CompuSyn Report

Experiment Name: MDA-231
Date: 14-08-21
File Name: C:\Users\User\Desktop\Esha-MDA-Compusyn (14-08-21).cse
Description Combination of Curcumin and Doxorubicin against breast cancer cell-line (MDA-MB-231), in vitro study

Drug: Curcumin (Cur.) [µM]
Drug: Doxorubicin (Doxo.) [µM]
Drug Combo: Combination (Combi) (Cur.+ Doxo. [100:1])

Data for Drug: Cur. [µM]

Data points usually from the average 'Fa' values of duplicate or triplicate assays

Dose	Effect
10.0	0.05
15.0	0.09
20.0	0.15
25.0	0.21
30.0	0.27
35.0	0.32
40.0	0.35
45.0	0.43
50.0	0.45
55.0	0.54
60.0	0.58
65.0	0.62
70.0	0.65
75.0	0.69
80.0	0.72
85.0	0.76
90.0	0.81
95.0	0.85

Dm

Design Dose Range: Some doses above 'Dm', and some doses below the 'Dm' value (e.g. here Dm= 42.59µM)

The approximate 'Dm' value can be from the wet-lab experiments, preliminary data or from published literature

Fa

'Fa' values cannot be <0.01 or >0.99, unless the assay is very accurate. If, Fa=0 or 1 is entered, the software would crash

18 data points entered.

X-int: 1.62936

Y-int: -2.1288 +/- 0.63532

The 'slope' of the median-effect (ME) plot, the dynamic order, or the 'shape' of

m: 1.30653 +/- 0.38022

Dose-Effect curve; m=1, >1 and <1

Dm: 42.5955

Dm: The median effect dose, in this case it is IC50 value, which indicate “potency” The value can be obtained from the X-intercept of the ME-plot

r: 0.96163

r: The linear correlation coefficient of the ME-plot. It signifies the “conformity” of the data with the mass-action law, an indication of how good are the data, when r=1, it is perfect. For *in vitro* experiment, usually r>0.95 are considered good

Data for Drug: Doxo. [µM]

Dose	Effect
0.25	0.14
0.5	0.18
1.0	0.25
1.25	0.31
1.5	0.35
2.0	0.40
2.25	0.45
2.5	0.53
3.0	0.68
3.25	0.74
3.5	0.78
4.0	0.91

D1 has 18 doses (concentrations), and D2 has 12 concentrations, not the same number is OK as long as they provide m_1, Dm_1 and m_2, Dm_2 values from the Dose-Effect curves

12 data points entered.

X-int: 0.23966

Y-int: -0.3114 +/- 0.08591

m: 1.29928 +/- 0.20967

Dm: 1.73642

r: 0.89072

The m_1, Dm_1 as well as m_2, Dm_2 are absolute requirements for determining synergism or antagonism or additive effect since they are required for the calculation of the CI value

Data for Drug Combo: Combination (Cur.+Doxo. [100:1])

Dose A	Effect
25.0+	0.37
30.0+	0.49
35.0+	0.54
40.0+	0.58
50.0+	0.62
55.0+	0.71
60.0+	0.75
65.0+	0.81
70.0+	0.89

In this case 100:1 means Curcumin 100µM+ Doxorubicin 1µM, etc.

[NOTES]

The constant ratio combination allows computerized simulation of dose-effect curves, Fa-CI effect, Fa-DRI Plot, and isobologram based on the m_1, m_2 and Dm_1, Dm_2 values. When combinations are at non-constant ratios, each “data point” has a ratio, the CI and DRI value can still be calculated, but automated computer simulation can’t be carried out; therefore, the acquired conclusions are limited

9 data points entered.

X-int: 1.52437

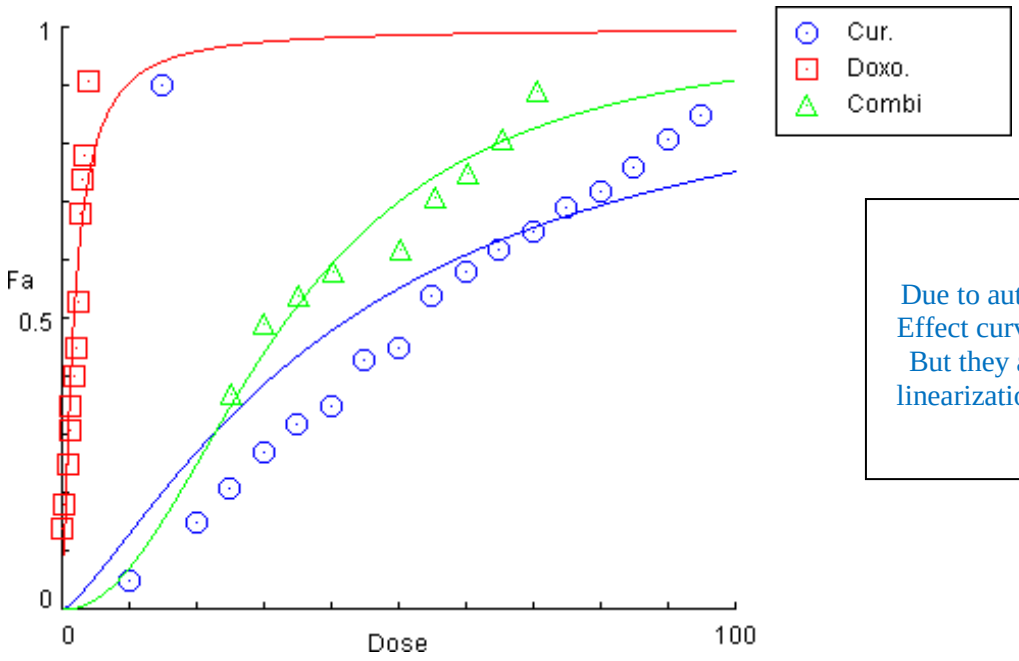
Y-int: -3.2306 +/- 0.43470

m: 2.11928 +/- 0.26090

Dm: 33.4478

r: 0.95083

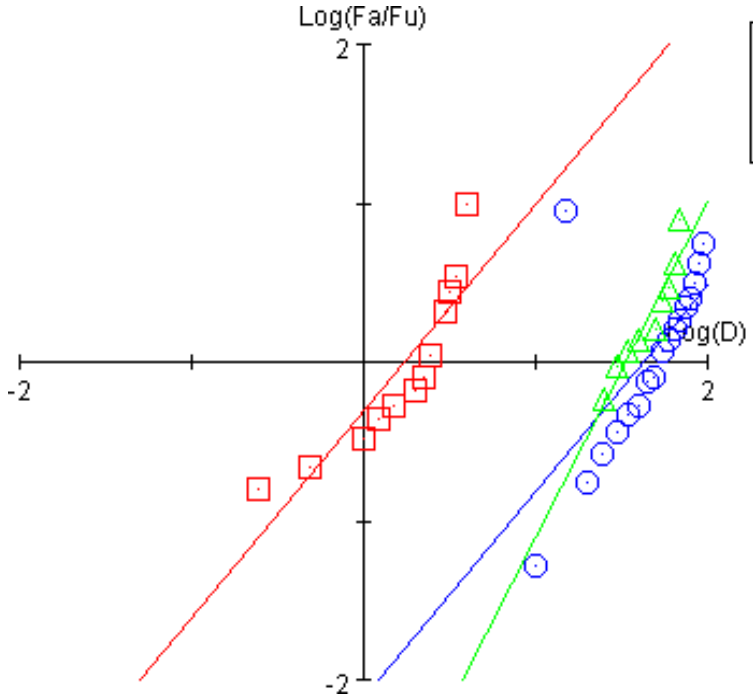
Dose-Effect Curve



[NOTES]

Due to automated scaling, the Dose-Effect curves are somewhat jammed. But they are clearly separated after linearization by the media-effect plot (Find below)

Median-Effect Plot



[NOTES]

The ‘slope’ (m) signifies the shape. The x-intercept (logDm) signifies the ‘potency’ (the antilog gives the Dm value)

CI Data for Drug Combo: Combination (Cur.+Doxo. [100:1])

Fa	CI Value	Total Dose
0.05	2.30351	8.33638
0.1	1.84868	11.8604
0.15	1.61331	14.7538
0.2	1.45609	17.3892
0.25	1.33786	19.9175
0.3	1.24245	22.4251
0.35	1.16171	24.9753
0.4	1.09092	27.6234
0.45	1.02710	30.4260
0.5	0.96819	33.4478
0.55	0.91265	36.7697
0.6	0.85926	40.5002
0.65	0.80690	44.7944
0.7	0.75446	49.8885
0.75	0.70066	56.1695
0.8	0.64377	64.3360
0.85	0.58104	75.8282
0.9	0.50706	94.3266
0.95	0.40695	134.201
0.97	0.34799	172.468

[NOTES]

CI<1indicates synergism,
CI=1 indicates additive effect and CI>1 indicates antagonism.

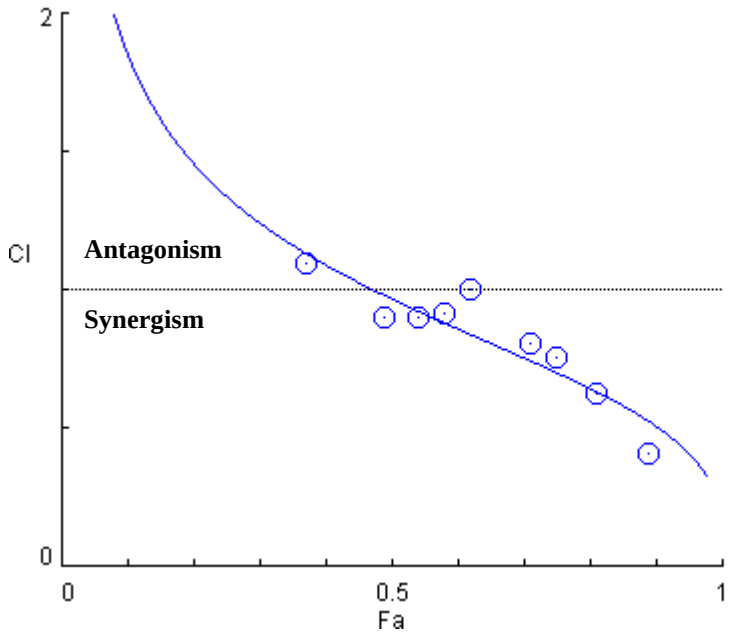
This is Fa-CI table with Fa increment of 0.05
At Fa>0.5 showed synergistic effect (CI<1)
For anti-cancer agents, synergism (CI<1) at high dose (high effect) is more relevant to the therapy than the CI values at low dose (low effect)

CI values for actual experimental points:

Total Dose	Fa	CI Value
25.25	0.37	1.09890
30.3	0.49	0.90437
35.35	0.54	0.90495
40.4	0.58	0.91320
50.5	0.62	1.00456
55.55	0.71	0.80969
60.6	0.75	0.75593
65.65	0.81	0.62562
70.7	0.89	0.41236

The CI values for each individual combination data point without a simulation

Combination Index Plot



Combi | 100:1 (100μM vs 1μM)

[NOTES]

Among 9 combination data points 7 of them are on the synergy side (CI<1), the other 2 points are nearly additive

The simulation at low Fa showed substantial antagonism. This is of less concern since CI values for synergism is 0-1, and for antagonism is 1-∞ low Fa is less relevant to therapy than high Fa (i.e. Killing cancer cells in small fraction is not useful in cancer therapy)

DRI Data for Drug Combo: Combination (Cur.+Doxo. [100:1])

Fa	Dose Cur.	Dose Doxo.	DRI Cur.	DRI Doxo.
0.05	4.47325	0.18008	0.54196	2.18171
0.1	7.92500	0.32005	0.67487	2.72544
0.15	11.2922	0.45693	0.77303	3.12802
0.2	14.7420	0.59741	0.85624	3.46990
0.25	18.3731	0.74548	0.93168	3.78027
0.3	22.2699	0.90456	1.00301	4.07405
0.35	26.5212	1.07829	1.07252	4.36061
0.4	31.2310	1.27094	1.14190	4.64697
0.45	36.5309	1.48792	1.21265	4.93920
0.5	42.5955	1.73642	1.28623	5.24336
0.55	49.6669	2.02643	1.36426	5.56625
0.6	58.0953	2.37239	1.44879	5.91629
0.65	68.4123	2.79624	1.54252	6.30480
0.7	81.4719	3.33328	1.64941	6.74827
0.75	98.7519	4.04460	1.77569	7.27270
0.8	123.076	5.04702	1.93214	7.92323
0.85	160.676	6.59871	2.14013	8.78921
0.9	228.943	9.42095	2.45140	10.0875
0.95	405.605	16.7439	3.05259	12.6015
0.97	609.297	25.2097	3.56815	14.7632

[NOTES]

DRI>1 and <1 indicate favorable and not favorable Dose-reduction,
DRI=1 indicates no Dose-reduction

This is Fa-DRI table with Fa increment of 0.05

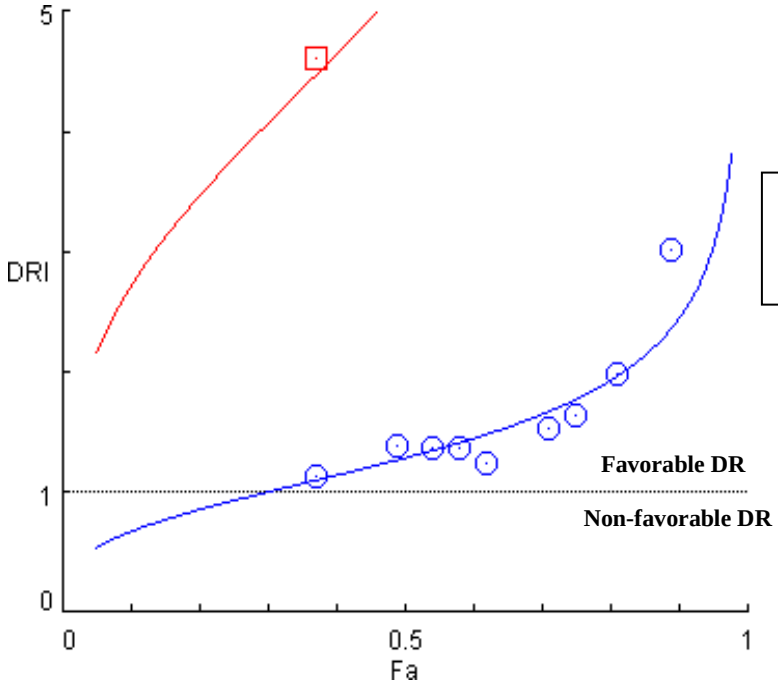
At 50% inhibition, it requires 42.5955μM of Curcumin, and requires 1.73642μM of Doxorubicin.
However, it requires 1.28623-fold less Curcumin plus 5.24336-fold less Doxorubicin to achieve the same 50% inhibition (i.e., **33.117μM Cur. + 0.331μM Doxo.**) (100:1 combination)

DRI values calculated at experimental points

Fa	Dose Cur.	Dose Doxo.	DRI Cur.	DRI Doxo.
0.37	28.3435	1.15281	1.13374	4.61124
0.49	41.3110	1.68377	1.37703	5.61258
0.54	48.1573	1.96450	1.37592	5.61285
0.58	54.5324	2.22610	1.36331	5.56525
0.62	61.9570	2.53099	1.23914	5.06198
0.71	84.5263	3.45895	1.53684	6.28900
0.75	98.7519	4.04460	1.64586	6.74099
0.81	129.226	5.30069	1.98810	8.15491
0.89	211.024	8.67964	3.01463	12.3995

DRI values of each drug at each combination data point

DRI Plot for Combo: Combination (Cur.+ Doxo. [100:1])

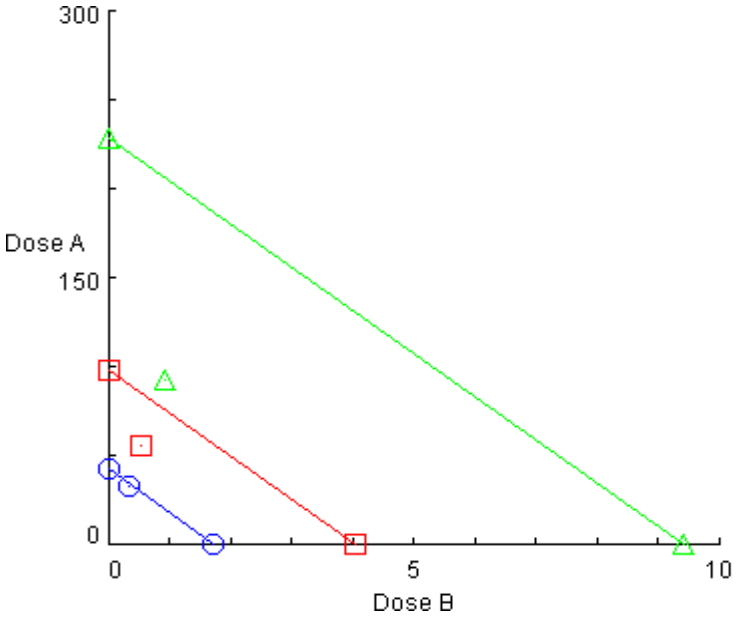


DRI values for Cur. (○) and Doxo. (□) are shown.
All combinations show favorable DRI (>1).
The simulation just to show the trends

In this case, Cur. (Blue circles) is very potent and toxic to cancer cells
Favorable Dose-reduction is very beneficial to Curcumin

Isobologram for Combo: Combi (Cur.+Doxo. [100:1])

Isobologram is for visual, not for quantitative determination of synergism or antagonism



[NOTES]

Isobolograms for 50%, 75% and 90% inhibition are shown. There are options in menu to select other Fa levels before the beginning of data entries Combination data point on the diagonal line indicates additive effects, on the lower left indicates synergism, and on the upper right indicates antagonism. In the present case, IC_{90}, IC_{75} showed highly synergism, and at IC_{50} showed the slightly synergism effect.

Summary Table

Most of the contents are used for constructing Table: 1

Experiment Name: Esha Sarkar
Date: 14-08-21
File Name: C:\Users\User\Desktop\Esha-MDA-Compusyn (14-08-21).cse
Description Combination of Curcumin and Doxorubicin against breast cancer cell-line (MDA-MB-231), in vitro study

Drug: Curcumin (Cur.) [µM]
Drug: Doxorubicin (Doxo.) [µM]
Drug Combo: Combination (Combi) (Cur.+Doxo. [100:1])

Drug/Combo	Dm	m	r
Cur.	42.5955	1.30653	0.65163
Doxo.	1.73642	1.29928	0.89072
Combi	33.4478	2.11928	0.95083

From 'Parameters' in Table: 1

CI values at:

Combo	ED50	ED75	ED90	ED95
Combi	0.96819	0.70066	0.50706	0.40695

From the 'Fa-CI Table' of simulation of CI values
For numbers given in CI column of Table 1 (at bottom)

Data for Fa = 0.5

Drug/Combo	CI value	Dose Cur.	Dose Doxo.
Cur.		42.5955	
Doxo.			1.73642
Combi	0.96819	33.1166	0.33117

These data are illustrated for the ED_{50}- Isobologram at Fa=0.5 (in Fig.1d)
For DRI at Fa=0.5 (bottom of Table: 1)
For Curcumin=42.5955/1.28623 =33.1166
For Doxorubicin=1.73642/5.24336=0.33117

Data for Fa = 0.75

Drug/Combo	CI value	Dose Cur.	Dose Doxo.
Cur.		98.7519	
Doxo.			4.04460
Combi	0.70066	55.6134	0.55613

For ED_{75}-isobologram in Fig. 1d

Data for Fa = 0.9

Drug/Combo	CI value	Dose Cur.	Dose Doxo.
Cur.		228.943	
Doxo.			9.42095
Combi	0.50706	93.3927	0.93393

For ED_{90}-isobologram in Fig. 1d

Data for Fa = 0.95

Drug/Combo	CI value	Dose Cur.	Dose Doxo.
Cur.		405.605	
Doxo.			16.7439
Combi	0.40695	132.873	1.32873

Synergy (CI<1) at high effect levels, (e.g., at Fa>0.90) is more relevant for anticancer (therapeutic) effect than the CI at low effect levels (e.g., at Fa<0.3)

Data for Fa = 0.97

Drug/Combo	CI value	Dose Cur.	Dose Doxo.
Cur.		609.297	
Doxo.			25.2097
Combi	0.34799	170.760	1.70760

[NOTES for manual calculation]

From the above Report, we obtain:

m_1=1.30653, $(Dm)_1$=42.5955μM;
m_2=1.29928, $(Dm)_2$=1.73642μM;
$m_{1,2}$=2.11928, $(Dm)_{1,2}$=33.4478; Cur.+ Doxo. [100:1]= **33.117μM Curcumin + 0.331μM Doxorubicin**

All parameters are calculated from the median-effect principle and equation of the mass-action law
$fa/fu = (D/Dm)^m$ m or $D = Dm\,[fa/(1-fu)]^{1/m}$ (Chou equation)
log(fa/fu) = m log (D) – m log (Dm)

Thus, the Median-effect Plot (MEP): x = log(D) y = log(fa/fu)
Gives the slope m, and the x-intercept log(Dm), then the antilog of the X-intercept gives the Dm value.

Based on the Combination index Theorem (CIT) and the Median-Effect Equation and Plot, when the combination $D_{1,2}$ for D_1 and D_2 is P/Q, we got:

$$CI = \frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2} = \frac{(D)_{1,2}[P/(P+Q)]}{(D_m)_1\,[f_a/(1-f_a)]^{1/m_1}} + \frac{(D)_{1,2}[Q/(P+Q)]}{(D_m)_2\,[f_a/(1-f_a)]^{1/m_2}}$$

Therefore, substituting, the m and Dm parameters, combination ratio P/Q into the corresponding equations given above, and setting fa=0.01-0.99, the CI values at all effect levels can be simulated as Fa-CI table or Fa-CI Plot. The default setting for the CompuSyn is fa=0.05, 0.1, 0.15…0.95 and 0.97

Based on the dose-reduction index (DRI) equations:

$$(DRI)_1 = \frac{(D_x)_1}{(D)_1}, \quad (DRI)_2 = \frac{(D_x)_2}{(D)_2}$$

$$(DRI)_1 = \frac{(D_m)_1[f_a/(1-f_a)]^{1/m}{}_1}{(D)_1}, \quad (DRI)_2 = \frac{(D_m)_2[f_a/(1-f_a)]^{1/m}{}_2}{(D)_2}$$

Similarly, $(DRI)_1$ and $(DRI)_2$ values at a particular combination data point can be determined or at different fa value can be simulated.