Combinatorial approaches for mitigating resistance to KRAS-targeted therapies

Hannah R. Warren¹, Sarah J. Ross², Paul D. Smith², Judy M. Coulson^{1,3} and Ian A. Prior^{1,3}

Supplementary Material

FIGURE LEGENDS

Supplementary Figure 1. *Sensitivity of NCI-H358 cells to Ras targeted therapeutics.* Antisense KRAS AZD4785 results in loss of KRAS and a concomitant decrease in Ras-MAP kinase output indicated by decreased DUSP6 expression. Higher sensitivity to drug is observed in 3D spheroid cell culture vs 2D culture following 7 days of incubation. Similar results are observed for the other drugs. Data are means ±SD from three independent experiments. Calculated IC₅₀ and IC₉₀ concentrations are summarized in the table.

Supplementary Figure 2: *Re-activation of Ras effectors is KRAS^{G12C} specific.* Acute treatments with IC₉₀ concentrations of inhibitor were carried out in (A) naïve NCI-H1792 *KRAS*^{G12C} cells and (B) naïve NCI-H1793 *KRAS*^{WT} cells. Treatments were redosed every 48 hours. NCI-H1792 cells exhibited rapid re-activation of Ras effectors following acute inhibitor treatments, whereas NCI-H1793 cells showed minimal changes in pERK levels. Blots are representative of two independent experiments.

Supplementary Figure 3. *Dose responses of combination therapeutics in resistant NCI-H358 cells.* Long-term resistant cell lines were treated with their previous IC₉₀ inhibitor doses in combination with dose responses of additional targeted inhibitors.

The change in confluency was recorded following 4 days of inhibitor treatment. Confluence data are means \pm SD from at least two independent experiments.

Supplementary Figure 4. *Synergy vs dose response matrices of triple combination therapeutics in resistant cells.* Synergy scores from double inhibitor combination assays were calculated using the Loewe model and plotted against % viability. Areas coloured blue indicate drug combinations that are synergistic. The final doses selected for triple combination analysis in Supplementary Figure 3 are indicated.









		Path inhib	Pathway 2D viability inhibition		3D viability		
Inhibitor	Target	IC50 (μΜ)	IC90 (μΜ)	IC50 (μΜ)	IC90 (μΜ)	IC50 (μΜ)	IC90 (μΜ)
AZD4785	KRAS	0.42	3.72	4.95	44.7	0.79	7.08
ARS1620	KRAS ^{G12C}	0.092	0.83	1.55	13.8	0.34	3.09
ARS1323	KRAS ^{G12C}	0.29	2.63	2.5	22.4	0.48	4.27
Selumetinib	MEK1/MEK2	0.0097	0.087	1.1	10.2	0.0062	0.055





	DMSO	ARS1620	AZD4785	SEL	DMSO	ARS1620	AZD4785	SEL
		RMC-4	450					
NVP-ADW742	0 2 10 50 250 1250 0 100 108 100 6 6 1 1 2 90 90 96 10	0 2 10 50 250 1250 100 408 92 79 47 12 99 41 80 68 43 80 99 41 80 68 43 80 98 48 40 40 80 80 88 89 84 80 84 80 85 80 84 80 84 80 85 80 84 80 84 80 85 80 84 80 84 80 85 80 84 80 84 80 84 85 80 84 80 84 80 84 80 84 80 84 80 84 80 84 80 84 80 84 80 84 80 84 80 84 80 84 80 84 80 84	0 2 10 50 250 1250 100 44 68 168 84 46 103 104 104 94 97 75 100 103 101 93 81 79 103 101 93 81 94 94 98 87 69 80 82 84 98 87 69 80 86 86 98 87 69 80 86 86 98 87 68 86 86 86 98 80 68 86 86 86 98 80 68 86 86 86 98 80 68 86 86 86 86 87 84 72 86 86 86 86 86 86 86 86 86 86 86 86 86 86 </th <th>0 2 10 50 250 1250 100 100 100 99 82 88 100 100 100 97 68 57 100 100 100 97 68 57 100 100 98 68 68 68 100 100 98 64 68 68 100 100 98 64 68 68 100 100 98 64 68 68 100 88 46 68 68 68 100 100 98 64 68 58 68 100 88 46 68</th> <th>2 10 50 250 1250 2 1 2 5 4 4 10 1 4 5 2 8 50 4 9 15 7 15 250 52 25 16 14 13 1250 8 12 15 17 17</th> <th>2 10 50 250 1250 7 7 6 5 2 3 11 3 4 6 2 4 21 5 4 2 4 21 5 4 3 15 17 17 13</th> <th>4450 2 10 50 250 1250 4 4 6 1 7 3 -4 7 7 4 13 11 20 14 14 14 20 14 14 14 15 14 20 14 14</th> <th>2 10 50 250 1200 0 0 3 4 11 0 0 2 4 10 0 0 5 10 9 2 6 10 2 2 4 9 3 2 3</th>	0 2 10 50 250 1250 100 100 100 99 82 88 100 100 100 97 68 57 100 100 100 97 68 57 100 100 98 68 68 68 100 100 98 64 68 68 100 100 98 64 68 68 100 100 98 64 68 68 100 88 46 68 68 68 100 100 98 64 68 58 68 100 88 46 68	2 10 50 250 1250 2 1 2 5 4 4 10 1 4 5 2 8 50 4 9 15 7 15 250 52 25 16 14 13 1250 8 12 15 17 17	2 10 50 250 1250 7 7 6 5 2 3 11 3 4 6 2 4 21 5 4 2 4 21 5 4 3 15 17 17 13	4450 2 10 50 250 1250 4 4 6 1 7 3 -4 7 7 4 13 11 20 14 14 14 20 14 14 14 15 14 20 14 14	2 10 50 250 1200 0 0 3 4 11 0 0 2 4 10 0 0 5 10 9 2 6 10 2 2 4 9 3 2 3
	0 2 10 50 250 1250	0 2 10 50 250 1250	145U 0 2 10 50 250 1250	0 2 10 50 250 1250		RMC-	4450	
Gefitinib	0 100 107 104 82 45 85 2 99 102 104 82 85 85 10 103 122 91 65 85 85 10 103 92 92 64 85 85 50 83 60 72 92 64 85 25 250 83 60 72 92 64 85 25 1250 83 60 72 80 86 82 85 1250 83 83 83 84 84 84 84 84 84	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	2 10 50 250 1250 2 2 8 17 5 6 10 5 3 14 11 12 50 30 12 14 15 15 250 6 11 9 12 14 1250 6 9 9 9	2 10 50 250 1250 a. b. b. b. b. b. a. 10 12 d. 2. b. a. 10 12 d. 2. d. 13. 12. 11. a. 10.	2 10 50 250 1250 2 .4 0 0 .1 0 0 .4 .3 .4 2 0 1 .2 .2 4 0 1 .2 .2 5 5 .5 .3 .6 3 4 4 4 .2	10 50 250 1250 -1 0 2 4 4 -2 -4 6 4 6 -2 -2 12 7 16 -3 20 12 5 16 -3 20 12 12 12 -4 -5 16 3 16
		AZD8	055					
	0 2 10 50 250 1250	0 2 10 50 250 1250	0 2 10 50 250 1250 100 90 62 42 38 36	0 2 10 50 250 1250	2 10 50 250 1250	AZD	2 10 50 250 1250	2 10 50 250 1250
Gefitinib	400 400 400 400 400 2 100 108 72 10 10 10 50 84 66 10 14 13 10 50 84 66 10 14 14 50 84 66 82 21 14 16 250 85 84 66 21 14 15 1250 85 84 86 66 61 66	44 4a a	na na na na na na 99 84 54 2 2 2 2 99 84 54 42 2 2 2 99 84 54 42 2 2 2 99 70 56 41 2 2 2 90 70 64 47 44 46 44 74 44 46 46	ια ια ια ια ια ια 101 90 89 10 10 21 98 99 91 62 10 10 21 98 99 91 62 10 10 10 10 99 91 62 63 10 10 10 10 99 91 62 63 10 10 10 10 10 99 91 62 63 10	2 10 50 220 1230 2 11 5 0 1 2 10 2 4 4 1 1 50 3 2 1 1 0 1250 1 8 1 4 4	2 10 30 20 123 4 2 6 2 5 19 1 6 3 4 2 4 11 5 4 4 8 2 3	10 30 230 120 7 5 5 2 2 6 8 -1 0 0 11 6 1 -2 4 13 -2 -6 -7 -8	2 10 30 230 230 0 4 9 3 0 3 0 3 0 2 6 9 14 3 4 1 -3 4 3 6
		RMC-4	450			DMC	4450	
	0 2 10 50 250 1250 0 100 100 97 75 64	0 2 10 50 250 1250 100 104 83 75 47 34	0 2 10 50 250 1250 100 100 101 96 92 92	0 2 10 50 250 1250 100 100 99 97 90 81	2 10 50 250 1250	2 10 50 250 1250	2 10 50 250 1250	2 10 50 250 1250
Lapatinib	10 -0.3 -0.3 -0.3 0.44 0.44 0	10 40 40 40 40 50 76 75 76 </th <th>1.42 .43 .44 .46 .46 .46 .46 9.9 9.8 9.6 9.3 9.6 9.4 9.6 9.3 9.1 9.8 1.42 1.42 1.42 1.42 1.42 1.42 9.8 1.00 9.8 9.6 9.3 9.1 1.42 1.42 9.9 9.4 90 9.0 8.8 1.44 1.42 1.42 9.9 9.4 9.0 9.0 8.8 1.44 1.42 1.42 9.9 9.8 9.4 9.2 9.2 9.8 8.8 1.44 9.9 9.8 9.4 9.2 9.9 8.8 1.44 1.44 1.44 1.44</th> <th>1-00 4-00 4-00 4-00 4-00 7-00 8-0 9-0 8-0</th> <th>1 3 18 16 13 10 20 7 20 20 250 15 16 4 7 21 1250 13 16 7 7 21</th> <th>0 0 1 2 4 3 4 9 7 4 7 5 8 7 4 1 2 1 1 3</th> <th>2 2 0 -1 2 0 2 1 -1 1 6 10 7 4 8 2 6 5 3 4</th> <th>0 1 1 7 0 -4 7 16 20 3 9 15 19 40 4 5 14 2 2</th>	1.42 .43 .44 .46 .46 .46 .46 9.9 9.8 9.6 9.3 9.6 9.4 9.6 9.3 9.1 9.8 1.42 1.42 1.42 1.42 1.42 1.42 9.8 1.00 9.8 9.6 9.3 9.1 1.42 1.42 9.9 9.4 90 9.0 8.8 1.44 1.42 1.42 9.9 9.4 9.0 9.0 8.8 1.44 1.42 1.42 9.9 9.8 9.4 9.2 9.2 9.8 8.8 1.44 9.9 9.8 9.4 9.2 9.9 8.8 1.44 1.44 1.44 1.44	1-00 4-00 4-00 4-00 4-00 7-00 8-0 9-0 8-0	1 3 18 16 13 10 20 7 20 20 250 15 16 4 7 21 1250 13 16 7 7 21	0 0 1 2 4 3 4 9 7 4 7 5 8 7 4 1 2 1 1 3	2 2 0 -1 2 0 2 1 -1 1 6 10 7 4 8 2 6 5 3 4	0 1 1 7 0 -4 7 16 20 3 9 15 19 40 4 5 14 2 2
	0 2 10 50 250 1250	AZD80 0 2 10 50 250 1250	0 2 10 50 250 1250	0 2 10 50 250 1250		AZD8	8055	
Lapatinib	0 100 40 112 40 93 40 412 40 93 40 413 40 93 40 43 40 1250 420 200	100 108 00 45 30 31 40 108 90 40 30 31 40 76 59 40 75 26 40 76 59 40 27 26 60 50 42 28 28 28 28 66 50 42 28 28 28 28 28 66 50 42 28 28 28 28 28 66 44 28 28 28 28 28 28 66 43 42 28 28 28 28 28 65 43 42 28 28 28 28 28 64 43 42 29 28 28 28 64 43 42 29 37 38 65 66 68 29 29 28	100 89 55 42	100 100 98 58 40 40 0.0 0.0 0.0 0.0 0.0 0.0 97 97 87 57 50 0.0 90 78 77 43 6.0 6.0 0.0 90 78 77 43 1.0 0.0 90 78 77 43 1.0 0.0 90 78 77 51 1.0 0.0 4.4 4.4 -0 -0 0.0 0.0 90 78 77 51 1.0 0.0 0.0 80 75 75 51 0.0 0.0 0.0	2 10 50 250 1250 2 1 2 6 3 4 10 7 14 4 2 2 20 10 10 8 2 1 50 10 10 8 2 1 1250 6 12 9 3 2	2 10 50 250 1250 7 17 9 2 9 4 17 9 2 9 4 11 12 4 2 1 10 3 4 2 1 12 4 2 3	2 10 50 250 1250 2* 6 -1 -2 -6 9 0 -2 -6 -4 13 9 -3 -6 -6 11 0 -7 -7 -6	2 10 50 250 1250 1 8 3 -3 1 3 7 2 -4 1 12 10 17 1 3 5 2 9 -4 4
		0/ vie	ability	100		tagonism	SVDO	
	0	/0 VIC	ability i	100	ai	layonishi	Syner	99

		830nM	2.34µM	87nM
	DMSO	ARS1620	AZD4785	SEL
NVP-ADW742	250	50	50	250
RMC-4550	50	50	50	250
Gefitinib	50	250	250	250
RMC-4550	10	10	250	250
Gefitinib	50	50	50	50
AZD8055	50	50	50	50
Lapatinib	50	50	50	250
RMC4550	50	50	50	250
Lapatinib	10	10	10	10
AZD8055	10	10	10	10