Supplementary

Table S1 Overview of potential biomarkers

Grouping	Variable
Clinical parameters	Age (</≥65 years), ALK status, best response to first-line therapy, differentiation grade, ECOG PS, EGFR status, LDH at baseline, metastatic sites, nintedanib plus docetaxel (line of therapy), PD-L1 positivity (tumor cells/immune cells), prior therapy, sex, smoking status, stage (UICC/AJCC) at diagnosis, time since first-line therapy
Genes analyzed by SeqCap EZ DNA sequencing	*AKT1, ALK, APC, ARHGEF4 (ASEF), ARID1A, ARID2, ATM, ATR, BCL2L11, BRAF, CCND1, CCNE1, CDKN2A, CDKN2B, CRKL, CTNNB1, DDR2, EGFR, ERBB2, ERBB3, ERBB4, FAT1, FBXW7, FGFR1, FGFR2, FGFR3, FLT1 (VEGFR1), FLT3, FLT4 (VEGFR3), GNAS, HRAS, KDM6A, KDR (VEGR2), KEAP1, KIT, KMT2C, KMT2D, KRAS, LCK, LRP1B, MAP2K1, MCL1, MDM2, MET, MGA, MTOR, MYC, NF1, NFKBIA, NKX2-1, NOTCH1, NRAS, NRG1, NTRK1, NTRK2, NTRK3, PBX1, PDGFRA, PDGFRB, PIK3CA, POU4F2, PTEN, RAF1, RB1, RBM10, RET, RIT1, ROS1, SETD2, SMAD4, SMARCA4, SOS1, SRC, STK11, TERT, TP53, U2AF1, ZKSCAN1*
RNA analyzed by NanoString nCounter System	
Cell cycle and proliferation	*ANLN, ATM, BAX, BCL2, BID, BRD4, CCNA1, CCNB1, CCND1, CCND3, CD70, CDC20, CDC25C, CDK2, CDK6, CDKN1A, CDKN1C, CDKN2A, CDKN2B, CENPF, CEP55, CXCR4, EXO1, IL10, KIF2C, LTB, MELK, MKI67, RB1, RRM2, TGFB2, THBS1, TNF, TNFAIP3, TNFRSF10B, TNFRSF10C, TNFRSF11A, TNFRSF11B, TNFRSF14, TNFRSF17, TNFRSF18, TNFRSF1A, TNFRSF1B, TNFRSF4, TNFRSF8, TNFRSF9, TNFSF10, TNFSF12, TNFSF13, TNFSF13B, TNFSF18, TNFSF4, TNFSF8, UBE2C*
Stromal factors	*ADAM12, ALDOA, ANGPT1, ANGPT2, ARG2, AXL, BBS1, CCL13, CCL2, CCL8, CCND2, CCNE1, CD14, CD44, CD74, CDH1, CDH11, CES3, COL11A1, COL11A2, COL17A1, COL4A5, COL5A1, COL6A3, CTSS, CXCL12, DLL4, E2F3, EDN1, EPCAM, EZH2, F2RL1, FAP, FGF18, FGFR1, FLT1, FSTL3, HEY1, HIF1A, HLA-DMA, HLA-DPA1, HLA-DPB1, HLA-DQB1, HLA-DRB5, ICAM1, ICAM2, ICAM3, IL10, ITGA1, ITGA2, ITGA4, ITGA6, ITGAE, ITGAL, ITGAM, ITGAV, ITGAX, ITGB2, ITGB3, ITPK1, JAG1, KDR, LAMB3, LOXL2, MMP1, MMP7, MMP9, MMRN2, NECTIN1, NFIL3, NID2, OLFML2B, P4HA1, P4HA2, PALMD, PDGFB, PDGFRB, PGPEP1, PIK3CA, PIK3R1, PLOD2, ROR2, RPL7A, RPS6KB1, SERPINB5, SERPINH1, SPP1, STC1, THBS1, THY1, TIE1, TNFAIP6, TPM1, TWIST1, TWIST2, TYMP, VCAM1, VCAN, VEGFA, VEGFB, VEGFC, VHL*
Immune cell localization to tumors	*A2M, ADGRE1, ANGPT2, APOE, ATF3, AXL, BATF3, BLK, CCL13, CCL14, CCL18, CCL19, CCL2, CCL20, CCL21, CCL22, CCL3/L1, CCL4, CCL5, CCL7, CCL8, CCR2, CCR4, CCR5, CD14, CD163, CD19, CD1C, CD2, CD209, CD244, CD247, CD27, CD274, CD276, CD36, CD38, CD3D, CD3E, CD3G, CD4, CD40LG, CD45RA, CD45RB, CD45RO, CD47, CD5, CD6, CD68, CD69, CD7, CD70, CD79A, CD79B, CD80, CD84, CD86, CD8A, CD8B, CDH1, CDH5, CEACAM3, CLEC14A, CLEC4E, CLEC5A, CLEC7A, CMKLR1, CPA3, CSF1, CSF1R, CSF2, CSF3, CSF3R, CTLA4, CTSW, CX3CL1, CX3CR1, CXCL1, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL16, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCL9, CXCR2, CXCR3, CXCR4, CXCR6, CXorf36, CYBB, DPP4, EGR1, EOMES, EPCAM, F2RL1, FAM30A, FAP, FAS, FCAR, FCGR1A, FCGR2A, FCGR3A/B, FCN1, FCRL2, FLT1, FOXP3, FPR1, FPR3, FYN, GNLY, GZMA, GZMB, GZMH, GZMK, GZMM, HCK, HDC, HLA-DMA, HLA-DMB, HLA-DOA, HLA-DOB, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DRA, HLA-DRB1, HLA-DRB5, HLA-E, HSD11B1, ICAM1, ICAM2, ICAM3, ICAM5, ICOS, ICOSLG, IDO1, IFI16, IFI27, IFI35, IFIH1, IFIT1, IFIT2, IFITM1, IFITM2, IFNA1, IFNAR1, IFNG, IFNGR1, IFNGR2, IHH, IL10, IL10RA, IL11, IL12RB2, IL15, IL16, IL17A, IL18, IL18R1, IL1A, IL1B, IL1R2, IL1RN, IL2, IL21R, IL22RA1, IL2RB, IL2RG, IL32, IL33, IL34, IL4, IL6R, IRF1, IRF4, IRF5, ITGA1, ITGA2, ITGA4, ITGA6, ITGAE, ITGAL, ITGAM, ITGAX, ITGB2, ITGB3, JAK3, KIR2DL3, KIR3DL1, KIR3DL2, KLRB1, KLRD1, KLRK1, LAG3, LCK, LDHA, LDHB, LILRA5, LILRB2, LOXL2, LRRC32, LY9, LY96, LYZ, MARCO, MFGE8, MICB, MMRN2, MS4A1, MS4A2, MS4A4A, MS4A6A, MYCT1, NCAM1, NCR1, NFAM1, NFATC2, NKG7, NLRP3, NOD2, NT5E, OAS3, OTOA, P2RY13, PDCD1, PDCD1LG2, PECAM1, PF4, PNOC, PRF1, PROM1, PSMB10, PSMB5, PSMB9, PTGER4, PTGS2, PTPRC, REN, ROBO4, ROR2, RORC, RUNX3, S100A12, S100A8, S100A9, SELE, SELL, SELP, SERPINA1, SH2D1A, SIGLEC1, SIGLEC5, SIRPA, SIRPB2, SLC11A1, SPIB, SPP1, STAT1, STAT4, TBX21, TCL1A, TGFB2, THBD, THY1, TIGIT, TLR1, TLR2, TLR4, TLR8, TLR9, TMEM140, TMEM173, TNF, TNFRSF17, TPSAB1/B2, TRAT1, TREM1, TSLP, TWF1, TWIST2, ULBP2, VCAM1, VEGFA, VEGFC, XCL1/2, ZAP70*
T cell priming and activation	*ADORA2A, BATF3, BTLA, CCL2, CCL22, CCL3/L1, CCL4, CCL5, CCR2, CCR5, CD1C, CD2, CD247, CD27, CD274, CD28, CD3D, CD3E, CD3G, CD4, CD40, CD40LG, CD44, CD48, CD5, CD68, CD69, CD70, CD80, CD86, CD8A, CLECL1, CTLA4, CX3CL1, CXCL10, CXCL11, CXCL13, CXCL9, CXCR3, DPP4, EGR1, EOMES, F2RL1, FOXP3, FYN, GNLY, GZMA, GZMB, GZMH, GZMK, GZMM, HAVCR2, HLA-DMA, HLA-DMB, HLA-DOA, HLA-DOB, HLA-DQA2, HLA-DQB1, HLA-DRA, HLA-DRB5, ICAM1, ICAM2, ICAM3, ICOS, ICOSLG, IDO1, IFNG, IFNGR1, IFNGR2, IHH, IL12RB2, IL15, IL17A, IL18, IL18R1, IL2, IL2RA, IL2RB, IL2RG, IL33, IL4, IRF1, IRF4, ITGA1, ITGAE, LAG3, LCK, LDHA, LDHB, LILRB2, LILRB4, LTB, LY9, MB21D1, MYD88, NECTIN2, NFATC2, PDCD1, PDCD1LG2, PF4, PRF1, PROM1, PSMB9, PTGS2, PVRIG, REN, RSAD2, SLC11A1, SPP1, STAT1, STAT4, TBX21, TGFB2, TIGIT, TLR1, TLR2, TLR3, TLR4, TLR5, TLR7, TLR8, TLR9, TMEM173, TNF, TNFAIP3, TNFRSF10B, TNFRSF10C, TNFRSF11A, TNFRSF11B, TNFRSF14, TNFRSF17, TNFRSF18, TNFRSF1A, TNFRSF1B, TNFRSF25, TNFRSF4, TNFRSF8, TNFRSF9, TNFSF10, TNFSF12, TNFSF13, TNFSF13B, TNFSF18, TNFSF4, TNFSF8, TNFSF9, TSLP, VEGFA, VTCN1, ZAP70*
Myeloid cell activity	*A2M, ANGPT1, ANGPT2, APOE, AREG, ARG1, ARG2, ATF3, AXL, BATF3, C1QA, C1QB, C2, C5, C5AR1, C7, CCL13, CCL19, CCL2, CCL20, CCL21, CCL22, CCL3/L1, CCL4, CCL5, CCL8, CCR2, CCR5, CD14, CD163, CD1C, CD2, CD247, CD274, CD36, CD3D, CD3E, CD3G, CD47, CD5, CD69, CD70, CD74, CD8A, CDH1, CDKN1A, CEBPB, CLEC4E, CLEC5A, CLEC7A, CMKLR1, COL11A1, COL17A1, CRABP2, CSF1, CSF1R, CSF2RB, CSF3R, CTLA4, CTSS, CTSW, CX3CL1, CXCL1, CXCL10, CXCL11, CXCL12, CXCL13, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCL9, CXCR3, CYBB, DAB2, DLL4, DPP4, EGR1, EOMES, F2RL1, FAP, FAS, FCAR, FCGR1A, FCGR2A, FCGR2B, FCGR3A/B, FCGRT, FCN1, FLT1, FOSL1, FPR1, FPR3, FYN, GNLY, GZMA, GZMB, GZMH, GZMK, GZMM, HCK, HLA-DMA, HLA-DPA1, HLA-DPB1, HLA-DQB1, HLA-DRB5, HMGB1, ICAM1, ICOS, IDO1, IER3, IFI27, IFI6, IFIT3, IFITM1, IFNA1, IFNAR1, IFNG, IFNGR1, IFNGR2, IHH, IL10, IL10RA, IL11, IL12RB2, IL15, IL16, IL17A, IL18, IL18R1, IL1A, IL1B, IL1R2, IL1RN, IL2, IL24, IL32, IL33, IL34, IL4, IL6, IL6R, IRF1, IRF4, IRF9, ISG15, ITGA1, ITGAE, ITGAL, ITGAM, ITGAX, LAG3, LAMB3, LCK, LDHA, LDHB, LGALS9, LIF, LILRA5, LILRB2, LOXL2, LTB, LY96, LYZ, MARCO, MB21D1, MFGE8, MICB, MMP1, MRC1, MX1, MXI1, MYD88, NFAM1, NFATC2, NLRC5, NLRP3, NOD2, NOS2, NT5E, OAS1, OAS2, OAS3, OASL, OLR1, P2RY13, PDZK1IP1, PECAM1, PF4, PRF1, PROM1, PTGS2, REN, ROR2, RUNX3, S100A12, S100A8, S100A9, SBNO2, SELE, SERPINA1, SIGLEC1, SIGLEC8, SIRPA, SIRPB2, SLC11A1, SOCS1, STAT1, STAT2, STAT3, STAT4, SYK, TBX21, TGFB1, TGFB2, TGFBR1, TGFBR2, TICAM1, TIE1, TLR1, TLR2, TLR3, TLR4, TLR5, TLR7, TLR8, TLR9, TMEM173, TNF, TNFAIP3, TNFAIP6, TNFRSF10B, TNFRSF10C, TNFRSF11A, TNFRSF11B, TNFRSF14, TNFRSF17, TNFRSF18, TNFRSF1A, TNFRSF1B, TNFRSF4, TNFRSF8, TNFRSF9, TNFSF10, TNFSF12, TNFSF13, TNFSF13B, TNFSF18, TNFSF4, TNFSF8, TREM1, TREM2, TRIM21, TSLP, TWIST2, ULBP2, VEGFA, VEGFC, ZC3H12A*
Natural killer cell activity	*CD96, GNLY, GZMA, GZMB, GZMH, GZMK, GZMM, IFI16, IFI35, IFIH1, IFIT2, IFITM2, KIR2DL3, KIR3DL1, KIR3DL2, KLRB1, KLRD1, KLRK1, MICB, NCAM1, NCR1, NKG7, PRF1, PVR, SELL, SELP, SLAMF7, TIGIT*
Cell types (abundance in the TME)	
B cells	*BLK, CD19, FCRL2, MS4A1, PNOC, SPIB, TCL1A, TNFRSF17*
CD45	*PTPRC*
CD8 T cells	*CD8A, CD8B*
Cytotoxic cells	*CTSW, GNLY, GZMA, GZMB, GZMH, KLRB1, KLRD1, KLRK1, NKG7, PRF1*
Dendritic cells	*CCL13, CD209, HSD11B1*
Exhausted CD8	*CD244, EOMES, LAG3, PTGER4*
Macrophages	*CD163, CD68, CD84, MS4A4A*
Mast cells	*CPA3, HDC, MS4A2*
Neutrophils	*CEACAM3, CSF3R, FCAR, FPR1, S100A12, SIGLEC5*
NK CD56dim cells	*IL21R, KIR2DL3, KIR3DL1, KIR3DL2*
NK cells	*NCR1*
T cells	*CD3D, CD3E, CD3G, CD6, SH2D1A, TRAT1*
Th1 cells	*TBX21*
Treg	*FOXP3*
SNP genotyping	*rs9582036 in the FLT1 gene*
Immunohistochemistry	*PD-L1, Ki-67*

AJCC, American Joint Committee on Cancer; ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; PD-L1, programmed death-ligand 1; SNP, single nucleotide polymorphism; TME, tumor microenvironment; UICC, Union for International Cancer Control.

https://dx.doi.org/10.21037/tlcr-24-326

Table S2 Overview of the biomarker sets used for the different multivariate analyses

Variables	IPF LASSO		Standard LASSO					Random forest
	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8
Predefined clinical parameters	M1	M1			X		X	X
DNA markers								
Genetic alterations	M2	M2	X		X		X	X
Tumor mutational burden	M2	M2	X		X		X	X
RNA markers								
Continuous pathways	M2	M2		X	X		X	X
Cell types	M2	M2		X	X		X	X
Individual angiogenesis genes	M2	M2		X	X		X	X
Immunohistochemistry								
% PD-L1-positive tumor and immune cells	M1					X	X	X
PD-L1 expression score (H-score)	M1					X	X	X
Ki67 score	M1					X	X	X
Location of PD-L1	M1					X	X	X
Genotyping								
Functional *FLT1* variant rs9582036	M1	M1					X	X

M1: clinical parameters, immunohistochemistry, genotyping; M2: RNA markers, genetic alterations by gene, tumor mutation burden. *FLT1*, Fms-related receptor tyrosine kinase 1; IPF LASSO, integrative LASSO with penalty factors; M1, modality 1; M2, modality 2; PD-L1, programmed death-ligand 1.

https://dx.doi.org/10.21037/tlcr-24-326

Table S3 Variables for LASSO and random forest analyses

Class	Variables	IPF LASSO		Standard LASSO					Random forest
		Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8
Clinical parameters									
Age		M1	M1			X		X	X
Best response to first line therapy	(PD or missing) *vs.* (non-PD)	M1						X	
	(Missing) *vs.* (non-PD or PD)		M1			X			
	(PD) *vs.* (non-PD or missing)		M1			X			X
	(Missing) *vs.* (non-PD or PD)								X
Differentiation grade	(Missing or well) *vs.* (moderately or poorly)	M1	M1			X		X	
	(Poorly) *vs.* (moderately or missing or well)	M1	M1			X		X	X
	(Moderately) *vs.* (well or poorly or missing)								X
	(Missing) *vs.* (well or moderately or poorly)								X
ECOG PS	(≥1) *vs.* (0 or missing)	M1	M1			X		X	X
	(Missing) *vs.* (0 or ≥1)								X
Metastatic sites	Adrenal (yes) *vs.* (no or missing)		M1			X			X
	Adrenal (missing) *vs.* (no or yes)		M1			X			X
	Brain (yes) *vs.* (no or missing)	M1	M1			X		X	X
	Brain (missing) *vs.* (no or yes)	M1	M1			X		X	X
	Liver (yes) *vs.* (no or missing)	M1	M1			X		X	X
	Liver (missing) *vs.* (no or yes)	M1	M1			X		X	X
	No. of metast. sites at baseline category (≤2 or missing) *vs.* (>2)	M1	M1			X		X	X
	No. of metast. sites at baseline category (missing) *vs.* (>2 or ≤2)								X
Nintedanib plus docetaxel (line of therapy)	(first- or second-line) *vs.* (third-line or higher)	M1	M1			X		X	X
Prior therapy	First-line chemo with bevacizumab (yes) *vs.* (no)								X
	Immunotherapy (yes) *vs.* (no)	M1	M1			X		X	X
Sex	(Male) *vs.* (female)	M1	M1			X		X	X
Smoking status	(Never) *vs.* (ex/current)								X
Stage (UICC/AJCC) at diag.	(≤III or missing) *vs.* (IV)	M1	M1			X		X	
	(≤III) *vs.* (IV or missing)								X
	(Missing) *vs.* (IV or ≤III)								X
Time since first-line therapy		M1	M1			X		X	X
DNA markers									
	ARID1A			X					X*
	CDKN2A	M2	M2	X		X		X	X*
	CDKN2B	M2	M2	X		X		X	X*
	DDR2	M2	M2	X		X		X	X*
	EGFR			X					X*
	ERBB2		M2	X		X			X*
	FAT1	M2	M2	X		X		X	X*
	FLT4		M2	X		X			X*
	KEAP1		M2	X		X			X*
	KMT2D	M2	M2	X		X		X	X*
	KRAS	M2	M2	X		X		X	X*
	LRP1B	M2	M2	X		X		X	X*
	MCL1	M2	M2	X		X		X	X*
	MDM2			X					X*
	MET	M2	M2	X		X		X	X*
	MYC	M2	M2	X		X		X	X*
	NF1		M2	X		X			X*
	NFKBIA	M2	M2	X		X		X	X*
	NKX2-1			X					X*
	NOTCH1	M2	M2	X		X		X	X*
	NTRK1		M2	X		X			X*
	RIT1	M2	M2	X		X		X	X*
	STK11		M2	X		X			X*
	TERT		M2	X		X			X*
	TP53	M2	M2	X		X		X	X*
Tumor mutational burden		M2	M2	X		X		X	X
RNA markers									
Continuous pathways	Angiogenesis	M2	M2		X	X		X	X
	Antigen presentation	M2	M2		X	X		X	X
	Apoptosis	M2	M2		X	X		X	X
	Autophagy	M2	M2		X	X		X	X
	Cell proliferation	M2	M2		X	X		X	X
	Costimulatory signaling	M2	M2		X	X		X	X
	Cytokine and chemokine signaling	M2	M2		X	X		X	X
	Cytotoxicity	M2	M2		X	X		X	X
	DNA damage repair	M2	M2		X	X		X	X
	Epigenetic regulation	M2	M2		X	X		X	X
	Hedgehog signaling	M2	M2		X	X		X	X
	Hypoxia	M2	M2		X	X		X	X
	Immune cell adhesion and migration	M2	M2		X	X		X	X
	Interferon signaling	M2	M2		X	X		X	X
	JAK-STAT signaling	M2	M2		X	X		X	X
	Lymphoid compartment	M2	M2		X	X		X	X
	MAPK	M2	M2		X	X		X	X
	Matrix remodeling and metastasis	M2	M2		X	X		X	X
	Metabolic stress	M2	M2		X	X		X	X
	Myeloid compartment	M2	M2		X	X		X	X
	NF-kappaB signaling	M2	M2		X	X		X	X
	Notch signaling	M2	M2		X	X		X	X
	PI3K-Akt	M2	M2		X	X		X	X
	TGF-beta signaling	M2	M2		X	X		X	X
	Wnt signaling	M2	M2		X	X		X	X
Cell types	B cells	M2	M2		X	X		X	X
	CD45 cells	M2	M2		X	X		X	X
	CD8 T cells	M2	M2		X	X		X	X
	Cytotoxic cells	M2	M2		X	X		X	X
	Dendritic cells	M2	M2		X	X		X	X
	Exhausted CD8 cells	M2	M2		X	X		X	X
	Macrophages	M2	M2		X	X		X	X
	Mast cells	M2	M2		X	X		X	X
	Neutrophils	M2	M2		X	X		X	X
	NK cells	M2	M2		X	X		X	X
	T cells	M2	M2		X	X		X	X
	Th1 cells	M2	M2		X	X		X	X
Individual angiogenesis genes	*CD274*	M2	M2		X	X		X	X
	VEGFA	M2	M2		X	X		X	X
	VEGFB	M2	M2		X	X		X	X
	VEGFC	M2	M2		X	X		X	X
Immunohistochemistry									
PD-L1	Percentage of PD-L1-positive tumor cells	M1					X	X	X
Ki67	Percentage of Ki67-positive tumor cells	M1					X	X	X
Genotyping									
FLT1	(Missing) *vs.* (G_T or T_T or G_G)								X
	(G_G) *vs.* (G_T or T_T or missing)								X

*, (alteration) *vs.* (no alteration or missing) and (missing) *vs.* (no alteration or alteration). M1: clinical parameters, immunohistochemistry, genotyping; M2: RNA markers, genetic alterations by gene, tumor mutation burden. AJCC, American Joint Committee on Cancer; ECOG PF, Eastern Cooperative Oncology Group performance status; IPF LASSO, integrative LASSO with penalty factors; M1, modality 1; M2, modality 2; metast., metastatic; NK, natural killer; PD, progressive disease; PD-L1, programmed death-ligand 1; UICC, Union for International Cancer Control.

https://dx.doi.org/10.21037/tlcr-24-326

Table S4 Variables for LASSO and random forest analyses in patients with prior immunotherapy

Class	Variables	IPF LASSO		Standard LASSO					Random forest
		Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8
Clinical parameters									
Age		M1	M1			X		X	X
Best response to first line therapy	(PD or missing) *vs.* (non-PD)								X
ECOG PS	(≥1) *vs.* (0 or missing)								X
Metastatic sites	Adrenal (yes or missing) *vs.* (no)								X
	Brain (yes) *vs.* (no or missing)								X
	Brain (missing) *vs.* (no or yes)								X
	Liver (Yes or missing) *vs.* (no)								X
	No. of metast. sites at baseline category (≤2 or missing) *vs.* (>2)								X
Nintedanib plus docetaxel (line of therapy)	(First- or second-line) *vs.* (third-line or higher)								X
Sex	(Male) *vs.* (female)								X
Stage (UICC/AJCC) at diagnosis	(≤III *vs.* IV)								X
Time since first-line therapy		M1	M1			X		X	X
DNA markers									
Gene alteration	*KRAS*			X					X*
Tumor mutational burden		M2	M2	X		X		X	X
RNA markers									
Continuous pathways	Angiogenesis	M2	M2		X	X		X	X
	Antigen presentation	M2	M2		X	X		X	X
	Apoptosis	M2	M2		X	X		X	X
	Autophagy	M2	M2		X	X		X	X
	Cell proliferation	M2	M2		X	X		X	X
	Costimulatory signaling	M2	M2		X	X		X	X
	Cytokine and chemokine signaling	M2	M2		X	X		X	X
	Cytotoxicity	M2	M2		X	X		X	X
	DNA damage repair	M2	M2		X	X		X	X
	Epigenetic regulation	M2	M2		X	X		X	X
	Hedgehog signaling	M2	M2		X	X		X	X
	Hypoxia	M2	M2		X	X		X	X
	Immune cell adhesion and migration	M2	M2		X	X		X	X
	Interferon signaling	M2	M2		X	X		X	X
	JAK-STAT signaling	M2	M2		X	X		X	X
	Lymphoid compartment	M2	M2		X	X		X	X
	MAPK	M2	M2		X	X		X	X
	Matrix remodelling and metastasis	M2	M2		X	X		X	X
	Metabolic stress	M2	M2		X	X		X	X
	Myeloid compartment	M2	M2		X	X		X	X
	NF-kappaB signaling	M2	M2		X	X		X	X
	Notch signaling	M2	M2		X	X		X	X
	PI3K-Akt	M2	M2		X	X		X	X
	TGF-beta signaling	M2	M2		X	X		X	X
	Wnt signaling	M2	M2		X	X		X	X
Cell types	B cells	M2	M2		X	X		X	X
	CD45 cells	M2	M2		X	X		X	X
	CD8 T cells	M2	M2		X	X		X	X
	Cytotoxic cells	M2	M2		X	X		X	X
	Dendritic cells	M2	M2		X	X		X	X
	Exhausted CD8 cells	M2	M2		X	X		X	X
	Macrophages	M2	M2		X	X		X	X
	Mast cells	M2	M2		X	X		X	X
	Neutrophils	M2	M2		X	X		X	X
	NK cells	M2	M2		X	X		X	X
	T cells	M2	M2		X	X		X	X
	Th1 cells	M2	M2		X	X		X	X
Individual angiogenesis genes	*CD274*	M2	M2		X	X		X	X
	VEGFA	M2	M2		X	X		X	X
	VEGFB	M2	M2		X	X		X	X
	VEGFC	M2	M2		X	X		X	X
Immunohistochemistry									
PD-L1	Percentage of PD-L1-positive tumor cells	M1					X	X	X
Ki67	Percentage of Ki67-positive tumor cells	M1					X	X	X

M1: clinical parameters, immunohistochemistry, genotyping; M2: RNA markers, genetic alterations by gene, tumor mutation burden. AJCC, American Joint Committee on Cancer; ECOG PS, Eastern Cooperative Oncology Group performance status; IPF LASSO, integrative LASSO with penalty factors; M1, modality 1; M2, modality 2; NK, natural killer; PD, progressive disease; PD-L1, programmed death-ligand 1; UICC, Union for International Cancer Control.

https://dx.doi.org/10.21037/tlcr-24-326

Table S5 Patient disposition

Variables	Overall	TSFLT <9 months[§]	TSFLT ≥9 months[†]	First-line PD[‡]	Prior immunotherapy
Screened, n	295				
Entered, n	260	140	119	67	67
Treated, n (%)	257 (100)	138 (100)	118 (100)	66 (100)	65 (100)
Discontinued study medication, n (%)	257 (100)	138 (100)	118 (100)	66 (100)	65 (100)
Discontinued nintedanib, n (%)	257 (100)	138 (100)	118 (100)	66 (100)	65 (100)
Progressive disease	156 (60.7)	90 (65.2)	65 (55.1)	37 (56.1)	37 (56.9)
Adverse event	68 (26.5)	32 (23.2)	36 (30.5)	19 (28.8)	19 (29.2)
Lost to follow-up	1 (0.4)	1 (0.7)	0	1 (1.5)	0
Other	32 (12.5)	15 (10.9)	17 (14.4)	9 (13.6)	9 (13.8)
Discontinued docetaxel, n (%)	257 (100)	138 (100)	118 (100)	66 (100)	65 (100)
Progressive disease	92 (35.8)	59 (42.8)	33 (28.0)	28 (42.4)	19 (29.2)
Adverse event	46 (17.9)	26 (18.8)	20 (16.9)	14 (21.2)	13 (20.0)
Other	119 (46.3)	53 (38.4)	65 (55.1)	24 (36.4)	33 (50.8)

One patient is missing data for time since start of first-line therapy. [§], early progression group; [†], late progression group; [‡], patients with PD as best response to first-line therapy. PD, progressive disease; TSFLT, time since start of first-line therapy.

Table S6 Summary of treatment with nintedanib plus docetaxel

Variables	Overall (n=260)	TSFLT <9 months (n=140)	TSFLT ≥9 months (n=119)	First-line PD (n=67)	Prior immunotherapy (n=67)
Line of treatment with nintedanib plus docetaxel, n (%)					
First-line	1 (0.4)	0	1 (0.8)	0	0
Second-line	179 (68.8)	127 (90.7)	52 (43.7)	51 (76.1)	10 (14.9)
Third-line	67 (25.8)	13 (9.3)	54 (45.4)	15 (22.4)	46 (68.7)
Fourth-line or higher	13 (5.0)	0	12 (10.1)	1 (1.5)	11 (16.4)
Duration of treatment with nintedanib plus docetaxel (weeks), median [range]	12.9 [0–111]	11.0 [1–74]	15.4 [0–111]	9.9 [1–111]	16.9 [1–111]

PD, progressive disease; TSFLT, time since start of first-line therapy.

Table S7 Most common adverse events (preferred term)

Adverse events	All	Mild	Moderate	Severe
Any AE/ADR	205 (79.8)	20 (7.8)	63 (24.5)	122 (47.5)
Diarrhea	76 (29.6)	24 (9.3)	44 (17.1)	8 (3.1)
Malignant neoplasm progression	66 (25.7)	0	1 (0.4)	65 (25.3)
Nausea	27 (10.5)	15 (5.8)	12 (4.7)	0
Fatigue	20 (7.8)	11 (4.3)	8 (3.1)	1 (0.4)
Vomiting	19 (7.4)	10 (3.9)	9 (3.5)	0
Pneumonia	16 (6.2)	1 (0.4)	7 (2.7)	8 (3.1)
Decreased appetite	15 (5.8)	7 (2.7)	7 (2.7)	1 (0.4)

Data are presented as n (%). AE, adverse event; ADR, adverse drug reaction.

https://dx.doi.org/10.21037/tlcr-24-326

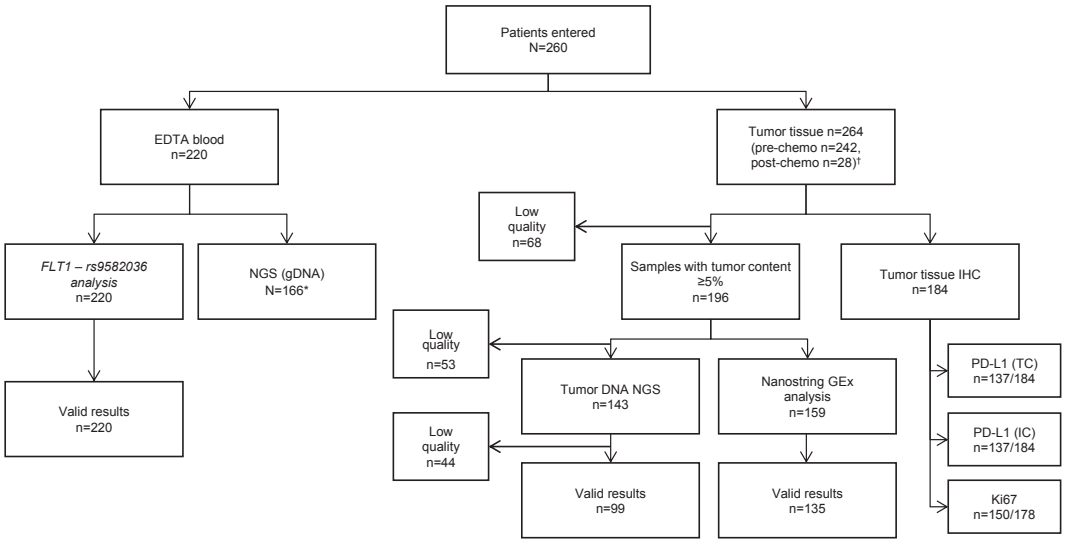


Figure S1 Biomarker samples and analysis flow. *, only samples with available matching FFPE tumor sample were analyzed. †, N=6 samples with matching pre- and post-chemotherapy samples available. *FLT1*, Fms-related receptor tyrosine kinase 1; gDNA, genomic DNA; GEx, gene expression; IC, immune cells; IHC, immunohistochemistry; PD-L1, programmed death-ligand 1; NGS, next-generation sequencing; TC, tumor cells.

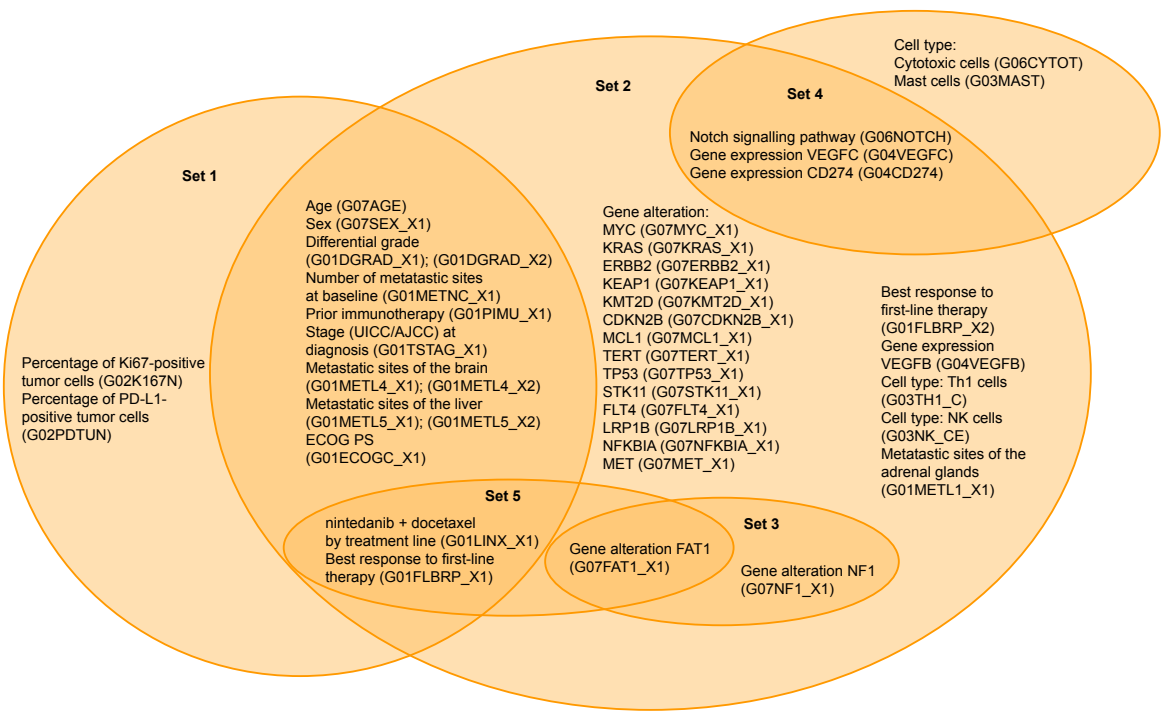


Figure S2 Variables with ≥30% relative frequency of IPF LASSO stability selection for time since first-line therapy (dichotomized)—sets 1 to 5. AJCC, American Joint Committee on Cancer; ECOG PS, Eastern Cooperative Oncology Group performance status; IPF LASSO, integrative LASSO with penalty factors; NK, natural killer; PD-L1, programmed death-ligand 1; UICC, Union for International Cancer Control.

https://dx.doi.org/10.21037/tlcr-24-326

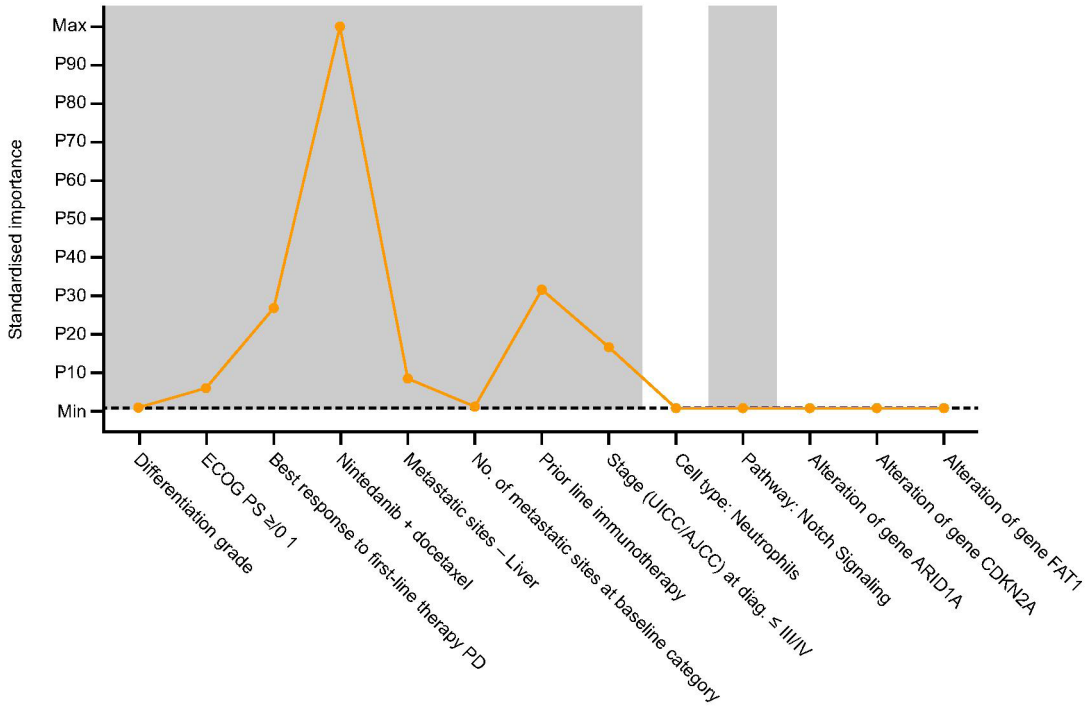
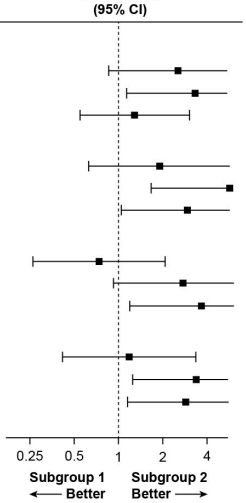


Figure S3 Random forest variable importance plot for time since start of first-line therapy (dichotomized) in set 8. AJCC, American Joint Committee on Cancer; diag, diagnosis; ECOG PS, Eastern Cooperative Oncology Group performance status; PD, progressive disease; UICC, Union for International Cancer Control.

https://dx.doi.org/10.21037/tlcr-24-326

A

Subgroup 1 versus Subgroup 2	Total Subgroup 1/ Subgroup 2	Events Subgroup 1/ Subgroup 2	Hazard Ratio (95% CI)	Interaction P-value	FDR P-value
Cell Proliferaton (cat)				0.66	0.33
≥ –0.22 (P33) and < 1.92 (P66) vs < –0.22 (P33)	11 / 10	10 / 8	2.587 (0.873, 7.665)		
≥ 1.92 (P66) vs < –0.22 (P33)	11 / 10	11 / 8	3.396 (1.153, 10.003)		
≥ 1.92 (P66) vs ≥ –0.22 (P33) and < 1.92 (P66)	11 / 11	11 / 10	1.313 (0.554, 3.108)		
DNA Damage Repair (cat)				0.008	0.20
≥ –0.90 (P33) and < 0.99 (P66) vs < –0.90 (P33)	11 / 10	9 / 6	1.916 (0.638, 5.755)		
≥ 0.99 (P66) vs < –0.90 (P33)	11 / 10	10 / 6	5.639 (1.683, 18.900)		
≥ 0.99 (P66) vs ≥ –0.90 (P33) and < 0.99 (P66)	11 / 11	10 / 9	2.943 (1.058, 8.188)		
Interferon Signaling (cat)				0.04	0.38
≥ –1.80 (P33) and < 1.54 (P66) vs < –1.80 (P33)	11 / 10	8 / 8	0.757 (0.282, 2.035)		
≥ 1.54 (P66) vs < –1.80 (P33)	11 / 10	9 / 8	2.636 (0.939, 7.401)		
≥ 1.54 (P66) vs ≥ –1.80 (P33) and < 1.54 (P66)	11 / 11	9 / 8	3.482 (1.195, 10.141)		
Neutrophils (cat)				0.01	0.16
≥ 189.76 (P33) and < 335.30 (P66) vs < 189.76 (P33)	11 / 10	8 / 6	1.181 (0.409, 3.411)		
≥ 335.30 (P66) vs < 189.76 (P33)	11 / 10	11 / 6	3.435 (1.253, 9.414)		
≥ 335.30 (P66) vs ≥ 189.76 (P33) and < 335.30 (P66)	11 / 11	11 / 8	2.908 (1.154, 7.331)		



B

Subgroup 1 versus Subgroup 2	Total Subgroup 1/ Subgroup 2	Events Subgroup 1/ Subgroup 2	Hazard Ratio (95% CI)	Interaction P-value	FDR P-value
DNA Damage Repair (cat)				0.03	0.24
≥ –0.90 (P33) and < 0.99 (P66) vs < –0.90 (P33)	11 / 10	9 / 9	1.360 (0.517, 3.575)		
≥ 0.99 (P66) vs < –0.90 (P33)	11 / 10	11 / 9	3.529 (1.268, 9.818)		
≥ 0.99 (P66) vs ≥ –0.90 (P33) and < 0.99 (P66)	11 / 11	11 / 9	2.595 (1.014, 6.642)		
Interferon Signaling (cat)				0.03	0.24
≥ –1.80 (P33) and < 1.54 (P66) vs < –1.80 (P33)	11 / 10	9 / 10	0.915 (0.359, 2.329)		
≥ 1.54 (P66) vs < –1.80 (P33)	11 / 10	10 / 10	2.793 (1.087, 7.174)		
≥ 1.54 (P66) vs ≥ –1.80 (P33) and < 1.54 (P66)	11 / 11	10 / 9	3.053 (1.185, 7.868)		
Neutrophils (cat)				0.02	0.18
≥ 189.76 (P33) and < 335.30 (P66) vs < 189.76 (P33)	11 / 10	10 / 8	1.370 (0.518, 3.627)		
≥ 335.30 (P66) vs < 189.76 (P33)	11 / 10	11 / 8	3.582 (1.336, 9.606)		
≥ 335.30 (P66) vs ≥ 189.76 (P33) and < 335.30 (P66)	11 / 11	11 / 10	2.614 (1.085, 6.296)		
Matrix Remodeling and Metastasis (cat)				0.03	0.24
≥ 0.46 (P33) and < 1.62 (P66) vs < 0.46 (P33)	11 / 10	11 / 10	0.541 (0.215, 1.362)		
≥ 1.62 (P66) vs < 0.46 (P33)	11 / 10	8 / 10	0.251 (0.085, 0.738)		
≥ 1.62 (P66) vs ≥ 0.46 (P33) and < 1.62 (P66)	11 / 11	8 / 11	0.464 (0.177, 1.216)		
TGF-beta Signaling (cat)				0.04	0.24
≥ –0.28 (P33) and < 1.13 (P66) vs < –0.28 (P33)	11 / 10	9 / 10	0.339 (0.128, 0.898)		
≥ 1.13 (P66) vs < –0.28 (P33)	11 / 10	10 / 10	0.344 (0.128, 0.925)		
≥ 1.13 (P66) vs ≥ –0.28 (P33) and < 1.13 (P66)	11 / 11	10 / 9	1.015 (0.401, 2.569)		
VEGFA (cat)				0.02	0.10
≥ 112.93 (P33) and < 203.05 (P66) vs < 112.93 (P33)	12 / 11	11 / 9	1.014 (0.407, 2.528)		
≥ 203.05 (P66) vs < 112.93 (P33)	12 / 11	10 / 9	2.971 (1.133, 7.788)		
≥ 203.05 (P66) vs ≥ 112.93 (P33) and < 203.05 (P66)	12 / 12	10 / 11	2.929 (1.193, 7.192)		

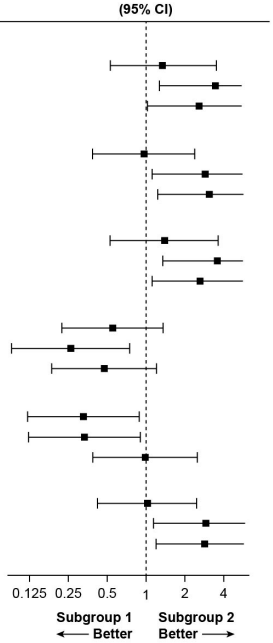


Figure S4 Molecular markers that demonstrated potential prognostic significance based on univariate analysis of patients who had previously received immunotherapy. (A) Molecular markers that demonstrated potential prognostic significance for OS. (B) Molecular markers that demonstrated potential prognostic significance for PFS. cat, category; CI, confidence interval; FDR, false discovery rate; OS, overall survival; P, percentile; PFS, progression-free survival; TGF, transforming growth factor; VEGFA, vascular endothelial growth factor A.

https://dx.doi.org/10.21037/tlcr-24-326