The synergistic combination of curcumin and polydatin improves temozolomide efficacy on glioblastoma cells

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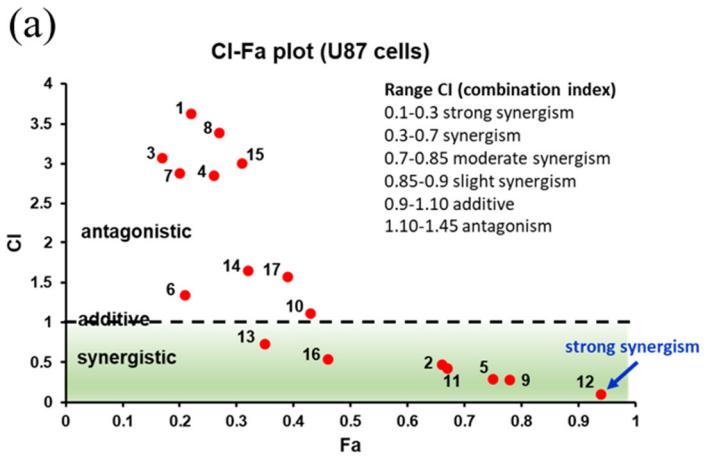
SUPPLEMENTARY FIGURE AND TABLES

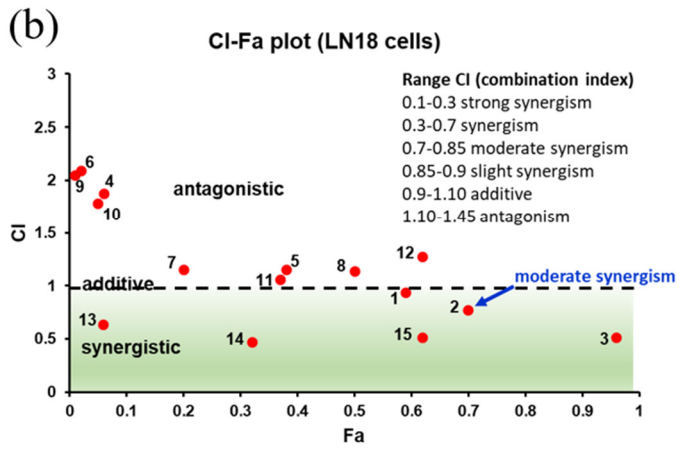
Data from CALCUSYN software



CI values for treatments on U87 cells

GROUP	PLD (µg/ml)	CUR (µg/ml)	CR	Fa	CI
1	8.75	5	1.75:1	0.216	3.626
2	17.5	10	1.75:1	0.661	0.467
3	8.75	2.5	3.5:1	0.171	3.074
4	17.5	5	3.5:1	0.262	2.85
5	35	10	3.5:1	0.747	0.292
6	8.75	1.25	7 :1	0.208	1.344
7	17.5	2.5	7 :1	0.2	2.882
8	35	5	7 :1	0.267	3.388
9	70	10	7 :1	0.777	0.282
10	35	3.5	10:1	0.435	1.11
11	70	7	10:1	0.669	0.426
12	100	10	10:1	0.942	**0.101**
13	17.5	1.25	14:1	0.335	0.731
14	35	2.5	14:1	0.317	1.643
15	70	5	14:1	0.331	3.002
16	35	1.25	28:1	0.458	0.537
17	70	2.5	28:1	0.393	1.570

CR: COMBINATION RATIO
CI: COMBINATION INDEX
Fa: FRACTION AFFECTED (percent of inhibition/100)



CI values for treatments on LN18 cells

GROUP	PLD (µg/ml)	CUR (µg/ml)	CR	Fa	CI
1	75	7.5	10:1	0.589	0.935
2	100	10	10:1	0.697	**0.772**
3	150	15	10:1	0.965	0.514
4	37.5	1.5	25:1	0.06	1.873
5	75	3	25:1	0.38	1.148
6	37.5	0.75	50:1	0.02	2.092
7	75	1.5	50:1	0.2	1.153
8	150	3	50:1	0.5	1.134
9	37.5	0.37 5	100:1	0.01	2.048
10	75	0.75	100:1	0.05	1.780
11	150	1.5	100:1	0.37	1.059
12	300	3	100:1	0.62	1.269
13	37.5	0.18	200:1	0.06	0.630
14	75	0.375	200:1	0.32	0.471
15	150	0.75	200:1	0.62	0.510

CR: COMBINATION RATIO
CI: COMBINATION INDEX
Fa: FRACTION AFFECTED (percent of inhibition/100)

Figure S1. Representative data from CalcuSyn software used to define the synergic concentrations of CUR and PLD in U87 (a) and LN18 (b) glioblastoma cells. Left panels report the CI-Fa plot in which values for synergistic, additive and antagonistic concentrations of CUR and PLD are illustrated; arrows point to the synergic combinations selected for the experiments in the two cell lines. The right tables report the most representative values of CUR and PLD concentrations and combination ratios (CR), faction affected (Fa) and combination index (CI) from which the left plots are obtained; the CI of the selected synergic combinations are marked in red.

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Table S1. Data from cytofluorometric analysis of cell cycle in U87 and LN18 glioblastoma cells. Values increased by the treatments are marked in red

U87 cells	Sub-G1	G1	S	G2/M
CTR	7	71	11	11
CUR+PLD 24h + 72h fresh-medium	4	66	14	16
CUR+PLD 24h + TMZ 72h	3	38	**18**	**40**
TMZ 72h	6	37	15	**42**
LN18 cells	**Sub-G1**	**G1**	**S**	**G2/M**
CTR	5	74	10	10
CUR+PLD 24h + 72h fresh-medium	11	67	13	10
CUR+PLD 24h + TMZ 72h	**20**	46	**20**	**14**
TMZ 72h	**15**	58	**15**	12

Table S2. Data from cytofluorometric analysis of apoptosis/necrosis by Annexin V and propidium iodide (PI) staining in U87 and LN18 glioblastoma cells. Values increased by the treatments are marked in red

U87 cells	% of Necrotic cells ($Annex^{-}/PI^{+}$)	% of Late apoptotic cells ($Annex^{+}/PI^{+}$)	% of Early apoptotic cells ($Annex^{+}/PI^{-}$)
CTR	5	6	1
CUR+PLD 24h + 72h fresh-medium	2	**15**	1
CUR+PLD 24h + TMZ 72h	3	**14**	3
TMZ 72h	3	8	5
LN18 cells	**% of Necrotic cells (PI+/Annex-)**	**% of Late apoptotic cells (Annex+/PI+)**	**% of Early apoptotic cells (Annex+/PI-)**
CTR	9	10	1
CUR+PLD 24h + 72h fresh-medium	**14**	**17**	2
CUR+PLD 24h + TMZ 72h	**10**	**19**	5
TMZ 72h	3	**27**	**10**

Table S3. List of antibodies/reagents used for Western blot analyses.

Antigen	Host	Cat. #	Working dilution	Supplier
MGMT	Mouse (monoclonal)	35-7000	1:500	Thermofisher Scientific
c-Myc	Rabbit (monoclonal)	32072	1:1000	Abcam
LC3B	Rabbit (monoclonal)	3868	1:1000	Cell Signalling Technology
PARP (total and cleaved)	Rabbit (polyclonal)	9542	1:1000	Cell Signalling Technology
GFAP	Mouse	25778	1:1000	BD Biosciences
GAPDH	Rabbit (polyclonal)	25778	1:15000	Santa Cruz