Characterizing In vitro Synergy using the Ray design methodology

Application to an Oncology combination study

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Outline

- Synergy definition and Loewe additivity model
- Ray design methodology applied to an Oncology combination study
  - Context and preliminary data
  - Experimental ray design
  - Statistical analysis and modeling
  - Model fitting and results validation
  - Conclusion
- Ray design methodology: pros and cons

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Synergy – definition (1/2)

- **Definition <pharmacology, physiology>:** The interaction of two or more treatments such that their combined effect is greater than the sum of the individual effects observed when each treatment is administered alone

- **Loewe additivity model (Loewe and Muischnek, 1926)**
  - Most suitable reference model
  - Reasoning at fixed effect: in a synergistic mixture, lower concentrations of the two products are needed to obtain a given effect, in comparison with additive situation

- **Equation for Loewe additivity model between products A and B**

$$\frac{C_A}{IC_{X,A}} + \frac{C_B}{IC_{X,B}} = 1$$

  - C_A, C_B: concentrations of each product in the mixture necessary to obtain X% of effect
  - $IC_{X,A}$, $IC_{X,B}$: concentrations of products A and B necessary to obtain X% of effect for each product alone (often relative IC50, concentration to obtain 50% of the delta between the maximum and the minimum effects)

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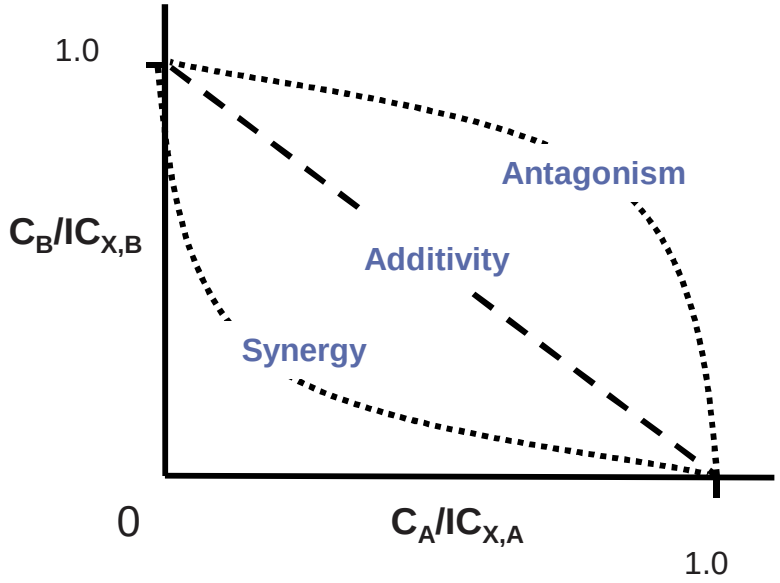
Synergy – definition (2/2)

- The left-hand term $K_i = \frac{C_A}{IC_{X,A}} + \frac{C_B}{IC_{X,B}}$ of the equation is significantly:
  - Inferior to 1 in case of synergy
  - Superior to 1 in case of antagonism



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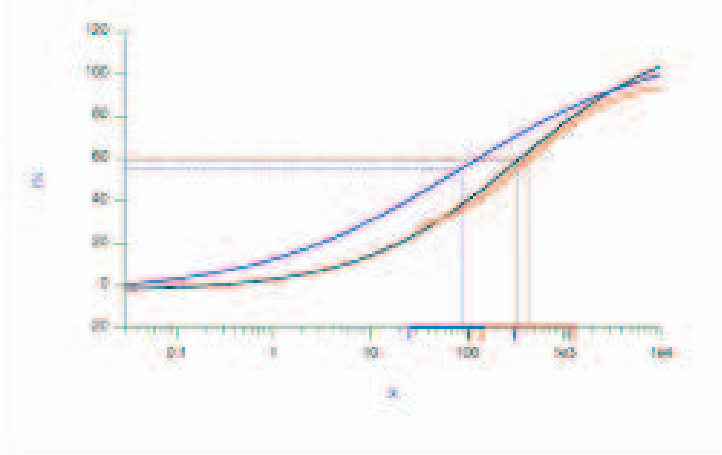
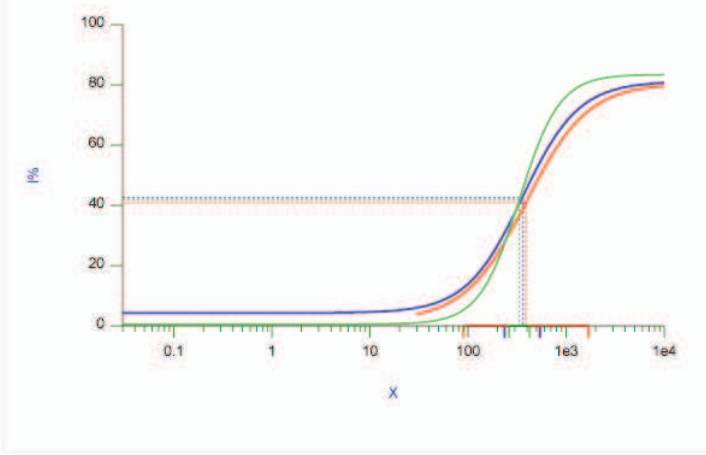
Study context and preliminary data

- Objective: study the In vitro combination of two anticancer agents, Prod.A and Prod.B, to detect their possible synergy on a given cancer cell line
- Parameter of interest: Percentage of inhibition of cancer cells growth
- Preliminary results on each product alone: relative IC50s, minimum and maximum concentrations

Product A: IC50=300nM
Min=0.03nM
Max=10000nM



Product B: IC50=300nM
Min=3nM
Max=10000nM



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Experimental Ray design (1/4)

- Each ray design contains at least 5 rays:
  - Two rays corresponding to each product alone
  - Other rays i consisting of couples of concentrations of products A and B, in a given proportion $c_i = C_{Prod.B}/C_{Prod.A}$, constant for each ray
  - Each couple of concentrations in duplicates, at least 6-7 successive dilutions by ray within the minimum and maximum concentrations range of each product
- The synergy zone is covered in a symmetric way from the equipotent ray, where $c_i = IC50_{Prod.B}/IC50_{Prod.A}$ and products are equally represented considering their respective potency
- At least three independent experiments (3 Ray designs) performed to ensure robustness of results

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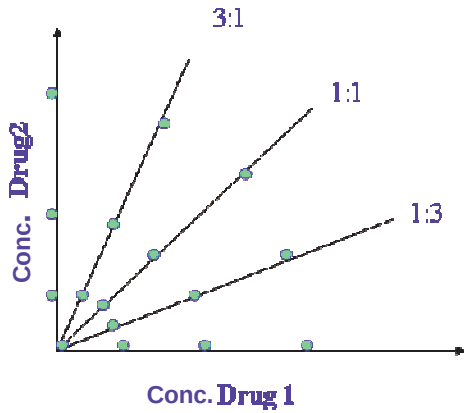
Experimental Ray design (2/4)

- **For each ray, each proportion $c_i = C_{Prod.B}/C_{Prod.A}$ translated into unit of effect of each product alone considering their respective IC50s' values, using the effective fraction f:** $f_i = \frac{1}{c_i \rho + 1}$ **where** $\rho = \frac{IC50_{Prod.A}}{IC50_{Prod.B}}$ **is the relative potency of the two products**
  - Ex1: f=0.5, effective equipotent ray (f ε]0,1[)
  - Ex2: f=0.75, ray where Prod.A is 3 times more represented than Prod.B considering their relative potency, also called ray « 3 for 1 »



- **In the study,** $\rho = \frac{300nM}{300nM} = 1$
- **With 5 rays, taking the two products alone and 3 rays with f=0.75 (ray 3 for 1), f=0.5 (equipotent ray) and f=0.25 (ray 1 for 3) permits to cover equally all the synergy zone**

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Experimental Ray design (3/4)

Example: Ray design for Experiment 1 (concentrations in nM)

Ray 1 : Product A alone

Mixture	1000	300	100	30	10	3	1	0.3	0.1	0.03
Prod.A	1000	300	100	30	10	3	1	0.3	0.1	0.03
Prod.B	0	0	0	0	0	0	0	0	0	0

Ray 2 (1 for 30, f=0.03) :
300nM (Prod.A) + 10000nM (Prod.B)

Mixture	21000	10300	3100	1030	310	103	31	10.3	3.1
Prod.A	1000	300	100	30	10	3	1	0.3	0.1
Prod.B	20000	10000	3000	1000	300	100	30	10	3

Ray 3 (1 for 10, f=0.09) :
300nM (Prod.A) + 3000nM (Prod.B)

Mixture	10100	3300	1100	330	110	33	11	3.3
Prod.A	1000	300	100	30	10	3	1	0.3
Prod.B	10000	3000	1000	300	100	30	10	3

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Experimental Ray design (4/4)

Ray 4 (1 for 3, f=0.25) :
300nM (Prod.A) + 1000nM (Prod.B)

Mixture	4000	1300	400	130	400	13	4
Prod.A	1000	300	100	30	10	3	1
Prod.B	3000	1000	300	100	30	10	3

Ray 5 (1 for 1, f=0.50) :
300nM (Prod.A) + 300nM (Prod.B)

Mixture	2000	600	200	60	20	6
Prod.A	1000	300	100	30	10	3
Prod.B	1000	300	100	30	10	3

Ray 6 (3 for 1, f=0.75) :
1000nM (Prod.A) + 300nM (Prod.B)

Mixture	1300	400	130	40	13
Prod.A	1000	300	100	30	10
Prod.B	300	100	30	10	3

Ray 7 : Product B alone

Mixture	20000	10000	3000	1000	300	100	30	10	3
Prod.A	0	0	0	0	0	0	0	0	0
Prod.B	20000	10000	3000	1000	300	100	30	10	3

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Statistical analysis of the Ray design

- For each experiment, global modelling of all rays together, each ray following a 4-parameter concentration-effect (inhibition) logistic curve

- For each experiment and from this global model:
  - Estimation of the experimental values of the IC50s of Prod.A and Prod.B alone
  - Estimation of the experimental values of « f »
  - Estimation of the Loewe additivity index K_i for each ray i (i=2..6)

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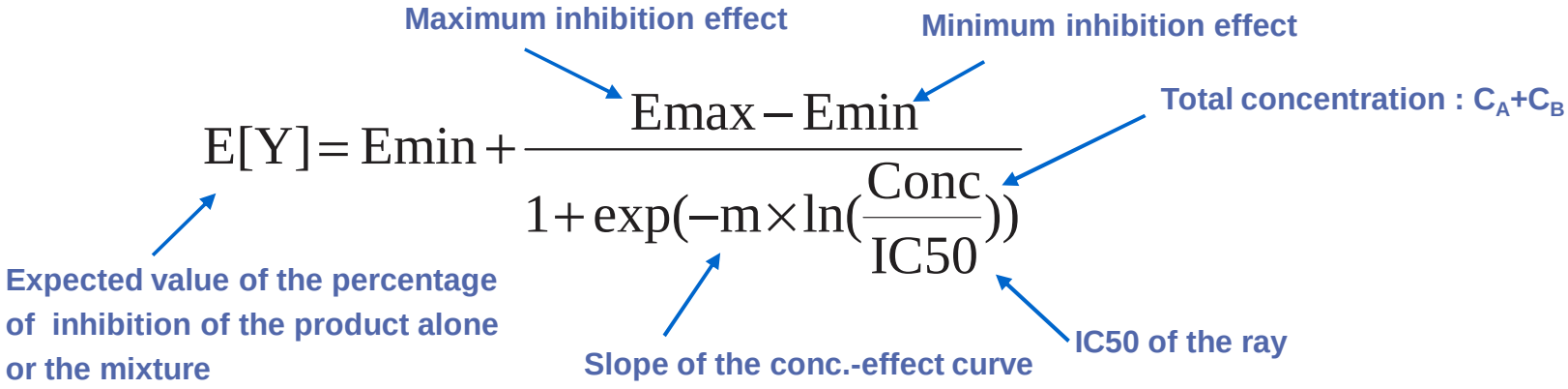
Modeling the Ray design

Concentration-effect model

- **A 4-parameter logistic model for each ray:**



- **Generalization to all rays i, i=1, …, 5 with different parameters Emax, Emin, m, IC50 for each ray:**

Global model:

$$E[Y] = Emin_i + \frac{Emax_i - Emin_i}{1 + \exp(-m_i \ln(\frac{Conc_i}{IC50_i}))}$$

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Measurement of interaction between drugs (1/2)

- K_i value, i=2, 3, 4, permits to measure the relationship (additivity, antagonism or synergy) between the 2 tested products A and B for Ray i:

$$K_i = \frac{IC50_i(IC50_A + c_i IC50_B)}{IC50_A IC50_B(1+c_i)}$$

where

  - $c_i = C_B / C_A$ for Ray i
  - $IC50_A$, IC50 for product A alone (ray 1)
  - $IC50_B$, IC50 for product B alone (ray 5)
  - $IC50_i$, with for rays i=2, i=3 and i=4, $c_i = C_B / C_A$

Obtained by the global model

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Measurement of interaction between drugs (2/2)

- K_i value is obtained with its corresponding CI95%
- K_i value is significantly:
  - Equal to 1 in case of additivity (CI95% contains '1')
  - Inferior to 1 in case of synergy (CI95% is strictly inferior to '1')
  - Superior to 1 in case of antagonism (CI95% is strictly superior to '1')
- $Emin_i$, $Emax_i$, m_i, $IC50_i$ and K_i estimates are obtained with NLMixed procedure

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Fitting the global concentration-effect model

- **NLMixed SAS software procedure**
- **Parameters initialization: use of estimations of parameters obtained from the fitting of each curve separately**
- **For each experiment, simultaneous estimation of adjusted curves parameters: $Emin_i$, $Emax_i$, $IC50_i$, slope m_i and K_i with i=1, …, 5**
- **Selection of the best model**
  - *First step*: full model with $Emin_i$, $Emax_i$ and m_i specific to each ray
  - *Second step*: for this model, test of equality of the parameters $Emin_i$, $Emax_i$ and m_i for all rays
  - *Third step*: new model in which the previously significant parameter(s) is(are) considered common to all rays

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Results validation

EXP. 1	Preliminary values	Experimental values
IC50 Prod.A	300	106.78
IC50 Prod.B	300	217.73
f Ray 2	0.03	0.06
f Ray 3	0.09	0.17
f Ray 4	0.25	0.40
f Ray 5	0.50	0.67
f Ray 6	0.75	0.86

Right shift of the conclusion domain, f values higher than expected

EXP. 2	Preliminary values	Experimental values
IC50 Prod.A	300	200.23
IC50 Prod.B	300	165.80
f Ray 2	0.23	0.20
f Ray 3	0.50	0.45
f Ray 4	0.77	0.73
f Ray 5	0.91	0.89
f Ray 6	0.97	0.96

Conclusion domain similar to the expected one

EXP. 3	Preliminary values	Experimental values
IC50 Prod.A	300	87.74
IC50 Prod.B	300	729.82
f Ray 2	0.23	0.71
f Ray 3	0.50	0.89
f Ray 4	0.77	0.97

Shift to the domain where Prod.A is 3 times more represented than Prod.B considering their relative potency

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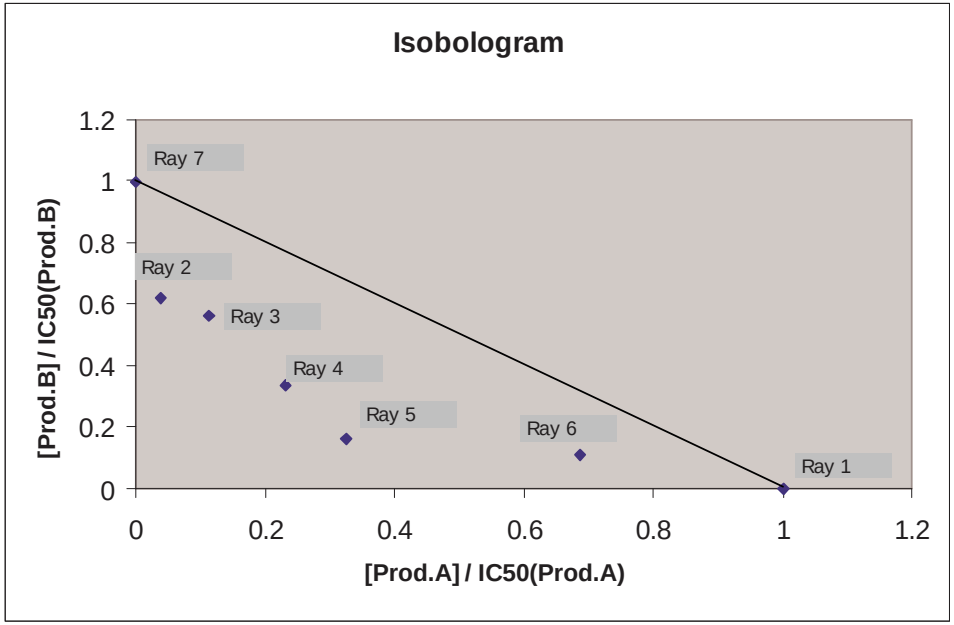
Statistical results: Experiment 1



EXP. 1	Experimental f values	Ki values and 95%CI	Conclusion
Ray 2	0.06	0.6585 [0.4641; 0.8528] (*)	Synergy
Ray 3	0.17	0.6756 [0.4802; 0.8709] (*)	Synergy
Ray 4	0.40	0.5702 [0.3656; 0.7748] (*)	Synergy
Ray 5	0.67	0.4865 [0.2316; 0.7414] (*)	Synergy
Ray 6	0.86	0.8006 [0.1445; 1.4568] (NS)	Additivity

(*): K is significantly different from 1 at 0.05 level NS: Non significant

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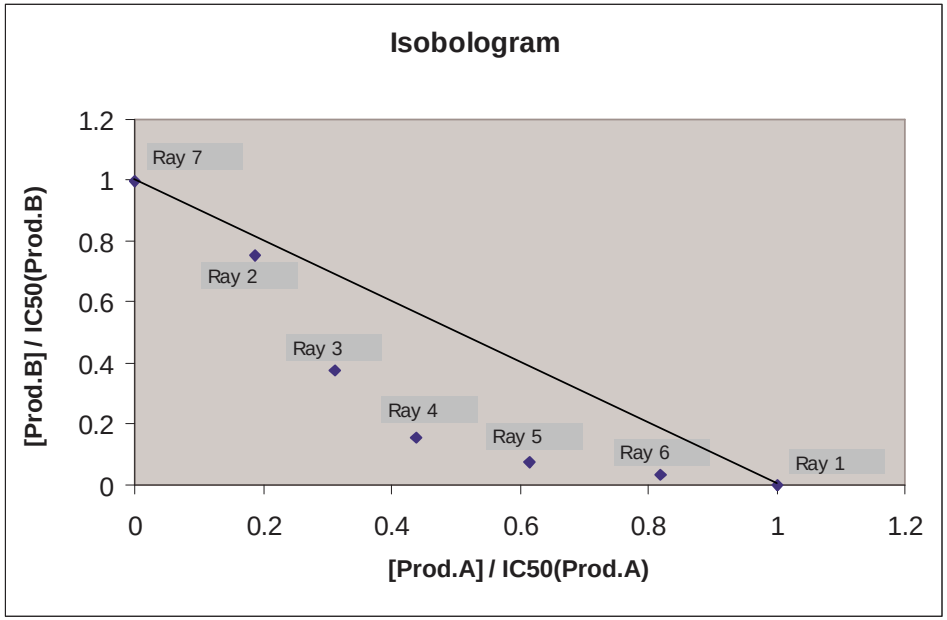
Statistical results: Experiment 2



EXP. 2	Experimental f values	Ki values and 95%CI	Conclusion
Ray 2	0.20	0.9441 [0.7094; 1.1788] (NS)	Additivity
Ray 3	0.45	0.6906 [0.4794; 0.9018] (*)	Synergy
Ray 4	0.73	0.5987 [0.3731; 0.8243] (*)	Synergy
Ray 5	0.89	0.6891 [0.3615; 1.0166] (NS)	Additivity
Ray 6	0.96	0.8523 [0.3127; 1.3918] (NS)	Additivity

(*): K is significantly different from 1 at 0.05 level NS: Non significant

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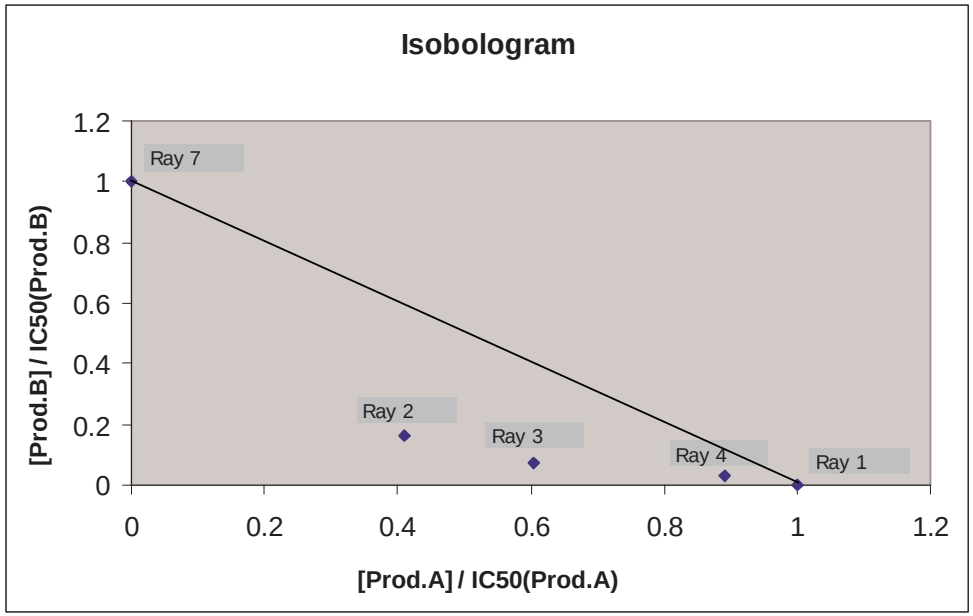
Statistical results: Experiment 3



EXP. 3	Experimental f values	Ki values and 95%CI	Conclusion
Ray 2	0.71	0.5746 [0.2503; 0.8989] (*)	Synergy
Ray 3	0.89	0.6770 [0.1806; 1.1734] (NS)	Additivity
Ray 4	0.97	0.9226 [0.2104; 1.6348] (NS)	Additivity

(*): K is significantly different from 1 at 0.05 level NS: Non significant

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Global conclusion

- Summary of the experimental f values and associated conclusions : synergy (syn), additivity (add) or antagonism (ant)

Experimental f values		
EXP.1	**EXP.2**	**EXP.3**
0.06 (syn)	0.20 (add)	**0.71 (syn)**
0.17 (syn)	**0.45 (syn)**	0.89 (add)
0.40 (syn)	**0.73 (syn)**	0.97 (add)
0.67 (syn)	0.89 (add)	
0.86 (add)	0.96 (add)	

- For these three experiments, synergy between Prod.A and Prod.B is observed in the three first quarters of the domain (except for Exp2, f=0.20)
- Additivity between Prod.A and Prod.B is observed for higher f values
- On the tested rays, 'synergistic' domain : relative concentrations of Prod.A and Prod.B so that Prod.A is never more than 3 times more represented than Prod.B considering their relative potency

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Ray design modeling methodology - Pros & cons

- **Adapted to the biological mechanism of synergy by:**
  - taking into account the relative proportions of products
  - allowing to consider the specificity of each ray
- **Less trial- and time-consuming than full designs like grid designs**
- **Permit an accurate research of the synergy zone based on the proportion condition of the two compounds**
- **Can be only applied on in-vitro multiple-dose experiments with at least 6-7 different doses by ray**
- **Require a good knowledge of the studied products : accurate products IC50s, max and min effects estimations**
- **Adequacy between the expected and observed zone is very sensitive to the reproducibility of the assay → Robustness of the methodology to be evaluated**

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