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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Turajlic S, Litchfield K, Xu H, et al. Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis. *Lancet Oncol* 2017; published online July 7. http://dx.doi.org/10.1016/S1470-2045(17)30516-8.

Supplementary Data for Turajlic, Litchfield et al. Insertion/deletion derived tumour specific neoantigens and the immunogenic phenotype: a pan-cancer analysis

Supplementary Table 1: Multivariate analysis of Jamal-Hanjani et al. dataset

Factor	Adjusted HR	Lower CI	Upper CI	P-value
indel_load	3.59	0.68	18.98	0.1318
Stage1b	2.86	0.44	18.58	0.2704
Stage2a	16.67	2.08	133.75	0.0081
Stage2b	21.95	3.23	149.14	0.0016
Stage3a	31.19	5.20	187.01	0.0002
Stage3b	164.27	7.27	3710.20	0.0013
Adjuvant therapy	4.48	1.23	16.31	0.0229
Age	0.98	0.92	1.03	0.4100
Histology Other	7.16	1.85	27.73	0.0044
Histology Squamous	1.66	0.57	4.84	0.3524

Supplementary Table 2: Immune signature gene sets as defined by Rooney et al.

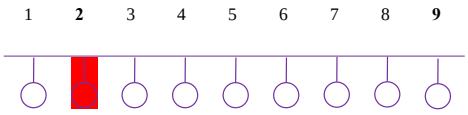
pDCs	Co-inhibition, APC	Type II IFN Reponse	Cytolytic Activity	MHC Class I	CD8+ T cells	Co-inhibition, T cell	Co-stimulation, APC
LILRA4	PDCD1LG2	GPR146	GZMA	HLA-A	CD8A	LAG3	ICOSLG
CLEC4C	CD274	SELP	PRF1	B2M		CTLA4	CD70
PLD4	C10orf54	AHR		TAP1		CD274	TNFSF14
PHEX	LGALS9					CD160	CD40
IL3RA	PVRL3					BTLA	TNFSF9
PTCRA						C10orf54	TNFSF4
IRF8						LAIR1	TNFSF15
IRF7						HAVCR2	TNFSF18
GZMB						CD244	TNFSF8
CXCR3						TIGIT	SLAMF1
							CD58

Supplementary Table 3: Summary of phase II data in non-CPI approved tumours under study

Tumour type	Phase/no of pts	Response rate	Line of Rx	Reference
TNBC	Ib/27*	18.5%	>2nd	Keynote-012[2]
UCEC	Ib/24*****	13%	>2nd	Keynote-028
PRAD	II/20	20%	2nd	Keynote-199
GBMLGG	Ib/26*	28%	2nd	Keynote- 028
BRCA (ER+/HER2-)	Ib/25*	12%	3rd	Keynote-028
Gastric ca	II/36*	21%	2nd	Keynote-012

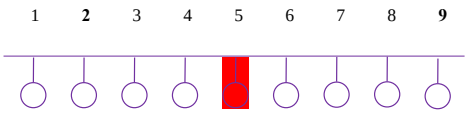
*PDL1 IHC with various cut-offs used as inclusion criteria

Supplementary Figure 1: Generation of neoantigens by different mutation types



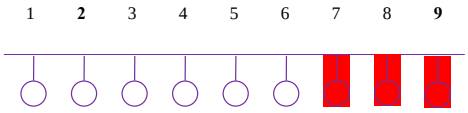
SNV: ATGG**G**TCCGCCGGAATGCGTAACTATA

Anchor residue mutated: **M** **G** P P E C V T I



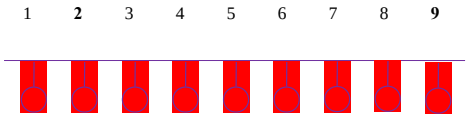
SNV: ATGG**G**TCCGCCGGAATGCGTAACTATA

Non- anchor residue mutated: **M** A P P **K** C V T I



3n indels: ATGGCTCCGCCGGAATGC**AAA**GTAACTATACCG

Anchor residue mutated: **M** A P P E C **K** V T I

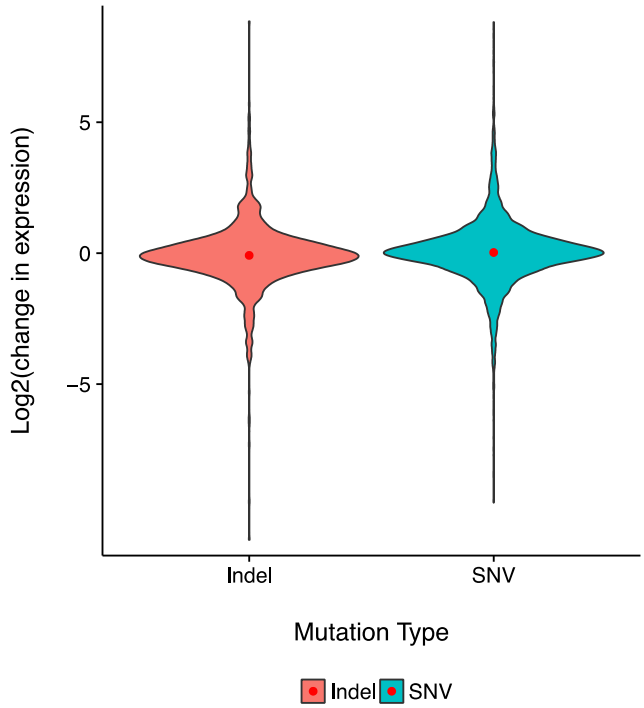


Frameshift: **ATGG**ATGGCTCCGCCGGAATGCGTAACTATA

All residues mutated: **M D G S A G M R N Y**

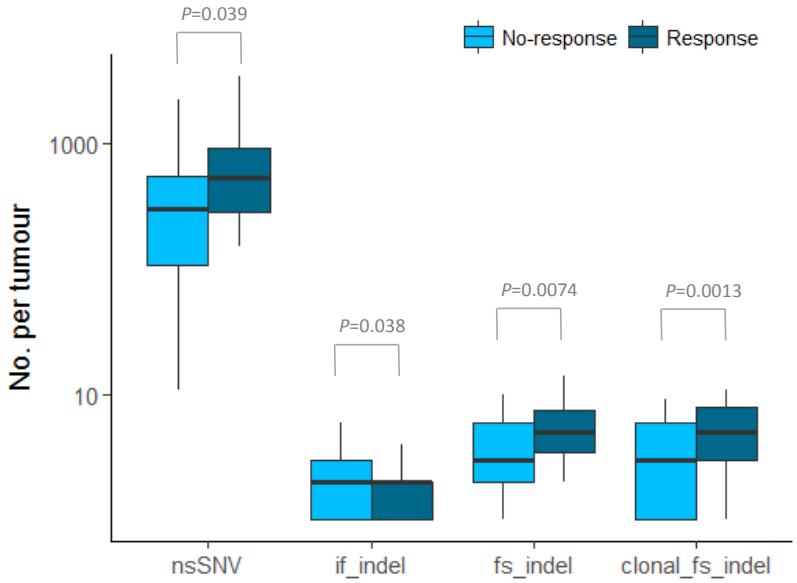
Different classes of mutations generate new peptide sequences (neoantigens) which may be presented on the tumour cells bound to MHC class I molecules. Sequence of 9 amino acids is shown with alterations relative to the wild type in red. Anchor residues in the peptide (usually positions 2 and 9) interact with MHC and when these are mutated they bind to MHC differentially compared to the wild type. Frameshift mutations create a novel peptide sequence which will differ at every residue and be highly distinct from the wild type sequence.

Supplementary Figure 2: Nonsense mediated decay (NMD) for indel and snv mutations.



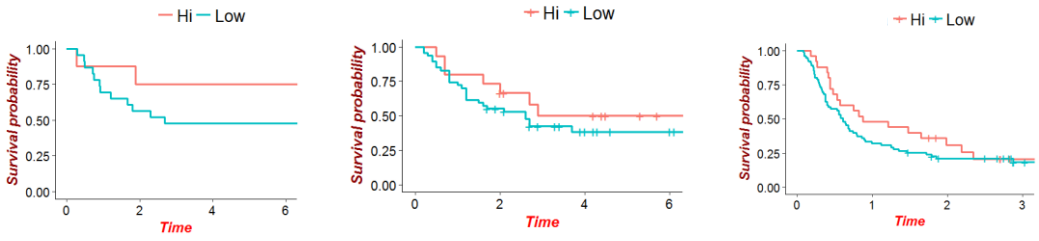
RNAseq expression changes in mutated versus wildtype samples. The extent of NMD was estimated for all indel and SNV mutations by comparing the mRNA expression level in samples with a mutation to the median mRNA expression level of the same transcript across all other tumour samples where the mutation was absent.

Supplementary Figure 3: The impact of clonality of frameshift indels on CPI response



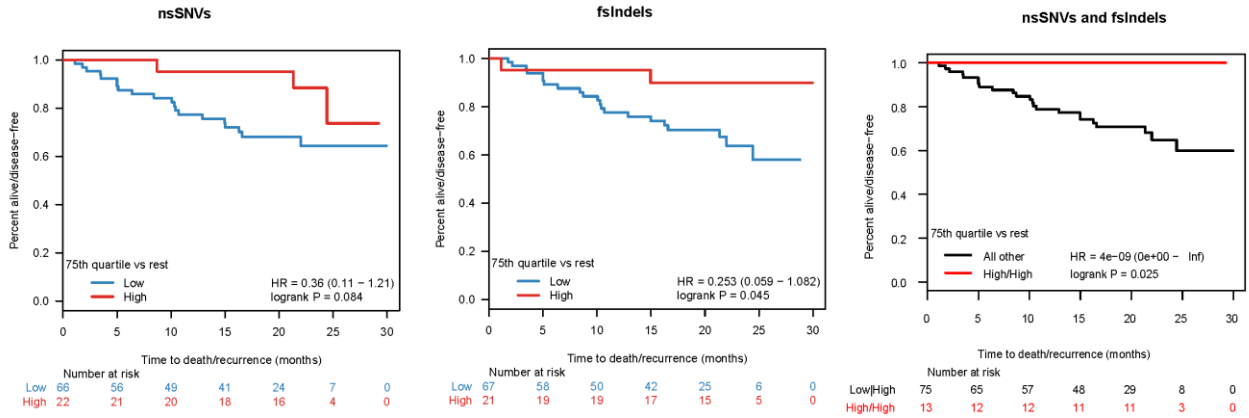
Non-synonymous SNV mutation burden (first), in-frame indel burden (second), frameshift indel burden (third) and clonal frameshift indel burden (fourth) are split by response to checkpoint inhibitor therapy in the Snyder et al., melanoma cohort.

Supplementary Figure 4: Overall survival based on frameshift indel mutation burden



Patients are split into high (upper quartile) and low (bottom 3 quartiles) groups, and data is presented for Hugo et al., Snyder et al. and Van Allen et al. cohorts.

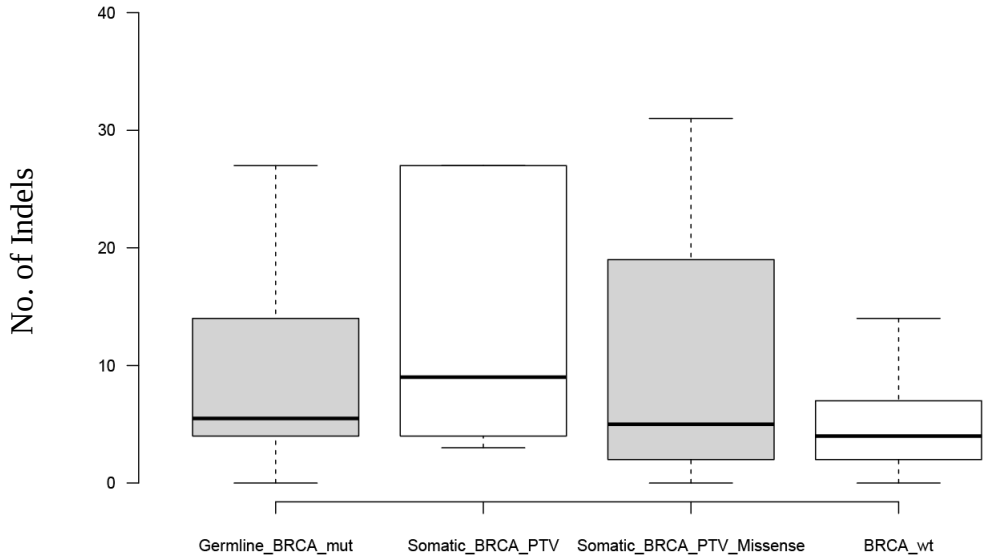
Supplementary Figure 5: Overall survival for non-CPI treated NSLC cohort from Jamal-Hanjani et al.



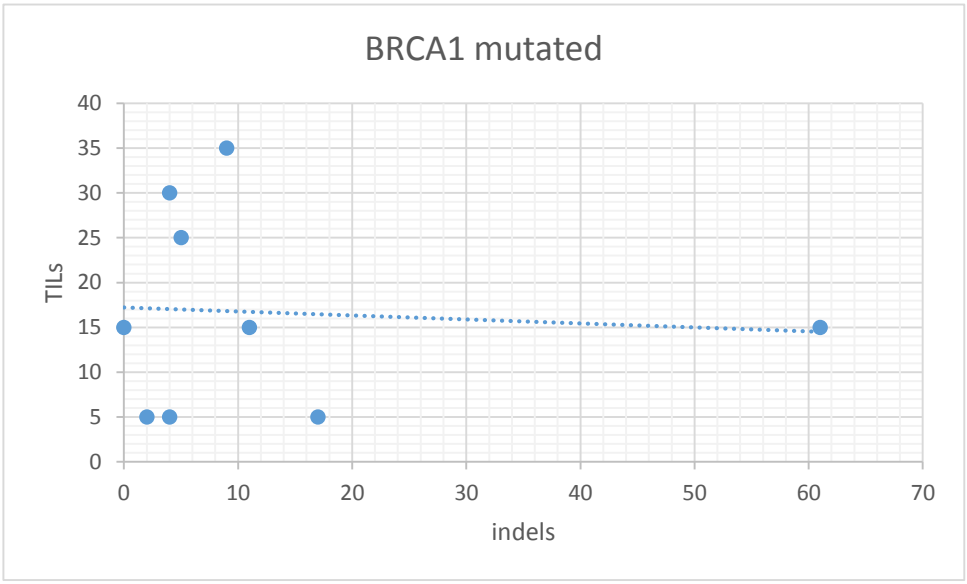
High (upper quartile) and low (bottom three quartiles) groups were compared for three measures: i) non-synonymous SNVs (left), ii) frameshift indels (middle) and iii) patients in the upper quartile for both measures i) & ii) (right).

Supplementary Figure 6: Indel load and TIL score by BRCA1 mutational status based on the data from Nolan et al.

A



B



Panel A: Indel load by BRCA1 mutational status (either germline or somatic; PTV=protein truncating; panel B: correlation between the indel load and TIL score (from Nolan et al.) in BRCA1 mutant TNBC