

Supplementary Tables S1-S10

“A multikinase and DNA-PK inhibitor combination immunomodulates melanomas, suppresses tumor progression, and enhances immunotherapies”

Tsai, et al.

Supplementary Table S1: Human melanoma cell lines and mutation status

Cell Line	BRAF	NRAS
624-Mel	V600E	WT
A375	V600E	WT
C32	V600E	WT
COLO-829	V600E	WT
G-361	V600E	WT
LOX-IMVI	V600E	WT
Malme-3M	V600E	WT
MNT-1	V600E	WT
RPMI-7951	V600E	WT
SK-MEL-5	V600E	WT
SK-MEL-24	V600E	WT
SK-MEL-28	V600E	WT
UACC-62	V600E	WT
UACC-257	V600E	WT
WM-115	V600D	WT
WM-266-4	V600D	WT
Mel-Juso	WT	Q61L
MM415	WT	Q61L
MM485	WT	Q61R
SK-MEL-2	WT	Q61R
SK-MEL-30	WT	Q61R
SK-MEL-103	WT	Q61R
SK-MEL-119	WT	Q61R
SK-MEL-147	WT	Q61R
SK-MEL-173	WT	Q61K
UCD-Mel-N	WT	Q61R
C8161	WT	WT

Abbreviations: WT = wild-type

Supplementary Table S2: Differences in PD-L1 and CD155 drug responses between BRAF- and NRAS-mutant cell lines

PD-L1				
Variable	BRAF (N = 16)	NRAS (N = 10)	Total (N = 26)	P-value
Reg				0.15 ¹
Mean±SD	0.82±0.2	0.72±0.13	0.79±0.18	
Median [Q1, Q3]	0.85 [0.7, 1.01]	0.7 [0.64, 0.77]	0.75 [0.68, 0.97]	
Min, Max	0.37, 1.05	0.58, 1.03	0.37, 1.05	
NU				0.07 ¹
Mean±SD	0.66±0.19	0.51±0.2	0.6±0.2	
Median [Q1, Q3]	0.65 [0.57, 0.74]	0.49 [0.39, 0.64]	0.59 [0.42, 0.73]	
Min, Max	0.36, 1.12	0.24, 0.81	0.24, 1.12	
Reg+NU				0.07 ¹
Mean±SD	0.52±0.27	0.36±0.16	0.46±0.24	
Median [Q1, Q3]	0.44 [0.38, 0.6]	0.33 [0.25, 0.47]	0.4 [0.31, 0.58]	
Min, Max	0.25, 1.4	0.15, 0.63	0.15, 1.4	
Vem				0.002¹ *
Mean±SD	0.96±0.23	1.43±0.39	1.14±0.37	
Median [Q1, Q3]	0.92 [0.83, 1.12]	1.49 [1.11, 1.56]	1.09 [0.86, 1.37]	
Min, Max	0.59, 1.41	0.97, 2.28	0.59, 2.28	
CD155				
Variable	BRAF (N = 16)	NRAS (N = 10)	Total (N = 26)	P-value
Reg				0.09 ¹
Mean±SD	0.74±0.11	0.61±0.22	0.69±0.17	
Median [Q1, Q3]	0.76 [0.67, 0.83]	0.6 [0.52, 0.71]	0.7 [0.6, 0.83]	
Min, Max	0.52, 0.9	0.21, 0.95	0.21, 0.95	
NU				0.06 ¹
Mean±SD	0.6±0.14	0.5±0.12	0.56±0.14	
Median [Q1, Q3]	0.6 [0.53, 0.66]	0.48 [0.42, 0.56]	0.58 [0.48, 0.64]	
Min, Max	0.35, 0.87	0.3, 0.71	0.3, 0.87	
Reg+NU				0.27 ¹
Mean±SD	0.47±0.21	0.41±0.26	0.45±0.23	
Median [Q1, Q3]	0.42 [0.35, 0.57]	0.36 [0.33, 0.42]	0.39 [0.33, 0.54]	
Min, Max	0.16, 0.96	0.13, 1.06	0.13, 1.06	
Vem				< 0.001¹ *
Mean±SD	0.72±0.18	1.1±0.16	0.86±0.25	
Median [Q1, Q3]	0.76 [0.53, 0.83]	1.15 [1.02, 1.22]	0.84 [0.73, 1.04]	
Min, Max	0.45, 1.01	0.77, 1.27	0.45, 1.27	

* p < 0.05

¹ Mann-Whitney U Test

Supplementary Table S3: Differences in HLA-I and CD73 drug responses between BRAF- and NRAS-mutant cell lines

HLA-I				
Variable	BRAF (N = 16)	NRAS (N = 9)	Total (N = 25)	P-value
Reg				0.65 ¹
Mean±SD	1.32±0.41	1.34±0.4	1.32±0.4	
Median [Q1, Q3]	1.14 [1.08, 1.34]	1.31 [1.09, 1.44]	1.15 [1.08, 1.44]	
Min, Max	0.95, 2.31	0.96, 2.27	0.95, 2.31	
NU				0.78 ¹
Mean±SD	1.05±0.17	1.09±0.21	1.06±0.18	
Median [Q1, Q3]	1.06 [0.99, 1.21]	1.1 [0.89, 1.27]	1.06 [0.95, 1.21]	
Min, Max	0.61, 1.28	0.79, 1.41	0.61, 1.41	
Reg+NU				0.73 ¹
Mean±SD	1.53±0.86	1.39±0.49	1.48±0.74	
Median [Q1, Q3]	1.32 [1.14, 1.58]	1.26 [1.03, 1.58]	1.28 [1.14, 1.58]	
Min, Max	0.97, 4.64	0.86, 2.32	0.86, 4.64	
Vem				0.005¹ *
Mean±SD	1.33±0.56	0.86±0.15	1.16±0.51	
Median [Q1, Q3]	1.16 [1.05, 1.47]	0.89 [0.84, 0.91]	1.07 [0.87, 1.25]	
Min, Max	0.67, 2.95	0.58, 1.07	0.58, 2.95	
CD73				
Variable	BRAF (N = 10)	NRAS (N = 10)	Total (N = 20)	P-value
Reg				0.15 ¹
Mean±SD	0.7±0.22	0.54±0.14	0.62±0.2	
Median [Q1, Q3]	0.67 [0.6, 0.86]	0.52 [0.51, 0.66]	0.63 [0.51, 0.69]	
Min, Max	0.32, 1.05	0.24, 0.71	0.24, 1.05	
NU				0.55 ¹
Mean±SD	0.67±0.3	0.54±0.13	0.61±0.24	
Median [Q1, Q3]	0.59 [0.42, 0.94]	0.5 [0.46, 0.67]	0.51 [0.45, 0.72]	
Min, Max	0.34, 1.09	0.35, 0.73	0.34, 1.09	
Reg+NU				0.13 ¹
Mean±SD	0.46±0.29	0.29±0.12	0.37±0.23	
Median [Q1, Q3]	0.33 [0.28, 0.48]	0.27 [0.24, 0.32]	0.29 [0.26, 0.41]	
Min, Max	0.22, 1.01	0.13, 0.57	0.13, 1.01	
Vem				0.001¹ *
Mean±SD	0.91±0.31	1.46±0.27	1.18±0.4	
Median [Q1, Q3]	1.02 [0.64, 1.14]	1.39 [1.29, 1.69]	1.2 [1.05, 1.38]	
Min, Max	0.36, 1.28	1.08, 1.84	0.36, 1.84	

* p < 0.05

¹ Mann-Whitney U Test

Supplementary Table S4: Differences in NGFR and CD55 drug responses between BRAF- and NRAS-mutant cell lines

NGFR				
Variable	BRAF (N = 8)	NRAS (N = 8)	Total (N = 16)	P-value
Reg				
Mean±SD	0.81±0.42	0.97±0.79	0.89±0.62	0.83 ¹
Median [Q1, Q3]	0.71 [0.53, 0.94]	0.66 [0.6, 1]	0.66 [0.56, 1]	
Min, Max	0.38, 1.63	0.29, 2.81	0.29, 2.81	
NU				
Mean±SD	0.76±0.13	0.65±0.12	0.7±0.14	0.14 ¹
Median [Q1, Q3]	0.77 [0.68, 0.85]	0.65 [0.56, 0.7]	0.7 [0.6, 0.8]	
Min, Max	0.54, 0.94	0.47, 0.84	0.47, 0.94	
Reg+NU				
Mean±SD	0.63±0.35	0.71±0.54	0.67±0.44	0.92 ¹
Median [Q1, Q3]	0.54 [0.4, 0.74]	0.65 [0.36, 0.86]	0.58 [0.39, 0.86]	
Min, Max	0.26, 1.3	0.11, 1.86	0.11, 1.86	
Vem				
Mean±SD	1.08±0.48	1.1±0.21	1.09±0.36	0.4 ¹
Median [Q1, Q3]	0.96 [0.87, 1.29]	1.05 [1, 1.15]	1.02 [0.93, 1.29]	
Min, Max	0.4, 2.03	0.83, 1.49	0.4, 2.03	
CD55				
Variable	BRAF (N = 15)	NRAS (N = 9)	Total (N = 24)	P-value
Reg				
Mean±SD	0.75±0.2	0.73±0.13	0.74±0.17	0.79 ¹
Median [Q1, Q3]	0.71 [0.6, 0.94]	0.69 [0.66, 0.83]	0.7 [0.61, 0.9]	
Min, Max	0.42, 1.07	0.54, 0.91	0.42, 1.07	
NU				
Mean±SD	0.88±0.42	0.63±0.1	0.78±0.36	0.07 ¹
Median [Q1, Q3]	0.83 [0.64, 1]	0.59 [0.57, 0.69]	0.67 [0.58, 0.84]	
Min, Max	0.49, 2.17	0.5, 0.81	0.49, 2.17	
Reg+NU				
Mean±SD	0.56±0.32	0.48±0.12	0.53±0.26	0.79 ¹
Median [Q1, Q3]	0.47 [0.4, 0.54]	0.49 [0.44, 0.56]	0.47 [0.42, 0.55]	
Min, Max	0.23, 1.48	0.25, 0.65	0.23, 1.48	
Vem				
Mean±SD	0.77±0.21	1.61±0.53	1.08±0.54	< 0.001 ¹ *
Median [Q1, Q3]	0.75 [0.64, 0.95]	1.41 [1.21, 1.67]	1.02 [0.74, 1.23]	
Min, Max	0.42, 1.06	1.15, 2.6	0.42, 2.6	

* p < 0.05

¹ Mann-Whitney U Test

Supplementary Table S5: Differences in PD-L1, CD155, and HLA-I drug responses in BRAF-mutant cell lines

PD-L1 (BRAF-mutant cell lines)						
Variable	NU (N = 16)	Reg (N = 16)	Reg+NU (N = 16)	Vem (N = 16)	Total (N = 64)	P-value ¹
Mean±SD	0.66±0.19	0.82±0.2	0.52±0.27	0.96±0.23	0.74±0.28	< 0.001 *
Median	0.65	0.85	0.44	0.92	0.7	
[Q1, Q3]	[0.57, 0.74]	[0.7, 1.01]	[0.38, 0.6]	[0.83, 1.12]	[0.58, 0.91]	
Min, Max	0.36, 1.12	0.37, 1.05	0.25, 1.4	0.59, 1.41	0.25, 1.41	
	•	•	•	•		0.019 *
	•					0.017 *
	•			•		< 0.001 *
		•	•			< 0.001 *
		•		•		0.149
			•	•		< 0.001 *
CD155 (BRAF-mutant cell lines)						
Variable	NU (N = 16)	Reg (N = 16)	Reg+NU (N = 16)	Vem (N = 16)	Total (N = 64)	P-value ¹
Mean±SD	0.6±0.14	0.74±0.11	0.47±0.21	0.72±0.18	0.63±0.19	< 0.001 *
Median	0.6	0.76	0.42	0.76	0.65	
[Q1, Q3]	[0.53, 0.66]	[0.67, 0.83]	[0.35, 0.57]	[0.53, 0.83]	[0.49, 0.8]	
Min, Max	0.35, 0.87	0.52, 0.9	0.16, 0.96	0.45, 1.01	0.16, 1.01	
	•	•	•	•		0.004 *
	•					0.035 *
	•			•		0.102
		•	•			< 0.001 *
		•		•		0.838
			•	•		0.001 *
HLA-I (BRAF-mutant cell lines)						
Variable	NU (N = 16)	Reg (N = 16)	Reg+NU (N = 16)	Vem (N = 16)	Total (N = 64)	P-value ¹
Mean±SD	1.05±0.17	1.32±0.41	1.53±0.86	1.33±0.56	1.31±0.57	0.02 *
Median	1.06	1.14	1.32	1.16	1.15	
[Q1, Q3]	[0.99, 1.21]	[1.08, 1.34]	[1.14, 1.58]	[1.05, 1.47]	[1.05, 1.38]	
Min, Max	0.61, 1.28	0.95, 2.31	0.97, 4.64	0.67, 2.95	0.61, 4.64	
	•	•	•	•		0.039 *
	•					0.001 *
	•			•		0.11
		•	•			0.184
		•		•		0.897
			•	•		0.27

* p < 0.05

¹ Kruskal-Wallis test or Mann-Whitney U Test

Supplementary Table S6: Differences in CD73, NGFR, and CD55 drug responses in BRAF-mutant cell lines

CD73						
(BRAF-mutant cell lines)						
Variable	NU (N = 10)	Reg (N = 10)	Reg+NU (N = 10)	Vem (N = 10)	Total (N = 40)	P-value¹
Mean±SD	0.67±0.3	0.7±0.22	0.46±0.29	0.91±0.31	0.68±0.32	0.01 *
Median	0.59	0.67	0.33	1.02	0.64	
[Q1, Q3]	[0.42, 0.94]	[0.6, 0.86]	[0.28, 0.48]	[0.64, 1.14]	[0.4, 0.95]	
Min, Max	0.34, 1.09	0.32, 1.05	0.22, 1.01	0.36, 1.28	0.22, 1.28	
	•	•				0.796
	•		•			0.035 *
	•			•		0.075
		•	•			0.043 *
		•		•		0.105
			•	•		0.002 *
NGFR						
(BRAF-mutant cell lines)						
Variable	NU (N = 8)	Reg (N = 8)	Reg+NU (N = 8)	Vem (N = 8)	Total (N = 8)	P-value²
Mean±SD	0.76±0.13	0.81±0.42	0.63±0.35	1.08±0.48	0.82±0.39	0.14 ²
Median	0.77	0.71	0.54	0.96	0.78	
[Q1, Q3]	[0.68, 0.85]	[0.53, 0.94]	[0.4, 0.74]	[0.87, 1.29]	[0.55, 0.95]	
Min, Max	0.54, 0.94	0.38, 1.63	0.26, 1.3	0.4, 2.03	0.26, 2.03	
CD55						
(BRAF-mutant cell lines)						
Variable	NU (N = 15)	Reg (N = 15)	Reg+NU (N = 15)	Vem (N = 15)	Total (N = 60)	P-value¹
Mean±SD	0.88±0.42	0.75±0.2	0.56±0.32	0.77±0.21	0.74±0.32	0.01 *
Median	0.83	0.71	0.47	0.75	0.68	
[Q1, Q3]	[0.64, 1]	[0.6, 0.94]	[0.4, 0.54]	[0.64, 0.95]	[0.5, 0.91]	
Min, Max	0.49, 2.17	0.42, 1.07	0.23, 1.48	0.42, 1.06	0.23, 2.17	
	•	•				0.539
	•		•			0.002 *
	•			•		0.624
		•	•			0.007 *
		•		•		0.744
			•	•		0.011 *

* p < 0.05

¹ Kruskal-Wallis test or Mann-Whitney U Test

² Kruskal-Wallis test

Supplementary Table S7: Differences in PD-L1, CD155, and HLA-I drug responses in NRAS-mutant cell lines

PD-L1						
(NRAS-mutant cell lines)						
Variable	NU (N = 10)	Reg (N = 10)	Reg+NU (N = 10)	Vem (N = 10)	Total (N = 40)	P-value¹
Mean±SD	0.51±0.2	0.72±0.13	0.36±0.16	1.43±0.39	0.75±0.48	< 0.001 *
Median	0.49	0.7	0.33	1.49	0.65	
[Q1, Q3]	[0.39, 0.64]	[0.64, 0.77]	[0.25, 0.47]	[1.11, 1.56]	[0.4, 0.99]	
Min, Max	0.24, 0.81	0.58, 1.03	0.15, 0.63	0.97, 2.28	0.15, 2.28	
	•	•				0.023 *
	•		•			0.105
	•			•		< 0.001 *
		•	•			< 0.001 *
		•		•		< 0.001 *
			•	•		< 0.001 *
CD155						
(NRAS-mutant cell lines)						
Variable	NU (N = 10)	Reg (N = 10)	Reg+NU (N = 10)	Vem (N = 10)	Total (N = 40)	P-value¹
Mean±SD	0.5±0.12	0.61±0.22	0.41±0.26	1.1±0.16	0.65±0.33	< 0.001 *
Median	0.48 [0.42, 0.56]	0.6 [0.52, 0.71]	0.36 [0.33, 0.42]	1.15 [1.02, 1.22]	0.58 [0.38, 0.94]	
[Q1, Q3]						
Min, Max	0.3, 0.71	0.21, 0.95	0.13, 1.06	0.77, 1.27	0.13, 1.27	
	•	•				0.166
	•		•			0.063
	•			•		< 0.001 *
		•	•			0.035 *
		•		•		< 0.001 *
			•	•		< 0.001 *
HLA-I						
(NRAS-mutant cell lines)						
Variable	NU (N = 9)	Reg (N = 9)	Reg+NU (N = 9)	Vem (N = 9)	Total (N = 36)	P-value¹
Mean±SD	1.09±0.21	1.34±0.4	1.39±0.49	0.86±0.15	1.17±0.39	0.003 *
Median	1.1	1.31	1.26	0.89	1.08	
[Q1, Q3]	[0.89, 1.27]	[1.09, 1.44]	[1.03, 1.58]	[0.84, 0.91]	[0.9, 1.31]	
Min, Max	0.79, 1.41	0.96, 2.27	0.86, 2.32	0.58, 1.07	0.58, 2.32	
	•	•				0.136
	•		•			0.258
	•			•		0.031 *
		•	•			1
		•		•		< 0.001 *
			•	•		0.004 *

* p < 0.05

¹ Kruskal-Wallis test or Mann-Whitney U Test

Supplementary Table S8: Differences in CD73, NGFR, and CD55 drug responses in NRAS-mutant cell lines

CD73						
(NRAS-mutant cell lines)						
Variable	NU (N = 10)	Reg (N = 10)	Reg+NU (N = 10)	Vem (N = 10)	Total (N = 40)	P-value¹
Mean±SD	0.54±0.13	0.54±0.14	0.29±0.12	1.46±0.27	0.71±0.48	< 0.001 *
Median	0.5	0.52	0.27	1.39	0.52	
[Q1, Q3]	[0.46, 0.67]	[0.51, 0.66]	[0.24, 0.32]	[1.29, 1.69]	[0.39, 0.82]	
Min, Max	0.35, 0.73	0.24, 0.71	0.13, 0.57	1.08, 1.84	0.13, 1.84	
	•	•				0.796
	•		•			< 0.001 *
	•			•		< 0.001 *
		•	•			0.004 *
		•		•		< 0.001 *
			•	•		< 0.001 *
NGFR						
(NRAS-mutant cell lines)						
Variable	NU (N = 7)	Reg (N = 7)	Reg+NU (N = 7)	Vem (N = 7)	Total (N = 28)	P-value¹
Mean±SD	0.66±0.13	1.03±0.84	0.75±0.57	1.12±0.22	0.89±0.53	0.04 *
Median	0.68	0.68	0.71	1.09	0.79	
[Q1, Q3]	[0.59, 0.72]	[0.62, 1.07]	[0.41, 0.87]	[1.02, 1.21]	[0.62, 1.04]	
Min, Max	0.47, 0.84	0.29, 2.81	0.11, 1.86	0.83, 1.49	0.11, 2.81	
	•	•				0.456
	•		•			0.71
	•			•		0.001 *
		•	•			0.535
		•		•		0.165
			•	•		0.053
CD55						
(NRAS-mutant cell lines)						
Variable	NU (N = 9)	Reg (N = 9)	Reg+NU (N = 9)	Vem (N = 9)	Total (N = 36)	P-value¹
Mean±SD	0.63±0.1	0.73±0.13	0.48±0.12	1.61±0.53	0.86±0.52	< 0.001 *
Median	0.59	0.69	0.49	1.41	0.66	
[Q1, Q3]	[0.57, 0.69]	[0.66, 0.83]	[0.44, 0.56]	[1.21, 1.67]	[0.56, 0.97]	
Min, Max	0.5, 0.81	0.54, 0.91	0.25, 0.65	1.15, 2.6	0.25, 2.6	
	•	•				0.077
	•		•			0.008 *
	•			•		< 0.001 *
		•	•			< 0.001 *
		•		•		< 0.001 *
			•	•		< 0.001 *

* p < 0.05

¹ Kruskal-Wallis test or Mann-Whitney U Test

Supplementary Table S9: Fractional product analysis of Reg, anti-CD40, and STING agonist *in vivo* results

Day	FTV: Reg	FTV: 40/S	40/S+Reg Expected	40/S+Reg Observed	Ratio
11	0.711	1.138	0.809	0.505	1.603
13	0.394	0.543	0.214	0.189	1.131
15	0.363	0.555	0.201	0.140	1.441
17	0.222	0.419	0.093	0.105	0.885
19	0.208	0.467	0.097	0.091	1.070
21	0.205	0.605	0.124	0.086	1.449
23	0.345	0.252	0.087	0.110	0.788
25	0.309	0.291	0.090	0.078	1.148

Synergy is defined as ratios > 1.0, additive effects as ratios = 1.0, and antagonistic effects as ratios < 1.0. Abbreviations: FTV = fractional tumor volume; 40 = anti-CD40; S = c-di-GMP (STING agonist).

Supplementary Table S10: Fractional product analysis of Reg and adoptive cell transfer *in vivo* results

Day	FTV: ACT	FTV: Reg	ACT+Reg Expected	ACT+Reg Observed	Ratio
14	0.874	0.567	0.496	0.478	1.037
16	0.776	0.524	0.407	0.381	1.067
18	0.661	0.417	0.276	0.301	0.917
20	0.675	0.549	0.371	0.285	1.299
22	0.658	0.700	0.460	0.260	1.773
24	0.754	0.866	0.653	0.276	2.362
26	0.955	1.110	1.060	0.415	2.555

Synergy is defined as ratios > 1.0, additive effects as ratios = 1.0, and antagonistic effects as ratios < 1.0. Abbreviations: FTV = fractional tumor volume; ACT: adoptive cell therapy.