

Optimizing therapeutic combinations of pimasertib, a selective MEK1/2 inhibitor, with PI3K/mTOR inhibitors or with multi-targeted kinase inhibitors in pimasertib-resistant human lung and colorectal cancer cells

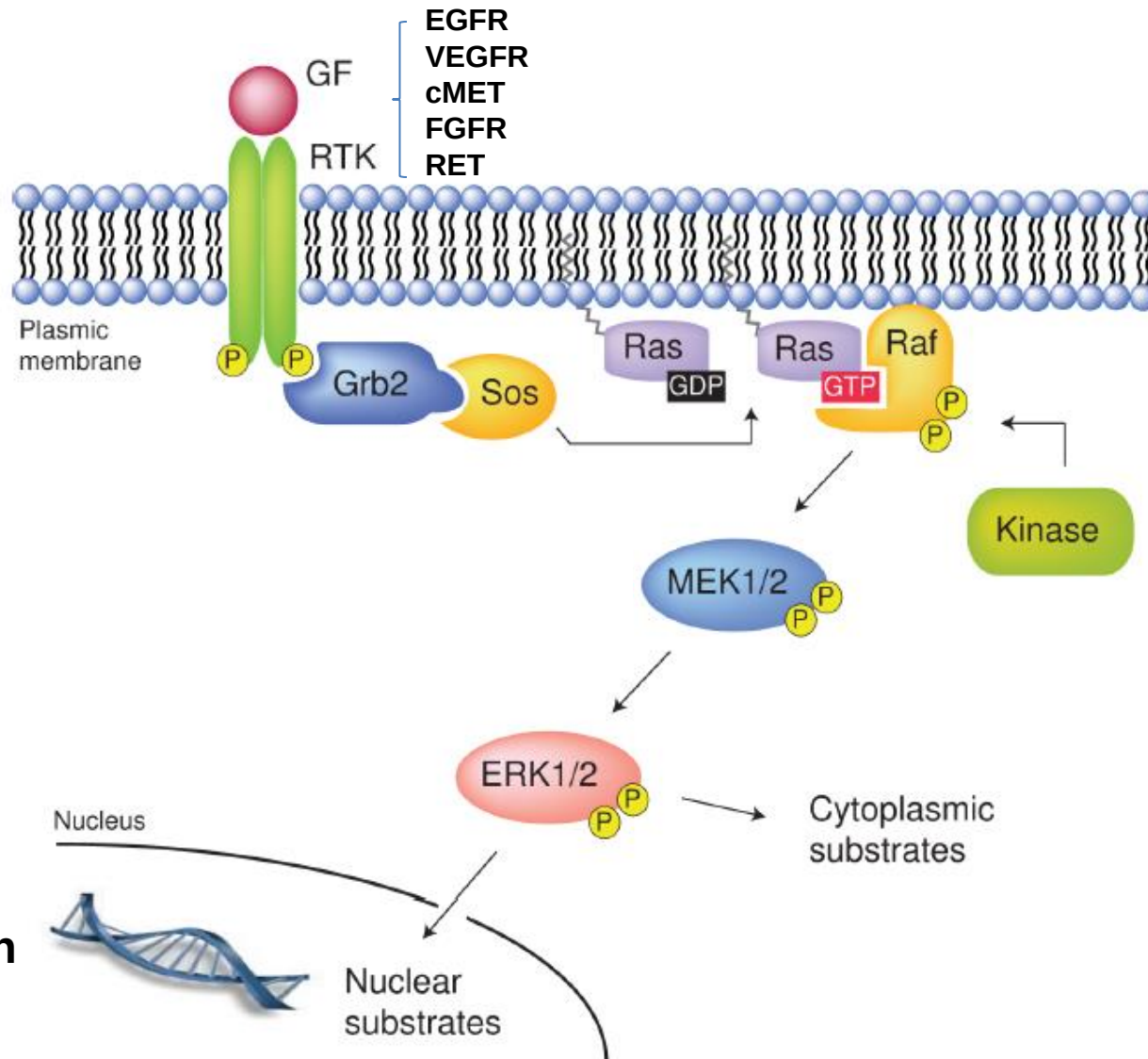
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Disclosure slide

- I have no conflict of interest to declare

The Ras-Raf-MEK-MAPK signaling



**Proliferation
Growth
Survival
Angiogenesis**

Background (1)

- ❖ MEK activation is found mostly in BRAF mutant cancer cells and in a subgroup of KRAS mutant cancer cells (*Davies BR, et al. Mol Cancer Ther 2007; Solit DB et al, Nature 2006*)
- ❖ Activating mutations of PI3CA and hyperactivity of the PTEN/PI3K/AKT pathway are associated to reduced activity or to resistance to MEK inhibitors in human cancer cells. (*Mirzoeva OK et al, Cancer Res 2009; Wee S et al, Cancer Res 2009*)
- ❖ Specific gene expression profiles have been suggested to predict sensitivity or resistance to MEK inhibitors (selumetinib, AZD6244) (*Dry J et al. Cancer Res 2010*)
- ❖ Pimasertib (a biaryl amine derivative) has a high selectivity for MEK1/2, with anti-proliferative effects in NSCLC cell lines and efficacy in xenograft models (*Morgillo et al, Br J Cancer 2011; Price S et al, Expert Opin Ther Patents 2008*)

Background (2)



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Intrinsic resistance to selumetinib, a selective inhibitor of MEK1/2, by cAMP-dependent protein kinase A activation in human lung and colorectal cancer cells

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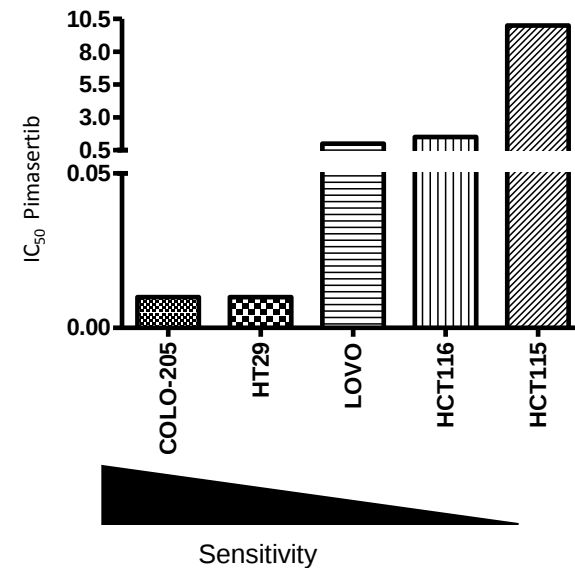
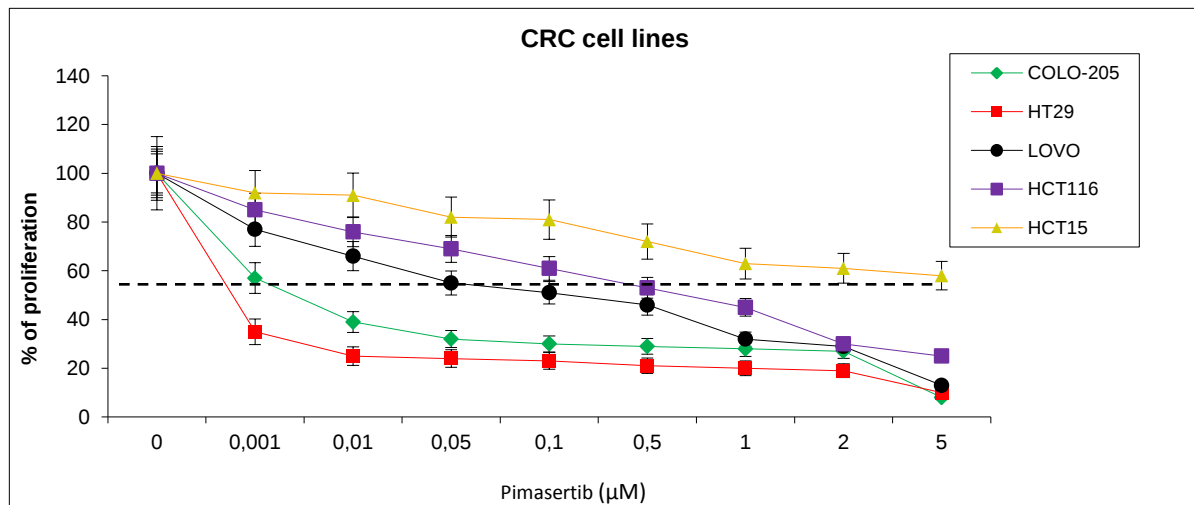
List of mutations according to COSMIC

NSCLC	Human cancer cell lines	KRAS	NRAS	BRAF	PI3KCA	EGFR
	H1299	WT	Mut (Q61K)	WT	WT	WT
	Calu-3	WT	WT	WT	WT	WT
	A549	Mut (G12S)	WT	WT	WT	WT
	H460	Mut (Q61H)	WT	WT	Mut (E545K)	WT
	H358	Mut(G12C)	WT	WT	WT	WT
	H1975	WT	WT	WT	Mut (G118D)	Mut (T790M) Mut (L858R)
CRC	COLO205	WT	WT	Mut (V600E)	WT	WT
	HT29	WT	WT	Mut (V600E)	Mut (P449T)	WT
	Lovo	Mut (G13D)	WT	WT	WT	WT
	HCT116	Mut (G13D)	WT	WT	Mut (H1047R)	WT
	HCT15	Mut (G13D)	WT	WT	Mut (E545K) Mut (D549N)	WT

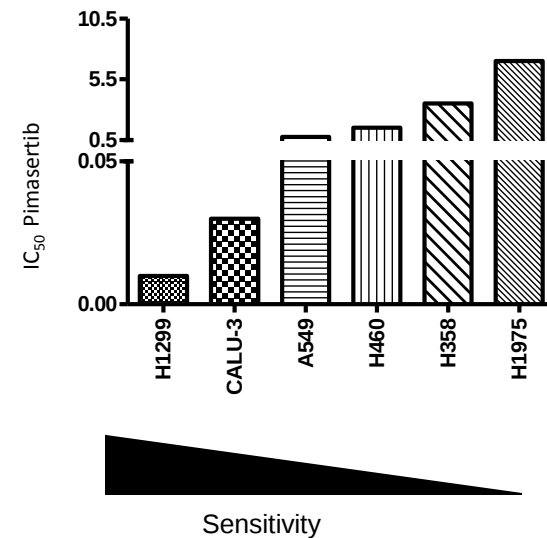
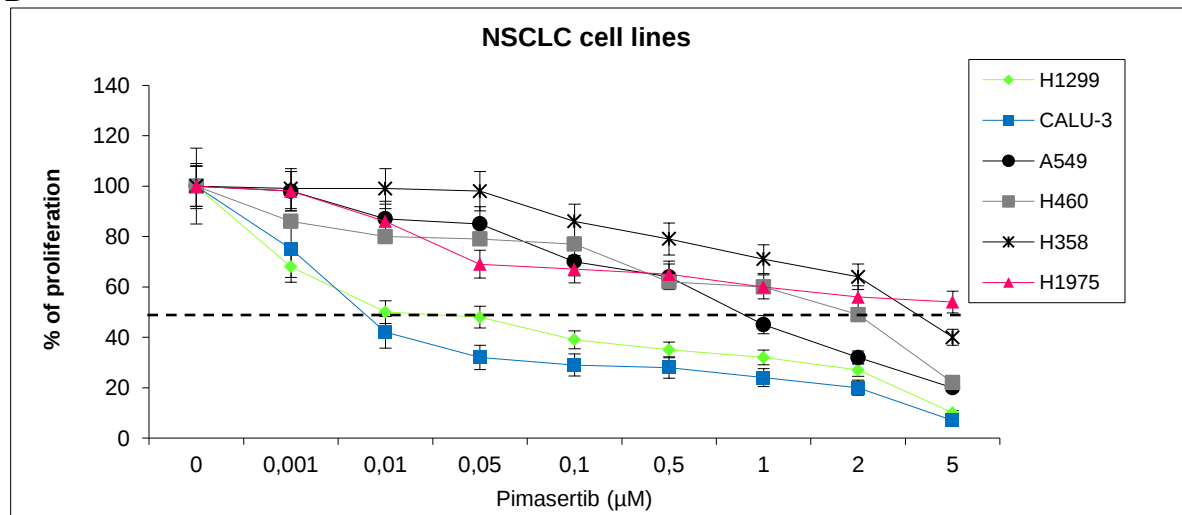
WT: wild type; Mut: mutated

Proliferation assay

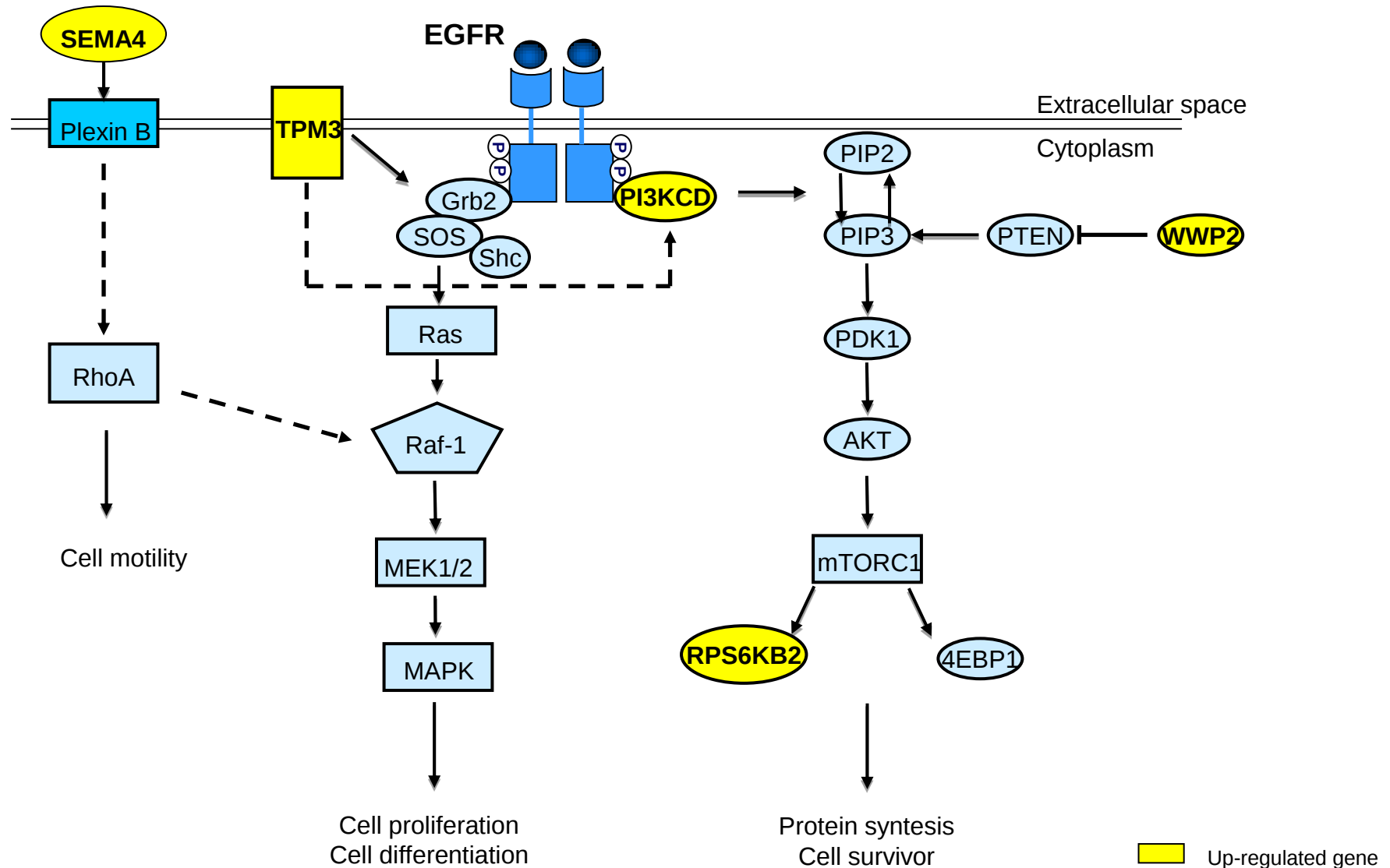
A



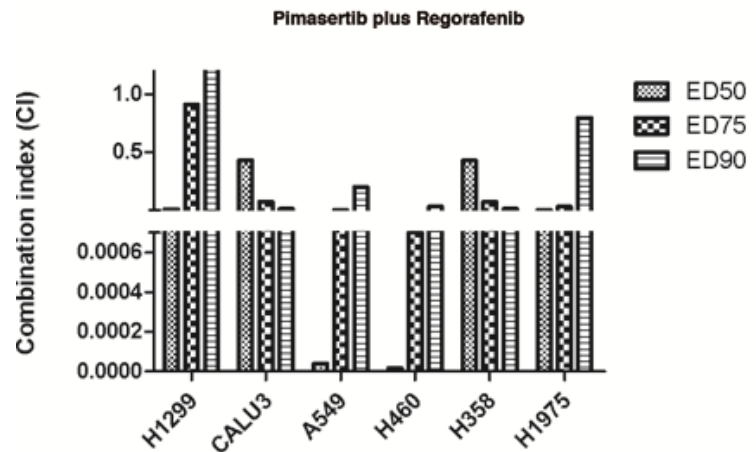
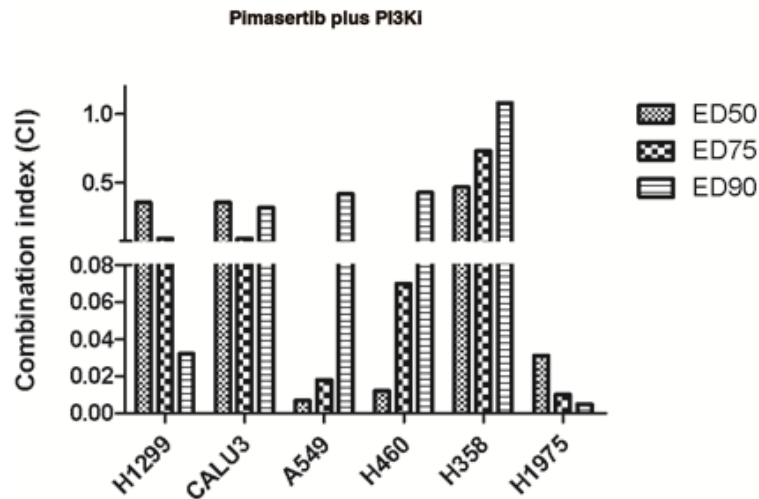
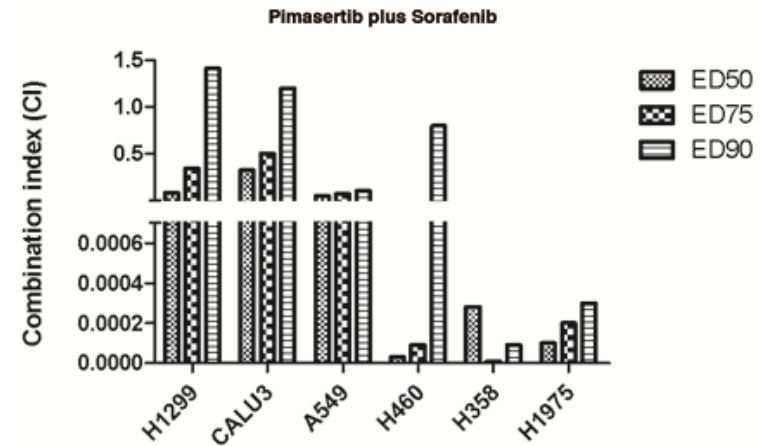
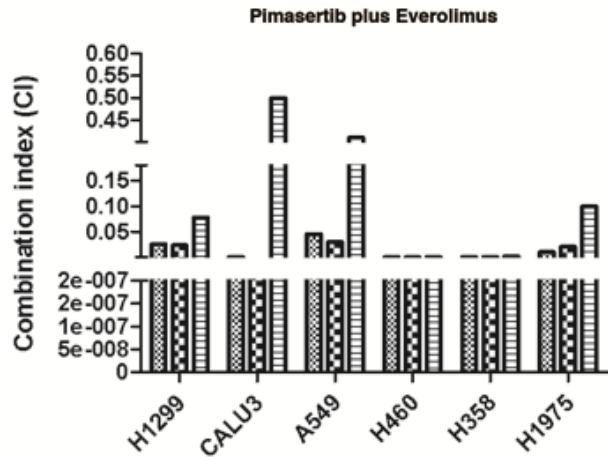
B



Five core genes involved in MEK resistance in our system

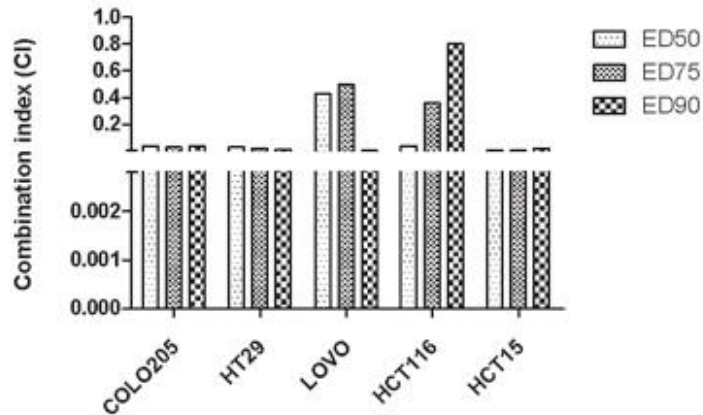


Synergistic activity of pimasertib in combination with selective PI3K/AKT/mTOR inhibitors or with multi-targeted inhibitors in NSCLC cells growth *in vitro*

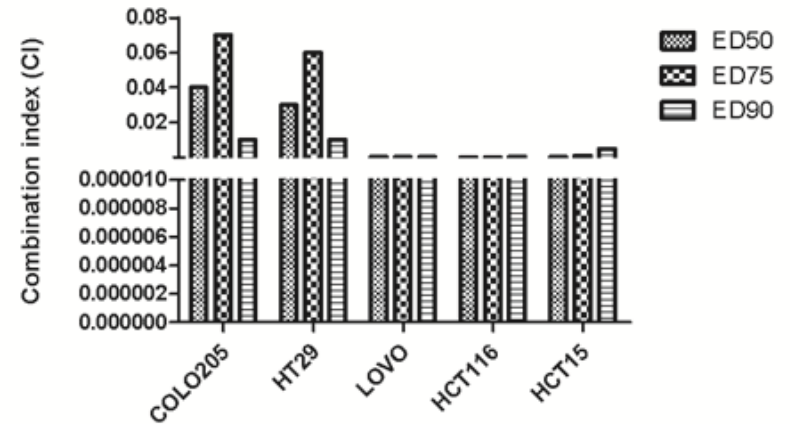


Synergistic activity of pimasertib in combination with selective PI3K/AKT/mTOR inhibitors or with multi-targeted inhibitors in CRC cells growth *in vitro*

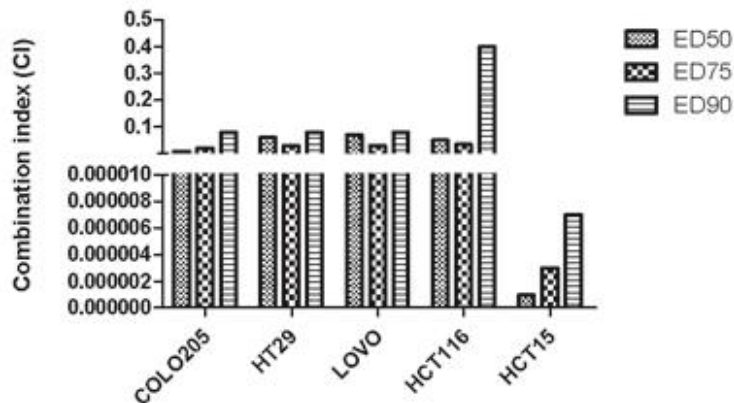
Pimasertib plus Everolimus



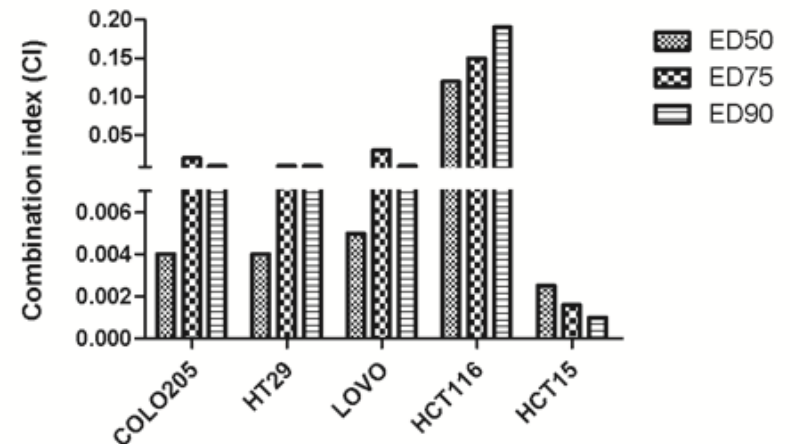
Pimasertib plus Sorafenib



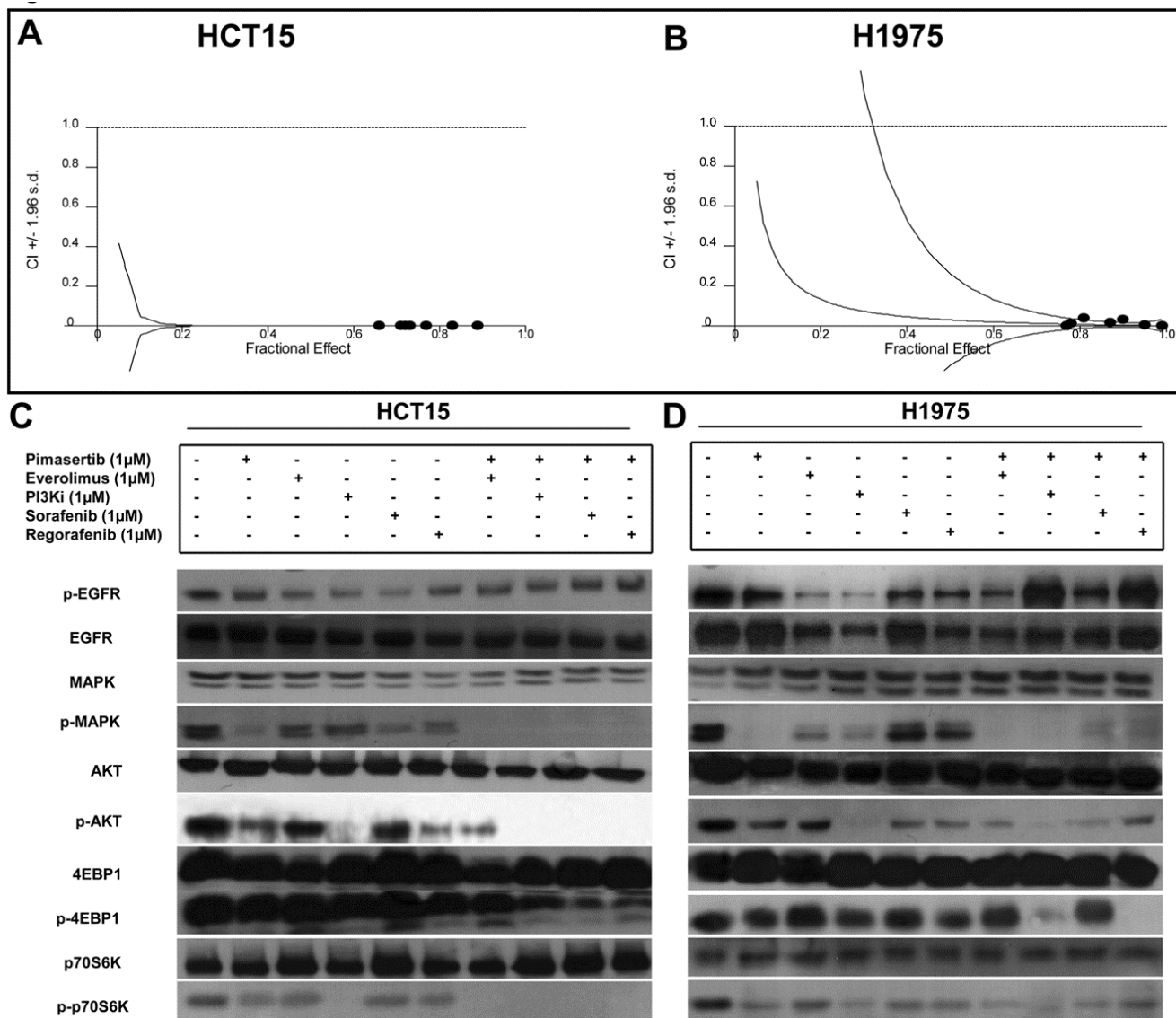
Pimasertib plus PI3KI



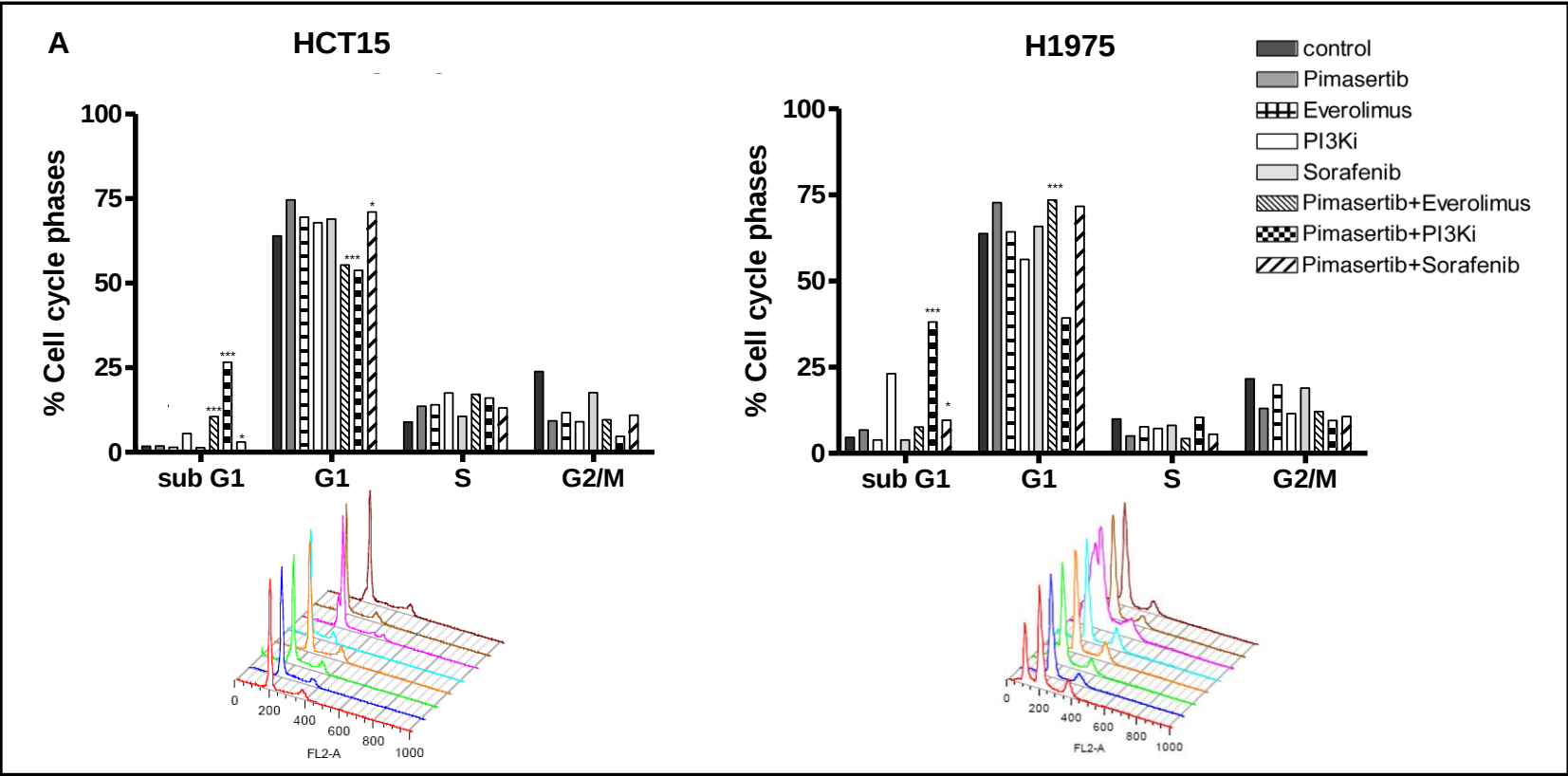
Pimasertib plus Regorafenib



Effects of combined treatment on cell proliferation and on intracellular signaling pathways in pimasertib resistant cancer cell lines



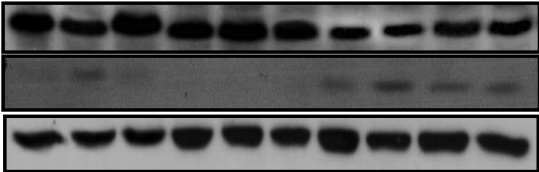
Effects of combined treatment on cell cycle distribution in pimasertib resistant cancer cell lines



B

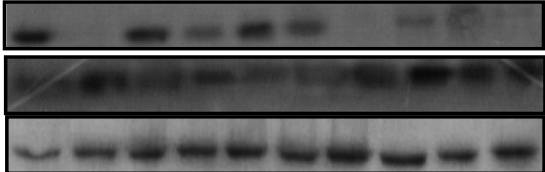
Pimasertib (1μM)
Everolimus (1μM)
PI3Ki (1μM)
Sorafenib (1μM)
Regorafenib (1μM)

-	+	-	-	-	-	+	+	+	+
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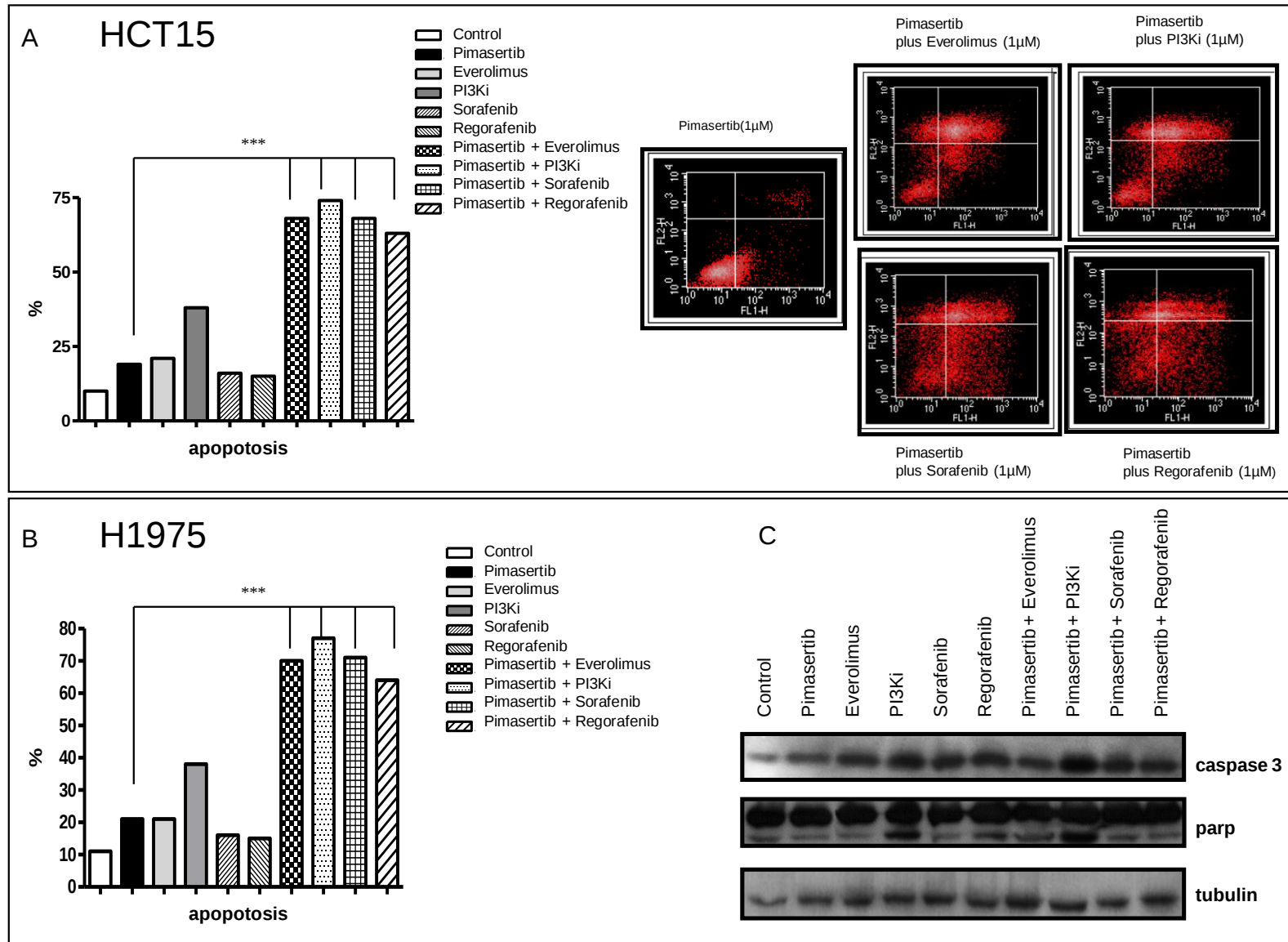


Pimasertib (1μM)
Everolimus (1μM)
PI3Ki (1μM)
Sorafenib (1μM)
Regorafenib (1μM)

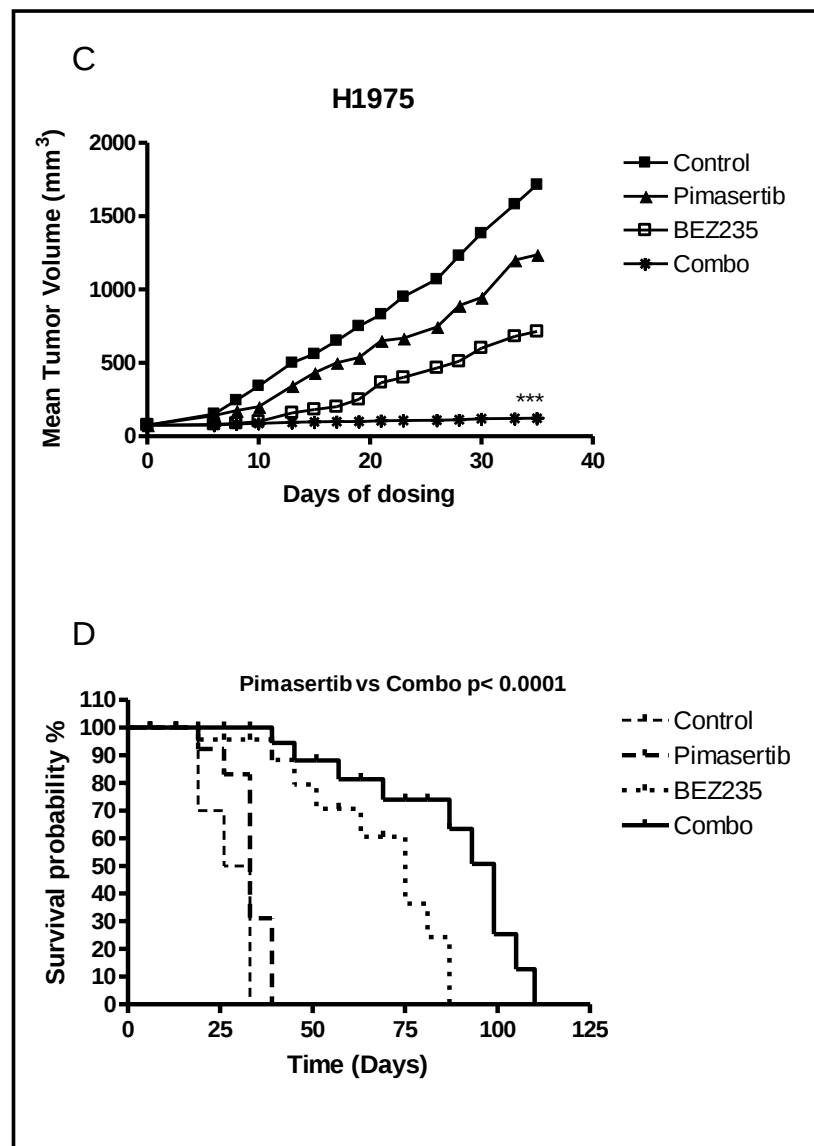
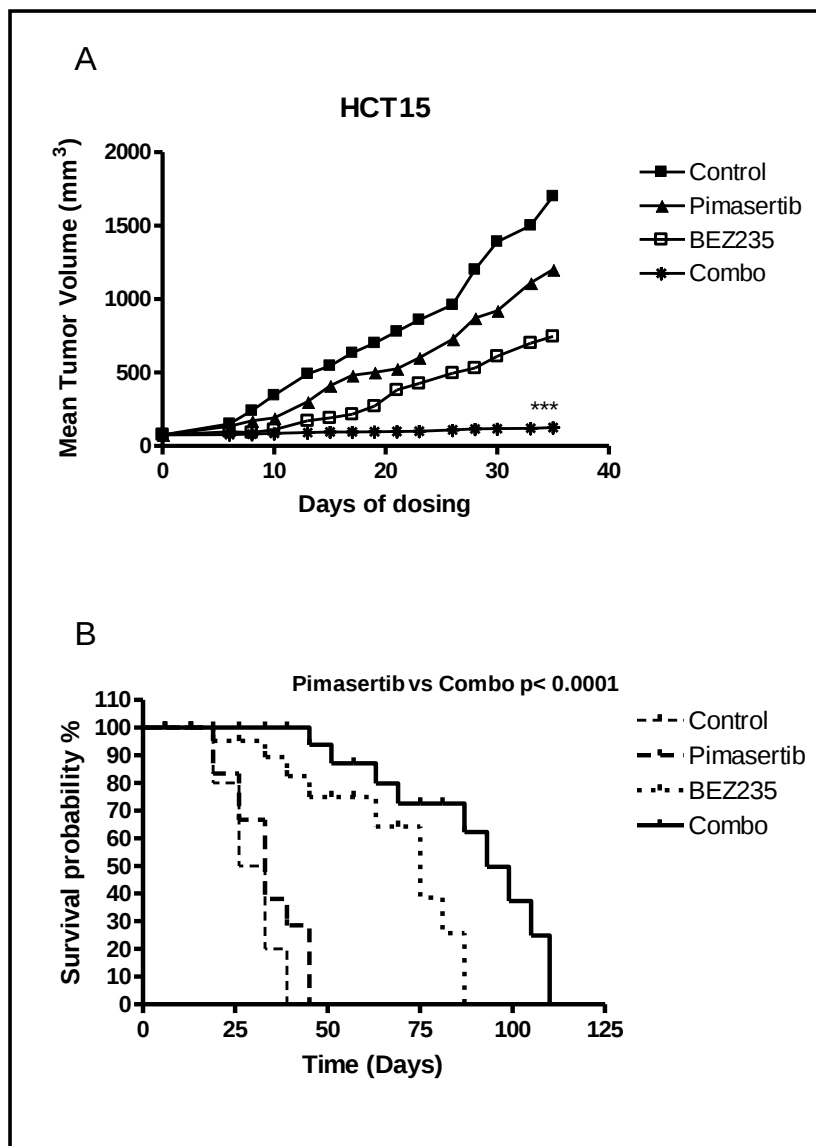
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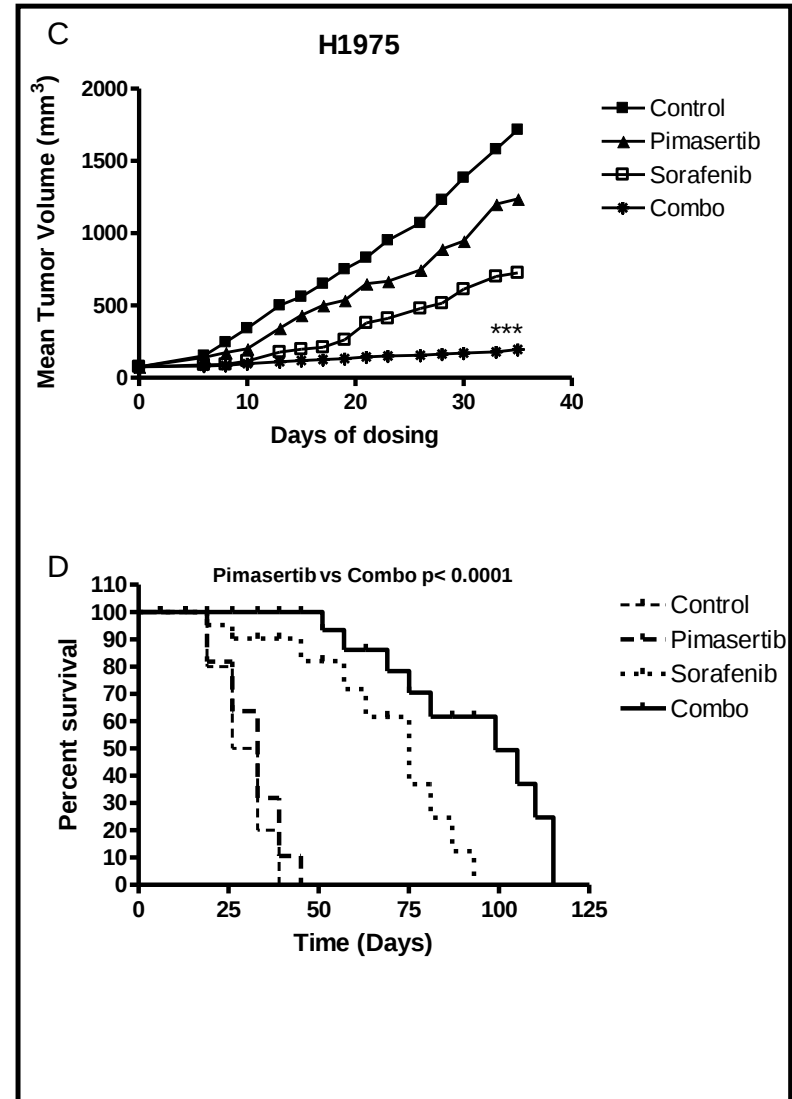
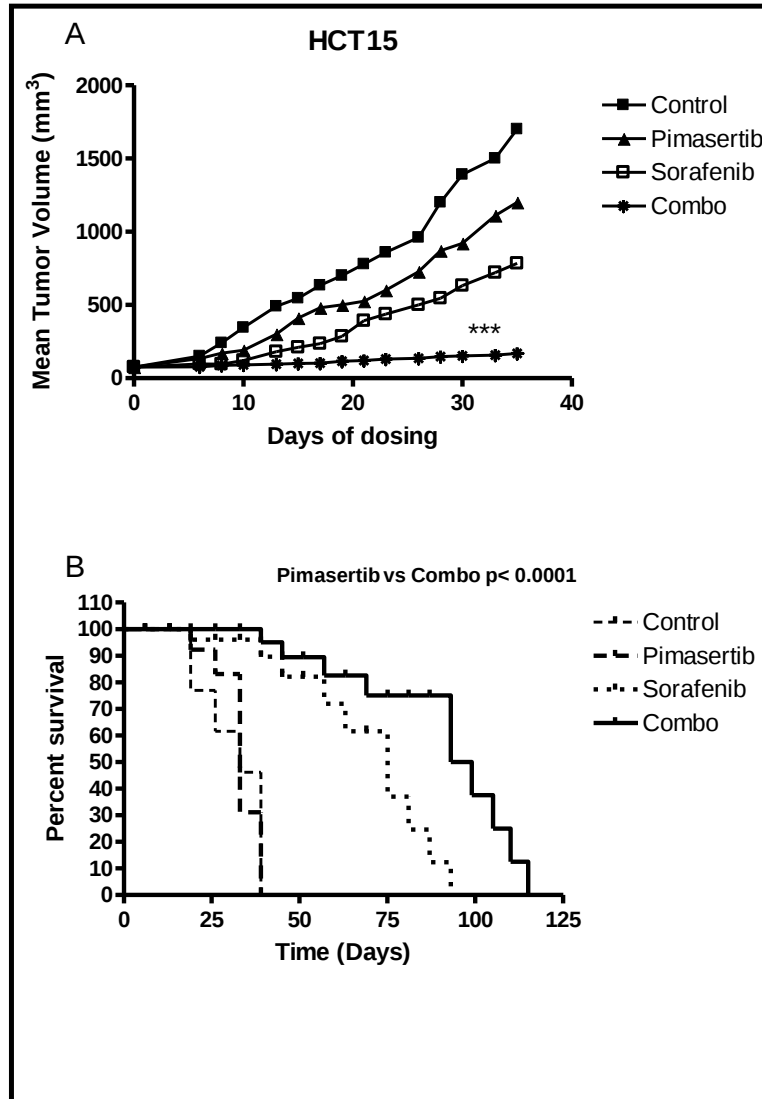
Induction of apoptosis in HCT15 and H1975 cancer cell lines



Pimasertib in combination with BEZ235, a dual PI3K/mTOR inhibitor on HCT15 and H1975 tumor xenografts



Pimasertib in combination with sorafenib inhibitor on HCT15 and H1975 tumor xenografts



Conclusions

- ❖ Basal gene expression profiles identified several up-regulated genes in pimasertib-resistant cancer cells involved in both RAS/RAF/MEK/MAPK and PTEN/PI3K/AKT/mTOR pathways.
- ❖ Combination with PI3K/mTOR inhibitors or with multi-targeted kinase inhibitors is able overcome pimasertib intrinsic resistance in CRC HCT15 and NSCLC H1975 cells both *in vitro* and *in vivo*
- ❖ These data provide the rational of evaluating the therapeutic activity of these combinations in cancer patients in order to better use selective MEK inhibitors, that have shown modest single agent antitumor activity in early clinical trials.

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