

# 5-hydroxymethylcytosine in cell-free DNA predicts immunotherapy response in lung cancer

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**Table S1. Patient demographics and baseline characteristics.**

<b>Characteristics</b>	<b>All patients (N=83)</b>	<b>Training (n=24)</b>	<b>Validation (n=16)</b>	<b>Test (n=18)</b>	<b>Non-ICI group (n=27)</b>
<b>Age, median (range), y</b>	69 (48-92)	70 (50-92)	72 (48-90)	67 (53-83)	69 (51-89)
<b>Female, No. (%)</b>	45 (54.2)	9 (37.5)	9 (56.2)	8 (44.4)	20 (74.1)
<b>Race, No. (%)</b>					
<b>Asian</b>	4 (4.8)	0	0	1 (5.6)	3 (11.1)
<b>Black</b>	18 (21.7)	6 (25.0)	4 (25.0)	1 (5.6)	7 (25.9)
<b>Caucasian</b>	60 (72.3)	18 (75.0)	12 (75.0)	16 (88.9)	16 (59.3)
<b>Unavailable</b>	1 (1.2)	0	0	0	1 (3.7)
<b>ECOG PS, No. (%)</b>					
<b>0</b>	6 (7.2)	1 (4.2)	2 (12.5)	2 (11.1)	1 (3.7)
<b>1</b>	62 (74.7)	19 (79.2)	13 (81.2)	16 (88.9)	16 (59.3)
<b>2</b>	1 (1.2)	1 (4.2)	0	0	0
<b>3</b>	2 (2.4)	1 (4.2)	0	0	1 (3.7)
<b>4</b>	3 (3.6)	1 (4.2)	0	0	2 (7.4)
<b>Unavailable</b>	9 (10.8)	1 (4.2)	1 (6.2)	0	7 (25.9)
<b>Smoking status, No. (%)</b>					
<b>Current</b>	13 (15.7)	3 (12.5)	2 (12.5)	3 (16.7)	5 (18.5)
<b>Former</b>	57 (68.7)	20 (83.3)	13 (81.2)	15 (83.3)	11 (40.7)
<b>Never</b>	13 (15.7)	1 (4.2)	1 (6.2)	0	11 (40.7)
<b>Histology, No. (%)</b>					
<b>NSCLC</b>					
<b>Adenocarcinoma</b>	59 (71.1)	18 (75.0)	8 (50.0)	13 (72.2)	22 (81.5)
<b>Squamous</b>	19 (22.9)	6 (25.0)	6 (37.5)	4 (22.2)	3 (11.1)
<b>Other subtypes</b>	3 (3.6)	0	2 (12.5)	1 (5.6)	0
<b>SCLC</b>	2 (2.4)	0	0	0	2 (7.4)
<b>Disease stage, No. (%)</b>					
<b>III</b>	12 (14.5)	2 (8.3)	5 (31.2)	0	5 (18.5)
<b>IV</b>	71 (85.5)	22 (91.7)	11 (68.8)	18 (100.0)	22 (81.5)
<b>PD-L1 expression, No. (%)</b>					
<b>&lt;1%</b>	42 (50.6)	14 (58.3)	9 (56.2)	10 (55.6)	11 (40.7)
<b>≥1%</b>	32 (38.6)	9 (37.5)	5 (31.2)	8 (44.4)	10 (37.0)
<b>Unavailable</b>	9 (10.8)	1 (4.2)	2 (12.5)	0	6 (22.2)
<b>PFS, median (range), mo</b>	6.6 (0.2-63.1)	7.0 (1.4-34.1)	6.3 (0.5-42.4)	8.7 (1.8-63.1)	5.4 (0.2-56.2)
<b>OS, median (range), mo</b>	12.4 (0.2-63.1)	14.1 (1.4-40.5)	9.8 (2.5-42.4)	17.8 (2.1-63.1)	9.1 (0.2-56.2)

ECOG PS, Eastern Cooperative Oncology Group performance status score; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; ICI, immune checkpoint inhibitor; PFS, progression-free survival; OS, overall survival.

**Table S4. Multivariate Cox regression analysis in patients treated with immune checkpoint inhibitors.**

	<b>HR (95% CI)</b>	<b>P</b>
Wp-score (Low vs High)	0.11 (0.04-0.29)	9.30×10 <sup>-6</sup>
Age (≥ 60 y vs <60 y)	0.69 (0.26-1.90)	0.47
Sex (Female vs Male)	0.57 (0.27-1.20)	0.13
Race (Caucasian vs Black)	0.88 (0.39-2.00)	0.76
Smoker (Current vs Former)	2.60 (1.00-6.20)	0.04
PD-L1 TPS (≥ 1% vs <1%)	0.73 (0.36-1.50)	0.38

Progression-free survival multivariate Cox regression analysis were performed in 51 patients treated with immune checkpoint inhibitors, considering wp-score (low vs high), age (≥ 60 vs < 60-year-old), sex (female vs male), race (Caucasian vs Black), smoking status (current vs former), and tumor PD-L1 TPS (≥ 1% vs <1%). Wp-score, weighted-predictive score. TPS, PD-L1 tumor proportion score. HR, hazard ratio. CI, confidence interval.

**Table S5. Therapeutic response to immune checkpoint inhibitors predicted by tumor PD-L1 expression.**

**Non-STOMP**

	<b>Responders (No.)</b>	<b>Non-responders (No.)</b>	<b>Objective response rate (95% CI)</b>
PD-L1 TPS ≥ 1%	7	6	53.9% (25.1%-80.8%)
PD-L1 TPS < 1%	11	8	57.9% (33.5%-79.8%)

**STOMP clinical trial**

	<b>Responders (No.)</b>	<b>Non-responders (No.)</b>	<b>Objective response rate (95% CI)</b>
PD-L1 TPS ≥ 1%	3	5	37.5% (8.5%-75.5%)
PD-L1 TPS < 1%	5	5	50.0% (18.7%-81.3%)

Responders: patients with complete or partial response to immune checkpoint inhibitor treatment who did not progress within six months after the treatment. Non-responders: patients with progression or stable disease or with response but progressed within six months after ICI treatment. TPS, PD-L1 tumor proportion score.

**Table S6. Prediction accuracy by the 5hmC signature and tumor PD-L1 expression.**

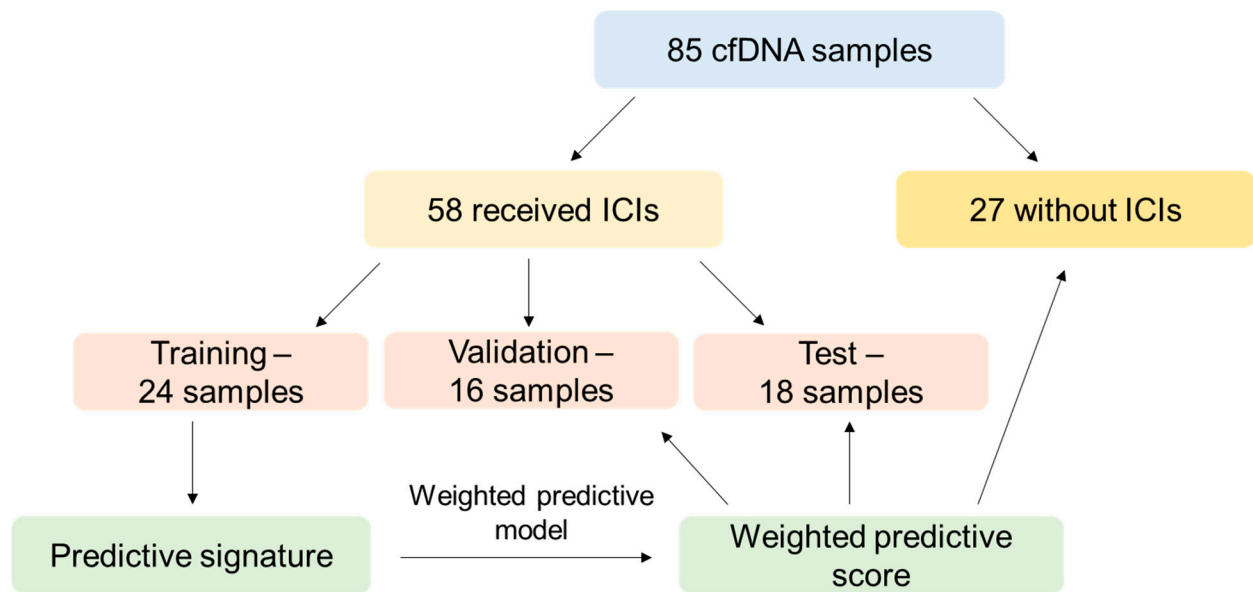
**Non-STOMP**

	<b>Consistent (No.)</b>	<b>Inconsistent (No.)</b>	<b>Overall accuracy (95% CI)</b>
Wp-score	27	5	84.4% (67.2%-94.7%)
PD-L1	15	17	46.9% (29.1%-65.3%)

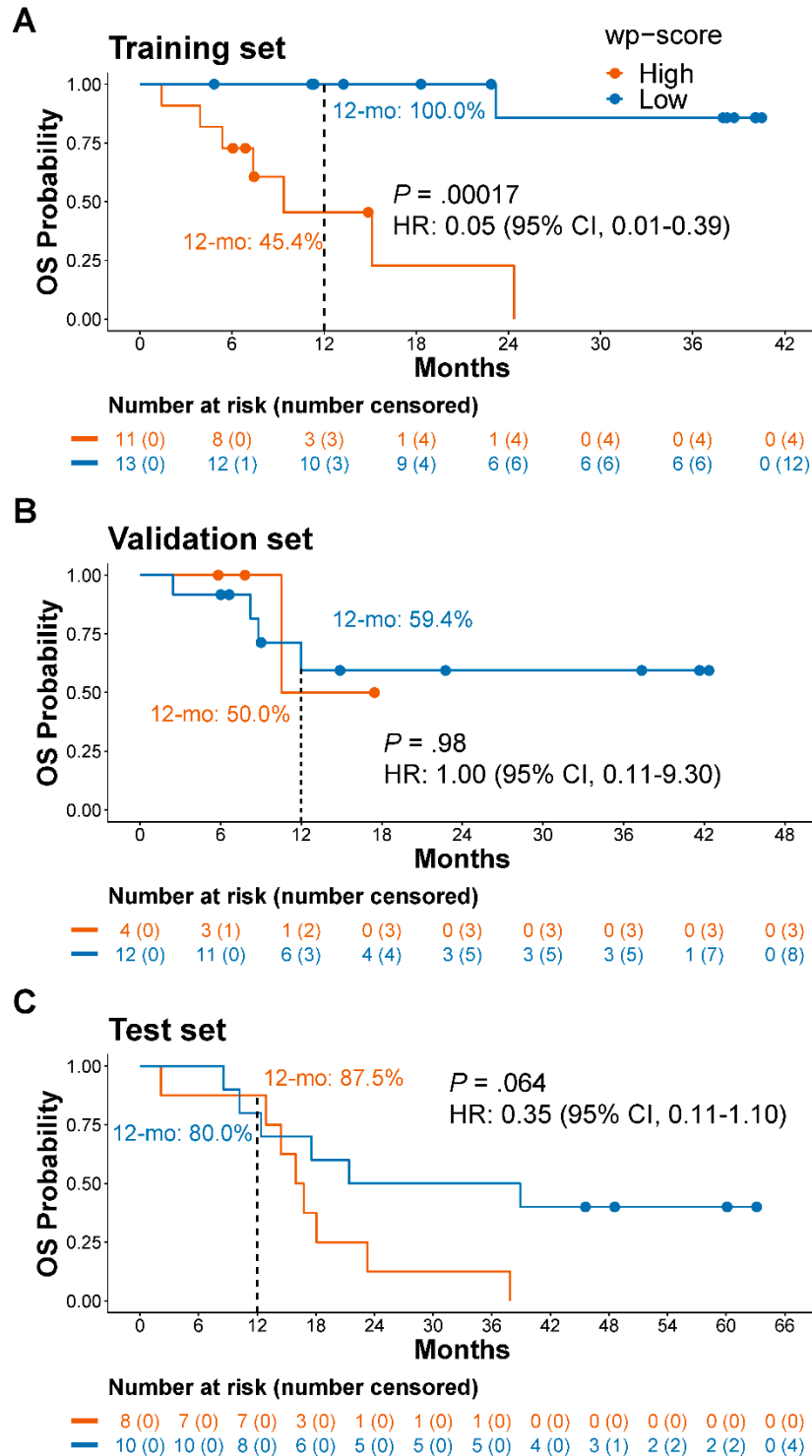
**STOMP clinical trial**

	<b>Consistent (No.)</b>	<b>Inconsistent (No.)</b>	<b>Overall accuracy (95% CI)</b>
Wp-score	16	2	88.9% (65.3%-98.6%)
PD-L1	8	10	44.4% (21.5%-69.2%)

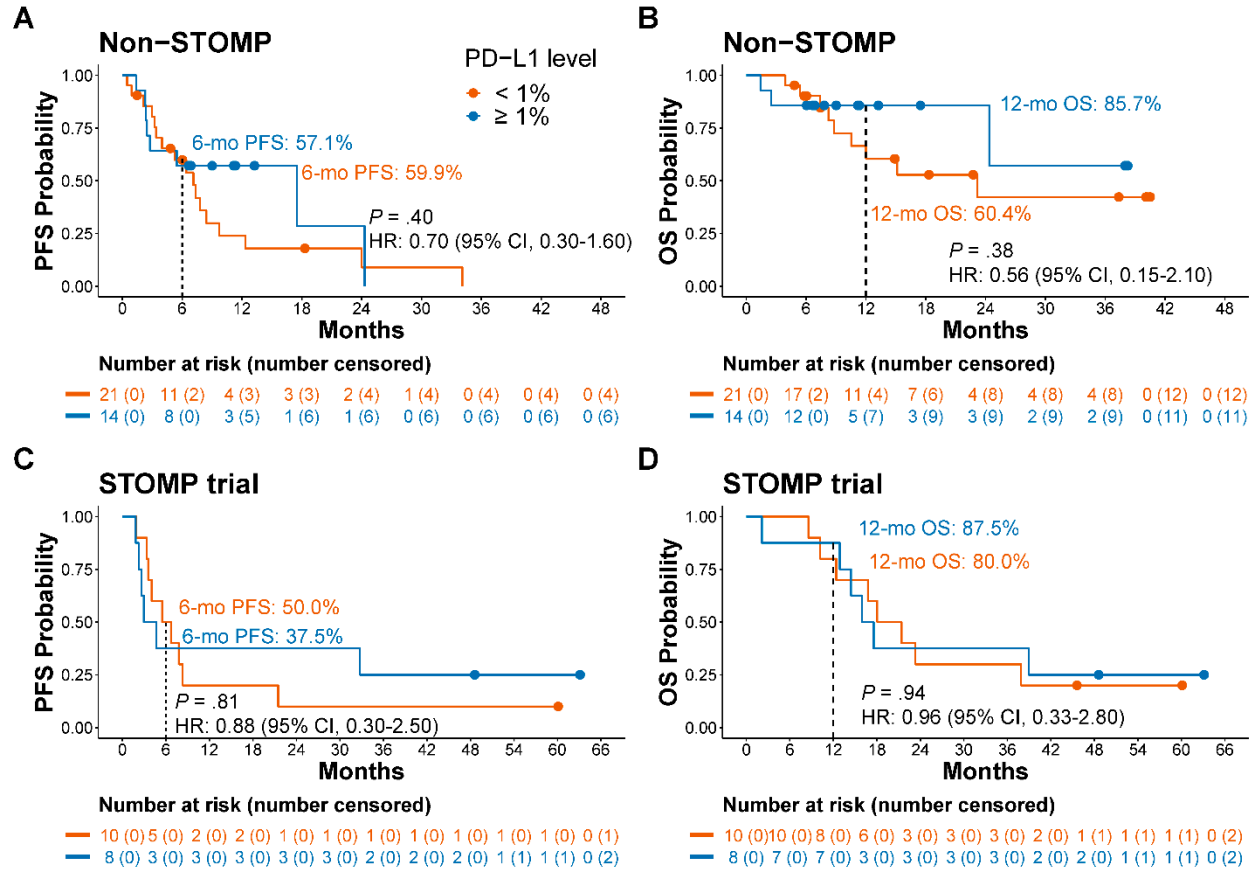
Consistent indicates the prediction is consistent with clinical response status. Inconsistent indicates the prediction is not consistent with clinical response status. CI, confidence interval.



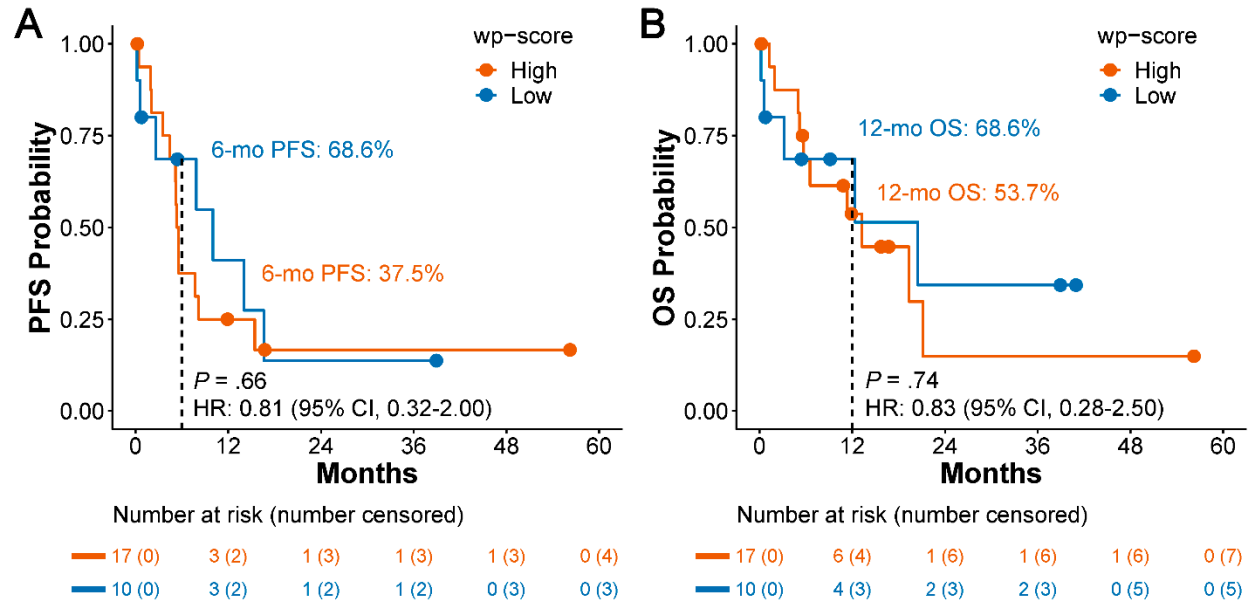
**Figure S1. Study design for cell-free DNA 5hmC predictive model for lung cancer immunotherapy.**



**Figure S2. Prediction of overall survival by a 5hmC predictive signature in lung cancer patients receiving immune checkpoint inhibitor treatment.** (A, B, C) Kaplan-Meier analysis of overall survival (OS) based on weighted predictive (wp)-scores in the training set (A), the validation set (B), and the test set (C). 12-mo, estimated OS in 12 months. Dots on the survival curve indicate that a patient was censored. HR, hazard ratio. CI, confidence interval.



**Figure S3. Immune checkpoint inhibitor treatment response predicted by tumor PD-L1 expression.** Kaplan-Meier analysis of progression-free survival (PFS, A) and overall survival (OS, B) in non-STOMP patients treated with immune checkpoint inhibitors based on tumor PD-L1 expression. Kaplan-Meier analysis of PFS (C) and OS (D) in STOMP clinical trial patients treated with immune checkpoint inhibitors based on tumor PD-L1 expression. HR, hazard ratio. CI, confidence interval.



**Figure S4. Treatment response predicted by the 5hmC predictive signature in lung cancer patients not receiving immune checkpoint inhibitors.** Kaplan-Meier analysis of progression-free survival (PFS, A) and overall survival (OS, B) in patients who did not receive immune checkpoint inhibitor treatment based on weighted prediction (wp)-scores.