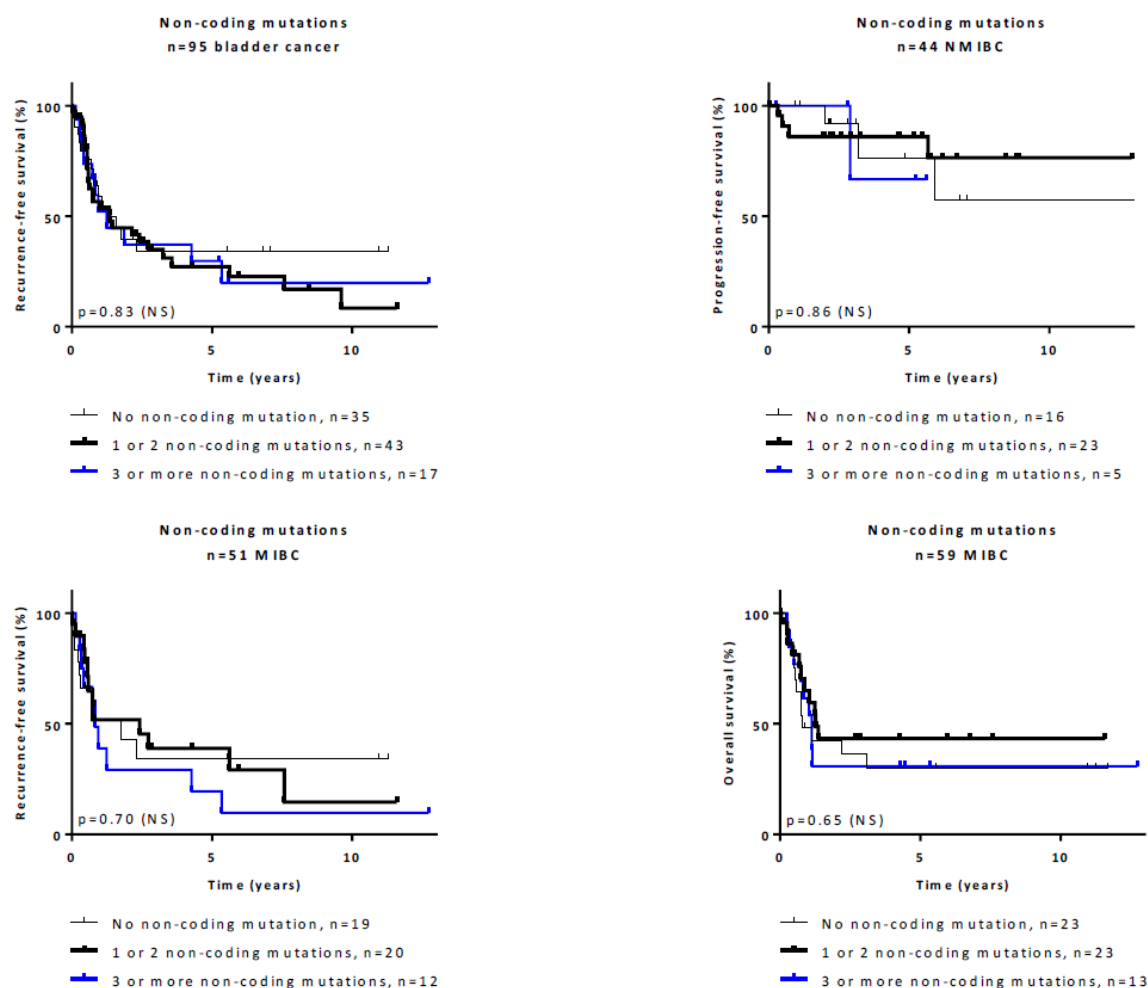


Supplementary Materials

# Genomic Instability Signature of Palindromic Non-Coding Somatic Mutations in Bladder Cancer.

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**Figure S1.** Survival curves of bladder patients according to non-coding mutation status.

**Table S1.** Primer sequences used for DNA mutation analysis.

Name	Forward Primer (5'–3')	Reverse Primer (5'–3')	Product (bp)
<i>TGAACA core motif identified by Nik-Zainal et al. [13]</i>			
GPR126	CCAGTGCATATTTACATGGACTCT	CAGTAGAGGATGTGTCAATCCTGGA	134
PLEKHS1	CTTCCAAGGCTGGGATGATCTA	AAGAAAGTGCCCATACAGAAATACA	107
Intron ADM	GTGCTTGCTGTGGGAATGATAC	AGCAAAGTGCTAAAAAAGGAATCTGT	150
Chr7:11	TGCACTACTGTCCACTTCATCAGACT	TGGGCCAGGGTCACTCGTAT	186
Chr15:96	GGGCTTGTTGATTTTTTCACTCATC	CTGACTACCCGAAGCAGAAAGAAC	190
Intron RNF169	GCCAAATTAAGGAATTTCCAGAAG	CTGAAGGTTTTTCCCAATTAACAGA	177
Chr7:17	GAGCAAATTTTTGTGCCACTACAC	TTTGCACTGACCGCAGCACT	127
Chr10:82	AGAAAGGCCAGAAGTGGGATAGA	AGGAAACTTTTACTCTCACTCGCT	132

<b>Chr18:42</b>	CAAAAGCTGAGATGATGGTCCTG	ATTCTTATCTCACCTGCAAAGCCT	123
<b>Chr3:82</b>	GCTTAAAATTGGTGGCAAGT	CCATATGGTTCTAAGATGGCTA	200
<i>TGAACA core motif identified by in silico analysis (present study)</i>			
<b>GABRG3</b>	TTTTGTAAAACAACAAGAAATAGTC	GTATAATTTTGTGCAATACGTGA	170
<b>PCDH18</b>	CAAGTTTATTGGAAAGAAGAGTA	TGAGAGATTCTTAATATCTTGTGTA	92
<b>EYS</b>	ATTATATTTGGTTTATGTTTACAC	TGCTTTAATTTCTGTAGTCTGA	186
<b>DAOA</b>	AGTACTGAAGCTCTACCAAGCTA	GCAATGTTGCTTATTCTCGT	161
<b>ZNF717</b>	TGAATTCCTTCTTACTCAATTACT	AGGATCATTACCTTTCTTACAT	130
<b>NGLY1</b>	CTTTTTTAGTATTAGACAATGTGAT	AATTACCAAAAACATACAGAGAT	133
<b>AP3B1</b>	GAAACTAAACCATTAGGTATTAGACA	CAGTTATATCCAAAGGCTTGAC	193
<b>CLVS2</b>	TTCTGTATTTGGATCTCGTCT	AAATATATGTCTCTCAAATAAAGTCT	159
<b>ZNF621</b>	TGCCATCTAAGTCCTTCATC	TTTTCAAGTTTGAGAATCACTG	186
<b>SCHIP1</b>	GGTTTTCTGTGCCTGGATAC	CTGAATTCCTTTGAAACACTG	125
<b>ARAP2</b>	CACCGTTGAAAAACAGCAG	TCAGGCAATATTTAACCAGTAG	143
<b>LOC100422737</b>	TAACCACATGGTTCAGACATC	CCAATACCGGGTAACTCAG	136
<b>KIAA1324L</b>	TATCTGTACTTTCTGTTACATACT	TTTTGTTATACTTTGTTTCATTCA	178
<b>DKK1</b>	TGAGGAATATTGTATTCAAATGTG	CTACCTTCCAAATGTTGCTATG	181
<b>RTN3</b>	GAGTATATTTTGCAGAAAGACAC	ATAAAGTTAACACAAACTATAAGCTA	165
<b>OR7E156P</b>	GCCTATGTGTGAAGATTTTATGA	TGGGACATATGCTTTTAATGAC	113
<b>LOC644649</b>	CACCTTTATTGGTCTTATTAGTACA	CAAATTTTAATGCAATTATTAAGTCT	111
<b>MIR645</b>	TGATGGAATTGCCTAGTGTGAGTCT	GGACCTCCCCCAACTCTGCT	182
<i>AGATCA core motif</i>			
<b>RAD51B</b>	TGCCAGAAAAACACTTACAC	AGATGAGGATCTCCCACAG	152

Table S2. Sequences of primers used for real-time quantitative RT-PCR.

Gene	Upper Primer (5' to 3')	Lower Primer (5' to 3')	Amplicon Size (bp)
<i>TBP</i>	TGCACAGGAGCCAAGAGTGAA	CACATCACAGCTCCCCACCA	132
<i>APOBEC3A</i>	GACAATGGCACCTCGGTCAAGA	GGGTCCAAGTCAAAGAAGGAAC	137
<i>APOBEC3B</i>	ATTCTTGCACCGCACGCTA	GTCTGAAGGACCAAAGGGTCATTA	95
<i>APOBEC3H</i>	CGAGGAAGGCCCTCTTGTGTTA	TTCTGTCATGGCACTTTTCTTGT	96

**Table S3.** Mutations in palindromic non-coding sites in the cohort of 103 bladder cancers.

See Excel document.

**Table S4.** Loci with a TGAACA core motif of palindromic sequences of 9-, 10- or 11-base pair (bp). The 18 loci selected are indicated in blue characters.

See Excel document.

**Table S5.** TMB, MSI status, and protein-coding gene alterations in bladder tumors showing high levels of non-coding TGAACA mutations.

Tumor Sample	Number of non-Coding Mutations	TMB	MSI	Number of Non-Synonymous Variants after Filtering	Focal Amplification	Genes with Pathogenic Variants (PV)					
						Genes	Chromosome	Transcript	NUCLEOTIDIC variant	proteic Variant	Allelic Ratio
T331	8	17.7	MSI-H	78	-	CDKN1A	chr6	NM_001291549.1	c.424dup	p.(Glu142GlyfsTer21)	73.68
						TERT	chr5	NA	c.-124C>T	NA	42.52
						KDM6A	chrX	NM_001291415.1	c.1973del	p.(Asn658ThrfsTer19)	40.92
						PTEN	chr10	NM_000314.6	c.492+2T>A	NA	25.62
						RXRA	chr9	NM_002957.4	c.1280C>T	p.(Ser427Phe)	25.34
						ROS1	chr6	NM_002944.2	c.5027G>A	p.(Trp1676Ter)	23.92
						FGFR3	chr4	NM_000142.4	c.1118A>G	p.(Tyr373Cys)	21.85
						PIK3CA	chr3	NM_006218.3	c.1624G>A	p.(Glu542Lys)	21.5
						MLH1	chr3	NM_000249.3	c.958G>T	p.(Glu320Ter)	20.89
						FOXP1	chr3	NM_032682.5	c.1160C>G	p.(Ser387Ter)	20.52
						MLH1	chr3	NM_000249.3	c.2005G>T	p.(Glu669Ter)	19.85
						NSD1	chr5	NM_022455.4	c.1262G>A	p.(Trp421Ter)	16.96
						TP53	chr17	NM_000546.5	c.574C>T	p.(Gln192Ter)	16.8
						PIK3CA	chr3	NM_006218.3	c.277C>T	p.(Arg93Trp)	16.41
						PPM1D	chr17	NM_003620.3	c.859G>T	p.(Glu287Ter)	15.92
						TP53	chr17	NM_000546.5	c.1164_1182del	p.(Glu388AspfsTer28)	13.47
						KMT2A	chr11	NM_005933.3	c.2318del	p.(Pro773ArgfsTer8)	5.78
T254	8	12.7	MSS	65	ERBB2	KDM6A	chrX	NM_001291415.1	c.4340dup	p.(Leu1448IlefsTer14)	78.68
						TERT	chr5	NA	c.-124C>T	NA	60.82
						TP53	chr17	NM_000546.5	c.574C>T	p.(Gln192Ter)	43.94
						ERBB3	chr12	NM_001982.3	c.994G>A	p.(Glu332Lys)	37.97
						KMT2D	chr12	NM_003482.3	c.4468G>T	p.(Glu1490Ter)	32.69
						FOXA1	chr14	NM_004496.3	c.412_415dup	p.(Ser139AsnfsTer89)	20.17
T206	5	10.4	MSS	93	-	PIK3CA	chr3	NM_006218.3	c.1633G>A	p.(Glu545Lys)	62.62
						ATM	chr11	NM_000051.3	c.7308-1G>C	NA	52.12
						TERT	chr5	NA	c.-146C>T	NA	45.07
						FGFR3	chr4	NM_000142.4	c.1118A>G	p.(Tyr373Cys)	43.56

T238	4	7.5	MSS	52	ERBB2	ETV6	chr12	NM_001987.4	c.313C>T	p.(Arg105Ter)	40.43
						STAG2	chrX	NM_001042749.1	c.2264del	p.(Lys755ArgfsTer7)	37.95
						PBRM1	chr3	NM_001350075.1	c.237-1G>T	NA	8.74
						ARID2	chr12	NM_152641.3	c.706-1G>T	NA	7.36
						RAD51C	chr17	NM_058216.2	c.1026+1G>T	NA	5.43
						ERBB2	chr17	NM_004448.2	c.2301C>G	p.(Ile767Met)	79.77
						TP53	chr17	NM_000546.5	c.672+1G>T	NA	38.67
						ASXL2	chr2	NM_018263.4	c.760G>T	p.(Glu254Ter)	25.0
						ARID1A	chr1	NM_006015.5	c.5393C>G	p.(Ser1798Ter)	23.53
						ERBB2IP	chr5	NM_018695.3	c.131_135del	p.(Phe44Ter)	18.62
T272	0	5.6	MSS	38	ERBB2	NIPBL	chr5	NM_015384.4	c.2509C>T	p.(Gln837Ter)	15.79
						RB1	chr13	NM_000321.2	c.1694C>A	p.(Ser565Ter)	34.5
						TERT	chr5	NA	c.-124C>T	NA	31.82
						TP53	chr17	NM_000546.5	c.536A>G	p.(His179Arg)	27.86

MSI: Microsatellite Instability; MSS: Microsatellite Stable; MSI-H: Microsatellite Instability High; NA: non applicable

**Table S6.** Associations between TGAACA core motif mutational signature and mRNA expression levels of immune-related genes.

Gene	Gene ID	Aliases	Group 1			Group 2			Group 3			p-Value *
			No non-coding mutations			One or two non-coding mutations			Three or more non-coding mutations			
			(n = 39)			(n = 46)			(n = 18)			
Median	Min	Max	Median	Min	Max	Median	Min	Max				
Immune cell population genes												
CD2	914		1.00	0.01	7.47	0.56	0.06	6.81	0.67	0.16	2.33	0.39 (NS)
CD3E	916		1.00	0.08	9.68	0.65	0.06	7.19	0.87	0.15	2.78	0.23 (NS)
CD4	920		1.00	0.17	5.36	0.73	0.11	4.11	0.99	0.13	1.97	0.11 (NS)
CD8A	925		1.00	0.05	29.1	0.65	0.07	13.5	1.22	0.15	8.95	0.68 (NS)
CTLA4	1493		1.00	0.00	8.63	0.58	0.00	5.65	1.27	0.08	3.10	0.32 (NS)
FOXP3	50943		1.00	0.12	6.67	0.78	0.08	5.64	0.94	0.07	3.43	0.22 (NS)
XCR1	2829		1.00	0.09	10.8	0.73	0.05	14.2	0.97	0.00	15.3	0.15 (NS)
MERTK	10461		1.00	0.24	3.55	1.07	0.27	2.41	0.98	0.14	3.11	0.90 (NS)
PTPRC	5788	CD45	1.00	0.02	7.18	0.58	0.02	6.14	0.97	0.13	2.47	0.19 (NS)
MS4A1	931	CD20	1.00	0.00	28.6	0.43	0.00	39.9	1.45	0.00	25.2	0.79 (NS)
NCAM1	4684	CD56	1.00	0.00	108.4	1.11	0.00	47.3	1.41	0.22	64.4	0.84 (NS)
PDGFRB	5159		1.00	0.07	7.29	0.50	0.08	6.42	0.87	0.07	3.23	0.17 (NS)
PECAM1	5175		1.00	0.14	6.14	0.66	0.10	2.83	0.83	0.19	2.74	0.14 (NS)
T cell activation genes												
PRF1	5551		1.00	0.09	16.8	0.47	0.05	10.4	0.83	0.10	3.08	0.34 (NS)
GZMA	3001		1.00	0.03	31.7	0.43	0.01	9.42	1.03	0.12	4.65	0.11 (NS)
GZMB	3002		1.00	0.01	40.8	0.41	0.02	20.1	1.51	0.02	11.1	0.38 (NS)
Checkpoint T cell genes												
CD28	940		1.00	0.01	5.45	0.64	0.02	4.89	0.87	0.08	1.61	0.062 (NS)
ENTPD1	953	CD39	1.00	0.10	4.13	0.66	0.10	3.55	0.75	0.12	2.32	0.28 (NS)
NT5E	4907	CD73	1.00	0.05	8.6	0.55	0.06	4.58	0.39	0.16	6.06	0.061 (NS)
CD96	10225		1.00	0.07	4.88	0.82	0.06	3.21	0.78	0.03	5.67	0.76 (NS)
TIGIT	201633		1.00	0.07	12.1	0.74	0.06	8.35	1.64	0.13	3.62	0.40 (NS)
CD226	10666		1.00	0.02	5.13	0.46	0.04	3.18	0.70	0.10	2.32	0.081 (NS)
TNFRSF14	8764		1.00	0.35	3.06	0.94	0.24	1.87	0.81	0.07	2.31	0.23 (NS)
TNFRSF18	8784	GITR	1.00	0.09	8.99	1.01	0.03	11.33	0.86	0.03	2.79	0.61 (NS)
TNFRSF4	7293	OX40, CD134	1.00	0.09	8.03	0.66	0.08	4.24	1.22	0.17	3.00	0.37 (NS)
CD27	939	TNFRSF7	1.00	0.00	17.4	0.68	0.00	10.7	1.14	0.10	5.02	0.44 (NS)
TNFRSF9	3604	CD137, 4-1BB	1.00	0.00	13.7	0.81	0.00	16.75	1.37	0.12	5.25	0.59 (NS)
HAVCR2	84868	TIM3	1.00	0.08	14.2	0.67	0.05	7.03	1.04	0.11	6.17	0.31 (NS)
ICOS	29851		1.00	0.00	8.44	0.50	0.02	5.36	0.80	0.10	1.74	0.15 (NS)
LAG3	3902		1.00	0.04	64.8	0.95	0.04	19.39	1.17	0.08	14.4	0.94 (NS)
PDCD1	5133		1.00	0.00	9.75	0.66	0.00	10.64	1.25	0.13	4.04	0.66 (NS)
Checkpoint tumor cell genes												
IDO1	3620		1.00	0.05	109.3	0.89	0.03	121.8	1.52	0.08	78.9	0.93 (NS)
CD80	941	B7-1	1.00	0.00	25.2	0.73	0.03	5.55	1.45	0.09	9.01	0.56 (NS)
CD86	942	B7-2	1.00	0.07	9.99	0.80	0.07	6.02	1.36	0.27	5.76	0.55 (NS)
CD276	80381	B7-H3	1.00	0.44	3.14	1.00	0.32	3.20	1.30	0.49	5.01	0.82 (NS)
LGALS9	3965	LGALS9	1.00	0.07	7.68	1.10	0.16	5.66	1.34	0.10	2.29	0.48 (NS)
CD274	29126	PDL1	1.00	0.05	24.1	0.89	0.06	18.1	1.49	0.09	7.78	0.84 (NS)
PDCD1LG2	80380		1.00	0.03	17.2	0.58	0.04	17.7	1.15	0.10	4.17	0.30 (NS)
ICOSLG	23308		1.00	0.20	3.31	0.80	0.18	3.55	1.29	0.39	4.81	0.22 (NS)
PVR	5817	CD155	1.00	0.26	3.00	0.93	0.00	3.40	0.99	0.23	3.01	0.78 (NS)
PVRIG	79037	CD112R	1.00	0.21	6.71	0.56	0.21	5.05	0.96	0.25	4.06	0.15 (NS)
TNFSF4	7292	OX40L	1.00	0.00	44.0	1.42	0.09	27.9	4.66	0.00	39.2	0.75 (NS)
Interferon inducible genes												
CXCL10	3627		1.00	0.02	269.9	1.20	0.02	85.7	2.60	0.04	44.6	0.69 (NS)
RSAD2	91543		1.00	0.24	42.0	3.21	0.11	22.5	3.48	0.31	87.7	0.044
IFI44L	10964		1.00	0.04	31.2	1.91	0.04	21.3	1.67	0.07	33.6	0.15 (NS)
IFI6	2537		1.00	0.11	16.6	1.96	0.05	16.5	1.90	0.19	47.0	0.045
IFIT1	3434		1.00	0.11	18.1	1.16	0.08	15.8	1.03	0.09	39.1	0.26 (NS)
IRF8	3394		1.00	0.06	7.88	0.82	0.02	6.47	1.23	0.15	3.04	0.37 (NS)
MX1	4599		1.00	0.12	15.5	1.52	0.18	17.4	1.14	0.11	30.1	0.21 (NS)
OAS1	4938		1.00	0.13	4.16	1.27	0.36	9.48	1.47	0.02	5.24	0.035

<i>IFI27</i>	3429		<b>1.00</b>	0.10	34.1	<b>3.64</b>	0.12	50.1	<b>1.03</b>	0.24	26.6	<b>0.015</b>
<i>G1P2</i>	9636	<i>ISG15</i>	<b>1.00</b>	0.12	11.9	<b>1.53</b>	0.09	7.78	<b>1.75</b>	0.18	27.3	<b>0.025</b>
<b>Major histocompatibility complex genes</b>												
<i>HLA-A</i>	3105		<b>1.00</b>	0.14	7.81	<b>0.98</b>	0.03	5.85	<b>0.91</b>	0.25	6.66	0.32 (NS)
<i>HLA-B</i>	3106		<b>1.00</b>	0.12	36.1	<b>1.07</b>	0.12	13.4	<b>0.73</b>	0.12	13.9	0.52 (NS)
<i>HLA-C</i>	3107		<b>1.00</b>	0.00	5.25	<b>0.38</b>	0.00	4.85	<b>0.12</b>	0.00	8.23	0.48 (NS)
<i>HLA-DRA</i>	3122		<b>1.00</b>	0.02	7.09	<b>0.50</b>	0.02	4.98	<b>0.82</b>	0.10	3.07	0.14 (NS)
<i>HLA-DRB1</i>	3123	<i>HLA-DRB</i>	<b>1.00</b>	0.08	6.16	<b>0.65</b>	0.02	5.36	<b>0.88</b>	0.11	3.71	0.37 (NS)

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\* Mann-Whitney test (no *vs.* 1 or more non-coding mutations).



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