Supplementary Data

Cell lines

Cell lines were obtained from commercial vendors and academic laboratories. Experiments were conducted within 3 months of cells being established in continuous culture. Malme-3M, NCI-H441, NCI-H1734, NCI-H1651, WM266.4, NCI-H23, BT-549, BT-474, A2058, MCF7, HUVEC and Ker-CT were all purchased from the American Type Culture Collection (LGC standards, Teddington, UK). CACO2, A2780, SKOV3 and A549 were purchased from the Health Protection Agency (Porton Down, Salisbury, UK). PEO1, T47D and SW620 came from Sigma Aldrich (Gillingham, UK), SKMEL-28 and SKMEL-5 from Cell Line Services (Eppelheim, Germany). IGROV-1 was obtained in-house and authenticated at the London Research Institute (London, UK).

Drugs

PD0325901, AZD6244 and AZD5363 were purchased from Selleck Chemicals (Munich, Germany) and AKT1/2 kinase inhibitor from Sigma Aldrich (Poole, UK).

Calculation of the degree of inhibition of signalling output

Cells were incubated with PD0325901 (Selleck Chemicals, S1036) and AKT 1/2 kinase inhibitor (Sigma Aldrich, A6730), ELISAs were used to quantify phosphorylation of ERK 1/2 and S6 (ser235/236) (Meso Scale Discovery, K151DWD-2 and K150DFD-2 respectively, Rockville, USA).

Calculation of the degree of inhibition of signalling output

Cells were incubated with 5 ml of the appropriate media at 37 ^{o}C with 5% CO_2 in 25 cm^2, 2 µm vented cap flasks (430639, Corning, Corning, NY, USA). Upon reaching 80-90%

confluence, cells were exposed to a DMSO control, 5, 10, 50, 100, 500, 1000 or 5000 nm of PD0325901 (MEK inhibitor, Selleck Chemicals, S1036) and a DMSO control, 10, 50, 100, 500, 1000, 5000, or 10,000 nm of AKT 1/2 kinase inhibitor (Sigma Aldrich, A6730), respectively, for 24 hrs. All experiments were conducted in triplicate. ELISAs were used to quantify phosphorylation of ERK 1/2 and S6 (ser235/236) (Meso Scale Discovery, K151DWD-2 and K150DFD-2 respectively, Rockville, USA) at each dosing concentration. 10 ug of protein lysate was added to each well. Manufacturers' protocols were followed in all instances. Plates were read on a SECTOR 6000 Imager (Meso Scale Discovery, Discovery Workbench 2006 v3.0.17.3).

Growth inhibition of 4 cell lines to maximal MEK and AKT inhibition caused by clinically used MEK (AZD6244) and AKT (AZD5363) respectively

Four cell lines ie SKMEL-28 (*BRAF*^{M}), T47D (*PIK3CA*^{M}), A549 (*KRAS*^{M}) and A2780 (*BRAF/PIK3CA/KRAS*^{WT}) were exposed to a clinically used MEK inhibitor (AZD6244) and AKT inhibitor (AZD5363) that caused maximal MEK and AKT inhibition respectively. Consistent with the growth inhibition pattern caused by PD0325901 and AKT 1/2 kinase inhibitor, maximal MEK inhibition caused by AZD6244 caused statistically greater growth inhibition in BRAF^{M} cell line SKMEL-28 compared to maximal AKT inhibition caused by AZD5363 (85.1% vs 44.1%; P <0.001, respectively). Also, maximal AKT inhibition caused by AZD5363 caused significantly greater growth inhibition in the *PIK3CA*^{M} cell line T47D compared to maximal MEK inhibition caused by AZD6244 (46.2% vs 7.04*%; P* = 0.001, respectively). Further, there was no statistical difference between growth inhibition caused by maximal MEK inhibition by AZD6244 and maximal AKT inhibition by AZD5363 in the *KRAS*^{M} (A549) and *BRAF/PIK3CA/KRAS*^{WT} (A2780) cell lines (40.8% vs 27.8*%; P* = 0.23 and 72.2% vs 62.6*%; P* = 0.06, respectively).

Growth inhibition of 2 normal human cell lines (Ker-CT and HUVEC) to maximal MEK and AKT inhibition caused by PD0325901 and AKT 1/2 kinase inhibitor

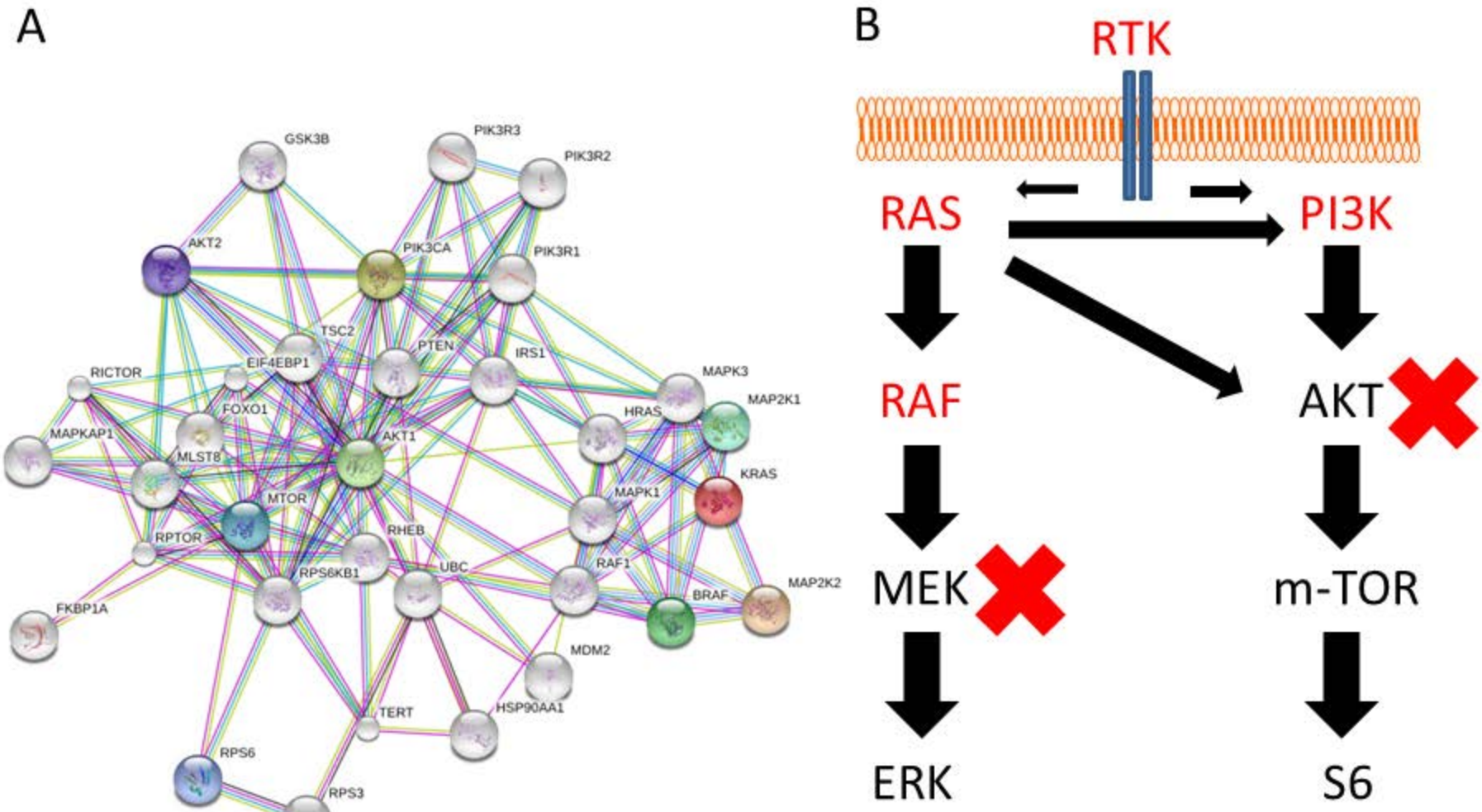
In an attempt to understand the effects of the combination of MEK and AKT inhibition in normal tissues, growth inhibition relative to inhibition of MEK and AKT signalling was studied in HUVEC (endothelial) and Ker-CT (immortalized keratinocyte) cells. There was no significant difference between growth inhibition caused by maximal MEK inhibition or AKT in HUVEC or Ker-CT cells; 22% (SD 16.9) vs 34.5 (SD 6.25); P= 0.3 and 68.4% (SD 11.4) vs 80.2% (SD 1.5); P= 0.15, respectively. Additional AKT inhibition to maximal MEK inhibition or additional MEK inhibition to maximal AKT inhibition did not cause a significant increase in growth inhibition in the Ker-CT cells but did so in HUVEC cells (Supplementary Figure 2A). Interestingly, in both HUVEC and Ker-CT cells suboptimal inhibition of MEK or AKT did not cause significantly more growth inhibition compared to maximal AKT or MEK inhibition alone (Supplementary Figure 2B).

Cell line	Tissue of Origin	Mutation
WM266.4	Melanoma	*BRAF* V600E
SKMEL-28	Melanoma	*BRAF* V600E
A2058	Melanoma	*BRAF* V600E
SKMEL-5	Melanoma	*BRAF* V600E
MALME-3M	Melanoma	*BRAF* V600E
BT474	Breast	*PIK3CA* K111N
SKOV-3	Ovary	*PIK3CA* H1047R
MCF-7	Breast	*PIK3CA* E545K
IGROV-1	Ovary	*PIK3CA* R38C
T47D	Breast	*PIK3CA* H1047R
A549	Lung	*KRAS* G12S
H23	Lung	*KRAS* G12C
SW620	Colon	*KRAS* G12V
H441	Lung	*KRAS* G12V
H1734	Lung	*KRAS* G13C
CACO2	Colon	
PEO1	Ovary	
H1651	Lung	
A2780	Lung	
BT549	Breast	
HUVEC	Endothelium	
Ker-CT	Skin	

Supplementary Table 1

Cell line	Mutation	PD0325901 ED_{50} p-ERK	AKT 1/2 inhibitor ED_{50} p-S6
A2058	*BRAF*	19.5	33.5
MALME3M	*BRAF*	6	33
SKMEL28	*BRAF*	3.5	1053
SKMEL5	*BRAF*	29.5	891
WM266.4	*BRAF*	2.56	1597
BT474	*PIK3CA*	3.5	46.5
IGROV1	*PIK3CA*	10	794
MCF7	*PIK3CA*	2.5	367.3
Skov3	*PIK3CA*	2.5	1328
T47D	*PIK3CA*	4.4	21
A549	*KRAS*	23	273
H1734	*KRAS*	11	5623
H23	*KRAS*	3.5	2399
H441	*KRAS*	3.5	5370
SW620	*KRAS*	2.55	132
A2780	*BRAF/PIK3CA/KRAS* WT	3	178
BT549	*BRAF/PIK3CA/KRAS* WT	42	1860
Caco2	*BRAF/PIK3CA/KRAS* WT	2.5	1546
H1651	*BRAF/PIK3CA/KRAS* WT	4.8	631
PEO1	*BRAF/PIK3CA/KRAS* WT	3	39.8
HUVEC	Normal cell	5.5	1905
Ker-CT	Normal cell	3.2	1659
Cell line	**Mutation**	**AZD6244 ED_{50} p-ERK**	**AZD5363 ED_{50} p-S6**
SKMEL28	*BRAF*	69.2	158.5
T47D	*PIK3CA*	20	141
A549	*KRAS*	4	229
A2780	*BRAF/PIK3CA/KRAS* WT	12.3	60

Suplementary Table 2



Supplementary Figure 1

A

	MEK 100	MEK100	MEK100	MEK100	MEK100	
	AKT 0	AKT 25	AKT 50	AKT 75	AKT 100	Significant?
HUVEC	21.99	38.27	46.47	54.78	57.36	Y
Ker-CT	68.41	75.82	79.26	81.79	82.82	N
	AKT 100	AKT100	AKT100	AKT100	AKT100	
	MEK 0	MEK 25	MEK 50	MEK75	MEK100	
HUVEC	34.47	39.81	42.84	49.13	57.36	Y
Ker-CT	80.18	80.5	81.39	82.03	82.82	N

B

	MEK 100	MEK 0	MEK 25	MEK 50	MEK 75
	AKT 0	AKT 100	AKT 25	AKT 50	AKT 75
HUVEC	21.99	34.47	20.5	28.73	44.41
Ker-CT	68.41	80.18	60.18	72.66	79.1

Supplementary Figure 2