# Recommendations for Application of ctDNA to Clinical Practice

Tuesday March 10, 2026
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Heme/Onc Fellow, PGY-4

Beth Israel Lahey Health

Beth Israel Deaconess Medical Center

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# Case presentation:
## Ms. KNM is a 53-year-old female

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**Presented May 2025 with:**
**Upper abdominal pain**
**Nausea**
**Vomiting**
**Malignant large bowel obstruction**

**Laparoscopic left colectomy**
**Invasive adenocarcinoma**
**Stage 2B (G2, pT4a, pN0, cM0)**
**pMMR, MTP+, TBS high, R0, LVI-**
**HRDsig-, KRAS G12D, NRAS wt, TP53 R175H**

**DPYD Intermediate (*1 / *2A)**

# Clinical Dilemma

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## Should this patient with Stage 2B colon adenocarcinoma s/p left colectomy receive adjuvant chemotherapy?

**TABLE 1.** Effect of ACT in Patients With T4 Stage II Colon Cancer

**Population: T4 stage II colon cancer**
**Intervention: ACT**
**Comparator: surgery alone**

| Outcome | Results | Absolute Effect Estimates | | Quality of Evidence (heterogeneity) | Plain Language Summary |
|---|---|---|---|---|---|
| | | Surgery Alone | ACT | | |
| OS | HR: 0.64 (95% CI, 0.56 to 0.75)<br>(18,517 patients in six studies)<br>Follow-up: 5 years | 483 deaths per 1,000 | 344 deaths per 1,000 | Low ($I^2$ = 60%) | ACT probably improves OS for patients with pT4 tumors |
| | | Difference: 139 fewer per 1,000 (95% CI, 174 fewer to 93 fewer) | | | |
| RFS | HR: 0.7 (95% CI, 0.63 to 0.77)<br>(7,711 patients in two studies)<br>Follow-up: 5 years | 541 recurrences or deaths per 1,000 | 420 recurrences or per 1,000 | Low ($I^2$ = 0) | ACT probably improves RFS for patients with pT4 tumors |
| | | Difference: 121 fewer per 1,000 (95% CI, 153 fewer to 90 fewer) | | | |

Abbreviations: ACT, adjuvant chemotherapy; HR, hazard ratio; OS, overall survival; RFS, recurrence-free survival.

# Broader Questions



Can ctDNA assess for the presence of residual malignancy with better specificity and sensitivity than conventional methods such as tumor markers, radiography, pathological evaluation, and overall clinical impression?

If so, does this improved testing result in better outcomes?

# Learning Objectives

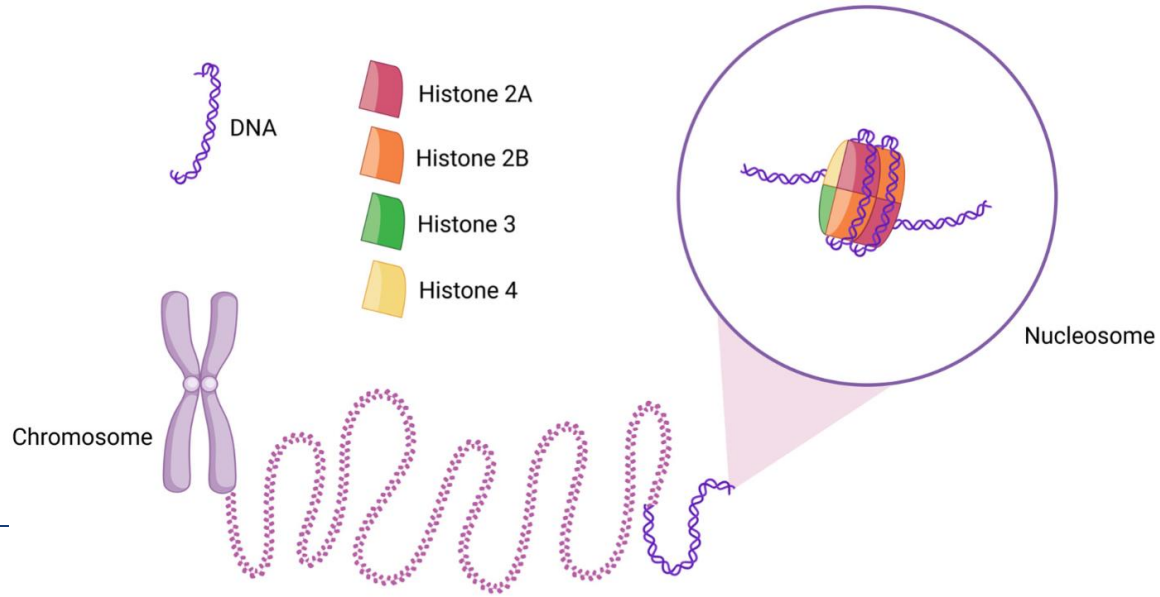


- Identify the scientific principles and create a mental model for cfDNA
- Explain the difference between tumor-informed and tumor-agnostic ctDNA testing
- Name 3 different ctDNA tests and their respective categories
- Weigh the pros and cons of ctDNA testing
- Evaluate the impact of ctDNA-guided treatment for patients with Stage 2 colon adenocarcinoma

# cfDNA (cell-free) Principles



- cfDNA = DNA from normal cells + tumor DNA (ctDNA) + fetal DNA (in pregnancy)
- Comes from apoptosis, necrosis, active secretion
- 70-90% cfDNA comes from hematopoietic cells (rest endothelium, liver, others)
- Healthy individuals 1-10ng/mL (like insulin, cortisol levels)
  - 300-3,000 genomic equivalents
- Fragments are 100-500bp in length (depends on # of nucleosomes)
- Half life is 15min-2hrs (cleared by liver, kidney, nucleases, macrophages)



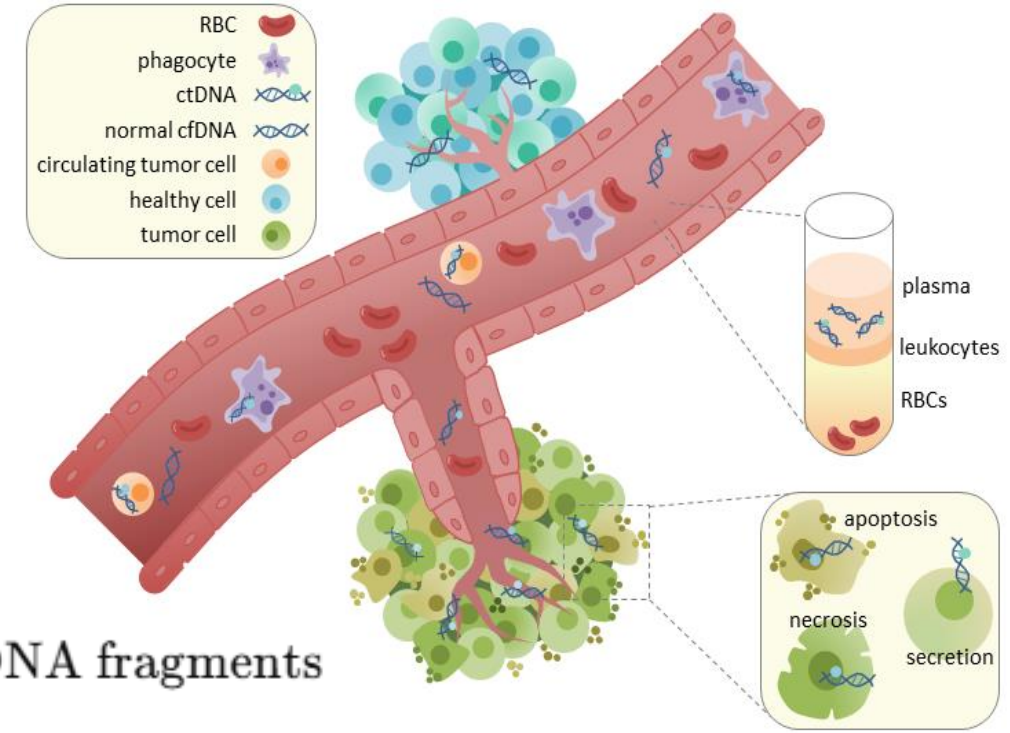
# ctDNA (circulating tumor) Principles



- From tumor cells, and as such has genetic alterations like the tumor
- ctDNA fragments are slightly shorter (140bp) than normal cfDNA (166-500bp)
- Cancer patients 10-1000ng/mL
  - 3,000-300,000 genomic equivalents
- Half life is 15min to 2hrs
- ctDNA Fraction ranges from 0.01%-50%+
  - % refers to proportion of fragments

$$\text{ctDNA fraction} = \frac{\text{number of mutant DNA fragments}}{\text{total DNA fragments}}$$

  - 0.01% = 1 tumor fragment per 10,000 total cfDNA fragments



# Mental Model

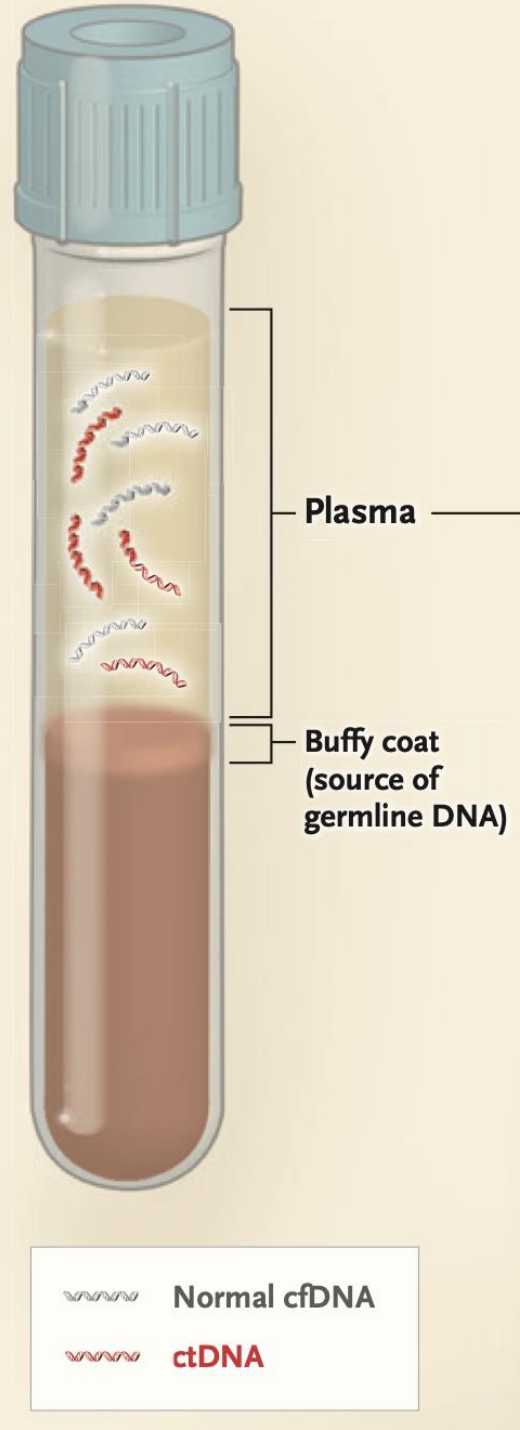
For detection of 0.01% Tumor Fraction of Heterozygous EGFR L858R mutation:

If you draw 10,000 cfDNA genomic equivalents from plasma

- 9,999 genomic equivalents come from normal cells
- 1 genomic equivalent comes from the tumor (ctDNA)

1 of these genomic equivalents may carry a mutation in EGFR

PCR-based enrichment or Hybrid-capture (probe-based) enrichment is used to identify and amplify this low number



# Types of ctDNA Tests



- Tumor-Agnostic
  - ctDNA test does not use any prior information about the patient's cancer
  - Uses a panel of "common culprit genes" to measure ctDNA

- Tumor-Informed
  - The company identifies specific gene variants that are present in the tumor
  - The company then searches for these same gene variants in the plasma in the form of ctDNA

# Examples of ctDNA Tests



- Tumor-Agnostic
  - Foundation Medicine
    - 324-gene panel
  - Guardant 360
    - 55-gene panel
  - Others (PGDx elio plasma, cobas EGFR, therascreen PIK3CA)

- Tumor-Informed
  - Signatera
    - 16 tracked variants
  - Others (Radar)

# To the board




WHY ARE YOU
WORKING SO HARD?
a Because
b. Umm, well...
RESULTS
a. 51%
b. 31%

# Possible Applications

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| Setting | Clinical position |
|---|---|
| **Early stage** | |
| Prior to treatment | ctDNA-positive patients to be treated with SOC. |
| After surgery | ctDNA could be evaluated for prognostication, ideally starting two weeks after definitive therapy; adjuvant therapy should be given per standard guidelines. |
| After completion of best SOC including systemic therapy | Persistent ctDNA-positive patients could be considered for clinical trials that accept ctDNA-positive patients. More intense imaging surveillance should be considered. |
| **Treatment-response monitoring** | |
| Neoadjuvant treatment monitoring | In cases where clinical complete response to neoadjuvant therapy permits consideration of non-operative management, persistent detection of ctDNA positivity may deter a non-operative approach. |
| Unresectable or advanced disease | Early assessment of response to systemic therapy with potential to switch treatment regimens if ctDNA does not decrease. |

| **Immunotherapy setting** | |
|---|---|
| True progression | Consideration should be given to altering the treatment regimen for patients who may have radiological and molecular (ctDNA) evidence of progression. |
| Pseudoprogression | Consideration should be given to not cease immunotherapy regimen prematurely for patients who have unconfirmed radiological progression accompanied by a ctDNA decrease. |
| Hyperprogression | Understanding whether large fluctuations in DNA could potentially identify hyperprogression requires additional research. |
| Exceptional responders | Treatment discontinuation could be considered for ctDNA-negative patients with continued surveillance monitoring with ctDNA monitoring added to SOC approaches. |
| IRAEs | Treatment could be discontinued for ctDNA-negative patients with continued monitoring using ctDNA and SOC approaches. |

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# Literature Review: DYNAMIC Trial

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JUNE 16, 2022 VOL. 386 NO. 24

## Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer

Jeanne Tie, M.D., Joshua D. Cohen, M.Phil., Kamel Lahouel, Ph.D., Serigne N. Lo, Ph.D.,

# Paper presentation: DYNAMIC Trial

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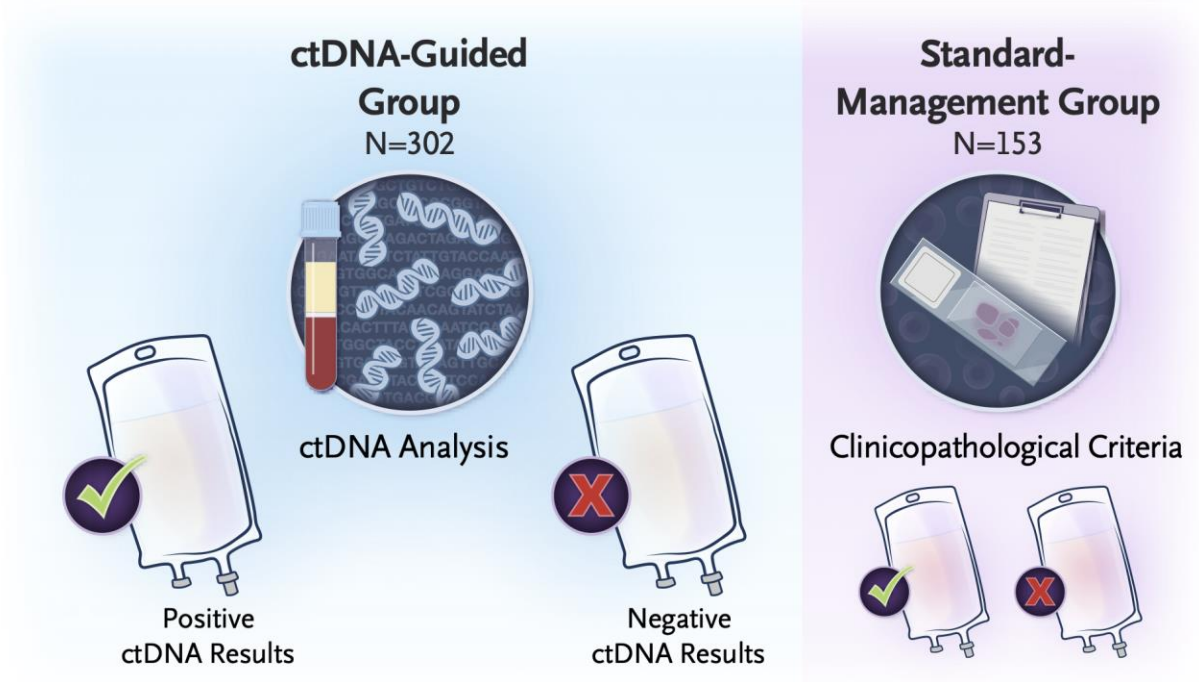
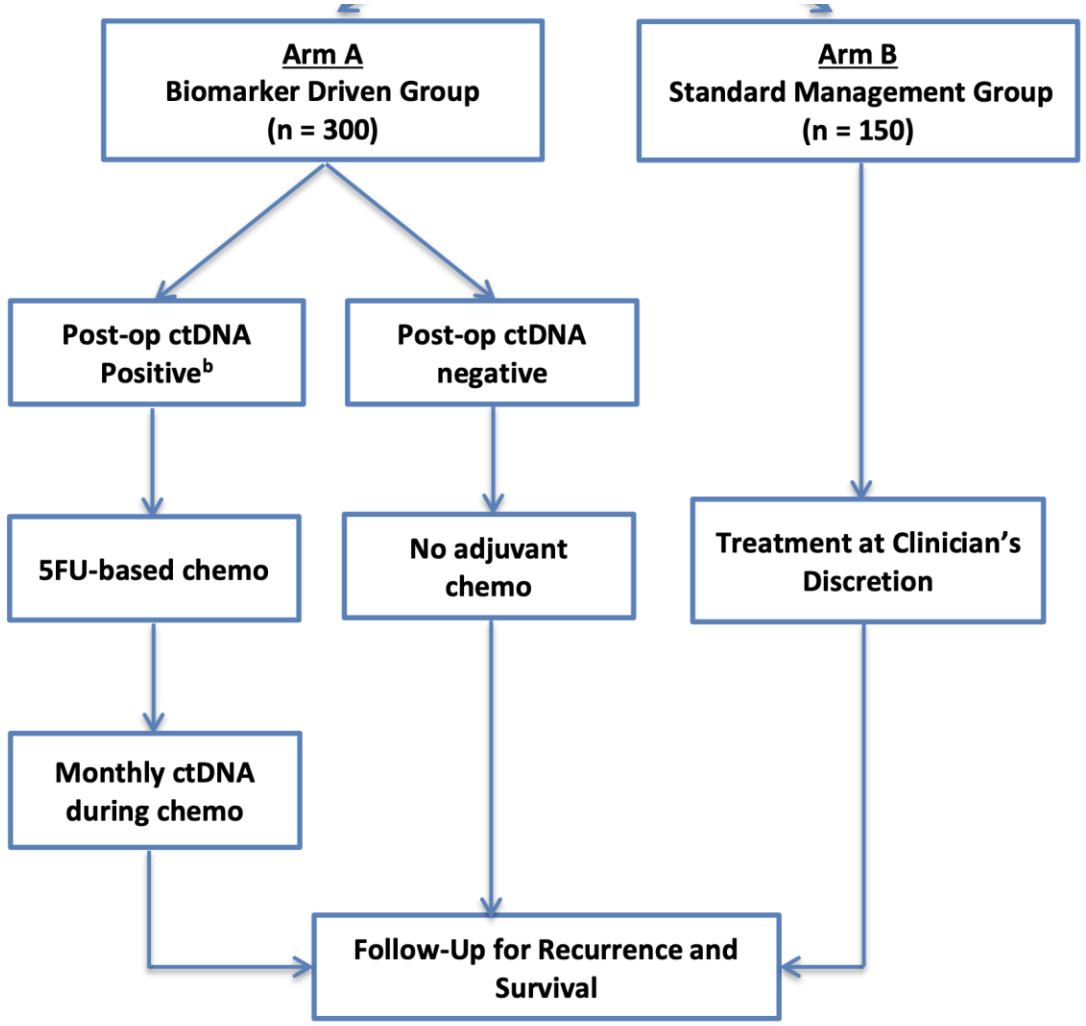
| | |
|---|---|
| **Design** | Phase 2, randomized, controlled noninferiority trial |
| **Population** | Patients with Stage 2 colon cancer |
| **Intervention** | ctDNA guided treatment |
| **Comparator** | Standard management |
| **Outcome** | Recurrence-free survival, 2yrs |



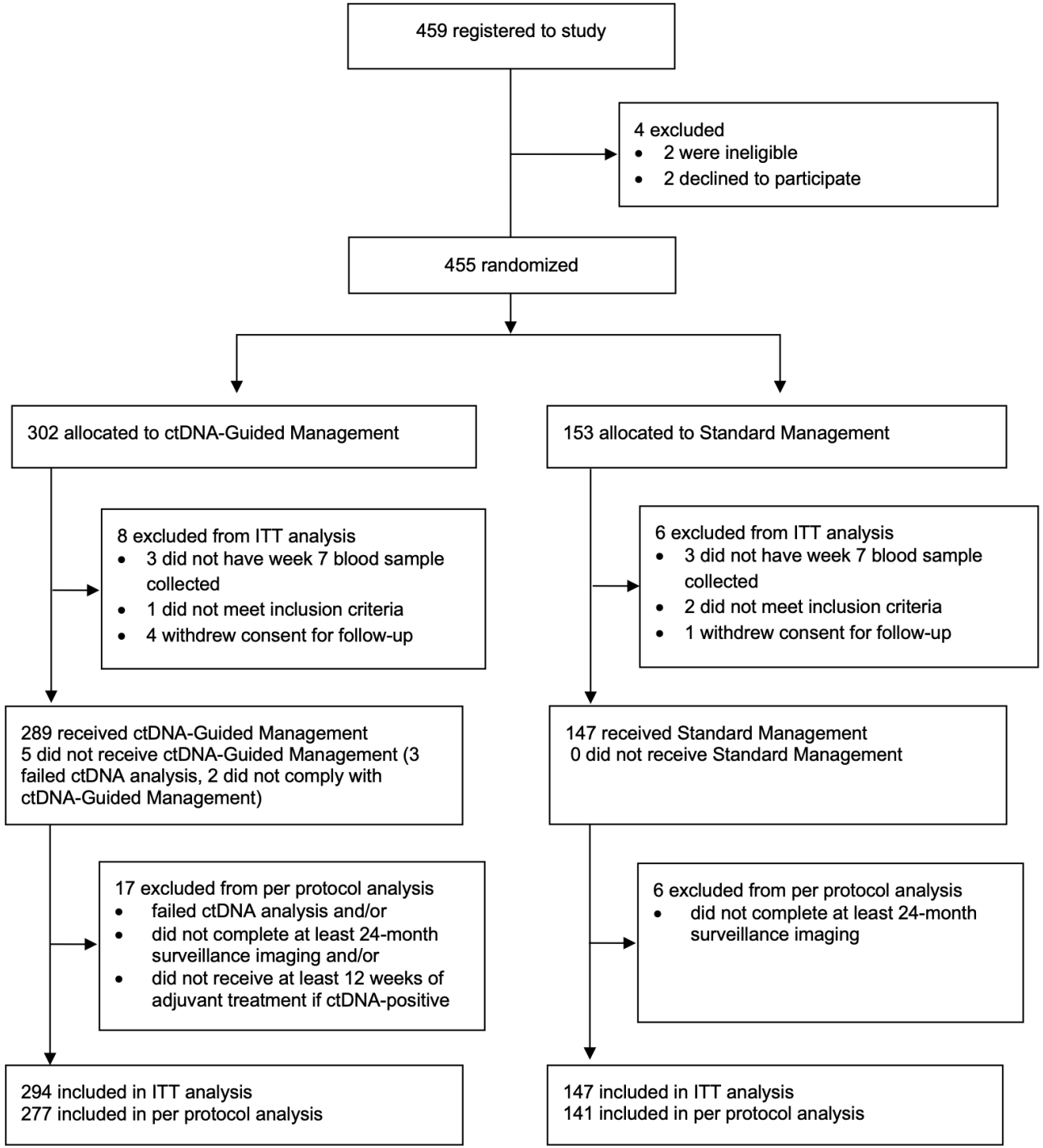
# Methods: ctDNA guided treatment



- Week 4 ctDNA
  - Available at Week 8
- Weed 7 ctDNA
  - Available at Week 10
- Assay: Illumina Safe SeqS



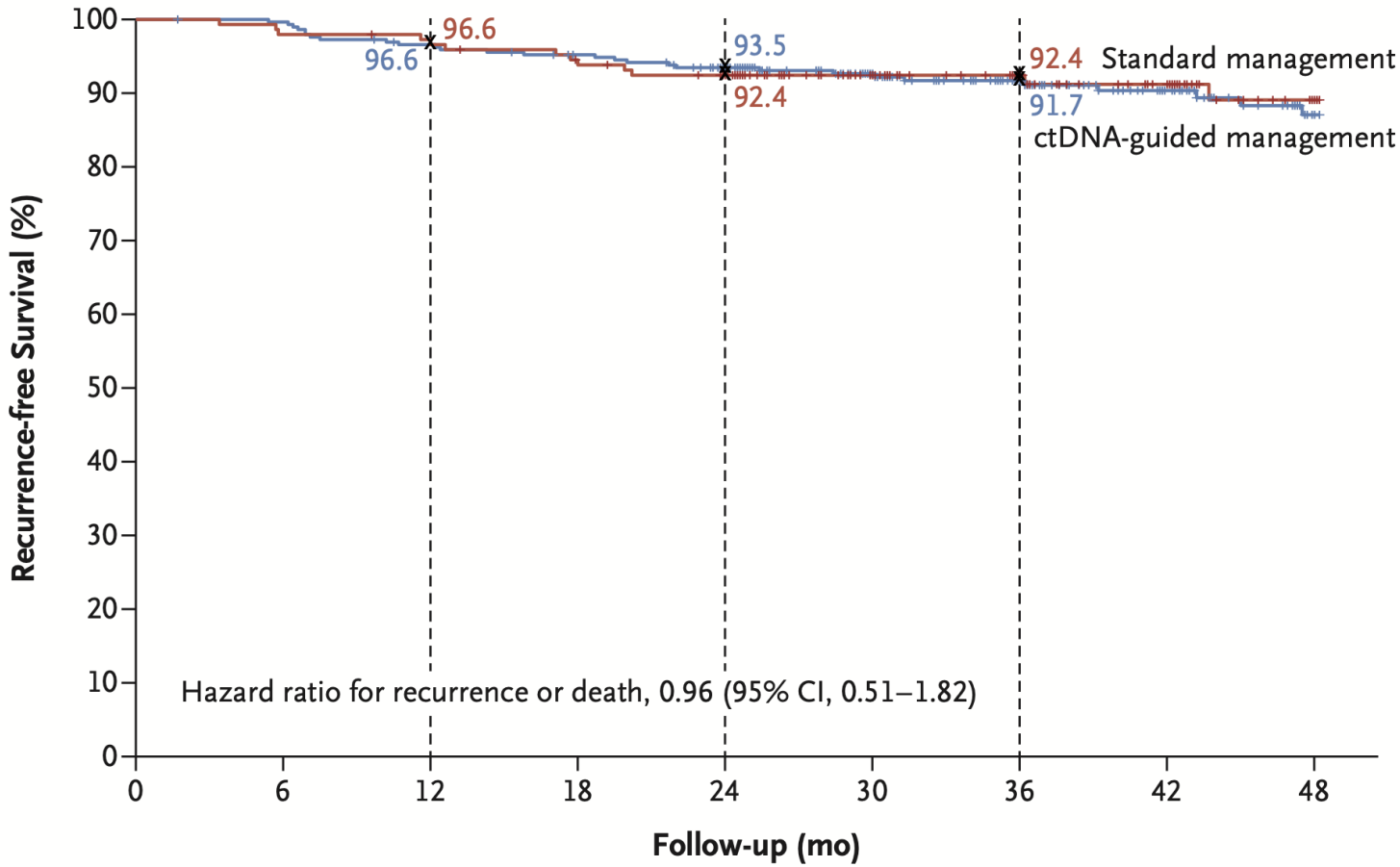
# Consort Diagram: DYNAMIC Trial



# Results:
# DYNAMIC Trial



**B** **Kaplan–Meier Estimates of Recurrence-free Survival**




**No. at Risk**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Standard management | 147 | 144 | 142 | 136 | 128 | 97 | 78 | 57 | 33 |
| ctDNA-guided management | 294 | 292 | 281 | 273 | 259 | 207 | 155 | 109 | 64 |

# Results:
# DYNAMIC Trial

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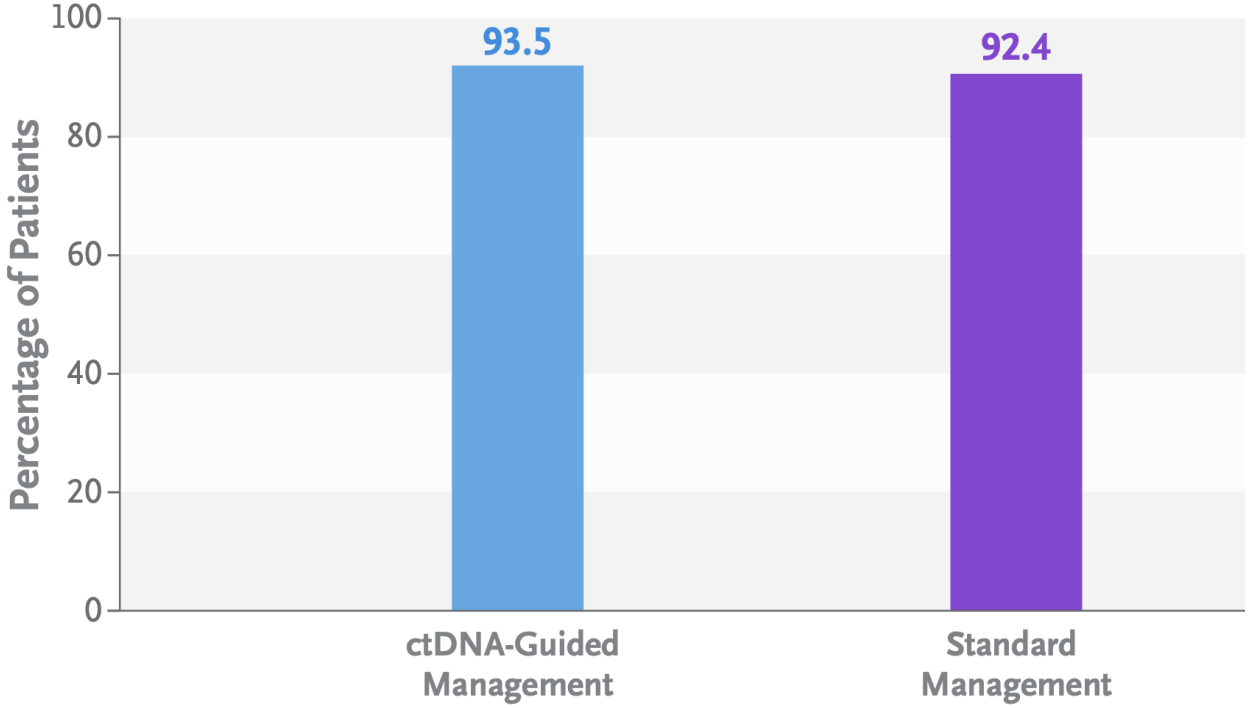
## 2-Year Recurrence-free Survival

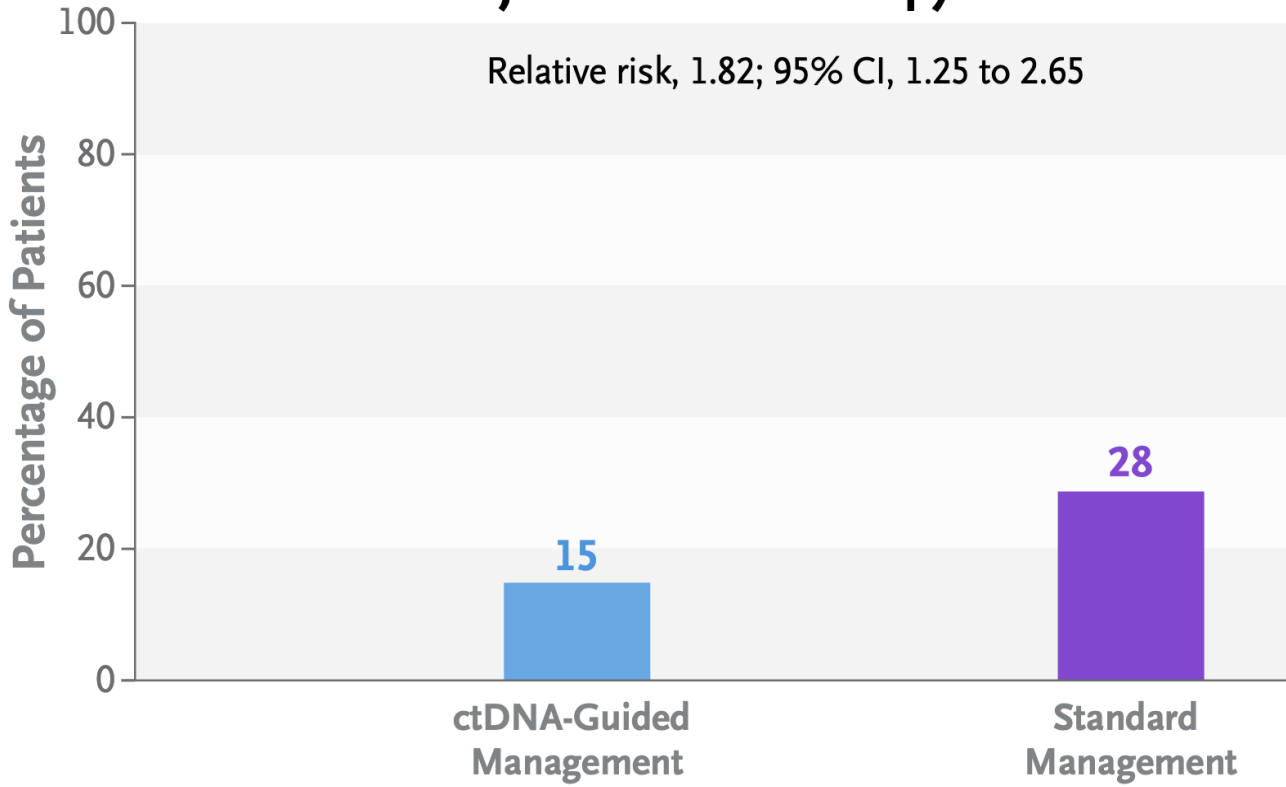
Absolute difference, 1.1 percentage points; 95% CI, –4.1 to 6.2

Noninferiority margin, –8.5 percentage points




## Adjuvant Chemotherapy Received

Relative risk, 1.82; 95% CI, 1.25 to 2.65



# Results:
# DYNAMIC Trial

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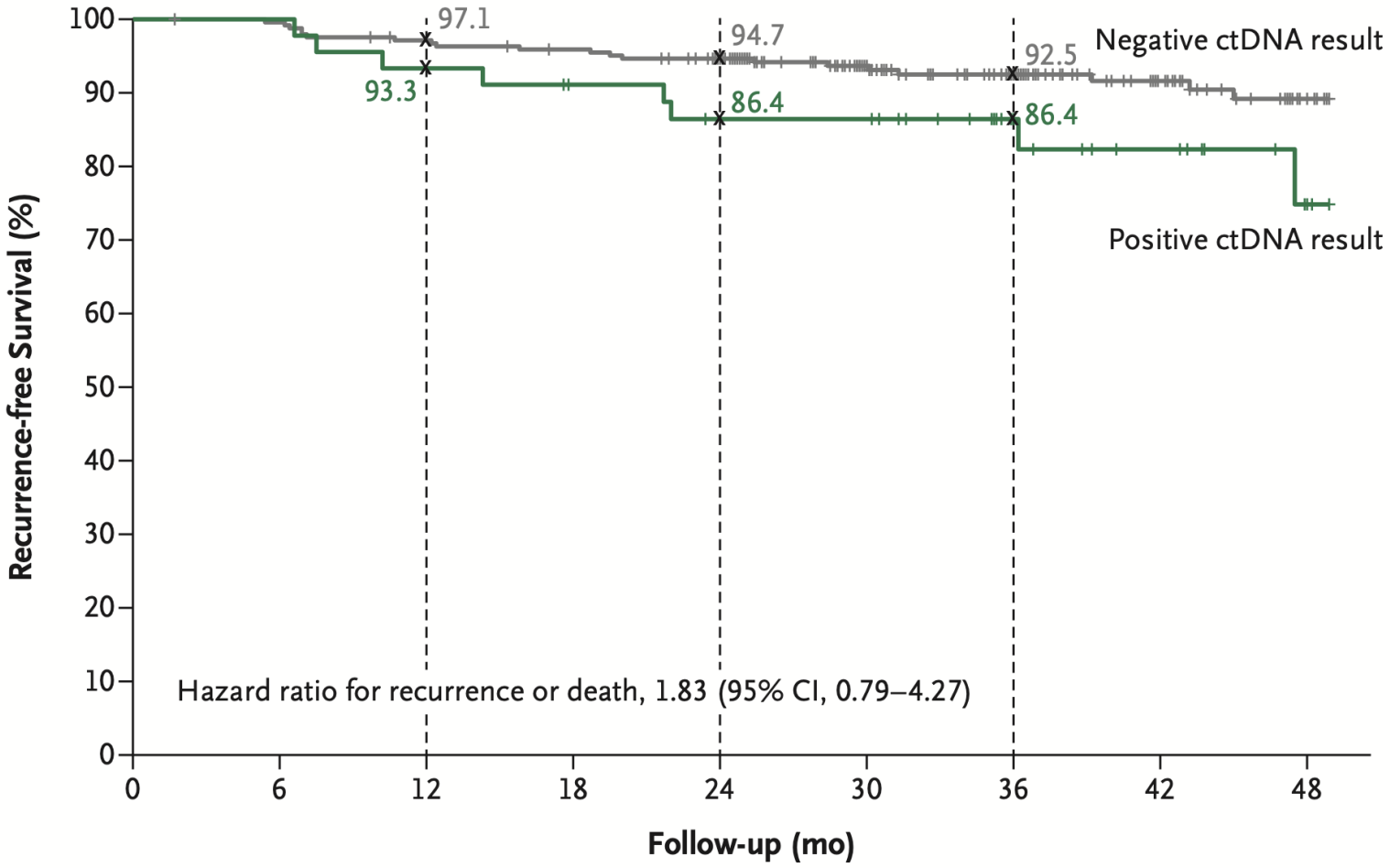


**No. at Risk**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Negative ctDNA result | 246 | 244 | 236 | 231 | 220 | 169 | 131 | 93 | 55 |
| Positive ctDNA result | 45 | 45 | 42 | 39 | 36 | 36 | 22 | 16 | 9 |

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# Summary:
# DYNAMIC Trial



- ctDNA-guided arm had significantly fewer patients receiving adjuvant chemo vs. standard management arm (15% vs. 28%), demonstrating ctDNA results meaningfully influenced treatment decisions
- At a median follow-up of ~37 months, 2-year recurrence-free survival in the ctDNA-guided arm was non-inferior to that in the standard arm (93.5% vs. 92.4%)
- Limitations:
  - The trial was too small to provide definitive findings for subgroups
  - Patients in the ctDNA +/- groups were not randomized
  - The effect of a ctDNA strategy was not assessed beyond the initial decision for adjuvant chemotherapy

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# Patient Course

# Treatment Course

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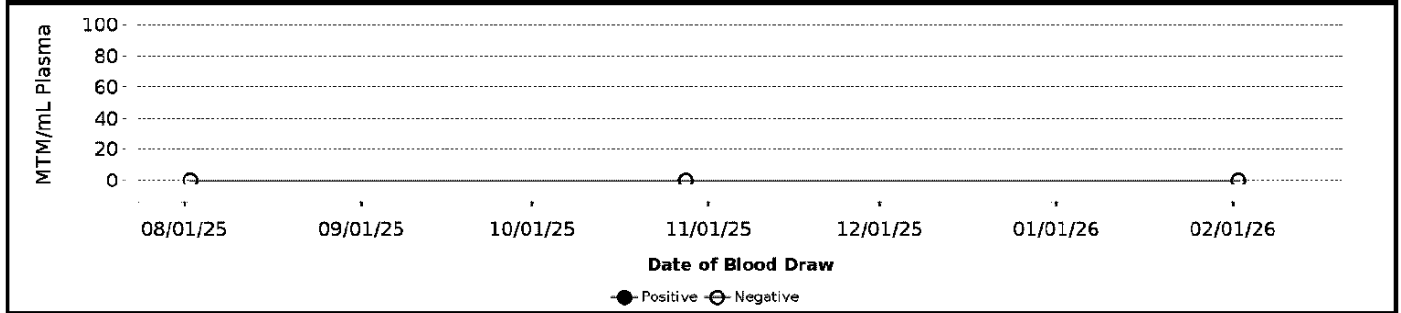
| | |
|---|---|
| 06/16/2025 | Laparoscopic left colectomy |
| 08/02/2025 | Signatera MRD negative |
| 08/07/2025 | C1 CAPOX |
| 08/19/2025 | Hospital admission for typhlitis (likely due to capecitabine) |
| 09/11/2025 | C2 CAPOX |
| 10/02/2025 | C3 CAPOX |
| 10/14/2025 | Hospital admission for enterocolitis (likely due to capecitabine) |
| 10/23/2025 | Patient declined cycle 4 |
| 10/28/2025 | Signatera MRD negative |
| 02/02/2026 | Signatera MRD negative |

**Historical Results**




| Date | Reported MTM/mL |
|---|---|
| Feb 02, 2026 | 0.00 |
| Oct 28, 2025 | 0.00 |
| Aug 02, 2025 | 0.00 |

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# Questions?

# Learning Objectives



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