Oncolines

How to select the best platform for testing drug combinations?

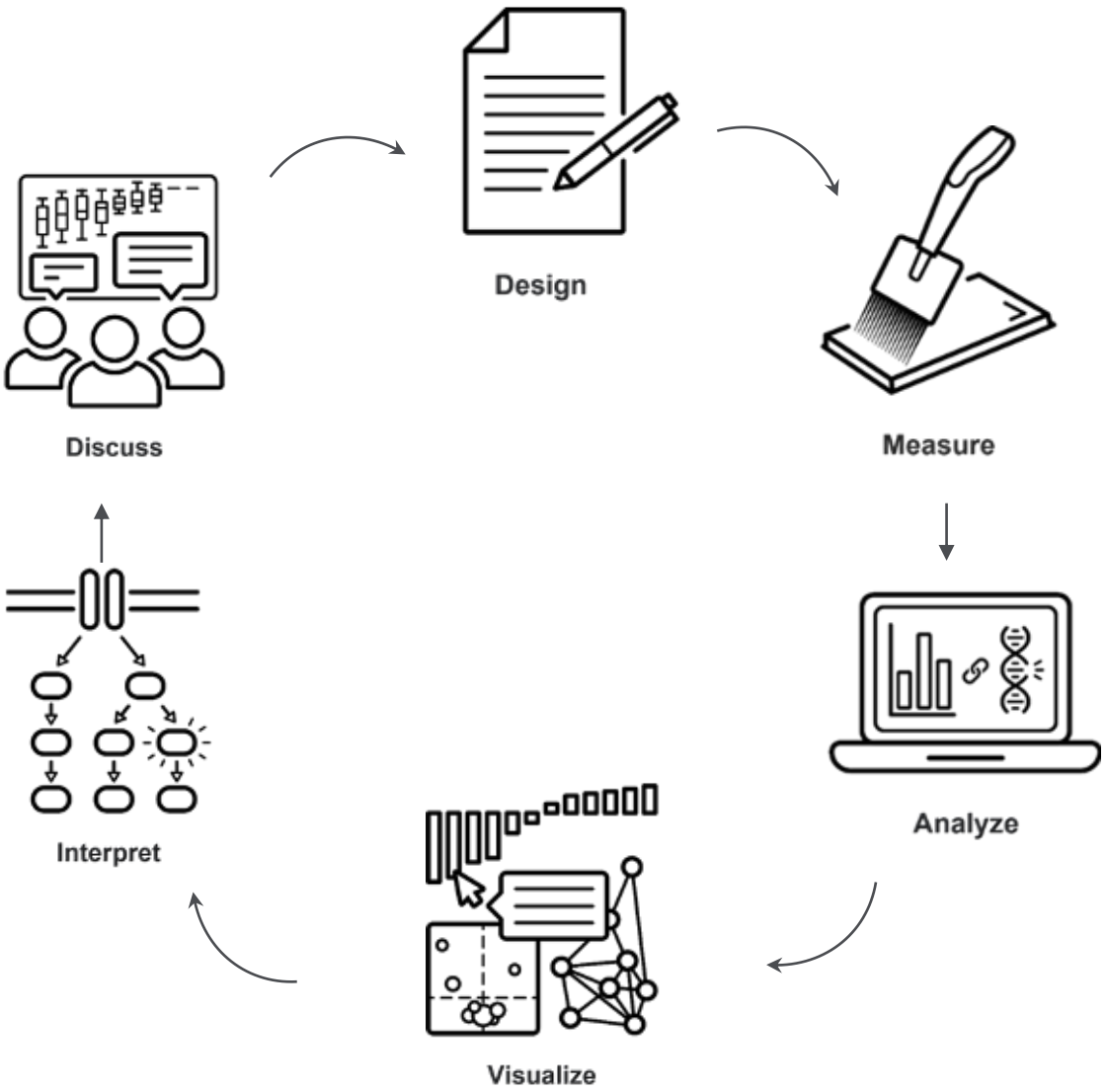
Webinar Oncolines
June 9th, 2022

Oncolines - Introduction

- We are a precision medicine services company in oncology

Oncolines' Team in April 2022

Oncolines - Adding Value

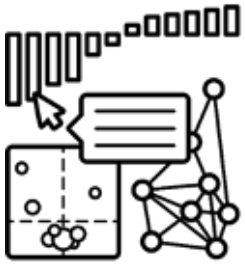


Oncolines - Adding Value

Design

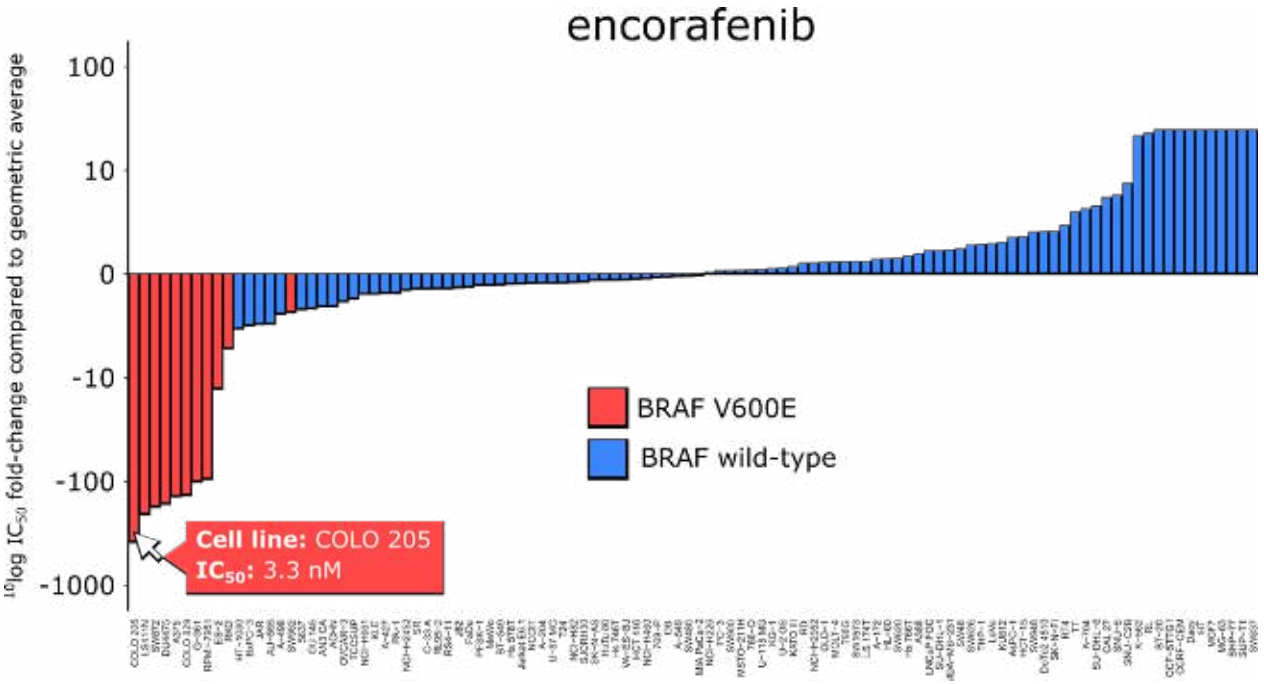
- Selection of cell lines
- Flexibility in incubation time

Oncolines - Adding Value



Visualize

- Interactive reporting
- Preparation of figures for filing purposes



Oncolines - Adding Value

Discuss

- Once reports are shared, we are open to discuss data together

- Support regarding interpretation of study results and follow-up studies

Oncolines – Core Services

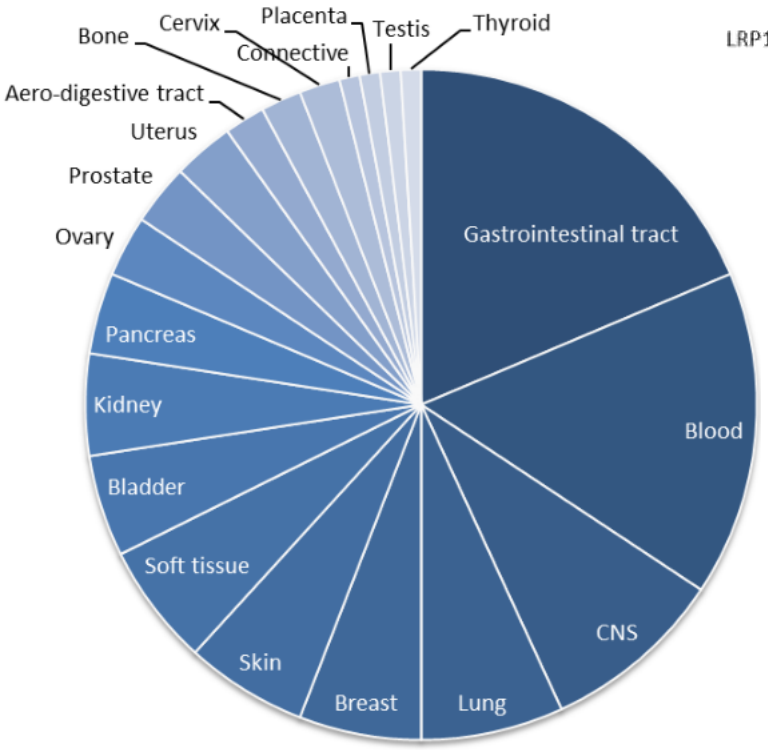
Oncolines Profiling

Combination Studies

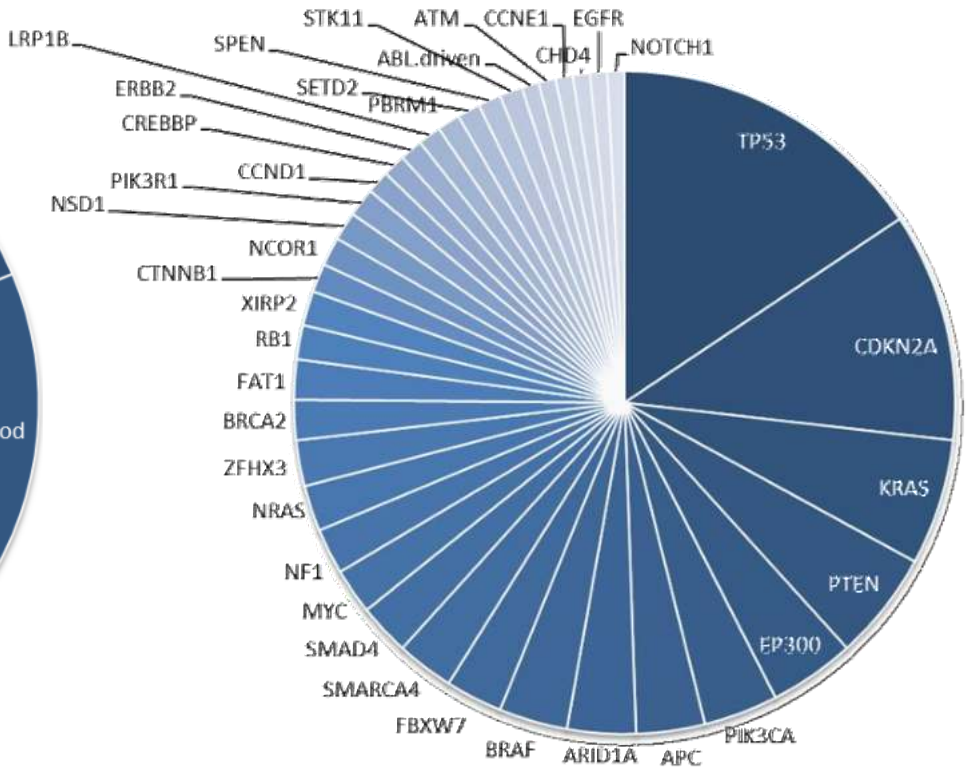
Mechanistic Cell Biology

Oncolines Profiling

- More than 200 cell lines available for proliferation assays
  - 102 Oncolines cancer cell line panel



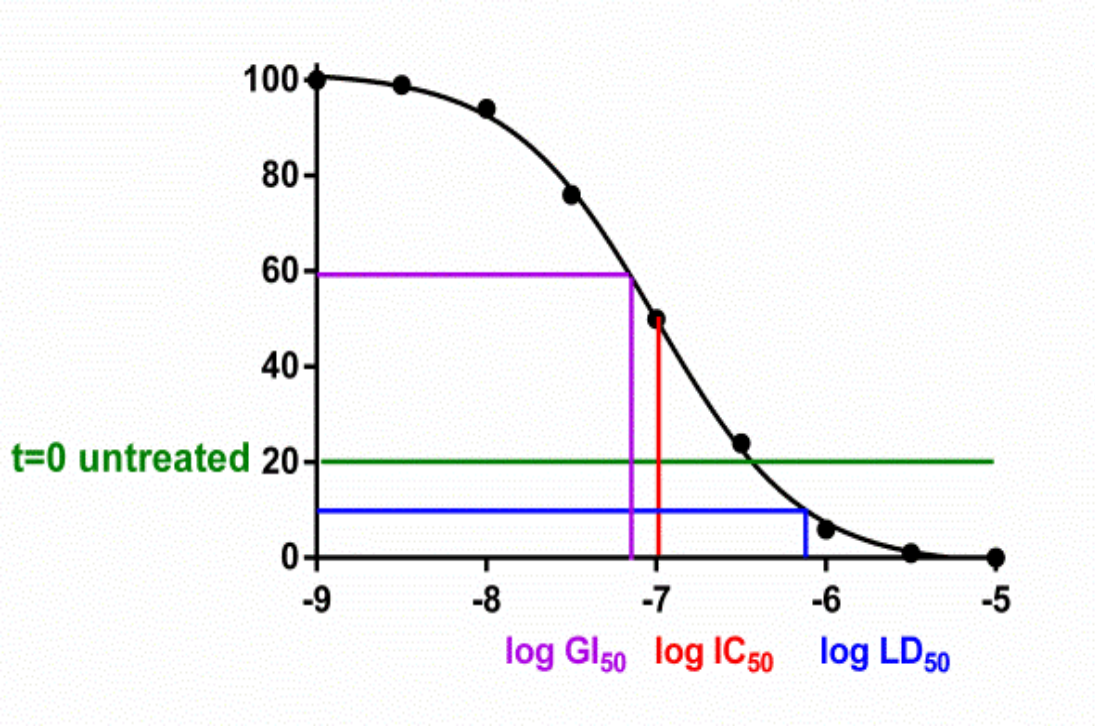
Distribution of tissue types



Distribution of mutations

Oncolines Profiling

- Nine-points dose range
- Read-out ATPLite™: intracellular ATP content as an indirect readout of cell number
- Determination of half-maximal inhibitory concentration (IC_{50}), half-maximal growth inhibitory concentration (GI_{50}), half-maximal lethal dose (LD_{50}), and maximum effect (efficacy)

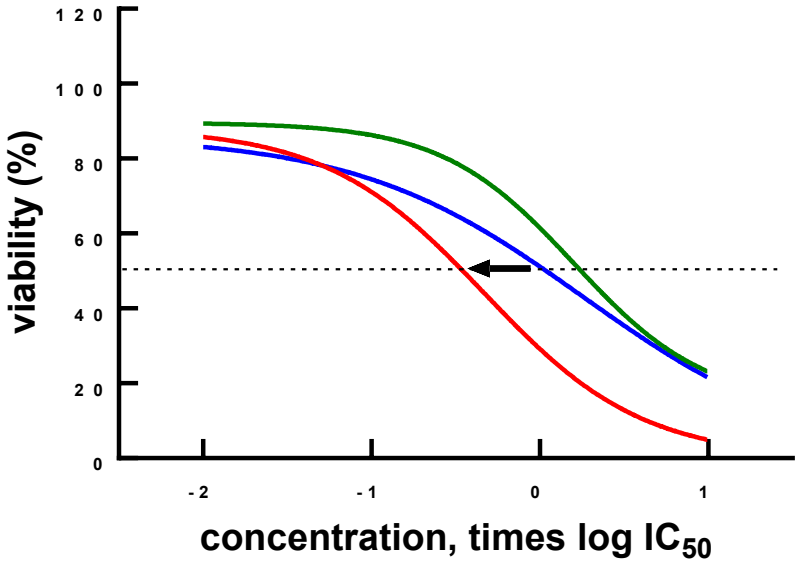


Combination Therapies

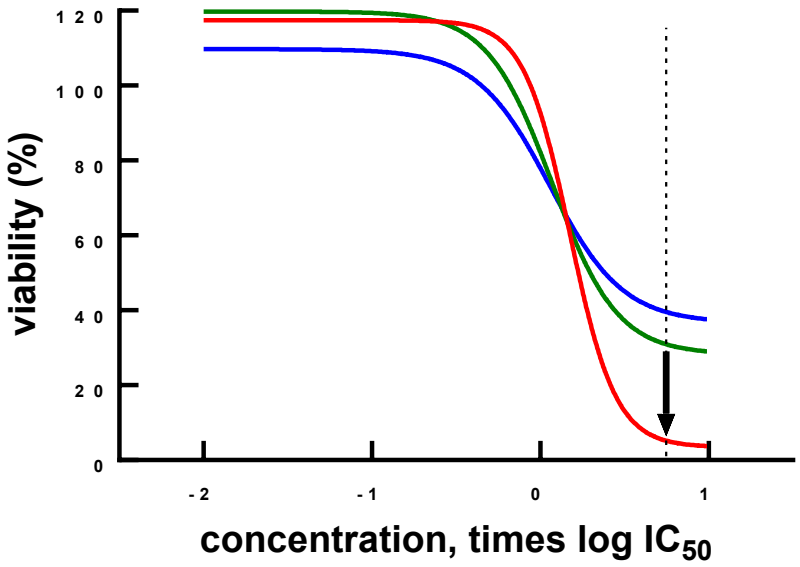
- Treatment modality that combines two or more therapeutic agents.
- In 1960's, one of the first combination experiments with cancer drugs was performed in pediatric leukemia patients with the aim to prolong remission.
- Nowadays, many cancer treatments are combination therapies.
- Combining drugs can improve therapy response, prevent drug resistance, or reduce toxicity.
- Combination experiments *in vitro* can help in providing a rationale for combination experiments *in vivo.*

Explaining synergy

Dose-based synergy



Effect-based synergy

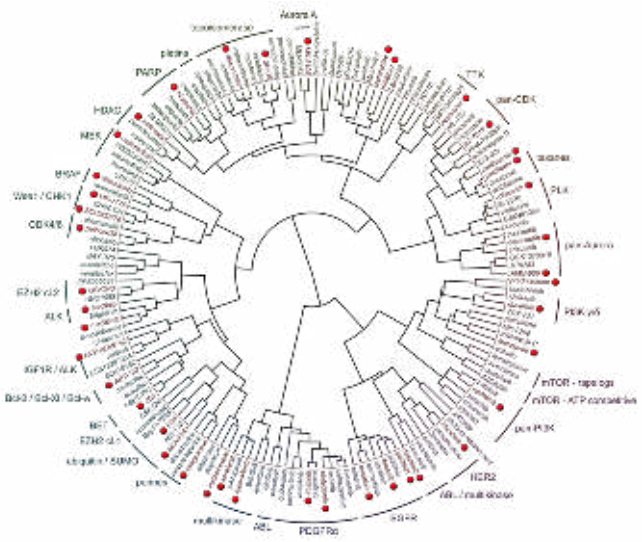


single curve cmpd 1

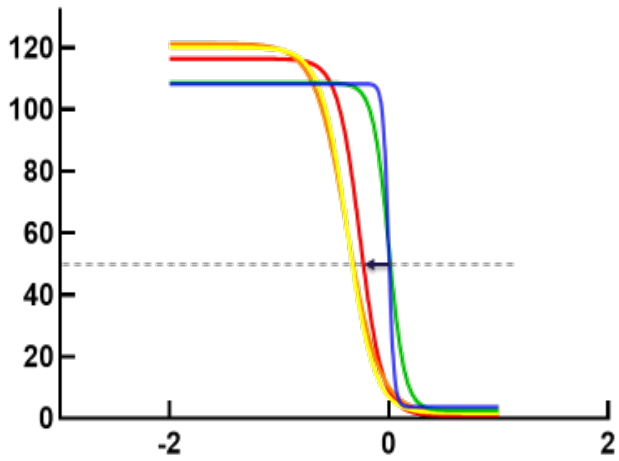
single curve cmpd 2

mixture of cmpd 1 + cmpd 2

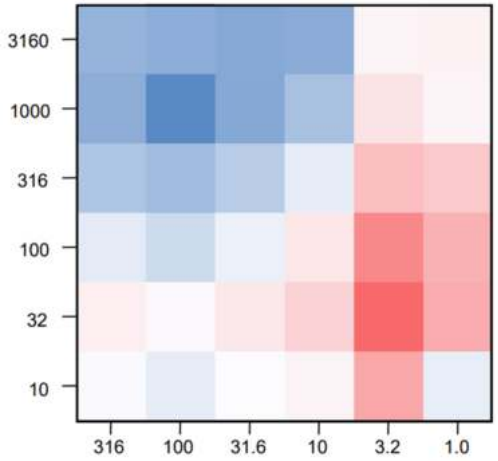
Overview of Oncolines' combination studies



SynergyScreen



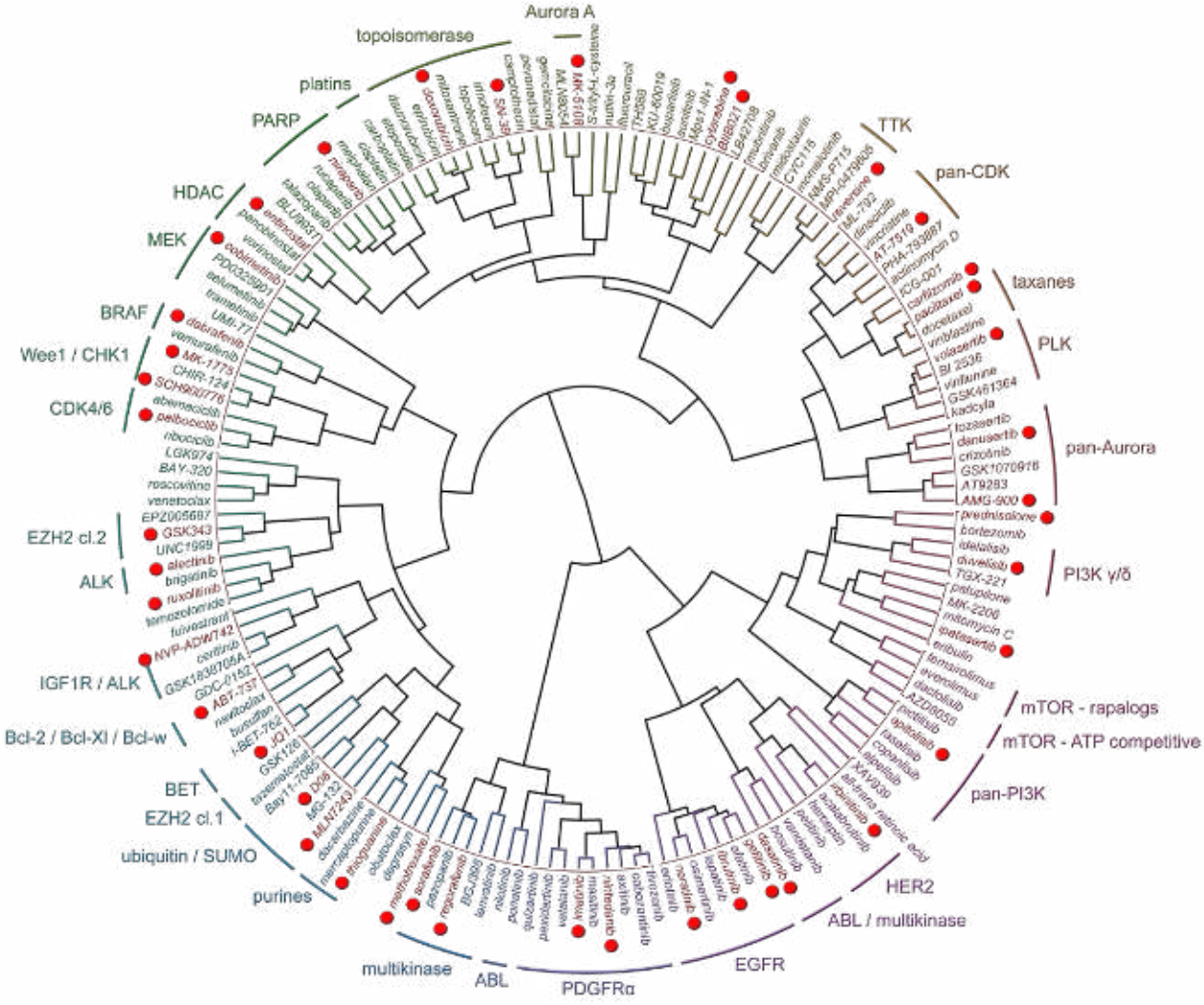
SynergyFinder



Combination matrix

Overview of SynergyScreen

- High througput screening with 42 reference anti-cancer agents
- Ideal to determine the synergistic ‘drug groups’ of your drug
- Selection from 190 anti-cancer agents tested in 102 Oncolines Cell Panel
- Full dose-response curve of reference agent + fixed dose of Client compound

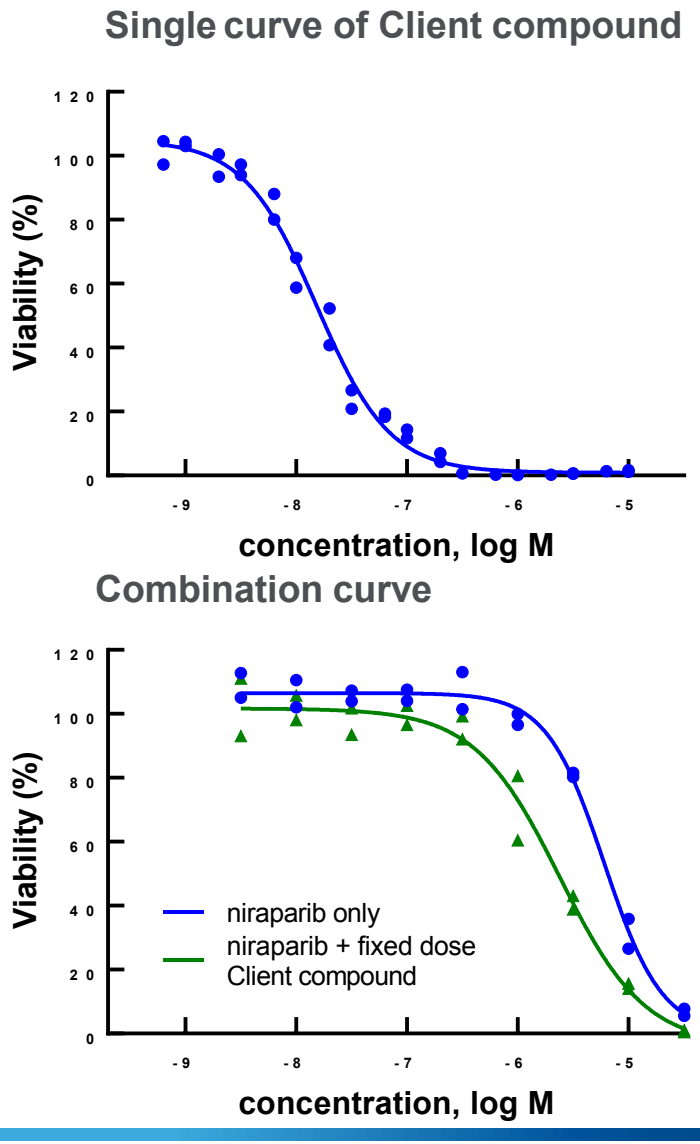


SynergyScreen – Experimental procedure

1. Determination of fixed dose of client compound

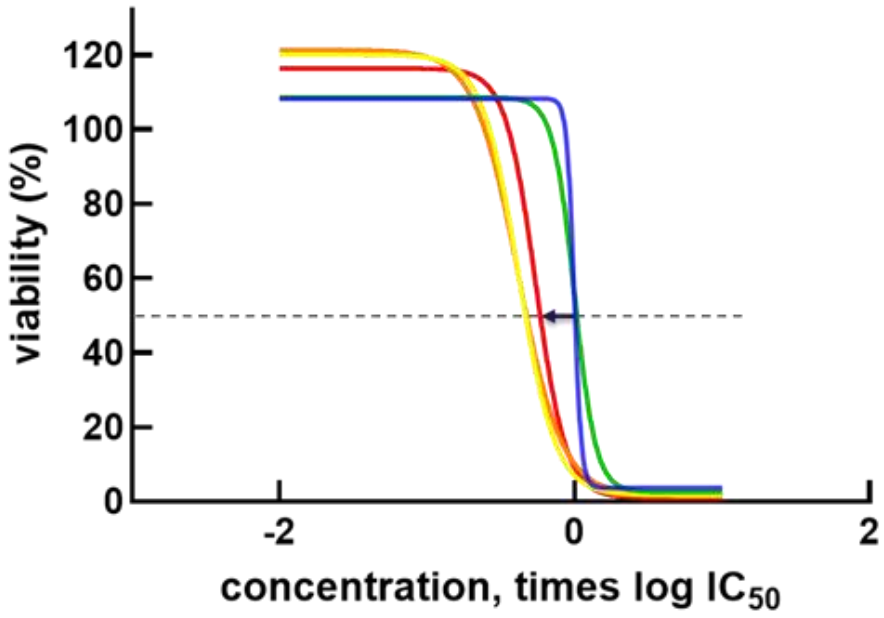
2. Based on full dose response curve of single reference agent and mixture-curve of that agent with a fixed concentration of the client compound

3. Determination of synergy via curve-shift analysis. If a synergistic hit is observed (Δ IC_{50} > 2), an independent repeat experiment is performed.



Overview of SynergyFinder

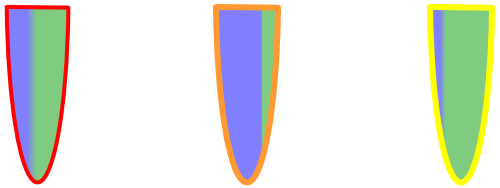
- Three mixtures of two compounds, based on equipotency
- Suitable for determining dose-based or effect-based synergies
- Suitable for confirmation of synergies



SynergyFinder – Experimental procedure

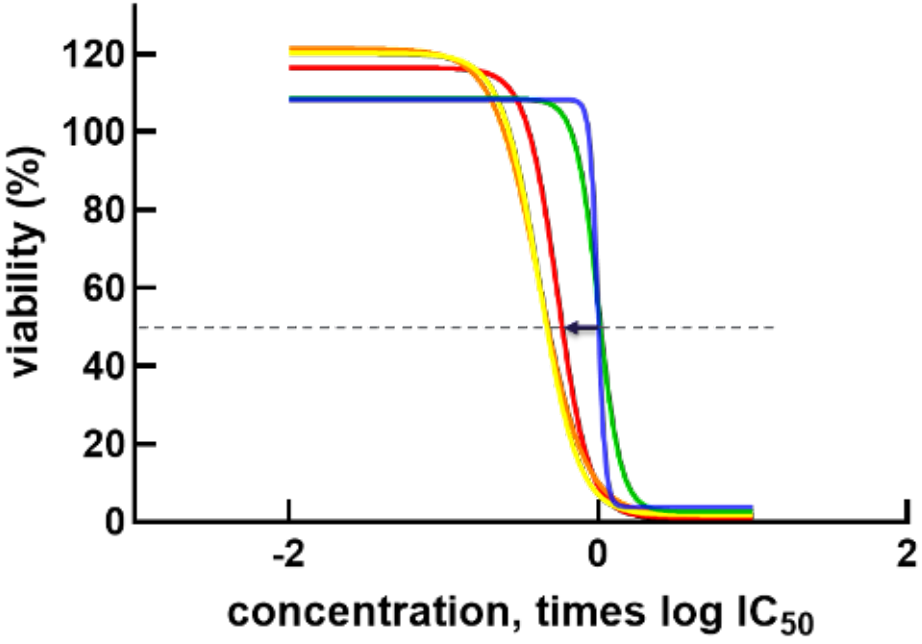
1. Pre-experiment: determination of IC_{50} of client compound and anti-cancer agent in selected cell lines.

2. Measuring three equipotent mixtures of IC_{50} of client compound and IC_{50} of anti-cancer agent.



1:1 4:1 1:4

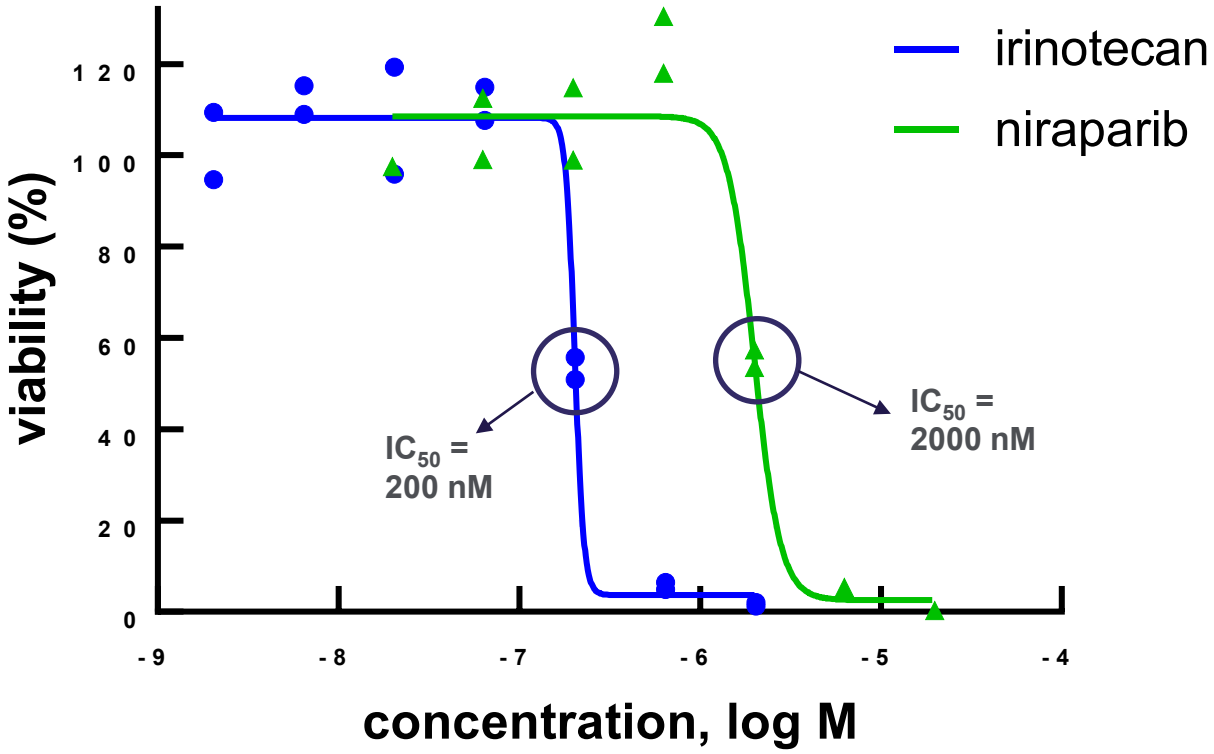
Equipotent = having equal potency



3. Calculation of Δ logIC_{50} and Combination Index (CI).

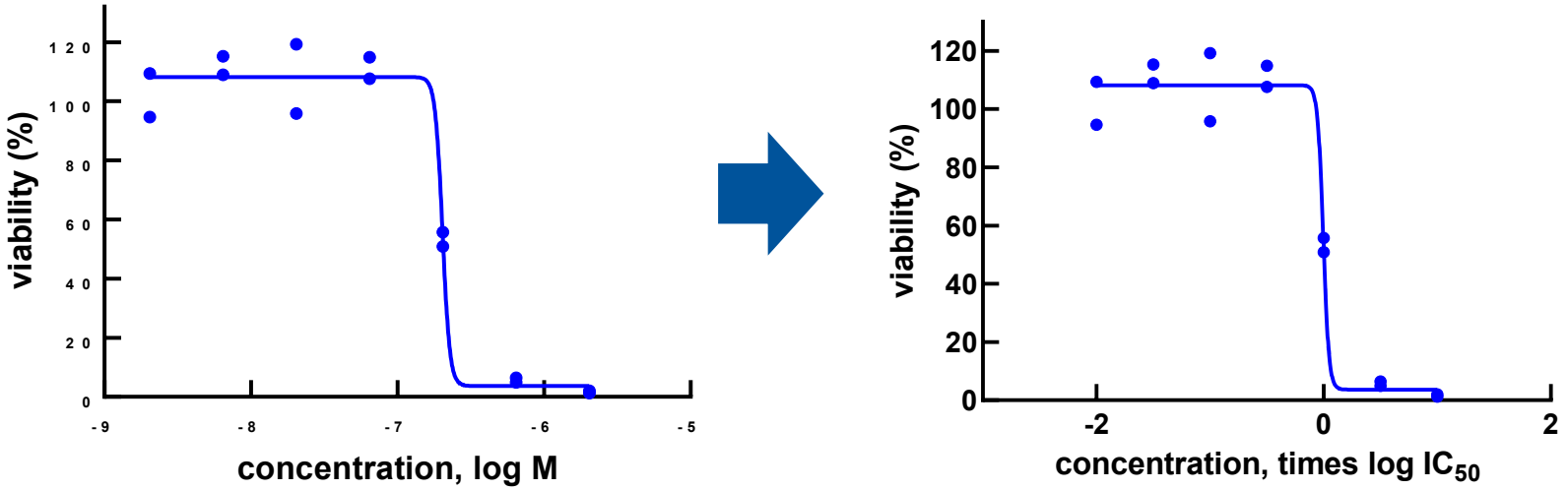
SynergyFinder – Explanation

- Dose-range in SynergyFinder is based on the IC_{50}: 0.01x IC_{50} – 10x IC_{50}
- Transformation of X-axis from ‘log M’ to ‘times log IC_{50}’



SynergyFinder – Explanation

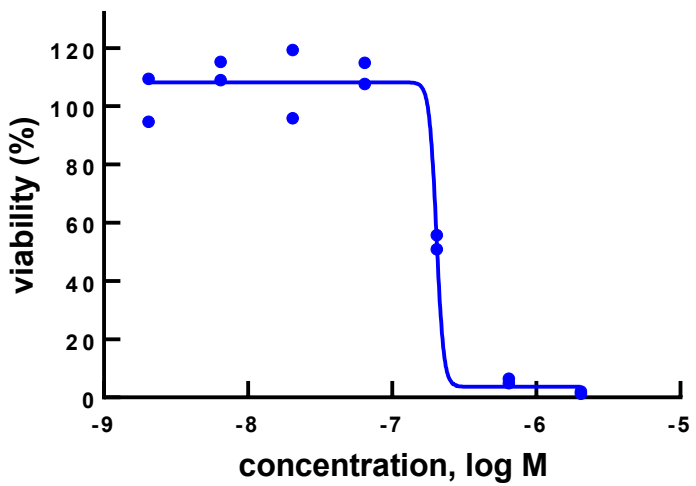
Dose curve of irinotecan in SynergyFinder

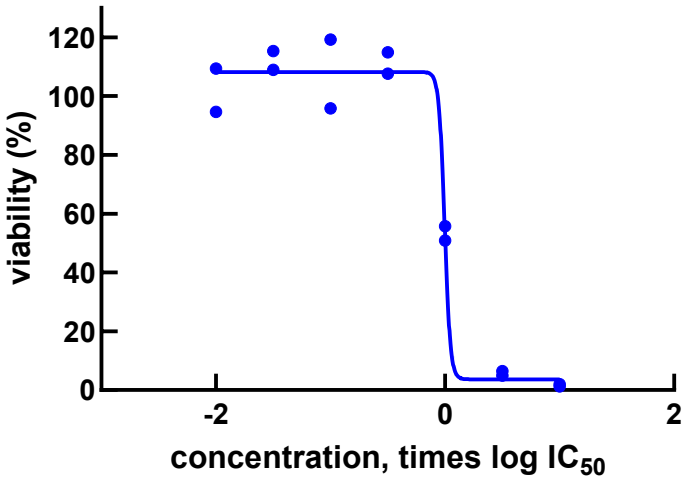


Concentration, log M	-8.7	-7.7	-6.7	-5.7
Dose-range single curve (nM)	2	20	200	2,000
Times IC_{50}	0.01x	0.1x	1	10x
Times log IC_{50}	-2	-1	0	1

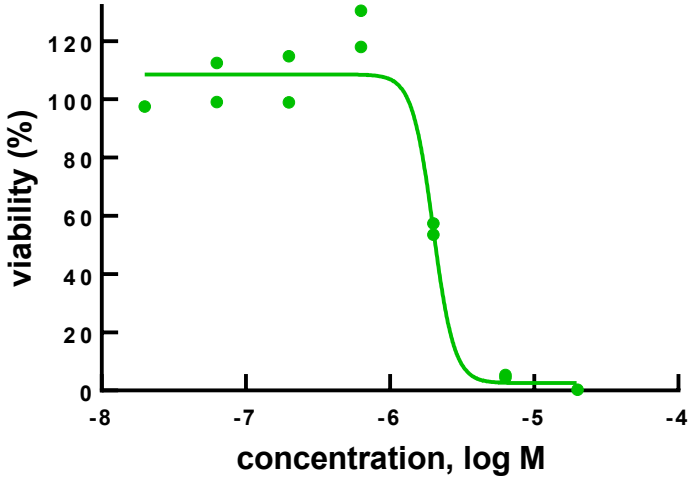
SynergyFinder – Explanation

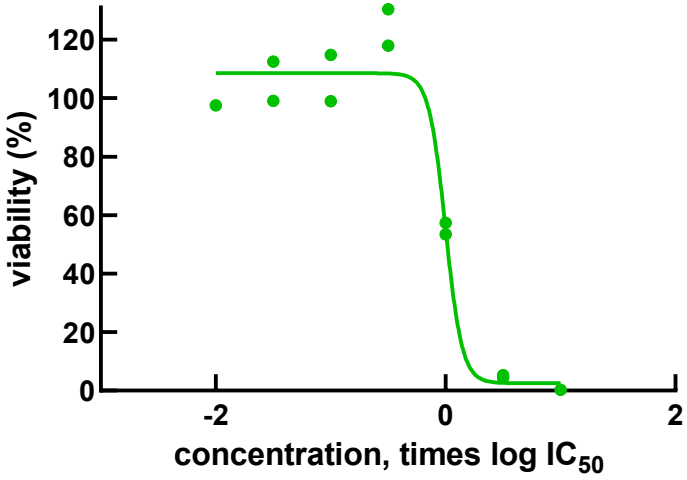
Single curve irinotecan



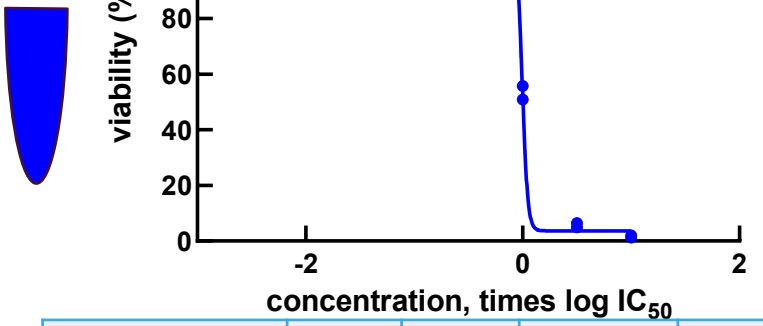


Single curve niraparib

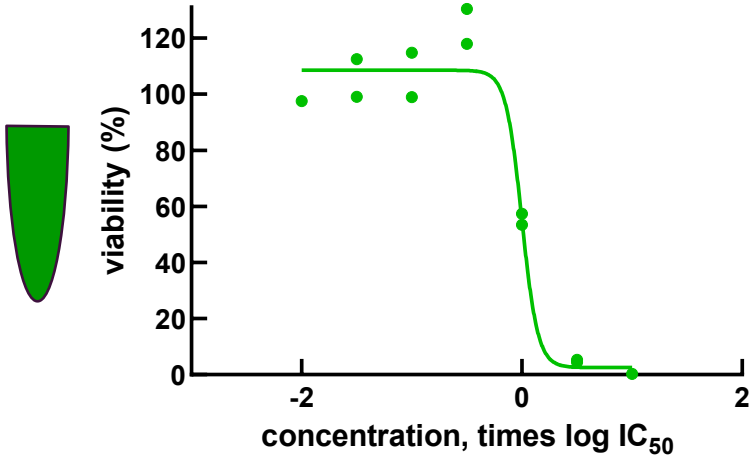




SynergyFinder – Explanation

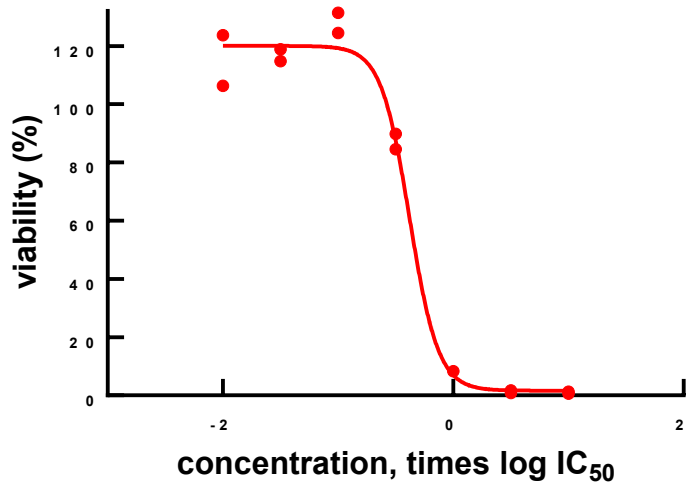


Times log IC_{50}	-2	-1	0	1
Concentration (nM)	2	20	200	2000



Times log IC_{50}	-2	-1	0	1
Concentration (nM)	20	200	2000	20,000

Equipotent mixture in 1:1 ratio

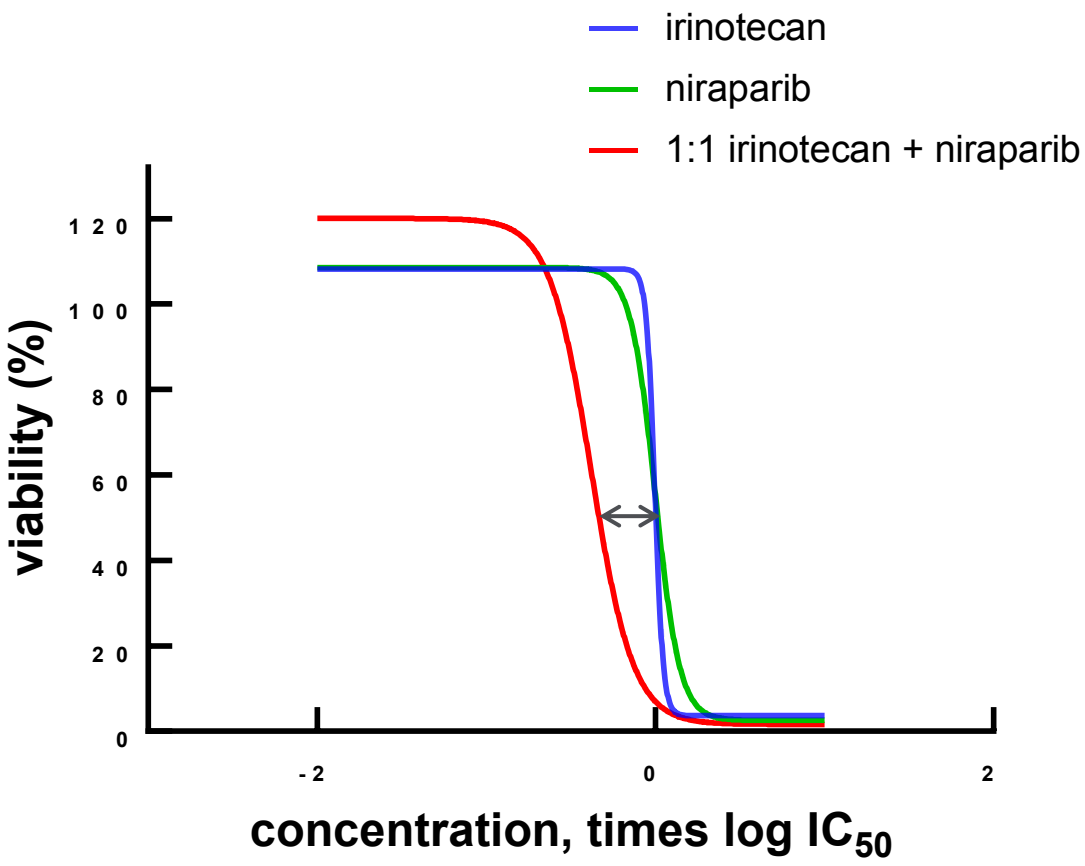


Times log IC_{50}	-2	-1	0	1
Concentration (nM)	1	10	100	1,000
Concentration (nM)	**10**	**100**	**1000**	**10,000**

SynergyFinder - Data analysis

- **Curve shift analysis**

$\Delta \log IC_{50}$



SynergyFinder - Data analysis

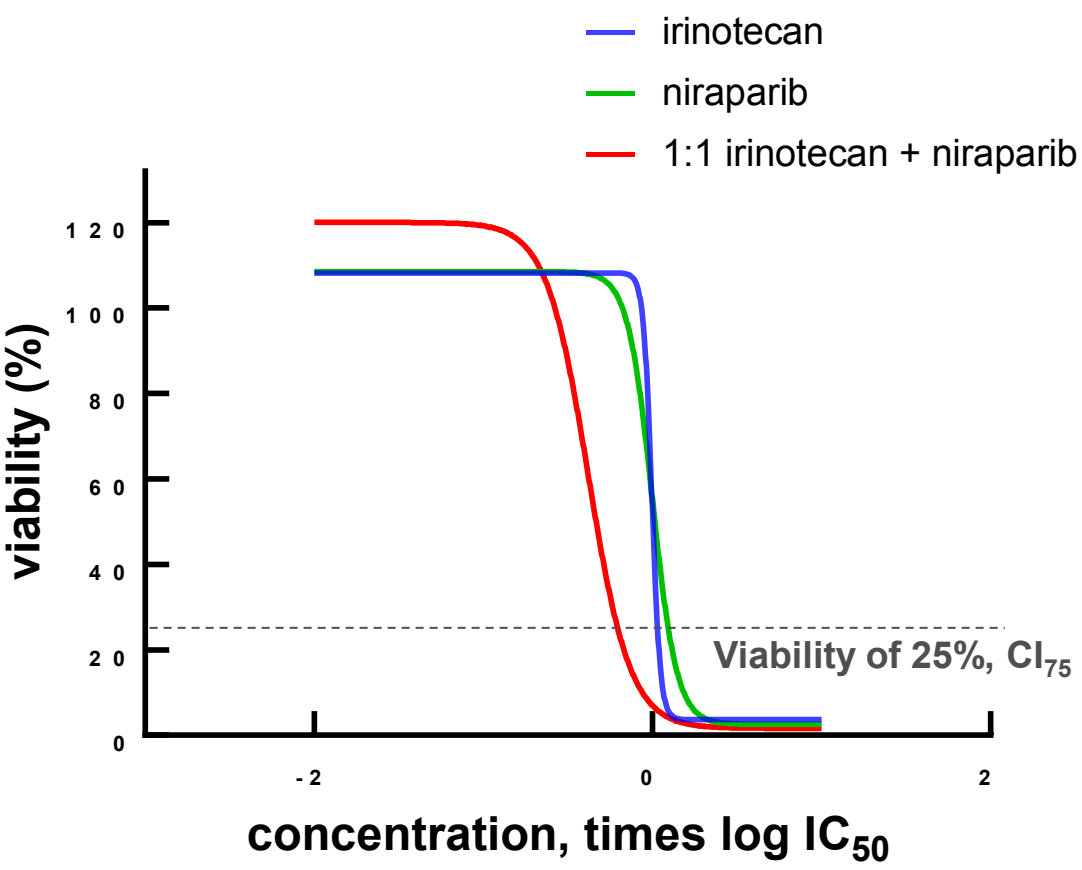
- **Combination Index (CI)**

$$\text{Viability} = \frac{treated\ t = end}{untreated\ t{=}end}$$

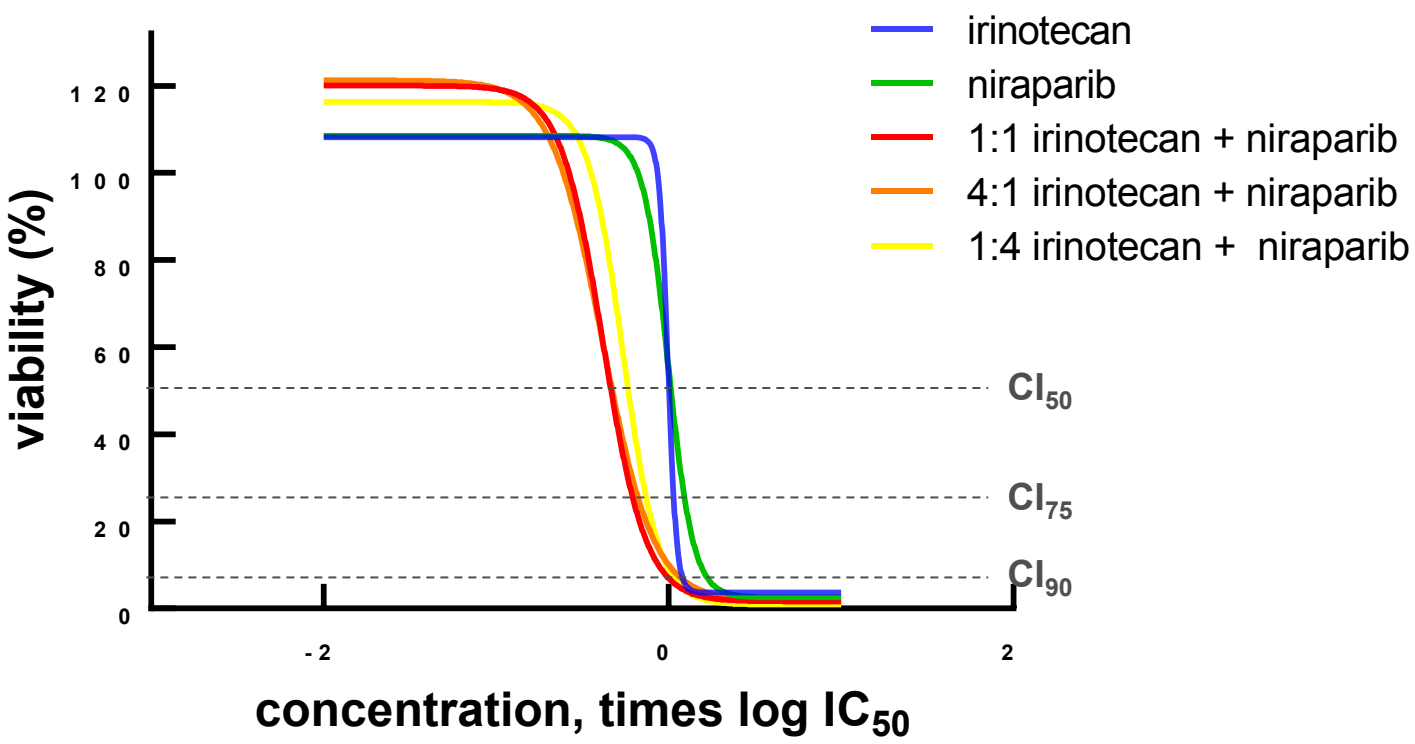
$$CI_{(100\text{-}V)} = \frac{[A_{mixture}]_V}{[A_{single}]_V} + \frac{[B_{mixture}]_V}{[B_{single}]_V}$$

CI < 1: synergistic effect
CI = 1 : additive effect
CI > 1 : antagonistic effect

CI < 0.88 is considered as significant synergy



SynergyFinder - Data analysis

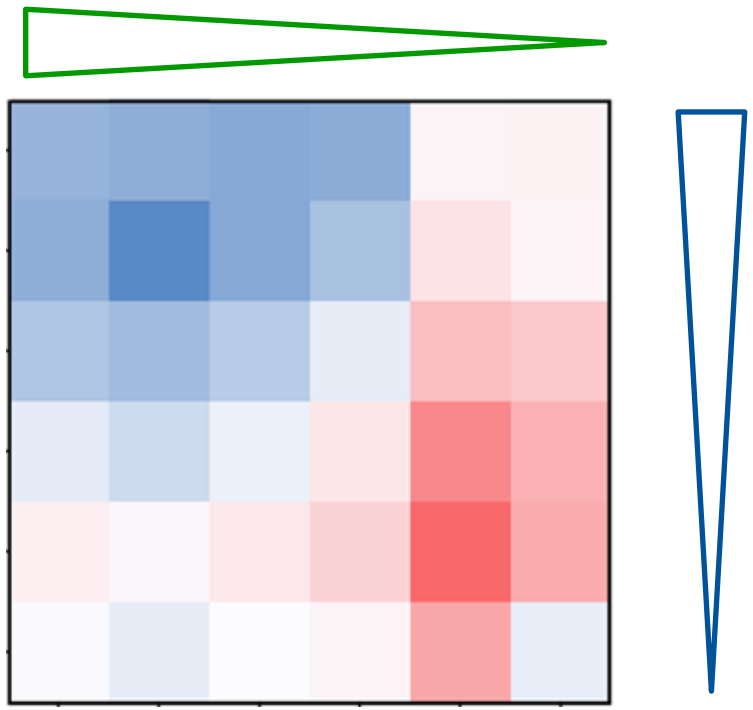


irinotecan + niraparib			
Compound	**ratios**		
irinotecan	1	4	1
niraparib	1	1	4
Δlog IC50	-0.38	-0.38	-0.26
Factor IC50 shift	2.38	2.39	1.83

	CI_{50}	CI_{75}	CI_{90}
1:1 irinotecan + niraparib	0.45	0.49	0.54
4:1 irinotecan + niraparib	0.46	0.52	0.61
1:4 irinotecan + niraparib	0.57	0.60	0.62

Overview of Combination Matrix

- Combination matrix is useful if you want to measure the best effect of your compound in combination
- Several synergy scores can be calculated based on a combination matrix. We focus here on the Bliss Independence Score
- Invented by C.J. Bliss in 1953.

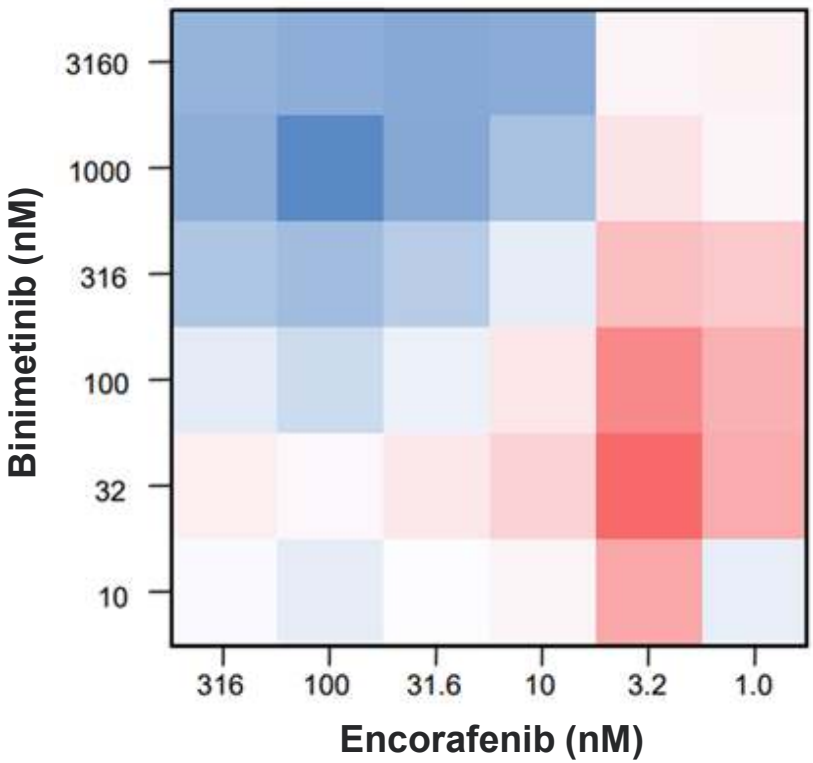


Combination Matrix – experimental procedure

1. Pre-experiment to determine optimal dose-range.

2. Combine two compounds in a 6 by 6 matrix

3. Calculation of Excess over Bliss score for each well. Sum of Bliss scores and highest Bliss score are reported per matrix.



Combination Matrix – data analysis

Plate lay-out

Encorafenib (nM)

316	100	31	10	3	1	blanc
						Single dose response curve binimetinib
Single dose response curve encorafenib						untreated

Binimetinib (nM)

3160
1000
316
100
31
10
blanc

- 6 by 6 combination matrix of encorafenib and binimetinib in G361 cell line
- Measurements are performed in quadruplo

Combination Matrix – data analysis

Raw data

Encorafenib (nM) / **Binimetinib (nM)**

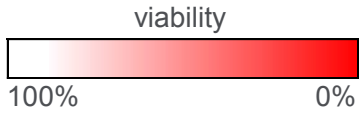
316		100		31		10		3		1		blanc		
428373	399493	438507	449867	424093	525200	519627	602707	529840	537253	550587	582120	591827	539427	3160
385827	497173	473227	524907	555493	583413	543013	840693	529813	576373	480653	564427	559773	486667	
458907	544907	577947	576787	574853	511467	527493	628267	463000	532413	602520	598893	590320	587240	1000
394600	440787	713067	612867	553800	518240	602813	532413	483387	525733	563200	656960	607667	562813	
448733	473520	613840	455320	464120	591013	635267	603613	692280	737093	776507	739813	930253	938613	316
408960	517800	542427	508987	534867	501493	567773	580320	651307	500227	580787	764533	820760	933480	
438827	423093	550733	635720	526427	573373	697787	722587	925360	952920	1063933	1201813	1452920	1445907	100
497653	464093	547453	541173	605773	574533	690973	723800	794960	880453	1149547	1095640	1318360	1392067	
470947	481293	647760	594587	579080	577067	712773	948320	1295013	1432893	1550053	1962467	1998080	1966027	31
515053	494773	642600	575987	799893	665587	1163080	1107587	1315280	1576413	1639853	2014053	1926987	2278187	
562373	602120	749947	617160	765533	675307	1065507	980680	1585947	1943107	2304080	2426600	2094213	2388787	10
606493	668267	779867	831920	907587	910973	1356227	1541333	2009293	2005720	2153213	2848453	2090160	2174507	
679373	677013	702013	708120	852667	948533	1416267	1442467	2546613	2722187	2695627	2497373	2130440	2229880	blanc
627827	548200	697533	788547	817347	822160	1116587	1473573	2211880	2462600	1957573	2965053	2647240	2334973	

- Each number represents the counts of ATP in the well

Combination Matrix – data analysis

Viability per well (% of untreated)

Encorafenib (nM) 316	100	31	10	3	1	blanc	Binimetinib (nM)
18	20	22	27	23	23	23	3160
20	27	23	25	21	26	25	1000
20	23	22	26	28	31	39	316
20	24	24	30	38	48	60	100
21	26	28	42	60	77	87	31
26	32	35	53	81	104	94	10
27	31	37	58	106	108	100	blanc



Combination Matrix – data analysis

Excess over Bliss score:

observed inhibtion rate – predicted inhibition rate

3	1	blanc	
60	77	87	31
81	104	94	10
106	108	100	blanc

Observed viability per well (% of untreated)

Combination Matrix – data analysis

Excess over Bliss score:

observed inhibtion rate – predicted inhibition rate

3	1	blanc	
60	77	87	31
81	104	94	10
106	108	100	blanc

Observed viability per well (% of untreated)

Bliss predicted inhibition rate:

$A_{single} + B_{single} – (A_{single} \times B_{single})$

A_{single} = inhibition rate of compound A
B_{single} = inhibition rate of compound B

3	1	blanc	
0.40	0.23	0.13	31
0.19	-0.04	0.06	10
-0.06	-0.08	100	blanc

Observed inhibition per well

Example:
Bliss predicted inhibition rate = -0.064 + 0.126 – (-0.064 x 0.126) = 0.070

3	1	blanc	
0.07		87	31
		94	10
106	108	1.00	blanc

Combination Matrix – data analysis

3	1	blanc	
60	77	87	31
81	104	94	10
106	108	100	blanc

Viability per well (% of untreated)

Bliss predicted inhibition rate based on single DRCs

3	1	blanc	
0.07	0.05	0.87	31
0.00	-0.01	0.94	10
1.06	1.08	1.00	blanc

Observed inhibition rate

3	1	blanc	
0.40	0.23	0.87	31
0.19	-0.04	0.94	10
1.06	1.08	1.00	blanc

Combination Matrix – data analysis

3	1	blanc	
60	77	87	31
81	104	94	10
106	108	100	blanc

Viability per well (% of untreated)

Bliss predicted inhibition rate based on single DRCs

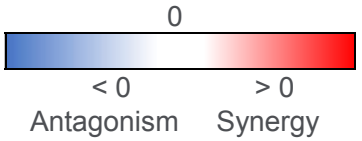
3	1	blanc	
0.07	0.05	0.87	31
0.00	-0.01	0.94	10
1.06	1.08	1.00	blanc

Observed inhibition rate

3	1	blanc	
0.40	0.23	0.87	31
0.19	-0.04	0.94	10
1.06	1.08	1.00	blanc

Excess over Bliss score (observed rate – predicted rate)

3	1	blanc	
0.33	0.18	0.87	31
0.19	-0.03	0.94	10
1.06	1.08	1.00	blanc

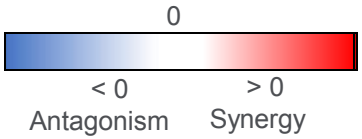


Combination Matrix – data analysis

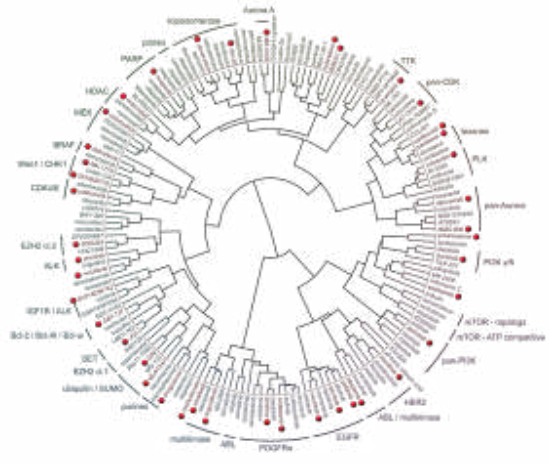
Excess over Bliss score

Encorafenib (nM) → / Binimetinib (nM) ↓	316	100	32	10	3	1	blanc
3160	-0.12	-0.13	-0.14	-0.13	0.02	0.02	0.23
1000	-0.13	-0.19	-0.14	-0.10	0.05	0.01	0.25
316	-0.09	-0.11	-0.08	-0.03	0.14	0.11	0.39
100	-0.03	-0.06	-0.02	0.05	0.26	0.17	0.60
32	0.03	0.01	0.04	0.09	0.33	0.18	0.87
10	-0.01	-0.03	0.00	0.02	0.19	-0.03	0.94
blanc	0.27	0.31	0.37	0.58	1.06	1.08	1.00

- Excess over Bliss score sum: 0.14
- Highest excess over Bliss score: 0.33

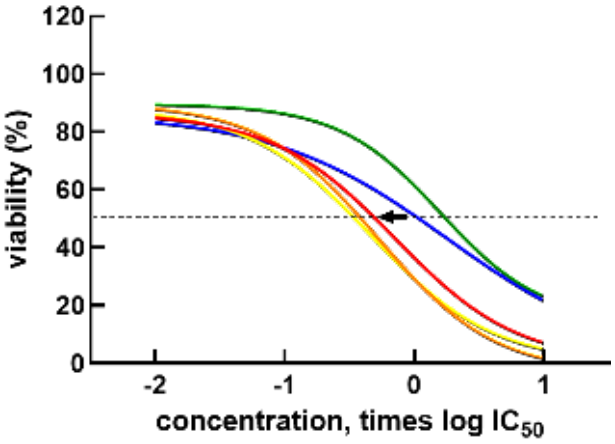


Overview of Oncolines' combination studies



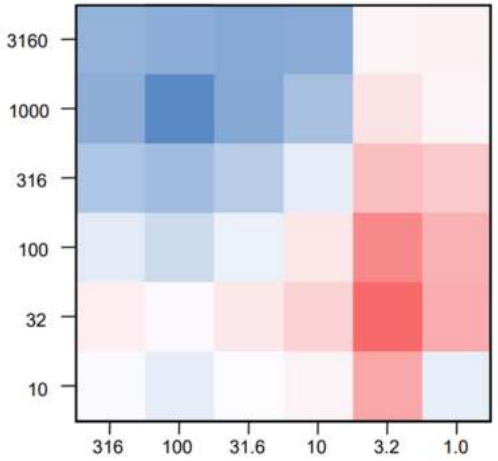
SynergyScreen

- Screening against a library of 42 anti-cancer representatives
- Fixed dose of compound
- Ideal to determine the synergistic 'drug groups' of your drug



SynergyFinder

- Based on equipotency
- Suitable for confirmation of synergies
- Suitable for measuring up to 100 combinations
- Suitable for full dose response curves



Combination matrix

- Six by six combination matrix
- Suitable to measure many combinations
- Suitable for partial effects

Case Study: translation *in vitro* results to the clinic

Concept of synthetic lethality

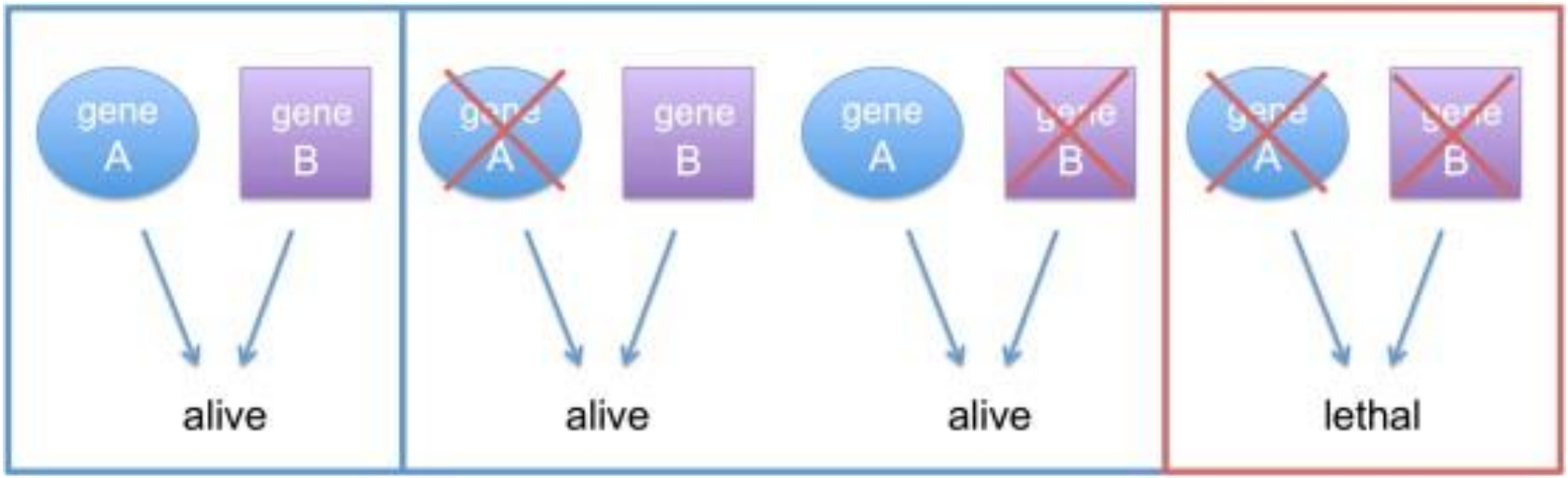


Figure from: Nijman, S. (2011) Synthetic lethality: General principles, utility and detection using genetic screens in human cells FEBS letters

Case study: translation *in vitro* results to the clinic

Concept of synthetic lethality

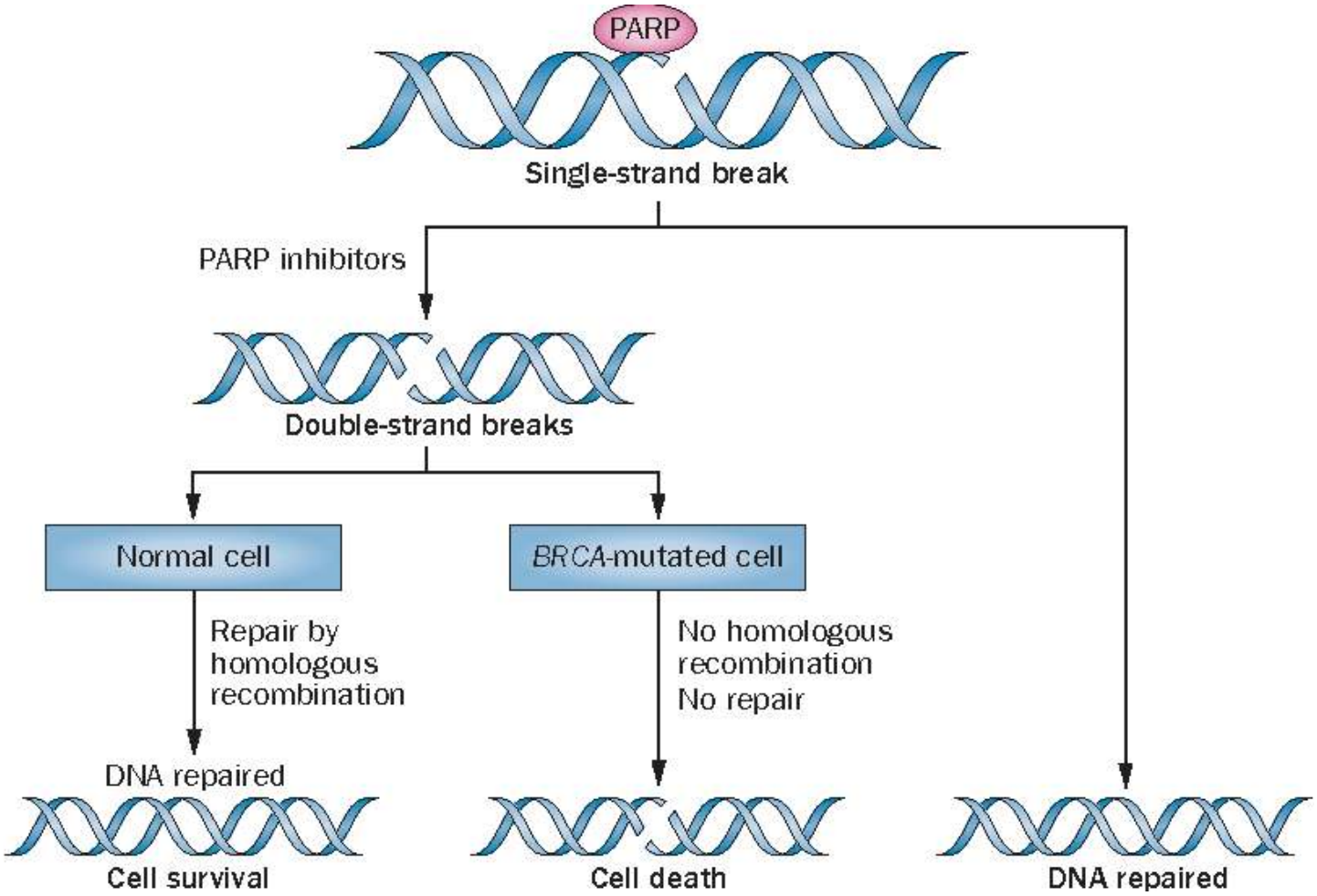
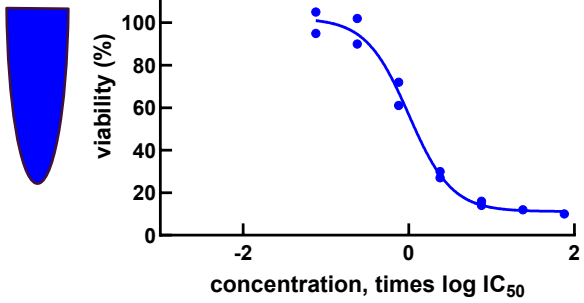


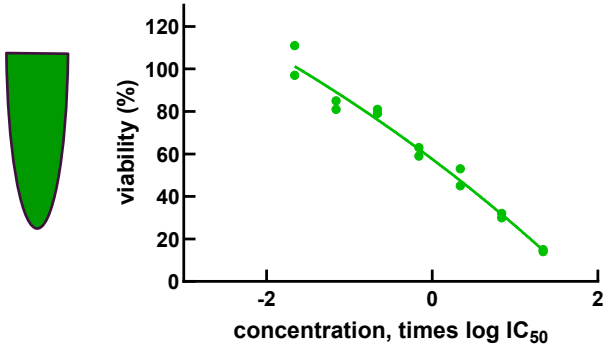
Figure from: Sonnenblick, A. *et al*. (2014) An update on PARP inhibitors – moving to the adjuvant setting Nat Rev. Clin. Oncol

Case study: translation *in vitro* results to the clinic

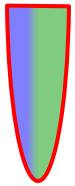
Single curve cisplatin

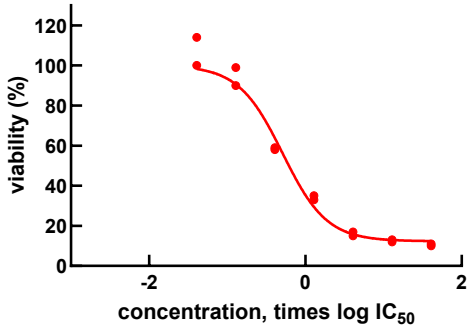


Single curve olaparib

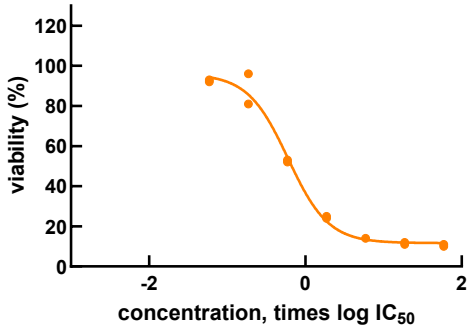


Equipotent mixture in 1:1 ratio

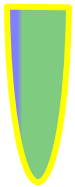


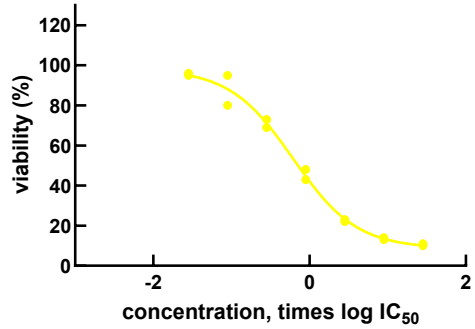


Equipotent mixture in 4:1 ratio



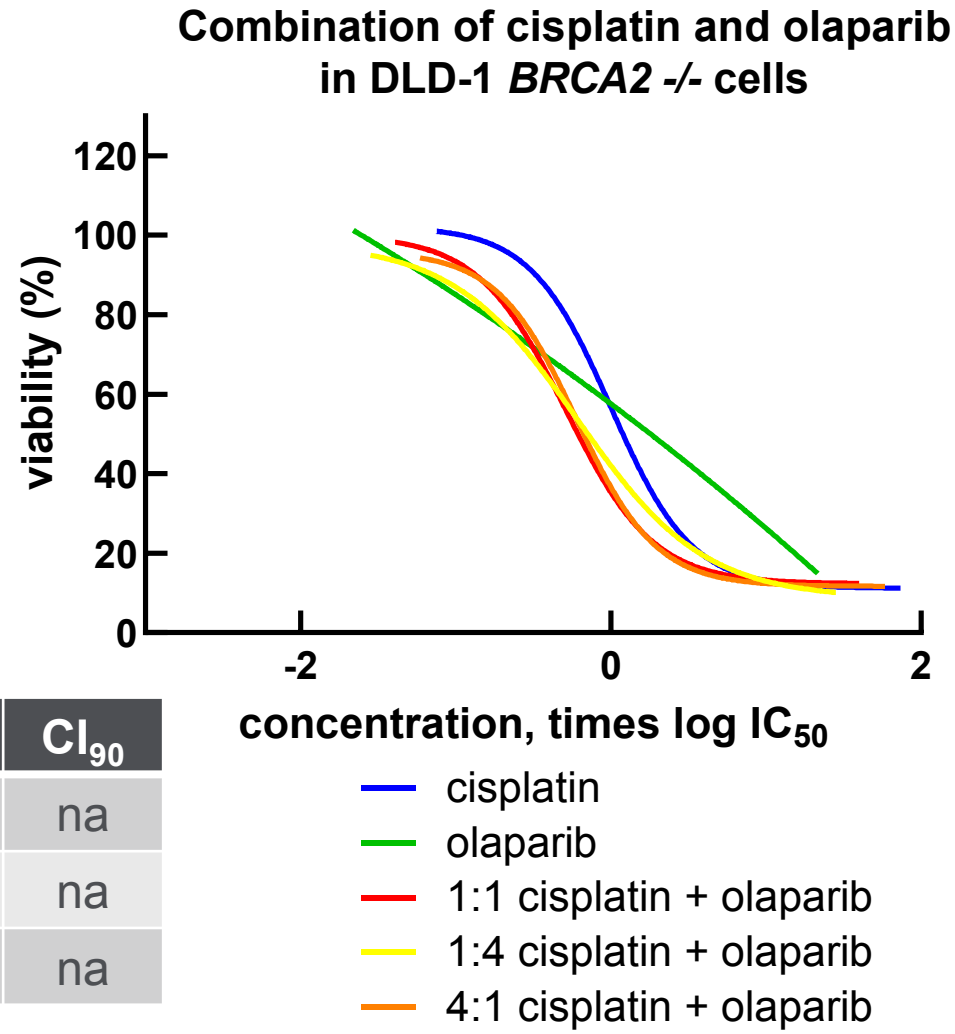
Equipotent mixture in 1:4 ratio





Case study: translation *in vitro* results to the clinic

- The combination of olaparib and cisplatin is approved in the clinic.
- In SynergyFinder, it was confirmed that the combination of olaparib and cisplatin was synergistic.



	CI_{50}	CI_{75}	CI_{90}
1:1 cisplatin + olaparib	0.59	0.58	na
4:1 cisplatin + olaparib	0.61	0.60	na
1:4 cisplatin + olaparib	0.60	0.60	na

Q & A

How to select the best platform for testing drug combinations?

For questions after this session, please contact us as services@oncolines.com

Example Combination Index

Example: calculation of CI at the concentration of which 50% of cells is death

- $[A_{sinlge}]$ = IC_{50} compound A = 100 nM
- $[B_{sinlge}]$ = IC_{50} compound B = : 200 nM

$$\mathbf{CI} = \frac{[\mathrm{Ami}_{\mathrm{xture}}]}{[A_{single}]} + \frac{[B_{mixture}]}{[B_{single}]}$$

In case of no synergy and no antagonistic effect:

- $[A_{mixture}]$ = 50 nM
- $[B_{mixture}]$ = 100 nM

$$\mathbf{CI_{50}} = \frac{50}{100} + \frac{100}{200} = 0.5 + 0.5 = 1$$

In case of a synergy with CI_{50} = 0.5, several scenarios are possible:

- $[A_{mixture}]$ = 25 nM
- $[B_{mixture}]$ = 50 nM

$$\mathbf{CI_{50}} = \frac{25}{100} + \frac{50}{200} = 0.25 + 0.25 = 0.5$$

Or

- $[A_{mixture}]$ = 12.5 nM
- $[B_{mixture}]$ = 75 nM

$$\mathbf{CI_{50}} = \frac{12.5}{100} + \frac{75}{200} = 0.125 + 0.375 = 0.5$$