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ABSTRACT #474

Longitudinal Circulating Tumor DNA Dynamics During & After First-line Therapy in a National Cohort of Large B-cell Lymphoma Patients

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HOVON

Circulating Tumor DNA (ctDNA) in Large B-Cell Lymphoma is an Increasingly Important Biomarker

- EOT response evaluation with ctDNA-MRD by ultrasensitive assays is supported by multiple validation studies

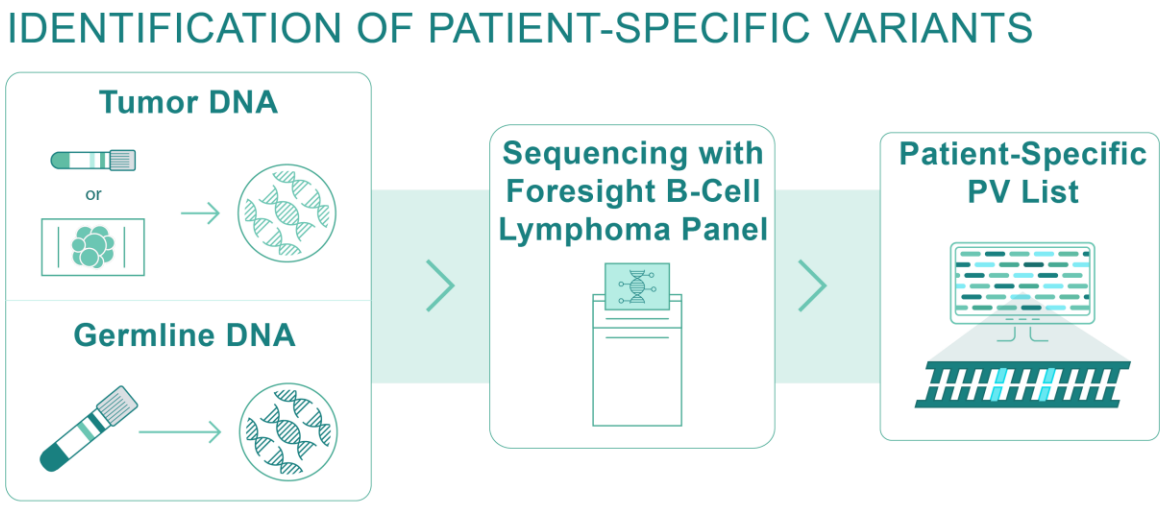
- Risk stratification by ctDNA (in combination with PET) now guides interventional trials:
  - Glofitamab + R-CHOP (NCT04980222): Therapy Escalation based on Molecular Response
  - SHORTEN-ctDNA (NCT06693830): Therapy De-Escalation based on Interim PET + MRD
  - SAKK 38/19 (NCT04604067): Therapy Escalation and De-Escalation based on Interim PET + MRD
  - ALPHA-3 (NCT06500273): Consolidation with CAR-T based on EOT ctDNA-MRD

- Post-remission disease surveillance relies mostly on clinical examination

- Longitudinal ctDNA dynamic during and after 1L treatment in real-world LBCL cohort is lacking

Roschewski et al. JCO 2025., Wang et al. JCO 2025 (in press), Krupka et al. medRxiv 2025., Meriranta et al. ResearchSquare 2025.
Nijland et al. ICML 2025. Cherng et al. ASCO 2025. Stathis et al. ICML 2025. Westin et al. ASCO 2025. Kumar et al. ASH 2020.

PhasED-seq: Analytical Performance for MRD Detection

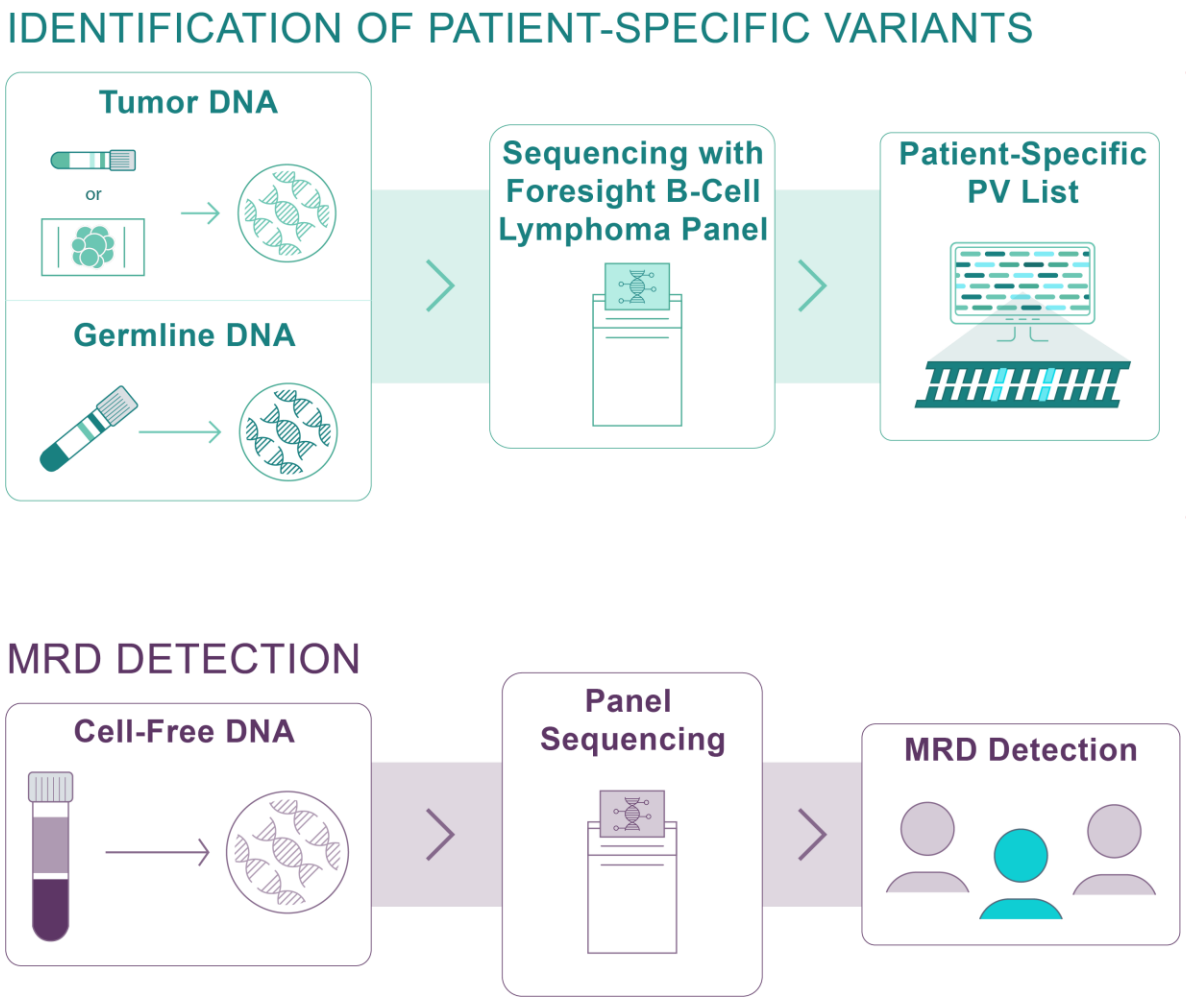


- **Phased Variants (PVs)** are identified from FFPE or pretreatment plasma
  - Buffy coat was used as paired normal
  - Used FFPE and Plasma in 53% and 47% of the cases for PV identification in this study

*PhasED-seq is performed as a laboratory developed test (LDT). This test has not been cleared or approved by the FDA.

Klimova et al. Oncotarget 2025.
Kurtz et al. Na Biotechnol 2021.

PhasED-seq: Analytical Performance for MRD Detection



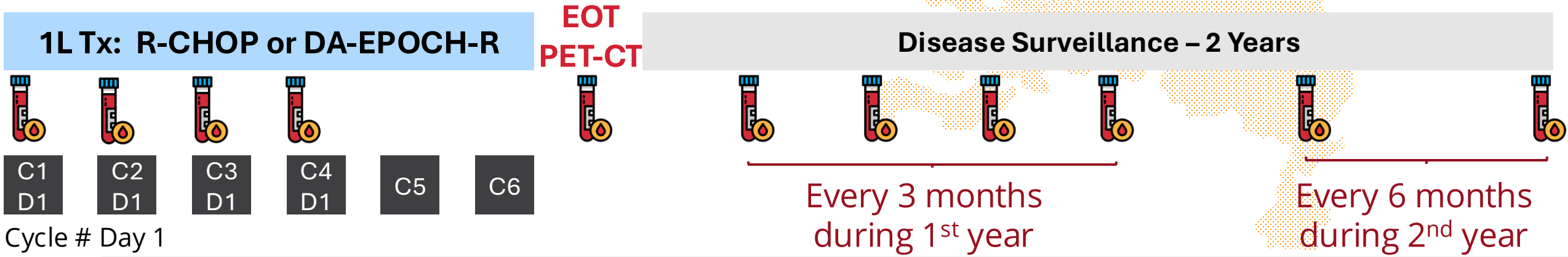
- **Phased Variants (PVs)** are identified from FFPE or pretreatment plasma
  - Buffy coat was used as paired normal
  - Used FFPE and Plasma in 53% and 47% of the cases for PV identification in this study
- **Sample-Level Sensitivity** depends on:
  - Quantity of DNA input
    - Plasma input in this study: 3-6 mL
  - Analytical performance
    - Median number of informative molecules in this study: 120,000 molecules/sample

*PhasED-seq is performed as a laboratory developed test (LDT). This test has not been cleared or approved by the FDA.

Klimova et al. Oncotarget 2025.
Kurtz et al. Na Biotechnol 2021.

A National Cohort* of 1L LBCL Patients

- ~ 50 academic and community centers in the Netherlands
- Newly diagnosed DLBCL or HGBL and received curative intent 1L therapy: R-CHOP or DA-EPOCH-R
- Blood was collected in PAXgene ccfDNA tubes



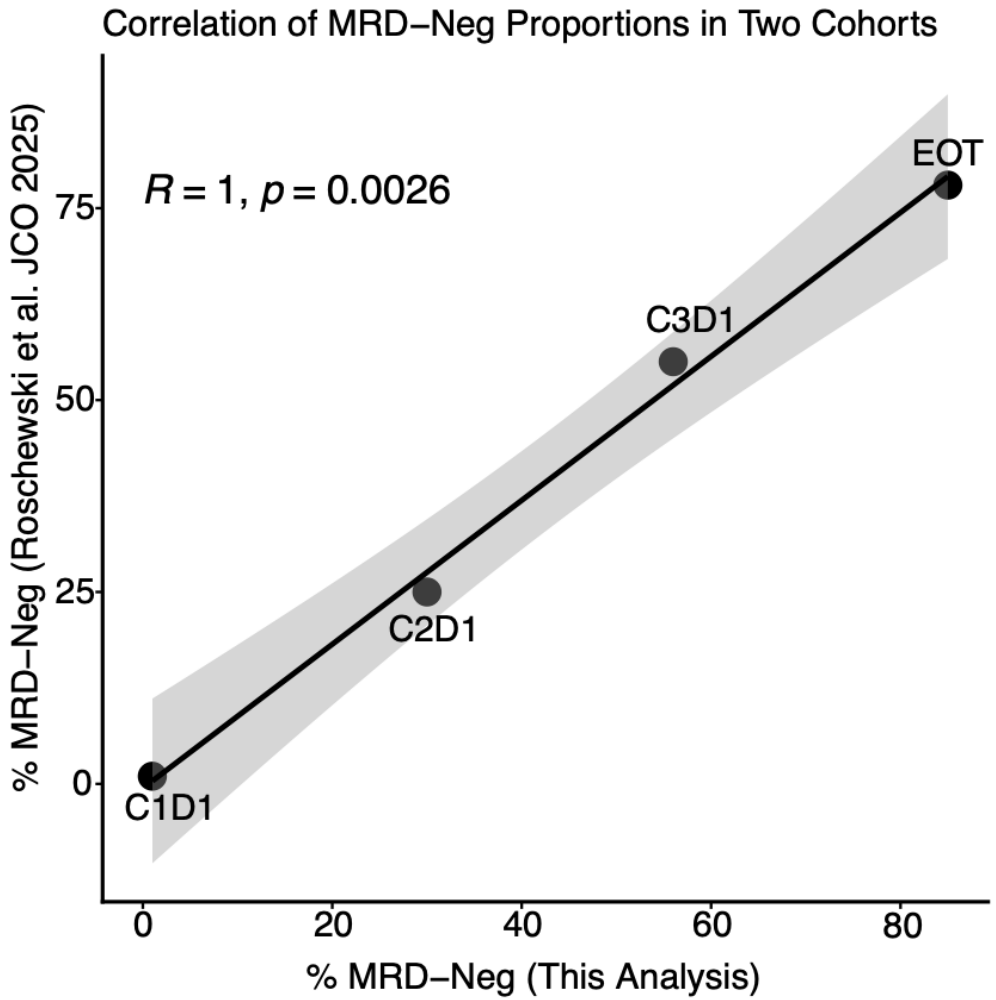
*HOVON-902 (NCT04139252)

Patient Selection and Characteristics

- **166** evaluable patients with surveillance and/or on-treatment samples
- PVs were identified in **164/166 (99%)**
- Median FU time was **33 months** (1.4-53.9 months)

Characteristic	Frequency (%)
Age, median (range)	69 (19-88)
Sex	
Male	106 (65%)
Female	58 (35%)
Diagnosis	
DLBCL	155 (95%)
HGBL	9 (5%)
Stage	
I-II	34 (21%)
III-IV	130 (79%)
IPI	
0-1	34 (21%)
2	53 (32%)
3	44 (27%)
4-5	33 (20%)
1L Treatment	
R-CHOP	156 (95%)
DA-EPOCH-R	8 (5%)

On-Treatment MRD Status is Prognostic for Outcomes at All Time Points Assessed



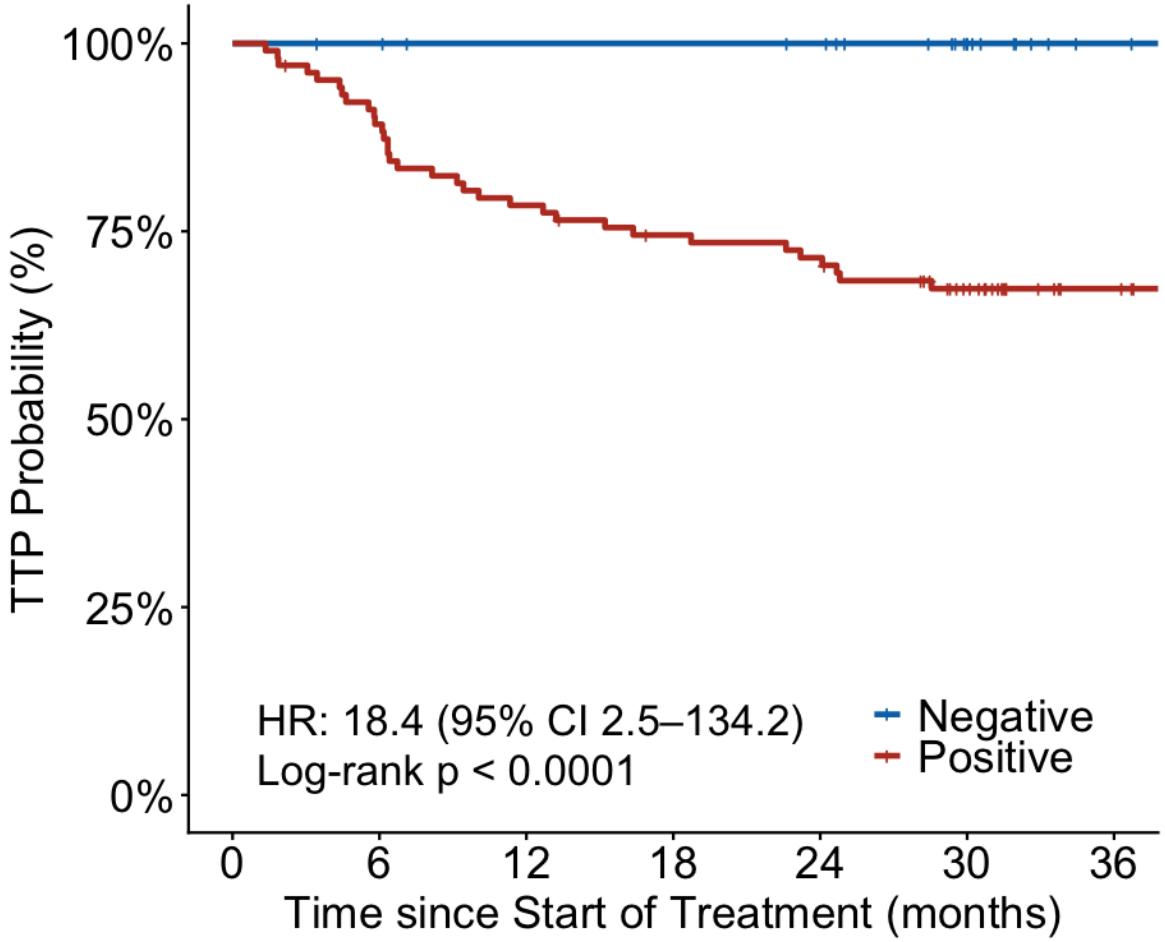
At each landmark assessed, ctDNA-MRD clearance was prognostic for PFS:

- C2D1: **HR 2.2** (95% CI 1.1-4.6), p < 0.01
- C3D1: **HR 2.5** (95% CI 1.4-4.5), p < 0.05
- C4D1: **HR 3.0** (95% CI 1.7-5.3), p < 0.001
- EOT: **HR 6.9** (95% CI 3.6-13.1), p < 0.0001

Roschewski et al. JCO 2025.

MRD Negativity at C2D1 is Associated with Low-Risk for Relapse

Time to Progression by C2D1* MRD Status



- **30%** of C2D1 samples was MRD-neg (n = 44)
- At the 3-year landmark:
  - **ALL** MRD-negative patients remained **relapse-free**
- Early MRD clearance at C2D1 is independently prognostic for TTP when controlled for IPI

*Analysis includes all evaluable patients with an available C2D1 sample.

Post 1L Treatment Disease Surveillance

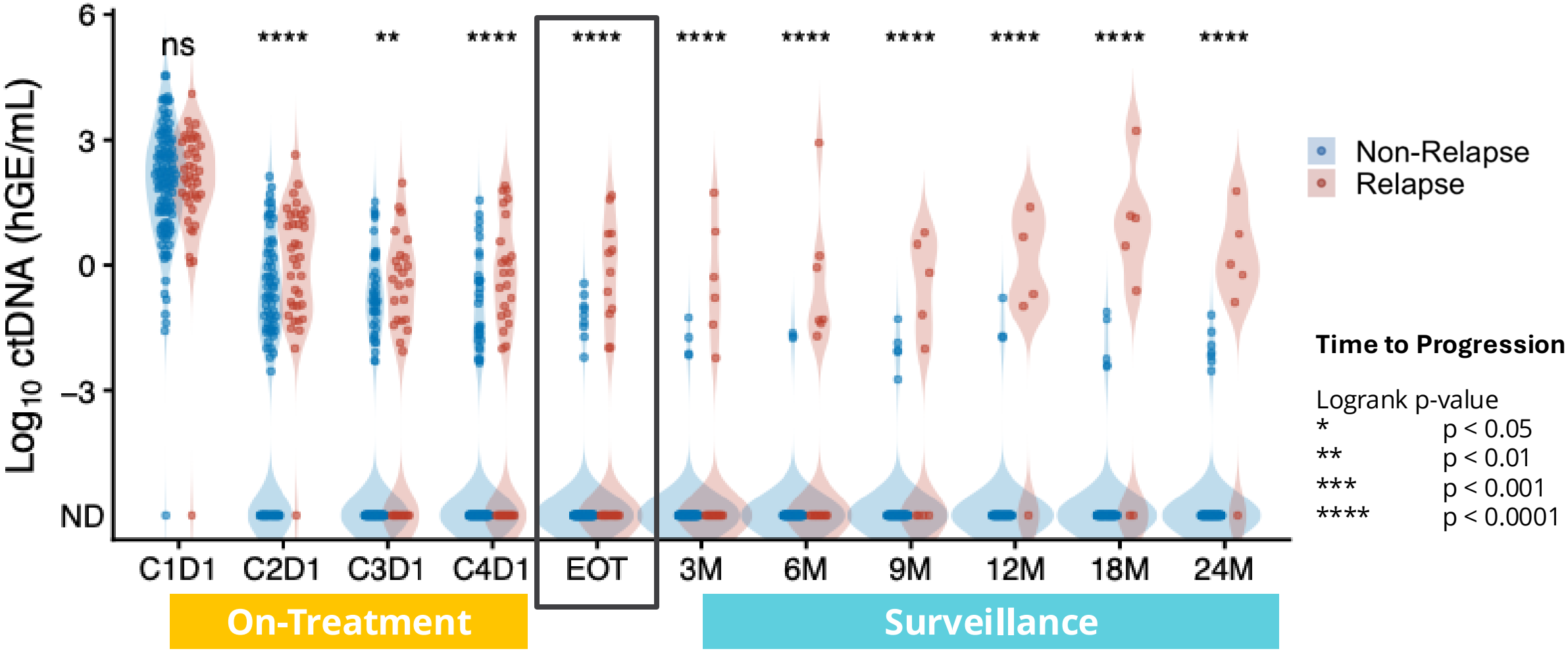
- Post-remission Surveillance
  - Dutch National Guideline recommends 2-year clinical follow-up without routine scans

- MRD assay for disease surveillance
  - IgHTS detected relapse early in a retrospective analysis[1]
  - Prospective[2] performance has shown variable sensitivity and lead time
  - **No data on the performance of MRD surveillance using PhasED-Seq in a real-world cohort**

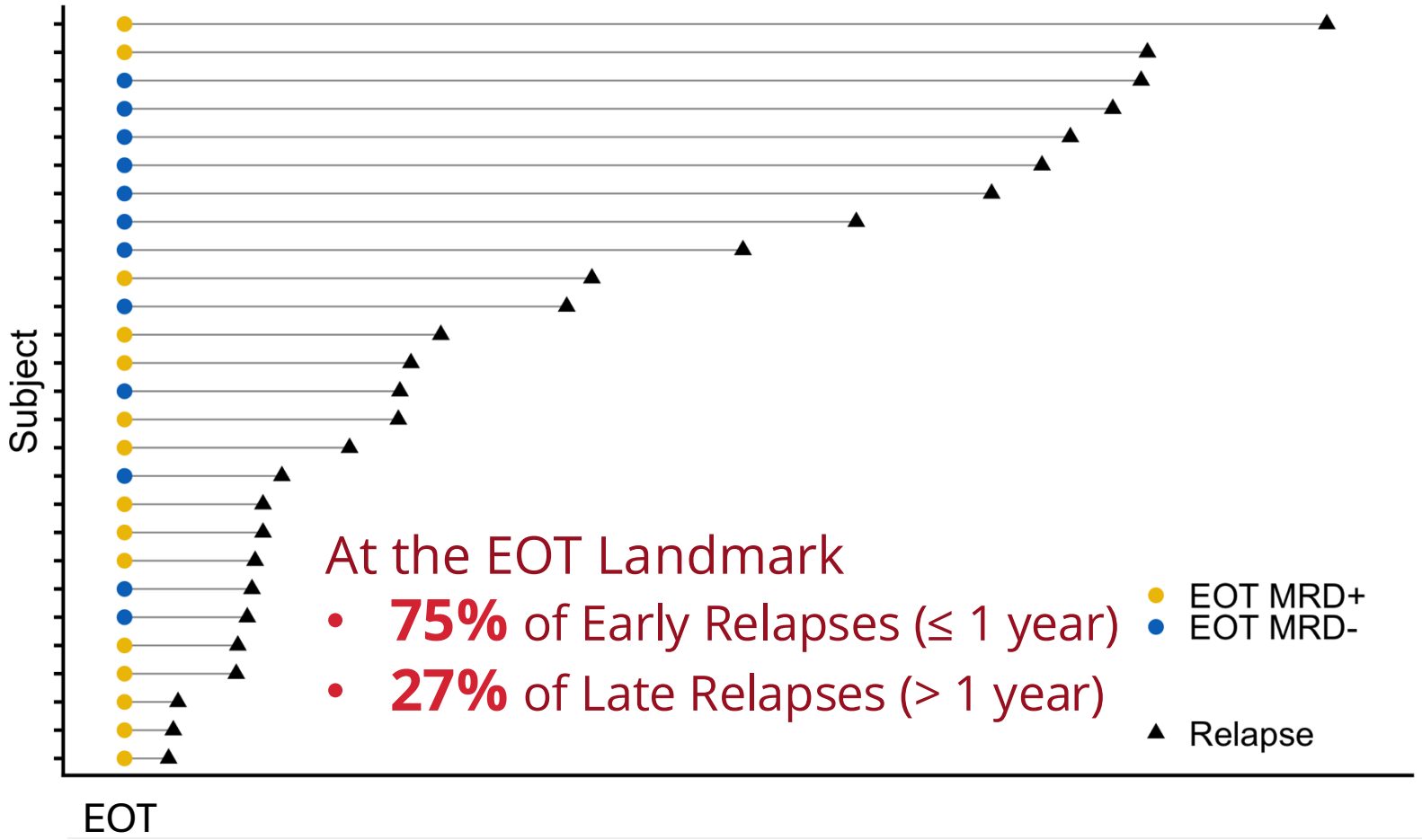
[1]Roschewski et al., Lancet Oncol 2015. [2]Kumar et al., ASH 2020.

ctDNA Dynamic During & After 1L Therapy

All Evaluated Time Points are Prognostic for Outcomes



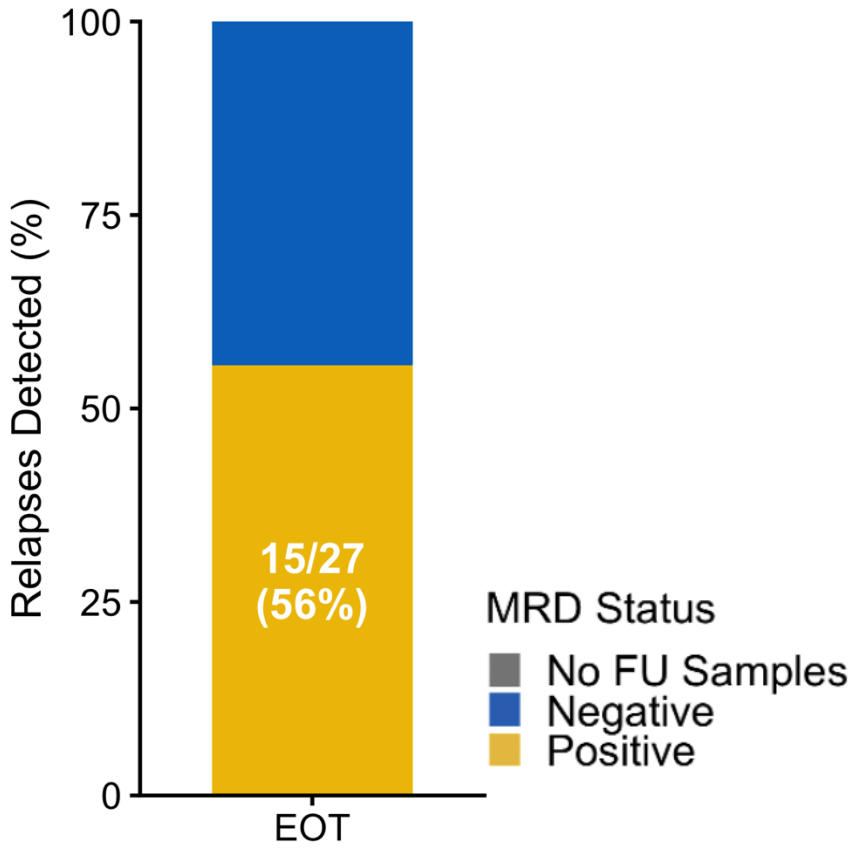
EOT ctDNA MRD Detects Early Relapses



At the EOT Landmark

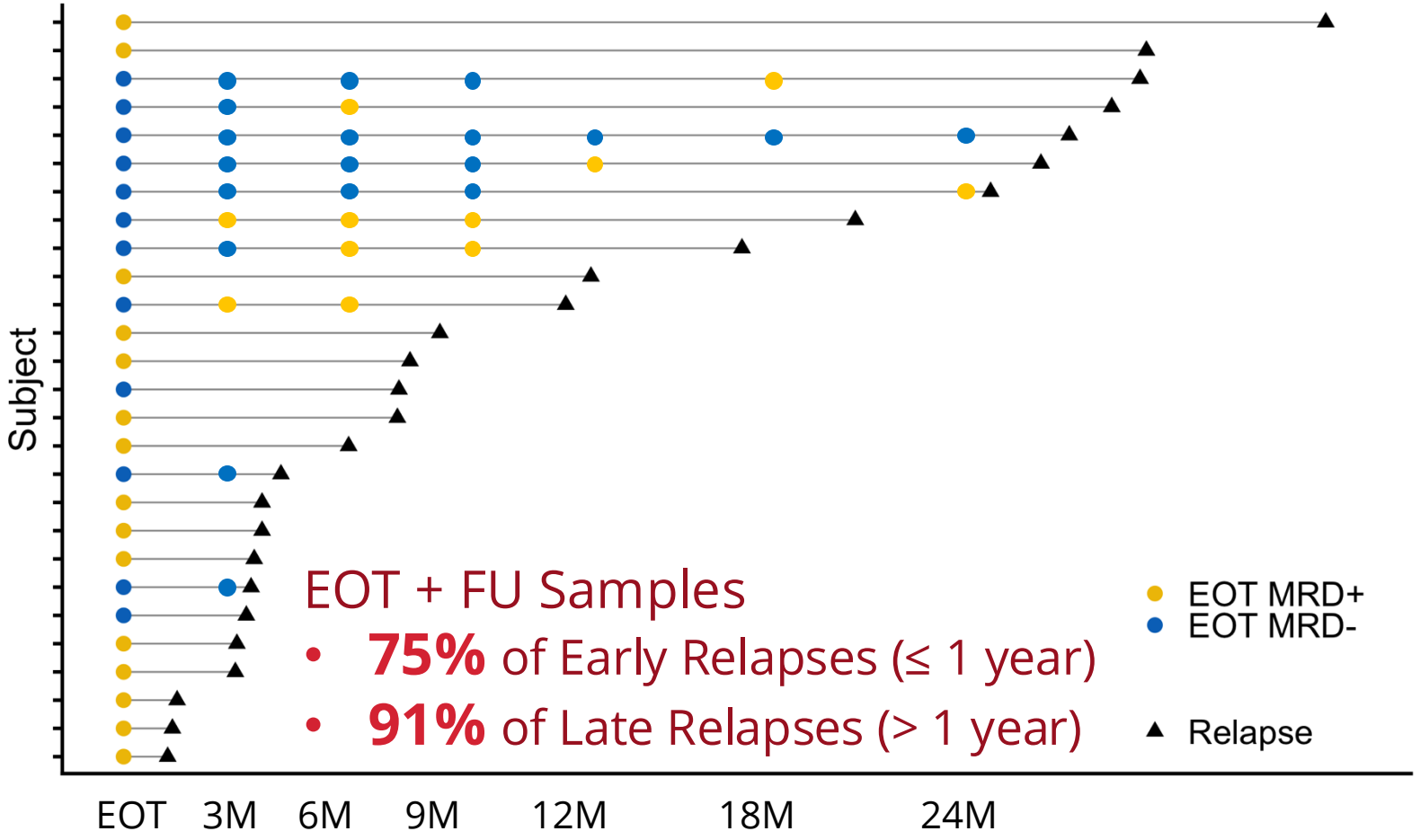
- **75%** of Early Relapses (≤ 1 year)
- **27%** of Late Relapses (> 1 year)

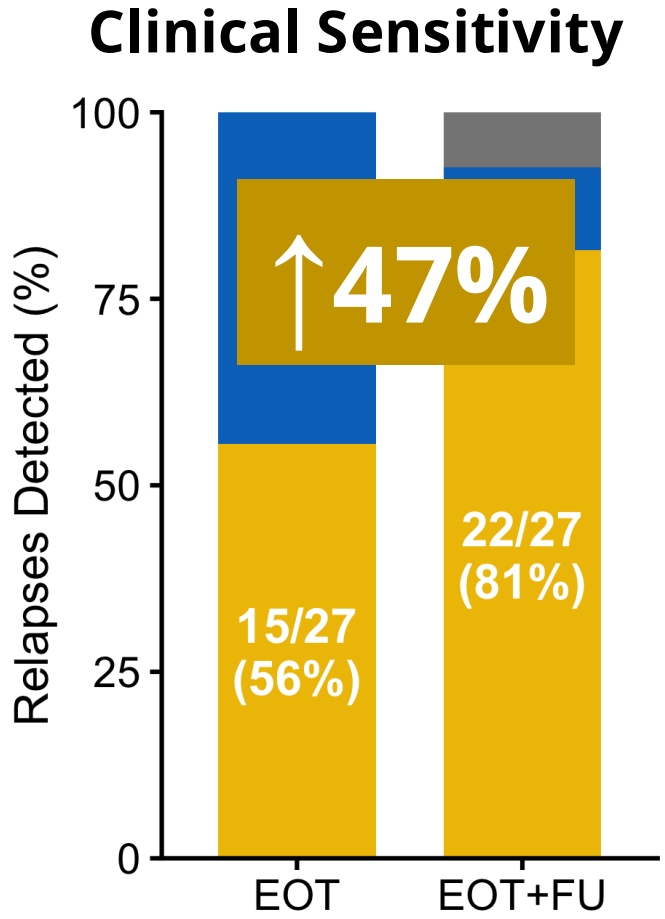
Clinical Sensitivity



Wang et al. JCO 2025. In Press.

Addition of Surveillance Samples Improves Detection – Especially in Late Relapses





Wang et al. JCO 2025. In Press.

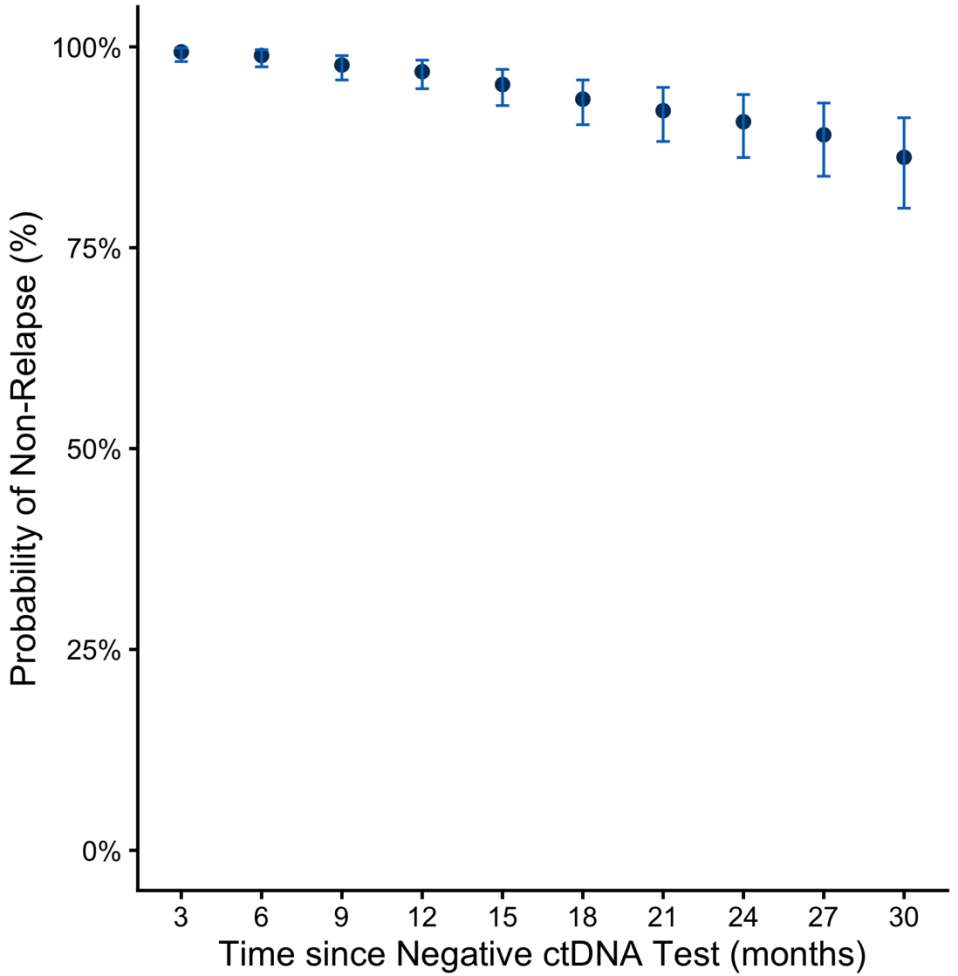
MRD Testing for Disease Surveillance

- "Surveillance testing" poses unique challenges for analytical evaluation and clinical practice
  1. **Frequency of surveillance testing** matters for "lead-time" (ie, testing every 3 vs 12 months)
  2. **Number of repeated tests** (ie, 1 test vs 10 tests) affects the chance of having a false positive result
  3. Tests are not acquired as a "series of tests" in clinical practice, but are **encountered one at a time**

- Given the challenges considering the performance of a "series of tests" as a single unit, better to consider the question:

  For any given test during surveillance, what does the result of a single MRD result mean?

Interpreting Any Given Negative Test during Surveillance



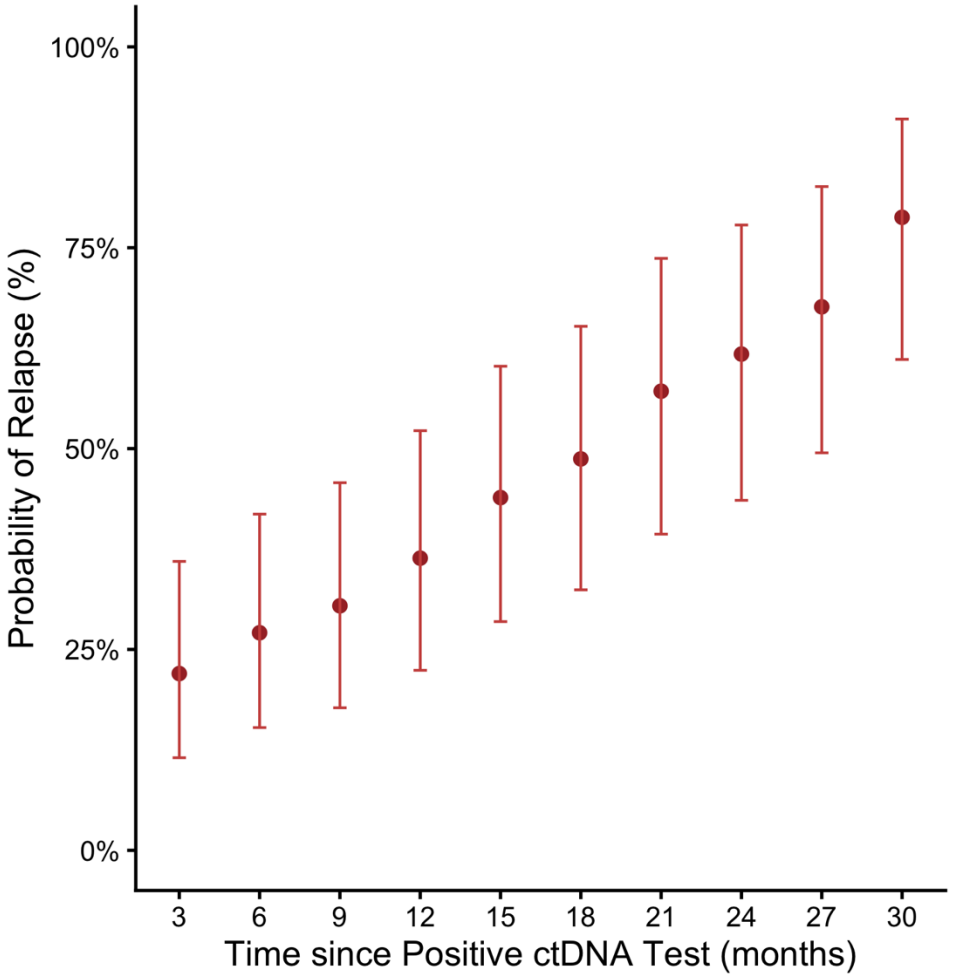
During surveillance, what does any given single negative test mean for future risk?

After a negative test at any given time, the chance* of remaining relapse-free:

- 6 months: **99%** (95% CI: 97%-100%)
- 12 months: **97%** (95% CI: 95%-98%)
- 24 months: **91%** (95% CI: 86%-94%)
- 30 months: **86%** (95% CI: 80%-91%)

*Values represent time-dependent conditional relapse probabilities in this cohort and are not diagnostic performance metrics.

Interpreting Any Given Positive Test during Surveillance



During surveillance, what does any given <u>single positive test</u> mean for future risk?

After a positive test at any given time, the risk* of a relapse:

- 6 months: **27%** (95% CI: 15%-42%)
- 12 months: **36%** (95% CI: 22%-52%)
- 24 months: **62%** (95% CI: 44%-78%)
- 30 months: **79%** (95% CI: 61%-81%)

*Values represent conditional relapse probabilities in this cohort and are not diagnostic PPV.

Conclusions & Future Perspectives

- **Early MRD clearance by C2D1 identifies a low-risk group (no relapse at 3 -year landmark)**
  - Potential as an early on-treatment biomarker for future therapy de-escalation trials

- MRD test performance during surveillance is time-dependent
  - **Any negative test is associated with very low risk for short-term relapse**
    - Probability of remaining relapse-free at 1 year after a negative test was at 97%
    - Yearly MRD testing warrants prospective evaluation in patients in remission at EOT
  - **The risk of a relapse after any positive test increases over time**
    - 27% at 6 months to nearly 80% at 30 months, reflecting ctDNA biological lead time
    - Future trials should evaluate whether early intervention upon MRD detection improves outcomes

Thank you to all patients and their families and local investigators.

Collaborator Foresight Diagnostics
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