

INTRODUCTION

- Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related mortality, and identifying EGFR mutations is essential for guiding targeted therapy in metastatic disease.
- Liquid biopsy using circulating tumour DNA (ctDNA) provides a minimally invasive alternative to tissue biopsy but frequently yields non-informative results, limiting its clinical utility.
- ctDNA is contained within circulating cell-free DNA (cfDNA), and lower cfDNA concentrations may reflect reduced tumour DNA shedding, decreasing the likelihood of detecting actionable mutations.

STUDY QUESTION:

Does plasma cfDNA concentration predict the likelihood of obtaining an informative EGFR liquid biopsy result in patients with EGFR-mutant metastatic NSCLC?

METHODS

DESIGN AND POPULATION:

- Retrospective cohort of adult patients with pathologically confirmed EGFR-mutant metastatic NSCLC, previously treated with 1st- or 2nd-generation EGFR TKIs, who underwent plasma EGFR testing at a tertiary cancer genomics laboratory (2018-2024) following clinically confirmed disease progression.

RESULT CATEGORIES:

- Informative:** The liquid biopsy detected one or more target EGFR mutations, providing clinically actionable molecular information.
- Uninformative:** The assay was technically successful, but no EGFR mutation was detected.
- Fail:** The assay could not be successfully completed because of technical or sample-related issues, resulting in no interpretable result.
- Indeterminate:** The assay produced a result that could not be definitively classified as positive or negative, requiring additional clinical interpretation or repeat testing.

TESTING WORKFLOW:

- Patient selection:** Confirmed EGFR-mutant metastatic NSCLC with radiographic progression following first- or second-generation EGFR TKI
- Plasma draw:** Blood collected and plasma cfDNA quantified
- EGFR plasma testing:** Blood collected and plasma cfDNA quantified
- Result classification:** Informative, uninformative, fail, and indeterminate

RESULTS

- A total of 641 plasma samples from 418 unique patients were included; 157 patients contributed multiple samples
- Results were informative in 36.3%, uninformative in 58.7%, failed in 4.2%, and indeterminate in 0.8% (Figure 1)
- Median cfDNA concentration was significantly higher in informative versus uninformative samples (1.19 ng/mL vs 0.80 ng/mL; $p < 0.001$)
- Higher cfDNA concentration was significantly associated with increased odds of an informative result across all model specifications, including unadjusted, adjusted, and mixed-effects models (all $p < 0.001$; Figure 3)
- A clear dose-response relationship was observed across cfDNA tertiles (Figure 2).
- Non-linear modeling showed a significant deviation from linearity ($p = 0.002$), with stronger effects at lower cfDNA concentrations and diminishing returns at higher concentrations (Figure 4)
- Higher cfDNA concentration was also significantly associated with detection of the T790M resistance mutation specifically (OR 1.50; $p < 0.001$, Figure 3)

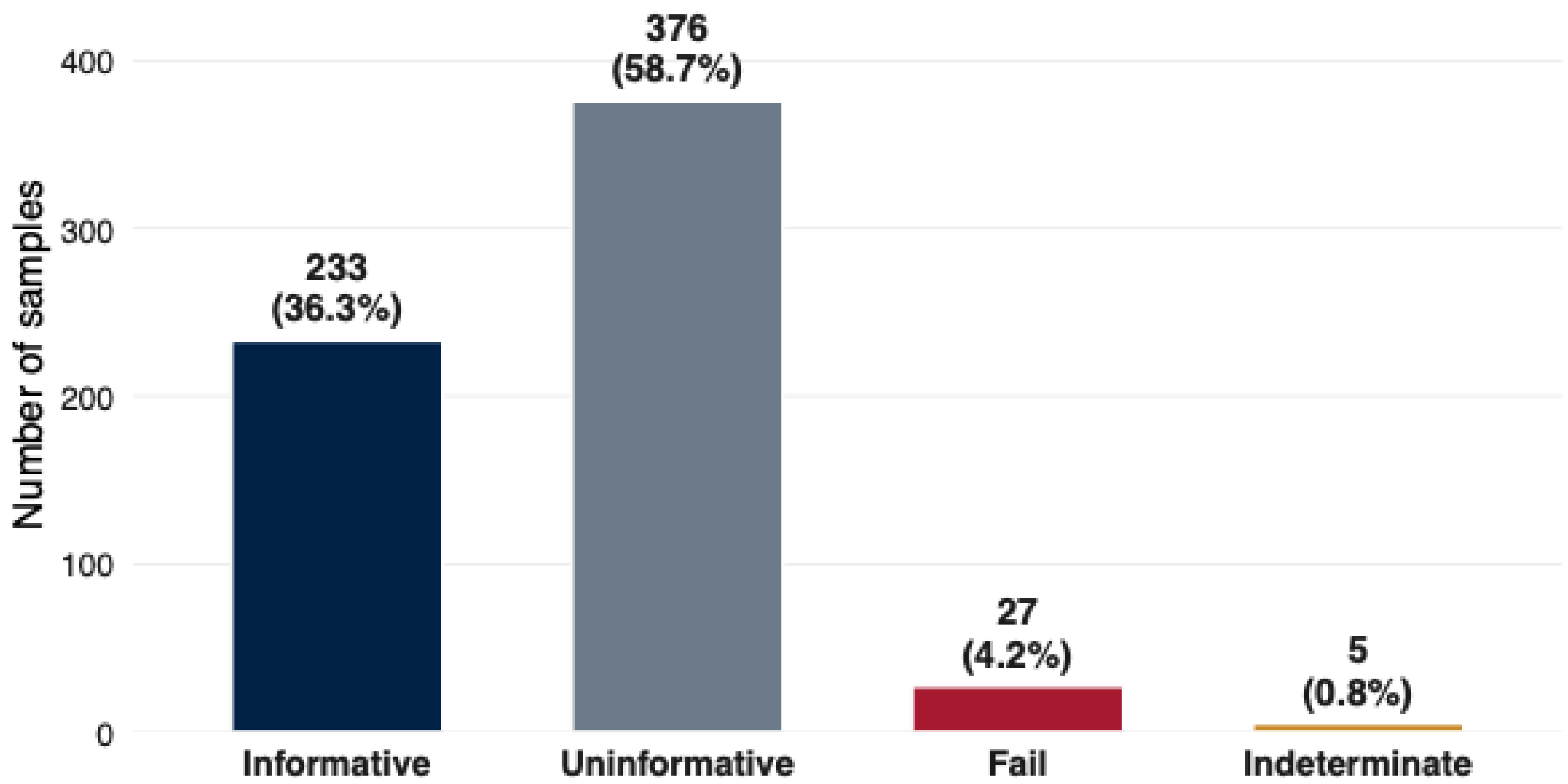


Figure 1. Liquid Biopsy Result Distribution

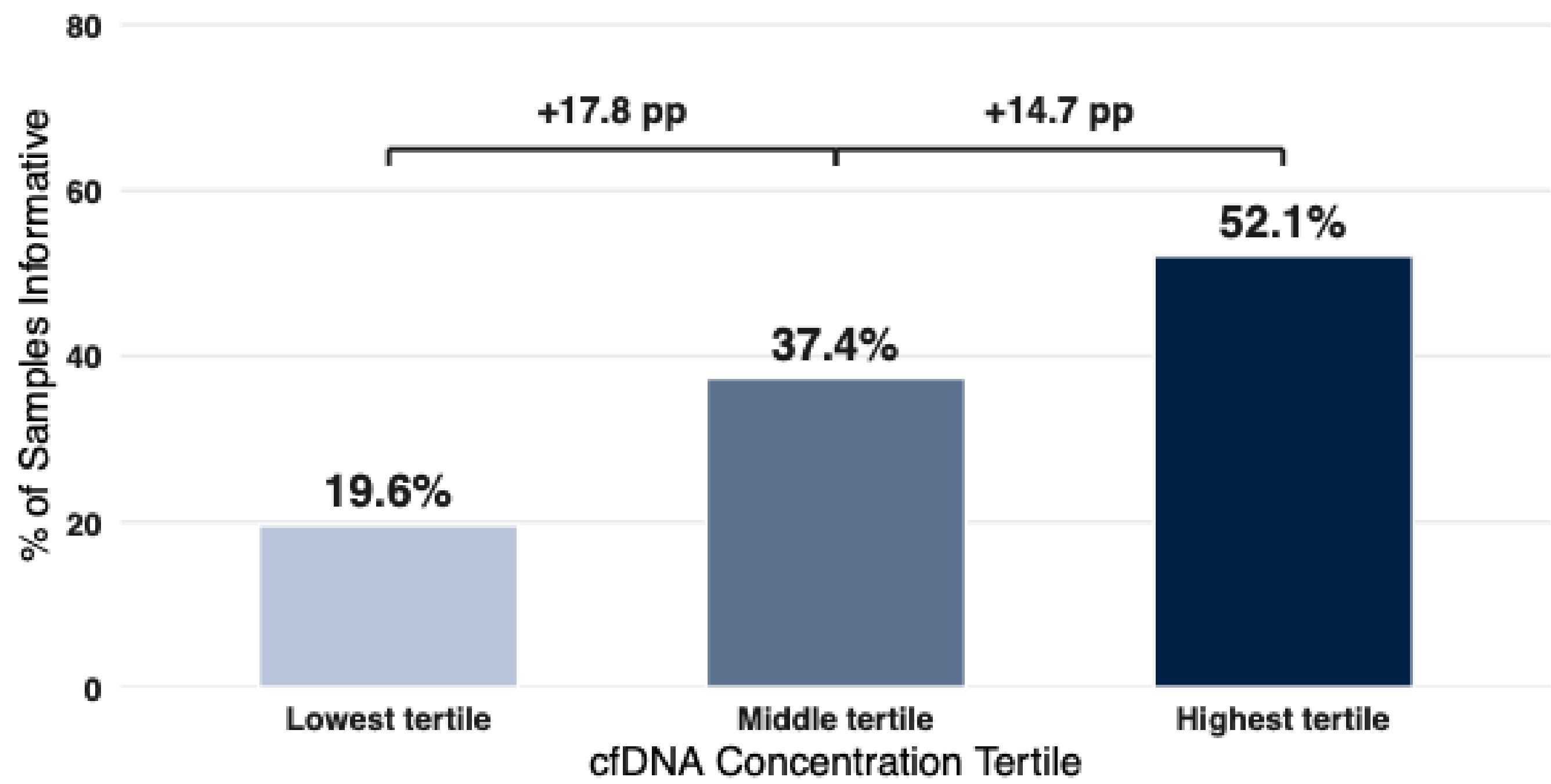


Figure 2. Dose-Response: Informative Rate by cfDNA Tertile

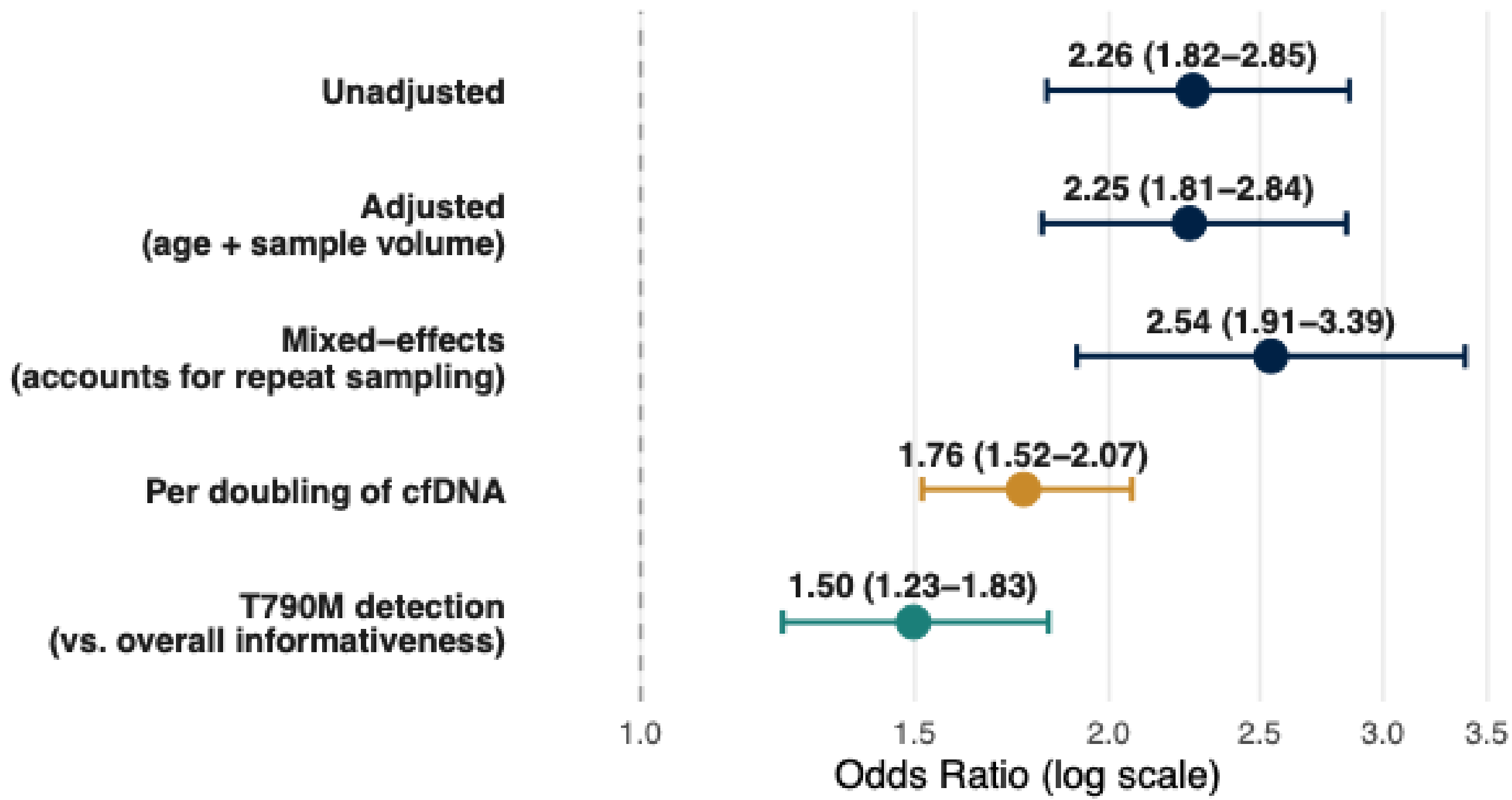


Figure 3. Higher cfDNA Concentration Predicts Both Informative Results and T790M Detection

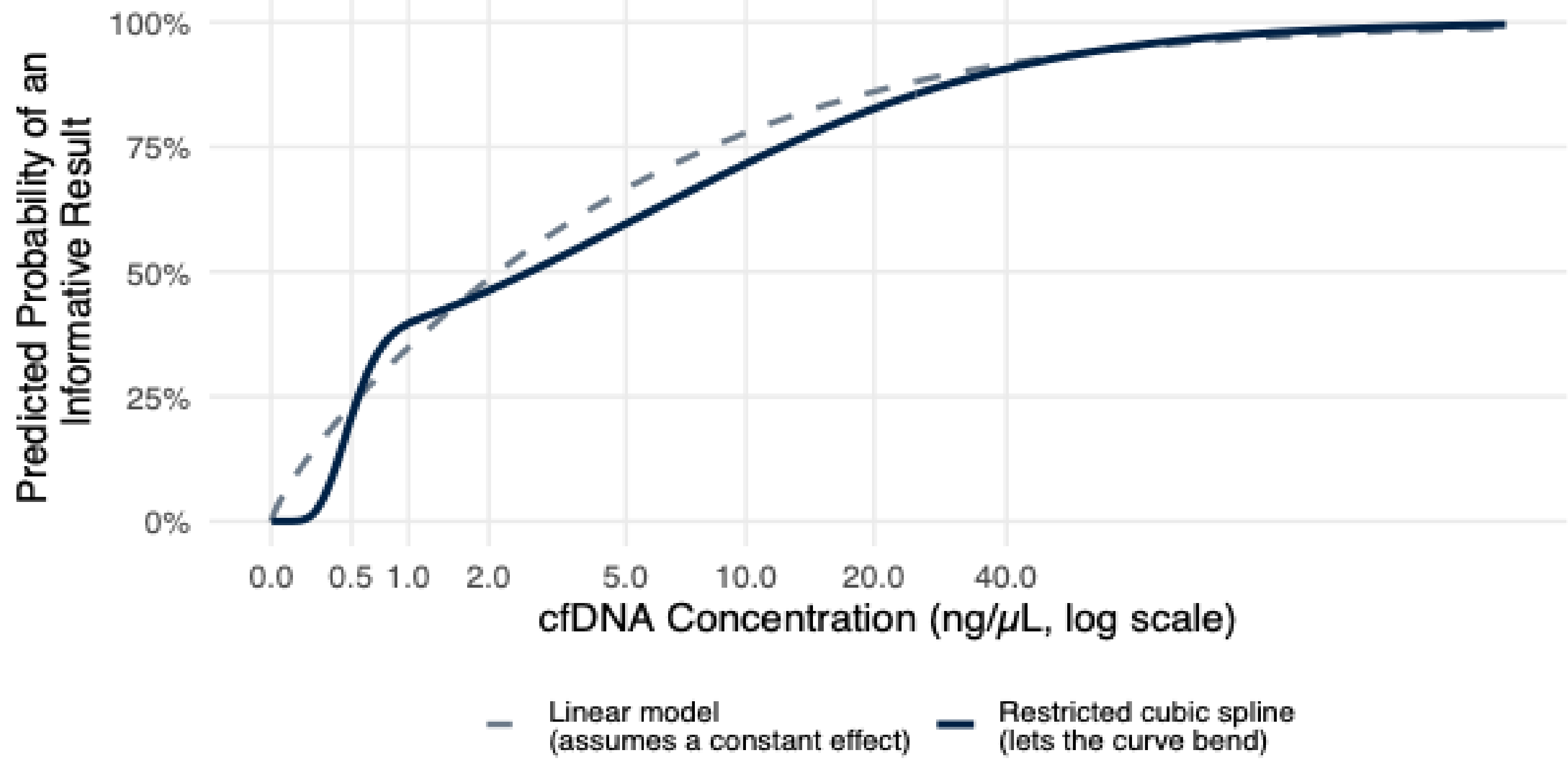


Figure 4. Non-Linearity of the cfDNA-Informativeness Relationship

CONCLUSION

- cfDNA concentration is a strong, independent, and non-linear predictor of informative EGFR liquid biopsy results in metastatic NSCLC
- A clear dose-response relationship exists, with stronger effects at lower cfDNA concentrations and diminishing returns at higher concentrations
- Discrimination was moderate (AUC 0.68), limiting cfDNA concentration's utility as a standalone clinical decision tool
- These findings support cfDNA concentration as a biologically relevant marker of liquid biopsy adequacy
- Results may inform patient selection strategies in future studies