Supplemental Tables

Summary

Table S1. PubMed Search strategy.

#1	Search "Urinary Bladder Neoplasms"[Mesh]
#2	Search "Bladder Neoplasm"
#3	Search "Bladder Tumor"
#4	Search "Urinary Bladder Cancer"
#5	Search "Malignant Tumor of Urinary Bladder"
#6	Search "Bladder Cancer"
#7	Search "Carcinoma, Transitional Cell"[Mesh]
#8	Search "Transitional Cell Carcinoma"
#9	Search "Urothelial Carcinoma"
#10	Search "Urothelial Cancer"
#11	Search "Upper Tract Urothelial Carcinoma"
#12	Search "Ureter"
#13	Search "Pelvis"
#14	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13
#15	Search "Mutation"[Mesh]
#16	Search "Mutations"
#17	Search "Mutant"
#18	Search "Alteration"
#19	Search "Alterations"
#20	Search "Variant"
#21	Search "Variants"
#22	#15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21
#23	Search "Receptor, Fibroblast Growth Factor, Type 3"[Mesh]
#24	Search "Fibroblast Growth Factor Receptor 3"
#25	Search "FGFR3"
#26	Search "Fibroblast Growth Factor Receptor"
#27	Search "FGFR"
#28	#23 OR #24 OR #25 OR #26 OR #27
#29	#14 AND #22 AND #28

Table S2. Clinical and pathological information of 6 UC tumors.

Patient ID	Sample ID	Tumor location	FGFR3 status	Age	Gender	Smoking status	Tumor stage	Tumor Grade	Single/Multiple	Tumor size (cm)	Surgical treatment
P1	FGFR3−Mutated−1	Bladder	Mutated	86	Male	Never	$T_3N_xM_x$	High	Single	4.0	Radical cystectomy
P2	FGFR3−Mutated−2	Bladder	Mutated	77	Male	Never	$T_2N_0M_x$	High	Single	1.5	Radical cystectomy
P3	FGFR3−Mutated−3	Bladder	Mutated	57	Male	Never	$T_1N_xM_x$	Low	Multiple	2.0	Radical cystectomy
P4	FGFR3−Wildtype−1	Bladder	Wildtype	48	Male	Never	$T_1N_0M_x$	High	Multiple	2.0	Radical cystectomy
P5	FGFR3−Wildtype−2	Bladder	Wildtype	67	Male	Never	$T_1N_xM_x$	High	Single	0.5	Transurethral resection of bladder tumor
P6	FGFR3−Wildtype−3	Bladder	Wildtype	75	Male	Never	$T_xN_xM_x$	High	Multiple	1.0	Transurethral resection of bladder tumor

Table S3. Association between FGFR3 mutation and PD-L1 tumor infiltrating IC level in IMvigor210 cohort. Data were analyzed by chi-square test.

PD-L1 tumor infiltrating IC level, n (%)	FGFR3-Mutated (n = 39)	FGFR3-Wildtype (n = 39)
IC0	15 (38.5)	35 (20.8)
IC1	17 (43.6)	62 (36.9)
IC2+	7 (17.9)	71 (42.3)

PD-L1, programmed death ligand 1; IC, immune cell.

Table S4. Univariable Cox regression analysis for OS after PSM.

Variables	Univariable Cox regression model HR (95% CI)	p value
Sex		
Female	Reference	
Male	0.99 (0.50-1.96)	0.979
Intravesical BCG use		
No	Reference	
Yes	0.92 (0.51-1.68)	0.791
Platinum-based chemotherapy use history		
No	Reference	
Yes	0.99 (0.49-2.00)	0.97
Metastatic site		
Lymph node only	Reference	
Liver	2.31 (0.52-10.22)	0.268
Visceral	1.19 (0.28-4.99)	0.815
Race		
Non-White	Reference	
White	1.35 (0.18-9.78)	0.77
Baseline ECOG Score		
0	Reference	
1	1.44 (0.78-2.67)	0.241
2	2.48 (0.73-8.46)	0.146
Tobacco		
Never	Reference	
Ever	1.33 (0.71-2.56)	0.368
FGFR3 status		
No	Reference	
Yes	2.11 (1.16-3.85)	**0.015**
TMB Group		
Low (<10)	Reference	
High (≥10)	0.54 (0.27-1.07)	0.079
TMB (mut/MB)	0.97 (0.93-1.01)	0.107
PD-L1 tumor infiltrating IC level		
IC0	Reference	
IC1	1.03 (0.54-2.00)	0.921
IC2+	0.37 (0.16-0.88)	**0.024**

OS, overall survival; PSM, propensity score matching; BCG, Bacillus Calmette-Guérin; ECOG, Eastern Cooperative Oncology Group; TMB, tumor mutation burden; PD-L1, programmed death ligand 1; IC, immune cell; HR, hazard ratio; CI, confidence interval.

Table S5. Multivariable Cox regression analysis for FGFR3 mutation status on OS after PSM.

Multivariable Cox regression models		HR (95% CI)	p value
Multivariable model 1 (adjusted for TMB Group)[a]			
FGFR3 status			
	No	Reference	
	Yes	1.99 (1.08-3.64)	**0.026**
Multivariable model 2 (adjusted for TMB [mut/MB])[b]			
FGFR3 status			
	No	Reference	
	Yes	2.01 (1.10-3.68)	**0.023**
Multivariable model 3 (adjusted for PD-L1 tumor infiltrating IC level)[c]			
FGFR3 status			
	No	Reference	
	Yes	1.94 (1.06-3.56)	**0.032**

[a] TMB group (categorical data) was adjusted in multivariable Cox regression model 1.

[b] TMB [mut/MB] (continuous data) was adjusted in multivariable Cox regression model 2.

[c] PD-L1 tumor infiltrating IC level (categorical data) was adjusted in multivariable Cox regression model 3.

The univariable Cox regression analysis was displayed in Table S4.

All variables used in the multivariable Cox regression models are described in Table 1.

Table S6. Summary of TMB levels between patients with driver oncogenic mutations in FGFR3 and patients with non-driver mutations in FGFR3 based on IMvigor210 immunotherapy, MSKCC (mBC) immunotherapy, and MSKCC (mUTUC and mUUC) immunotherapy cohorts.

Trial name/Cohort name	Alteration	Oncogenic, n	Mutation Effect	TMB (mut/MB), median (IQR)	TMB Group, n (%)	
					High (≥10)	Low (<10)
IMVigor210						
	R248C (n = 6) S249C (n = 26) G370C (n = 2) Y373C (n = 4) K650E (n = 1)	Driver oncogenic mutation (n = 39)	Gain of function	7.2 (5.0-11.3)	11 (28.2)	28 (71.8)
MSKCC cohort (mBC)						
	R248C (n = 1) S249C (n = 15) S371C (n = 1) Y373C (n = 3) M528I (n = 1) FGFR3-TACC3 Protein fusion (n = 2)	Driver oncogenic mutation (n = 23)	Gain of function	8.8 (5.0-16.2)[a]	10 (43.5)	13 (56.5)[b]
	S84L (n = 1) A168S (n = 1) R175H (n = 1) D244E (n = 1) S415F (n = 1) L563V (n = 1)	Non-driver mutation (n = 6)	Neutral	14.1 (12.5-15.3)	5 (83.3)	1 (16.7)
MSKCC cohort (mUTUC and mUUC)						
	R248C (n = 4) S249C (n = 5) Y373C (n = 2) G380R (n = 1)	Driver oncogenic mutation (n = 12)	Gain of function	8.3 (5.4-40.6)	5 (41.7)	7 (58.3)

MSKCC, Memorial Sloan Kettering Cancer Center; mBC, metastatic bladder cancer; mUTUC= metastatic upper tract urothelial carcinoma; mUUC, urethral urothelial carcinoma; TMB, tumor mutation burden; IQR, interquartile range.

[a] Wilcoxon test P=0.374.

[b] Fisher's exact test P=0.169.

Table S7. The Newcastle-Ottawa Scale evaluated the quality of enrolled retrospective cohort studies.

First author	Trial name/Cohort name	Year	Selection				Comparability	Outcomes			Total scores
			Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Outcome of interest was not present at start of study	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Follow-up long enough	Adequacy of follow up	
Wang et al.	CheckMate275	2019	1	1	1	1	0	1	1	1	7
Wang et al.	IMVigor210	2019	1	1	1	1	0	1	1	1	7
Samstein et al.	MSKCC cohort (mBC)	2019	1	1	1	1	0	1	1	1	7
Samstein et al.	MSKCC cohort (mUTUC and mUUC)	2019	1	1	1	1	0	1	1	1	7
Szabados et al.	Clinico-Genomic Database	2022	1	1	1	1	0	1	1	1	7
Rose et al.	-	2021	1	1	1	1	2	1	1	1	9
Chawla et al.	-	2022	1	1	1	1	0	1	1	1	7
Tully et al.	-	2021	1	1	1	1	2	1	1	1	9

MSKCC, Memorial Sloan Kettering Cancer Center; mBC, metastatic bladder cancer; mUTUC, metastatic upper tract urothelial carcinoma; mUUC, urethral urothelial carcinoma.

Table S8. Cell number of each major cell type in every tumor sample.

Sample ID	B/Plasma cell	Endothelial cell	Epithelial cell	Fibroblast cell	Mast cell	Myeloid cell	T/Nautral Killer cell	Total
FGFR3-Mutated-1	106	110	2252	52	16	1218	2397	6151
FGFR3-Mutated-2	1049	185	2382	537	82	1872	5951	12058
FGFR3-Mutated-3	89	308	8871	433	29	629	3682	14041
FGFR3-Wildtype-1	1540	901	226	1081	302	2124	7437	13611
FGFR3-Wildtype-2	18	132	112	770	37	3555	1480	6104
FGFR3-Wildtype-3	925	57	768	170	85	1208	2891	6104

Table S9. Up-regulated genes of each T and natural killer cell subtype.

Natural_ Killer	CD8_Terminal_Exhausted_Cytotoxic	CD8_Effector	CD4_Treg	CD4_Naive	CD4_Exhausted	CD4_Memory	Cycling_T
GNLY	GZMB	GZMK	TNFRSF4	ANK3	CXCL13	IL7R	STMN1
TYROBP	CCL5	AOAH	IL2RA	TSHZ2	PDE7B	PLCB1	HMGB2
TRDC	MYO1E	CCL5	RTKN2	LTB	TSHZ2	ANK3	TUBA1B
NKG7	CD8A	GZMH	IKZF2	IL7R	NMB	BACH2	TOP2A
XCL2	CD8B	GZMA	TNFRSF18	CCR7	RBPJ	NR3C2	MKI67
GZMB	ITGAE	CST7	FOXP3	KLF2	PTPN13	ANXA1	HIST1H4C
KLRD1	GZMA	THEMIS	CRADD	FAAH2	FKBP5	KLRB1	ASPM
KLRC1	HAVCR2	CD8A	TBC1D4	SESN3	NR3C1	DPP4	CENPF
FCER1G	CCL3	DTHD1	CARD16	BACH2	GNG4	KLF2	TUBB
NCAM1	HOPX	CD8B	STAM	SERINC5	AHI1	TC2N	HIST1H1B
CCL3	PLPP1	NKG7	IL32	LEF1	TOX	BTBD11	RRM2
CCL4	ALOX5AP	IFNG	TIGIT	RPS6	MAF	CXCR4	HIST1H1D
XCL1	CXCL13	SAMD3	BATF	RPS20	GADD45G	TNFAIP3	PCLAF
KLRC2	NKG7	TC2N	ICA1	TCF7	ELMO1	ERN1	UBE2C
AOAH	KLRD1	CLDND1	CADM1	GPR183	TRPS1	MGAT4A	HMGN2
MCTP2	KLRC2	PPP2R2B	CTLA4	PRKCA	GAB2	MYBL1	NUSAP1
PRF1	ITGA1	KLRG1	PLCL1	RPL34	CD200	FOSB	HIST1H1E
CCL5	KLRC1	LYAR	VAV3	RPS12	AGFG1	RPS12	CENPP
HOPX	IFNG	CCL4	MAGEH1	RPS8	GEM	EEF1B2	H2AFZ
TXK	KLRK1	SYNE1	FANK1	RPS3A	PDCD1	PLAC8	DIAPH3
KLRF1	SRGAP3	ARHGAP26	ICOS	RPL32	RDH10	ZFP36L2	TPX2
CTSW	ATP8B4	ANXA1	LAIR2	RPL21	NELL2	TPT1	TYMS
FCGR3A	FUT8	KLRK1	IL1R1	RPS18	DLEU1	SBF2	HIST1H1C
GZMA	DAPK2	TRGC2	THADA	RPS17	TOX2	MGAT5	CENPE
CCL4L2	CCL4	PARP8	BACH1	RPL13	GRAMD1B	P2RY8	KNL1
KLRC3	ENTPD1	SLAMF7	LTB	TPT1	PPARG	RPS14	KIF14
PLCG2	NELL2	CMC1	TNFRSF1B	CMTM8	SNX9	RPL3	DUT
ATP8B4	TRGC2	GZMM	PHACTR2	SELL	JARID2	HSPA6	HMGB1
MATK	CTSW	CLEC2B	GK	RPL11	MYO1E	RBMS1	KIF11
CLIC3	LAG3	GIMAP7	CD27	RPL13A	PAM	AUTS2	TMPO

Table S10. Up-regulated genes of each myeloid cell subtype.

TREM2+Macrophage	Cycling_Macrophage	cDC1	cDC2	cDC3	Monocyte	Neutrophil
APOE	STMN1	CCSER1	S100B	CCL17	VCAN	IFITM2
CCL18	TUBA1B	CST3	HLA-DQB1	CCL22	EREG	FCGR3B
APOC1	TUBB	WDFY4	HLA-DPB1	BIRC3	CCL20	PDE4B
SELENOP	TOP2A	HDAC9	HLA-DPA1	CCL19	TIMP1	G0S2
RNASE1	MKI67	CPVL	HLA-DRA	GPC5	CXCL3	CMTM2
C1QA	SPP1	IDO1	HLA-DQA2	FSCN1	FCN1	AQP9
C1QB	HMGN2	CLNK	HLA-DRB1	LAMP3	PID1	PROK2
CTSB	CENPF	DNASE1L3	HLA-DQA1	LIMCH1	BAG3	S100A8
CTSD	CENPP	CADM1	CD74	CRIP1	CCL3	LUCAT1
CTSL	HIST1H4C	IRF8	CST3	KIF2A	MT2A	NAMPT
LGMN	H2AFZ	AUTS2	FCER1A	MARCKSL1	PLIN2	LCP2
C1QC	HMGB2	CPNE3	HLA-DQB2	LY75	CD300E	HCAR3
F13A1	HIST1H1D	SNX3	CLEC10A	CCR7	MT1X	KATNBL1
GPNMB	NUSAP1	HLA-DPA1	HLA-DMA	SLCO5A1	CRADD	PLEK
KCNMA1	RRM2	CLEC9A	LTB	CERS6	HSPD1	KCNJ15
SPP1	PCLAF	HLA-DPB1	PPA1	TXN	THBS1	IL1R2
MSR1	HIST1H1B	LGALS2	RPS19	IDO1	KYNU	BCL2A1
FMNL2	NUCKS1	CD74	GPR183	ARHGAP10	ATP1B3	CPD
CCL2	ASPM	S100B	YWHAH	FLT3	MAP2K1	CSF3R
CTSZ	DIAPH3	RGCC	AFF3	SLC22A23	CXCL2	PELI1
FRMD4A	HMGB1	CAMK2D	GSN	ENOX1	PLCB1	LITAF
SLCO2B1	DUT	NEGR1	CXCL9	L3MBTL4	AREG	CXCL8
NRP1	UBE2C	SRSF7	CD1C	WNT5B	S100A10	LIMK2
CD163	RAN	HLA-DQB1	CALHM6	VOPP1	LYZ	BCL6
CTSC	PTMA	RAB7B	RPS23	NUB1	DNAJA4	IVNS1ABP
PLTP	TMPO	RGS10	LYZ	AUTS2	NR4A1	S100A9
FOLR2	KNL1	HLA-DRA	CTSH	MALT1	HSPA6	HCAR2
PSAP	ATAD2	NAAA	RPS2	RFTN1	HSPH1	ACSL1
MRC1	PCNA	SHTN1	TMSB10	PLAAT3	HSPA1B	ALDH1A2
TREM2	MIR924HG	TMSB4X	RPS18	KDM2B	GSTO1	MCTP2

Table S11. Up-regulated genes of each firoblast cell subtype.

mCAF	iCAF
RGS5	PTGDS
NDUFA4L2	CFD
CCDC102B	DCN
CRIP1	FBLN1
ADAMTS9	LUM
MCAM	TNFAIP6
ADIRF	CXCL1
CACNA1C	CXCL12
ACTA2	CXCL14
EBF1	ABI3BP
PRKG1	POSTN
INPP4B	VCAN
EPS8	CTHRC1
COL18A1	RARRES2
MEF2C	MMP2
ID4	FN1
NOTCH3	BNC2
LHFPL6	C3
SPARCL1	IER3
GUCY1A2	ACSL4
IGKC	THBS2
CACNB2	SERPINF1
MYH11	CCDC80
SLIT3	FTH1
RGS16	FTL
CPE	CXCL6
TINAGL1	UCHL1
UTRN	BASP1
DLC1	KYNU
PDGFA	PTGS2