CrownSyn™ Service
Fixed Ratio Design Application Guide

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Combination therapies are an increasingly important treatment modality for several complex conditions including cancer. To make it easier for researchers to evaluate and quantify two-drug combination effects (synergistic, antagonistic, and additive) for *in vitro* cell-based assays, CrownBio has built a bioinformatics service, CrownSyn.

This application guide demonstrates how the CrownSyn service is applied to drug combination studies following a Fixed Ratio Design.

Fixed Ratio Design Principle

In this experiment, efficacy is measured by percent inhibition of cancer cell proliferation for two drugs individually, and in combination for a series of concentrations. In the combination treatment, the concentration ratio of the two drugs is fixed. Dose-response data, as shown in **Table 1**, are the input data used by the CrownSyn service to evaluate combination effects.

Table 1: Dose-Response Data for Drug A, B, and the Combination of Drugs A and B (Combo).
Percent cancer cell proliferation inhibition is provided as a measure of efficacy.

Drug	Drug A Concentration (nM)	Drug B Concentration (nM)	% Inhibition
A	40	0	100.00
A	20	0	100.00
A	10	0	99.96
A	5	0	64.41
A	2.5	0	35.61
A	1.25	0	20.03
A	0.625	0	12.54
B	0	40	100.00
B	0	20	99.94
B	0	10	96.32
B	0	5	48.10
B	0	2.5	27.99
B	0	1.25	19.71
B	0	0.625	13.43
Combo	40	40	100.00
Combo	20	20	99.99
Combo	10	10	99.97
Combo	5	5	99.93
Combo	2.5	2.5	91.60
Combo	1.25	1.25	29.97
Combo	0.625	0.625	21.66

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Methods and Results

The combination index (CI) is calculated based on the IC_{50} equivalence method (Zhao *et al*).
The combination effect is readily visualized by examining the shift of the combination curve with respect to the two individual drug curves:

- A left shift indicates a synergistic effect (as shown in **Figure 1**)
- A right shift means an antagonistic effect
- No shift means an additive effect.

To further characterize combination effects, the CrownSyn service generates the CI, along with its 95% confidence interval, which is plotted against efficacy (% cancer cell proliferation inhibition) of the combination treatment **(Figure 2)**.

Figure 1: Resulting Dose-Response Curves using IC_{50}-Normalized Drug Concentrations.

Three individual curves are displayed together to show the response for Drug A, Drug B, and the Drug A and B combination (Combo).

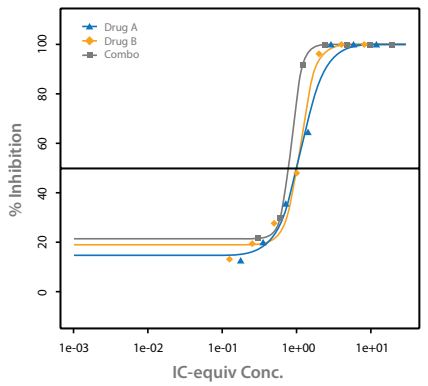
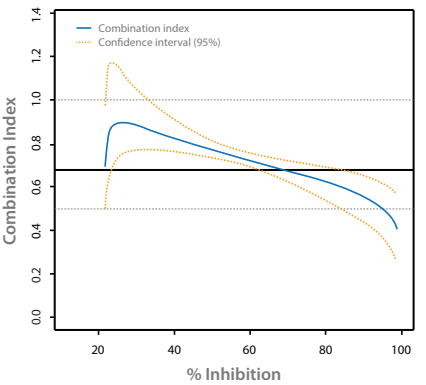


Figure 2: Combination Treatment Combination Index vs Efficacy.

Drug A and B CI and 95% confidence interval shown against percent cancer cell proliferation inhibition for the combination treatment.



Conclusions

Drug A and B work synergistically to inhibit cancer cell proliferation, with the synergistic effect becoming stronger with increased concentrations of Drug A and Drug B. Downstream pharmacology analysis should therefore be conducted to further understand the dosing parameters, safety, and efficacy of the combination therapy.

Acknowledgements

He *et al*. (2018) Methods for High-throughput Drug Combination Screening and Synergy Scoring. In: von Stechow L. (eds) Cancer Systems Biology. Methods in Molecular Biology, vol 1711. Humana Press, New York, NY.
Zhao *et al*. Evaluation of combination chemotherapy: integration of nonlinear regression, curve shift, isobologram, and combination index analyses. *Clinical Cancer Research* 2004;10(23):7994-8004.