Patients Across Multiple MRD Platforms: A Retrospective Real-World Analysis.

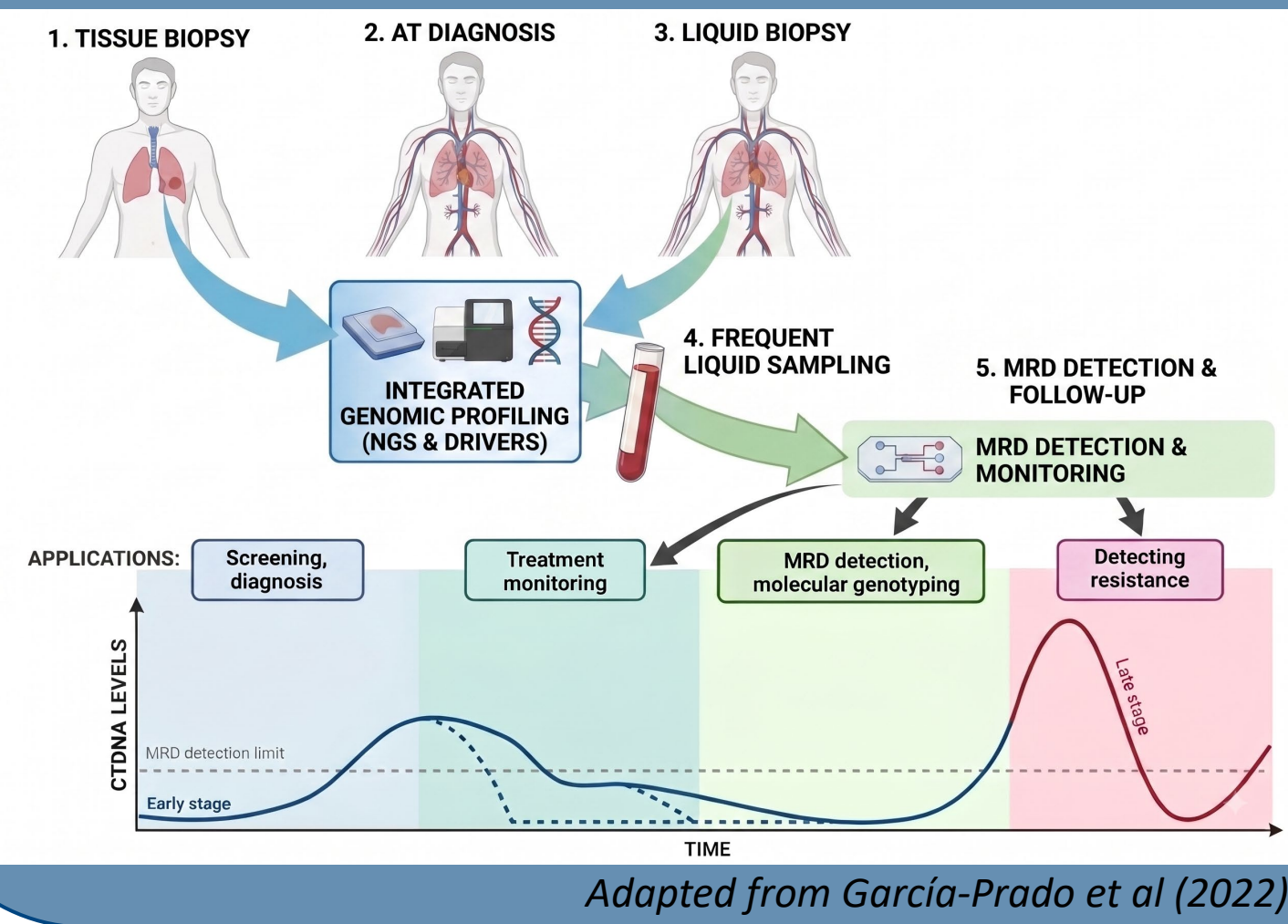
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BACKGROUND

Circulating tumor DNA (ctDNA) assays for minimal residual disease (MRD) detection are increasingly used to predict recurrence after curative-intent treatment in non-small cell lung cancer (NSCLC). Current clinical practice largely relies on a binary interpretation; either ctDNA is detected (positive) or not detected (negative), without accounting for longitudinal trends or quantitative levels over time. We evaluated whether longitudinal ctDNA patterns stratify recurrence risk consistently across distinct MRD platforms.”

Integration of tissue and liquid biopsy in Non-Small Cell Lung Cancer management and longitudinal ctDNA dynamics.



Top panel: Sequential workflow combining diagnostic **tissue biopsy** for genomic profiling with longitudinal **liquid biopsy** (blood sampling) for minimal residual disease (MRD) monitoring. **Bottom panel:** Timeline of ctDNA levels relative to clinical milestones across disease management. Dashed horizontal line indicates the threshold for ctDNA detection.

METHODS

We conducted a retrospective study of patients with stage I–III NSCLC treated at Mount Sinai Medical Center who underwent ctDNA MRD monitoring during a 5-year period a using one of two ctDNA MRD platforms based on whole-exome sequencing (WES) or whole-genome sequencing (WGS). Patients with ≥ 3 serial ctDNA assessments were classified according to longitudinal ctDNA dynamics as **Stable Negative**, **Baseline Positive**, **Transient Positive**, or **Persistent Positive**. Clinical outcomes were assessed from diagnosis through last clinical follow-up. Recurrence-free probability was estimated using Kaplan–Meier analysis, with time measured from **NSCLC diagnosis to recurrence or last clinical follow-up**. Cox proportional hazards models were used to estimate hazard ratios relative to the Stable Negative group.

CLASSIFICATION SYSTEM

- Stable Negative** ctDNA undetected at all evaluable serial timepoints, from baseline (pre-treatment, if available) through the entire monitoring period.
- Persistent Positive** ctDNA detected at the majority ($\geq 50\%$) of evaluable timepoints, regardless of whether levels are stable or rising over time.
- Baseline Positive** ctDNA detected at diagnosis or early in the treatment course, then cleared following curative-intent treatment and remaining undetectable.
- Transient Positive** ctDNA detected at ≥ 1 but $< 50\%$ of evaluable timepoints, with intervening or subsequent negative results and no sustained upward trend.

SUPPORT

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COHORT CHARACTERISTICS

Patient and Disease Characteristics by ctDNA MRD Platforms

Cohort characteristics differed across MRD platforms, particularly in stage distribution and monitoring duration. Despite these differences, longitudinal ctDNA categories showed consistent prognostic patterns across cohorts.

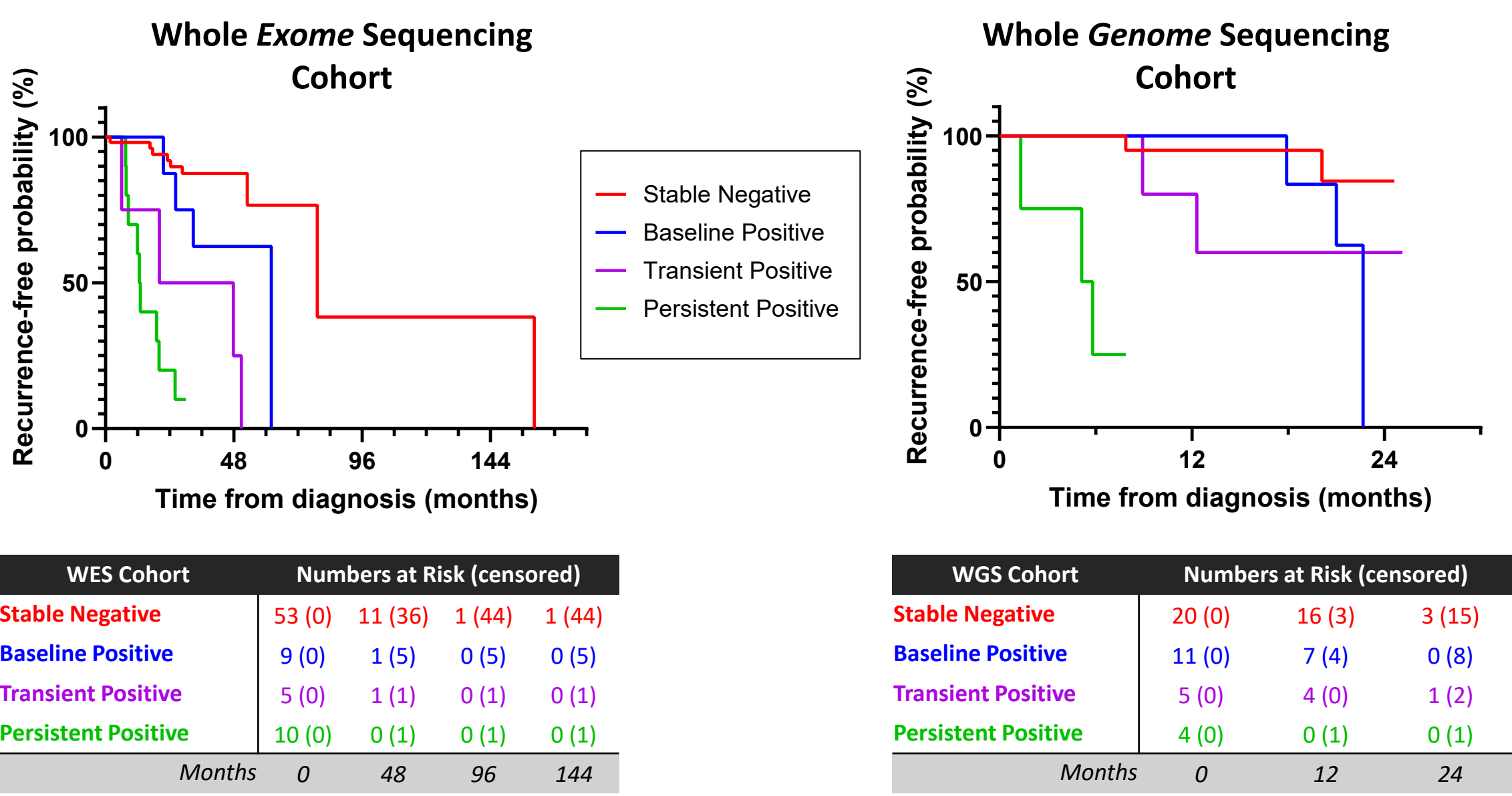
Variable	Category	Whole Exome Seq	Whole Genome Seq	Pooled cohort
Age, median (range), years		72 (39–89)	72 (53–92)	72 (39–92)
Sex, n (%)	Male	35 (45)	22 (55)	57 (49)
	Female	42 (55)	18 (45)	60 (51)
Histology, n (%)	Adenocarcinoma	57 (74)	28 (70)	85 (73)
	Squamous	18 (23)	12 (30)	30 (26)
	Adenosquamous	2 (3)	0 (0)	2 (2)
Ethnicity, n (%)	Hispanic	54 (70)	21 (52)	75 (64)
	Non-Hispanic	23 (30)	19 (48)	42 (36)
Race, n (%)	American Indian	0 (0)	0 (0)	0 (0)
	Asian	1 (1)	0 (0)	1 (1)
	Black	2 (2)	4 (10)	6 (5)
	Native Hawaiian	1 (1)	0 (0)	1 (1)
	White	66 (86)	32 (80)	98 (84)
	Undisclosed	7 (9)	4 (10)	11 (9)
	Multiracial	0 (0)	0 (0)	0 (0)
Clinical Stage, n (%)	I	39 (51)	15 (38)	54 (46)
	II	18 (23)	6 (15)	24 (21)
	III	20 (26)	19 (47)	39 (33)
ctDNA Classification, n (%)	Stable Negative	53 (69)	20 (50)	73 (62)
	Baseline Positive	9 (12)	11 (28)	20 (17)
	Transient Positive	5 (6)	5 (13)	10 (9)
	Persistent Positive	10 (13)	4 (10)	14 (12)
MRD monitoring duration, median (range), months		30.3 (3–47)	14.3 (4.8–22.7)	21 (3.4–47)

Data are presented as n (%) unless otherwise indicated. ctDNA, circulating tumor DNA; MRD, minimal residual disease.

PROGNOSTIC IMPACT OF LONGITUDINAL ctDNA

Cross-platform Longitudinal ctDNA Patterns Stratify Recurrence Risk

Kaplan–Meier estimates of recurrence-free probability stratified by longitudinal ctDNA classification for the WES and WGS cohorts. Time was measured from NSCLC diagnosis to recurrence or last clinical follow-up. Cox proportional hazards analyses are shown below each plot, with hazard ratios (HRs), 95% confidence intervals (CIs), and p-values for each ctDNA category relative to the Stable Negative group. Longitudinal ctDNA categories were assigned using serial MRD assessments obtained during follow-up.



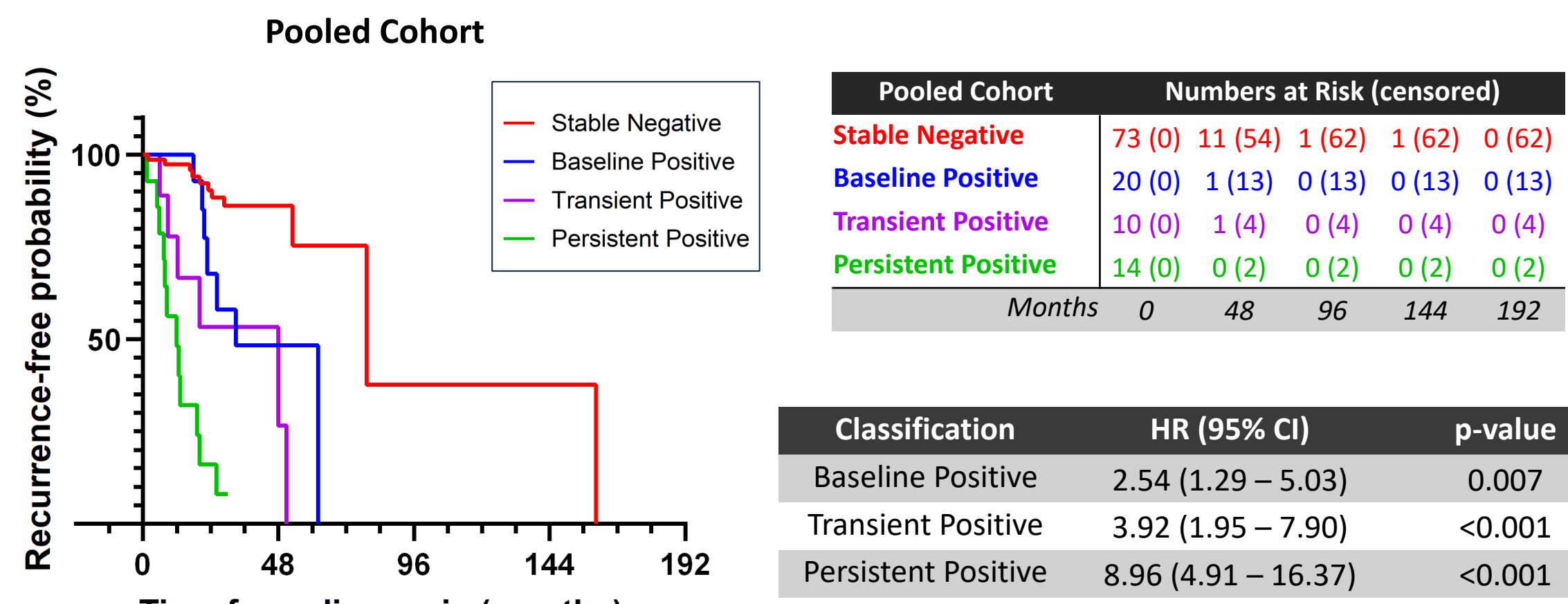
Cox Proportional Hazards Analysis

WES Cohort			WGS Cohort		
Classification	HR (95% CI)	p-value	Classification	HR (95% CI)	p-value
Baseline Positive	3.49 (1.02 – 11.99)	0.0470	Baseline Positive	2.89 (0.47 – 17.60)	0.250
Transient Positive	10.25 (2.85 – 36.81)	<0.001	Transient Positive	3.69 (0.52 – 26.31)	0.192
Persistent Positive	24.21 (8.10 – 72.42)	<0.001	Persistent Positive	100.23 (7.44 – 1350.01)	<0.001

POOLED ANALYSIS OF LONGITUDINAL ctDNA

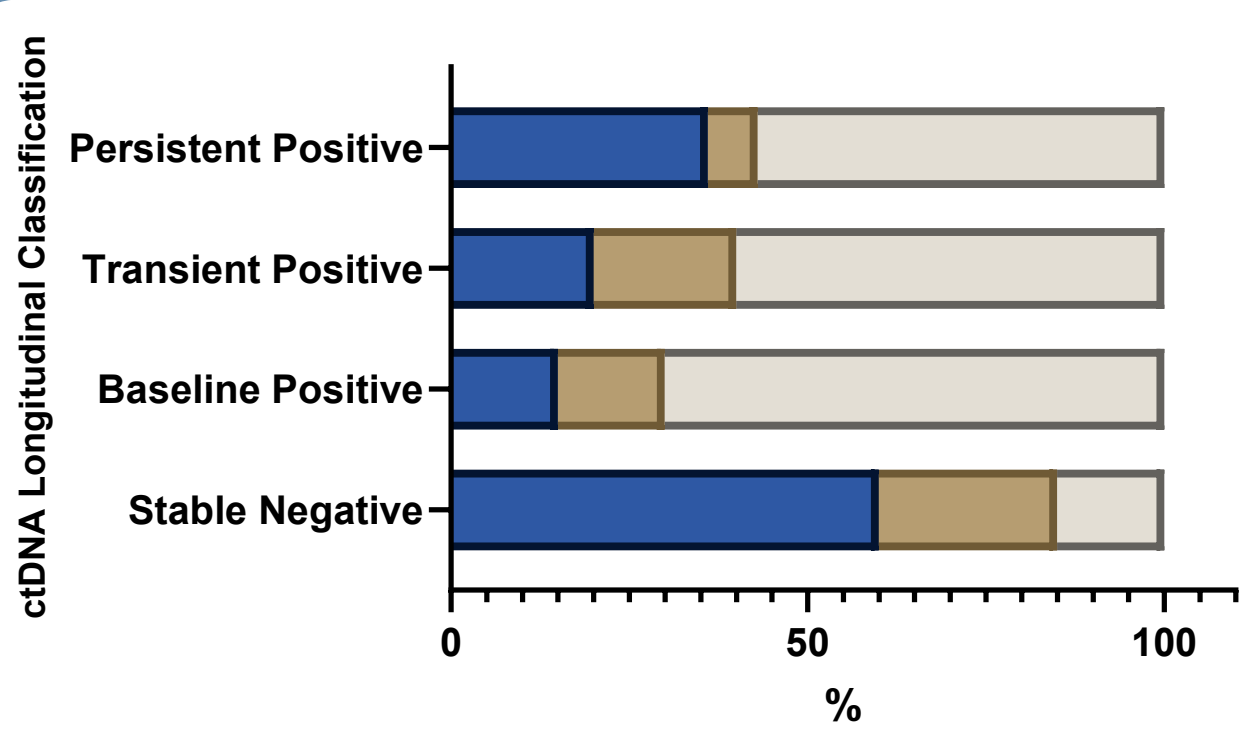
Longitudinal ctDNA Patterns Stratify Recurrence Risk in the Pooled Cohort

Kaplan–Meier estimates of recurrence-free probability stratified by longitudinal ctDNA classification in the pooled cohort. Time was measured from NSCLC diagnosis to recurrence or last clinical follow-up. Cox proportional hazards analysis reports hazard ratios (HRs), 95% confidence intervals (CIs), and p-values relative to the Stable Negative group. Recurrence risk increased progressively across Baseline Positive, Transient Positive, and Persistent Positive groups.



CLINICAL STAGE DISTRIBUTION BY LONGITUDINAL ctDNA CLASSIFICATION

Longitudinal ctDNA Patterns Are Observed Across Clinical Stages I–III



Clinical stage distribution varied across longitudinal ctDNA categories, with Stage III disease more prevalent among ctDNA-positive groups and Stage I disease more prevalent among Stable Negative patients. Nevertheless, all four longitudinal ctDNA patterns were represented across stages I–III. Percentages are calculated within each ctDNA category.

CONCLUSIONS

- Longitudinal ctDNA dynamics provided clinically meaningful risk stratification beyond a single positive/negative result, with recurrence risk increasing as ctDNA positivity became more sustained over time.
- Persistent ctDNA positivity consistently identified the highest-risk patients across both MRD platforms, despite differences in cohort characteristics and assay methodology.
- In the pooled cohort, recurrence risk increased stepwise from **Baseline Positive (HR 2.54)** to **Transient Positive (HR 3.92)** and **Persistent Positive (HR 8.96)** relative to Stable Negative patients.
- Serial ctDNA patterns may provide a cross-platform framework for refining post-treatment risk assessment in NSCLC, warranting prospective validation and evaluation for ctDNA-guided treatment strategies.

The pattern of ctDNA over time — not simply whether ctDNA is ever detected — may be critical for identifying patients at greatest risk of recurrence.