Tumor biomarker analyses in PURE-01:

DNA was extracted from formalin fixed paraffin embedded tissue obtained from pre-therapy TURB samples. Comprehensive genomic profiling (CGP) was performed in a Clinical Laboratory Improvement Amendments (CLIA)-certified, College of American Pathologists (CAP)-accredited laboratory (Foundation Medicine, Cambridge, MA). CGP was performed on hybridization-captured, adaptor ligation-based libraries to a median coverage depth of 743× for 395 cancer-related genes plus select introns from 31 genes frequently rearranged in cancer (Supplementary Material). Custom filtering was applied to remove benign germline events as described.[1,2] To determine microsatellite status, 114 intronic homopolymer repeat loci on the FoundationOne panel were analyzed for length variability and compiled into an overall microsatellite instability (MSI) score via principal components analysis. TMB was calculated as the number of somatic base substitutions or indels per megabase (Mb) of the coding region target territory of the test (1.1 Mb) after filtering to remove known somatic and deleterious mutations and extrapolating that value to the exome or genome as a whole.[2] For purposes of mutation burden estimation, all base substitutions and indels, including synonymous alterations, are counted. Subtracted from this number are functionally oncogenic or germline alterations, as defined below. Germline alterations are those listed in the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP), those with two or more counts in the ExAC database (http://exac.broadinstitute.org), or those predicted by a somatic-germline zygosity algorithm to be germline in the specimen being assessed.[3] TMB is reported as mutations per megabase (mut/mb).

Microsatellite instability (MSI) was measured by evaluating the changes to 114 loci selected from a total set of 1,897 that have adequate coverage. In a large training set of data from clinical specimens, we then used principal components analysis (PCA) to project the 228-dimension data onto a single dimension (the first principal component) that maximizes the data separation, producing an NGS-based "MSI score".[4]

PD-L1 expression was determined by IHC (Dako 22C3 PharmDx assay; Agilent Technologies, Carpinteria, CA, USA) at the local laboratory, with expressions scored using the CPS as previously described.[5,6] Approval for this study, including a waiver of informed consent and a HIPAA waiver

of authorization, was obtained from the Western Institutional Review Board (Protocol No. 20152817).

References:

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4. Hall MJ, Gowen K, Sanford EM, et al. Evaluation of microsatellite instability (MSI) status in 11,573 diverse solid tumors using comprehensive genomic profiling (CGP). J Clin Oncol. 2016;34(15_suppl):1523-1523. doi:10.1200/JCO.2016.34.15_suppl.1523

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6. Balar AV, Castellano D, O'Donnell PH, et al. First-line pembrolizumab in cisplatin-ineligible patients with locally advanced and unresectable or metastatic urothelial cancer (KEYNOTE-052): a multicentre, single-arm, phase 2 study. Lancet Oncol. 2017;18:1483-1492.

SUPPLEMENTARY MATERIAL: Genes sequenced using the FoundationOne assay (version T7)

Exonic capture (n=395)

HUGO SYMBOL	HUGO SYMBOL	HUGO SYMBOL	HUGO SYMBOL	HUGO SYMBOL	HUGO SYMBOL	HUGO SYMBOL	HUGO SYMBOL	HUGO SYMBOL	HUGO SYMBOL
ABL1	BRD4	CRLF2	FANCF	GLI1	KDM5A	MST1R	PHLPP2	RB1	SYK
ABL2	BRIP1	CSF1R	FANCG	GNA11	KDM5C	MTOR	PIK3C2B	RBM10	TAF1
ACVR1B	BTG1	CTCF	FANCI	GNA13	KDM6A	MUTYH	PIK3C2G	REL	TBX3
AKT1	BTK	CTNNA1	FANCL	GNAQ	KDR	MYC	PIK3C3	RET	TEK
AKT2	C11orf30 (EMSY)	CTNNB1	FANCM	GNAS	KEAP1	MYCL (MYCL1)	PIK3CA	RICTOR	TERC
AKT3	CARD11	CUL3	FAS	GPR124	KEL	MYCN	PIK3CB	RNF43	TERT (promoter only)
ALK	CASP8	CUL4A	FAT1	GREM1	KIT	MYD88	PIK3CG	ROS1	TET2
ALOX12B	CBFB	CUL4B	FAT3	GRIN2A	KLHL6	NBN	PIK3R1	RPA1	TGFBR2
AMER1 (FAM123B)	CBL	CYLD	FBXW7	GRM3	KMT2A (MLL)	NCOR1	PIK3R2	RPTOR	TIPARP
APC	CCND1	CYP17A1	FGF10	GSK3B	KMT2C (MLL3)	NF1	PLCG2	RUNX1	TNF
APCDD1	CCND2	DAXX	FGF12	H3F3A	KMT2D (MLL2)	NF2	PMS2	RUNX1T1	TNFAIP3
AR	CCND3	DDR1	FGF14	HGF	KRAS	NFE2L2	PNRC1	SDHA	TNFRSF14
ARAF	CCNE1	DDR2	FGF19	HLA-A	LMO1	NFKBIA	POLD1	SDHB	TNKS
ARFRP1	CD274	DICER1	FGF23	HLA-B	LRP1B	NKX2-1	POLE	SDHC	TNKS2
ARID1A	CD79A	DIS3	FGF3	HLA-C	LRP6	NOTCH1	PPARG	SDHD	TOP1
ARID1B	CD79B	DNMT3A	FGF4	HNF1A	LTK	NOTCH2	PPP2R1A	SETD2	TOP2A
ARID2	CDC73	DOT1L	FGF6	HOXB13	LYN	NOTCH3	PRDM1	SF3B1	TP53
ASXL1	CDH1	EGFR	FGF7	HRAS	LZTR1	NOTCH4	PREX2	SH2B3	TP53BP1
ATM	CDH2	EP300	FGFR1	HSD3B1	MAGI2	NPM1	PRKAR1A	SLIT2	TRRAP
ATR	CDH20	EPHA3	FGFR2	HSP90AA1	MAP2K1	NRAS	PRKCI	SMAD2	TSC1
ATRX	CDH5	EPHA5	FGFR3	IDH1	MAP2K2	NSD1	PRKDC	SMAD3	TSC2
AURKA	CDK12	EPHA6	FGFR4	IDH2	MAP2K4	NTRK1	PRSS1	SMAD4	TSHR
AURKB	CDK4	EPHA7	FH	IGF1	MAP3K1	NTRK2	PRSS8	SMARCA4	TYRO3
AXIN1	CDK6	EPHB1	FLCN	IGF1R	MAP3K13	NTRK3	PTCH1	SMARCB1	U2AF1
AXL	CDK8	EPHB4	FLT1	IGF2	MCL1	NUDT1	PTCH2	SMARCD1	VEGFA
BACH1	CDKN1A	EPHB6	FLT3	IGF2R	MDM2	NUP93	PTEN	SMO	VHL
BAP1	CDKN1B	ERBB2	FLT4	IKBKE	MDM4	PAK3	PTPN11	SNCAIP	WISP3
BARD1	CDKN2A	ERBB3	FOXL2	IKZF1	MED12	PAK7	PTPRD	SOCS1	WT1
BCL2	CDKN2B	ERBB4	FOXP1	IL7R	MEF2B	PALB2	QKI	SOX10	XPO1
BCL2A1	CDKN2C	ERCC4	FRS2	INHBA	MEN1	PARK2	RAC1	SOX2	XRCC2
BCL2L1	CEBPA	ERG	FUBP1	INPP4B	MERTK	PARP1	RAD50	SOX9	XRCC3
BCL2L2	CHD2	ERRFI1	GABRA6	INSR	MET	PARP2	RAD51	SPEN	ZBTB2
BCL6	CHD4	ESR1	GALNT12	IRF2	MITF	PARP3	RAD51B (RAD51L1)	SPOP	ZNF217
BCOR	CHEK1	EZH2	GATA1	IRF4	MKNK1	PARP4	RAD51C	SPTA1	ZNF703
BCORL1	CHEK2	FAM175A	GATA2	IRS2	MKNK2	PAX5	RAD51D (RAD51L3)	SRC	ZNRF3
BLM	CHUK	FAM46C	GATA3	JAK1	MLH1	PBRM1	RAD52	STAG2	
BMPR1A	CIC	FANCA	GATA4	JAK2	MPL	PDCD1LG2	RAD54L	STAT3	
BRAF	CRBN	FANCC	GATA6	JAK3	MRE11A	PDGFRA	RAF1	STAT4	
BRCA1	CREBBP	FANCD2	GEN1	JUN	MSH2	PDGFRB	RANBP2	STK11	
BRCA2	CRKL	FANCE	GID4 (C17orf39)	KAT6A (MYST3)	MSH6	PDK1	RARA	SUFU	

Select intronic capture for rearrangement analysis (n=31 genes)

HUGO SYMBOL	HUGO SYMBOL	HUGO SYMBOL	HUGO SYMBOL	HUGO SYMBOL	HUGO SYMBOL	HUGO SYMBOL	HUGO SYMBOL	HUGO SYMBOL	HUGO SYMBOL
ALK	BRCA1	EGFR	ETV5	FGFR1	KIT	MYB	NTRK1	RAF1	ROS1
BCL2	BRCA2	ETV1	ETV6	FGFR2	KMT2A (MLL)	MYC	NTRK2	RARA	RSPO2
BCR	BRD4	ETV4	EWSR1	FGFR3	MSH2	NOTCH2	PDGFRA	RET	TMPRSS2
BRAF									

GENE PATHWAY	pT0N0 (N=42)		pT2-4/pN+ or NR (N=47)		p-value	Corrected-p*	Odds ratio
	N	%	N	%			
Cell-cycle regulators**	25	59.5	30	63.8	0.8	1.0	0.83
Chromatin remodelling	32	76.2	33	70.2	0.6	1.0	1.36
FGFR1/2	1	2.4	2	4.3	1.0	1.0	0.55
FGFR3	7	16.7	6	12.8	0.8	1.0	1.37
HER2/3	11	26.2	9	19.2	0.5	0.9	1.50
HRD	14	33.3	9	19.2	0.15	0.8	2.11
PI3K/AKT/MTOR	18	42.8	23	48.9	0.7	1.0	0.78
RAS/RAF/MEK	10	23.8	15	31.9	0.5	0.9	0.67

Supplementary Table 1. Comparison of baseline molecular alterations identified in outlier pathologic responders subgroups (N=112 evaluable TURBT samples)

Abbreviations: HRD: homologous recombination defect genes; NR: non responders; TURBT: transurethral resection of the bladder tumor.

*Bonferroni adjustment was used for multiple hypothesis adjustment.
**RB1 gene alterations alone: corrected p-value=0.91.

Supplementary Figure 1

CONSORT diagram.

Abbreviations: CGP: comprehensive genomic profiling; CPS: combined positive score; RC: radical cystectomy; TMB: tumor mutational burden; TURBT: transurethral resection of the bladder tumor.

Supplementary Figure 2

Targetable genomic alterations and signatures identified in PURE-01 samples. Genomic alterations were ranked using the ESCAT actionability scale. Each case was assigned a tier according to the highest ranked genomic alteration/signature. ESCAT rankings were performed without TMB/MSI genomic signatures considered on the actionability scale.

Supplementary Figure 3

Longtail plot showing the comparison of genomic alterations in pT0N0 responders (A) versus non-responders (pT2-4 and/or pN+ and/or clinically non-responders).

Supplementary Figure 4
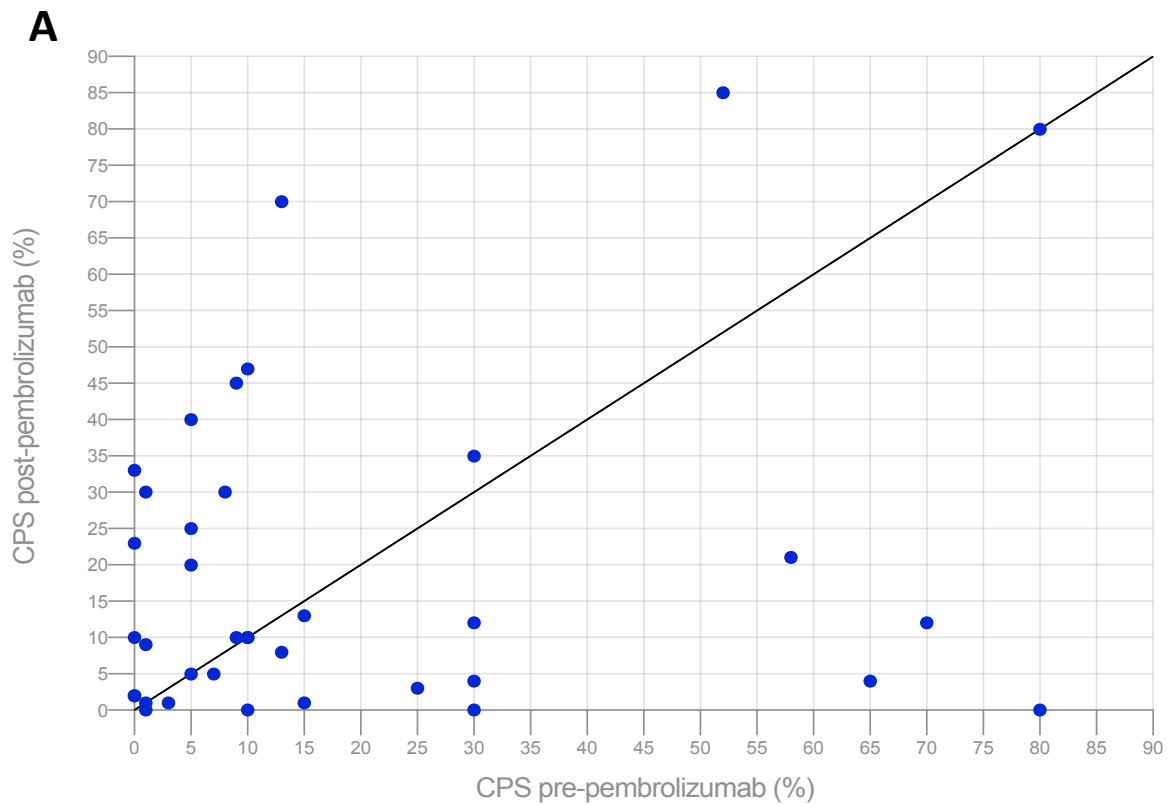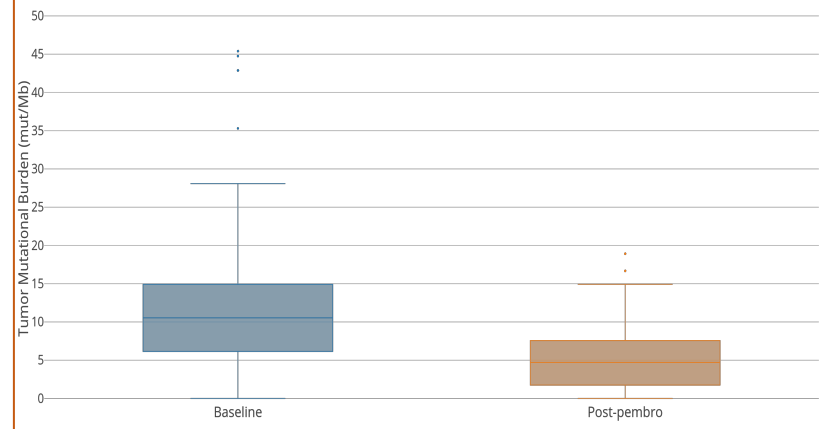

Logarithmic probabilities of pT0N0 — derived from a logistic single-variable model — were plotted according to the continuously coded value of the TMB (A) and CPS (B), respectively.

Abbreviations: CPS: combined positive score; PD-L1: programmed cell-death ligand-1; T0: pathologic complete response; TMB: tumor mutational burden.

Supplementary Figure 5

Logarithmic probabilities of pT0N0 — derived from a logistic single-variable model — were plotted according to the continuously coded value of CPS for patients with TMB values below the median (≤11Mut/Mb) (A) and for patients above the median (>11Mut/Mb) (B), respectively.
Abbreviations: CPS: combined positive score; PD-L1: programmed cell-death ligand-1; T0: pathologic complete response; TMB: tumor mutational burden.
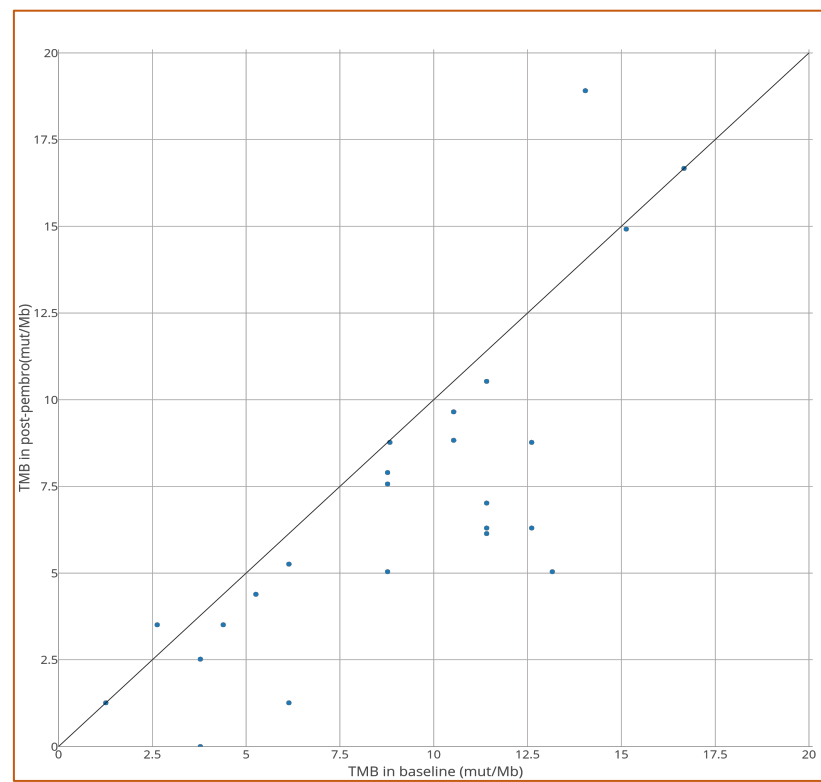
Supplementary Figure 6

Scatter plot illustrating the matched pre-therapy and post-therapy values of CPS (A) and TMB (B). Linear regression curve is shown in the plot.
Abbreviations: CPS: combined positive score; TMB: tumor mutational burden.