Dynamic bTMB combined with residual ctDNA improves survival prediction in locally advanced NSCLC patients with chemoradiotherapy and consolidation immunotherapy

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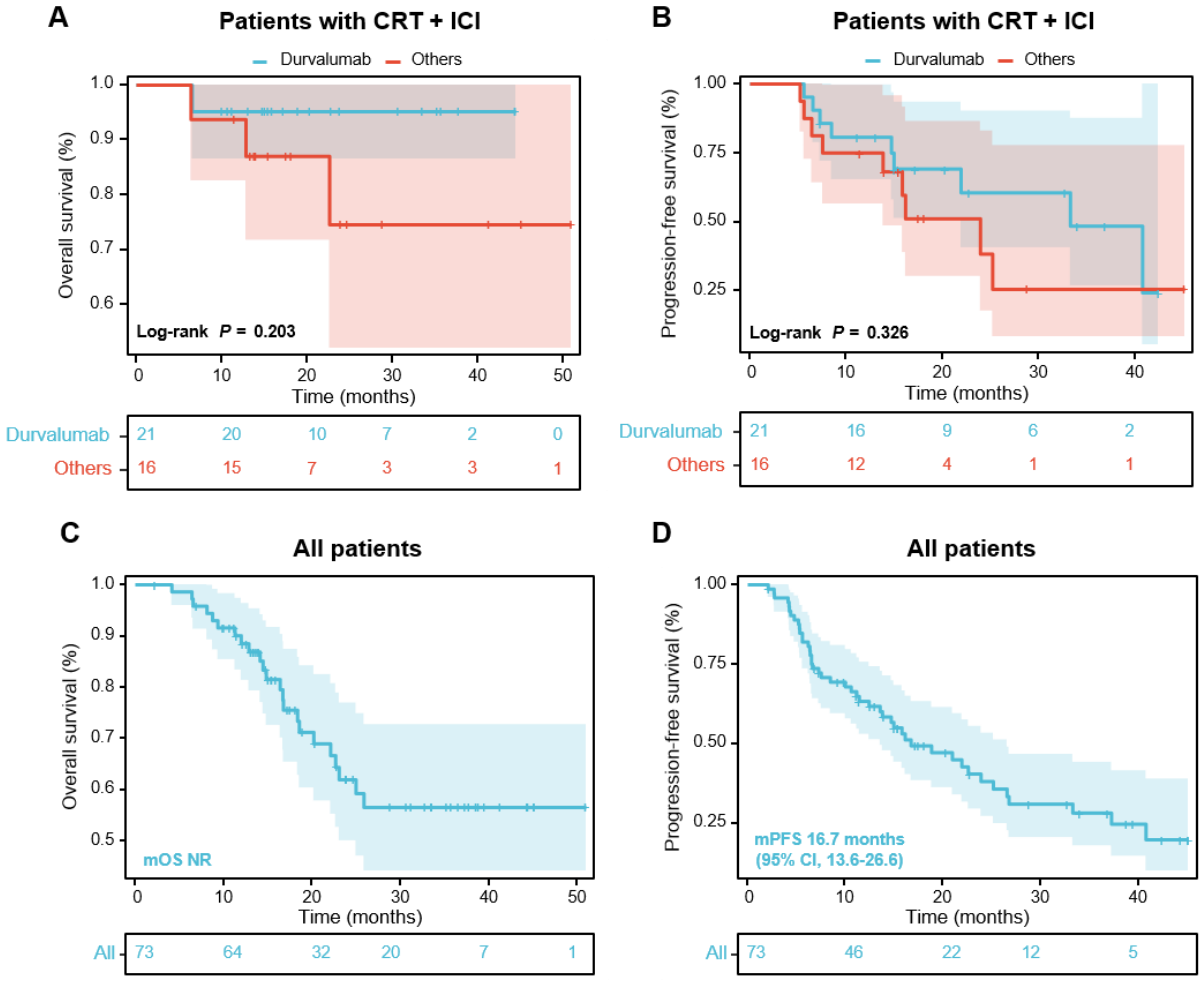
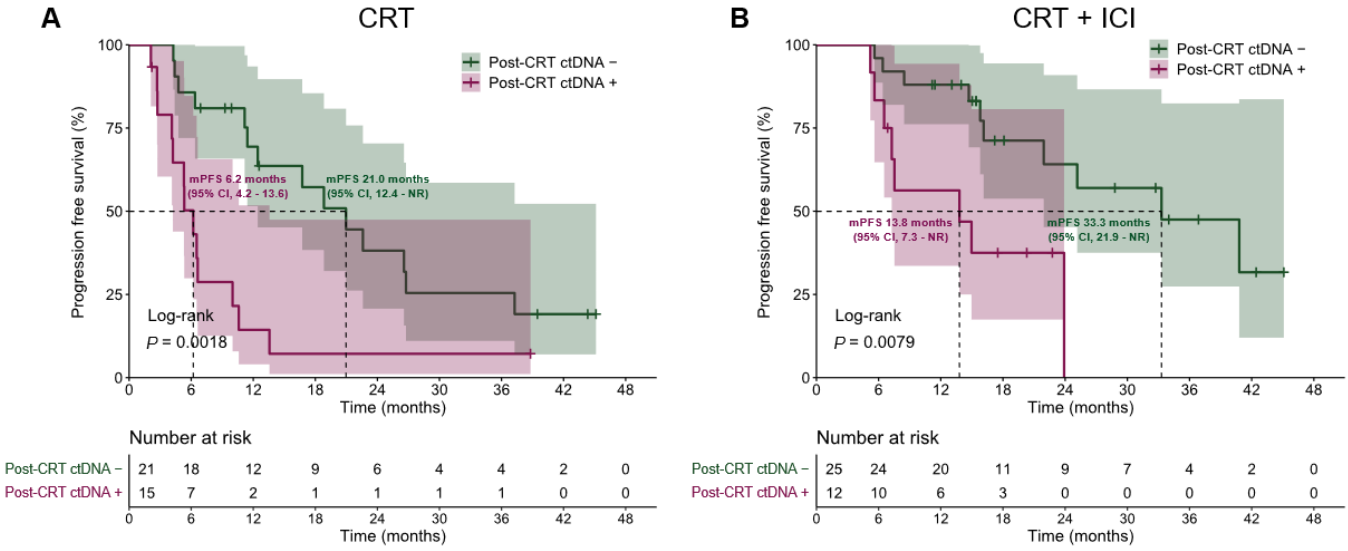
Supplementary materials

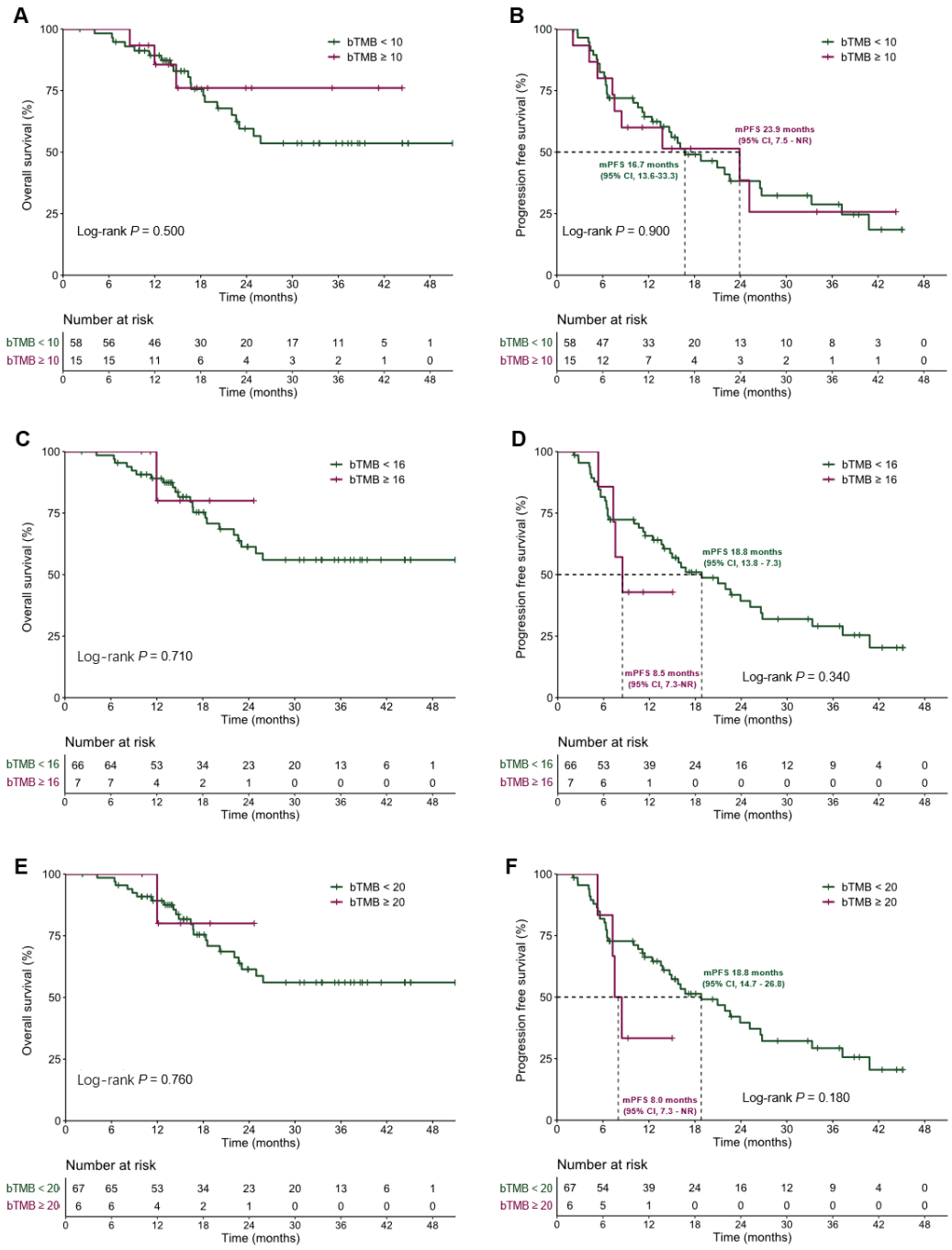
Supplementary figures



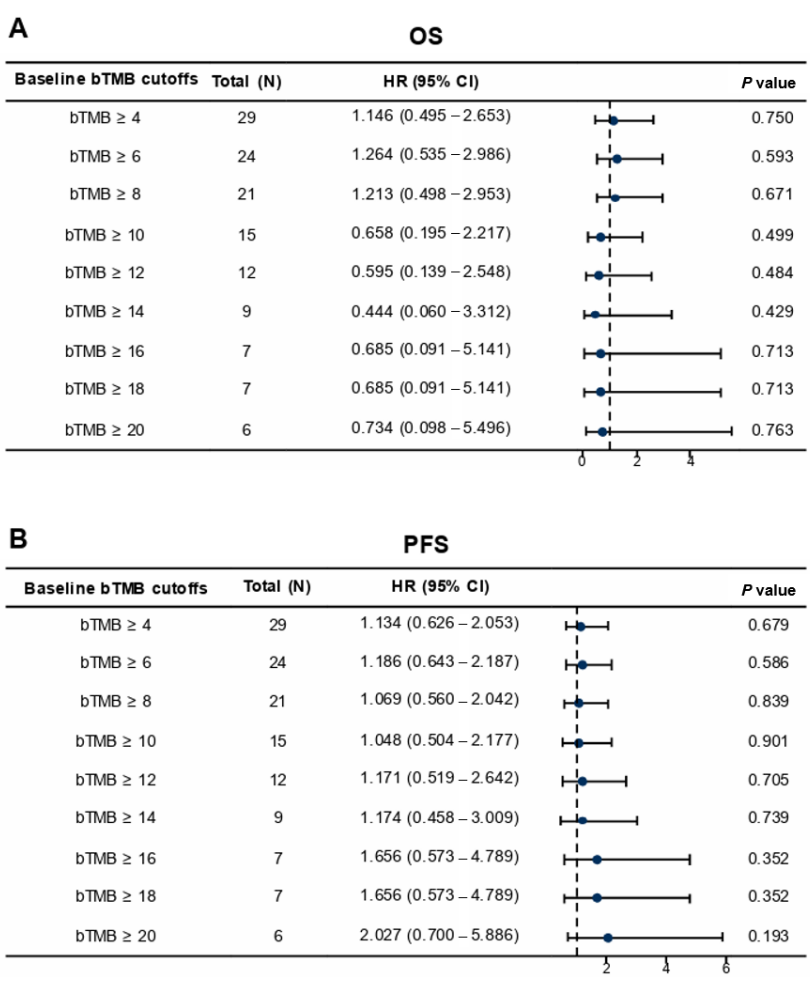
Supplementary Fig. 1. Survival outcomes for patients treated with consolidation durvalumab versus other agents in the discovery cohort. Kaplan-Meier curves of overall survival (A) and progression-free survival (B) for patients treated with consolidation durvalumab versus other ICI agents. Overall survival (C) and progression-free survival (D) for all patients in the discovery set. CI, confidence interval; CRT, chemoradiotherapy; ICI, immune checkpoint inhibitor; mOS, median overall survival; mPFS, median progression-free survival; NR, not reached.



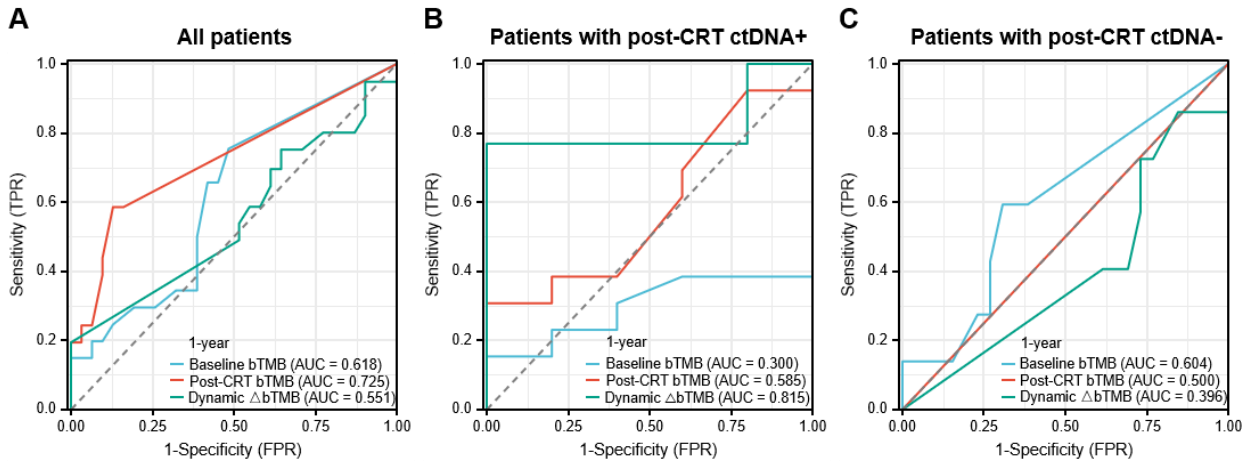
Supplementary Fig. 2. The predictive effect of post-CRT residual ctDNA detection on survival in patients with or without consolidation ICI. In the discovery set, residual ctDNA after CRT was associated with significantly shorter progression-free survival for patients treated with CRT alone (A) or treated with CRT + consolidation ICI (B). CI, confidence interval; CRT, chemoradiotherapy; ctDNA, circulating tumor DNA; ICI, immune checkpoint inhibitor; mPFS, median progression-free survival; NR, not reached.



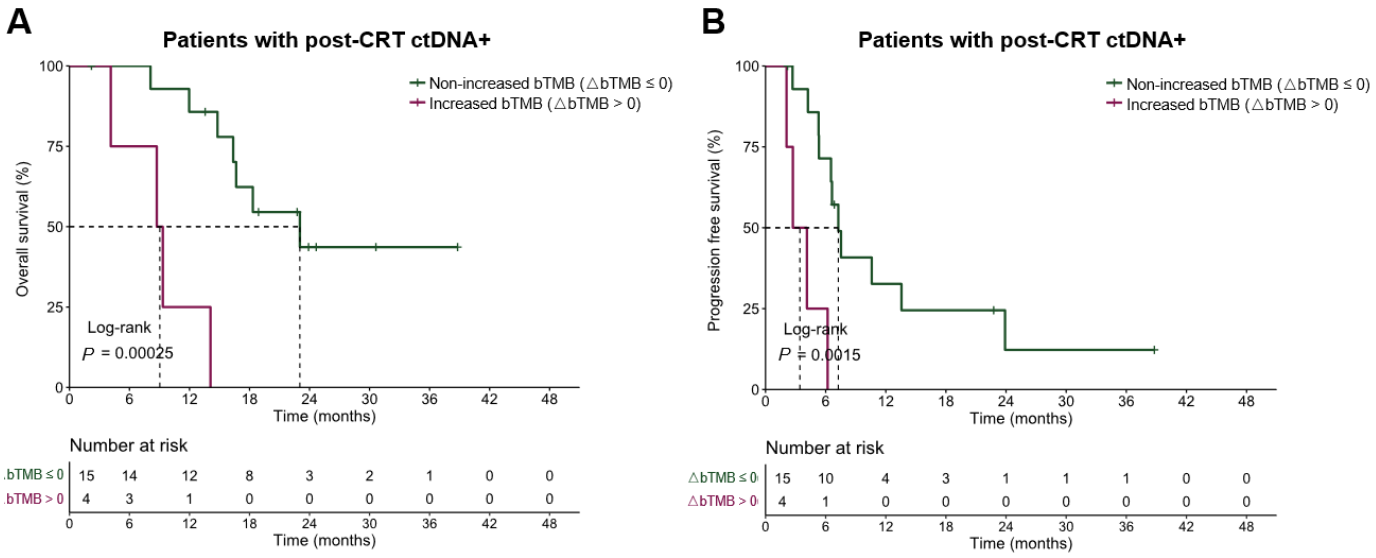
Supplementary Fig. 3. Baseline bTMB at various cutoffs failed to predict survival. No significant differences in overall survival or progression-free survival stratified by baseline bTMB at cutoff values of 10 mut/Mb (A, B), 16 mut/Mb (C, D), and 20 mut/Mb (E, F). bTMB, blood tumor mutational burden; CI, confidence interval; mPFS, median progression-free survival.



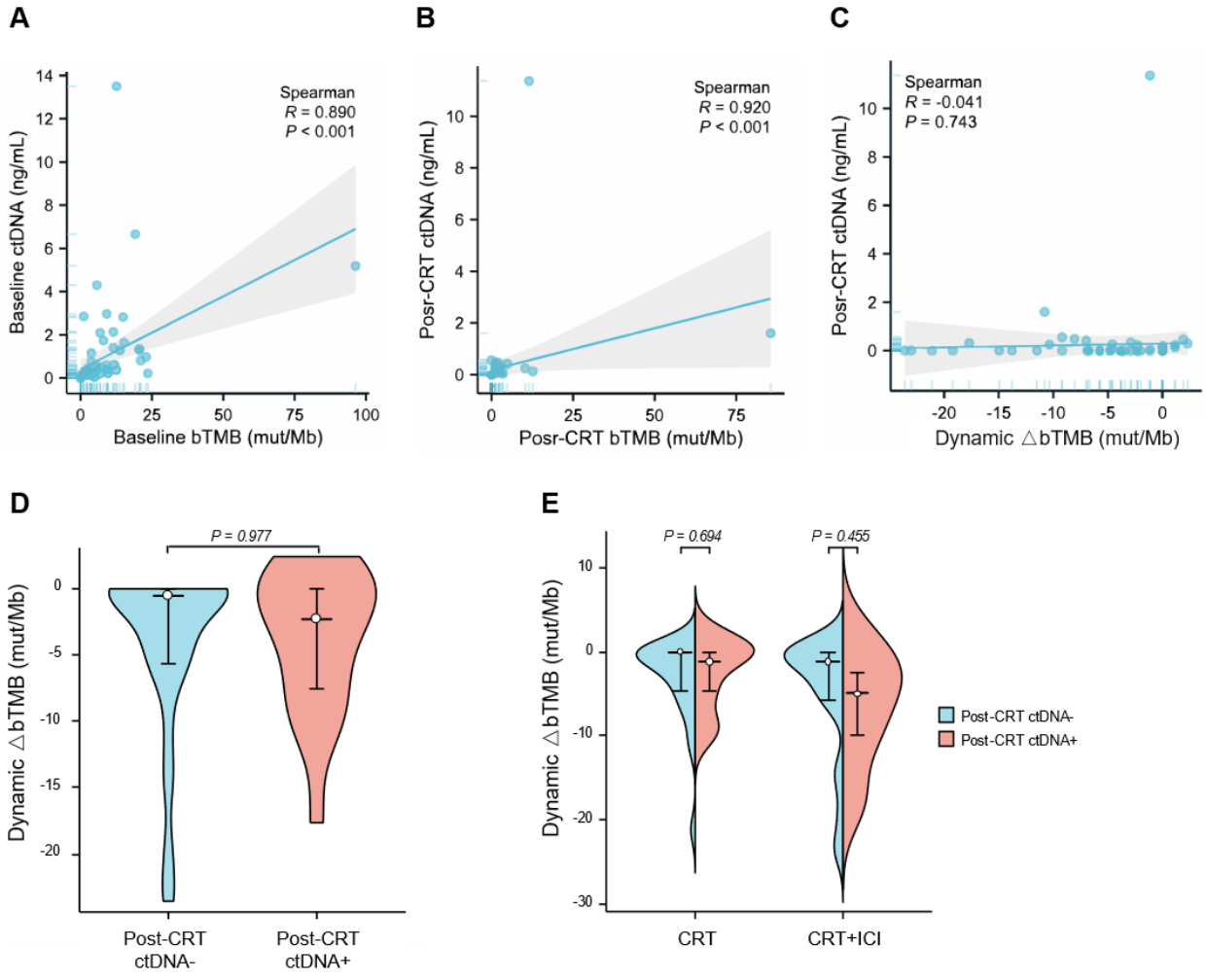
Supplementary Fig. 4. Forest plots for Cox proportional hazards regression analysis. Baseline bTMB at the cutoff points ranging from 4 to 22 mut/Mb was not predictive of overall survival (A) or progression-free survival (B). bTMB, blood tumor mutational burden; CI, confidence interval; HR, hazard ratio; N, number; OS, overall survival; PFS, progression-free survival.



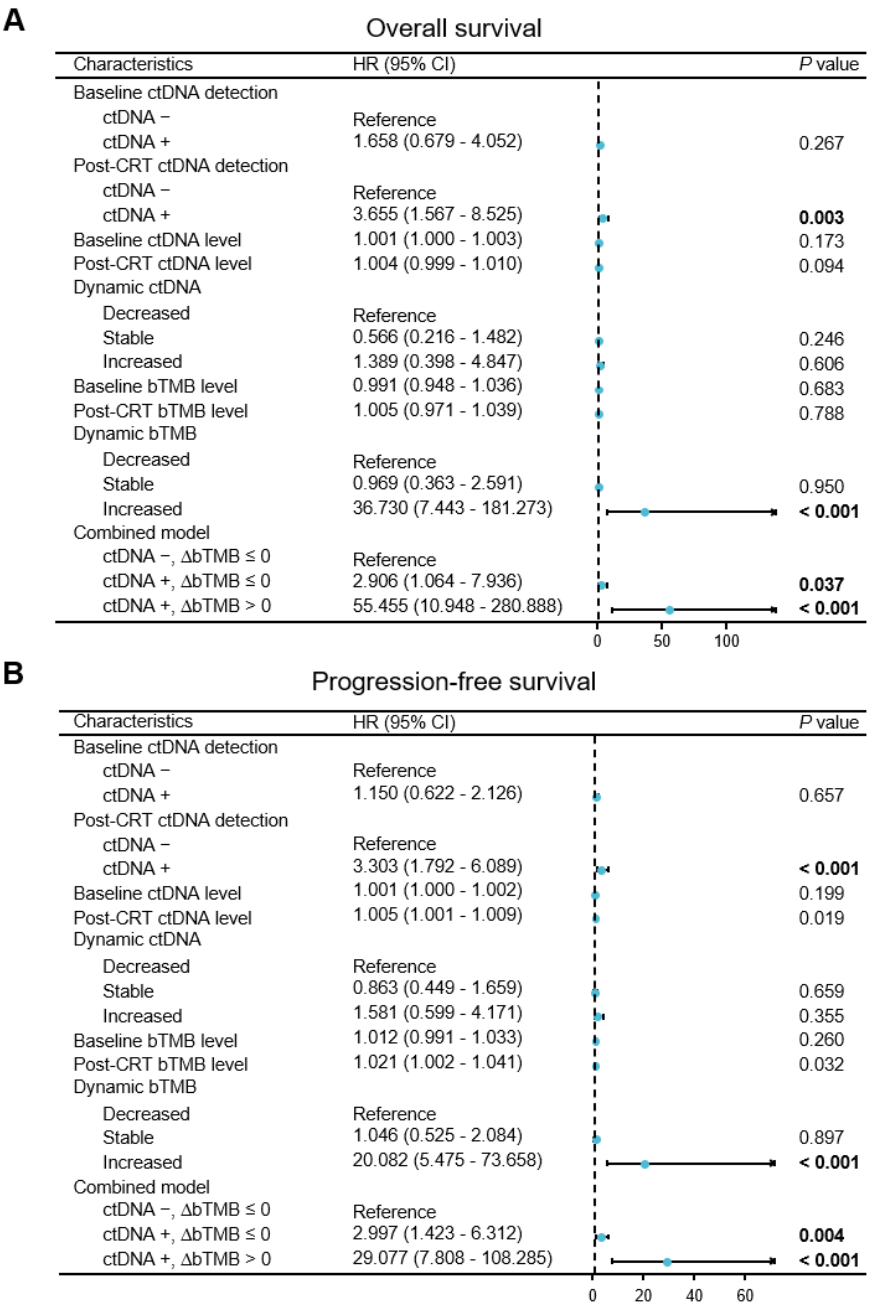
Supplementary Fig. 5. The predictive effect of dynamic ΔbTMB in the discovery set. (A) Time-dependent receiver operating characteristic curves showed, neither baseline bTMB, nor post-CRT bTMB, nor ΔbTMB levels, could effectively predict progression-free survival of patients in the discovery set (none of them with AUC > 0.75). However, ΔbTMB (AUC = 0.815) was predictive of survival in patients with residual ctDNA (B), rather than in patients with ctDNA clearance (C). AUC, area under the curve; bTMB, blood tumor mutational burden; CRT, chemoradiotherapy; ctDNA, circulating tumor DNA; FPR, false positive rate; TPR, true positive rate.



Supplementary Fig. 6. Dynamic ΔbTMB was an independent stratification factor of post-CRT ctDNA prediction. For patients with residual ctDNA after CRT, increased bTMB (ΔbTMB > 0) could further identify patients with significantly poorer overall survival (A) and progression-free survival (B). bTMB, blood tumor mutational burden; CRT, chemoradiotherapy; ctDNA, circulating tumor DNA.



Supplementary Fig. 7. Dynamic ΔbTMB was independent of post-CRT ctDNA. Scatter plots and linear fits of Spearman's correlation tests showing the strong correlation between baseline bTMB and baseline ctDNA levels (A), and between post-CRT bTMB and post-CRT ctDNA levels (B), but the negligible correlation between ΔbTMB and post-CRT ctDNA levels (C). No significant correlation between ΔbTMB and post-CRT ctDNA status (D), in both the CRT and the CRT + ICI cohorts (E). bTMB, blood tumor mutational burden; CRT, chemoradiotherapy; ctDNA, circulating tumor DNA; ICI, immune checkpoint inhibitor.



Supplementary Fig. 8. Forest plots for univariate Cox proportional hazards regression analysis. Combining post-CRT ctDNA detection with dynamic ΔbTMB could significantly differentiate survival outcomes into 3 groups. Patients with residual ctDNA plus increased ΔbTMB had significantly worse overall survival (A) and progression-free survival (B). bTMB, blood tumor mutational burden; CI, confidence interval; CRT, chemoradiotherapy; ctDNA, circulating tumor DNA; HR, hazard ratio.

Supplementary tables

Supplementary Table 1. 486 cancer-related genes in next-generation sequencing panel.

ABCB1	ABCC2	ABL1	ADH1B	AIP	AKT1	AKT2	AKT3	TET2
ALDH2	ALK	AMER1	APC	APEX1	AR	ARAF	ARID1A	TFG
ARID1B	ARID2	ARID5B	ASCL4	ASXL1	ATF1	ATIC	ATM	THADA
ATR	ATRX	AURKA	AURKB	AXIN2	AXL	B2M	BAD	TNF
BAI3	BAK1	BAP1	BARD1	BAX	BCL2	BCL2L1	BCL2L11	TNFAIP3
BCL3	BCR	BIRC3	BLM	BMPR1A	BRAF	BRCA1	BRCA2	TNFRSF11A
BRD4	BRIP1	BTG2	BTK	BUB1B	c11orf30	CASP8	CBL	TMEM127
CBLB	CCND1	CCNE1	CD274	CD74	CDA	CDC73	CDH1	TMEM167A
CDK10	CDK12	CDK4	CDK6	CDK8	CDKN1A	CDKN1B	CDKN1C	TMPRSS2
CDKN2A	CDKN2B	CDKN2C	CEBPA	CEP57	CHD4	CHD8	CHEK1	TNFRSF14
CHEK2	CREBBP	CRKL	CSF1R	CTCF	CTLA4	CTNNB1	CUL3	TNFRSF19
CUX1	CXCL8	CXCR4	CYLD	CYP19A1	CYP2A13	CYP2A6	CYP2A7	TNFRSF1B
CYP2B6*6	CYP2C19*2	CYP2C9*3	CYP2D6	CYP3A4*4	CYP3A5	CYSLTR2	DAXX	TNFSF11
DDR2	DENND1A	DHFR	DICER1	DLL3	DNMT3A	DOT1L	DPYD	TOP1
DTL	DUSP2	EGFR	EIF1AX	EML4	EP300	EPAS1	EPCAM	TOP2A
EPHA2	EPHA3	EPHA5	ERBB2	ERBB2IP	ERBB3	ERBB4	ERCC1	TP53
ERCC2	ERCC3	ERCC4	ERCC5	ESR1	ETV1	ETV4	ETV5	TP63
ETV6	EWSR1	EXT1	EXT2	EZH2	EZR	FANCA	FANCC	TPMT
FANCD2	FANCE	FANCF	FANCG	FANCI	FANCL	FANCM	FAT1	TSC1
FBXW7	FGF19	FGFR1	FGFR2	FGFR3	FGFR4	FH	FLCN	TSC2
FLT1	FLT3	FLT4	FOXA1	FOXL2	FOXO1	FOXO3	FOXP1	TSHR
FRG1	GATA1	GATA2	GATA3	GATA4	GATA6	GNA11	GNAQ	TTF1
GNAS	GREB1	GREM1	GRIN2A	GRM3	GRM8	GSTM1	GSTM4	TUBB3
GSTP1	GSTT1	HDAC1	HDAC2	HDAC9	HGF	HLA-A	HMOX1	TYMS
HNF1A	HNF1B	HOXB13	HRAS	HSPB1	IDH1	IDH2	IFNA6	U2AF1
IFNB1	IFNE	IFNG	IFNGR1	IFNGR2	IGF1R	IGF2	IKBKE	UGT1A1
IKZF1	IL13	IL1A	IL7R	INPP4B	IRF1	IRF2	ITGB6	UNG
JAK1	JAK2	JAK3	JARID2	JUN	KDM5A	KDR	KEAP1	VAMP2
KIF1B	KIT	KITLG	KLLN	KMT2A	KMT2B	KMT2C	KMT2D	VEGFA
KRAS	LHCGR	LIG3	LIG4	LIN28B	LMO1	LRP1B	LYN	VHL
LZTR1	MALT1	MAP2K1	MAP2K2	MAP2K4	MAP3K1	MAP3K4	MAPK1	WAS
MAPK3	MAX	MC1R	MCL1	MDM2	MDM4	MECOM	MED12	WISP3

MEF2B	MEN1	MET	MGMT	MIF	MITF	MLH1	MLH3	WRN
MLLT1	MLLT3	MLLT4	MMP1	MPL	MRE11A	MSH2	MSH6	WT1
MTAP	MTHFR	MTOR	MTRR	MUC5B	MUTYH	MYBL1	MYC	XPA
MYCL	MYCN	MYD88	MYH9	NAT1	NBN	NCOR1	NEIL1	TAP2
NF1	NF2	NFE2L2	NFKB1	NFKBIA	NKX2-1	NOS2	NOS3	TBK1
NOTCH1	NOTCH2	NOTCH3	NPM1	NQO1	NRAS	NRG1	NSD1	TEK
NTHL1	NTRK1	NTRK2	NTRK3	NUTM1	PAK2	PAK3	PALB2	TEKT4
PALLD	PARK2	PARP1	PARP2	PAX5	PBRM1	PDK1	PDCD1	TERC
PDCD1LG2	PDE11A	PDGFRA	PDGFRB	PGR	PHOX2B	PIK3C3	PIK3CA	TERT
PIK3CD	PIK3R1	PIK3R2	PKHD1	PLAG1	PLCB4	PLK1	PMAIP1	TGFB1
PMS1	PMS2	PNKP	POLD1	POLD3	POLE	POLH	POT1	TGFBR2
PPARD	PPM1D	PPP2R1A	PPP2R2A	PRDM1	PREX2	PRF1	PRKACA	XPC
PRKAR1A	PRKCB	PRKDC	PRKCI	PRSS1	PRSS3	PTCH1	PTEN	XRCC1
PTK2	PTPN11	PTPN13	QKI	RAC1	RAC3	RAD50	RAD51	XRCC2
RAD51B	RAD51C	RAD51D	RAD54L	RAD9A	RAF1	RARA	RARG	XRCC3
RASGEF1A	RB1	RCC1	RECQL4	RELA	RELN	RET	RHBDF2	XRCC4
RHOA	RICTOR	RNF43	ROS1	RPTOR	RRM1	RUNX1	RUNX1T1	XRCC5
SBDS	SDC4	SDHA	SDHB	SDHC	SDHD	9-Sep	SERPINE1	YAP1
SETBP1	SETD2	SF3B1	SGK1	SKP2	SLC34A2	SLC3A2	SMAD2	ZNF2
SMAD3	SMAD4	SMAD7	SMARCA4	SMARCB1	SMO	SOCS1	SOS1	ZNF217
SOX2	SPOP	SPRED1	SPRY4	SRC	SRSF2	SRY	STAG2	ZNF703
STAT1	STAT3	STK11	STMN1	SUFU	SUMO1	TACC3	TAP1	

Supplementary Table 2. Univariate Cox regression analysis exploring clinical-pathological factors associated with progression-free survival.

Characteristics	Hazard ratio (95% CI)	*P* value
Sex		
Female	Reference	
Male	0.925 (0.412–2.077)	0.851
Smoking		
No	Reference	
Yes	0.855 (0.440–1.661)	0.644
ECOG		
0	Reference	
1	3.188 (0.985–10.319)	0.053
Pathology		
LUAD	Reference	
LUSC	1.032 (0.556–1.916)	0.920
Others	1.720 (0.579–5.114)	0.329
Stage		
II	Reference	
IIIA	0.660 (0.140–3.099)	0.598
IIIB	0.763 (0.179–3.248)	0.714
IIIC	1.060 (0.231–4.865)	0.940
Initial tumor size, cm	1.073 (0.934–1.233)	0.319
T stage		
1	Reference	
2	1.372 (0.531–3.541)	0.514
3	0.865 (0.345–2.169)	0.757
4	1.262 (0.506–3.149)	0.617

N stage		
0	Reference	
1	0.839 (0.139–5.065)	0.848
2	2.026 (0.468–8.768)	0.345
3	1.968 (0.457–8.463)	0.363
Baseline ctDNA abundance, ng/mL	1.155 (0.988–1.350)	0.070
Baseline ctDNA concentration, hGE/mL	1.001 (1.000–1.002)	0.199
Baseline bTMB level, mut/Mb	1.012 (0.991–1.033)	0.260
Treatment cohort		
CRT	Reference	
CRT + ICI	0.468 (0.257–0.853)	0.013

Abbreviations: bTMB, blood tumor mutational burden; CI, confidence interval; CRT, chemoradiotherapy; ctDNA, circulating tumor DNA; ECOG, Eastern Cooperative Oncology Group; ICI, immune checkpoint inhibitor; LUAD, lung adenocarcinoma; LUSC, lung squamous carcinoma.

Supplementary Table 3. Univariate and multivariate Cox proportional hazards regression of quantitative and qualitative biomarkers predicting progression-free survival.

Characteristics	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Baseline ctDNA				
ctDNA −	Reference			
ctDNA +	1.150 (0.622–2.126)	0.657		
Post-CRT ctDNA				
ctDNA −	Reference		Reference	
ctDNA +	3.303 (1.792–6.089)	< 0.001	5.068 (1.009–25.461)	0.049
Baseline ctDNA level	1.001 (1.000–1.002)	0.199		
Post-CRT ctDNA level	1.005 (1.001–1.009)	0.019	0.994 (0.980–1.009)	0.456
Dynamic ctDNA				
Decreased	Reference			
Stable	0.863 (0.449–1.659)			
Increased	1.581 (0.599–4.171)			
Baseline bTMB level	1.012 (0.991–1.033)	0.260		
Post-CRT bTMB level	1.021 (1.002–1.041)	0.032	1.021 (0.994–1.049)	0.133
Dynamic bTMB				
Decreased	Reference		Reference	
Stable	1.046 (0.525–2.084)	0.897	1.583 (0.700–3.581)	0.270
Increased	20.082 (5.475–73.658)	< 0.001	11.382 (2.612–49.592)	0.001

Abbreviations: bTMB, blood tumor mutational burden; CI, confidence interval; CRT, chemoradiotherapy; ctDNA, circulating tumor DNA; HR, hazard ratio.

Supplementary Table 4. Baseline characteristics of patients in the validation set.

Validation set	Overall	CRT	CRT + ICI	P^*
	n = 30	n = 17	n = 13	
Age, years, median (IQR)	60 (54–66)	62 (53–66)	60 (57–62)	0.557
Gender, n (%)				
Male	25 (83)	14 (82)	11 (85)	1.000
Female	5 (17)	3 (18)	2 (15)	
Smoking, n (%)				
Current/former	23 (77)	13 (77)	10 (77)	1.000
Never	7 (23)	4 (23)	3 (23)	
Histology, n (%)				
LUSC	17 (57)	9 (53)	8 (62)	0.721
LUAD	13 (43)	8 (47)	5 (38)	
ECOG, n (%)				
0	7 (23)	2 (12)	5 (38)	0.190
1	23 (77)	15 (88)	8 (62)	
Stage, n (%)				
II	5 (17)	4 (24)	1 (8)	0.355
IIIA	4 (13)	1 (6)	3 (23)	
IIIB	12 (40)	8 (47)	4 (31)	
IIIC	9 (30)	4 (24)	5 (38)	
PD-L1 status¶, n (%)				
≥ 1%	7 (64)	3 (75)	4 (57)	1.000
< 1%	4 (36)	1 (25)	3 (43)	
Baseline ctDNA, median (IQR)				
ctDNA abundance, ng/mL	0.1 (0–0.7)	0 (0–0.3)	0.2 (0.1–0.7)	0.175
ctDNA concentration, hGE/mL	34.4 (0–65.9)	0 (0–76.3)	47.2 (18.9–65.9)	0.232
Baseline bTMB, mut/Mb, median (IQR)	1.5 (0–4.4)	0 (0–1.2)	2.9 (0.8–9.2)	0.055

***** Comparisons of baseline characteristics between patients treated with CRT and patients with CRT plus consolidation ICI.

¶ Nineteen patients had unknown PD-L1 expression data, including 13 in the CRT cohort and 6 in the CRT plus consolidation ICI cohort.

Abbreviations: bTMB, blood tumor mutational burden; CRT, chemoradiotherapy; ctDNA, circulating tumor DNA; ECOG, Eastern Cooperative Oncology Group; ICI, immune checkpoint inhibitor; IQR, interquartile range; LUAD, lung adenocarcinoma; LUSC, lung squamous carcinoma; PD-L1, programmed death–ligand 1.

Supplementary Table 5. Comparisons of baseline features between the discovery and validation sets.

Characteristics	Discovery set (n = 73)	Validation set (n = 30)	*P* value
Sex			
Male	61 (59.2%)	25 (24.3%)	1.000
Female	12 (11.7%)	5 (4.9%)	
Smoking			
No	19 (18.4%)	7 (6.8%)	0.775
Yes	54 (52.4%)	23 (22.3%)	
Pathology			
LUAD	30 (29.1%)	13 (12.6%)	0.339
LUSC	38 (36.9%)	17 (16.5%)	
Others	5 (4.9%)	0 (0%)	
ECOG			
0	13 (12.6%)	7 (6.8%)	0.520
1	60 (58.3%)	23 (22.3%)	
Stage			
II	3 (2.9%)	5 (4.9%)	0.079
III	70 (68%)	25 (24.3%)	
Treatment cohort			
CRT	36 (35%)	17 (16.5%)	0.498
CRT + ICI	37 (35.9%)	13 (12.6%)	

Abbreviations: CRT, chemoradiotherapy; ECOG, Eastern Cooperative Oncology Group; ICI, immune checkpoint inhibitor; LUAD, lung adenocarcinoma; LUSC, lung squamous carcinoma.