

Table S1 Detailed search strategy for PubMed

Concept	Search terms	Number of articles
Diagnosis	ctDNA; methylation; lung cancer; diagnosis	78
	Liquid biopsy; methylation; lung cancer; diagnosis	101
Prognosis	ctDNA; methylation; lung cancer; prognosis	34
	Liquid biopsy; methylation; lung cancer; prognosis	36
Treatment	ctDNA; methylation; lung cancer; treatment	67
	Liquid biopsy; methylation; lung cancer; treatment	62

Articles were then selected based on the information provided in the abstract and subsequently in the full text. cfDNA, circulating free DNA; ctDNA, circulating tumor DNA.

Table S2 Clinical applications of Met-ctDNA for prognosis, treatment response, and detection of minimal residual disease or recurrence

References	Cancer type	Samples	Methylation study method	Genes	Clinical application	Main findings
Chen <i>et al.</i> , 2020	NSCLC	Plasma	MS PCR, SB conversion	Multiple methylated genes	Tumor staging	Identification of specific methylation markers for occult lymph node metastasis
Wang <i>et al.</i> , 2007	NSCLC patients with no surgical resection	Plasma	MS PCR, SB conversion	<i>RASSF1A</i>	Tumor staging, treatment response	<i>RASSF1A</i> hypermethylation associated with relative poor differentiation and late stage
						Tendency to have higher <i>RASSF1A</i> hypermethylation frequency in absence of surgical resection
						No methylation differences between patient who received chemotherapy or/and radiotherapy and those who did not
Xia <i>et al.</i> , 2019	Stage IV osimertinib-treated EGFR T790M-LUAD	Plasma	Bisulfite sequencing, use of a MR prediction model	NP, multiple genes	Treatment response	Decrease in methylation level reflected treatment efficacy Gradual methylation increase reflected disease progression prior radiological imaging
Wang <i>et al.</i> , 2015	Advanced lung cancer	Plasma before and 24 hours after chemotherapy	MS PCR	<i>APC</i> , <i>RASSF1A</i>	Treatment response	High plasma methylation levels after chemotherapy predict sensitivity to cisplatin and is associated with improved outcomes
Peng <i>et al.</i> , 2019	Stage IV lung cancer	Plasma	MS PCR, SB conversion	<i>SHOX2</i>	Treatment response, survival	Correlation between tumor size and the plasma methylated <i>SHOX2</i> level
						Methylated <i>SHOX2</i> levels before and after two cycles therapy were predictors of the OS, while the methylated <i>SHOX2</i> level change were not predictor of the OS
						Decreased plasma methylated <i>SHOX2</i> level in patients with partial response, but not in those with stable disease
Shen <i>et al.</i> , 2021	NSCLC patients	Plasma	MS PCR, SB conversion	<i>WIF1</i>	Treatment response, survival	Methylated <i>SHOX2</i> level change reflected the degree of response
						Patients with hypomethylated <i>WIF1</i> showed better PFS and OS
						Lower DNA methylation level of <i>WIF1</i> promoter in patients with a complete or partial response to gefitinib
Juergens <i>et al.</i> , 2011	Surgically resected stage I NSCLC	Plasma (prior and after one cycle therapy)	MS PCR	<i>APC/RASSF1A/CDH13/CDKN2A</i>	Treatment response, survival	Methylation associated with shorter OS and PFS Early demethylation could predict clinical benefit from therapy (azacitidine, entinostat)
Hsiao <i>et al.</i> , 2024	NSCLC (majority advanced stage)	Plasma	NGS, establishment of TMS	>500 genomic locations	Treatment response, survival	Possible TMS correlation with PFS Change in TMS after therapy correlated with anti-PD1 response
Schmidt <i>et al.</i> , 2015	Lung cancer (LUAD, SCLC, NSCLC, SCC)	Plasma	MS PCR, SB conversion	<i>SHOX2</i>	Treatment response, survival	Correlation between radiological data and the change of plasma methylated <i>SHOX2</i> level
						Baseline plasma methylated <i>SHOX2</i> level below the median had a slightly longer survival time
						Fast and strong decline of plasma methylated <i>SHOX2</i> DNA in patients responding to therapy (chemo/radio-chemotherapy)
Ramirez <i>et al.</i> , 2005	Advanced NSCLC	Serum (before treatment)	MS PCR, SB conversion	14-3-3σ	Treatment response, survival	Patients treated with cisplatin and gemcitabine: Longer median survival in the methylation-positive group Methylation-negative responders
						had a four times greater risk of death
Belinsky <i>et al.</i> , 2017	Stage IA or IB NSCLC	Plasma	MS PCR, SB conversion	<i>CDKN2</i> , <i>MGMT</i> , <i>DAPK1</i> , <i>RASSF1</i> , <i>GATA4</i> , <i>GATA5</i> , <i>PAX5α</i> , <i>PAX5β</i>	Prediction of recurrences and treatment response	Methylation of one or more genes is associated with increased risk for local or distant recurrence
						No significate association between recurrence and methylation
		Sputum				No significant difference of the eight genes methylation levels was observed between LC patients with recurrence after selenium treatment and those without recurrence after selenium treatment
Liu <i>et al.</i> , 2024	EGFR-TKI-treated advanced NSCLC	Plasma	Bisulfite genomic sequencing	Multiple locations including snoRNA, <i>SNORD3F</i>	Survival and recurrences	Lower methylation levels of snoRNA genes associated with longer PFS
						Methylation level of snoRNA genes is an independent risk factor to predict patient's recurrences
						High-level methylation of <i>SNORD3F</i> associated with higher risk of recurrence and poorer PFS
Thanou <i>et al.</i> , 2024	NSCLC	Plasma	MS PCR, SB conversion	<i>NALCN</i>	Relapse detection	Incidence of relapse higher when NALCN was hypermethylated in tissue, but these results were not confirmed in ctDNA
Bossé <i>et al.</i> , 2024	LUAD, SCC	Plasma	MCED test	NS	Relapse detection	LUAD, SCC: association between MCED positivity and 5-year relapse
						LUAD: Increased rate of MCED ctDNA detection in patients who relapsed ≤2 years post-surgery
Ponomaryova <i>et al.</i> , 2013	NSCLC patients	Plasma	MS PCR, SB conversion	<i>RARB2</i> , <i>RASSF1A</i>	Relapse detection, treatment response	Decreased methylation of <i>RARB2</i> and <i>RASSF1A</i> after chemotherapy and lung resection Increased methylation of <i>RARB2</i> was correlated with an advanced stage and associated with disease relapse
Chen <i>et al.</i> , 2023	LC	Plasma	Bisulfite genomic sequencing (timMRD score)	Multiple genes	Survival, follow-up, relapse detection	Higher timMRD-scores associated with shorter DFS in patients with stage I and LUAD
						Negative baseline ctDNA but high postoperative timMRD-score associated with shorter DFS
						Elevated timMRD-scores associated with relapse, before radiological detection of relapse
Wen <i>et al.</i> , 2023	Advanced NSCLC treated with PD-1/PD-L1 inhibitors	Plasma	Digital PCR, SB conversion	<i>HOXA9</i>	Survival	<i>HOXA9</i> methylation associated with poorer OS and PFS
						Biomarker score combining the levels of NK cell activity (IFN gamma) and methylated <i>HOXA9</i> after the first cycle of treatment stratify the prognosis
Tian <i>et al.</i> , 2023	LUAD	Plasma	Bisulfite genomic sequencing	<i>SMAD7</i>	Survival	Higher <i>SMAD7</i> methylation levels in patients with metastatic cancer than those with only primary tumor
						Reduction of <i>SMAD7</i> ctDNA methylation levels after tumor resection
						Higher <i>SMAD7</i> methylation is associated with shorter survival
Janke <i>et al.</i> , 2022	ALK positive NSCLC	Plasma	DNA methylation immune-precipitation sequencing	Multiple genomic location	Survival	Increased methylation score associated with shorter survival
						Methylation score concordant with radiological assessment
						Methylation score elevated at disease progression
Mastoraki <i>et al.</i> , 2021	Operable and metastatic NSCLC patients	Plasma	Real-time MS PCR, SB conversion	<i>KMT2C</i>	Survival	Operable patients: <i>KMT2C</i> promoter methylation-positive patients had a lower DFS time and OS time Metastatic patients: <i>KMT2C</i> promoter methylation-positive patients had a lower PFS time and OS time
Liu <i>et al.</i> , 2020	SCC	Plasma	Bisulfite targeting sequencing	Multiple location including <i>DMRTA2</i> , <i>PAX3</i> , <i>BARHL2</i> , <i>LINC01475</i>	Survival	Higher methylation levels associated with worse survival outcomes
Wen <i>et al.</i> , 2020	NSCLC stage III and IV	Plasma	MS PCR, SB conversion	<i>HOXA9</i>	Survival	Detection of methylated <i>HOXA9</i> at baseline is a negative prognostic factor
						Shorter median OS for patients with detectable methylated <i>HOXA9</i>
						Shorter median OS and PFS for patients with detectable methylated <i>HOXA9</i> after treatment
Guo <i>et al.</i> , 2020	NSCLC	Plasma	NGS, SB conversion, sonication	NR, multiple genes	Survival	Combination of genetic and methylated DNA decreased had high reliability in predicting OS
Ooki <i>et al.</i> , 2017	Stage IA LUAD and NSCLC	Plasma	MS PCR, SB conversion	<i>CDO1</i> , <i>HOXA9</i> , <i>PTGDR</i> , <i>AJAP1</i>	Survival	Promoter methylation of <i>CDO1</i> and <i>HOXA9</i> showed a trend of a poor outcome
						AJAP1 methylation showed a trend of a favorable outcome
						Combination of both <i>CDO1</i> and <i>HOXA9</i> promoter methylation significantly associated with poor outcomes
Powrózek <i>et al.</i> , 2016	LC	Plasma	MS PCR, SB conversion	<i>DCLK1</i>	Survival	Combination of both <i>PTGDR</i> and <i>AJAP1</i> promoter methylation associated with a better outcome
Zhang <i>et al.</i> , 2016	LC	Whole blood	Human methylation 450K BeadChip Assay, SB conversion	smoCpGs	Survival	DCLK1 methylation (several regions of the gene promoter): significantly shorter median OS
						Methylation levels at baseline significantly lower among patients who died from LC
						Methylation at the vast majorities of smoCpGs (77 of 81) inversely associated with LC mortality
Balgkouranidou <i>et al.</i> , 2014	Operable and advanced NSCLC patients	Plasma	Real-time MS PCR, SB conversion	<i>BRMS1</i>	Survival	4 smoCpGs exhibited the opposite patterns with LC death status, and showed positive associations with LC mortality
						Operable patients: Methylation associated with shorter OS time and DFI time
						Advanced patients: Methylation associated with shorter OS time and PFS time
Balgkouranidou <i>et al.</i> , 2014	Operable and advanced NSCLC patients	Plasma	MS PCR, SB conversion	<i>SOX17</i>	Survival	Operable patients: Marked but not statistically significant trend of <i>SOX17</i> promoter methylation on patient survival time
						Advanced patients: OS was significantly different in patients with <i>SOX17</i> promoter methylation (risk of death 1.8 higher for patients with <i>SOX17</i> promoter methylation)
Ko <i>et al.</i> , 2013	Node-negative stage I-II NSCLC	Plasma	MS PCR	<i>RASSF1A</i>	Survival	Hypermethylation of <i>RASSF1A</i> with negative expression of p63: higher recurrence risk and poorer RFS
Botana-Rial <i>et al.</i> , 2012	LUAD patients	Pleural effusion	MS PCR	MGMT, BRCA1, RARβ, p16/INKα	Survival	Better prognosis in case of methylation of one or more genes
						Shorter survival when the number of hypermethylated genes increase
Vinayanuwattikun <i>et al.</i> , 2011	Advanced NSCLC	Plasma	MS PCR, SB conversion	<i>SHP1P2</i>	Survival	Methylation of less than 700 pg/mL associated with improve PFS and OS
Ng <i>et al.</i> , 2002	NSCLC	Plasma, pleural lavage	MS PCR, SB conversion	p16	Survival	p16 methylation in plasma associated with poor survival and shorter DFS
						Preresectional p16 methylation in pleural lavage associated with poor survival and shorter DFS
						Postresectional p16 methylation in pleural lavage associated with tumor involving the visceral pleura

BAL, bronchoalveolar lavage; DFI, disease-free interval; DFS, disease-free survival; LUAD, lung adenocarcinoma; MR, methylation ratio; MS PCR, methylation-specific PCR; NGS, next-generation sequencing; NS, not specified; OS, overall survival; PFS, progression-free survival; RFS, recurrence-free survival; SBC, sodium bisulfite conversion; SCC, squamous cell carcinoma; smoCpGs, smoking-associated CpGs; TMS, tumor methylation score.