CrownSyn™ Service
Matrix Design Application Guide

CrownBio
CONNECTING SCIENCE TO PATIENTS

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 matrix design, also called grid or checkerboard design.

Matrix Design Principle

In this experiment, efficacy is measured for two drugs individually at a series of concentrations, and for all concentration combinations of the two drugs.

Dose-response data, as shown in **Table 1**, are the input data used by the CrownSyn service to evaluate combination effects. The percent inhibition of cancer cell proliferation is given for each combination.

Table 1: Dose-Response Matrix. Percent inhibition of cancer cell proliferation is shown at combined doses of Drug A and Drug B. The bold text in the first column are the concentrations of Drug A, while the bold text in the first row are the concentrations for Drug B.

A:B	0	2.5	5	10	20	40	80	160
0	0.000	0.023	0.015	0.081	0.249	0.365	0.436	0.469
1.5625	0.227	0.234	0.257	0.266	0.415	0.493	0.556	0.541
3.125	0.122	0.150	0.153	0.181	0.331	0.407	0.504	0.496
6.25	0.212	0.211	0.233	0.272	0.420	0.474	0.551	0.563
12.5	0.120	0.127	0.186	0.232	0.392	0.450	0.509	0.538
25	0.315	0.347	0.374	0.459	0.597	0.640	0.638	0.636
50	0.666	0.678	0.699	0.757	0.785	0.783	0.777	0.750
100	0.851	0.853	0.854	0.854	0.854	0.848	0.845	0.837

Methods and Results

The CrownSyn service automatically chooses the appropriate method to calculate synergy based on the two single-drug dose-response curves. Specifically, if both curves are accurately estimated and their Hill slopes are not significantly different (constant relative potency), then CrownSyn uses the Loewe Additivity model. Otherwise, the CrownSyn service uses the Bliss Independence model.

The Loewe Additivity model rests on both the dose equivalence and sham combination principles, which cannot be satisfied if the two dose-response curves are not available or difficult to estimate. CalcuSyn®, a widely used commercial software, uses the Loewe Additivity model exclusively.

The CrownSyn service generates dose-response curves for the two drugs and an inhibition heat map for all concentration combinations. In addition, a 2D contour map and 3D response surface plot are created to further illustrate the combinations that achieve optimal efficacy.

CrownSyn Service
Matrix Design Application Guide

CrownBio
CONNECTING SCIENCE TO PATIENTS

Figure 1: Dose-Response Curves for Drugs A and B and Corresponding Dose-Response Matrix Heat Map for all Concentration Combinations

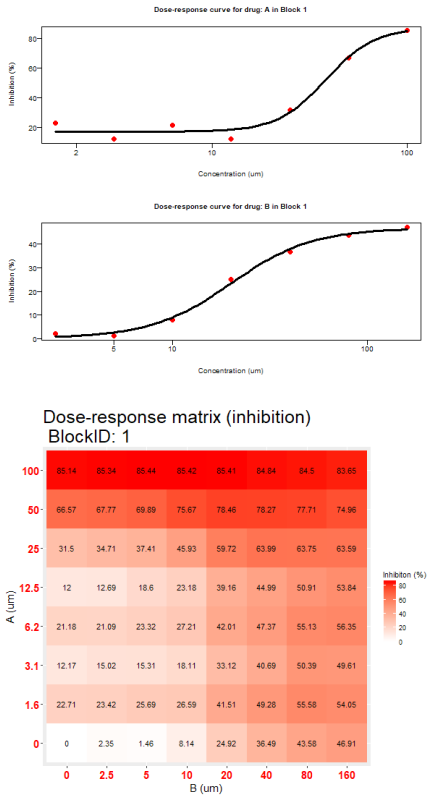
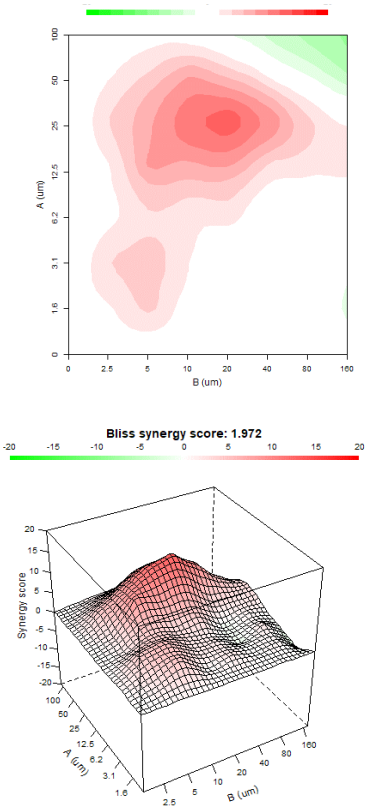


Figure 2: 2D Contour Map and 3D Response Surface Plot Demonstrating the Synergistic Effects of the Two Drugs at Various Concentration Combinations



Conclusions

Drug A and B work synergistically to inhibit cancer cell proliferation. The synergistic effect is optimized when Drug A is approximately 25μM and Drug B is approximately 20μM, while strong synergistic effect is seen when Drug A is 15-50μM and Drug B is 5-50μM. Downstream pharmacology analysis should 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.