Distinct Subtypes of Kidney Transplant-Associated Urothelial Carcinoma Harboring BK Polyomavirus Integration and Aristolochic Acid Mutational Signatures

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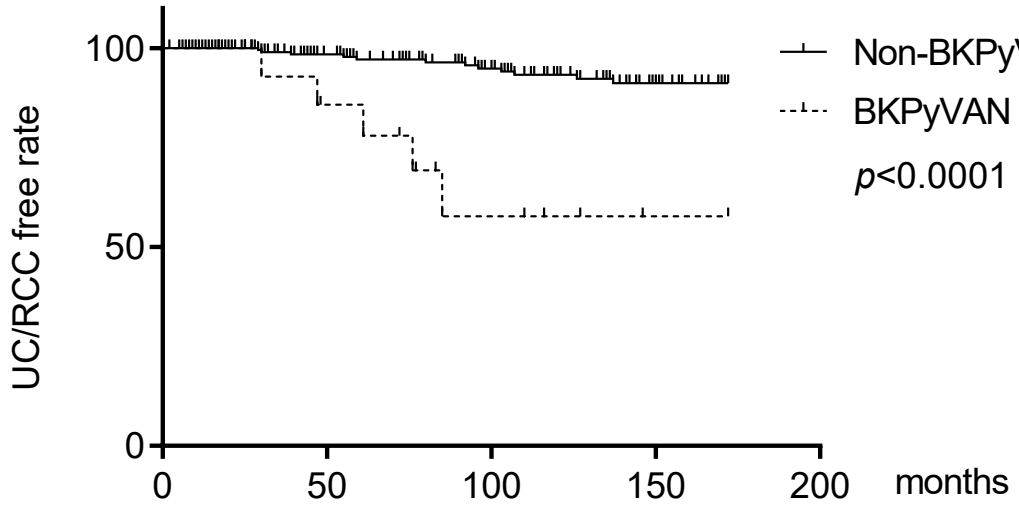
Polyomavirus Infection Associated Cancers

- Reactivation of BK Polyomavirus (BKPyV, BKV) in kidney transplant recipients may result in BKPyV-associated nephropathy (BKPyVN), contributing significantly to allograft failure.

- Although animal and in-vitro models demonstrate BKPyV-induced oncogenic alterations, its oncogenic role in humans remains unconfirmed.

High Incidence and Early Onset of Urinary Tract Cancers in Patients with BK Polyomavirus Associated Nephropathy

- 244 kidney transplant recipients received examination of BKPyV viruria from June 2000 to February 2020.



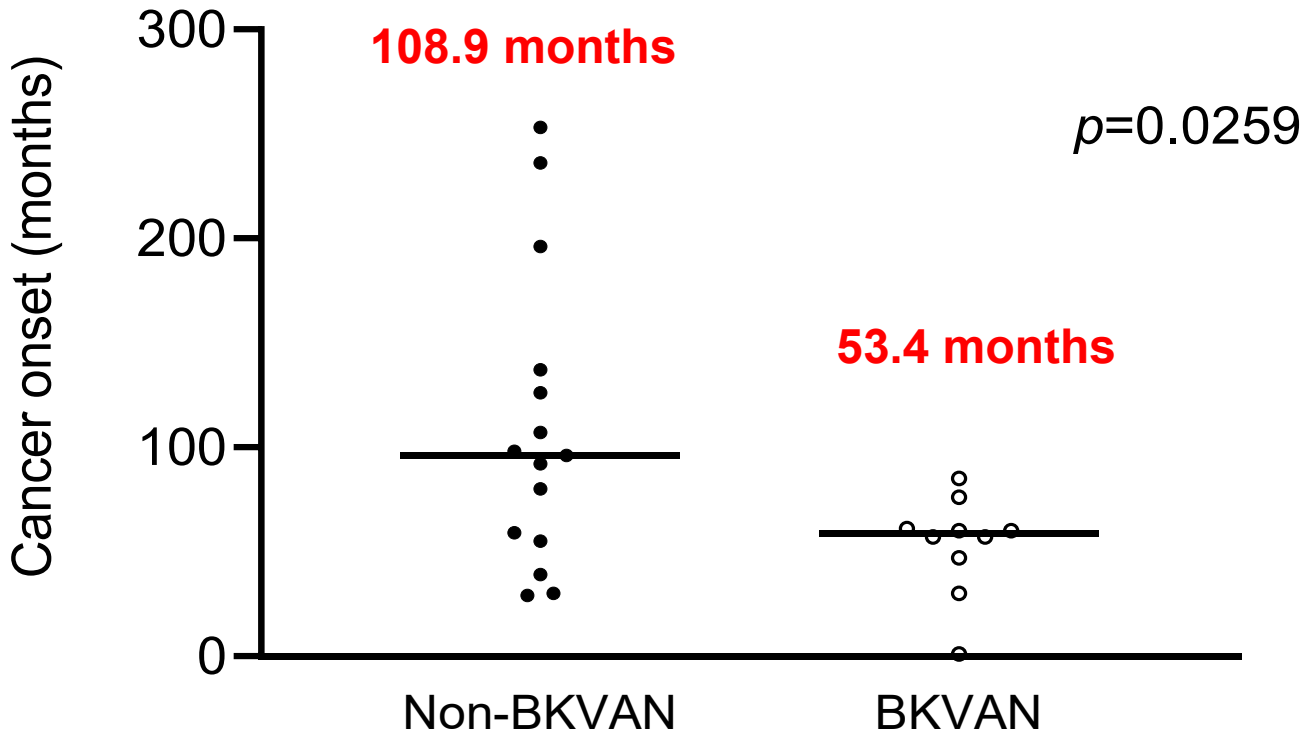
Non-BKPyVAN *n* = 227 → **8.2% had urinary tract cancers**

BKPyVAN *n* = 17 → **29.4% had urinary tract cancers**

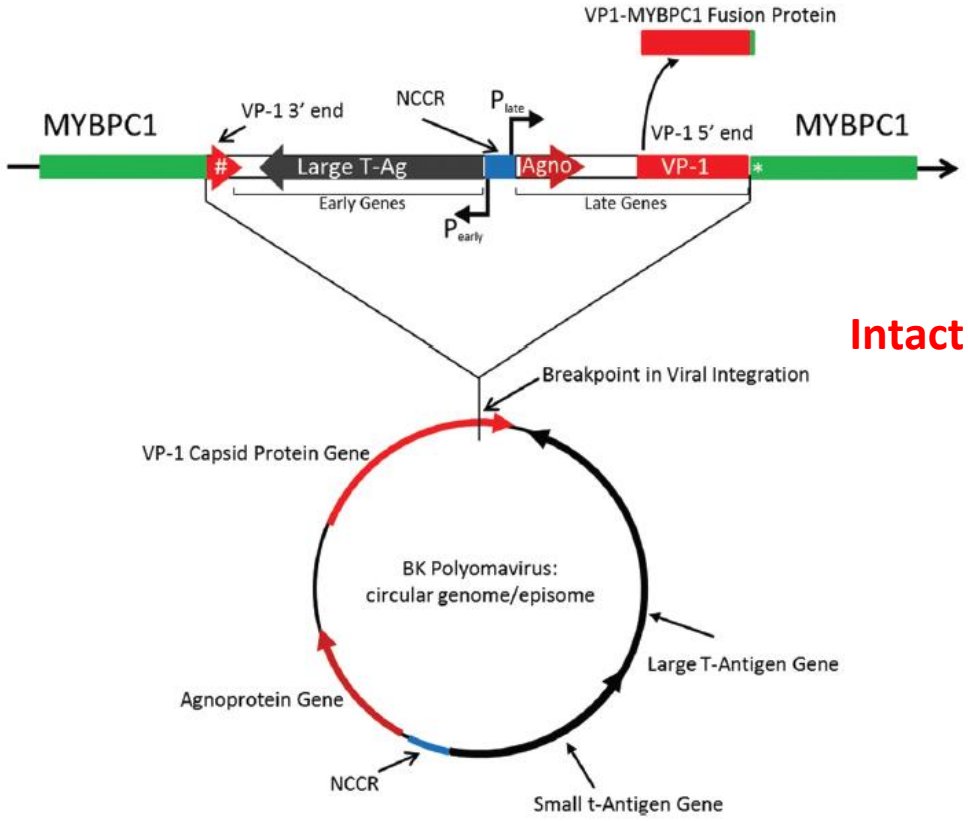
p<0.0001

Li YJ, CH Chen, HH Wang, YJ Chiang, Tian YC et al. Viruses 2021

Earlier Onset of Urinary Tract Cancers in BKPyVN patients



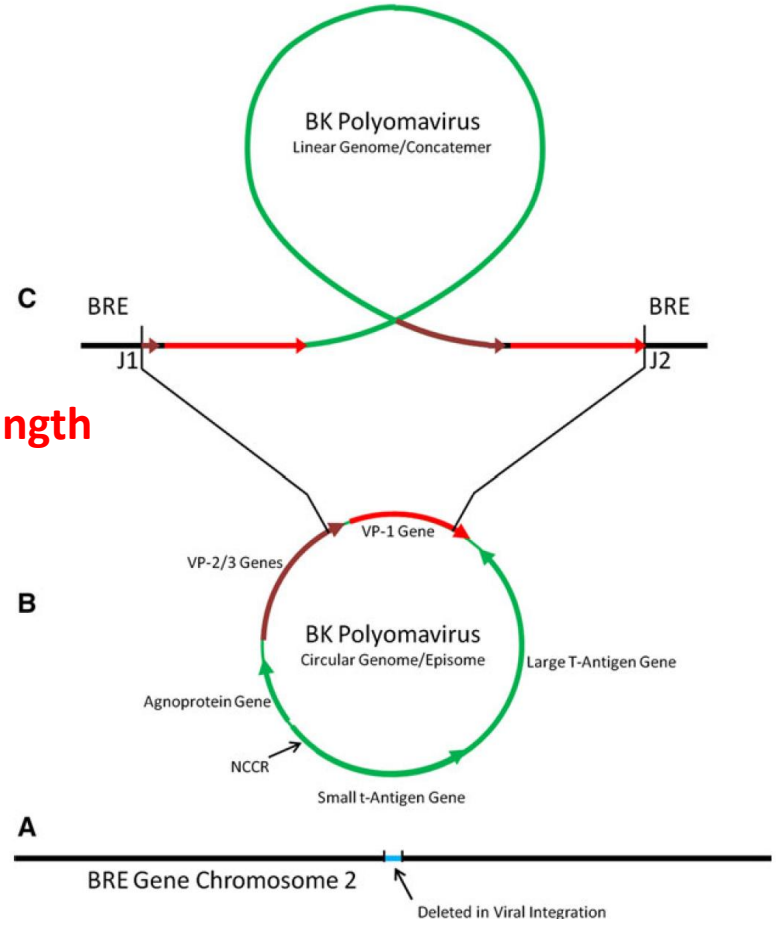
The Oncogenic Potential of BK-Polyomavirus is Linked to Viral Integration into the Human Genome (in one UC)



Kenan DJ et al. J Pathol; 2015; 237(3):379

BK Polyomavirus Genomic Integration and Large T Antigen Expression: Evolving Paradigms in Human Oncogenesis (in one RCC)

Intact TAg gene → full-length TAg protein



Kenan DJ et al. Am J Transplant; 2017;17(6):1674

Viral integration in BK polyomavirus-associated urothelial carcinoma in renal transplant recipients: multistage carcinogenesis revealed by next-generation virome capture sequencing

Table 1 Viral integration profile of the samples of BKPyV-associated urothelial carcinoma by virome capture sequencing.

Sample	Clean data, Mb (Aligned rate, %)	No. of integration sites	BKPyV genotype	Average depth[a], ×	Average supporting reads[b]	MMEJ		
						%	Average length ± SD, bp	Max length[c], bp
Case 1								
Primary	63.05 (99.0%)	5	AB217919.1(tw-3a/IVb-1)	6440	103	60.0	3.3 ± 1.5	5
Metastatic	106.79 (99.5%)	6	AB217919.1(tw-3a/IVb-1)	7853	542	83.3	4.0 ± 2.8	9
Case 2								
Primary	246.45 (54.2%)	100	DQ989813.1(PittNP1/Ib-1)	2406	68	85.0	7.9 ± 3.4	23
Metastatic	162.20 (56.3%)	42	DQ989813.1(PittNP1/Ib-1)	3065	108	71.4	7.4 ± 2.7	14
Case 3								
Primary	912.00 (91.8%)	174	AB211369.1(Dik/Ib-1)	1054	138	86.8	6.9 ± 2.9	16
Metastatic	519.82 (97.9%)	5	AB211369.1(Dik/Ib-1)	502	15050	60.0	7.3 ± 2.5	10

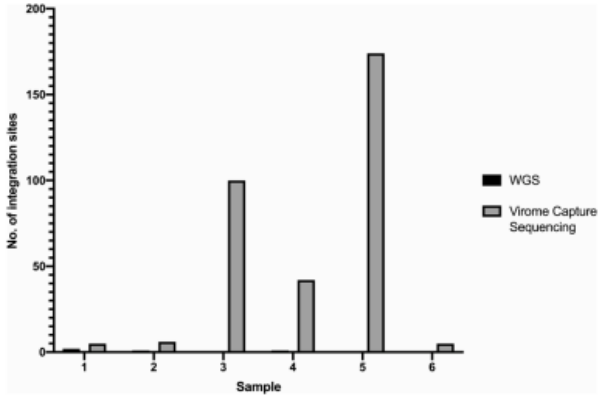


Fig. Number of deteacted integration sites in each sample by WGS and virome capture sequencing.

Wang Y et al. Oncogene 2020

Assessment of BKPyV Genome Integration and aristolochic acid mutational signature (AA) in Post-Transplant Urothelial Carcinoma

- Nineteen frozen urothelial carcinoma (UC) specimens from post–kidney transplant recipients were analyzed (5 from Linkou Chang Gung Memorial Hospital and 14 from the Taipei Veterans Hospital tissue bank; 2000–2018).

- Following DNA extraction, samples were submitted to the Laboratory of Cancer Epigenome, National Cancer Centre Singapore for whole-genome analysis.

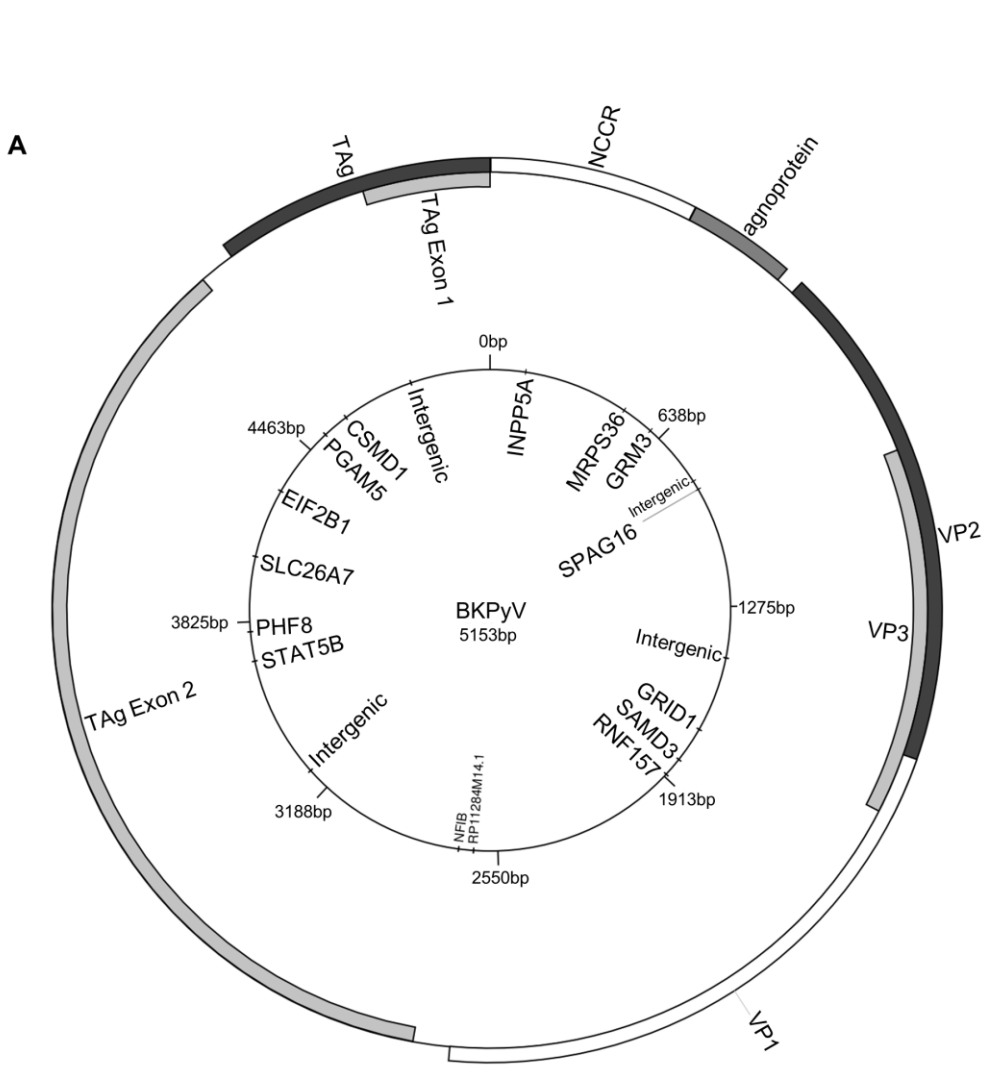
BKPyV genome integration and the aristolochic acid (AA) mutational signature in urothelial carcinoma

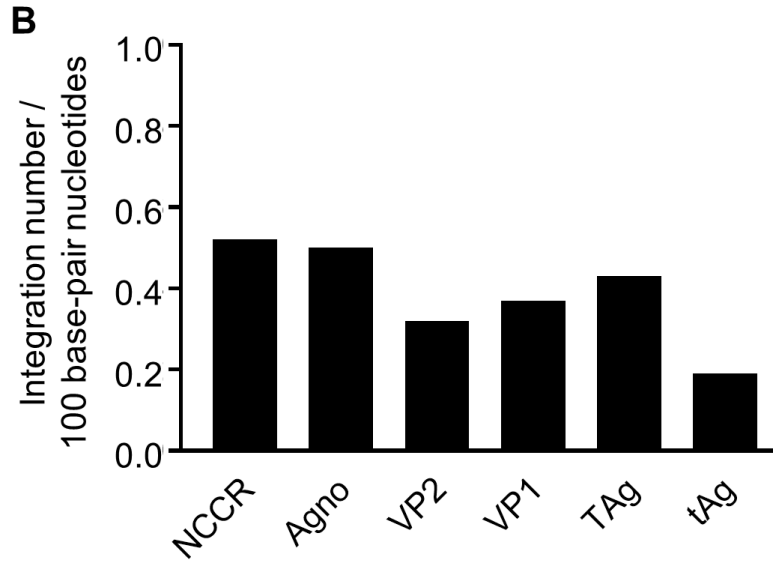
Sample	Hospital	Kidney transplant recipient	Biopsy-proven BKPyVAN	LTAg/VP1 immunostaining	BKPyV genome integration	AA mutational signature
L1	LCGMH[a]	Yes	+	+/-	+	-
L2	LCGMH	Yes	-	-/-	-	+
L3	LCGMH	Yes	+	+/-	+	-
L4	LCGMH	Yes	-	+/-	+	+
L5	LCGMH	No	-	+/-	+	+/-
T1	TVH[b]	Yes	unknown	-/-	-	+
T2	TVH	Yes	unknown	-/-	-	+
T3	TVH	Yes	unknown	-/-	-	+
T4	TVH	Yes	unknown	-/-	-	+
T5	TVH	Yes	unknown	-/-	-	+
T6	TVH	Yes	unknown	-/-	-	+
T7	TVH	Yes	unknown	-/-	+	-
T8	TVH	Yes	unknown	-/-	-	+
T9	TVH	Yes	unknown	-/-	-	+
T10	TVH	Yes	unknown	-/-	-	+
T11	TVH	Yes	unknown	-/-	+	+
T12	TVH	Yes	unknown	-/-	-	+
T13	TVH	Yes	unknown	-/-	-	+
T14	TVH	Yes	Unknown	-/-	-	+
n=19					**6 (32%)**	**16 (84%)**

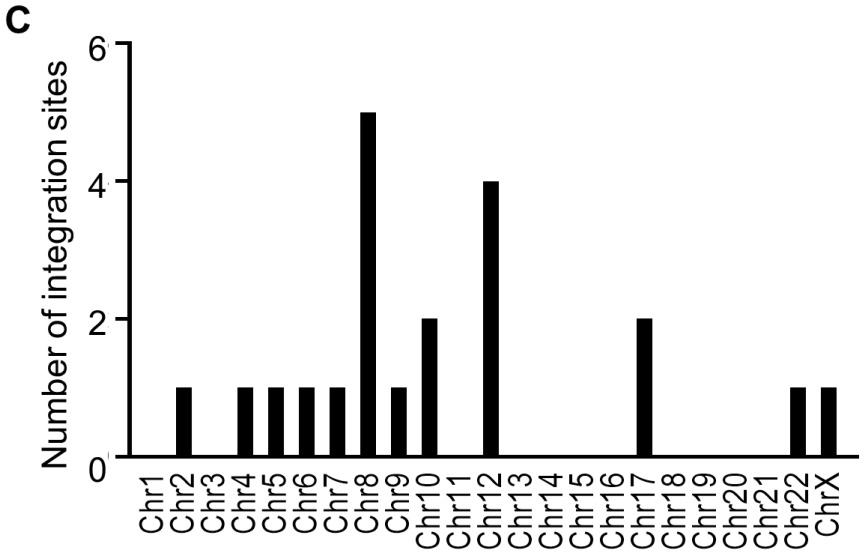
Sample (N)	Break point in BKPyV	Integration site in human	Tumor / Neighbor	Supporting reads
L1 (N=2)	TAg: 4289	EIF2B1 intron (Chr12:124115229 - 124115289)	T	2987
	TAg: 4535	PGAM5 exon (Chr12:133298040 - 133298070)	T	438
L3 (N=1)	TAg: 4049	SLC26A7 intron (Chr 8: 92303116 - 92303162)	T	46
L4 (N=6)	NCCR: 122	INPP5A intron (Chr10:134509463 - 134509406)	N	4
	Agn: 485	MRPS36 intron (Chr5: 68514904 - 68514843)	N	3
	Intergenic: 2684	NFIB intron (Chr9: 14215321 - 14215435)	N	4
	VP1: 1837	SAMD3 intron (Chr6: 130549272 - 130549179)	N	4
	TAg: 4289	EIF2B1 intron (Chr12:124115210-124115289)	N	14
	Intergenic: 4626	CSMD1 intron (Chr8: 4536112 - 4536011)	N	5
L5 (N=1)	TAg: 4289	EIF2B1 intron (Chr12:124115253-124115289)	N	15
T7 (N=1)	VP1: 1903	RNF157 intron (Chr17: 74218194 - 74218256)	T	5
T11 (N=10)	Intergenic: 598	GRM3 intron (Chr7: 86322907 - 86322840)	T	7
	VP2: 825	Intergenic (Chr 8: 77148747 - 77148864)	N	2
	VP2: 857	SPAG16 intron (Chr2: 214718887 - 214718784)	N	2
	VP2: 1452	Intergenic (Chr8: 20753050 - 20752944)	T	4
	VP1: 1713	GRID1 intron (Chr10: 87786943 - 87786852)	T	5
	VP1: 2633	RP11-284M14.1 intron	N	9
	TAg: 3267	Intergenic (Chr8: 58207410 - 58207317)	T	2
	TAg: 3690	STAT5B intron (Chr17: 40375866 - 40375753)	N	3
	TAg: 3791	PHF8 intron (ChrX: 53988989 - 53989049)	T	1
	tAg: 4878	Intergenic (Chr22: 19631038 - 19630965)	T	8

Total 21 integration sites

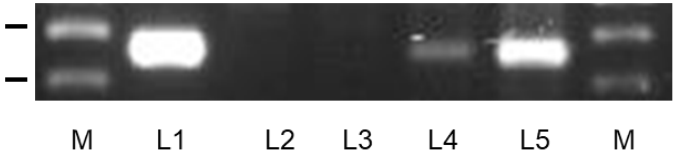
Distribution of BKPyV Genome Integration Site Breakpoints in the Viral and Human Genomes

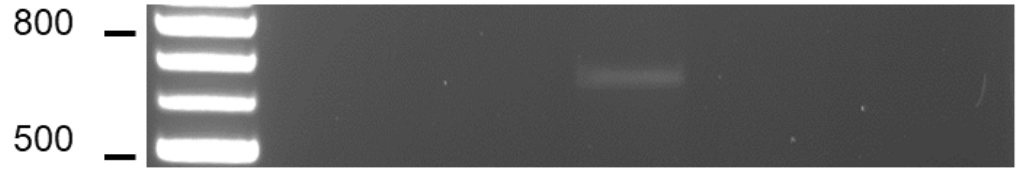




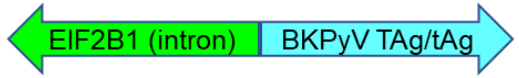


A





B



Case L1 Sanger sequence

CCGACCTGTATCTTCAATTCATTCATTCATTCAGTGA
ATATTTACTAGGTACATACTATATACCAGGCACTATTC
TAGATG***CTA***GGGATACATCAGTGAACAAGACAGGTA
GGGTTCTGCTGTGATGGAGTTTTGATTGTAATAGAG
GAAGCGGATAGTAAACAGAATTTGAGCTTTTTCTTTA
GTAGTATACACAGCAAAGCAGGCAAGGGTTCTATTA
CTAAATACAGCTTGACTAAGAAACTGGTGTAGATCA
GAAGGAAAGTCTTTAGGGTCTTCTACCTTTCTTTTTT
TCTTGGGTGGTGTTGAGTGTTGAGAATCTGCTGTT
GCTTCTTCATCACTGGCAAACATATCTTCATGGCAA
AA

Case L4 Sanger sequence

TCCGACCTGTATCTTCAATTCATTCATTCATTCAGTG
AATATTTACTAGGTACATACTATATACCAGGCACTATT
CTAGATG***CTA***GGGATACATCAGTGAACAAGACAGGT
AGGGTTCTGCTGTGATGGAGTTTTGATTGTAATAGA
GGAAGCGGATAGTAAACAGAATTTGAGCTTTTTCTT
TAGTAGTATACACAGCAAAGCAGGCAAGGGTTCTAT
TACTAAATACAGCTTGACTAAGAAACTGGTGTAGATC
AGAAGGAAAGTCTTTAGGGTCTTCTACCTTTCTTTTT
TTCTTGGGTGGTGTTGAGTGTTGAGAATCTGCTGTT
GCTTCTTCATCACTGGCAAACATATCTTCATGGCAAT
A

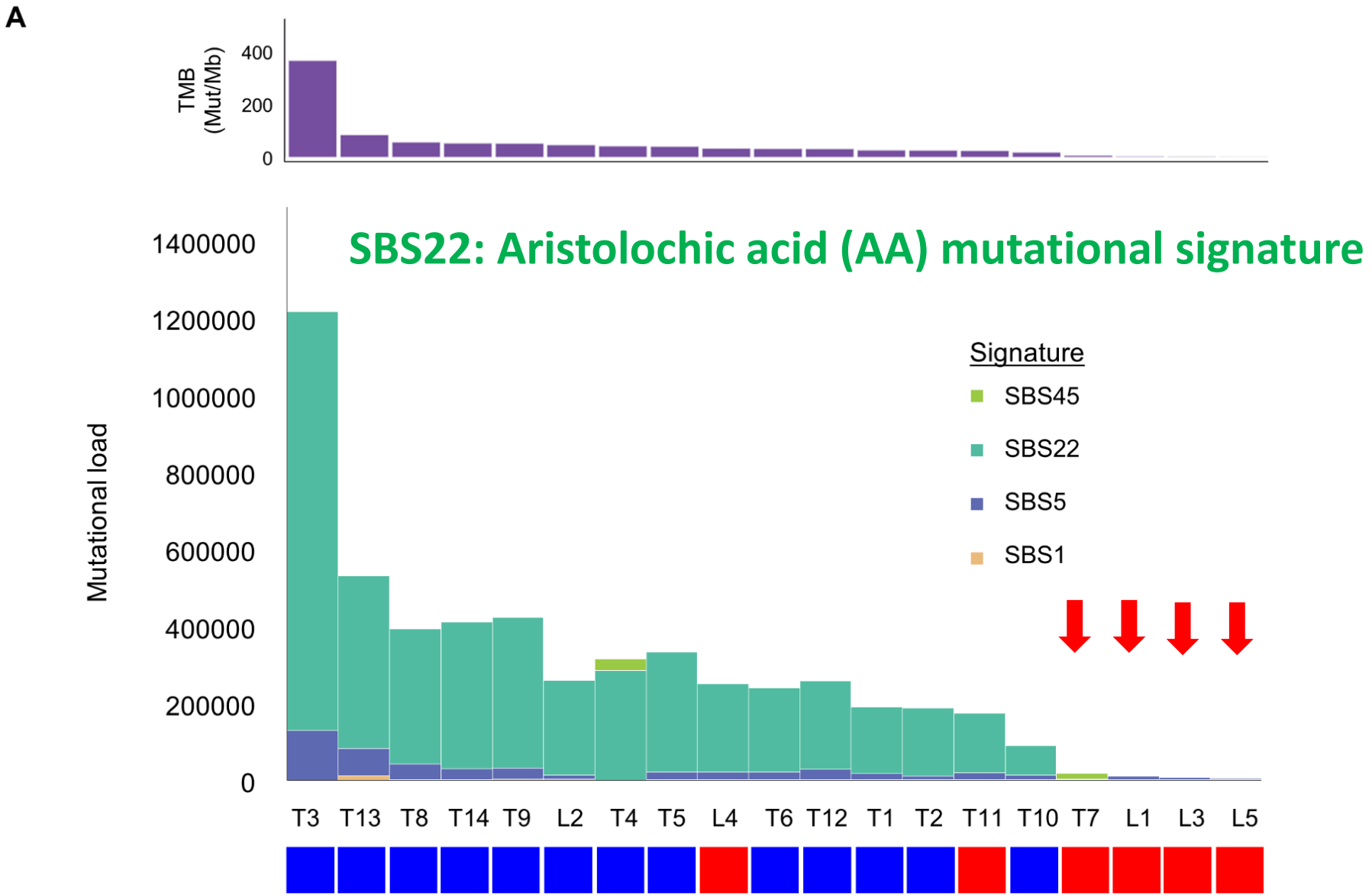
Case L5 Sanger sequence

TTTTGATTGTAATAGAGGAAGCGGATAGTAAACAGA
ATTTGAGCTTTTTCTTTAGTAGTATACACAGCAAAGC
AGGCAAGGGTTCTATTACTAAATACAGCTTGACTAA
GAAACTGGTGTAGATCAGAAGGAAAGTCTTTAGGG
TCTTCTACCTTTCTTTTTTTCTTGGGTGGTGTTGAGT
GTTGAGAATCTGCTGTTGCTTCTTCATCACTGGCAA
ACATATCTTCATGGC

Case L2 Sanger sequence

TTATGAGGGAAGGAACAACACTTATCAATCACTTCA
GTTTCTGAT**TTA**CTATTATCAAAAGCCTGGGAAAAAT
TATTTACTATAATTTACTATTATCTAAAGATAAATTAGTT
ACCTTAAAGCTTTAGATCTCTGAAGGGAGTCTCTCC
AATTATTTGGACCCACCATTGCAGAGTTTCTTCAGTT
AGGTCTAAGCCAAACCACTGTGTGAAGCAGTCAAT
GCAGTAGCAATCTATCCAAACCAAGGGCTCTTTTCT
TAAAAATTTTCTATTTAAATGTCTTAATCTTAGCTGAC
ACAGCATGCAAGGGCAGTGCACTGAAGGCTTTTTG
GAACAAATAGGCCATTCCTTGCAGTACAGGGTATCT
GGGCAAAGAGGAAAATCAGCACAAACCTCTGAGCT
ACTCCAGGTTCCAAAATCAGGCTGGTGAGCTACCT
TTACATCTTGCTCCATTTTTTTATATAAAGTATTCATTC
TCTTCATTTTATCCTCGTCGCCCCCTTTGTCAGGGT
GAAATTCCTTACACTTTCTTAAATAGGCTTTCCT**CAT**
TAGGGGAAGGTTTCCCCAGGCAGCTCTTCAAGGCC
TAAAAGTCCATGA

Low Total Mutation Burden in BKPyV Gene-Integrated UC



Low Oncogenic Mutation Genes in BKPyV Gene-Integrated UC

Chromatin modification

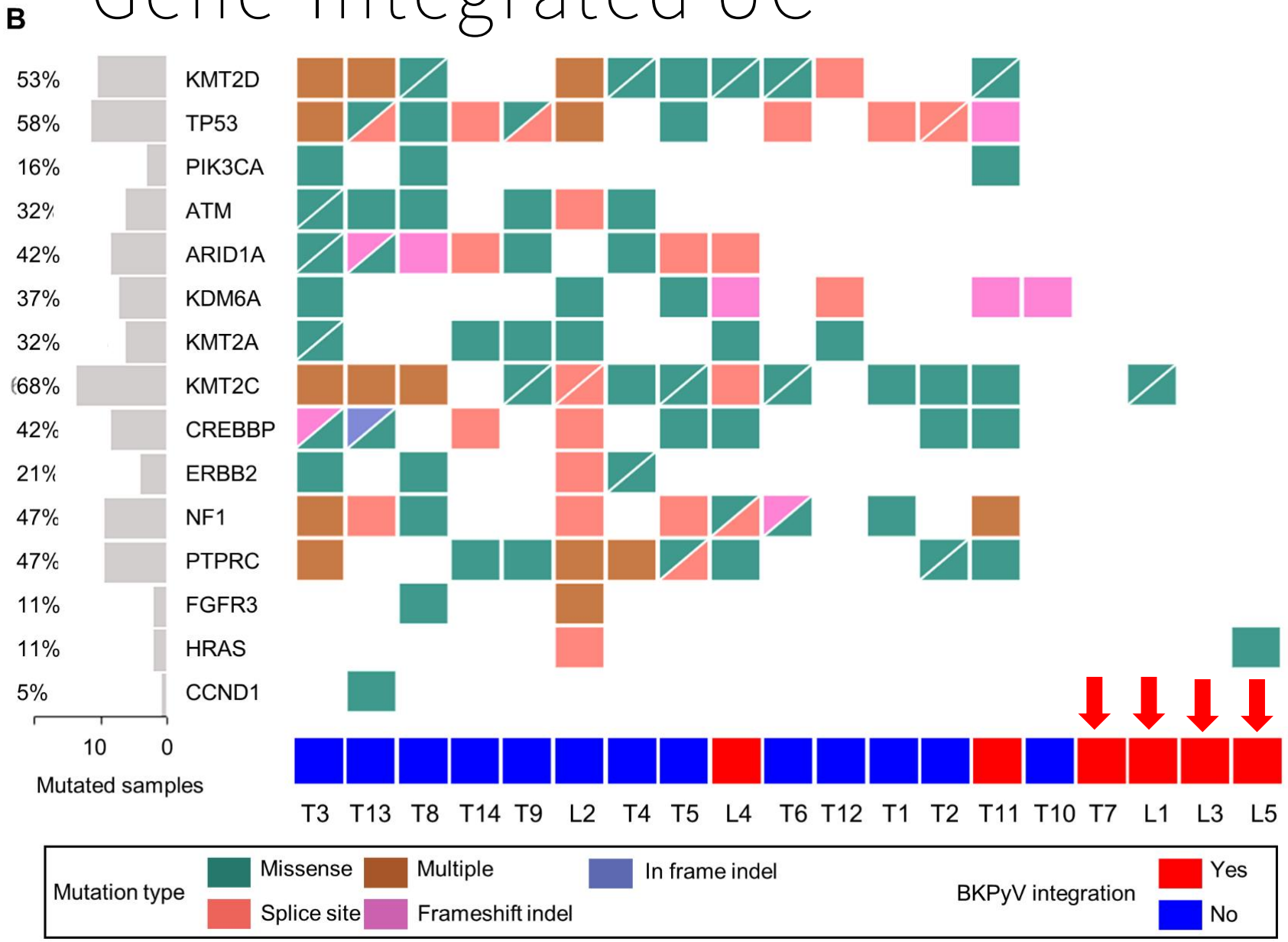
KMT2C, KMT2D, CREBBP, ARID1A

Cell-cycle regulation

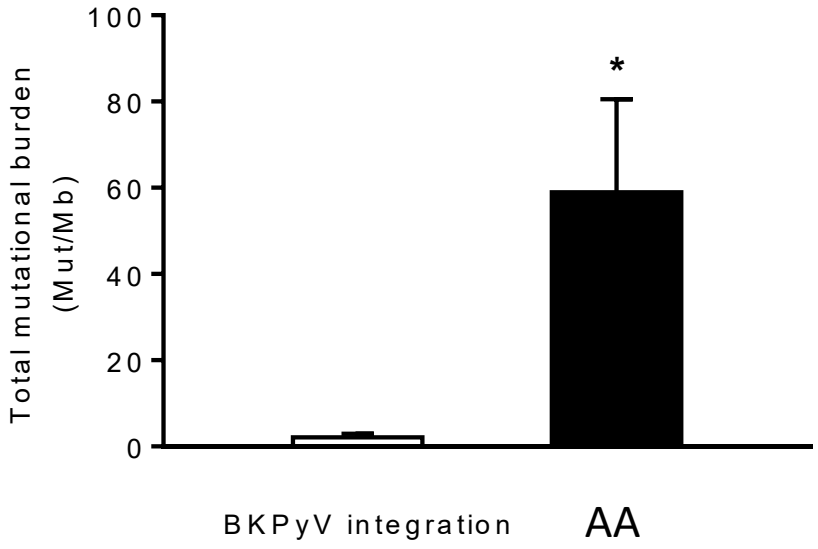
TP53, NF1, ATM, CCND1

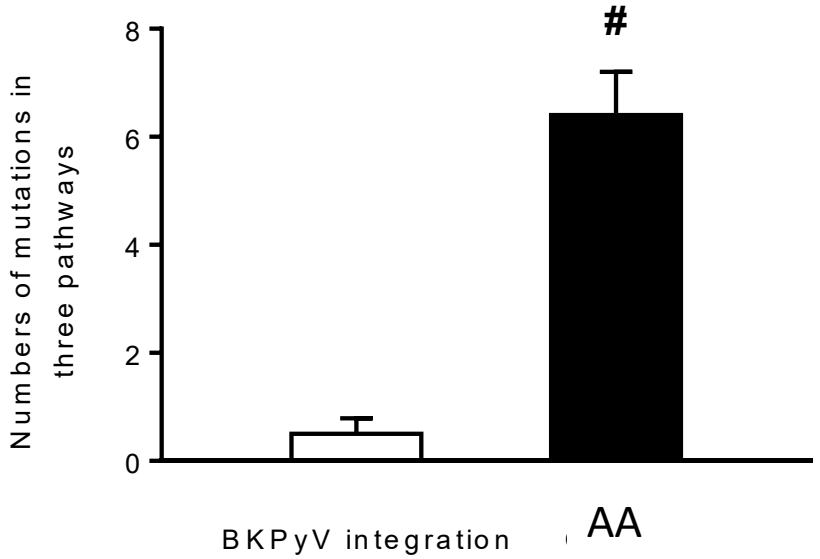
Oncogenic signaling pathways

ERBB2, PIK3CA, FGFR3, HRAS

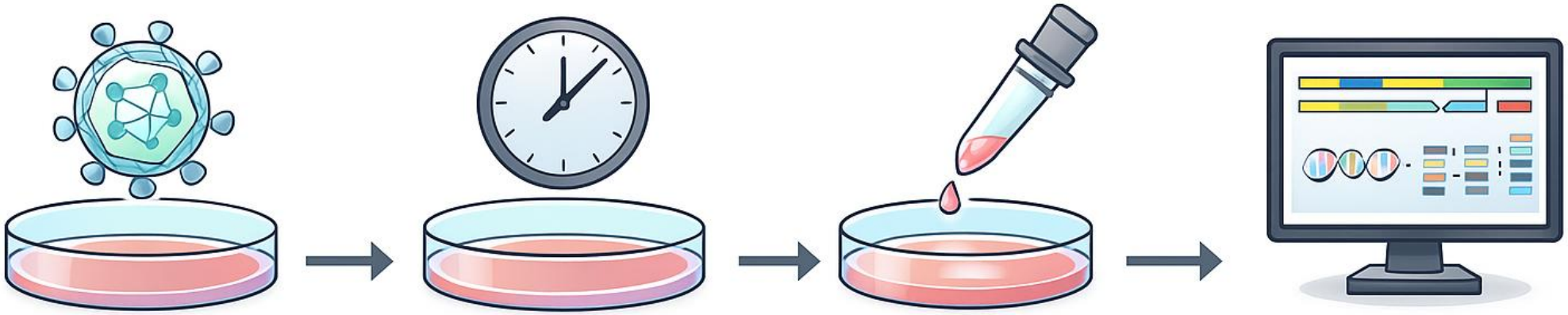


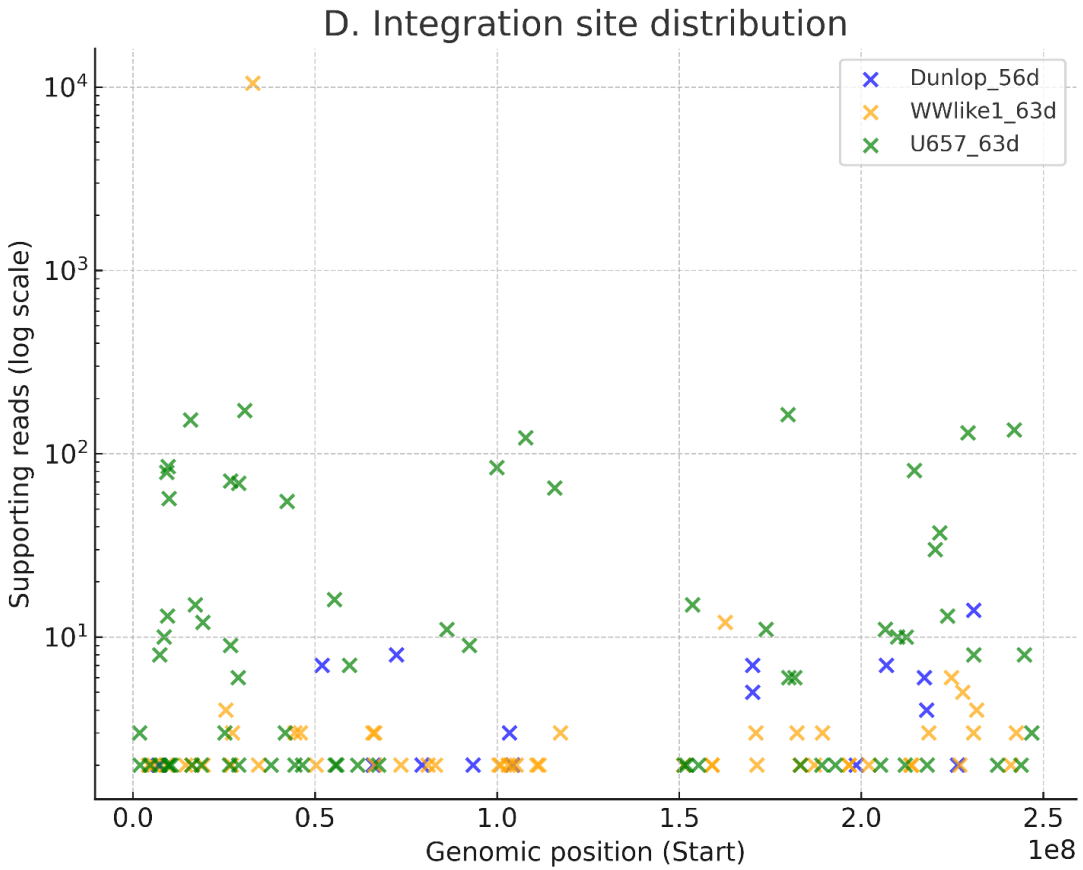
Distinct mutation patterns in BKPyV Gene-Integrated UCs and AA-positive UCs

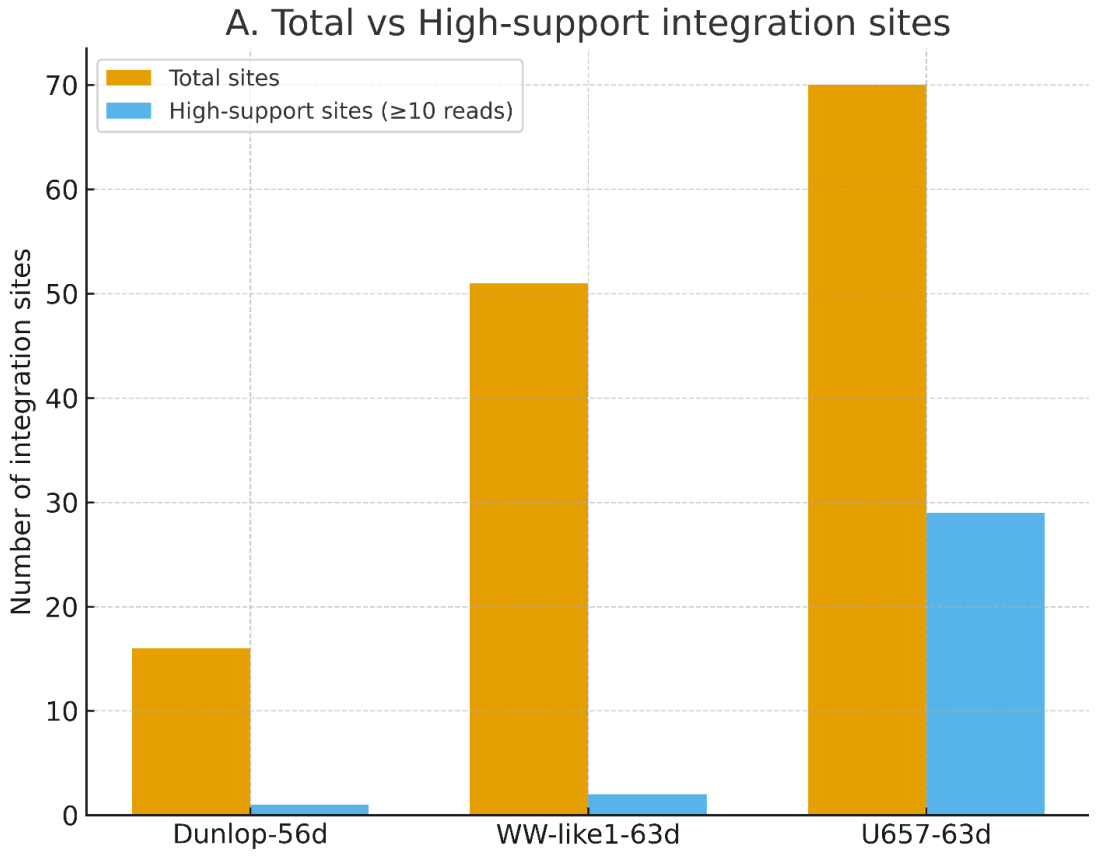




BKPyV Infection Culture Model to Assess Viral Integration into the Host Genome




D. Integration site distribution
Dunlop_56d
WWlike1_63d
U657_63d
Supporting reads (log scale)
10^4
10^3
10^2
10^1
0.0
0.5
1.0
1.5
2.0
2.5
Genomic position (Start)
1e8


A. Total vs High-support integration sites
Total sites
High-support sites (≥10 reads)
Number of integration sites
0
10
20
30
40
50
60
70
Dunlop-56d
WW-like1-63d
U657-63d

Conclusion

- Post-transplant UC commonly exhibits BKPyV integration and AA mutational signatures.

- These two distinct molecular subtypes suggest divergent tumorigenic pathways and may require different risk stratification and management strategies in post-transplant UC.

Thank you！ ！ ！