Assessing drug synergy in combination therapies

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Outline

Introduction

Pharmacokinetic and pharmacodynamic drug interactions

Drug interactions depend on the phenotype

Experimental design

Fixed doses Dose gradients Checkerboards

Analysis of drug interactions

Different null hypotheses:

- Pharmacological independence = Gaddum (1940)
- Pharmacological additivity = Loewe (1928) + Chou-Talalay (1984)
- Statistical independence = Bliss (1939)

Experimental design revisited

Clinical relevance

Demonstration of Isobologram analysis

Introduction - Why combination therapy?

Clinical Benefit

- Stronger pharmacologic effect
- Hinders evolution of drug resistance
  in viruses, bacteria, and cancers
- Clinical trials show superior outcomes *for the right combinations*
  some drug combinations are both more effective and less toxic
  some drug combinations are both less effective and more toxic

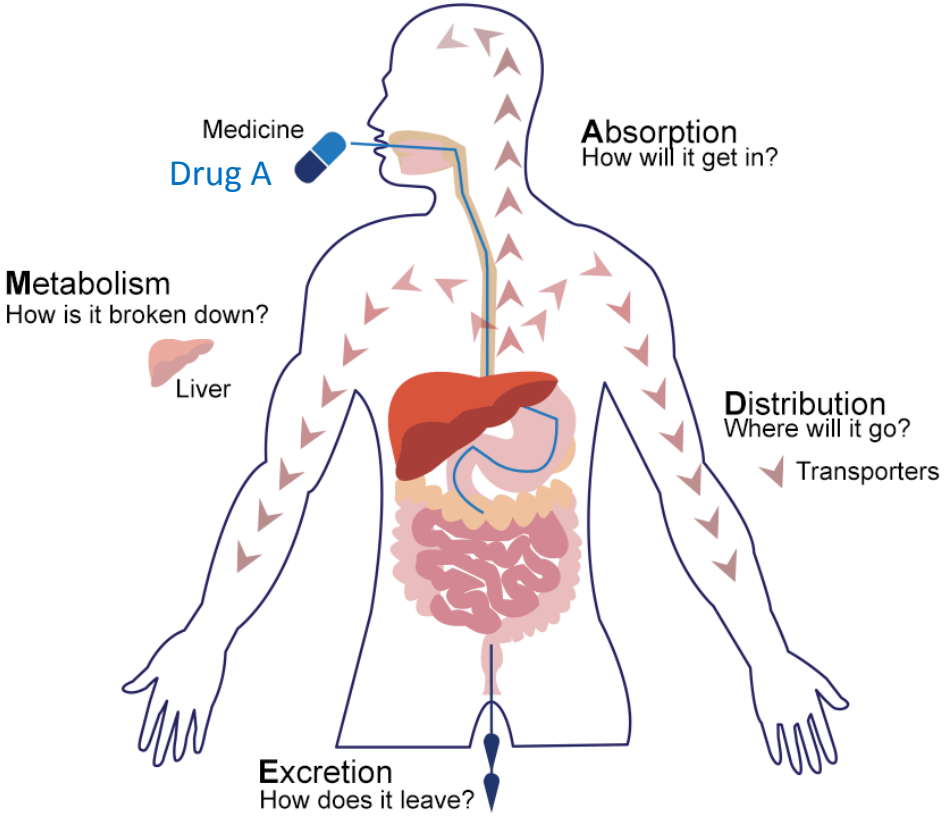
Research Use

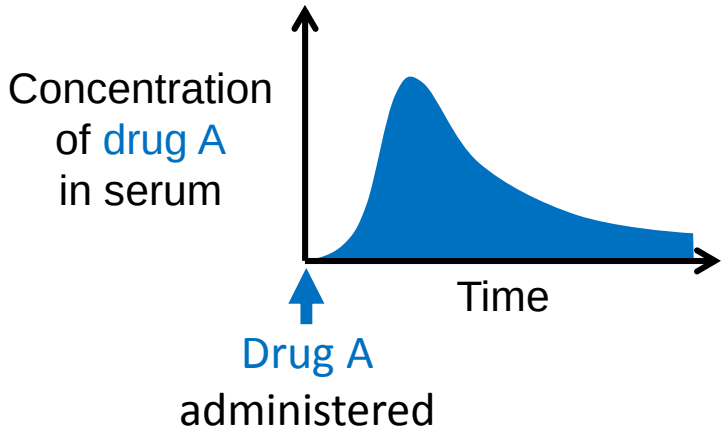
- Combined perturbations reveal functional interactions between cellular processes
- Drug interactions depend on mechanism of drug action

What do we mean by “drug interactions”?

Pharmacokinetics = What the body does to the drug

Absorption, Distribution, Metabolism, Excretion (ADME)



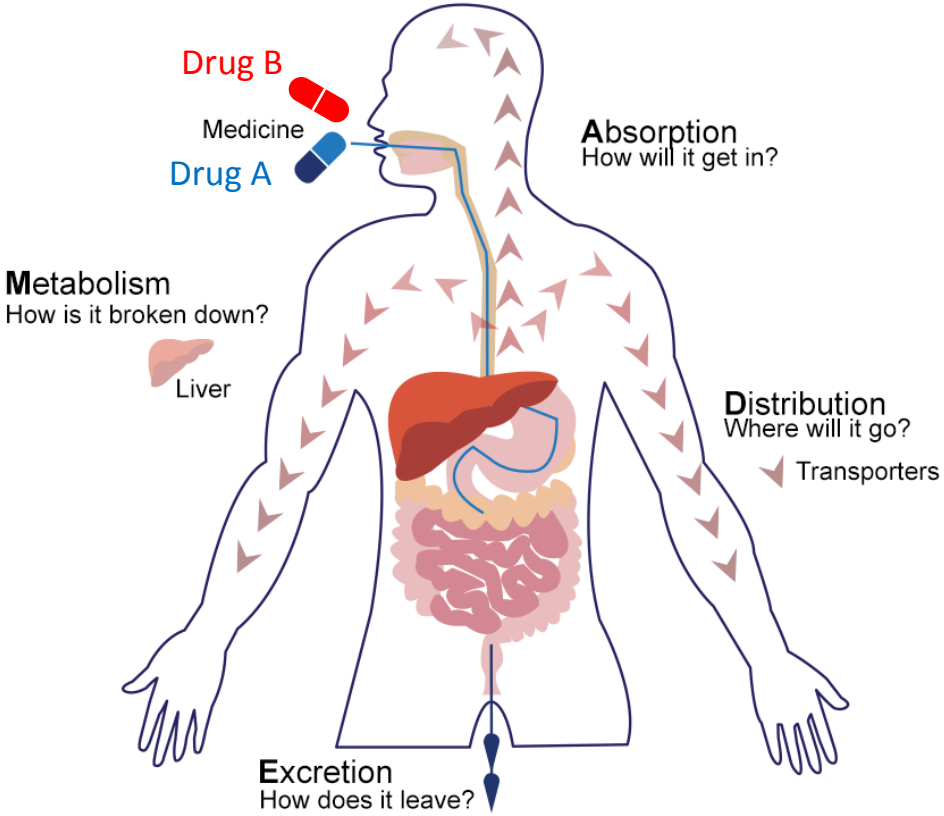


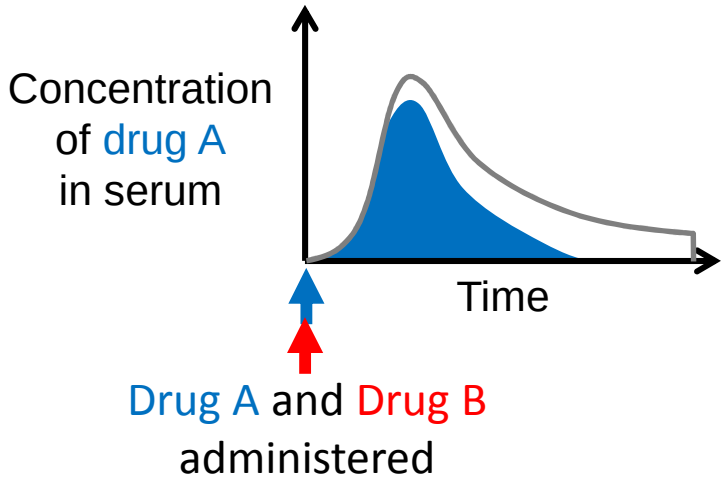
What do we mean by “drug interactions”?

Pharmacokinetics = What the body does to the drug

Absorption, Distribution, Metabolism, Excretion (ADME)

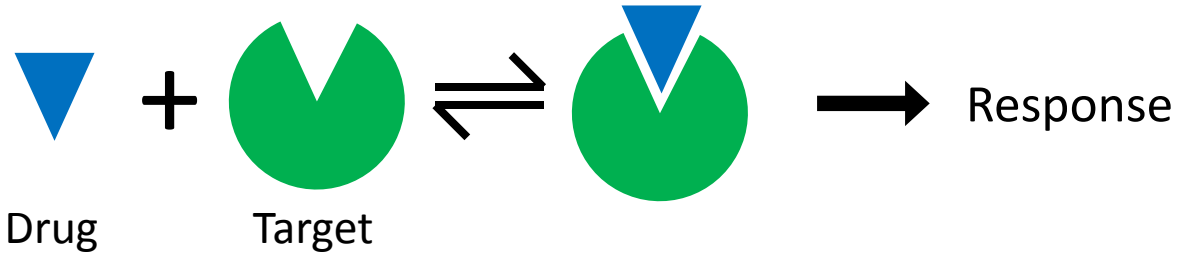
In medicine and physiology, “drug interaction” means pharmacokinetic interaction. The kinetics of drug A are changed in the presence of another drug B.



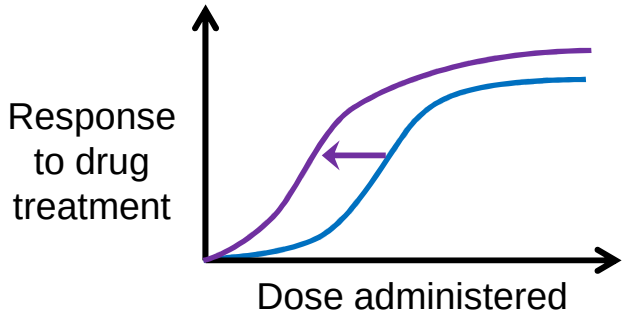


What do we mean by “drug interactions”?

Pharmacodynamics = What the drug does to the body (or cell)



To molecular biologists, “drug interaction” usually refers to *pharmacodynamic* interaction.



⇒ Drugs in combination have unexpected potency, e.g. by dose response function.

When both drugs exert the same effect, Interpreting drug interactions is complicated.

Caution: drug interactions can vary by endpoint

Drug treatments usually affect more than one phenotype.

A combination therapy could have:

Stronger effect on phenotype #1

Weaker effect on phenotype #2

⇒ Drug interactions must be understood in context of the phenotype.

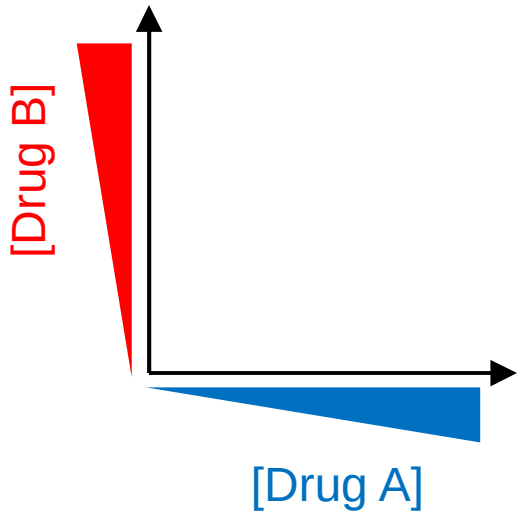
Examples:

In skin cancers with BRAF mutation,
Combination of BRAF + MEK inhibition has

- More tumor inhibition
- Less skin toxicity

To a toxicologist, “drug synergy” is bad because it refers to harmful effects (alcohol + barbiturates)

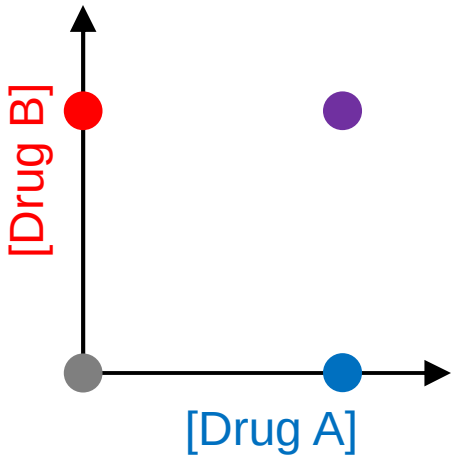
Experimental Design

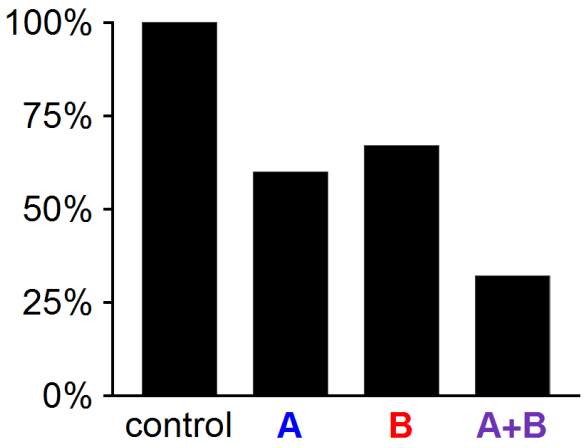


Experimental Design

Single doses

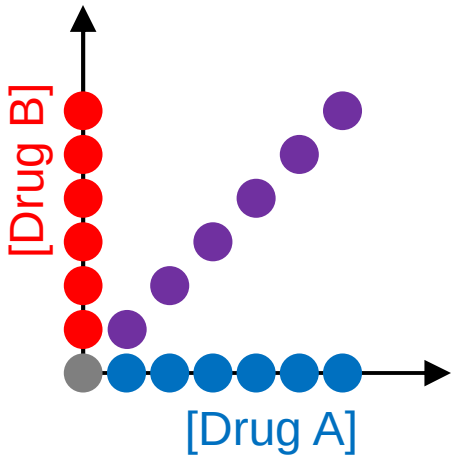
Least effort

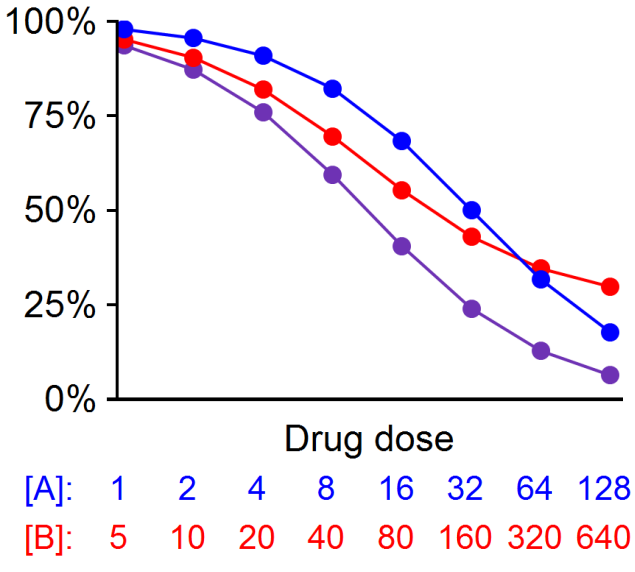




Dose gradients

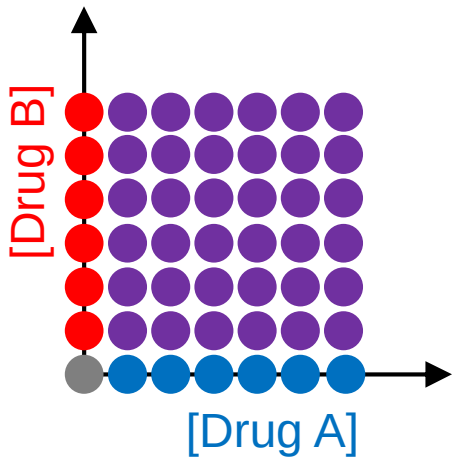
Efficient

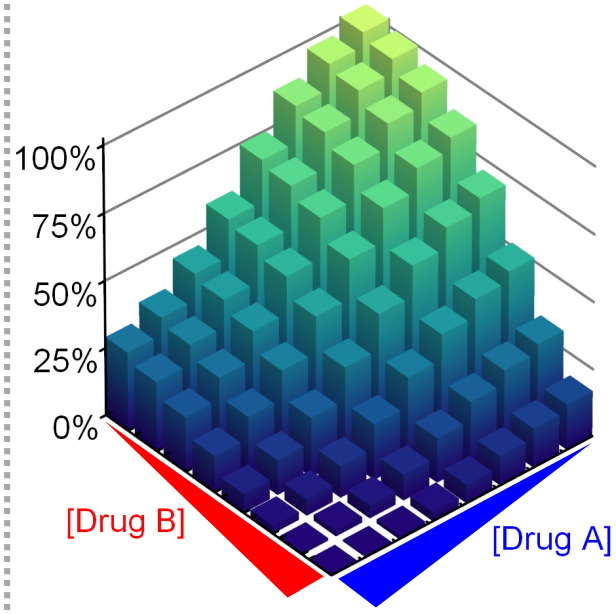




Checkerboard

Comprehensive





Experimental Design

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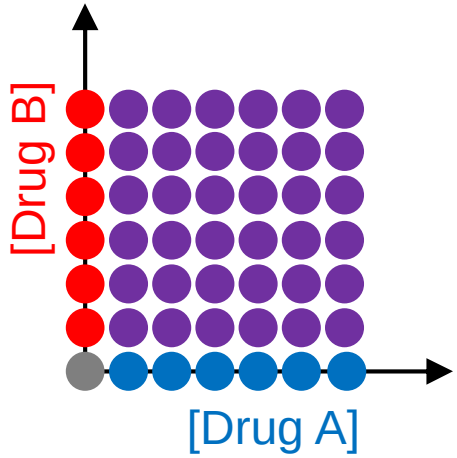
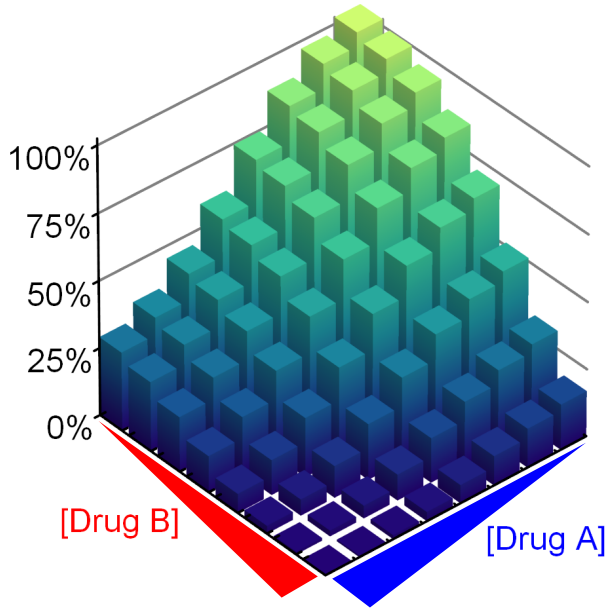


Practical for pairs only.

Most insightful data:
Reveals drug interactions
at all ratios between drugs



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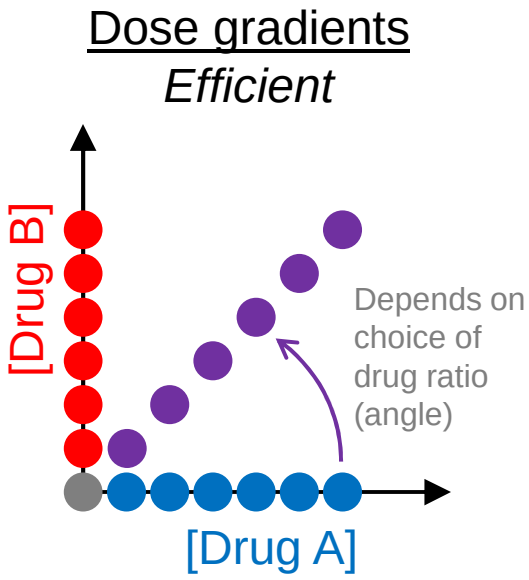
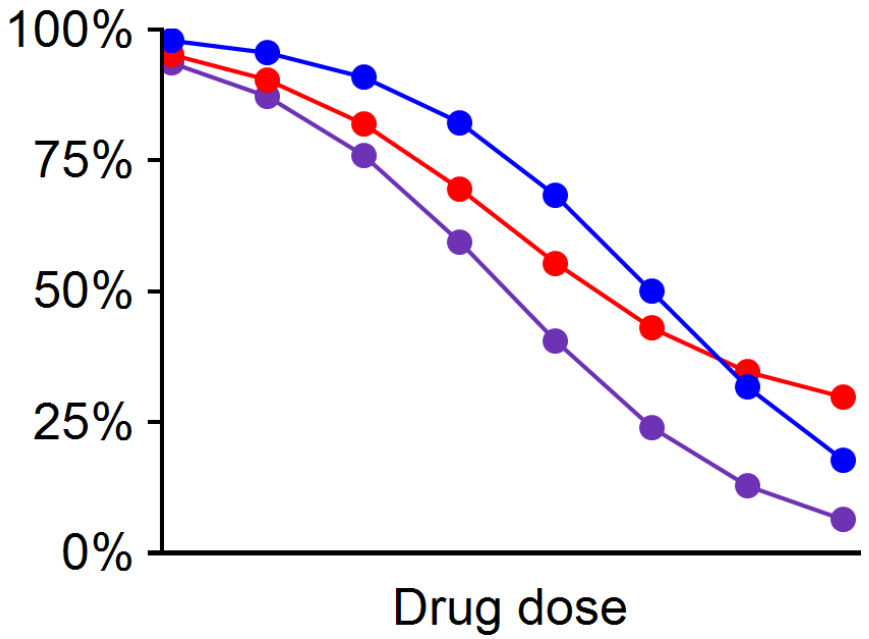




[A]:	1	2	4	8	16	32	64	128
[B]:	5	10	20	40	80	160	320	640

More effort than single-dose by a fixed factor (eg. 8× more points)

Easily scales to triple-, quadruple-drug combinations

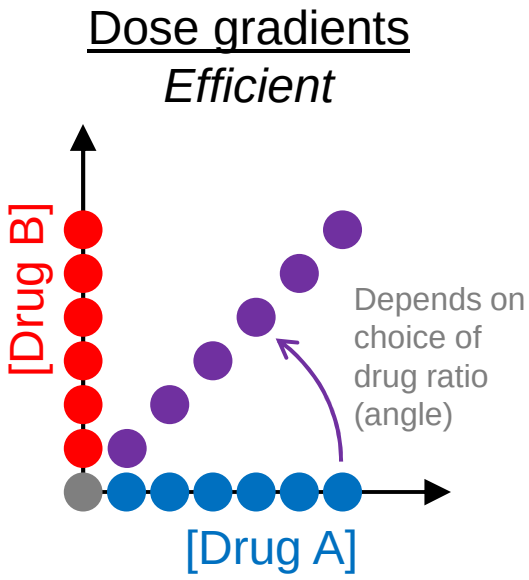
Need to chose a ratio between drugs (angle)

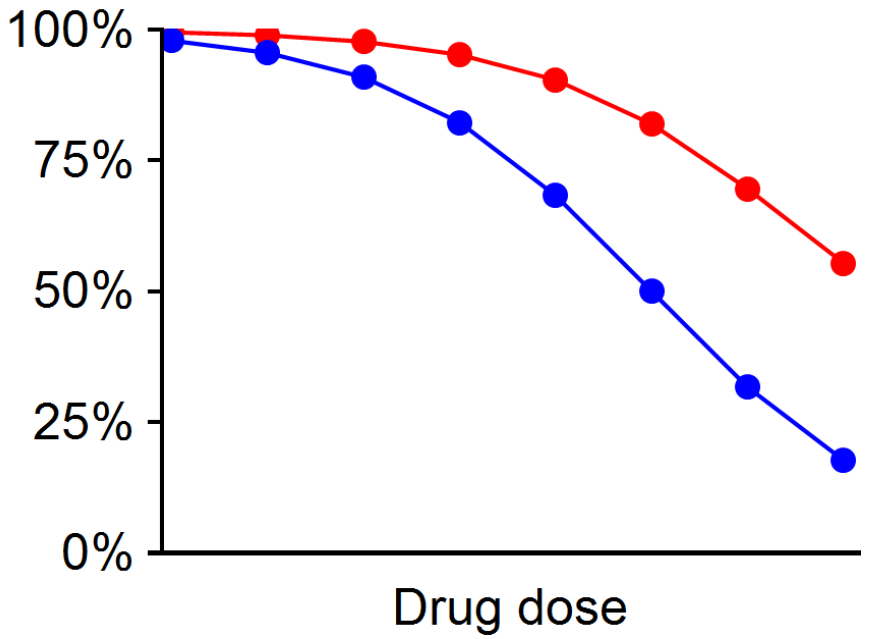
Analysis can be attentive to dose-response function
⇒ Robust assessment of interactions *at the chosen ratio*

Fixed ratio combination
[A] : [B] = 1 : 5

Mixed in equal potency
= “Equipotent” (on a chosen readout)

Experimental Design





Might miss interactions if potencies are imbalanced.

[A]:	1	2	4	8	16	32	64	128
[B]:	1	2	4	8	16	32	64	128

More effort than single-dose by a fixed factor (eg. 8× more points)

Easily scales to triple-, quadruple-drug combinations

Need to chose a ratio between drugs (angle)

Analysis can be attentive to dose-response function
⇒ Robust assessment of interactions *at the chosen ratio*

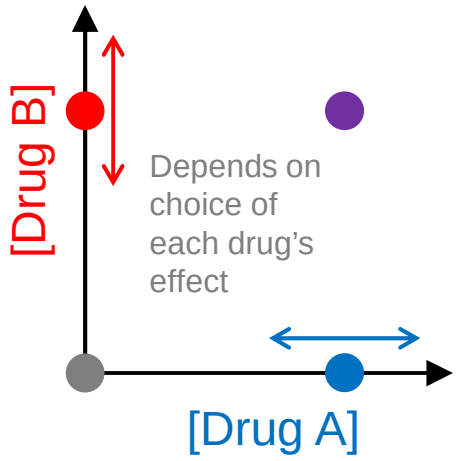
Fixed ratio combination
[A] : [B] = 1 : 1

Mixed in equal concentration
= “Equimolar”

Experimental Design

Single doses

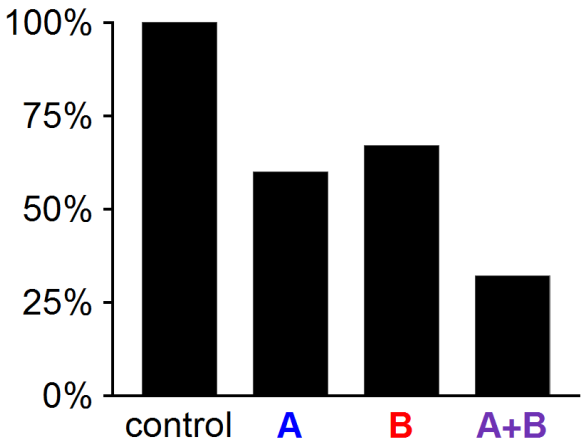
Least effort



Easily scales to triple- , quadruple-drug combinations

High-throughput screening

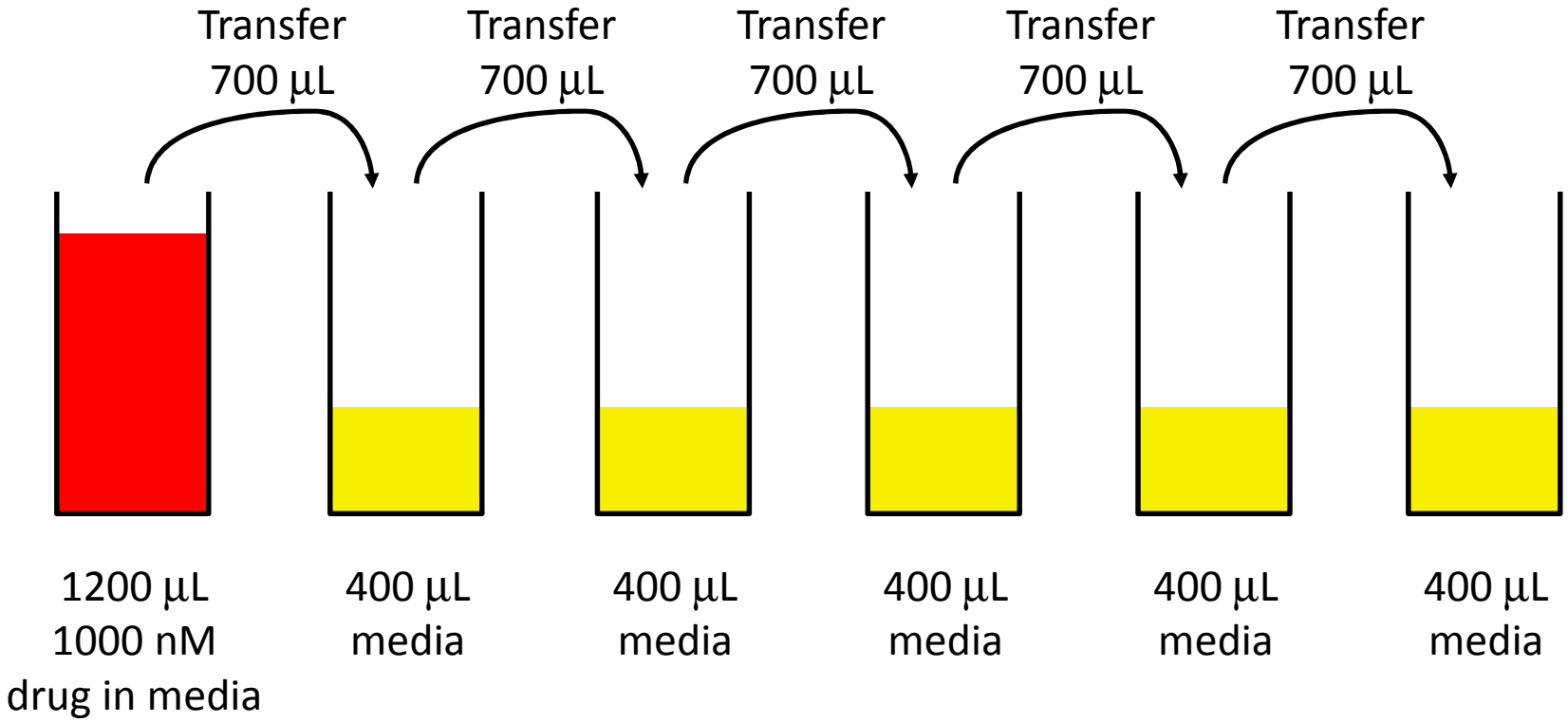
Data has limited value:
no analysis of dose-response
⇒ *prone to false-positive interactions*



Experimental Design

Cannot reliably detect a change in drug potency less than the increments in drug concentration.

Solution: Small-step serial dilutions



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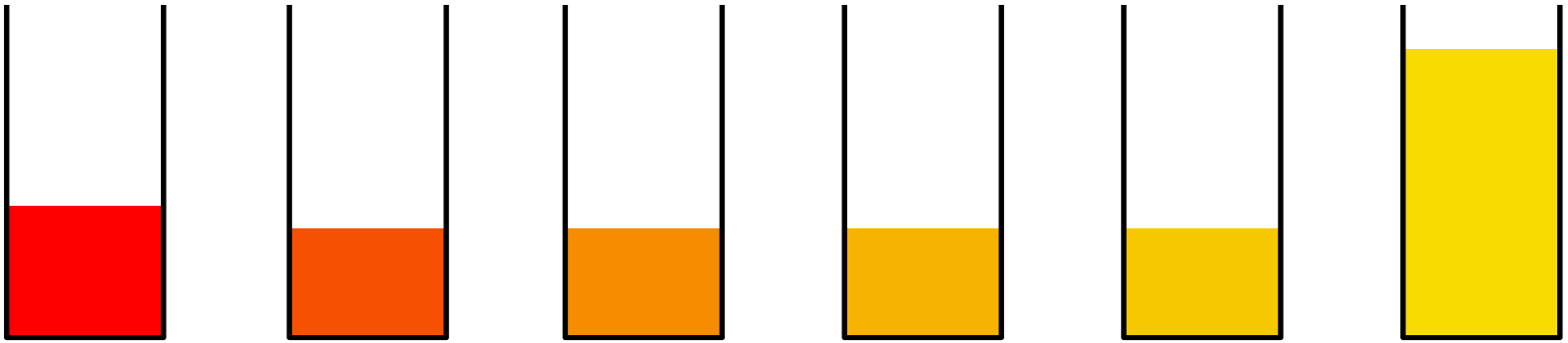
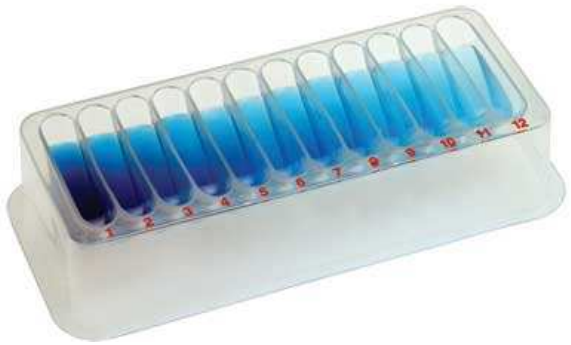


1000 nM drug in media | 636 nM drug | 405 nM drug | 258 nM drug | 164 nM drug | 104 nM drug



≈

$$\left(\frac{700}{700+400}\right)^5 = 0.104$$

Analysis of drug interactions

Identifying drug interactions depend on the “no interaction” null-hypothesis.

Null-hypotheses are based on the observed drug response
not a model of mechanism.

If “Synergy” means “stronger than my mechanistic model predicts”, then:
(1) a genuinely powerful combination isn’t classed as synergistic if it is predictable
(2) synergy/antagonism changes as your knowledge and model changes.

Descriptions based on the dose-response are empirical,
and do not depend on current state of understanding.

Explained well by Berenbaum (1989) *What is Synergy?* Pharmacological Reviews

Analysis of drug interactions

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Gaddum (1940): Pharmacological Independence

Are two drugs more or less powerful than one drug?

Loewe (1928): Pharmacological Additivity:

Are drugs (A+B) more or less powerful than (A+ *more* A), or (B+ *more* B)?

Also measured by the Chou-Talalay ‘Combination Index’

Bliss (1939): Statistical Independence of toxins:

In a population, is each individual’s probability of death from drug A statistically independent of the probability of death from drug B?

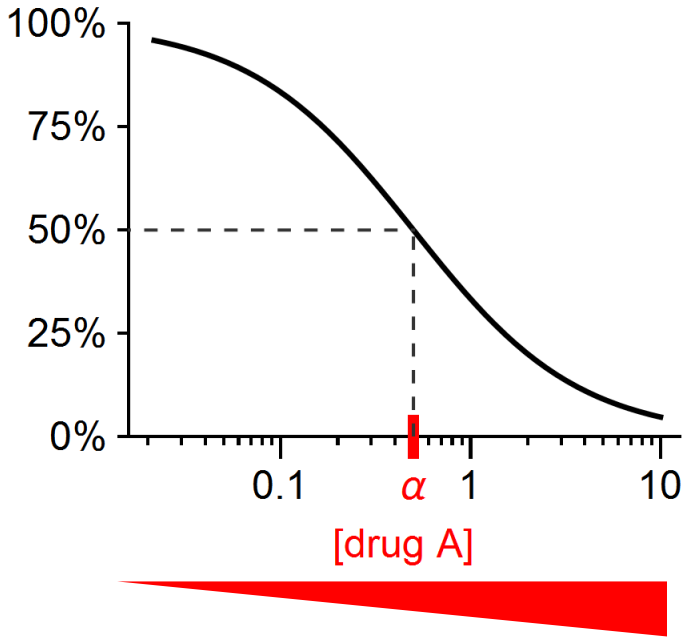
These are different questions.

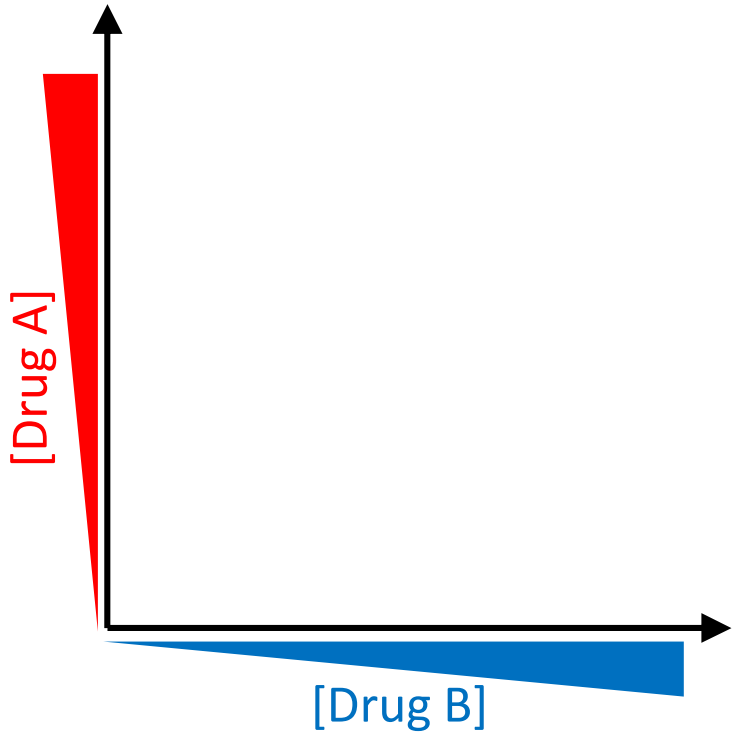
Not alternative ways to ask the same question.

Gaddum (1940) Pharmacological independence

Dose α of drug A has a certain strength of effect.

How much drug A is needed to have the same effect when drug B is added?





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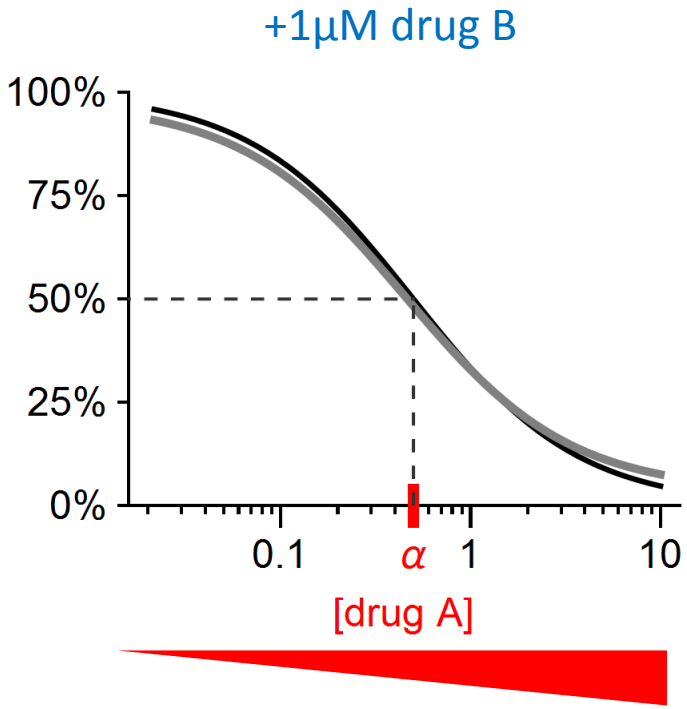

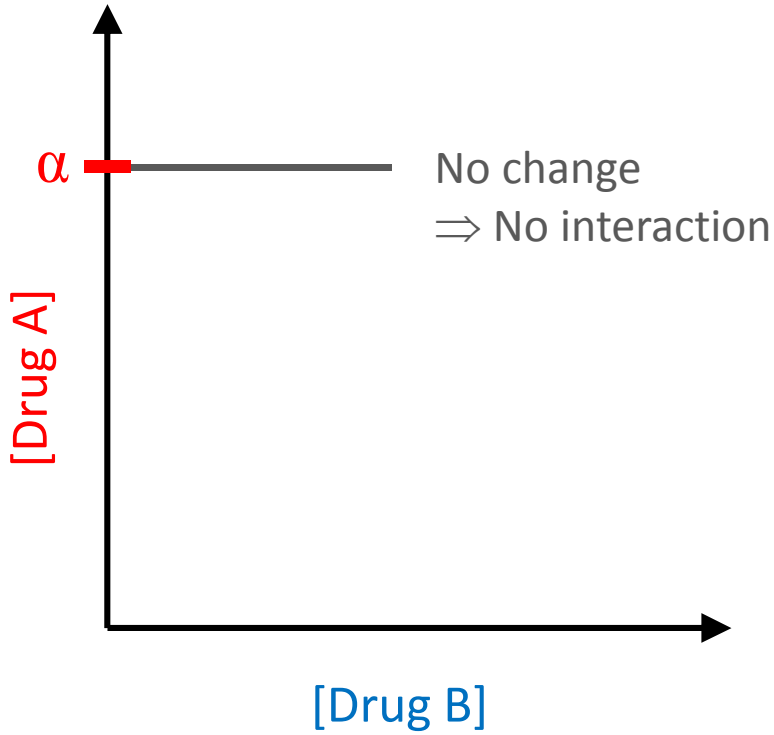

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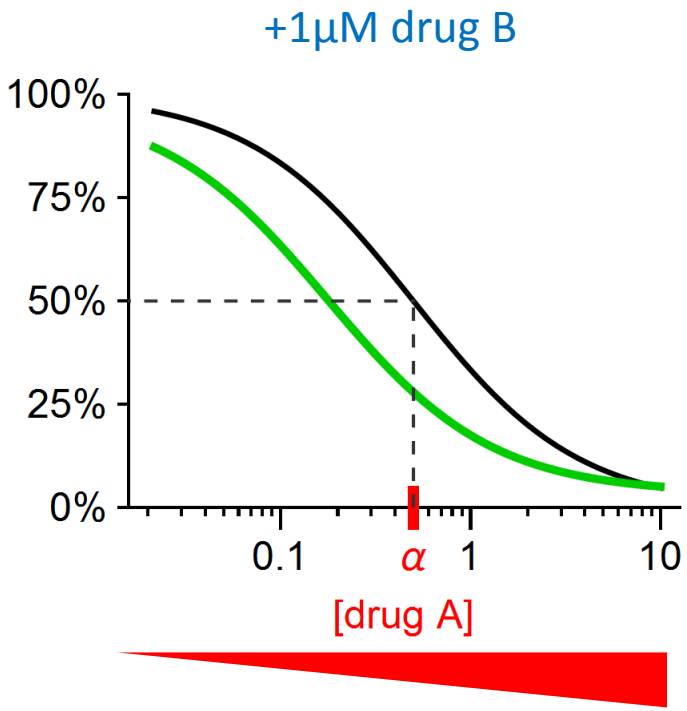

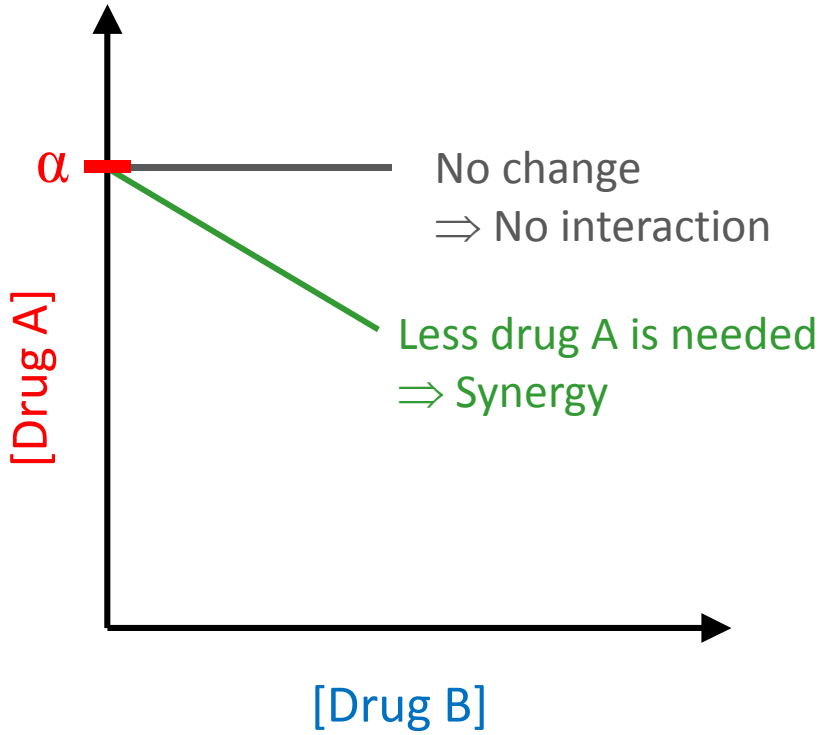

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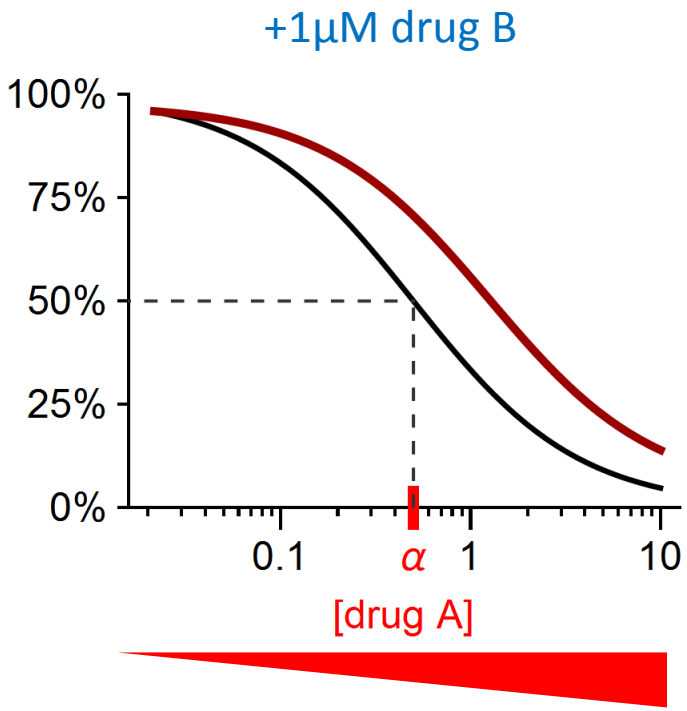
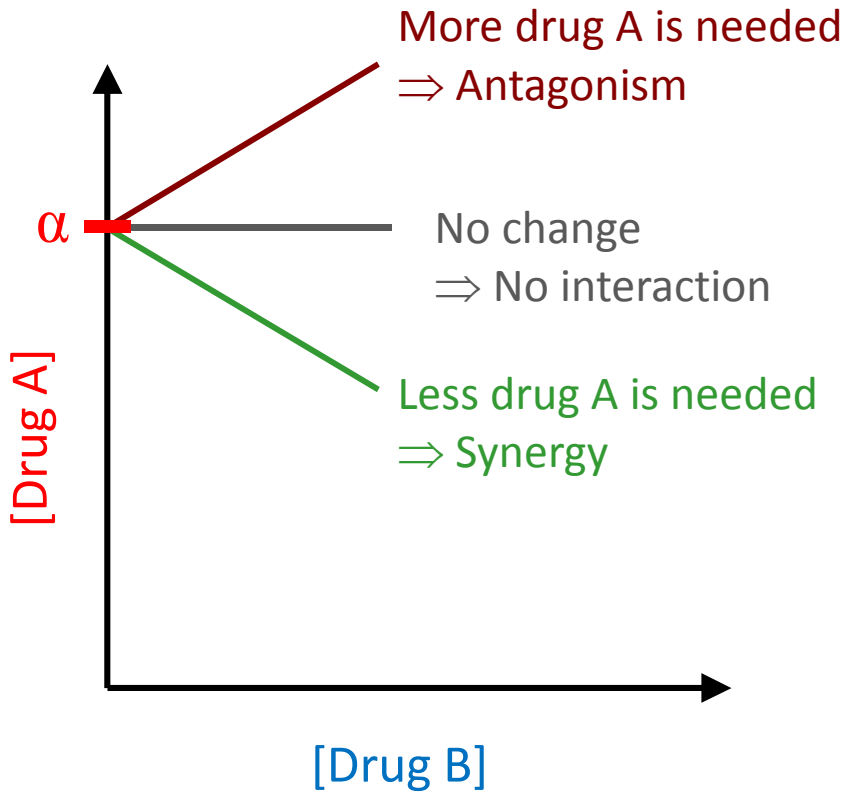
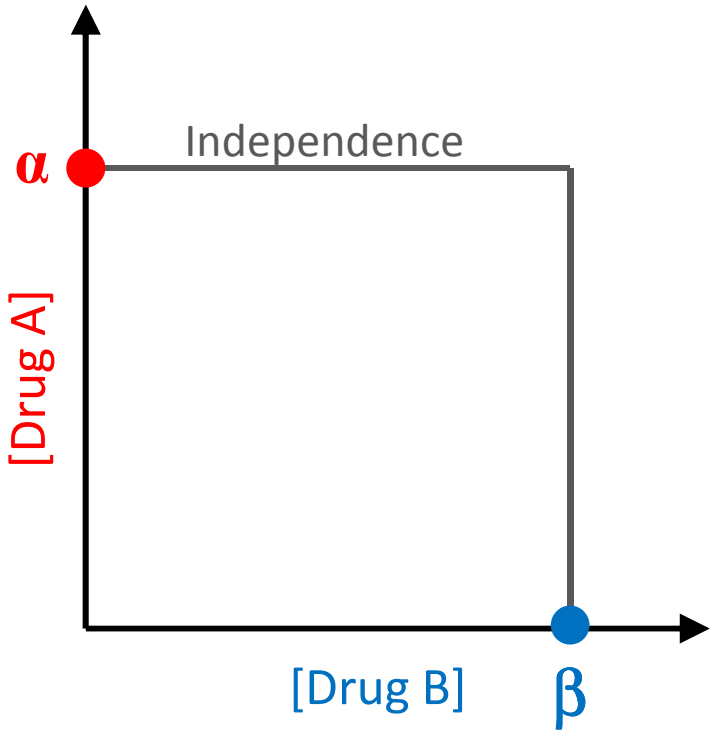




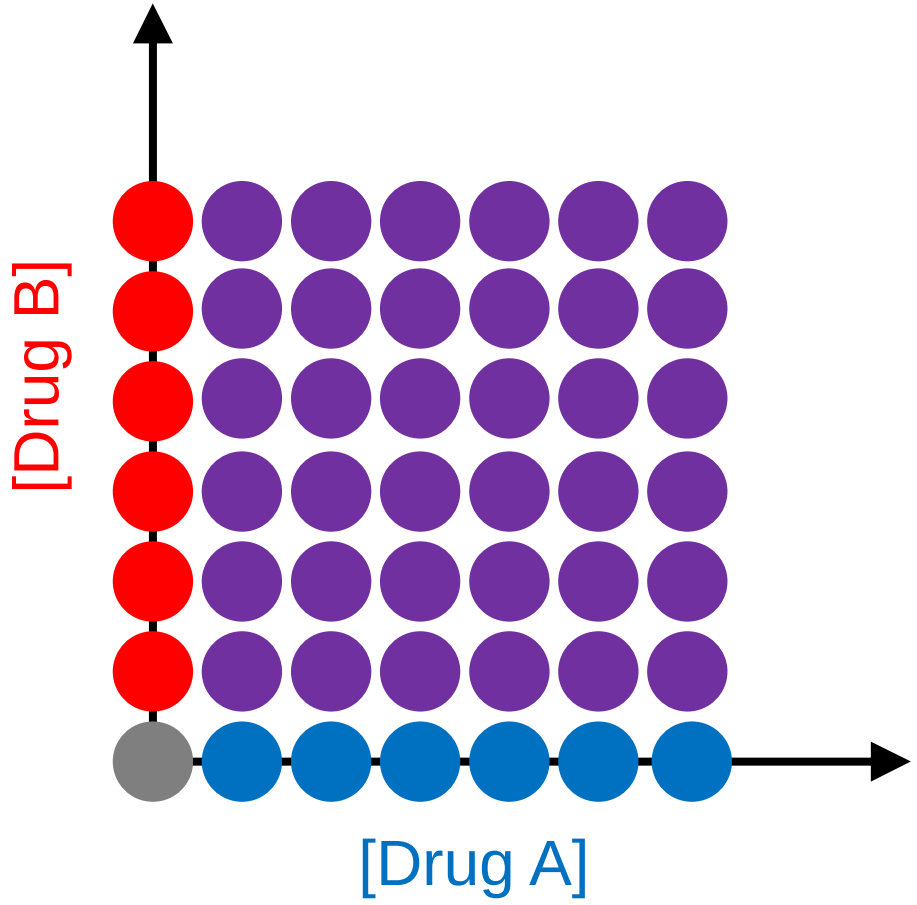
Gaddum (1940) Pharmacological independence

Simple analysis when only one drug produces the effect studied.

What if both drugs have this effect?

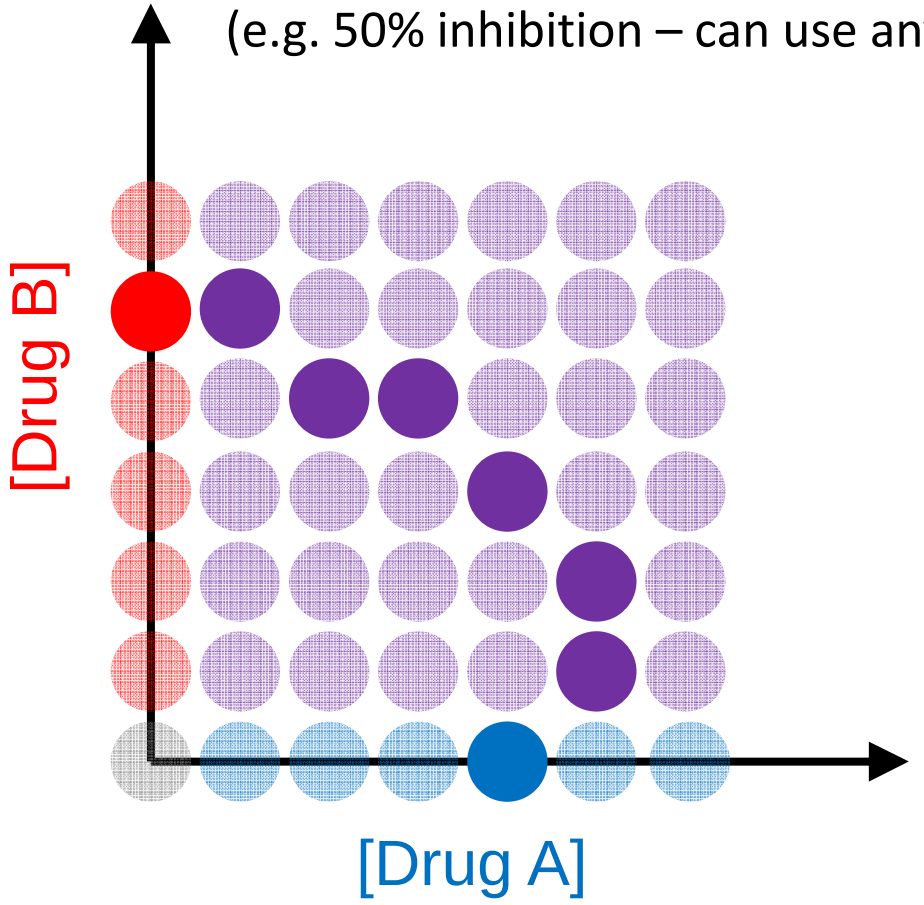


Isobologram analysis



Isobologram analysis

Points with a particular strength of drug effect
(e.g. 50% inhibition – can use any threshold)



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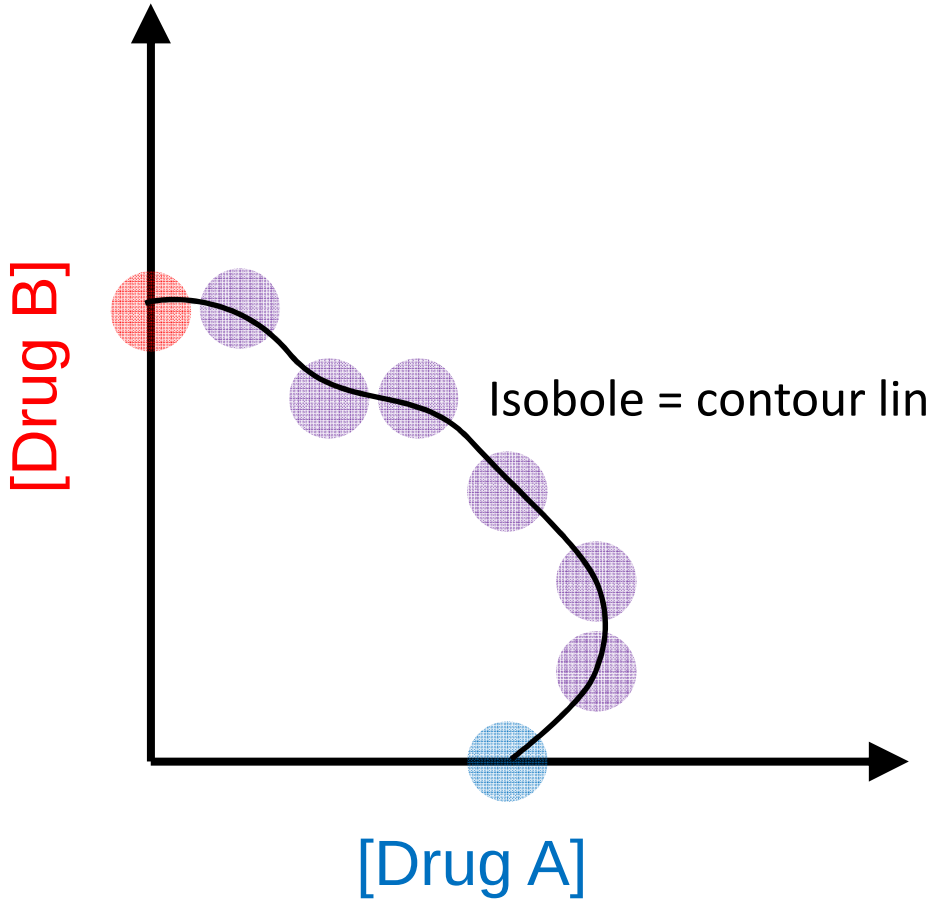
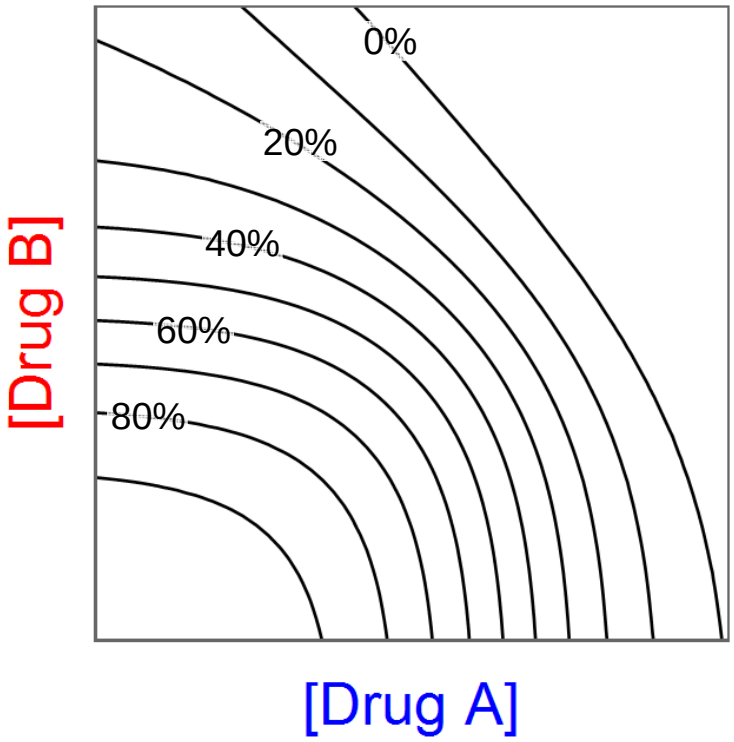
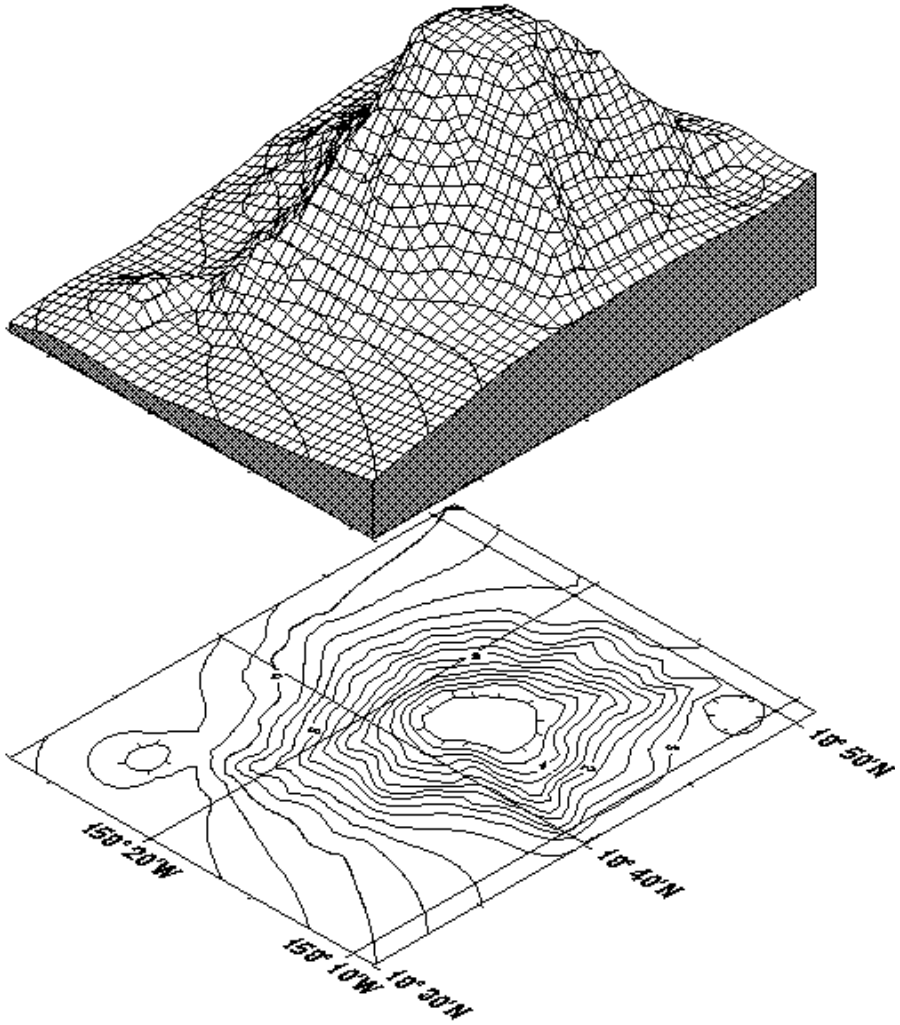


Isobole = contour line of equal drug effect

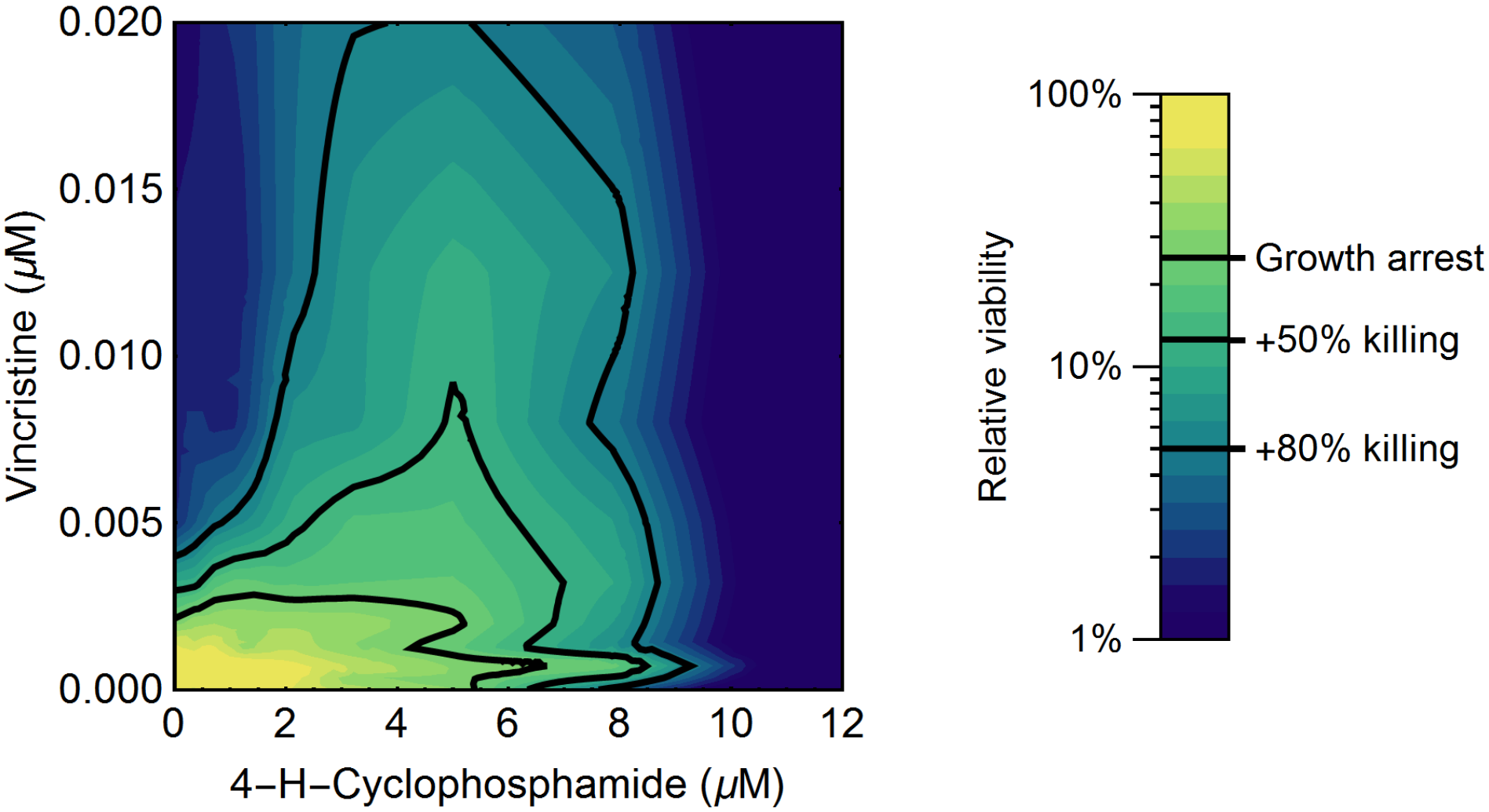
Isobologram analysis



Like contour maps in geography:

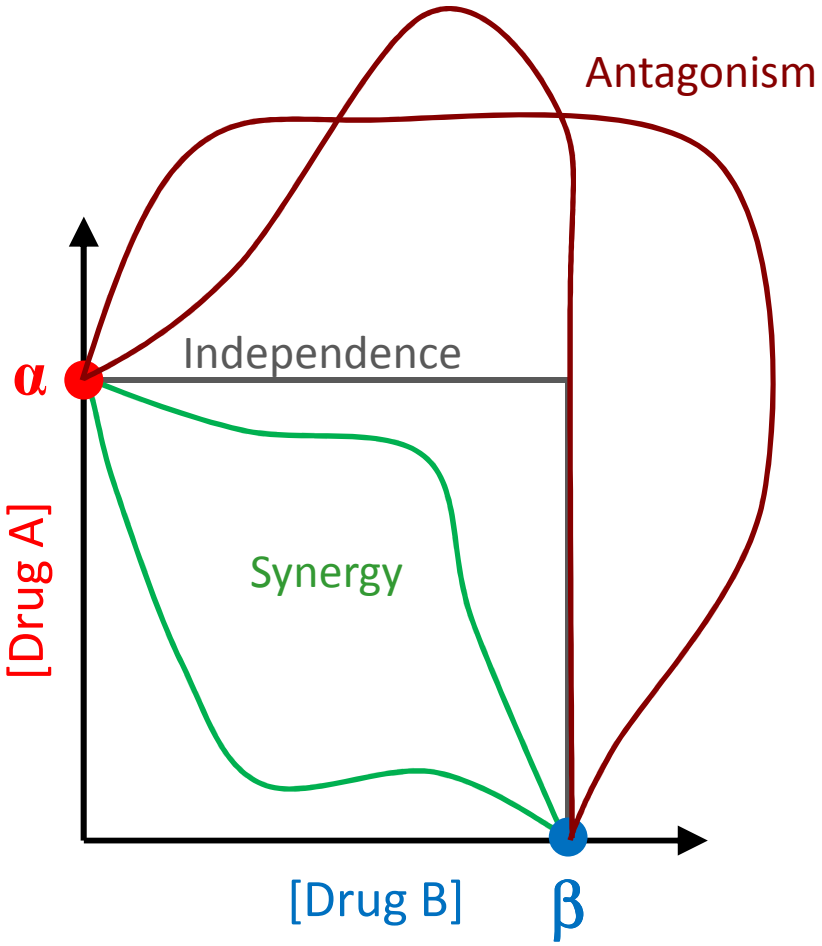


Example of isobologram analysis



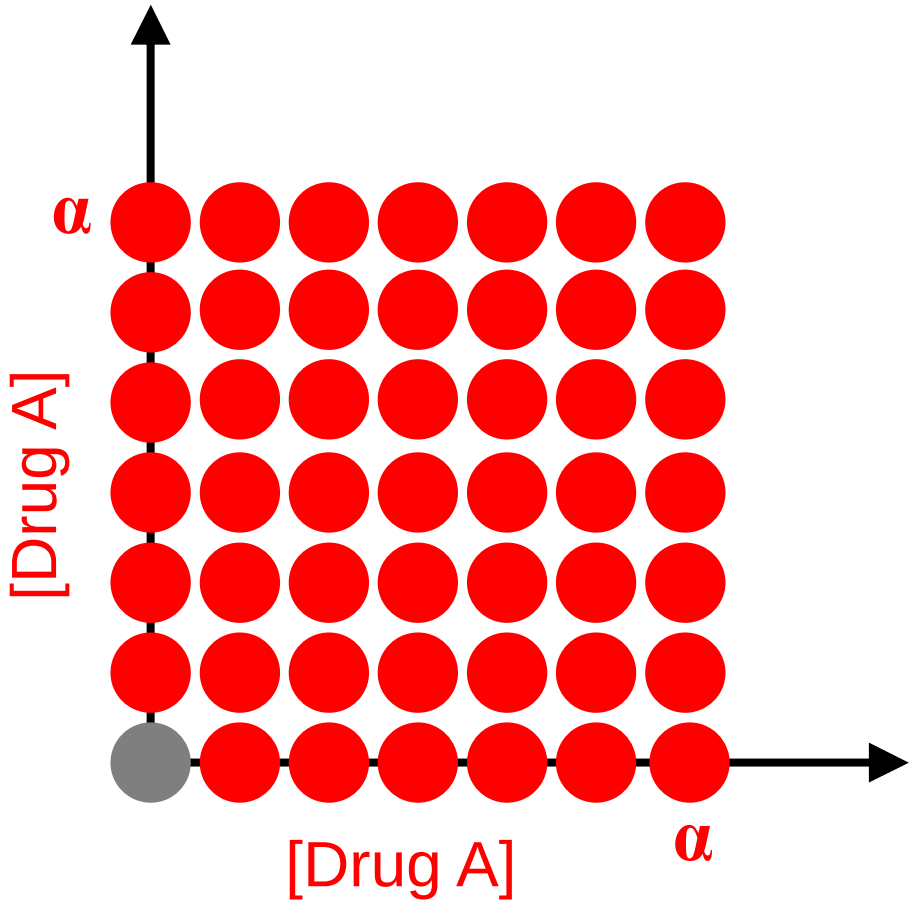
Interpretation after a break / questions

Gaddum (1940) Pharmacological independence



Loewe (1928) Pharmacological additivity

The only certain expectation is that a drug ‘combined’ with itself produces straight-line isoboles



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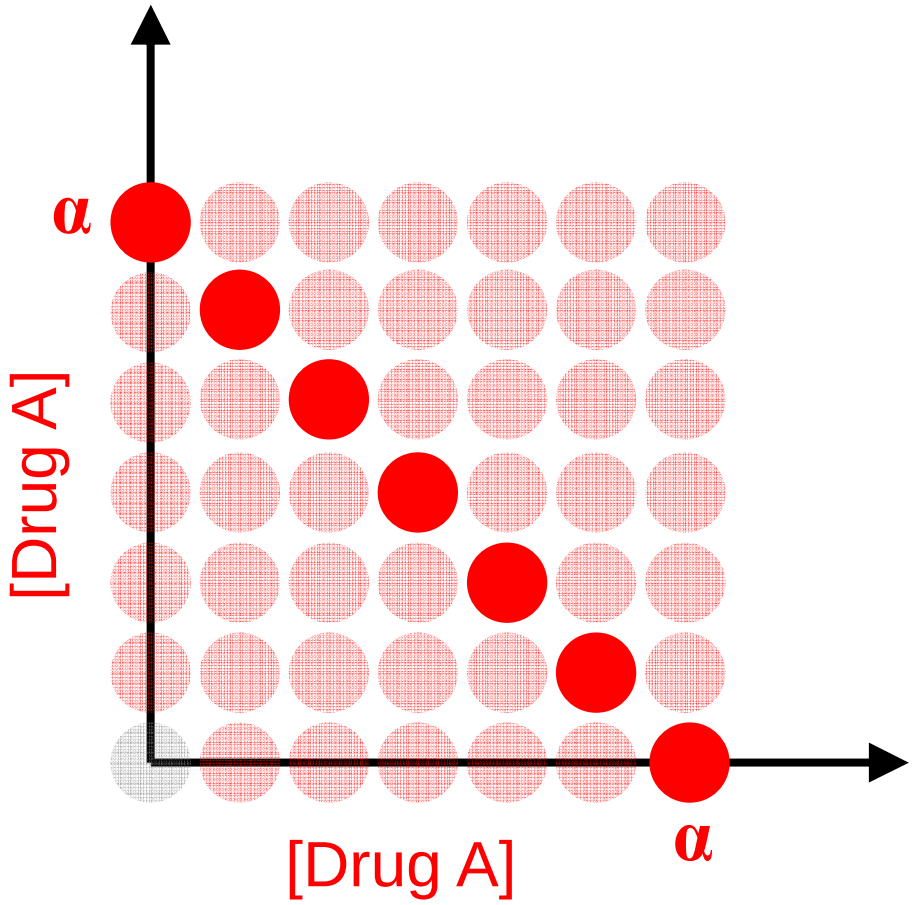

Linear drug scale

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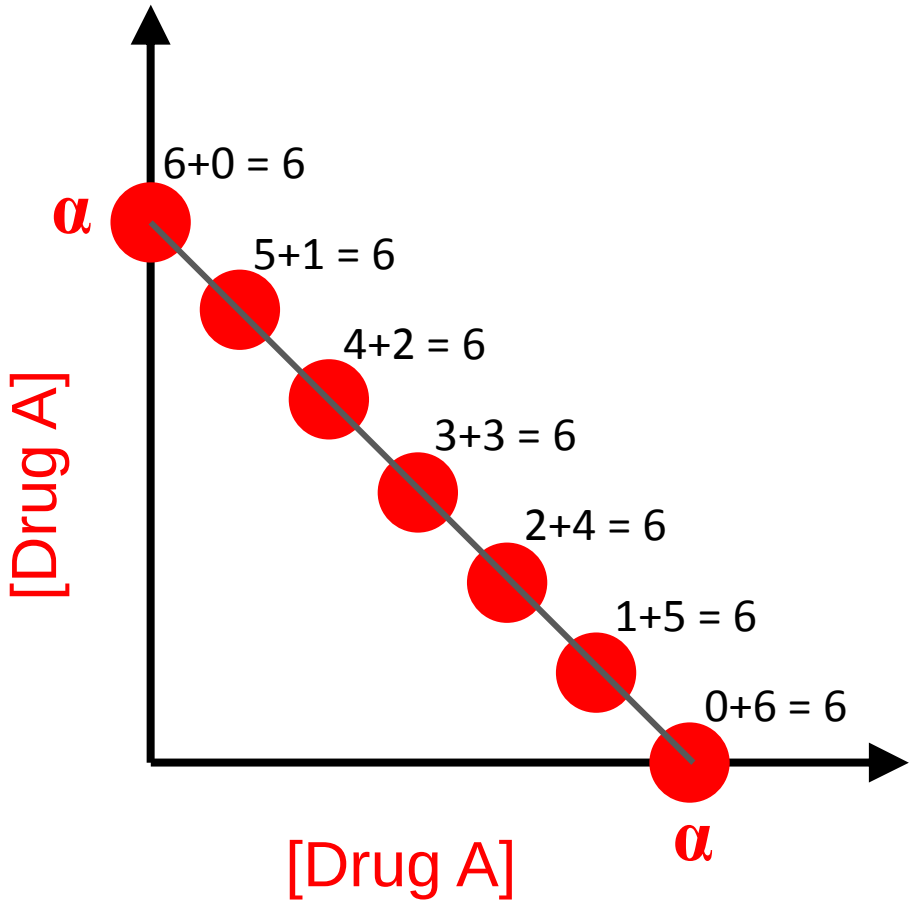
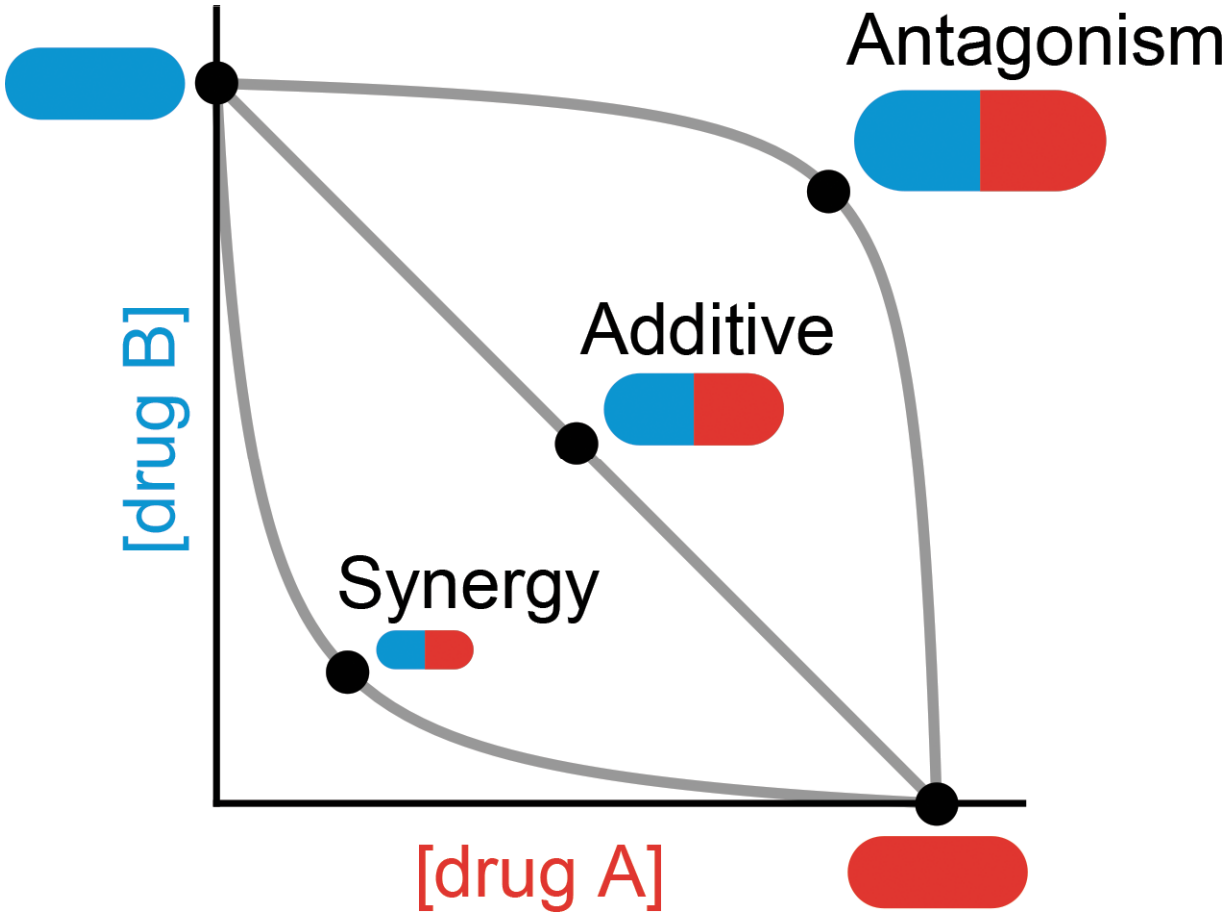


Linear drug scale

Loewe (1928) Pharmacological additivity

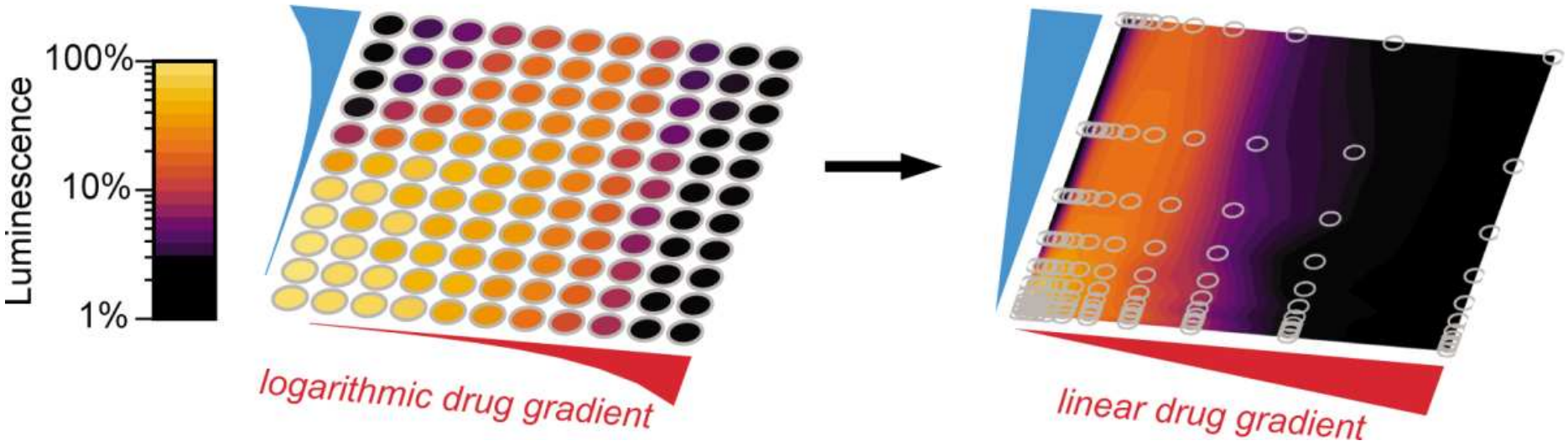


Linear drug scale

Loewe (1928) Pharmacological additivity

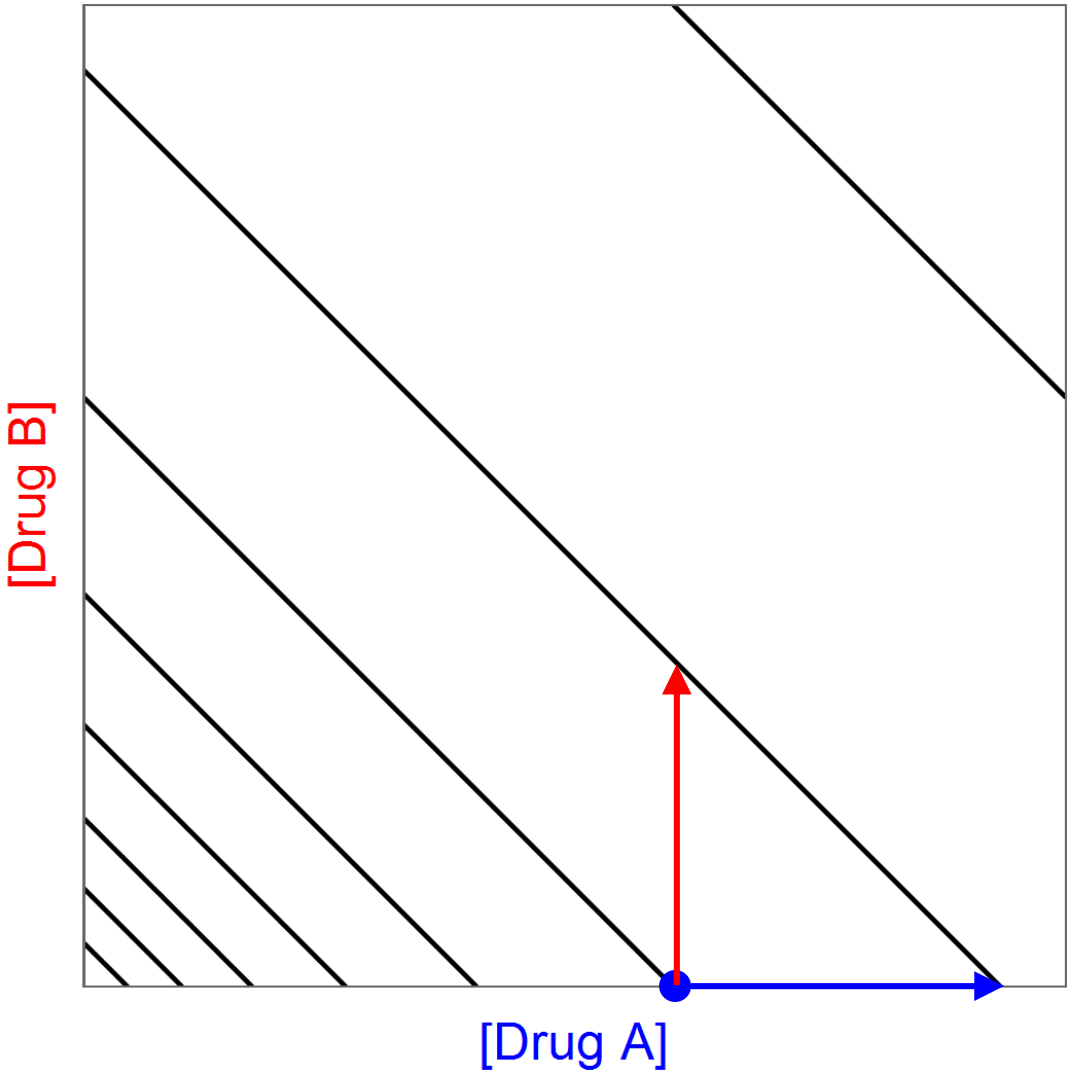
Can measure over logarithmic concentration gradient,

and analyze over linear concentration gradients



Loewe (1928) Pharmacological additivity

For two different drugs, additivity is not a prediction but a point of reference for the readout, regardless of mechanism.

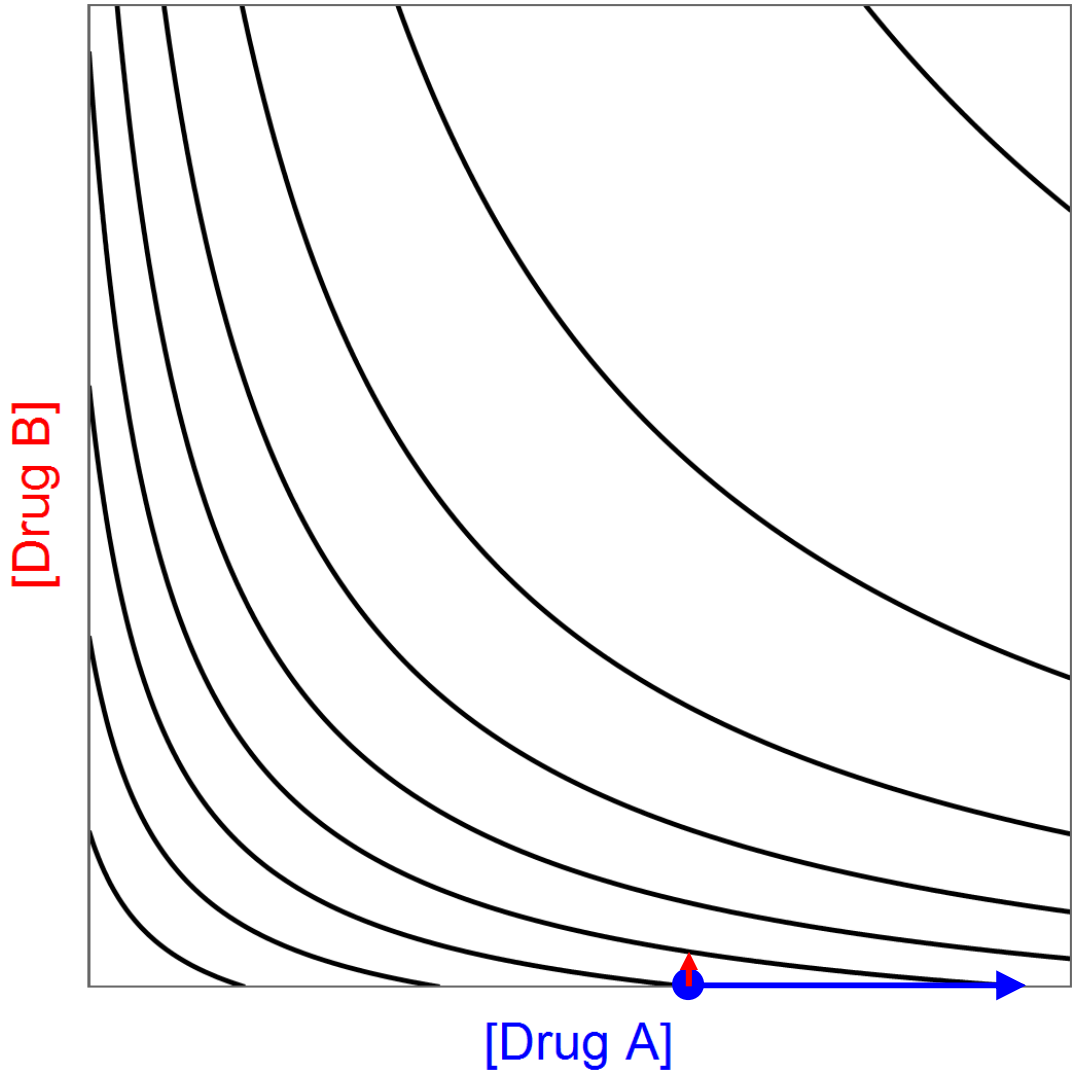


Additivity

⇒ achieving a stronger effect needs the same increase in drug A or drug B

Loewe (1928) Pharmacological additivity

Additivity is predicted when two drugs bind the same pocket with the same effect.
For two different drugs, additivity is not a prediction but a point of reference.



Synergy

⇒ achieving a stronger effect is easier with a second drug.

Loewe (1928) Pharmacological additivity

Additivity is predicted when two drugs bind the same pocket with the same effect.
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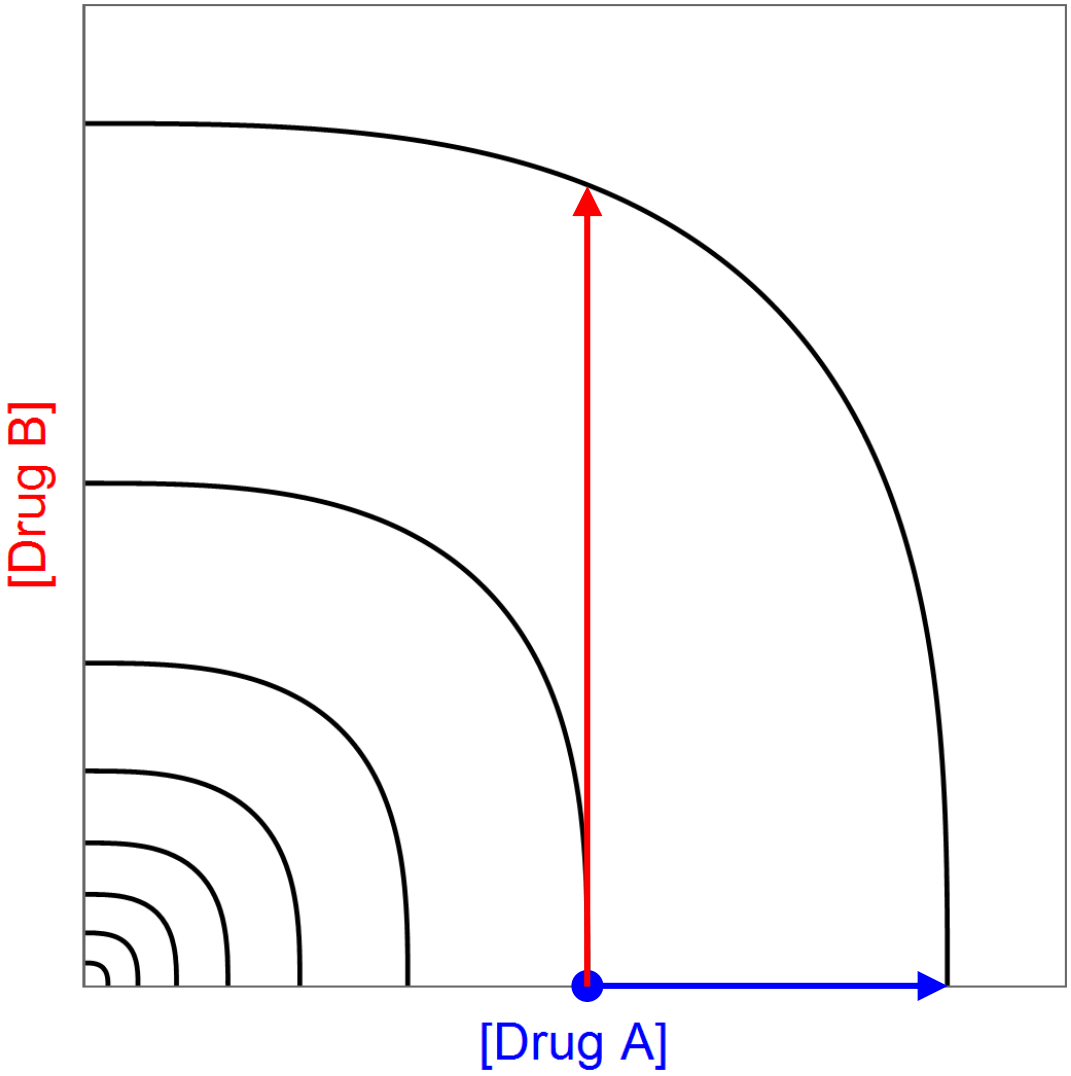


Antagonism

⇒ achieving a stronger effect is harder with a second drug.

Loewe (1928) Pharmacological additivity

Definitions of “independence”, “synergy”, “antagonism” are argued, but Loewe’s additivity is undisputed.

Gaddum called additivity a special case of synergy:

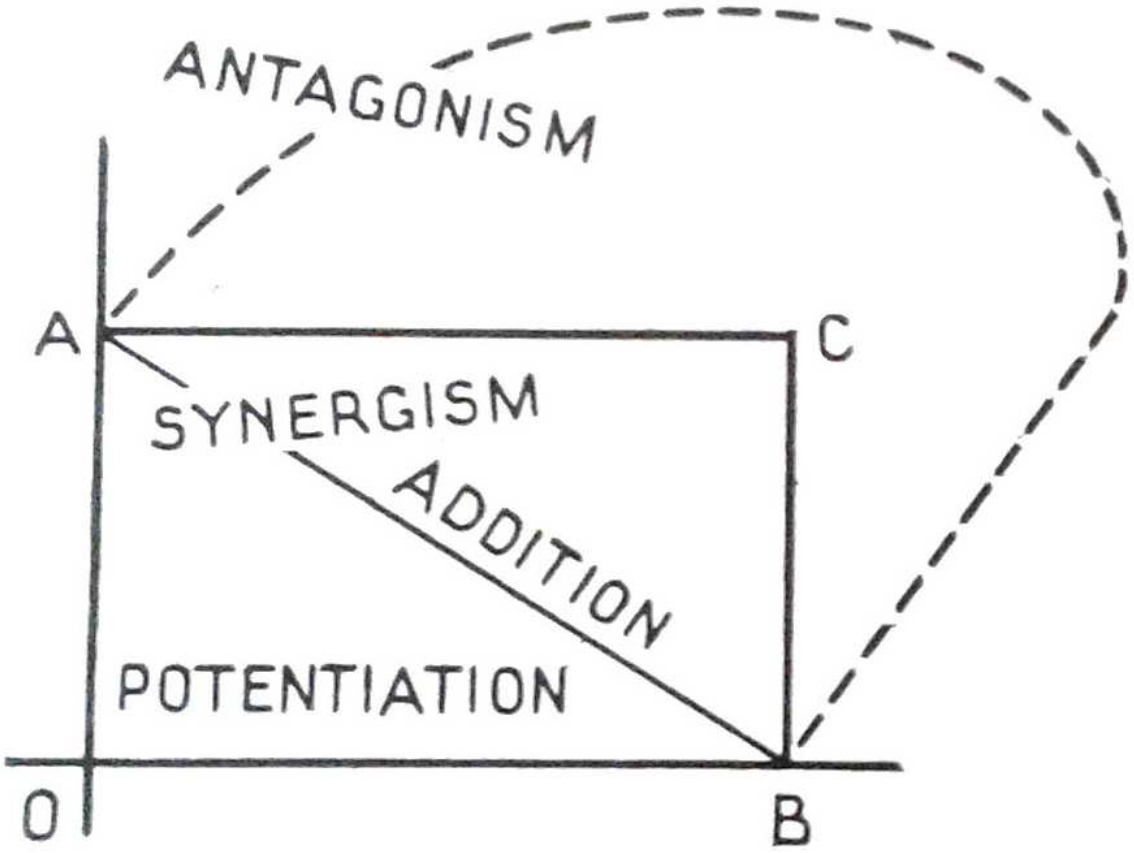
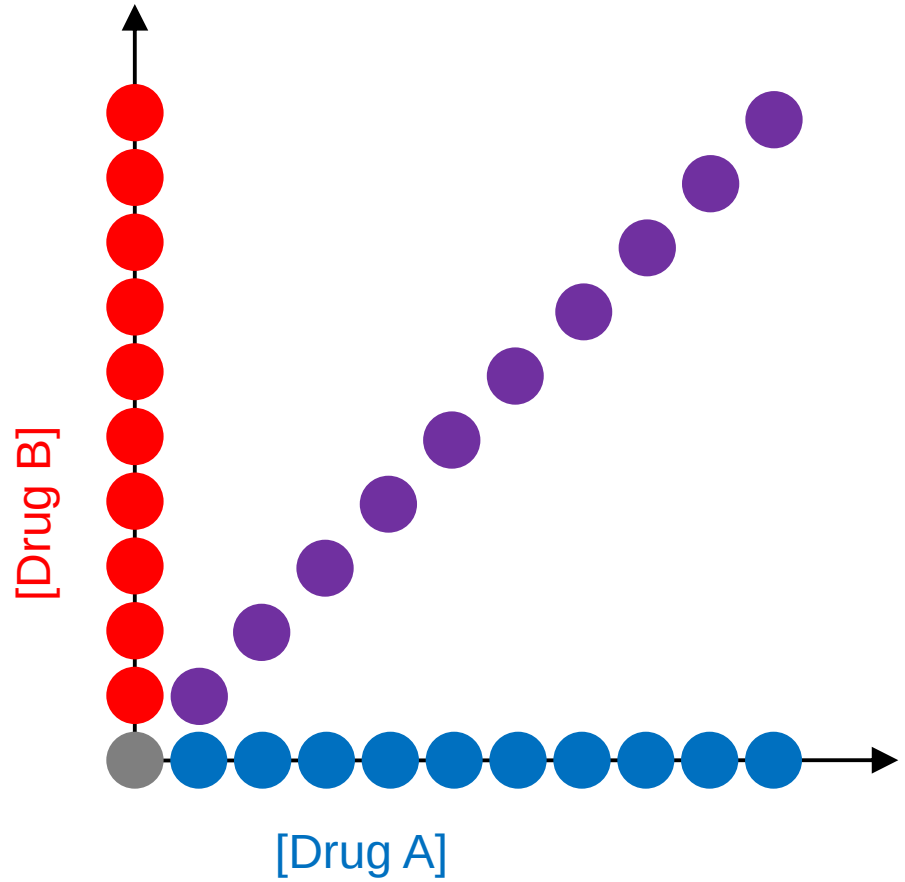


Fig. 65, Gaddum (1942) *Pharmacology*

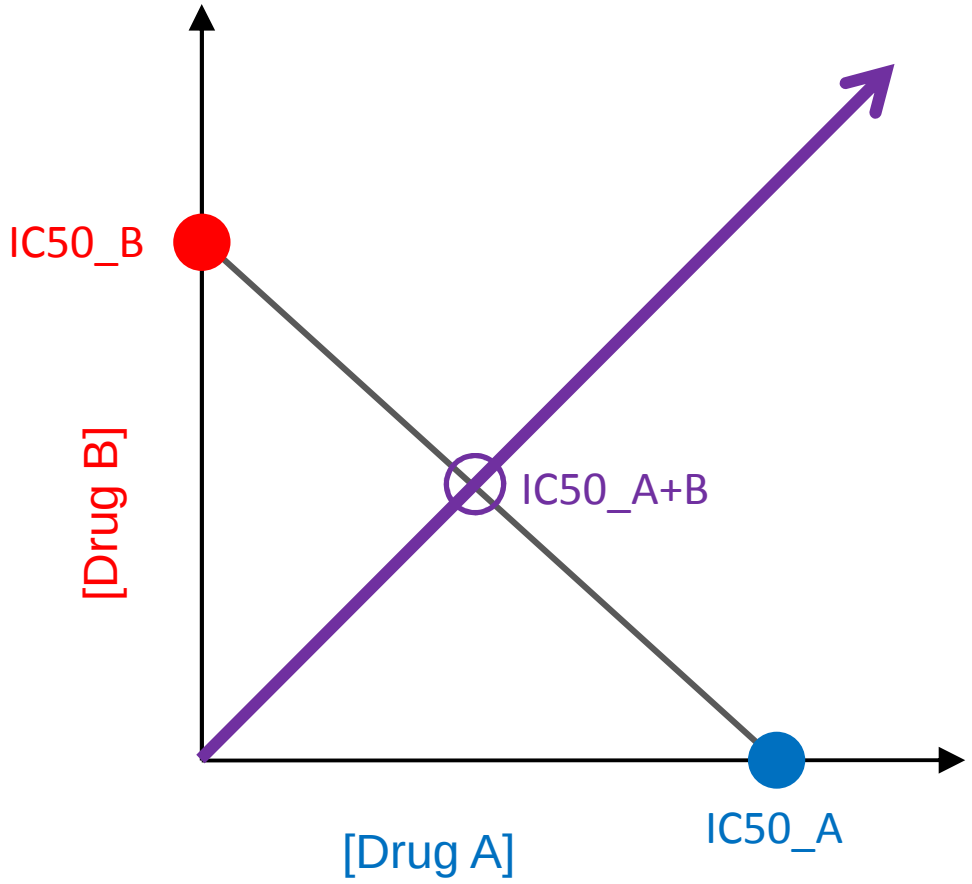
Chou-Talalay (1984): the *Combination Index*

Loewe's additivity model simplified for fixed-ratio combinations



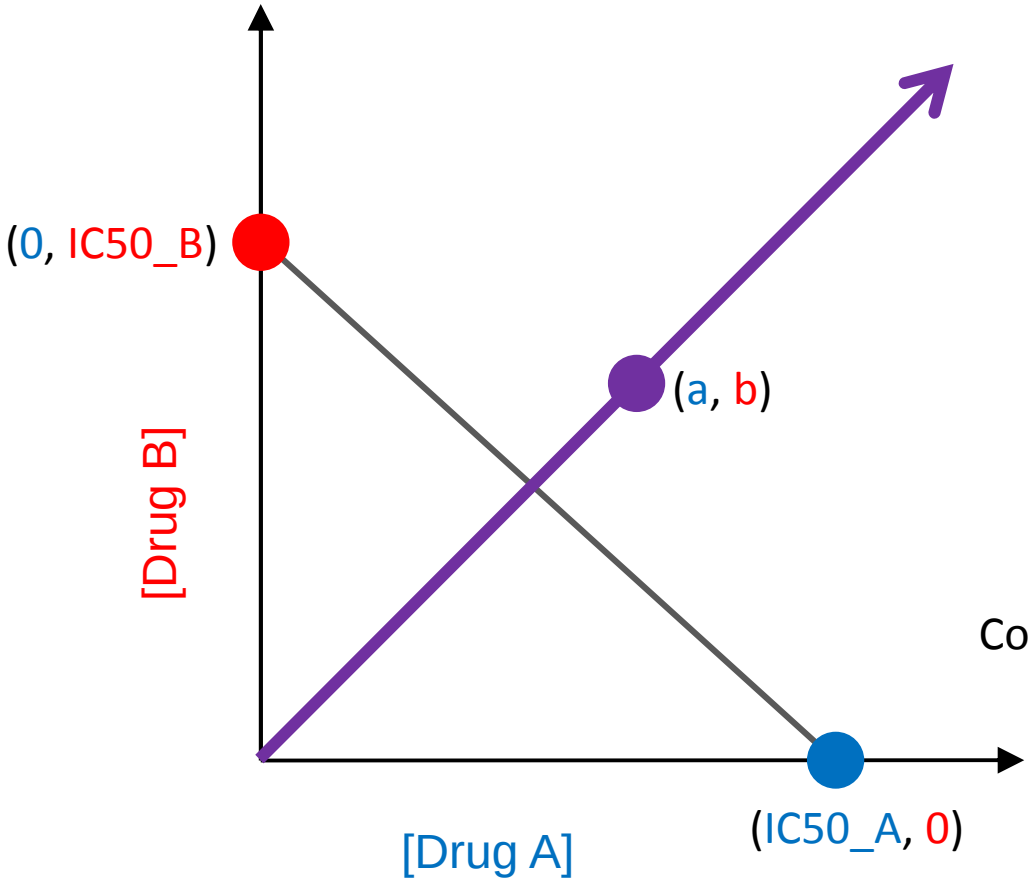
Chou-Talalay (1984): the *Combination Index*

Loewe's additivity model simplified for fixed-ratio combinations



Chou-Talalay (1984): the *Combination Index*

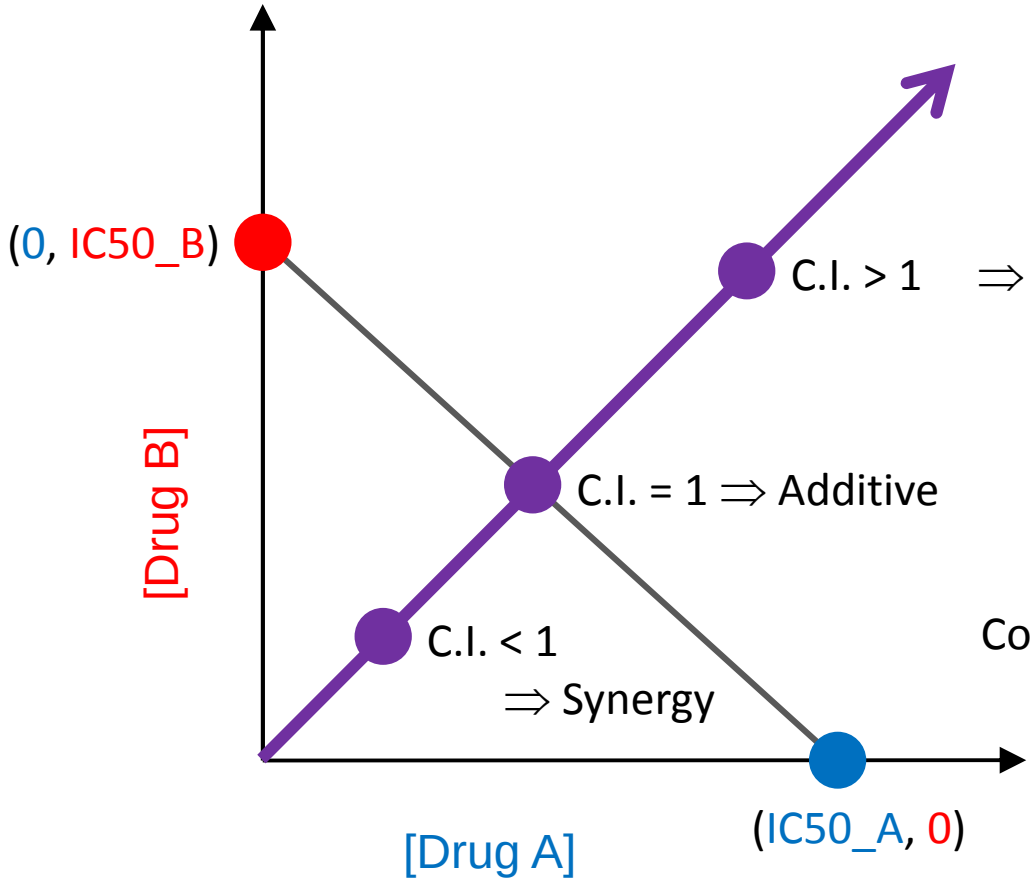
Loewe's additivity model simplified for fixed-ratio combinations



$$\text{Combination Index} = \frac{a}{IC50\_A} + \frac{b}{IC50\_B}$$

Chou-Talalay (1984): the *Combination Index*

Loewe's additivity model simplified for fixed-ratio combinations



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Chou-Talalay (1984): the *Combination Index*

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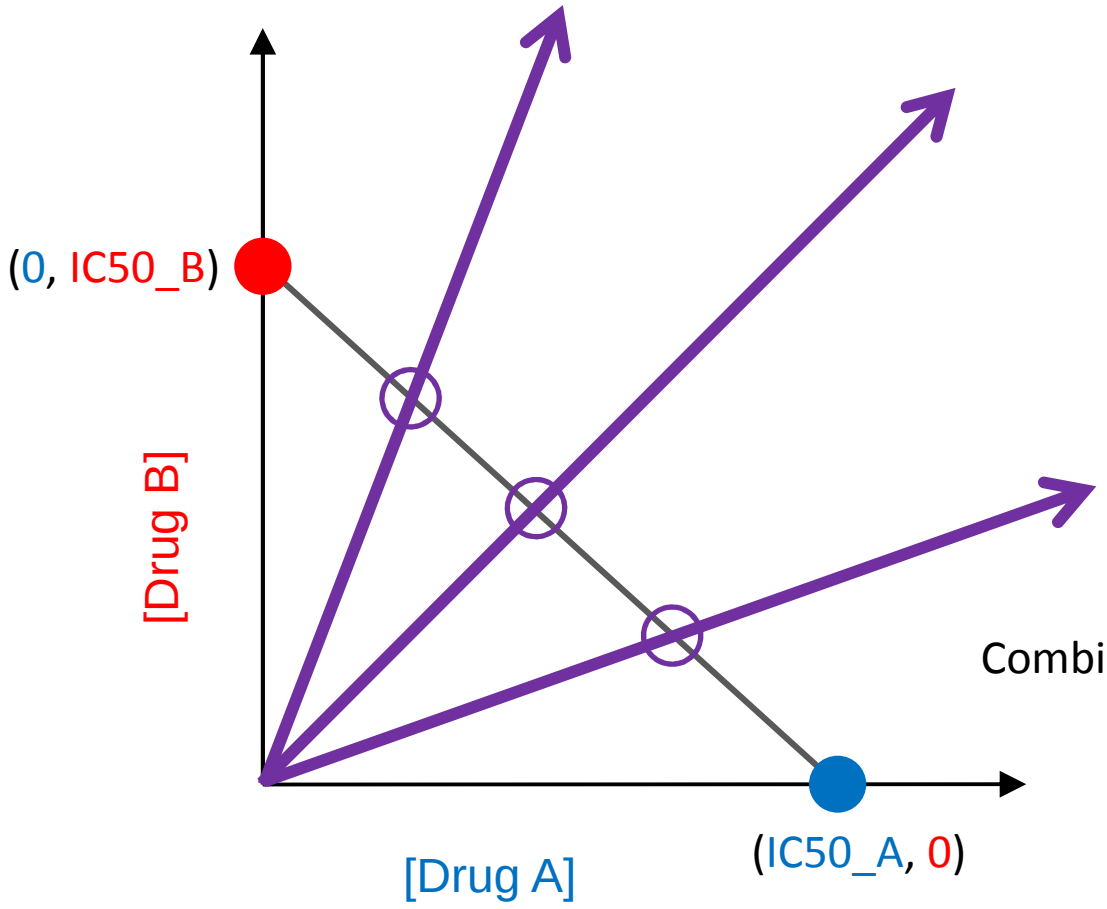
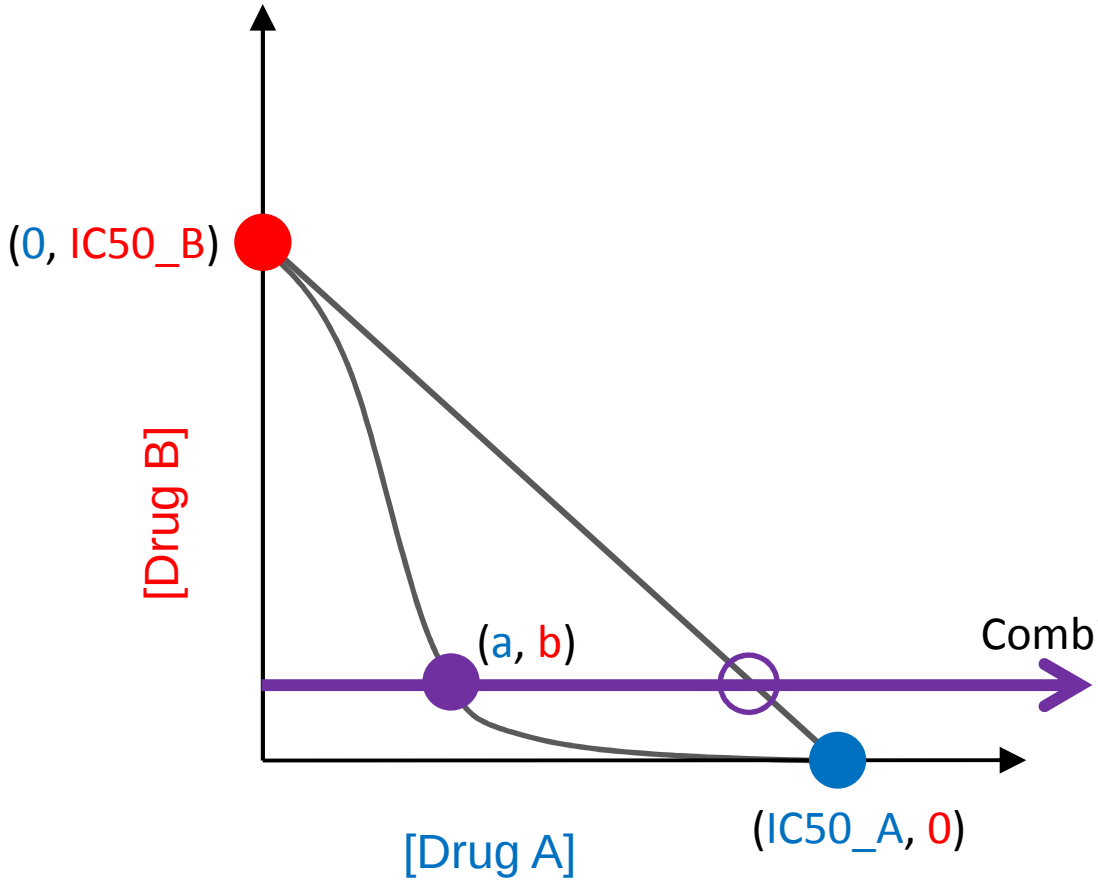


Analysis valid at any ratio

$$\text{Combination Index} = \frac{a}{IC50\_A} + \frac{b}{IC50\_B}$$

Chou-Talalay (1984): the *Combination Index*

Loewe's additivity model simplified for fixed-ratio combinations



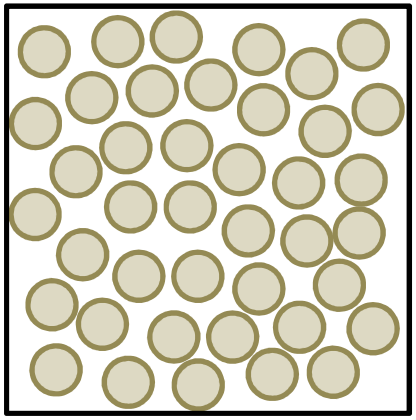
Analysis valid at any ratio

or with a fixed dose of drug B

$$\text{Combination Index} = \frac{a}{IC50\_A} + \frac{b}{IC50\_B}$$

Bliss (1939): Statistical Independence of toxins

Population of toxin-treated individuals
(e.g. cells, or people; originally insect eggs)



Bliss (1939): Statistical Independence of toxins

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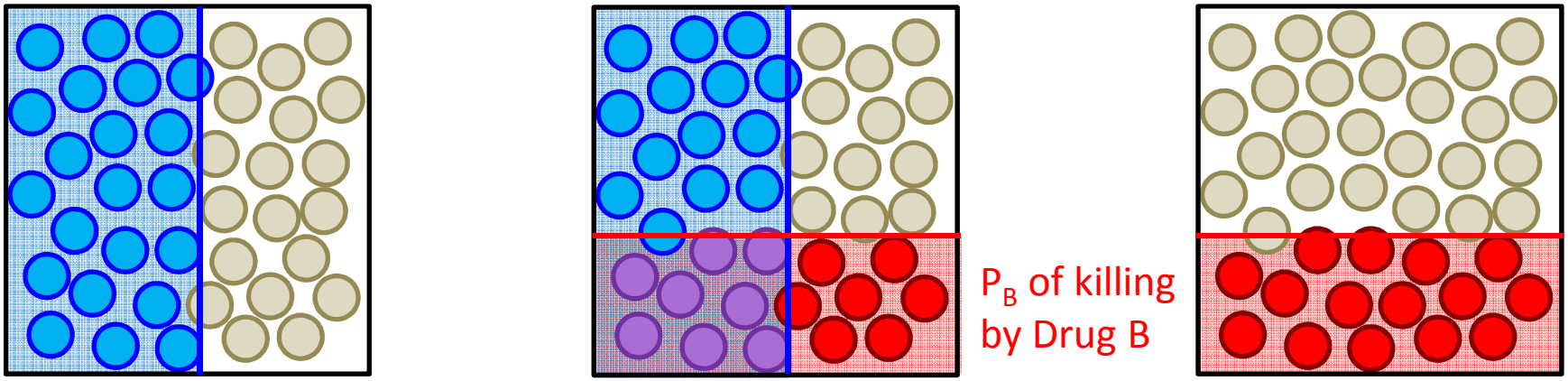


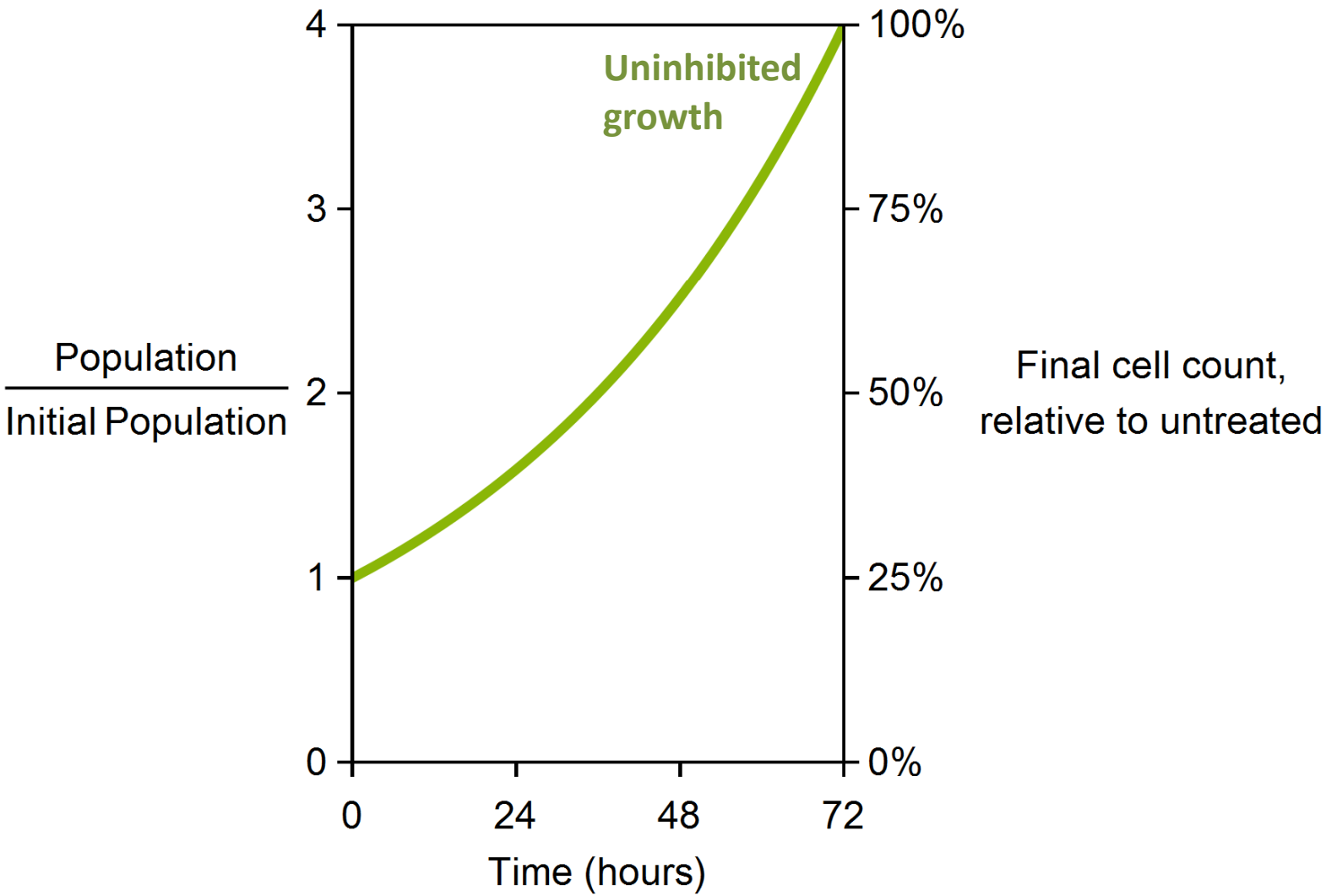
If probabilities of death are statistically independent, then $P_{A+B} = 1 - (1 - P_A) \times (1 - P_B)$

Bliss method can apply to other probabilities of "yes/no" events in populations, e.g. enzymes active or inhibited.

Not scientifically valid to analyze quantitative phenotypes, e.g. blood pressure, length of cell cycle.

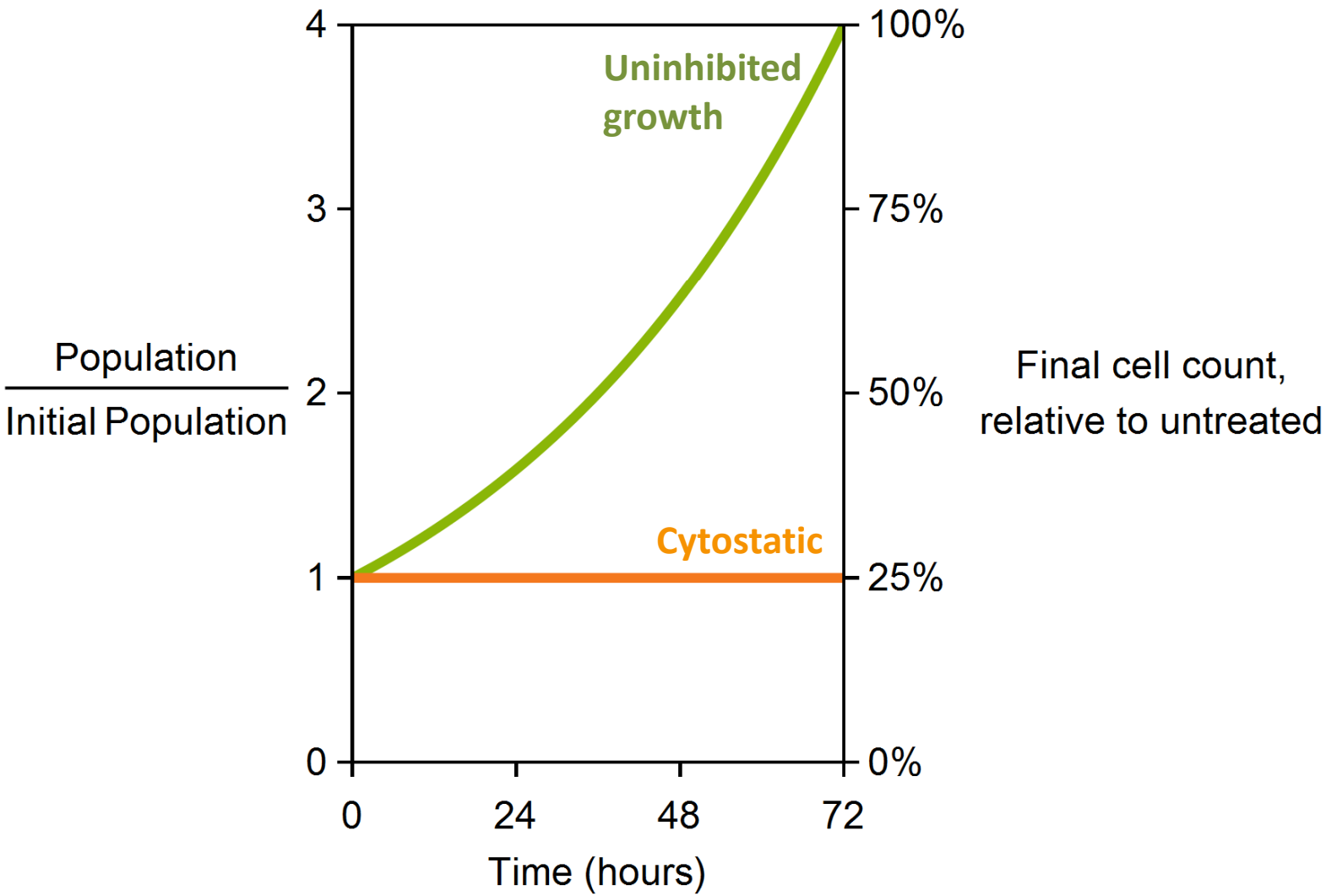
Bliss (1939): Statistical Independence of toxins

Bliss is not appropriate for growth inhibition:



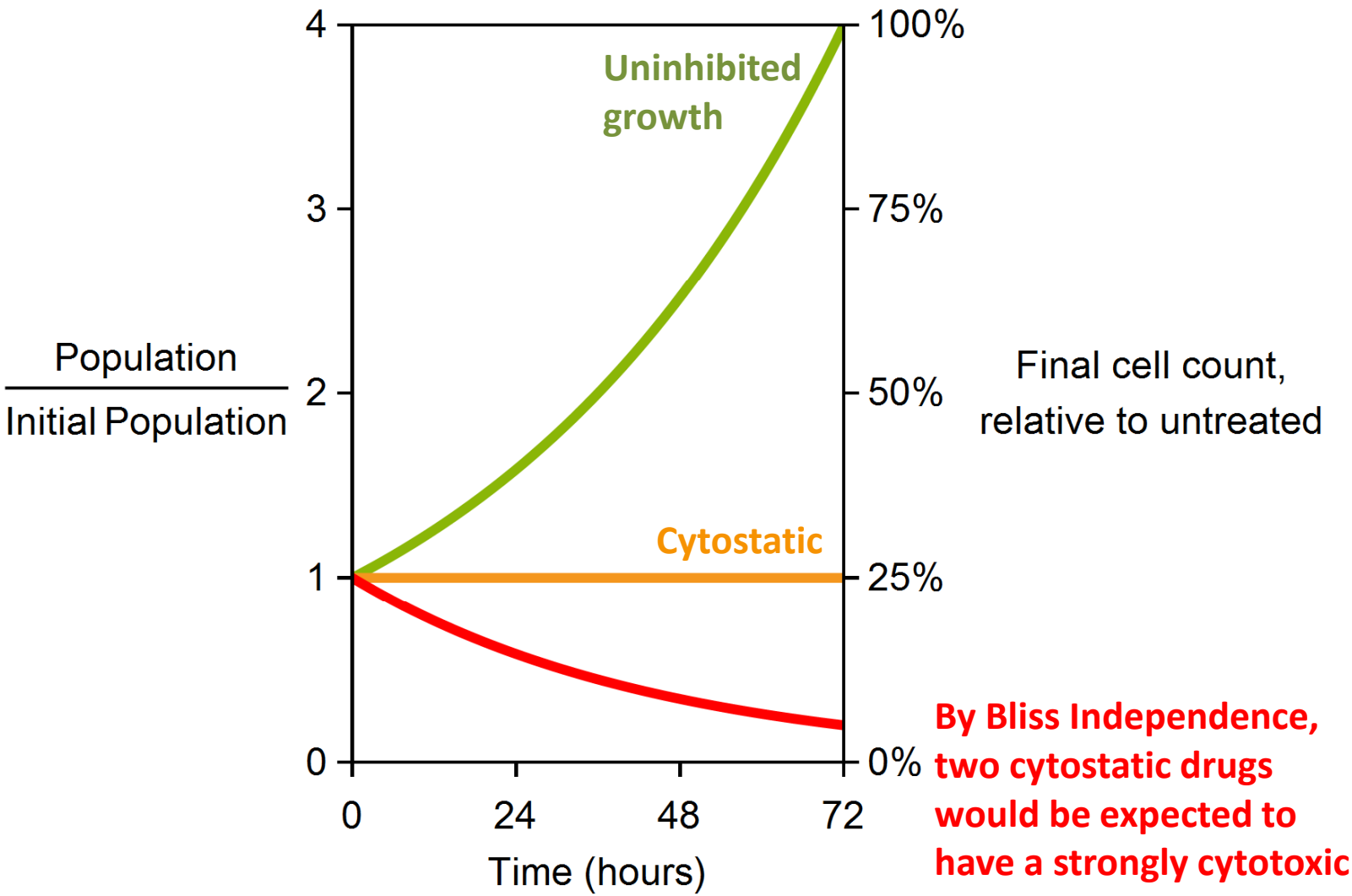
Bliss (1939): Statistical Independence of toxins

Bliss is not appropriate for growth inhibition:



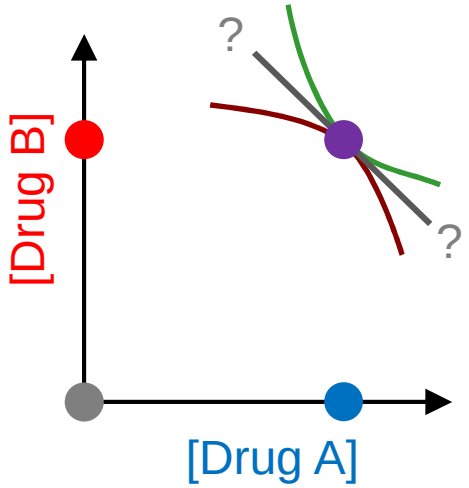
Bliss (1939): Statistical Independence of toxins

Bliss is not appropriate for growth inhibition:



By Bliss Independence, two cytostatic drugs would be expected to have a strongly cytotoxic effect in combination.

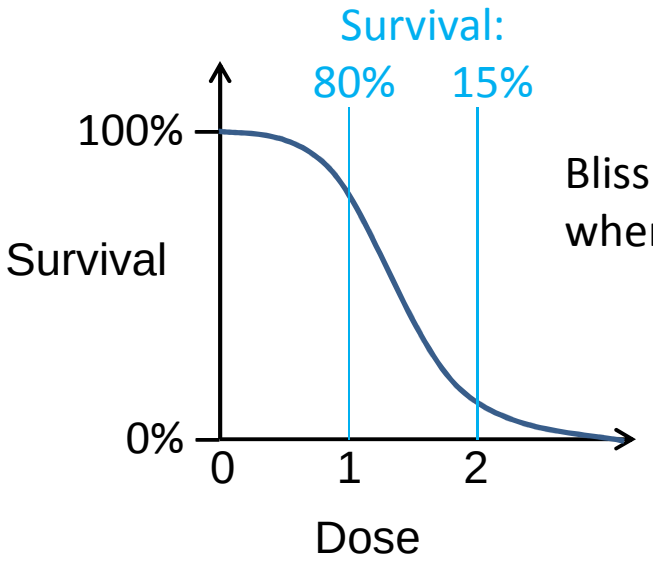
Bliss (1939): Statistical Independence of toxins



Statistical interaction assessed by Bliss gives
no information about pharmacological interaction.

Bliss synergy can occur with Loewe antagonism.
Bliss antagonism can occur with Loewe synergy.

These methods ask different questions,
and Bliss method lacks attention to dose response shape.



Bliss method identifies drugs as 'synergistic' with themselves,
when doses are at the tipping point of a steep dose-response.

These points explained well by Berenbaum (1989) *What is Synergy?* Pharmacological Reviews

Analysis of drug interactions

Synergy: “work together”

Antagonism: “struggle against”

Classical interpretation: (Gaddum)

Synergy:	Two drugs	>	Strongest one drug
No interaction:	Two drugs	=	Strongest one drug
Antagonism:	Two drugs	<	Strongest one drug

Modern interpretation: (Loewe, or Combination Index, or Bliss)

Synergy:	Two drugs	>	“sum of parts”
No interaction:	Two drugs	=	“sum of parts”
Antagonism:	Two drugs	<	“sum of parts”

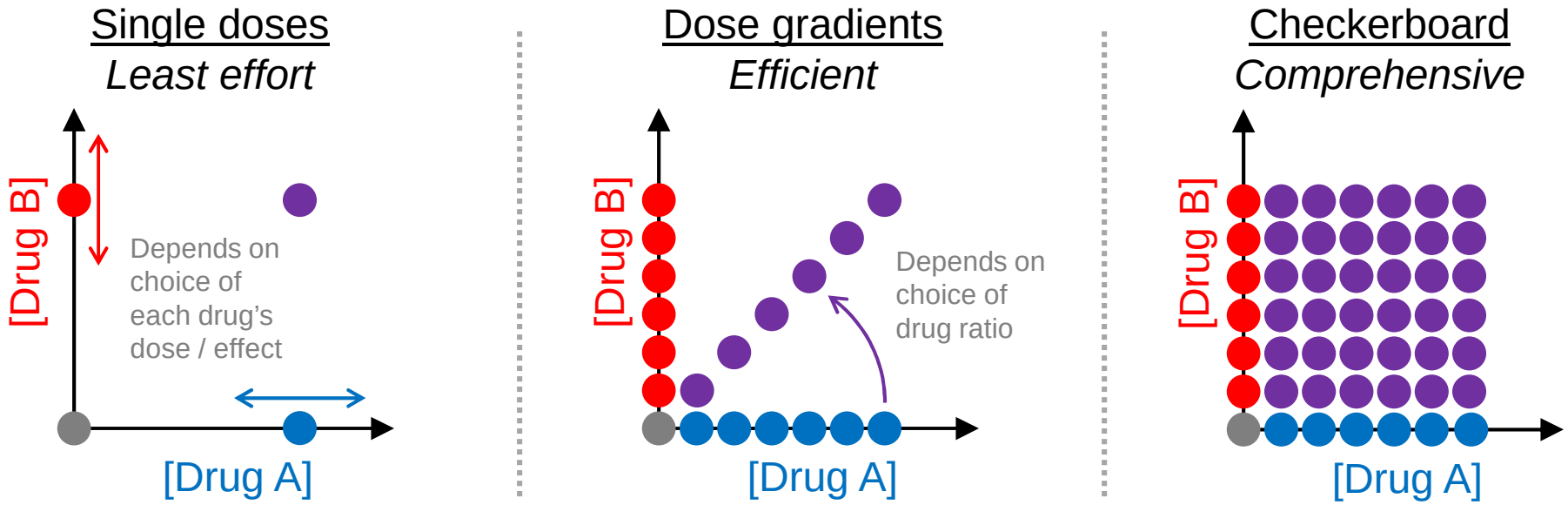
Modern “Antagonism” can produce classic “synergy”

Modern “synergy” is not necessary for stronger effect and clinical benefit

Definitions of ‘*no interaction*’ are different.

Modern use of ‘*synergy*’ or ‘*antagonism*’ depends on the expected sum of parts.

Experimental Design Revisited



Pharmacological Additivity (Loewe): **Isobologram analysis**

Combination Index (Chou-Talalay)

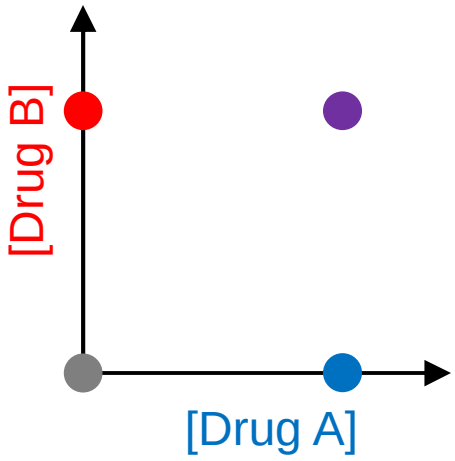
Pharmacological Independence (Gaddum)

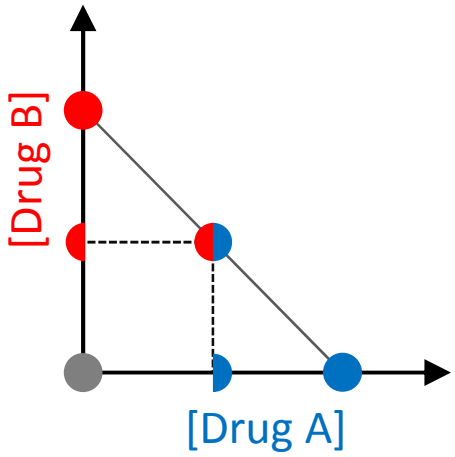
Statistical Independence (Bliss)

Experimental Design Revisited

Single doses

Least effort

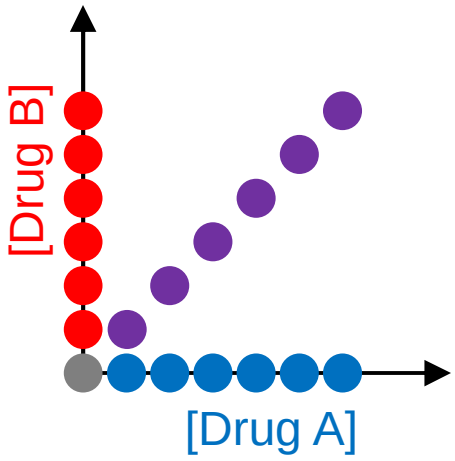


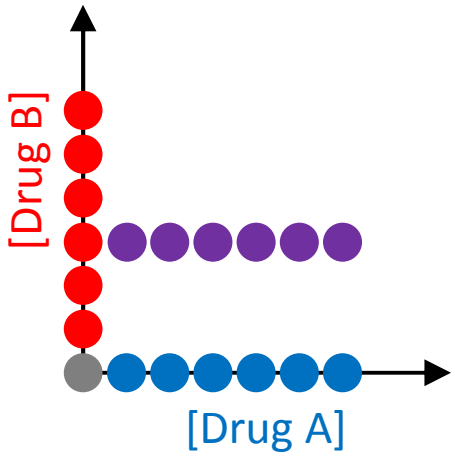


Can calculate
“excess over additive”

Dose gradients

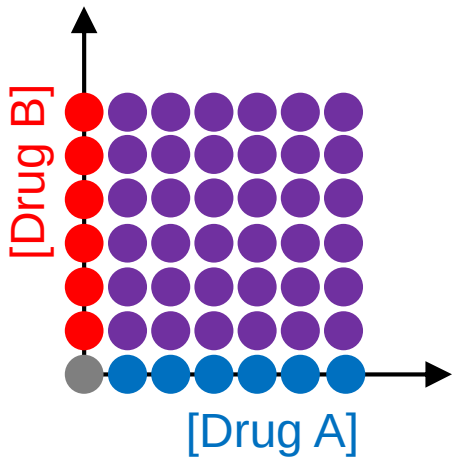
Efficient





Checkerboard

Comprehensive



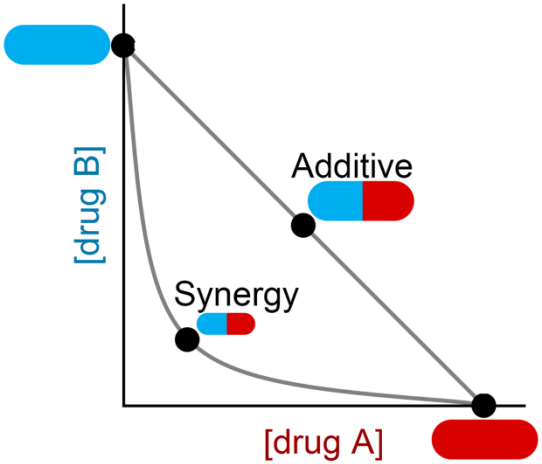
Clinical Relevance

Is drug synergy necessary for clinical benefit?

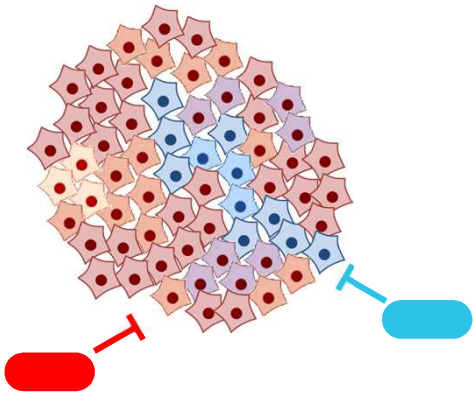
Complicating factors:

- Synergy of desired effect vs. Synergy of toxic side effects
- Synergy vs. Individual drug potency
- Synergy vs. Slowing evolution of drug resistance
- Synergy vs. Addressing heterogeneity

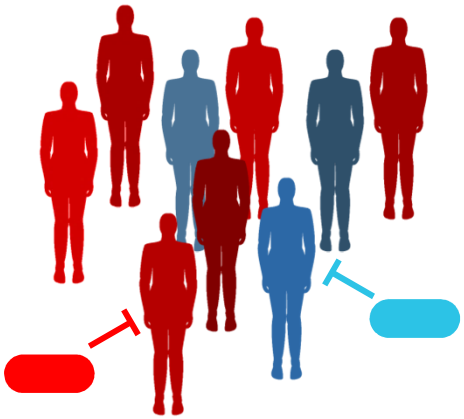
Three rationales for combination cancer therapy



Pharmacological additivity or synergy



Within-tumor heterogeneity

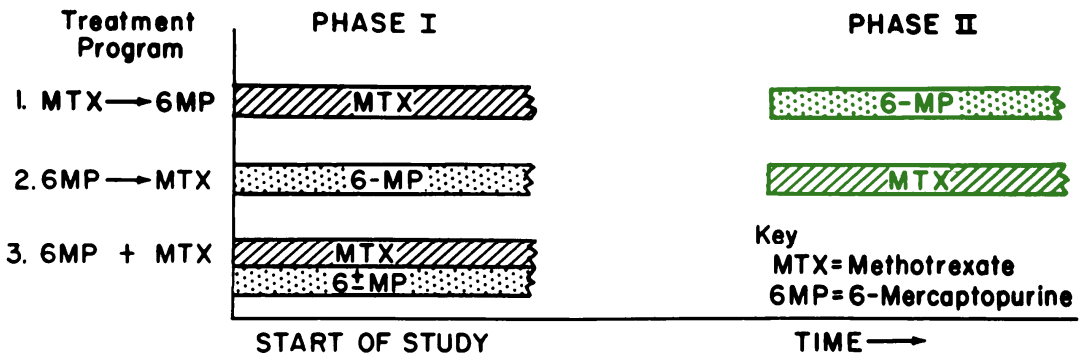


Between-tumor heterogeneity

S. Loewe (1928) *Ergeb. Physiol*.
Gaddum (1940) *Pharmacology*

L. Law (1952) *Cancer Research*

E. Frei 3rd, *et al.* (1961) *Blood*



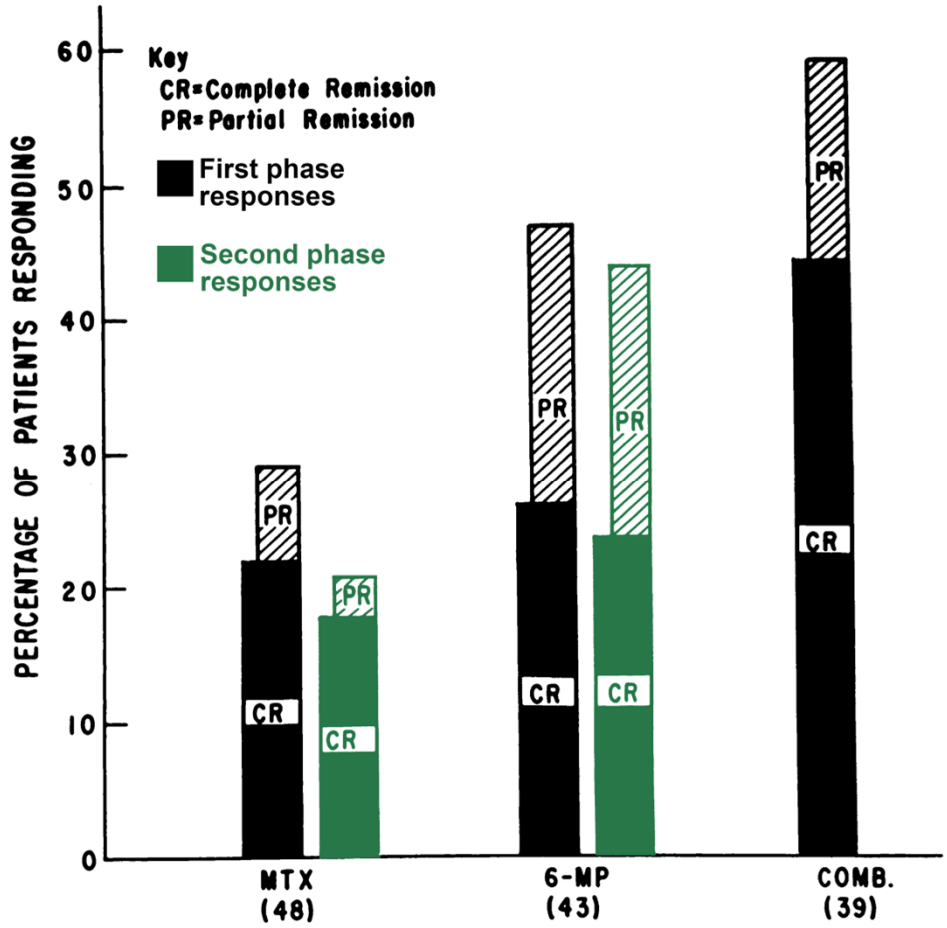
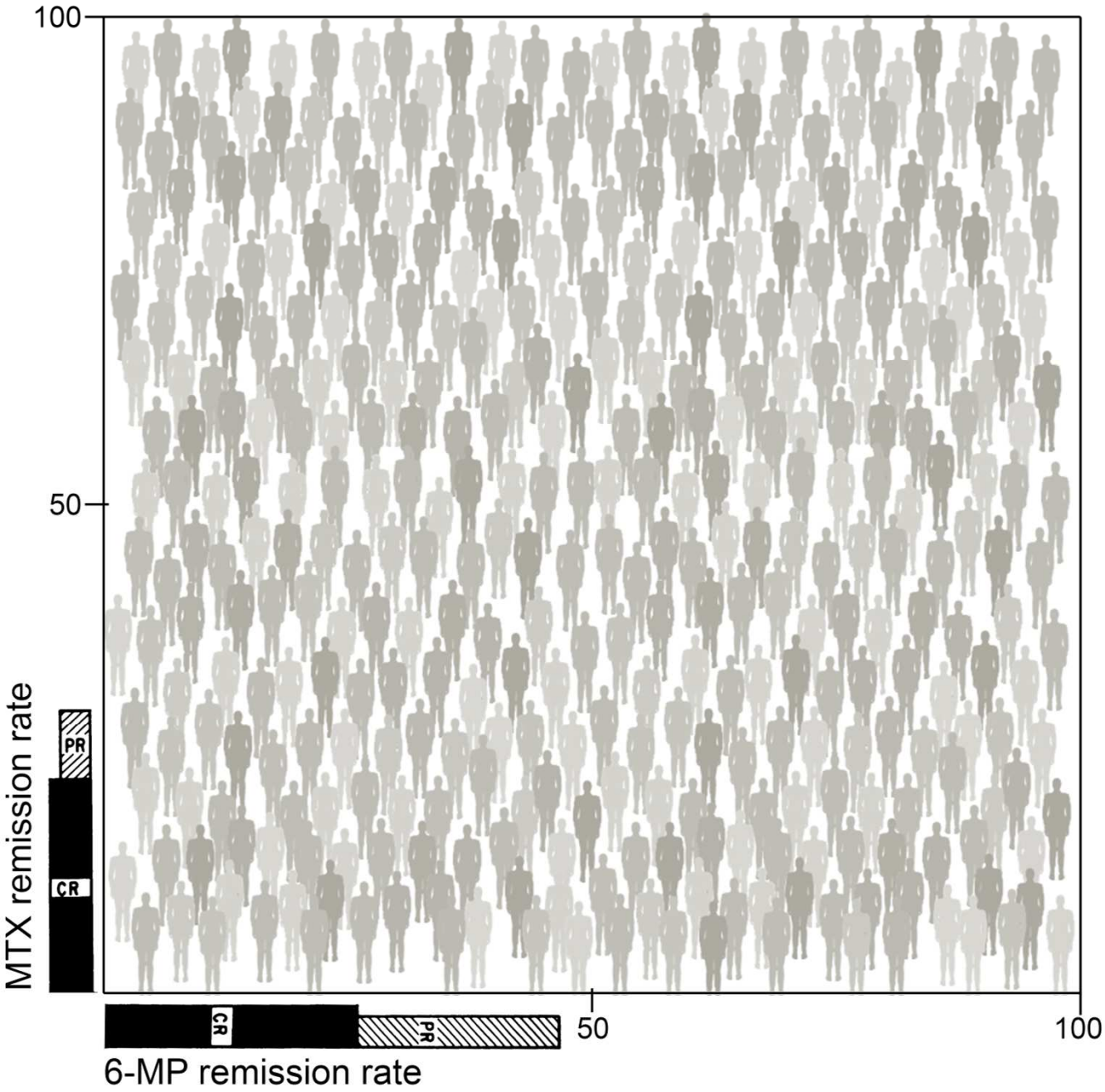
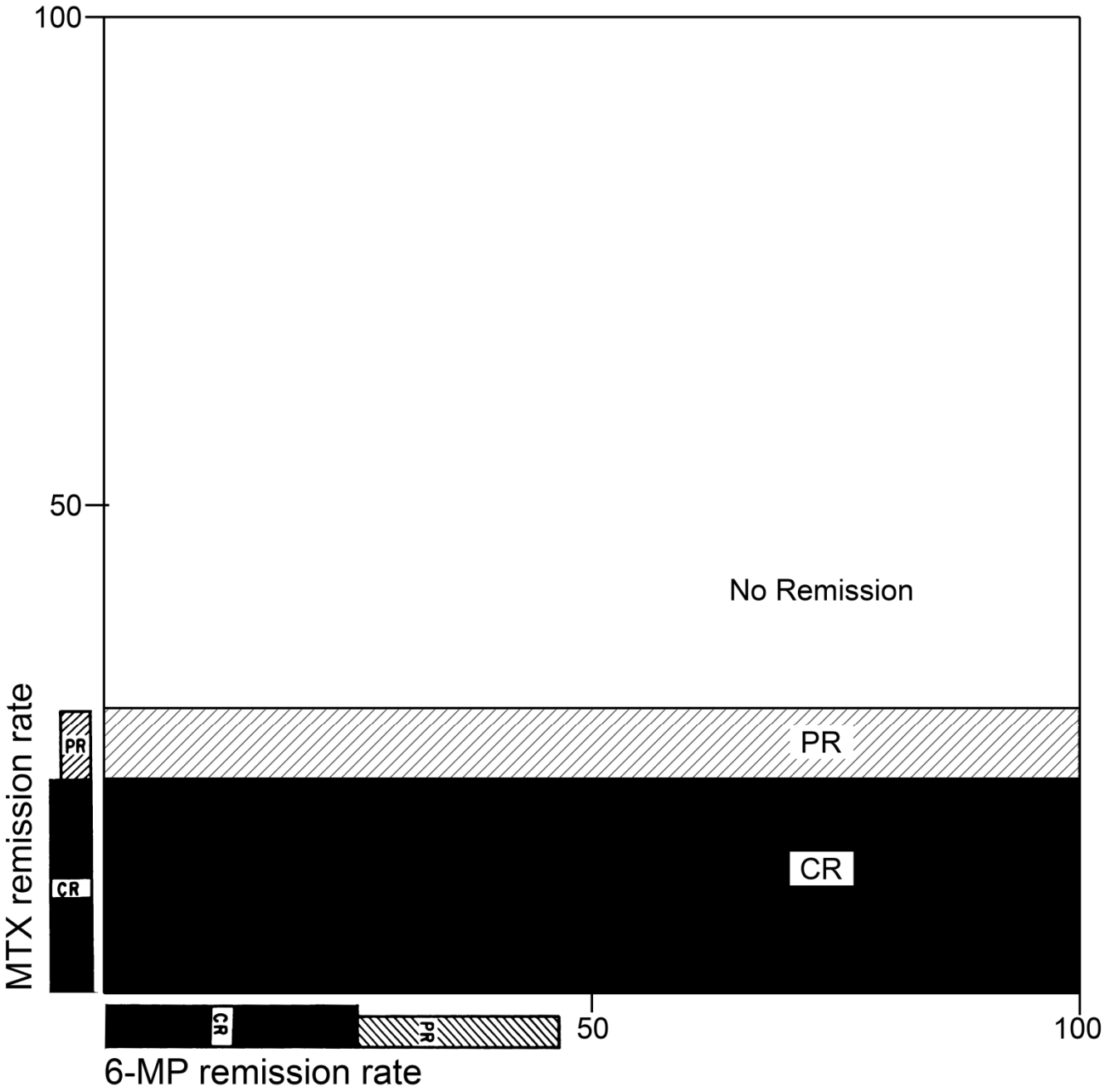
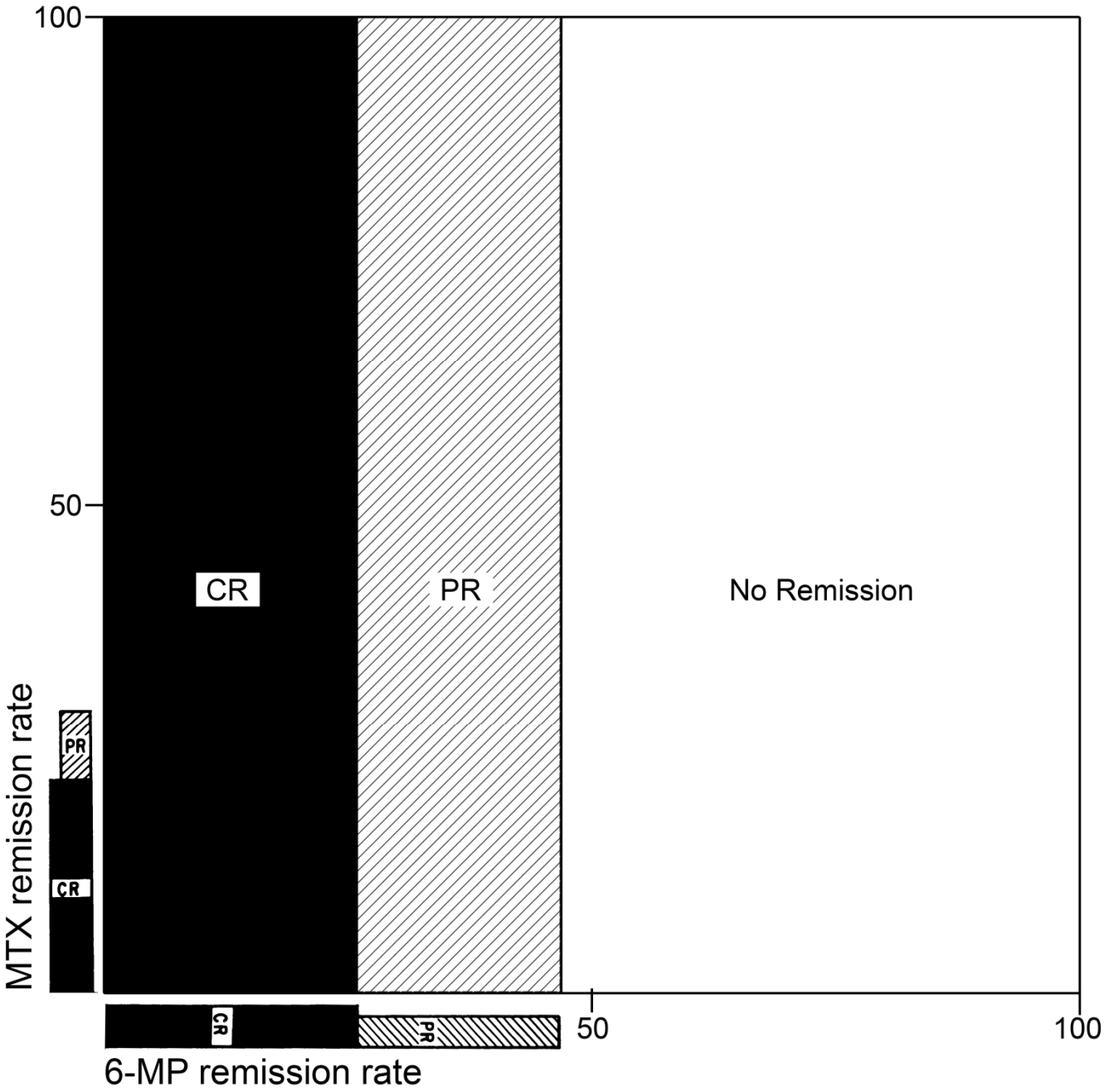


Fig. 2.—Acute lymphocytic leukemia in children. Response rates in Phase I by treatment.

Figure adapted from Emil Frei 3rd, *et al.* Blood (1961) **18**:431


100
50
MTX remission rate
PR
CR
CR
PR
50
100
6-MP remission rate


100
50
No Remission
PR
CR
MTX remission rate
PR
CR
CR
PR
50
100
6-MP remission rate


100
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CR
PR
50
100
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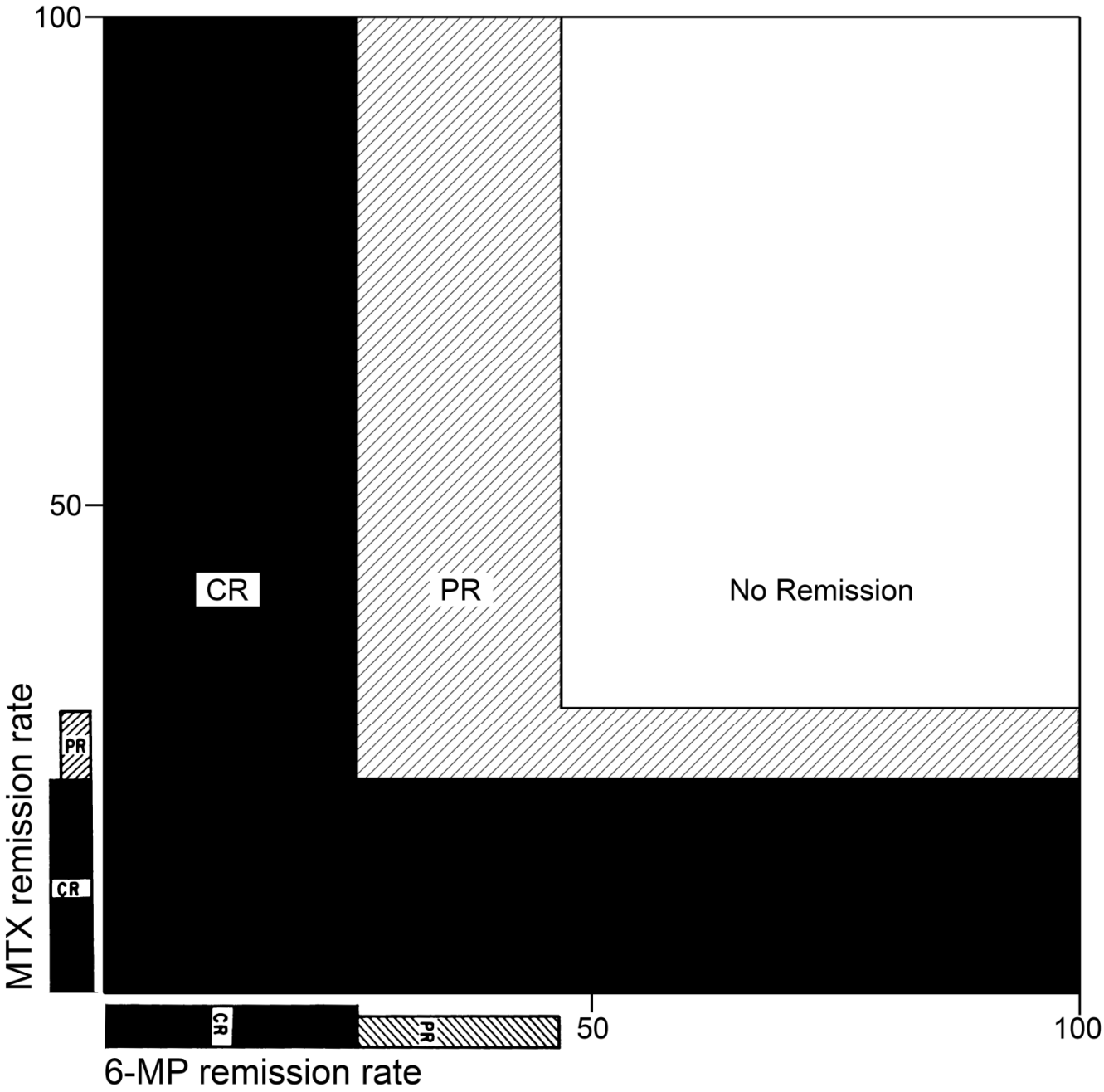
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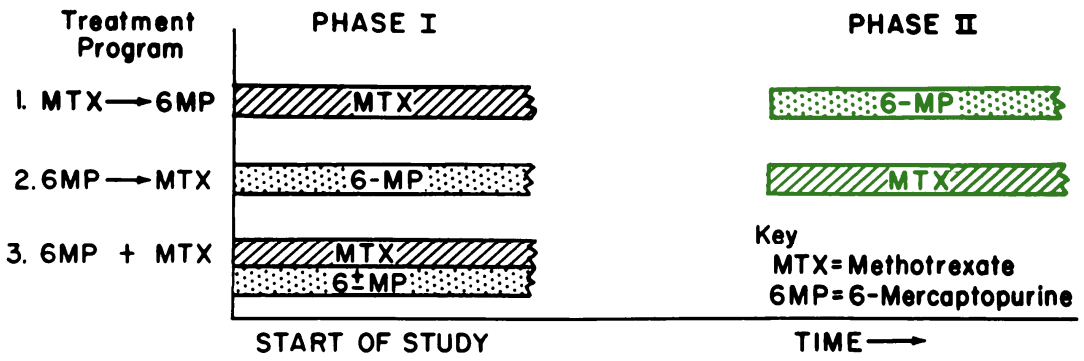


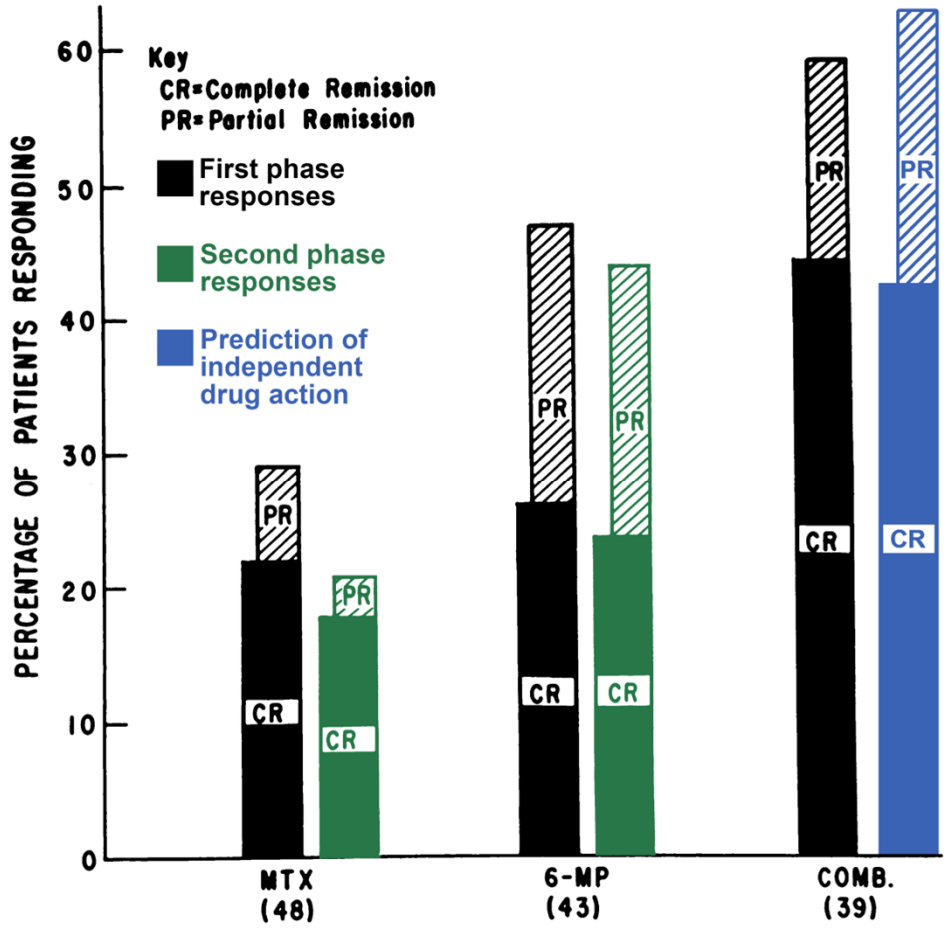
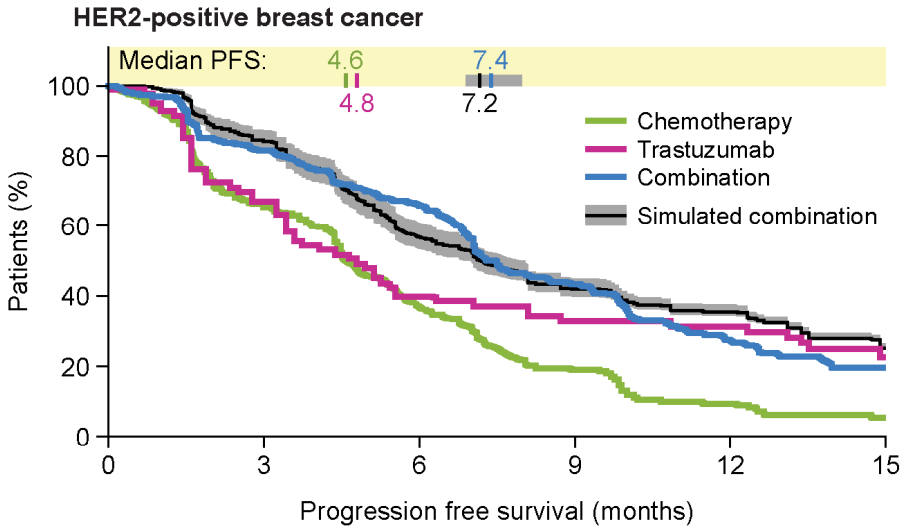


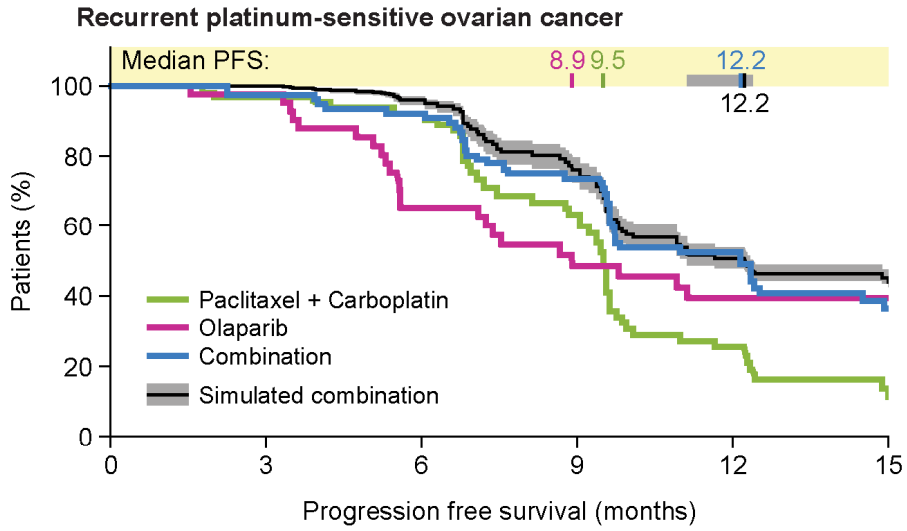
Fig. 2.—Acute lymphocytic leukemia in children. Response rates in Phase I by treatment.

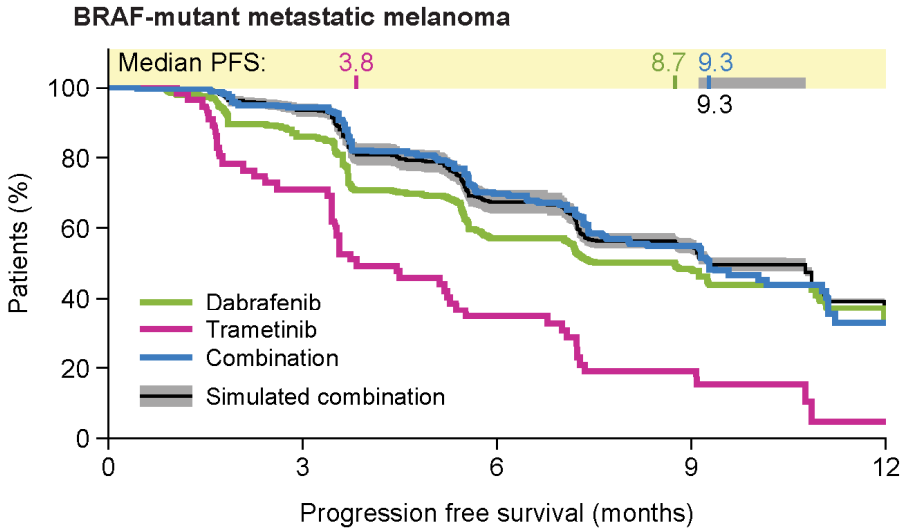
Independent action explains the clinical efficacy of many 2-drug combination therapies for acute leukemia

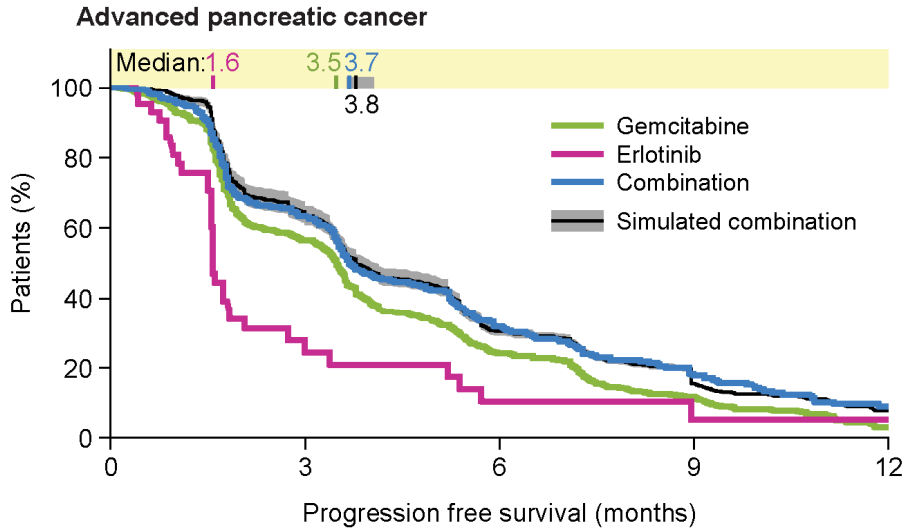
Figure adapted from Emil Frei 3rd, *et al.* Blood (1961) **18**:431

Independent action explains the clinical efficacy of many combination therapies for metastatic cancers









Palmer & Sorger (*in review*)

Conducting Isobologram analysis

	4-H-Cyclophosphamide (µM):										
	0.00	0.32	0.50	0.79	1.20	2.00	3.20	5.07	8.00	12.67	20.00
Vincristine (nM):	Log10(Viability)										
0.00	0.00	-0.28	-0.13	-0.09	-0.01	-0.19	-0.35	-0.50	-1.42	-3.00	-3.00
0.32	0.01	0.00	0.05	-0.10	-0.18	-0.13	-0.52	-0.56	-1.00	-3.00	-3.00
0.51	-0.05	-0.14	0.03	-0.21	0.05	-0.05	-0.32	-0.50	-0.77	-3.00	-3.00
0.80	-0.21	0.02	0.05	-0.09	-0.10	-0.18	-0.32	-0.53	-0.62	-3.00	-3.00
1.27	-0.11	-0.29	-0.16	-0.20	-0.45	-0.31	-0.51	-0.68	-1.22	-3.00	-3.00
2.00	-0.55	-0.40	-0.48	-0.38	-0.42	-0.41	-0.43	-0.57	-1.12	-3.00	-3.00
3.20	-0.98	-0.98	-0.84	-0.71	-0.65	-0.74	-0.70	-0.69	-1.01	-3.00	-3.00
5.07	-1.73	-1.53	-1.53	-1.29	-1.25	-0.97	-0.78	-0.78	-1.11	-3.00	-3.00
8.00	-1.75	-1.68	-1.60	-1.79	-1.70	-1.17	-1.13	-0.87	-1.40	-3.00	-3.00
12.53	-1.77	-1.75	-1.81	-1.79	-1.75	-1.43	-1.13	-0.95	-1.21	-3.00	-3.00
20.00	-1.89	-1.83	-1.79	-1.71	-1.68	-1.43	-1.31	-1.28	-1.51	-3.00	-3.00

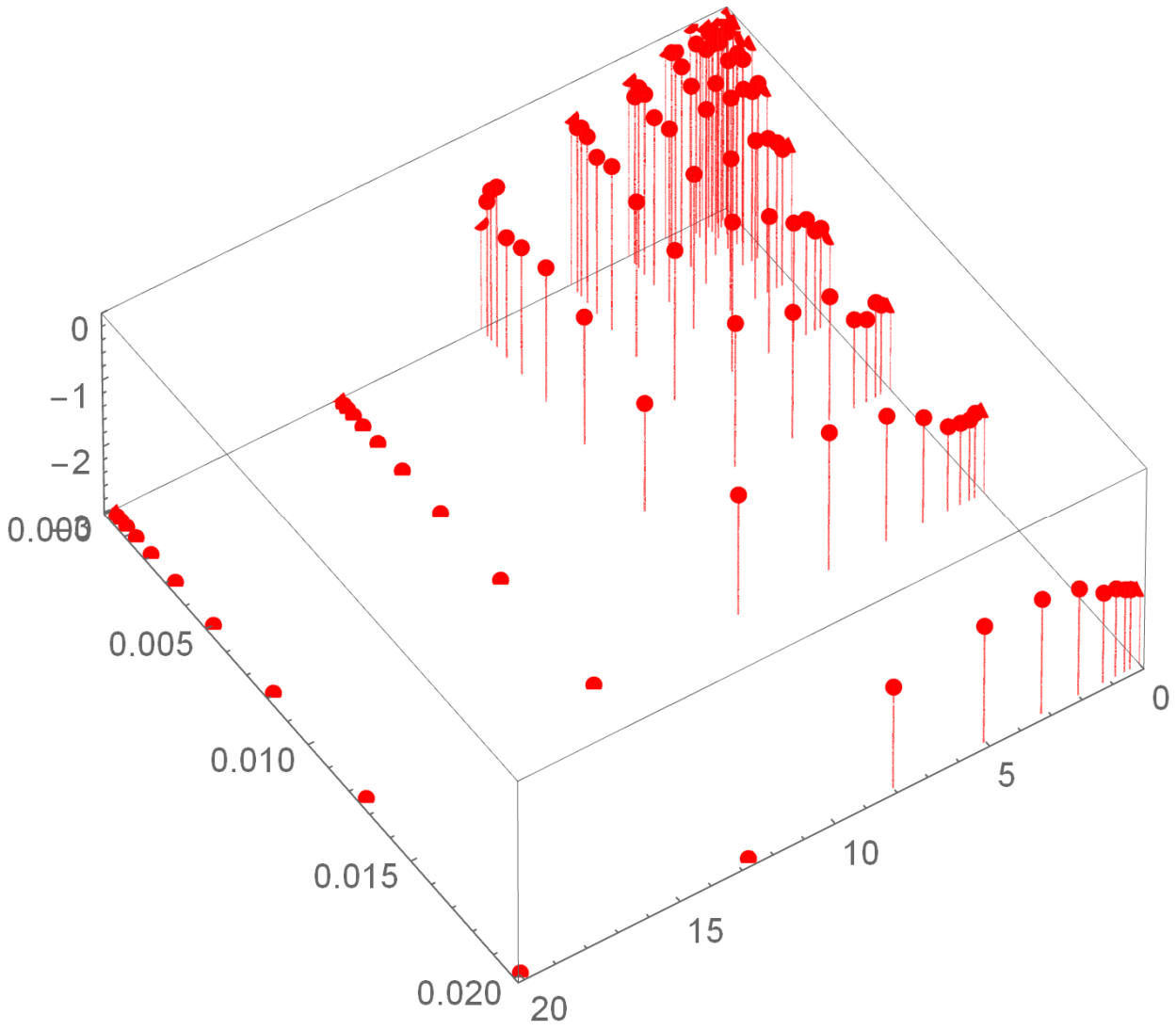
Response grid

Conducting Isobologram analysis

Vinc (nM)	4-H-C (μM)	Log10(viability)
0.00	0.00	0.00
0.00	0.32	-0.28
0.00	0.50	-0.13
0.00	0.79	-0.09
0.00	1.20	-0.01
0.00	2.00	-0.19
0.00	3.20	-0.35
0.00	5.07	-0.50
0.00	8.00	-1.42
0.00	12.67	-3.00
0.00	20.00	-3.00
0.32	0.00	0.01
0.32	0.32	0.00
0.32	0.50	0.05
0.32	0.79	-0.10
0.32	1.20	-0.18
0.32	2.00	-0.13
0.32	3.20	-0.52

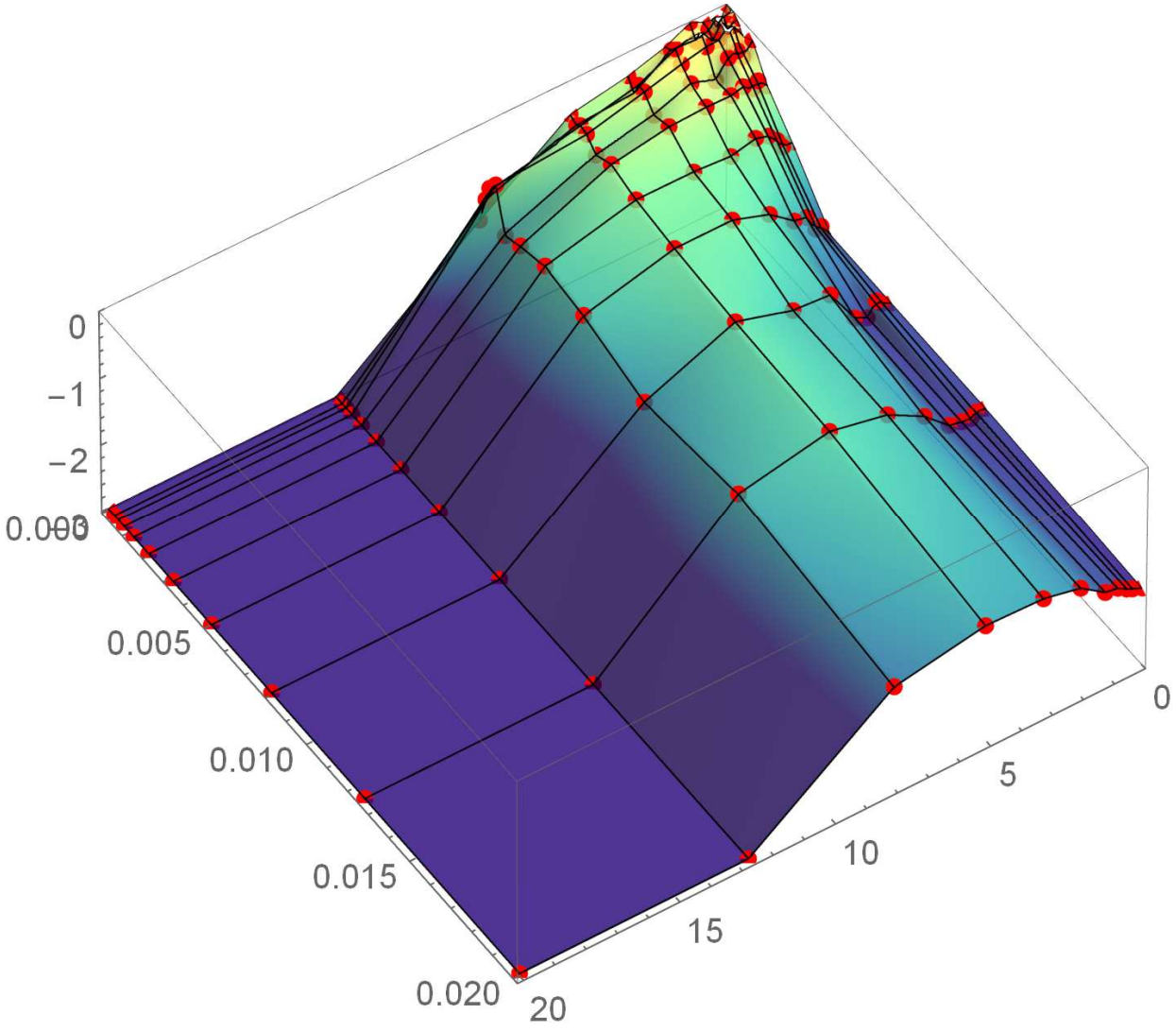
Response grid $\Rightarrow$ 3D coordinates

Conducting Isobologram analysis



Response grid $\Rightarrow$ 3D coordinates

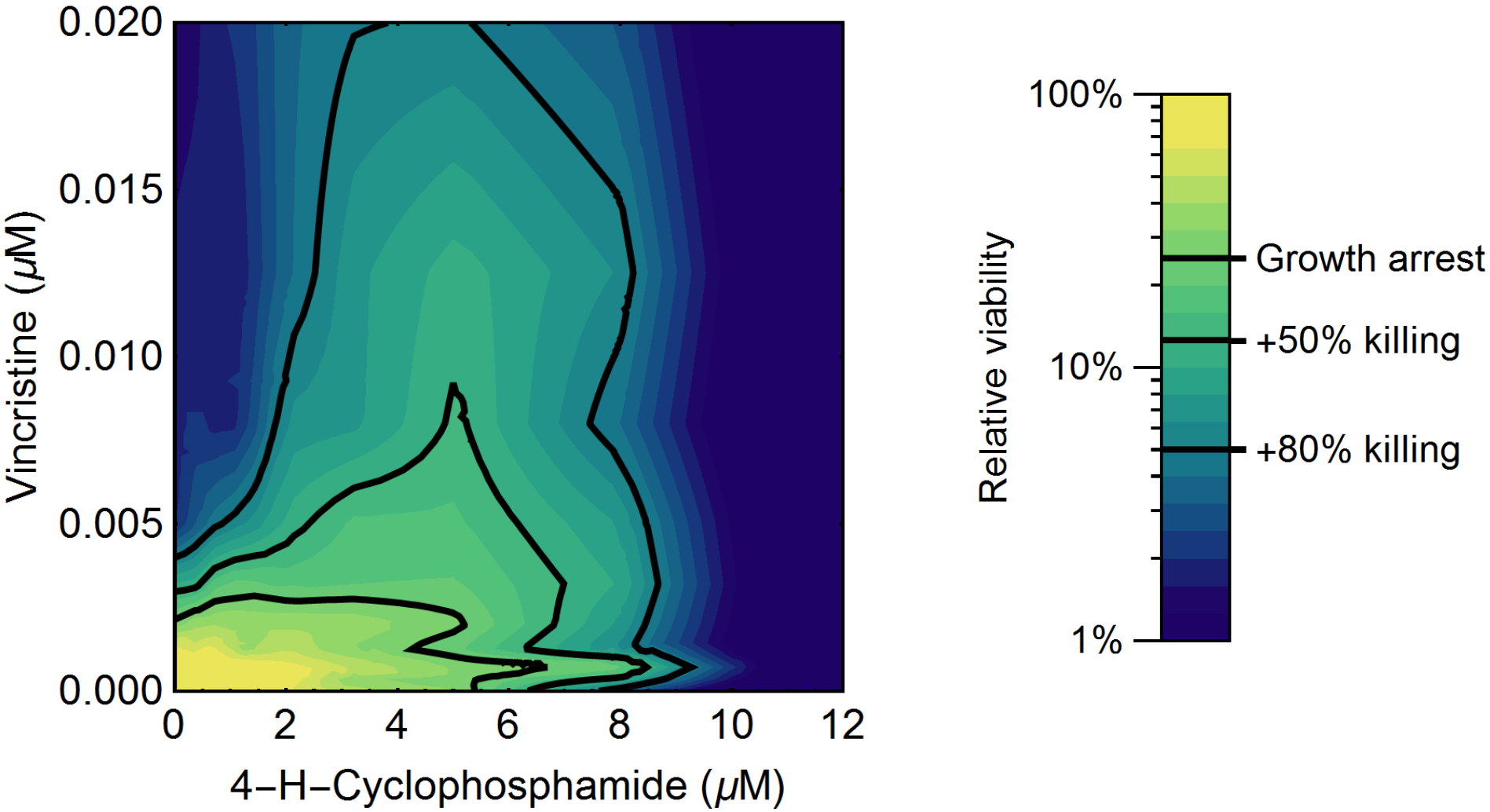
Conducting Isobologram analysis



Response grid ⇒ 3D coordinates ⇒ Interpolation

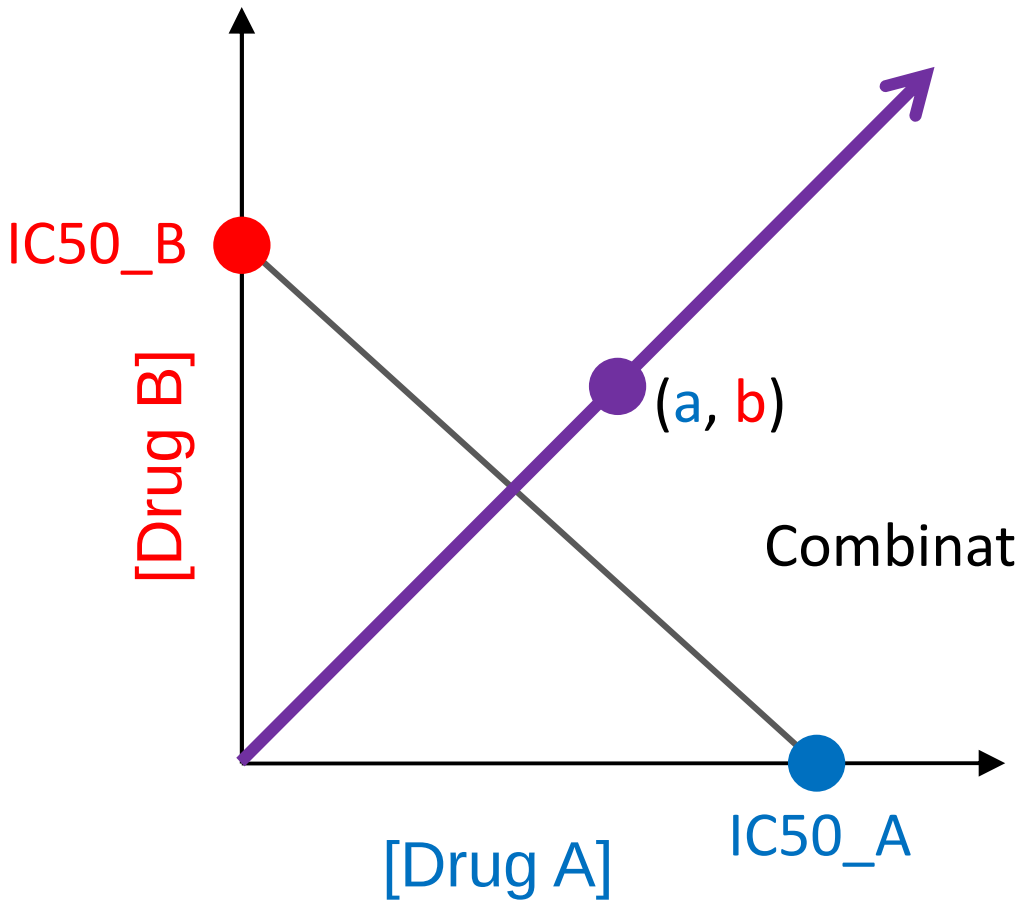
(recommend first-order interpolation)

Conducting Isobologram analysis



Response grid ⇒ 3D coordinates ⇒ Interpolation ⇒ Contour plot

Calculating Combination Index



$$\text{Combination Index} = \frac{a}{IC50\_A} + \frac{b}{IC50\_B}$$