

#3322

Non-invasive cfDNA methylation profiling to predict PD-L1 tumor proportion score status and clinical outcomes in NSCLC



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Introduction

- Programmed death-ligand 1 (PD-L1) expression, measured by tumor proportion score (TPS), is a key biomarker guiding immunotherapy (IO) selection in non-small cell lung cancer (NSCLC). Patients with high PD-L1 expression (TPS ≥50%) are more likely to benefit from IO monotherapy, while those with negative or low expression (<50%) are typically considered for IO in combination with chemotherapy.
- However, tissue-based PD-L1 immunohistochemistry (IHC) has several limitations, including insufficient tissue availability, sampling bias, and intratumoral heterogeneity, which can lead to inaccurate or incomplete assessment.
- Cell-free DNA (cfDNA) methylation profiling provides a non-invasive approach to detect tumor-derived epigenetic signals from plasma. Because methylation patterns reflect tumor biology at a genome-wide level, this approach may better capture PD-L1-associated biology compared to localized tissue sampling.
- Here, we developed a cfDNA methylation-based classifier to identify NSCLC patients with low PD-L1 TPS (<50%) using liquid biopsy, and assessed its clinical relevance using outcomes from the RADIOHEAD cohort.

Methods

- We analyzed cfDNA methylation profiles from over 500 NSCLC plasma samples with matched tumor PD-L1 IHC measurements.
- Methylation signals were quantified across thousands of regulatory regions spanning the genome
 - Features capturing tumor-associated epigenetic patterns were derived from cfDNA
 - A regularized logistic regression model was trained to predict PD-L1 TPS <50%
 - Model training and tuning were performed using cross-validation
 - Performance was evaluated on an independent analytical validation (AV) cohort (N=285), where predicted PD-L1 status was compared against tissue-based IHC results.
- We further assessed clinical relevance:
- The RADIOHEAD NSCLC cohort (N=188) with available treatment and survival data, including overall survival (OS), was evaluated using Kaplan-Meier analyses and multivariable Cox models adjusted for tumor fraction, age, sex, and stage

Results

- The cfDNA methylation-based PD-L1 prediction model demonstrated strong performance in identifying PD-L1 negative or low cases (TPS <50%) through cross-validation. The model achieves over 90% precision along with 50% sensitivity and 87% specificity at the selected threshold.
- AV results (n=285) were consistent with cross-validation findings, supporting the model’s ability to reliably identify PD-L1 negative or low cases with high positive predictive value.

Metric	Value (95% CI)
PPA	47.91% (41.63%, 54.26%)
Raw PPV	87.27% (79.57%, 92.86%)
Prevalence adjusted PPV	88.51% (83.19%, 93.31%)

Table 1. PD-L1 prediction AV performance

- Among RADIOHEAD cohort, cfDNA of 188 patients had enough signal to be evaluated at T0. Among these patients, 74 of them received IO combo therapy while the other 114 received monotherapy
- KM analysis showed trending better OS of patients with a PD-L1 TPS<50% prediction (Figure 1) with adjusted p-val=0.078 (adjusted by sex, age, cancer stage, tumor fraction).

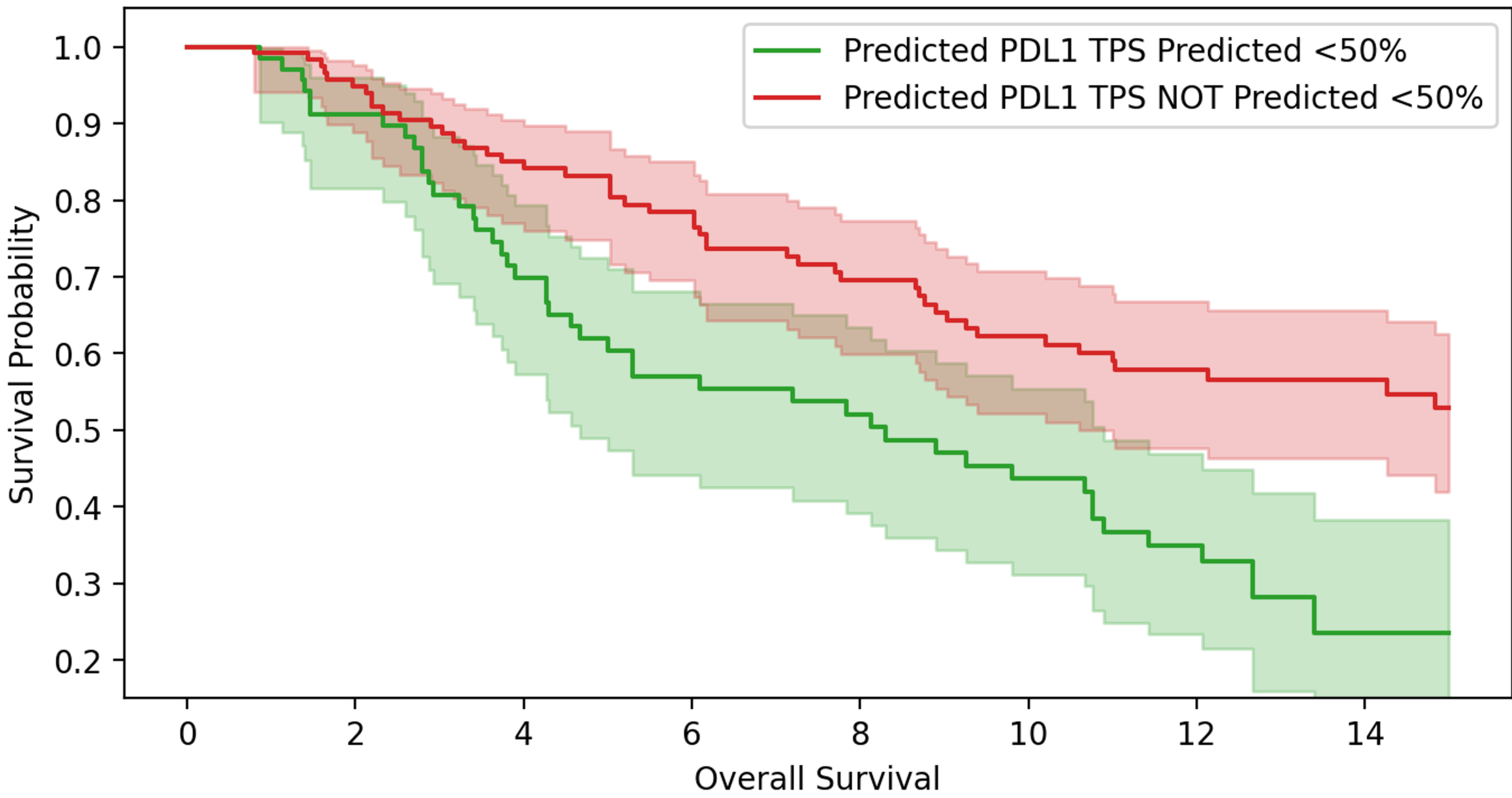


Figure 1. Figure 3. Kaplan-Meier curves of overall survival stratified by T0 model prediction

- In the patient group receiving IO monotherapy, significantly better OS was observed for patients with a PD-L1 TPS<50% prediction (Figure 1) with adjusted p-val=0.047.
- The median OS exceeded 15 months in patients without PD-L1 TPS<50% versus approximately 7-8 months in those with PD-L1 TPS<50% patients, with a hazard ratio of 1.81 (95% CI [1.02, 3.22])

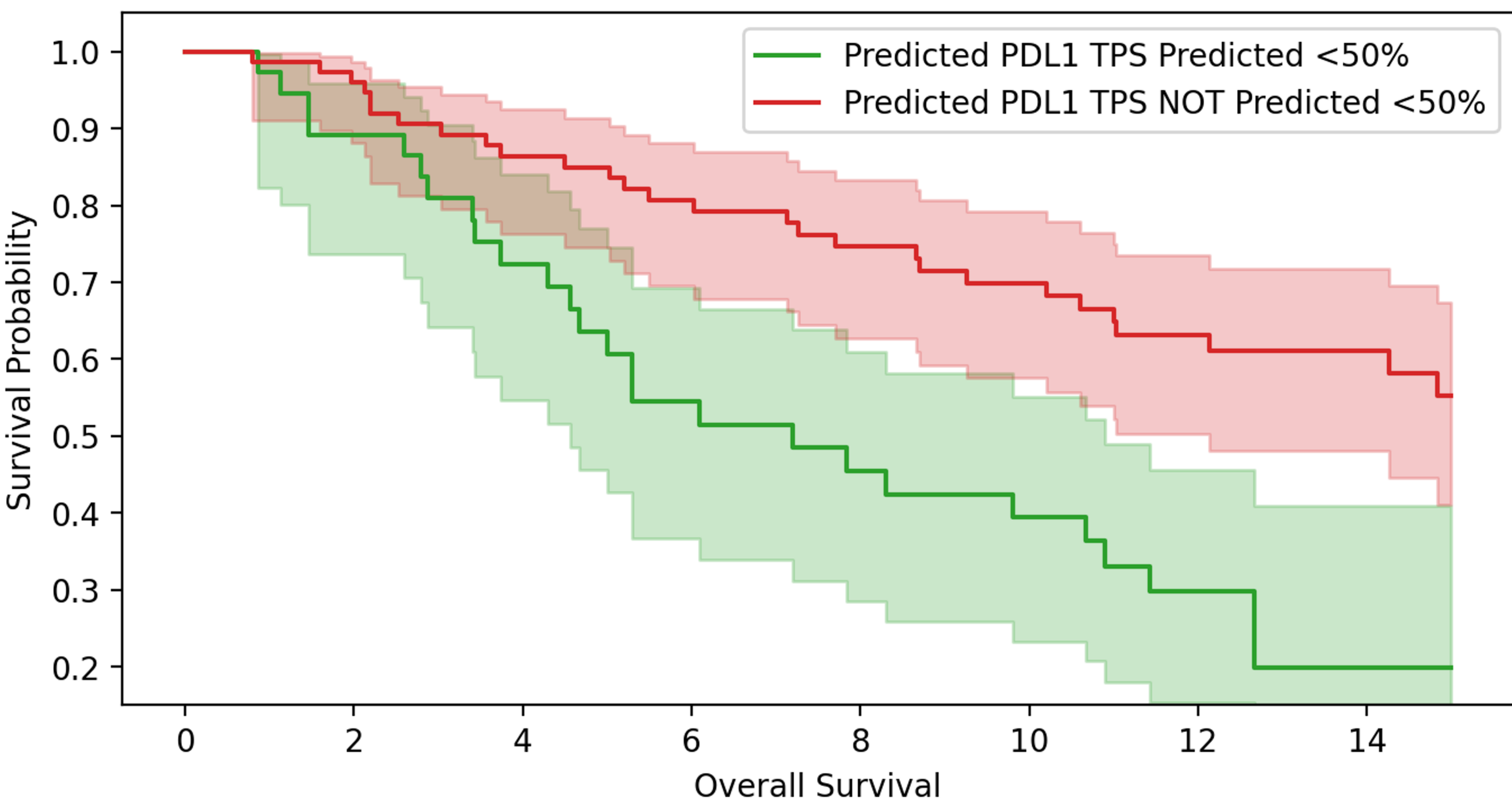


Figure 2. Figure 2. Kaplan-Meier curves of overall survival stratified by T0 model prediction among patients receiving monotherapy

Conclusions

We developed a cfDNA methylation-based predictor for identifying NSCLC patients with negative or low PD-L1 expression with robust performance. The prediction on RADIOHEAD cohort was associated with meaningful survival differences in NSCLC patients. These findings support the potential role of non-invasive epigenetic profiling to complement tissue PD-L1 testing, particularly in settings of limited tissue availability, and to support prognostic assessment of outcomes in NSCLC patients treated with immunotherapy

References

Quandt Z et al. J Immunother Cancer. 2025;13(5):e011545.