Determinants of Gemcitabine-Pemetrexed Synergism in Pancreatic Cancer Cell Lines

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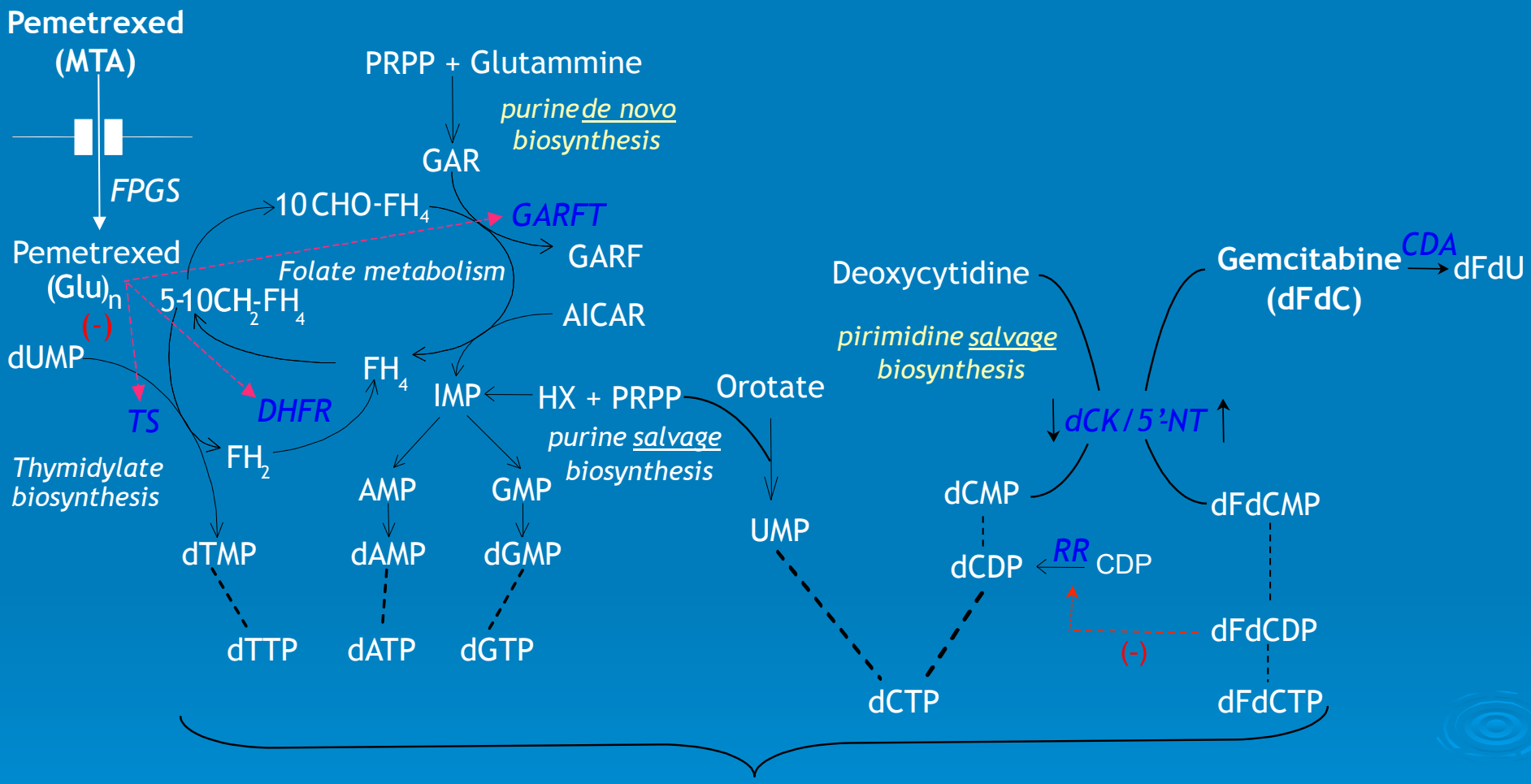
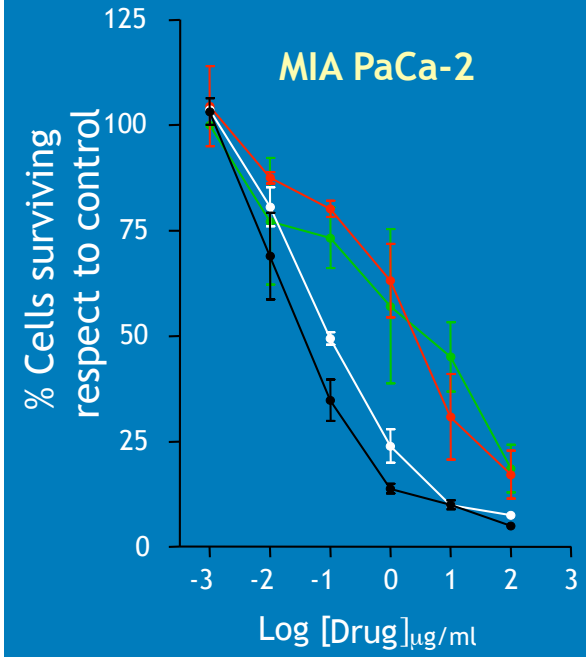
Mechanism of action of gemcitabine and pemetrexed



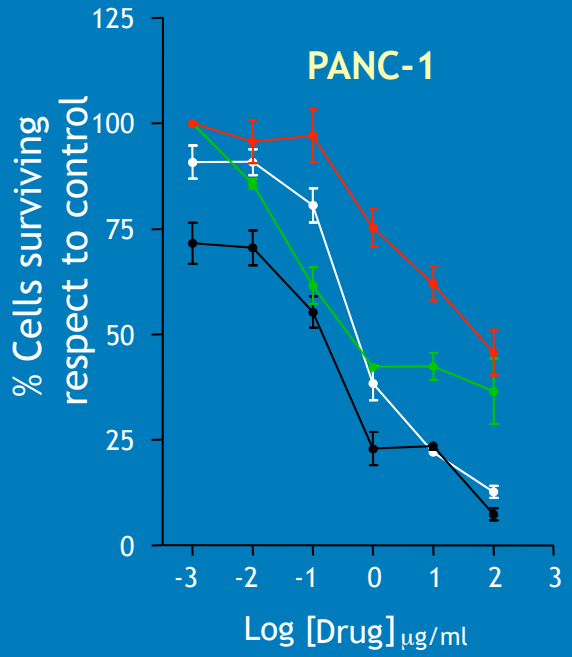
Adjei et al., J Clin Oncol 2000; 8:1748
Shih et al., Cancer Res 1997; 57:1116

Tonkinson et al., Cancer Res 1999; 59:3671
Tesei et al., Clin Cancer Res 2002; 8:233

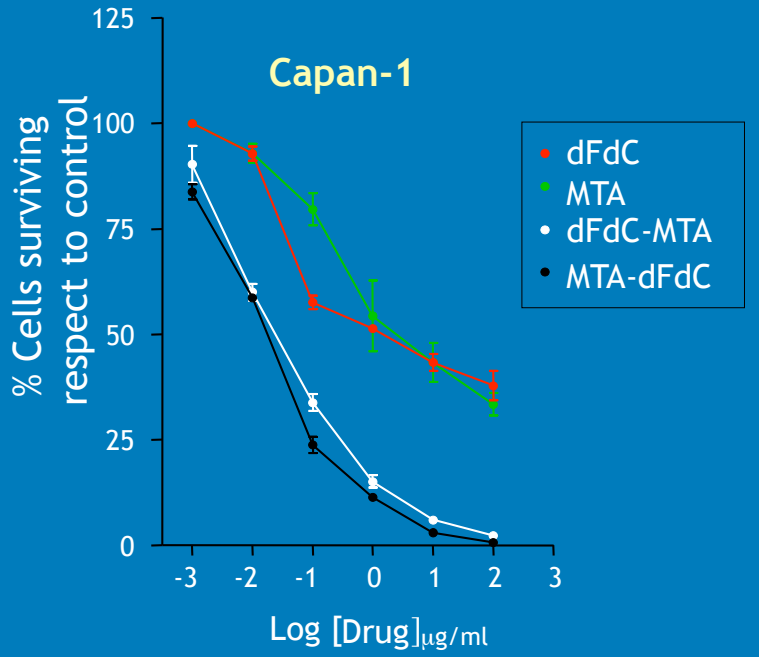
Cytotoxicity and pharmacologic interaction between gemcitabine and pemetrexed



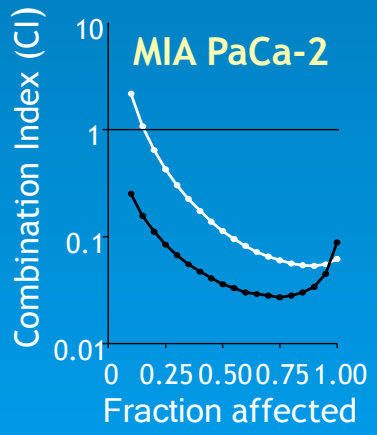
dFdC (IC_{50})= 2.90 µg/ml
MTA (IC_{50})= 1.58 µg/ml
dFdC-MTA (IC_{50})= 0.12 µg/ml
MTA-dFdC (IC_{50})= 0.04 µg/ml

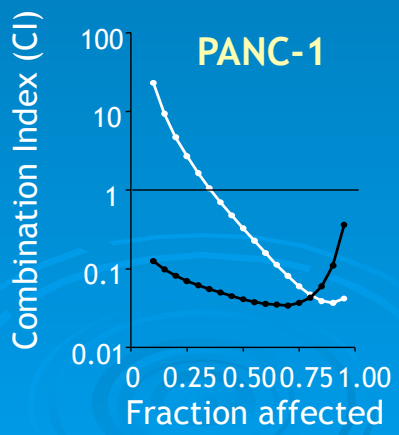


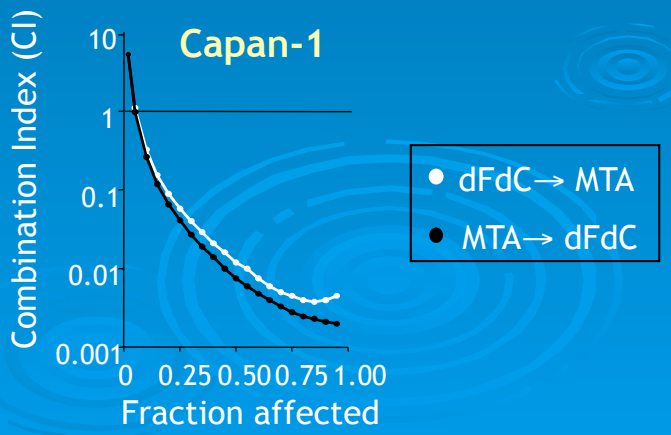
dFdC (IC_{50})= 42.21 µg/ml
MTA (IC_{50})= 2.42 µg/ml
dFdC-MTA (IC_{50})= 0.75 µg/ml
MTA-dFdC (IC_{50})= 0.09 µg/ml



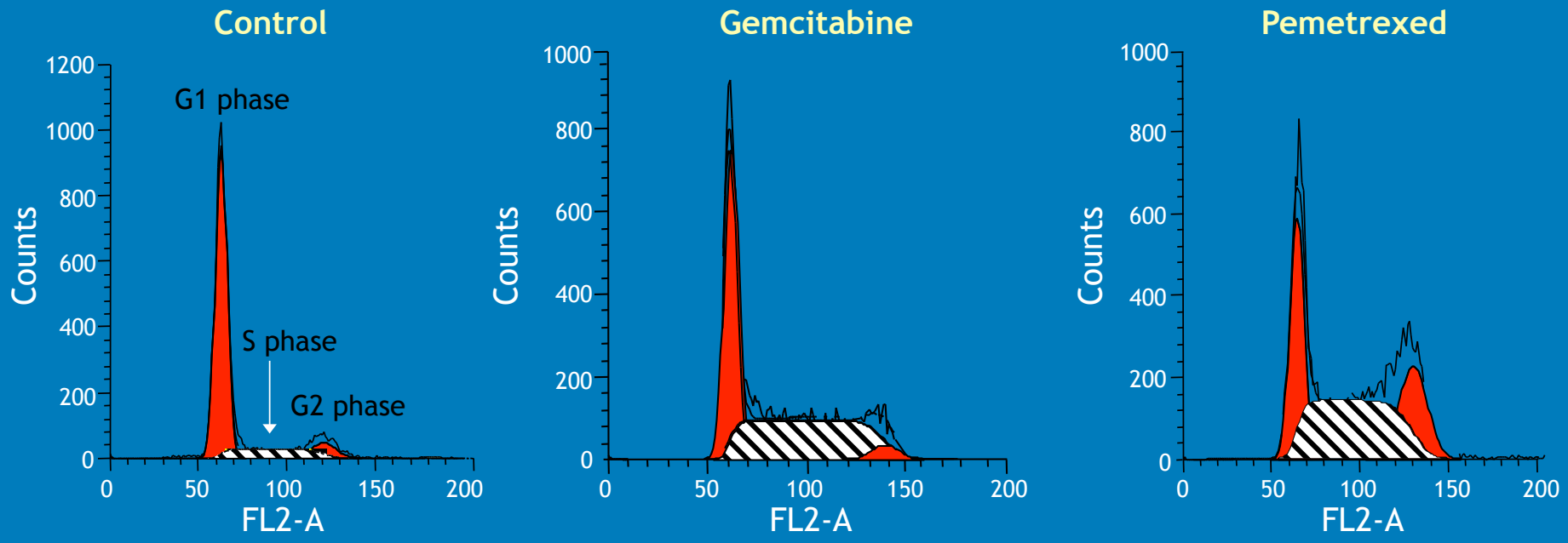
dFdC (IC_{50})= 4.75 µg/ml
MTA (IC_{50})= 7.33 µg/ml
dFdC-MTA (IC_{50})= 0.03 µg/ml
MTA-dFdC (IC_{50})= 0.02 µg/ml







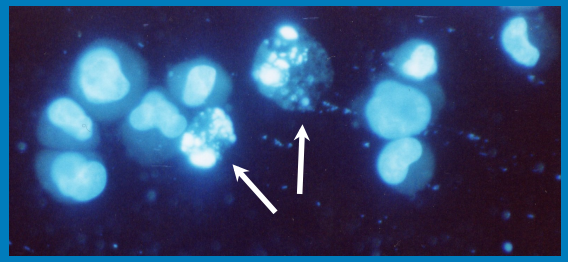
Cell cycle modulation by gemcitabine and pemetrexed

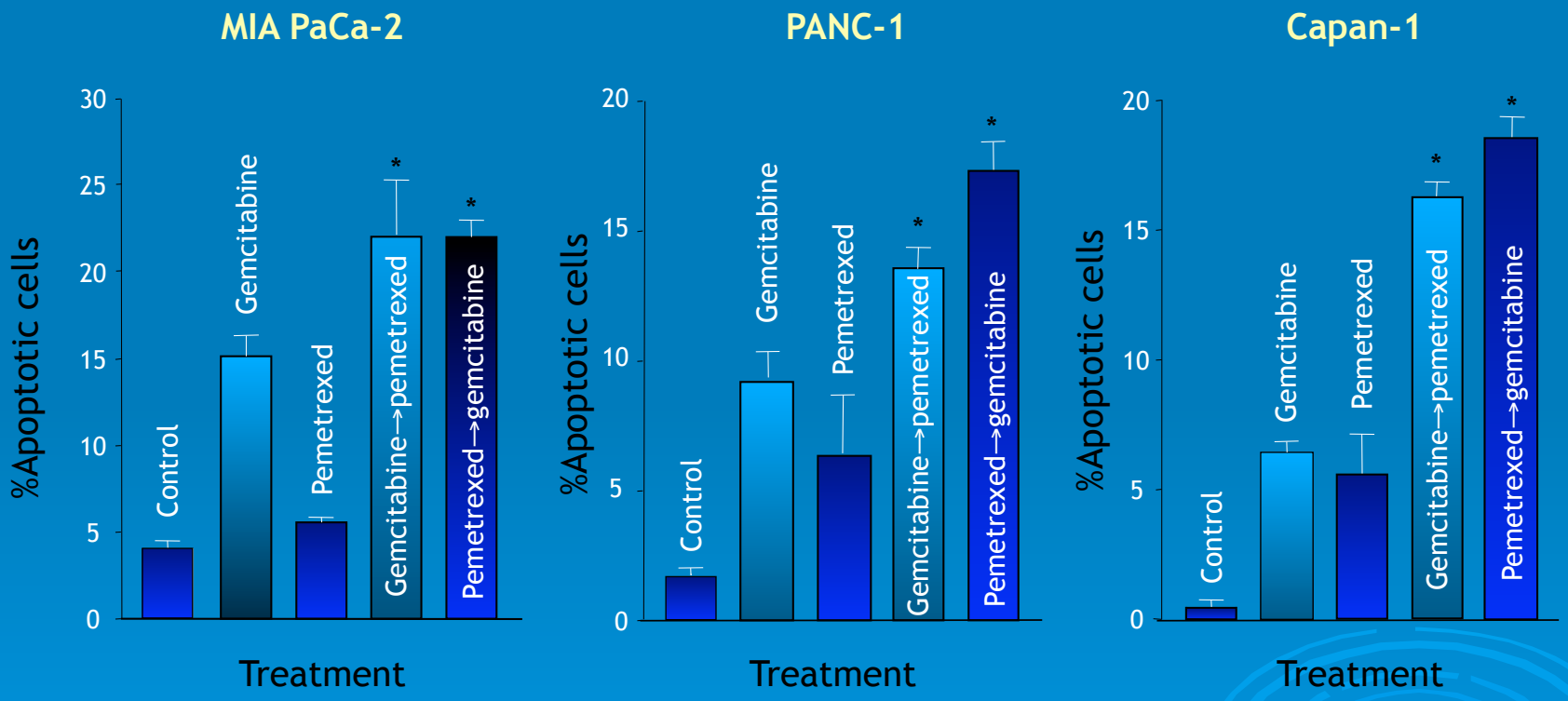


	Treatment	G1 (%)[a]	S (%)	G2 (%)
MIA PaCa-2	Control	77.01	15.30	7.69
	Gemcitabine	46.36	49.29	4.35
	Pemetrexed	30.12	46.63	23.25
PANC-1	Control	88.25	10.55	1.20
	Gemcitabine	66.98	29.29	3.73
	Pemetrexed	17.21	80.13	2.66
Capan-1	Control	50.73	31.13	18.14
	Gemcitabine	54.19	36.40	9.41
	Pemetrexed	31.10	63.21	5.69

[a] Mean percent values of total number of cells examined in three independent experiments

Induction of apoptosis by gemcitabine, pemetrexed and their combination



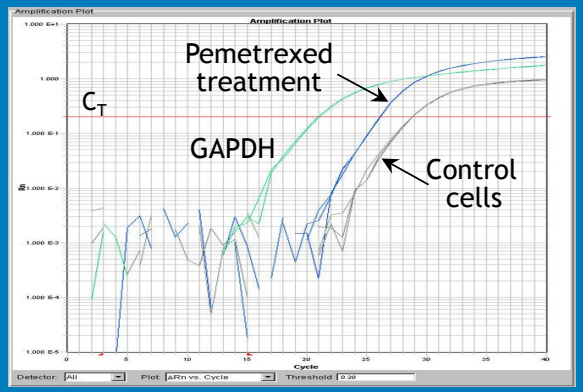


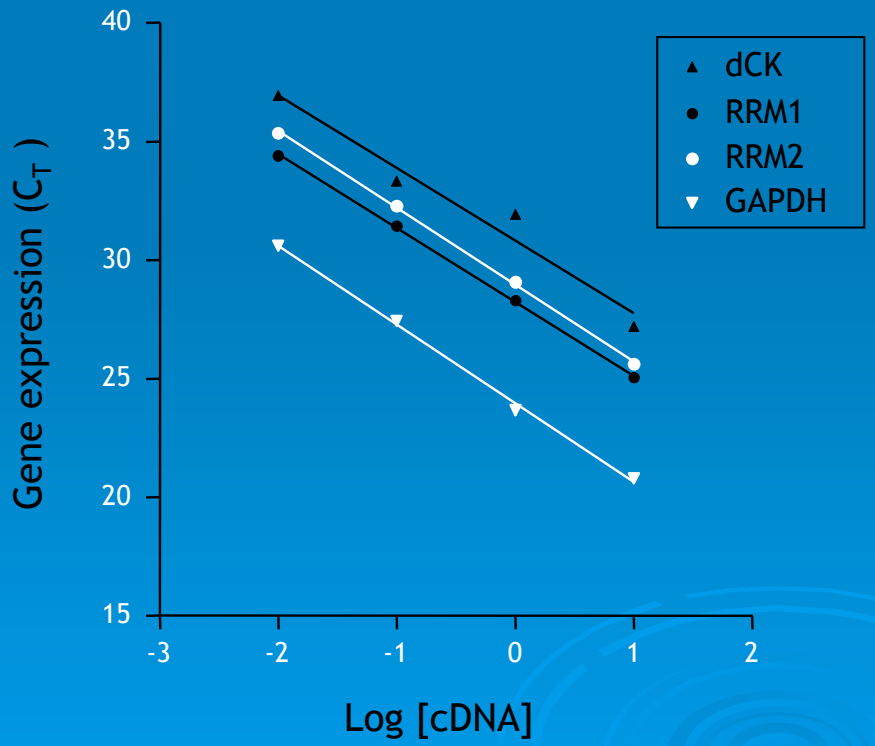
Columns, mean values obtained from three indipendent experiments; *bars*, SE
*Statistically significant different from controls ($P<0.05$)

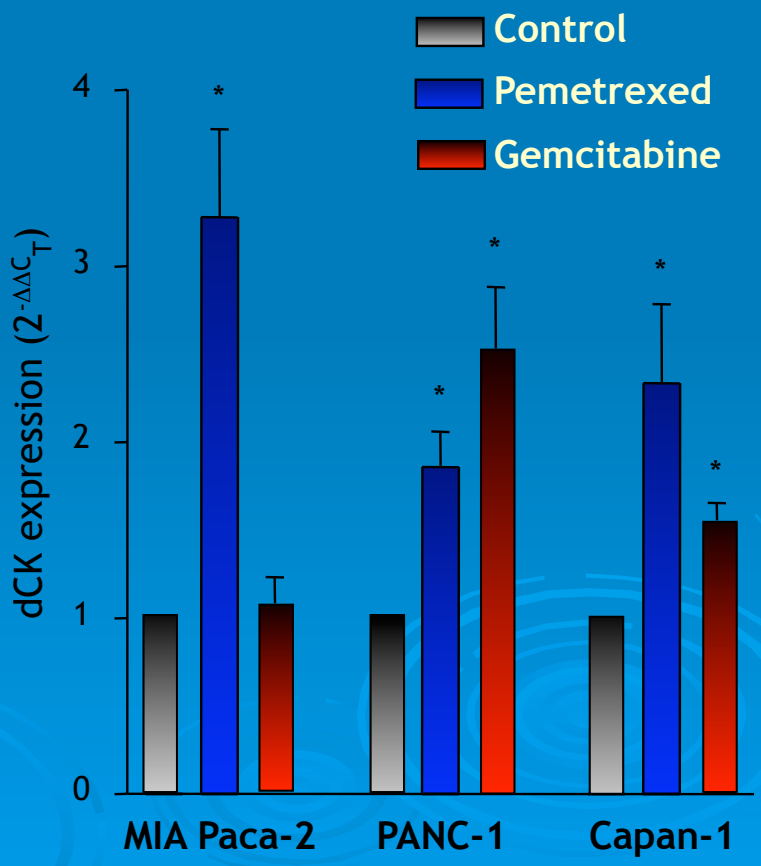
Modulation of dCK expression by pemetrexed

	IC_{50} (ng/ml) [a]			
	Gemcitabine	+dCyd	+DEPC	+THU
MIA PaCa-2	12.29	86.11	10.15	7.54
PANC-1	53.43	503.97	28.03	10.52
Capan-1	29.90	272.53	13.71	9.40

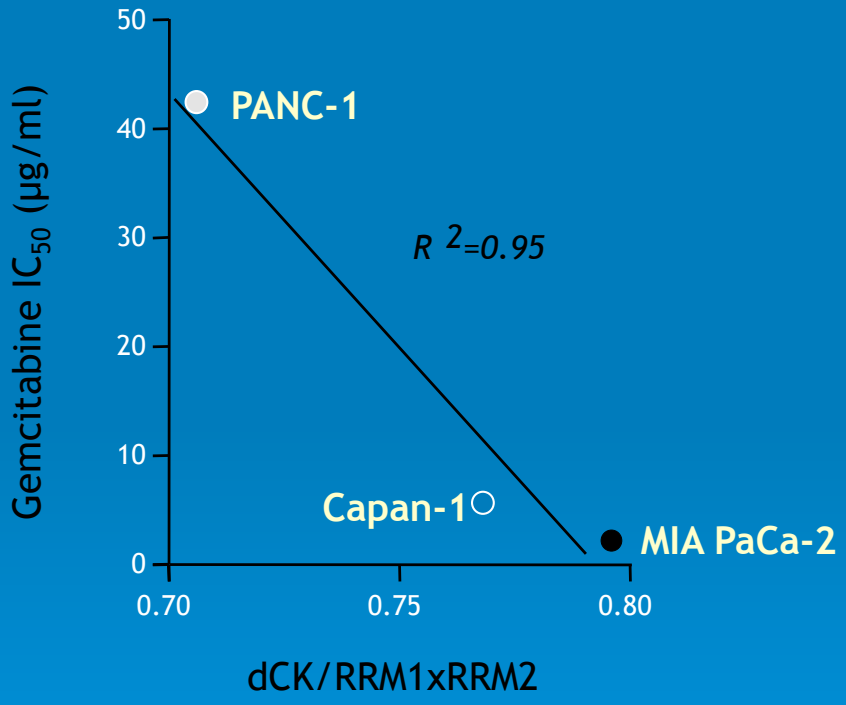
[a] Mean values ±SE of at least three independent experiments

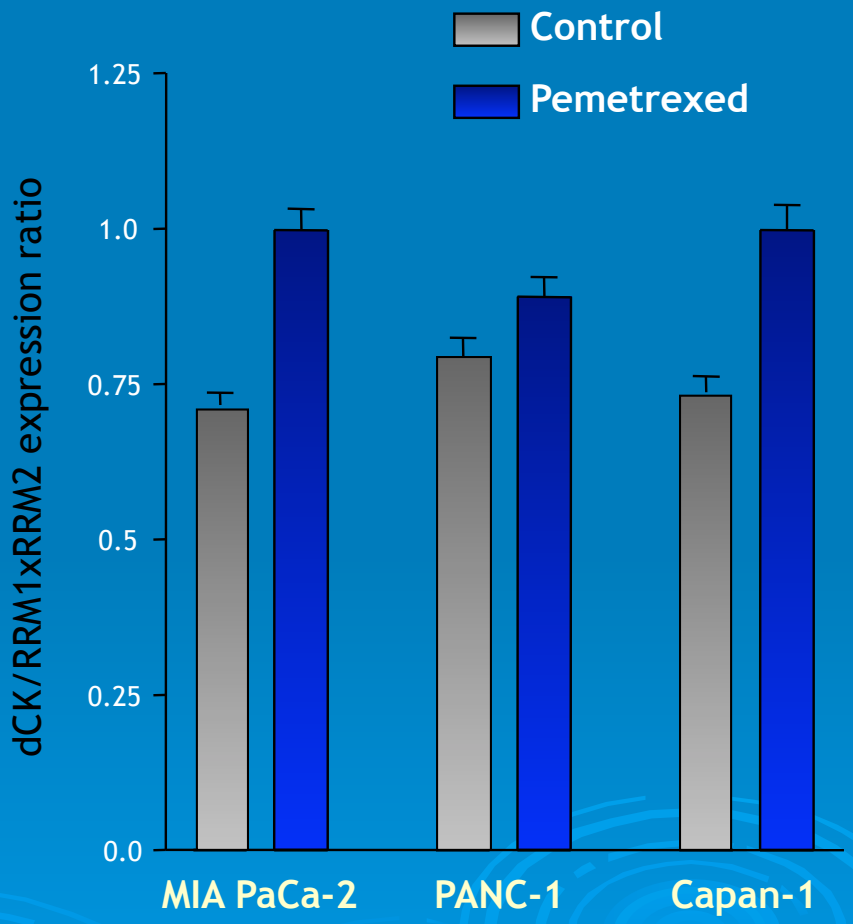






Enhancement of dCK/RRM1×RRM2 expression ratio after pemetrexed treatment





Conclusions

Gemcitabine and pemetrexed were cytotoxic against MIA PaCa-2, PANC-1 and Capan-1 cells and the combination index demonstrated that the drug sequence showing the maximum degree of synergism was pemetrexed→gemcitabine in all cell lines

Flow cytometric studies demonstrated that pemetrexed and gemcitabine enhanced cellular population in S phase in all cell lines

Gemcitabine-pemetrexed combinations increased the occurence of apoptosis

Quantitative RT-PCR analysis showed that pemetrexed significantly enhanced dCK expression in all cell lines, while there was only a minor increase of RR expression

These data provide evidence that the combination of gemcitabine and pemetrexed displays schedule-dependent synergistic cytotoxic activity against various pancreatic cancer cells, associated with favorable modulation of cell cycle, induction of apoptosis and inducible dCK gene expression.