Appendix I. Example I-B. Table of Crude Data for 2- and/or 3- Drug Combinations and Summary of Results. Cancer Cell System in Vitro.

Table 11. Example of experimental design and dose-effect relationships of paclitaxel, cisplatin, and topotecan and their two- and three-drug combinations on growth inhibition of 833K teratocarcinoma cells after 96 hours' exposure **(Adopted from Chou et al, 1994, *J Natl Cancer Inst* 86: 1517-1524. For Details of Analysis See CompuSyn Report for Appendix I-B)[*]**

Drug, μM				Parameter[†]			
Paclitaxel	Cisplatin	Topotecan	Fractional Inhibition, f_a	m	D_m, μM	r	CI[‡]
$(D)_1$							
0.002			0.429				
0.004			0.708				
0.005			0.761				
0.01			0.882				
0.02			0.932	1.248	0.00217	.990	
	$(D)_2$						
	0.05		0.055				
	0.1		0.233				
	0.2		0.301				
	0.5		0.559				
	1.0		0.821				
	2.0		0.953	1.459	0.320	.986	
		$(D)_3$					
		0.01	0.069				
		0.02	0.213				
		0.05	0.373				
		0.1	0.785				
		0.2	0.940				
		0.5	0.991	1.855	0.0462	.991	
$(D)_1$+$(D)_2$ (1:100)[§]							
0.001	0.1		0.450				0.900
0.002	0.2		0.701				0.815
0.005	0.5		0.910		0.0001147		0.681[‖]
0.01	1.0		0.968	1.572	+ 0.11471	.999	0.602
	$(D)_2$+$(D)_3$ (100:10)						
	0.05	0.005	0.304				0.445
	0.1	0.01	0.413				0.658
	0.2	0.02	0.675				0.669
	0.5	0.05	0.924		0.1053		0.561
	1.0	1.0	0.977	1.588	+ 0.01053	.989	0.522
$(D)_1$	+	$(D)_3$ (1:10)					
0.001		0.01	0.274				1.373
0.002		0.02	0.579				1.078
0.005		0.05	0.901		0.00166		0.719
0.01		0.1	0.965	1.891	+ 0.01661	.999	0.681
$(D)_1$+$(D)_2$+$(D)_3$ (1:100:10)							
0.001	0.1	0.01	0.456				1.121
0.002	0.2	0.02	0.806		0.001162		0.729
0.003	0.3	0.03	0.947		+ 0.11616		0.403
0.005	0.5	0.05	0.995	3.363	+ 0.011612	.984	0.136

[*] Experimental data were subjected to automated calculation of m, D_m and r parameters as well as plots simulations using a software, CompuSyn (Chou and Martin, 2005).

[†] The parameters m, D_m, and r are the slope, antilog of x-intercept, and the linear correlation coefficient of the median-effect plot, which signifies the shape of the dose-effect curve, the potency (IC_{50}), and the conformity of the data to the mass-action law, respectively. D_m and m values are used for calculating the CI values.

[‡] CI<1, C1=1, and CI>1 indicate synergism, additivity, and antagonism, respectively. As based on the classic isobologram equation, CI can be calculated by Eq. 16: $CI = [(D)_1/(D_x)_1] + [(D)_2/(D_x)_2]$, where $D_x = D_m[f_a/(1-f_a)]^{1/m}$ (Eq. 8).

§ Drug mixture was serially diluted and added to incubation mixture at 0 hour. The combination ratio was approximately equal to the D_m ratio of the component drugs (i.e., close to their equipotency ratio).

‖ Sample pocket calculator for calculation of the CI value of 0.005 μM paclitaxel + 5 μM cisplatin that inhibited 833K cell growth by 91.0% (f_a = 0.910). On the basis of Eq. 8, for paclitaxel alone to inhibit cell growth by 91% would require $[D_{0.91}]_{paclitaxel} = (D_m)_{paclitaxel}\,[0.91/(1\text{-}0.91)]^{1/1.248}$ = 0.00217 μM x 6.385 = 0.01385 μM and for cisplatin alone to inhibit cell growth by 91% would require $[D_{0.91}]_{cisplatin} = (D_m)_{cisplatin}\,[0.91/(1\text{-}0.91)]^{1/1.458}$ = 0.320 μM x 4.888 = 1.564 μM. Therefore,

$$CI = \frac{0.005\,\mu M}{0.01385\,\mu M} + \frac{0.5\,\mu M}{1.564\,\mu M} = 0.681 \text{ at 91\% inhibition.}$$

Comments by T.C. Chou on Example I-B:

1. **The left three columns actually showed experimental design for 3 drugs: A, B, and C. The dose-effect curves for A, B, and C each alone is absolutely required since their parameters m (shape), D_m (IC_{50} for potency) and r (how good are the data) can be automatically determined, where m and D_m values for each drug are required for CI calculation in combinations.**

2. **Note that A:B:C = 1:100:10 which is not too far from the IC_{50} ratios. So, each drug contributed the effect significantly or nearly equally in the combinations (in this case, the mixtures).**
   Also note the A:B = 1:100, B:C = 100:10, and A:C = 1:10, are all in constant ratio combinations and are in correspondence with A:B:C = 1:100:10 for most simple and efficient analysis.

3. **The 2- and 3- drug combinations can be done at the same time, and the single-drug parameters can be shared for combinations. We can do only 2-drug combinations only or 3-drug combinations only. But the present design allows the dissections of 3-drug outcome with 2-drug components combination outcomes (i.e., A+B+C dissect with A+B, B+C, and A+C).**

4. **It is suggested that this large size experiment to be carried out at one time so that the assay conditions would be nearly the same.**

5. **The right column only presents the CI values for each of the actual drug combination data points for synergism (CI<1) or antagonism (CI>1). The CI values at any effect (or dose) levels are shown in CompuSyn Report's Fa-CI tables and Fa-CI plot (Chou-Talalay plot).**