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Comparison of null models for combination drug therapy reveals Hand model as biochemically most plausible

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Null models for the effect of combination therapies are widely used to evaluate synergy and antagonism of drugs. Due to the relevance of null models, their suitability is continuously discussed. Here, we contribute to the discussion by investigating the properties of five null models. Our study includes the model proposed by David J. Hand, which we refer to as Hand model. The Hand model has been introduced almost 20 years ago but hardly was used and studied. We show that the Hand model generalizes the principle of dose equivalence compared to the Loewe model and resolves the ambiguity of the Tallarida model. This provides a solution to the persisting conflict about the compatibility of two essential model properties: the sham combination principle and the principle of dose equivalence. By embedding several null models into a common framework, we shed light in their biochemical validity and provide indications that the Hand model is biochemically most plausible. We illustrate the practical implications and differences between null models by examining differences of null models on published data.

Combination drug therapy is an advancing field of research in oncology, anaesthesiology and immunology^{1–6}. Treatments with multiple drugs are studied to distribute side effects and minimize toxicity while attaining the full efficacy⁷. The discovery of synergistic combinations can enhance the development of multi-drug therapies and was ranked second place in the principles of combination therapy after *decreased toxicity without decreased efficacy*⁸. The term *combination* refers to the choice of pairing agents *A* and *B*, to their specific concentration ratio as well as to their explicit doses. In the following we will use the terms *drug* and *agent* interchangeable.

Synergy is detected and quantified via the comparison of an experimentally obtained effect and the mathematical reference effect of a null model. If the measured effect to a combination therapy exceeds the reference effect based on the measured effects of the individual drugs, the dose pair is considered synergistic, otherwise antagonistic. In order to quantitatively assess the level of synergy, concepts such as combination indices⁹ or tools of statistical analysis^{10,11} were introduced. A careful choice of the null model used to study synergy is important to not over-interpret results of drug combination studies¹². However, this choice requires a detailed understanding of the null models and the differences between them.

Applied in combination, the single effects of an *A*-dose and a *B*-dose are expected to add up in a way the null model specifies. Although there exist cases in which the simple algebraic sum of the effects may serve as a null model¹³, in general this sum will exceed the maximal possible effect, e.g., when the effect is measured in percentage of bound agents. A variety of null models has been introduced in the past and each model tries to provide a plausible concept of additivity that can be biochemically justified. Conceptually, effect-based strategies and dose-effect based strategies are distinguished¹⁴. In the established Bliss model¹⁵, normalized effects are interpreted as probabilities of stochastically independent events, e.g., the event of an analgesic binding to a receptor. This effect-based strategy permits an additivity concept according to the addition rule of probabilities. Two agents which act on different components in a biochemical cascade can be modelled in this mutually non-exclusive way

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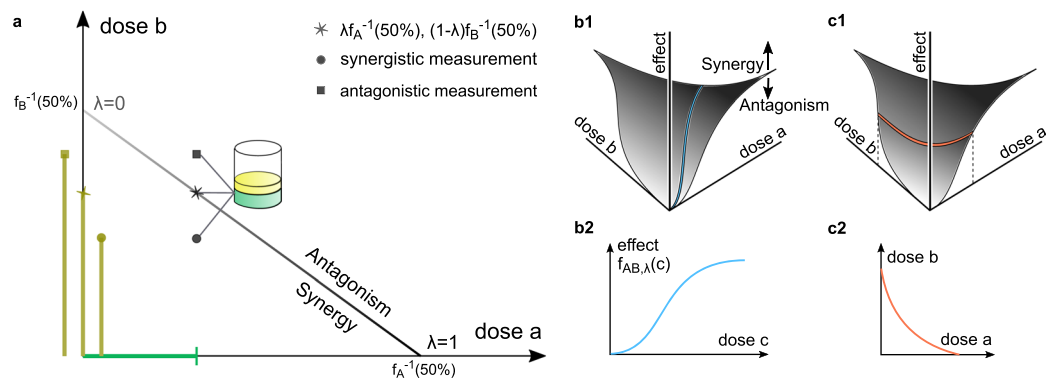


Figure 1. Loewe isobologram and the general effect surface. **(a)** Straight isobole at effect level 50% in the Loewe model. If the dose pairs located at star, circle, box induced 50%, the Loewe model would rate them non-interacting, synergistic and antagonistic, respectively. **(b,c)** visualize a general effect surface with informative cuts. **(b1)** Measured effects above and below the predicted effect E indicate synergy and antagonism, respectively. Vertical cuts through the effect surface along rays correspond to **(b2)** dose-effect curves of combined agents. **(c1)** Horizontal cuts through the effect surface provide **(c2)** isoboles.

(e.g., the HIV-1 entry inhibitors CoRA and FI⁵). However, the implicit assumption of stochastic independence does not hold in case of drugs that act through a similar mechanism. For example, cetuximab and afatinib both inhibit EGFR in the pathway of non-small cell lung cancer proliferation^{16,17}. In particular, a sham combination, i.e., a combination of an agent with itself, cannot be described by the Bliss model¹⁸.

Other null models incorporate knowledge on the functional relationship between dose concentration and effect for each single agent over a large range of doses. Such dose-effect based strategies shift the task of designing a null model towards specifying a plausible theory of addition of functions instead of a few numeric values¹⁹. This functional relationship between dose concentration and effect for the mono-therapies are frequently modelled as Hill curves, which can be fitted to experimental data by standard software²⁰. Here, we assume, that the dose-effect curves are increasing and have their maximal numerical value at the saturation level. However, minor changes allow an analogous treatment of decreasing dose-effect curves.

The classical dose-effect based model for mutually exclusive agents was first introduced by Loewe^{21,22} and popularized by Berenbaum²³, Chou and Talalay⁹. It states that doses of drug A and drug B may be linearly converted into each other inducing no modification in effect, where the conversion rate generally depends on the current effect level. For example, if both drugs attain the same maximal effect, then one third of the half-max concentration of A , i.e., the concentration at which the half-max effect is reached, combined with two thirds of the half-max concentration of B is predicted to yield an observed effect of 50%. The set of all dose combinations in the $([a], [b])$ -plane, that are predicted to induce the same effect level is called an isobole, where $[a]$ and $[b]$ are the doses of A and B , respectively. Being an indicator of zero interaction, the isobole separates the plane into a region of synergy of points located below the isobole and a region of antagonism located above it. For the Loewe model isoboles are straight lines, whose slopes are the conversion rates as depicted by the isobologram in Fig. 1a.

The Loewe model is broadly accepted and used in cases of constant potency ratio. The potency ratio is the ratio of the equipotent doses of two drugs, and two dose-effect curves are said to have a constant potency ratio if they are identical up to rescaling the dose axis. This means that they are parallel in the representation with a logarithmic dose scale. We will refer to this central but rare case as the *constant potency ratio case* or the *case of parallel dose-effect curves*. If the dose-effect-relations are given by Hill curves, two curves exhibit a constant potency ratio, exactly if they coincide in their maximal effects and Hill coefficients. In the prominent cases of a full and a partial agent or different cooperativities, i.e., maximal effect or the Hill coefficient differ, the potency ratio varies across effect levels.

The Loewe model is accepted for parallel dose-effect curves because this case implies that the conversion rate does not depend on the effect level, i.e., the isoboles for all effect levels in the Loewe model are parallel. Two principles were extracted from the constant potency ratio case that seem to permit a transfer to the varying potency ratio case: The principle of dose equivalence²⁴ and the principle of sham combinations²⁵. The former states that each A -dose can be assigned a B -equivalent, which induces the same effect. The latter states that, a drug is not expected to synergise in a combination with itself. The sham combination principle is rather specific to mutually exclusive agents and violated by several null models, including the Highest Single Agent (HSA) model, the Bliss model and the Fisher's dosage orthogonality²⁶.

The attempt to base a null model on coupling both principles for curves of varying potency ratio^{24,27,28} fails to identify unique isoboles, as objected by Lorenzo²⁹. The failure of this null model, which we call the Tallarida model in the following, has incorrectly been attributed to Loewe as the section *indeterminate Loewe additivity solution* in Geary¹⁹ indicates. In fact, the Loewe solution cannot be indeterminate, because the model, by definition, postulates a unique linear isobole. To the contrary, the Loewe additivity equation can very well be combined with existing theory on dose-effects^{30,31}, causing no mathematical contradiction. The indetermination in the varying potency ratio case results of ambiguously combining dose equivalence and sham combination principle in the way Grabovsky and Tallarida suggest²⁴.

While the Loewe model does not lack uniqueness, it is nonetheless justified to doubt its validity in several cases. Among the critics of its validity in the rather generic case of varying potency ratio one finds Loewe himself²². In particular, scepticism in the case of differing maximal effects when combining a full and a partial agent has led to an increasing amount of competitive models^{18,32–34}. Experimentalists emphasize that curved rather than straight isoboles are expected^{19,35} if dose-effect curves are not parallel, which is confirmed by examples of mechanistic models³⁶. To account for these deficits, Hand introduced an alternative and more general non-interaction model³⁷, which has unfortunately been overlooked in the reception of synergy detection models. Hand suggests to construct dose-effect curves for combined agents via an ordinary differential equation (ODE) in a way that both agents contribute linearly to the instantaneous gain in effect.

The conflict about the compatibility of sham combination principle and dose equivalence has been persisting for long. In this manuscript, we show how the Hand model can be obtained as a unique limit model of the Tallarida model. In doing so, we add an argument to dissolving this conflict. We introduce effect-sensitivity curves as a visualization tool, that provides the best insight into the Hand model's concept of additivity. This does not replace the dose-effect-visualization, but adds an alternative view, which confirms the biochemical plausibility of the Hand model in terms of the dynamical change in effect. We explore qualitative features of the Hand model that are not discussed in the original publication³⁷. Most strikingly, its isoboles are always convex, which reveals that the Hand model systematically provides a more conservative prediction than the Loewe model. Furthermore, we demonstrate how the models by Loewe, Hand and Tallarida can be interpreted as contrasting strategies for solving to some extent an unsolvable partial differential equation characterizing the effect-surface. Finally, we compare the reference effects of different null models for a set of published dose effect data.

Mathematical Background

Dose-effect curves and drug combinations. The dose-effect curves of the mono-therapies with drugs A and B are denoted by f_A and f_B . Both curves are assumed to be strictly increasing and their inverses are denoted by f_A^{-1} and f_B^{-1} . In practice, Hill functions are widely used²⁰, i.e., dose-effect curve

$$f_A(a) = E_{\min,A} + \frac{E_{\max,A} - E_{\min,A}}{1 + \left(\frac{EC_{50,A}}{a}\right)^{n_A}} \quad (1)$$

with inverse

$$f_A^{-1}(x) = EC_{50,A} \left(\frac{E_{\max,A} - 1}{x - E_{\min,A}} \right)^{n_A} \quad (2)$$

for drug A with dose a , effect level x , minimal effect $E_{\min,A}$, maximal effect $E_{\max,A}$, the half-max dose $EC_{50,A}$ and Hill coefficient n_A . The minimal effect $E_{\min,A}$ describes the untreated condition and is identical for different drugs, $E_{\min,A} = E_{\min,B}$. The maximal effects $E_{\max,A}$ and $E_{\max,B}$ can differ and the drug with the greater maximal effect is called the full agent, whereas the other is labelled as partial agent. In general $E_{\min,A}$ is normalized to zero, and the larger of $E_{\max,A}$ and $E_{\max,B}$ is normalized to one.

The potency ratio $\alpha(x)$ at effect level x quantifies how much more potent A is compared to B in generating effect x , i.e.,

$$\alpha(x) = \frac{f_A^{-1}(x)}{f_B^{-1}(x)}.$$

If the potency ratio is constant, both curves are identical up to rescaling the dose axis, or equivalently both curves are parallel, when plotted on a logarithmic dose axis.

We denote a combination drug of A and B at fixed ratio $\lambda \in [0, 1]$ by C_λ , i.e., one unit $[c]$ of the agent C_λ is composed as $[c] = \lambda[a] + (1 - \lambda)[b]$. Accordingly, the treatment with an amount c of the combination drug C_λ corresponds to a treatment with $a = \lambda c$ of drug A together with $b = (1 - \lambda)c$ of drug B .

Favourable properties of a null model. Given the dose-effect curves f_A and f_B of the single agents, a null model predicts the effect as a function $E: [0, \infty) \times [0, \infty) \rightarrow [0, \infty)$,

$$E(a, 0) = f_A(a), \quad E(0, b) = f_B(b).$$

Two cuts through the two-dimensional effect-surface $\{(a, b, E(a, b)) | a, b \geq 0\}$ visualize the combined effect¹⁸. Dose-effect curves of combination drugs C_λ vertically cut the surface along a ray directed according to the fixed ratio λ , i.e., the combined curve follows

$$f_{AB,\lambda}(c) = E(\lambda c, (1 - \lambda)c),$$

where we sometimes oppress the index λ for readability (Fig. 1b). Isoboles are horizontal cuts at level x , i.e., level sets of E , projected onto the $([a], [b])$ -plane (Fig. 1c).

A null model for drug combinations should ideally possess at least two properties to be biochemically plausible:

- The combination of a drug with itself should neither result in synergy nor antagonism, meaning that it does not interact with itself and meets the sham combination principle. In the above notation E satisfies the *sham combination principle* if for all $\lambda \in [0, 1]$

$$f_{AA,\lambda} = f_A.$$

Geometrically the isoboles of the effect surface are then straight lines with unit slope.

- The swap of drug A and B should not change the effective results. Mathematically, this implies that E is *commutative* in A and B , i.e., for all $\lambda \in [0, 1]$

$$f_{AB,\lambda} = f_{BA,1-\lambda}.$$

In addition, a model may satisfy an associative property, which allows to combine combination drugs:

- The combination of agents C_λ and C_μ at ratio ν should be equivalent to directly combining A and B at a suitable ratio. For $[c_\lambda] = \lambda[a] + (1-\lambda)[b]$ and $[c_\mu] = \mu[a] + (1-\mu)[b]$, the resulting combination is $\nu[c_\lambda] + (1-\nu)[c_\mu] = (\nu\lambda + (1-\nu)\mu)[a] + (\nu(1-\lambda) + (1-\nu)(1-\mu))[b]$. E satisfies the *associative principle* if for any $\lambda, \mu, \nu \in [0, 1]$

$$f_{C_\lambda C_\mu, \nu} = f_{AB, \nu\lambda + (1-\nu)\mu}.$$

The associativity principle is stronger than commutativity, because the latter is contained in the associativity property by the particular choice $\lambda=0, \mu=1$.

Geometrically, associativity means: C_λ and C_μ define new coordinate axes in the $([a], [b])$ -plane with angle smaller than 90° . The model prediction along these curves are taken to be the new mono-therapies while the rest of the effect surface is ignored. Bending these axes to an angle of 90° and applying the model to this new coordinate system should then span the same effect surface as bending the null model originally obtained from A and B . The associative property guarantees that the model is in this sense compatible with a change of the coordinate axes.

Dose-effect based null models. In this section, we introduce the null models proposed by Loewe, Tallarida and Hand. These null models are based on the dose-effect strategy and rely on the knowledge of the complete dose-effect curve.

The Loewe model. The classical approach for mutually exclusive drug combinations is the Loewe model^{22,23}. As its core, the model postulates that the isoboles are straight²¹. The isobole at effect level x can thus be characterized as the set of all combinations (a, b) satisfying the Loewe additivity equation

$$\frac{a}{f_A^{-1}(x)} + \frac{b}{f_B^{-1}(x)} = 1. \quad (3)$$

The effect $E_L(a, b)$ predicted by the Loewe model for an arbitrary combination (a, b) can be obtained by (numerically) solving (3) for x , i.e., $E_L(a, b)$ is the unique effect, that solves

$$\frac{a}{f_A^{-1}(E_L(a, b))} + \frac{b}{f_B^{-1}(E_L(a, b))} = 1.$$

A generic point on the isobole has the form $(\lambda f_A^{-1}(x), (1-\lambda)f_B^{-1}(x))$ (Fig. 1a). E_L is commutative and satisfies the sham combination principle. However, it is only defined for effects x , which are attained by both f_A and f_B . Hence, it is not applicable for effect sizes $x \geq \min\{E_{\max,A}, E_{\max,B}\}$.

The potency ratio $\alpha(x)$, which is geometrically the slope of the isobole, precisely expresses the conversion rate of equivalent doses in the Loewe model. If A and B exhibit a constant potency ratio, then all isoboles in the Loewe model are parallel. Tallarida²⁷, Geary¹⁹ and others argue that this property is necessary to ensure the validity of the Loewe model. Accordingly, the probable case of a varying potency ratio would prevent the use of the Loewe model.

The Tallarida model. In an attempt to transfer the principles of dose equivalence and sham combination to the case of varying potency ratio, Grabovsky and Tallarida suggested to convert a dose a into an equivalent B -dose $f_B^{-1}(f_A(a))$ generating the same effect $f_A(a)$ (Fig. 2a). Adding this B -equivalent to b should predict the effect via f_B^{-1} , i.e.,

$$E_{T,A \rightarrow B}(a, b) = f_B(b + f_B^{-1}(f_A(a))). \quad (4)$$

Since the model was further popularized by Tallarida^{27,28}, we refer to it as the Tallarida model. Conceptually, the Tallarida model decomposes the total effect $x = E_{T,A \rightarrow B}(a, b) = x_A + x_B$ as in the following way: dose a of A individually achieves an effect $x_A = f_A(a)$ on its lower range of doses, while dose b of B acts on its higher dose range, contributing on top of the effect achieved by A , i.e.,

$$x_A + x_B = f_B(b + f_B^{-1}(f_A(a))) \Leftrightarrow x_B = f_B(b + f_B^{-1}(x_A)) - x_A. \quad (5)$$

Note that the model with altered roles of A and B is

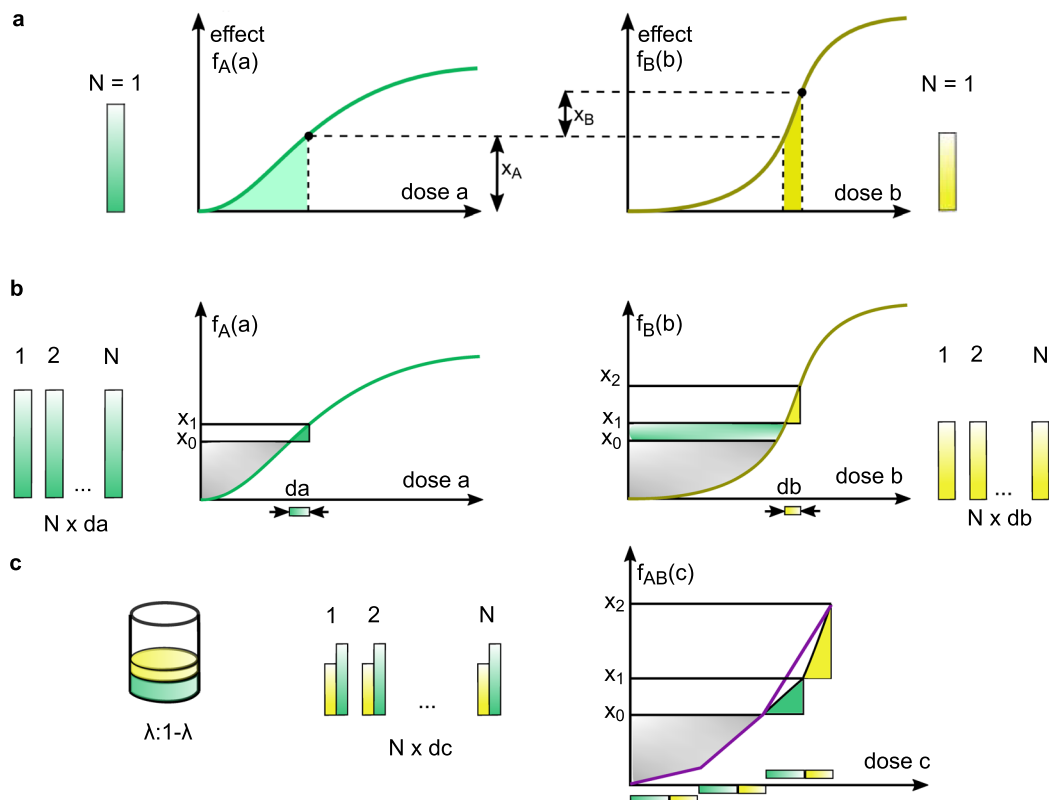


Figure 2. The effect decomposition in the Tallarida model forms the basis for the infinitesimal approach in the Hand model. (a) Dose a of A individually achieves an effect $x_A = f_A(a)$ on its lower range of doses, while dose b of B acts on its higher dose range, contributing on top of the effect achieved by A . The contribution of drug B to the effect is $x_B = f_B(b + f_B^{-1}(x_A)) - x_A$. (b) The doses a and b are split into N small pieces da and db , respectively. The effect level x_0 is elevated by da to x_1 , db raises the effect from x_1 to x_2 . (c) The combined dose-effect curve for the mixture C_λ is constructed by applying N pieces of $dc = da + db$. After twice applying dc , the effect level x_0 is attained. Applying a third mixture of da and db successively increases the effect to x_1 and finally x_2 .

$$E_{T,B \rightarrow A}(a, b) = f_A(a + f_A^{-1}(f_B(b))).$$

The models $E_{T,A \rightarrow B}$ and $E_{T,B \rightarrow A}$ might predict a different effect because in general

$$f_B(b + f_B^{-1}(f_A(a))) \neq f_A(a + f_A^{-1}(f_B(b))).$$

Accordingly, while the Tallarida model meets the sham combination principle, it does not fulfil the commutation property. The two model predictions $E_{T,A \rightarrow B}(a, b)$ and $E_{T,B \rightarrow A}(a, b)$ encompass a range of effect values. In the following, we will refer to the TallaridaLB model and the TallaridaUB model,

$$E_{T,LB}(a, b) := \min \{E_{T,A \rightarrow B}(a, b), E_{T,B \rightarrow A}(a, b)\}, \quad E_{T,UB}(a, b) := \max \{E_{T,A \rightarrow B}(a, b), E_{T,B \rightarrow A}(a, b)\}$$

that give a lower and an upper bound to this range.

The Hand model. It remains the problem that the Tallarida model, introduced in 2004 by Grabovsky and Tallarida, is not consistent with changing the roles of A and B . In 2000, Hand already anticipates the ambiguity in what we call the Tallarida model. He identifies as a solution to this problem that instead of adding the effect of b as an absolute quantity to the effect level reached by a in equation (4) one rather needs to account for the simultaneous nature of drug action³⁷. This hints at formulating additivity in terms of instantaneous effect gains rather than effect levels. Hand proposes to construct E by specifying the rate at which the effect is changing. Since the rate is a one dimensional quantity, it is advantageous to assign values to the entire plane of dose pairs by going along rays at fixed ratio λ . More precisely he suggested that the combined curve $f_{AB,\lambda}$ must satisfy the differential equation

$$f'_{AB,\lambda}(f_{AB,\lambda}^{-1}(x)) = \lambda f'_A(f_A^{-1}(x)) + (1 - \lambda) f'_B(f_B^{-1}(x)), \quad f_{AB,\lambda}(0) = 0. \tag{6}$$

This characterizing equation states that at each effect level both agents contribute linearly to the instantaneous gain in effect of the combined curve. For an arbitrary dose pair (a, b) , identify the ratio $\lambda \in [0, 1]$, such that

	Loewe	Tallarida	Hand	Bliss	HSA
uniqueness	✓	—	✓	✓	✓
commutativity in A and B	✓	—	✓	✓	✓
sham combination principle	✓	✓	✓	—	—
associative property	✓	—	✓	—	—
defined for full and partial agent	—	✓	✓	✓	✓

Table 1. Properties of null models.

$$\frac{a}{b} = \frac{\lambda}{1 - \lambda}, \quad (7)$$

then $(a, b) = (\lambda c, (1 - \lambda)c)$ is a dose c of the drug mixture C_λ . For f_{AB} solving (6) with λ satisfying (7), we assign

$$E_H(a, b) = f_{AB, \frac{a}{a+b}}(a + b).$$

Using the inverse derivative formula, we express the differential equation (6) as

$$\frac{1}{f_{AB, \lambda}^{-1'}(x)} = \frac{\lambda}{f_A^{-1'}(x)} + \frac{1 - \lambda}{f_B^{-1'}(x)}, \quad (8)$$

or in integral representation

$$f_{AB, \lambda}^{-1}(x) = \int_0^x \left(\frac{\lambda}{f_A^{-1'}(y)} + \frac{1 - \lambda}{f_B^{-1'}(y)} \right)^{-1} dy. \quad (9)$$

The Hand model was constructed to satisfy the sham combination principle and clearly it is commutative in A and B. Hand stresses that if an agent saturates at a lower level than the other, its derivative is set to zero above that level. This is a natural interpretation of (6) given that x is not in the range of either f_A or f_B . The Hand model thus comprises the case of a full and a partial agent. Moreover, Hand mentions that the isoboles will be straight in the constant potency ratio case. Coinciding with the Loewe model for this sub-case, it is regarded by Hand as a generalization of the Loewe model. We will demonstrate, by embedding three of the models into a common framework, that the Loewe model is justified also for cases of varying potency ratio and thus the term *generalization* should be used with care.

Effect-based null models. Alternatives to the dose-effect based null models are effect-based models. These null models require only the knowledge of the effect of the single doses a and b to provide a prediction of the combined effect $E(a, b)$. Commonly used effect-based models are the Bliss model and the HSA model. The Bliss model¹⁵ is based on a probabilistic framework, and defines the effect of a drug combination as

$$E_{\text{Bliss}}(a, b) = f_A(a) + f_B(b) - f_A(a) \cdot f_B(b)$$

For this definition it is necessary, that the greater of the maximum values of the two dose-effect curves, $E_{\text{max},A}$ and $E_{\text{max},B}$, are normalized to 1. The HSA model, often referred to as Gaddum's non-interaction²⁵, is defined as

$$E_{\text{HSA}}(a, b) = \max \{f_A(a), f_B(b)\}.$$

The Bliss and the HSA model do not fulfil the sham and the associative principle. For a detailed comparison of the properties of all null models we refer to the corresponding section and Table 1.

Results

Mathematical analysis of the Hand model. In this study, we consider five previously published null models. Amongst them, the Hand model is the least well known and studied one. To facilitate an intuitive understanding, we will provide a unified derivation of the Tallarida and the Hand model; and we will introduce the novel concept of effect-sensitivity curves which confirm the additivity principle. Subsequently, we will study the isoboles of the Hand model.

Unified derivation of the Tallarida and the Hand model. In the literature, the Tallarida and the Hand model are presented and discussed separately^{24,37}. Here, we establish a link and improve the understanding of the Tallarida and the Hand model by providing a unified derivation. By this derivation we extended the original paper's frank justification on addition of rates.

We start from the concept of Tallarida on equivalent doses (5). However, instead of applying a entirely and then b entirely, we apply portions of both, constantly alternating (Fig. 2b,c). Formally, we split both doses a and b into N pieces $da = \frac{a}{N}$ and $db = \frac{b}{N}$, respectively. In particular it is

$$\frac{da}{db} = \frac{\lambda}{1 - \lambda}$$

with λ satisfying (7). We define $dc := da + db$ as a small portion of the combination drug C_λ . By alternately applying portions da and db , we keep track of how the effect changes. Suppose, we have constructed the dose-effect curve $f_{AB,\lambda}$ of C_λ up to an effect level x_0 , induced by a mixture dose

$$c = f_{AB,\lambda}^{-1}(x_0). \quad (10)$$

Applying da elevates the effect level to $x_1 = f_A(da + f_A^{-1}(x_0))$ and db subsequently amplifies the effect level to $x_2 = f_B(db + f_B^{-1}(x_1))$. By the above procedure we obtain

$$\begin{aligned} x_A &= x_1 - x_0 = f_A(da + f_A^{-1}(x_0)) - f_A(f_A^{-1}(x_0)) \\ x_B &= x_2 - x_1 = f_B(db + f_B^{-1}(x_1)) - f_B(f_B^{-1}(x_1)) \\ x_{AB} &= x_2 - x_0 = f_{AB,\lambda}(dc + c) - f_{AB,\lambda}(c). \end{aligned}$$

For $N = 1$, this procedure yields the Tallarida model (Fig. 2a). To study $N \rightarrow \infty$ we take the first-order Taylor approximation, divide by dc and note that $da = \lambda dc$, $db = (1 - \lambda)dc$. This yields (Supplementary Information S1.1)

$$\frac{f_{AB,\lambda}(c + dc) - f_{AB,\lambda}(c)}{dc} = \lambda f'_A(f_A^{-1}(x_0)) + (1 - \lambda) f'_B(f_B^{-1}(x_1)) + o(1).$$

In the limit case $dc \rightarrow 0$, i.e., an infinite amount of infinitesimal pieces dc , the difference quotient tends to the derivative on the left side in (6) and we obtain the Hand model. Note that the roles of A and B in the derivation may be switched, approaching the same differential equation in the limit. This yields a unique limit model even though each approximative model is ambiguous.

Effect-sensitivity curves and an additivity principle for the Hand model. In the Hand model (6) the instantaneous gain in effect depends on the individual dose-effect curves $f_A(a)$ and $f_B(b)$ via the functions $s_A(x) = f'_A(f_A^{-1}(x))$ and $s_B(x) = f'_B(f_B^{-1}(x))$, respectively. We term the derivative $f'_A(a)$, which is measured in effect per dose, its *sensitivity*. As the functions $s_A(x)$ and $s_B(x)$ describe the sensitivity of the dose-effect relation subject to the effect level, we term $(x, s_A(x))$ and $(x, s_B(x))$ *effect-sensitivity curves*. The dose-effect curve f_A is then the solution to

$$\frac{d}{da}x(a) = s_A(x(a)).$$

The sensitivity is precisely the right-hand side of the autonomous ODE which guides the dynamics of the dose-effect curve, evolving over the dose range instead of time. For Hill curves as in (1) the effect-sensitivity curve is explicitly given by

$$s_A(x) = \frac{n_A}{EC_{50,A} \cdot (E_{\max,A} - E_{\min,A})} (x - E_{\min,A})^{1 - \frac{1}{n_A}} (E_{\max,A} - x)^{\frac{1}{n_A}}$$

for $x \in [E_{\min,A}, E_{\max,A}]$ (Supplementary Information S2.1). Under weak conditions, it is possible to retransform effect-sensitivity into dose-effect curves, rendering them an equivalent notion (Supplementary Information S2.2). The Hand model specifies the following additivity concept

$$s_{AB,\lambda}(x) = \lambda s_A(x) + (1 - \lambda) s_B(x), \quad (11)$$

where the quantities, which are added, are the sensitivities; i.e. the effect-sensitivity curve of the combined agent is a weighted average of the single effect-sensitivity curves with weights according to the mixture ratio (Fig. 3). More suggestively, we say that the Hand model summates the dynamics of the single dose-effect curves.

Geometric properties of the Hand model's isoboles. The isoboles of the null model are the basis for assessing synergy and antagonism of drugs. While the Loewe model's isoboles are easily graphically obtained and analytically given in closed form as soon as $f_A^{-1}(x)$ and $f_B^{-1}(x)$ are known, the Hand model's isoboles are non-trivial and might often have to be computed numerically. However, we could show that the Hand isoboles share a characteristic geometric feature: they are always convex (Supplementary Information S4). As a consequence, they systematically diminish the region of synergy compared to the Loewe model.

Combination drugs for partial and full agent. In practice the maximal effect of drug A might be smaller than the maximal effect of drug B , meaning that A acts as the partial agent and B as the full agent. Since the construction in the classical Loewe model requires effect levels that are attained by both agents²², this case has motivated to a large extent the search for alternative models^{18,32–34}. In the subsequent discussion of the Loewe and the Hand model we assume $E_{\max,A} < E_{\max,B}$.

Loewe model for partial and full agent. The Loewe model assigns effect values to all dose pairs (a, b) with $b < f_B^{-1}(E_{\max,A})$ (Fig. 4a). In fact, the slope of the isoboles approaches 0 as the effect level approaches $E_{\max,A}$.

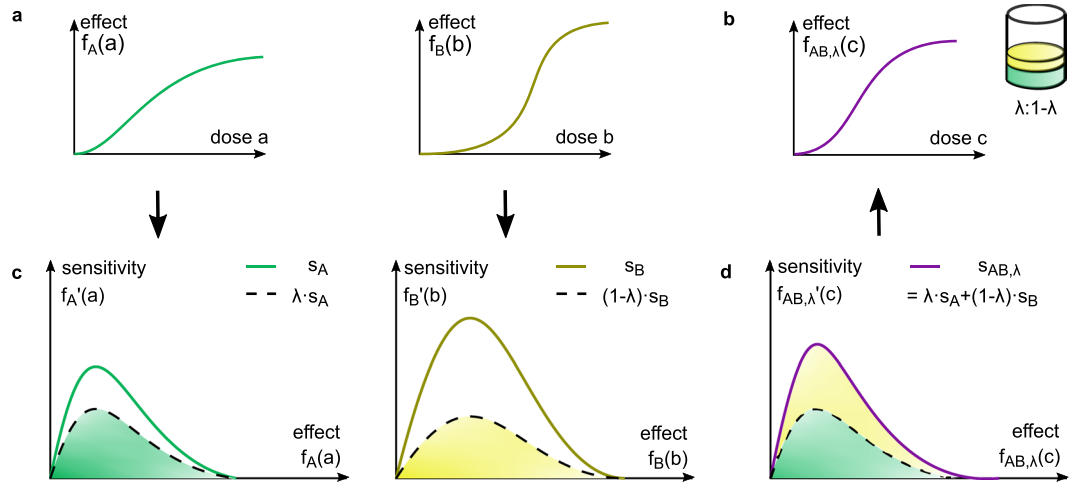


Figure 3. Concept of additivity according to the Hand model. (a) The Hand model specifies how to add the two dose-effect curves of drugs A and B to get the combined dose-effect curve at fixed dose ratio λ (b). (c) f_A and f_B are transformed to effect-sensitivity curves via $s_A(x) = f'_A(f_A^{-1}(x))$, reflecting the saturation dynamics. (d) The effect-sensitivity curve $s_{AB,\lambda}$ of the mixture C_λ is their weighted algebraic sum. $f_{AB,\lambda}$ is obtained from $s_{AB,\lambda}$ by integration.

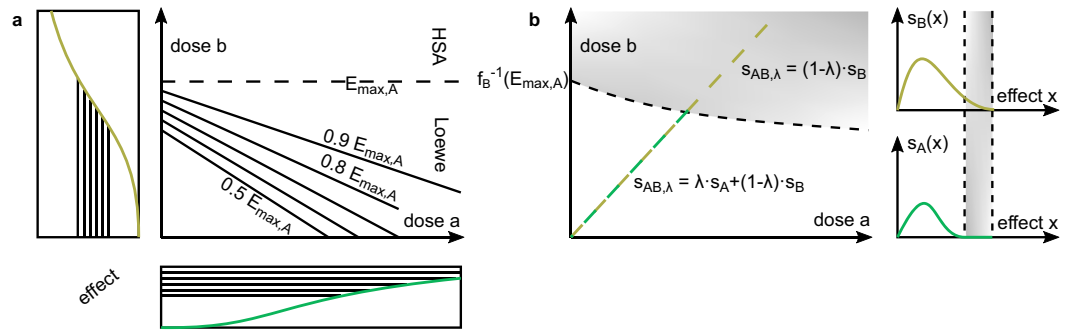


Figure 4. Model predictions for combination drugs of a partial agent A and full agent B. The isobolograms for the (a) Loewe model and (b) Hand model. (a) The Loewe model assigns reference effects only to dose pairs below the horizontal limit isobole at effect value $E_{max,A}$, beyond which it can be extended via the HSA model. (b) Above the partial agent A’s maximal effect value its sensitivity is set to 0. As soon as the combined curve passes the curved limit isobole (a^*, b^*) at effect level $E_{max,A}$ it is driven by the full agent alone.

This phenomenon arises because the Hill curve f_A saturates asymptotically. Hence the strip in the $([a], [b])$ -plane that is bounded by the horizontal line $b = f_B^{-1}(E_{max,A})$ is covered entirely by Loewe isoboles (Supplementary Information S5). The horizontal limit isobole $b = f_B^{-1}(E_{max,A})$ coincides with the HSA model’s isobole at level $E_{max,A}$, which suggests to extend the Loewe model by the HSA model.

Hand model for partial and full agent. As shown in the original publication³⁷, the Hand model comprises the case of a partial agent A and a full agent B. In terms of sensitivities, the effect-sensitivity curve s_A is extended by constant zero on the effect domain $[E_{max,A}, E_{max,B}]$ (Fig. 4b), i.e. the sensitivity of the combined curve is

$$s_{AB,\lambda}(x) = \begin{cases} \lambda s_A(x) + (1-\lambda)s_B(x), & 0 \leq x < E_{max,A} \\ (1-\lambda)s_B(x), & E_{max,A} \leq x < E_{max,B} \end{cases}$$

If $c^*(\lambda)$ denotes the dose of mixture C_λ at which $E_{max,A}$ is attained, then

- for $c \leq c^*(\lambda)$, the ODE guiding the evolution of $f_{AB,\lambda}$ is (6).
- For $c > c^*(\lambda)$, the evolution simplifies to the initial value problem

$$\frac{d}{dc} f_{AB,\lambda}(c) = (1-\lambda)s_B(f_{AB,\lambda}(c)), \quad f_{AB,\lambda}(c^*(\lambda)) = E_{max,A}.$$

The solution for $c > c^*(\lambda)$ is $f_{AB,\lambda}(c^*(\lambda) + \tilde{c}) = f_B(f_B^{-1}(E_{max,A}) + (1-\lambda)\tilde{c})$, $\tilde{c} > 0$, hence,

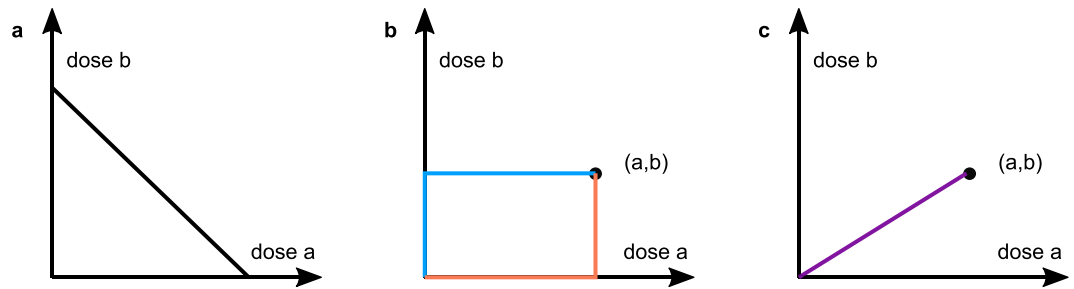


Figure 5. Paths for the three null models. The three null models solve equation (12) to some extent along different paths. (a) The Loewe model solves (12) along isoboles. This requirement forces the isoboles to be linear. (b) The Tallarida model solves (12) along rectangular paths. (c) The Hand model solves (12) along rays.

$$E_H(a, b) = f_B \left(f_B^{-1}(E_{\max,A}) + b - \frac{b}{a+b} c^* \left(\frac{a}{a+b} \right) \right)$$

for all $a + b \geq c^* \left(\frac{a}{a+b} \right)$. Accordingly, for effect sizes greater than $E_{\max,A}$ only B has an effect and the dose-effect curve for C_λ in this regime corresponds to the scaled and shifted dose-effect curve f_B . In contrast to the Tallarida model, the effect surface does not copy the dose-effect curve f_B in the direction parallel to the B-axis.

Using the integral representation, $c^*(\lambda)$ is explicitly given by

$$c^*(\lambda) = \int_0^{E_{\max,A}} \left(\frac{\lambda}{f_A^{-1'}(x)} + \frac{1-\lambda}{f_B^{-1'}(x)} \right)^{-1} dx.$$

Decomposing the combined dose $c^*(\lambda)$ into its contributions $a^*(\lambda) = \lambda c^*(\lambda)$ and $b^*(\lambda) = (1-\lambda)c^*(\lambda)$, the limit isobole at effect level $E_{\max,A}$ is parametrized by $\lambda \mapsto (a^*(\lambda), b^*(\lambda))$. Its B-component

$$b^*(\lambda) = \int_0^{E_{\max,A}} \left(\frac{\lambda}{1-\lambda} \frac{1}{f_A^{-1'}(x)} + \frac{1}{f_B^{-1'}(x)} \right)^{-1} dx.$$

is a decreasing continuous function with $\lim_{\lambda \rightarrow 1} b^*(\lambda) = 0$.

Let us contrast this finding with the Loewe model. Assuming Hill curves, which saturate asymptotically, as dose-effect-relations for both models, the Loewe model ignores the amount of A at the critical effect size $E_{\max,A}$, while in the Hand model a higher amount of A, and thus a larger λ , reduces the amount of $b^*(\lambda)$ necessary to generate the effect $E_{\max,A}$.

Common framework for the Loewe, the Tallarida and the Hand model. The dose-effect based null models for mutually exclusive interaction presented above pursue different approaches in constructing the effect surface $E(a, b)$. While the Loewe model imposes a condition on the isoboles' shape, the Tallarida model elevates the dose-effect curves to the respective initiating level of the other curve. Consequently it constructs the effect surface $E(a, b)$ by copying and attaching the existing curves of the single agents. The Hand model specifies the evolution of effect surface $E(a, b)$ for increasing amount of mixture dose at fixed ratio, hence constructs the surface along rays. While the models seem rather different, we could derive a novel common framework for all three, based on the concept of effect-sensitivity curves introduced in the above section.

Assuming an underlying mechanistic dynamical model for the effect of the individual drugs, it is plausible to request that the change in effect should only depend on the current effect level. If nothing but the knowledge about the single dose-effect curves is available, it is plausible that the single dynamics predict the dynamics of the combined effect. More precisely, we expect the sensitivities of f_A and f_B to translate changes of A- and B-doses respectively into changes in effect, i.e.,

$$\begin{aligned} \frac{\partial}{\partial a} E(a, b) &= s_A(E(a, b)) \\ \frac{\partial}{\partial b} E(a, b) &= s_B(E(a, b)). \end{aligned} \tag{12}$$

For given dose-effect curves f_A and f_B , and the derived effect-sensitivity curves s_A and s_B , such a function E does in general not exist. However, if we request the equations to hold to some extent, we recover the above null models.

The Loewe model is recovered, if we request (12) to hold along the isoboles (Fig. 5a): Let $\gamma = (\gamma_A, \gamma_B): [0, 1] \rightarrow [0, \infty) \times [0, \infty)$ parametrize the isobole at effect level x , then

$$0 = \frac{d}{dt}E(\gamma(t)) = \frac{\partial}{\partial a}E(\gamma(t))\dot{\gamma}_A(t) + \frac{\partial}{\partial b}E(\gamma(t))\dot{\gamma}_B(t).$$

Using $E(\gamma(t)) \equiv x$, we obtain

$$\frac{\dot{\gamma}_A(t)}{\dot{\gamma}_B(t)} = -\frac{f'_B(f_B^{-1}(x))}{f'_A(f_A^{-1}(x))} = -\frac{f_A^{-1'}(x)}{f_B^{-1'}(x)} = -\frac{f_A^{-1}(x)}{f_B^{-1}(x)}. \quad (13)$$

The last equality holds because $f_A^{-1}(0) = 0 = f_B^{-1}(0)$. The calculation (13) shows that the ratio $\frac{\dot{\gamma}_A(t)}{\dot{\gamma}_B(t)}$ does in fact not depend on t , but is constant for all t . Consequently, γ parametrizes a straight line with the slope given precisely by the potency ratio $\alpha(x)$. It is important to notice, that we did not request the isoboles to be straight lines in the first place, however, satisfying (12) forces them to be linear.

The **Tallarida model** $E_{T,A \rightarrow B}$ is recovered by following a rectangular path from $(0, 0)$ to (a, b) (Fig. 5b).

$$\gamma = \gamma_1 \oplus \gamma_2, \quad \gamma_i: [0, 1] \ni t \mapsto (ta, 0), \quad \gamma_2: [0, 1] \ni t \mapsto (a, tb)$$

where \oplus concatenates the paths, i.e. for $t \in [0, 2]$

$$\gamma(t) = \begin{cases} \gamma_1(t), & 0 \leq t \leq 1 \\ \gamma_2(t-1), & 1 < t \leq 2. \end{cases}$$

Then along γ_1 equation (12) integrates to $E_{T,A \rightarrow B}(\gamma_1(t)) = f_A(ta)$ and along γ_2 equation (12) with initial condition $E_{T,A \rightarrow B}(\gamma_2(0)) = E_{T,A \rightarrow B}(\gamma_1(1)) = f_A(a)$ integrates to $E_{T,A \rightarrow B}(a, tb) = f_B(f_B^{-1}(f_A(a)) + tb)$. Analogously, the model $E_{T,B \rightarrow A}$ is obtained by the rectangular path

$$\gamma_1(t) = (0, tb), \quad \gamma_2(t) = (ta, b).$$

The **Hand model** is recovered if we request (12) to hold along combined curves $\gamma(c) = (\lambda c, (1-\lambda)c)$ (Fig. 5c). In this case we compute by the chain rule

$$\frac{d}{dc}E(\gamma(c)) = \lambda \frac{\partial}{\partial a}E(\gamma(c)) + (1-\lambda) \frac{\partial}{\partial b}E(\gamma(c))$$

and set $E(\gamma(c)) = f_{AB,\lambda}(c)$, $c = f_{AB,\lambda}^{-1}(x)$.

In the constant potency ratio case, (12) admits a solution and all three models coincide. Indeed, for $f_A^{-1}(x) = \alpha f_B^{-1}(x)$ the solution E is given by

$$E(a, b) = f_A(a + \alpha b) = f_B\left(\frac{a}{\alpha} + b\right).$$

The representation of the Loewe, the Tallarida and the Hand model as alternative paths through an underlying null model (12) provides a novel perspective. It highlights the relation of the existing dose-effect based null model and the importance of the effect-sensitivity curves. Furthermore, this common framework might facilitate the basis for the development of alternative models with novel properties.

Comparison of the mathematical properties of the null models. The null models considered in this study possess different (combinations of) properties. In this section, we collect the available information, present results on the associative principle and outline the implications of our findings. In addition, we study the reference effect values calculated by different null models and prove rigorous orderings.

Properties of the Loewe, the Tallarida and the Hand models. Given a general framework for all three null models, we studied their properties in further detail. A summary is provided in Table 1. As outlined before,

- the Loewe, the Tallarida and the Hand model meet the sham combination principle (Supplementary Information S3.2), which is not the case for the Bliss and the HSA model (Supplementary Information S3.3); and
- the Loewe and the Hand model meet the commutation principle (Supplementary Information S3.4). Indeed, we could also prove here that
- the Loewe and the Hand model fulfil the strong property of associativity (Supplementary Information S3.6).

For drugs with a constant potency ratio, the models by Loewe, Hand and Tallarida coincide (Supplementary Information S3.1). Furthermore, the Tallarida and the Hand model are applicable to a combination of full and partial agents.

Our analysis reveals that the Hand model fulfils all the previously defined favourable properties (Supplementary Information S3 and original publication³⁷), while all other models only fulfil some of them. As the favourable properties are biochemically motivated, this implies that the Hand model is biochemically most plausible among the considered models.

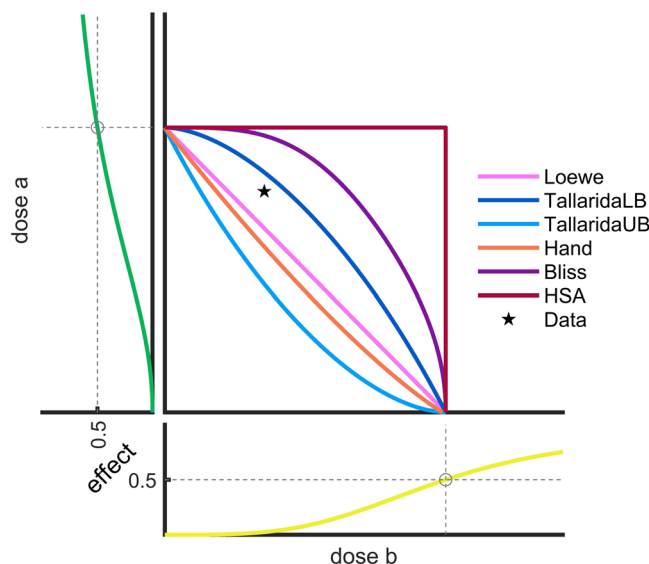


Figure 6. Assessment of synergy and antagonism using different null models. Dose-effect curves and isobologram at EC_{50} for different null models. A measured effect of 0.5 for a given dose pair (★) is considered as synergistic by the Bliss, the HSA and the TallaridaLB model, while it is antagonistic according to the Loewe, the Hand and the TallaridaUB model.

Systematic inequalities. The assessment of drug synergy or antagonism for a single dose of a combination drug is equivalent to the question: On which side of the isobole corresponding to the observed effect does the measured dose pair lie? Accordingly, the definition of drug synergy or antagonism depends directly on the isoboles, which potentially differ substantially among the null models. For the example shown in Fig. 6, the HSA, the Bliss, and the TallaridaLB model classify a dose pair to be synergistic, while the Loewe, the TallaridaUB, and the Hand model classify it as antagonistic. Note that a null model which tends to predict higher effects compared to measured values correspondingly yields an isobole closer to the origin, and less dose pairs are classified as synergistic.

Mathematically, an important implication of the Hand model's convex isoboles is that its reference effect bounds the one provided by the Loewe model from above. For a specific dose pair (a, b) , the Loewe model provides a reference effect $x = E_L(a, b)$. If the dose pair is expressed in terms of a quantity c of the mixture drug with ratio $\lambda = \frac{a}{a+b}$, i.e., $(a, b) = (\lambda c, (1 - \lambda)c)$, then the convexity of the Hand model's x -isobole implies that $E_H(\tilde{a}, \tilde{b}) = x$ for $(\tilde{a}, \tilde{b}) = (\lambda\tilde{c}, (1 - \lambda)\tilde{c})$ with some $\tilde{c} \leq c$. As the combined curve $f_{\lambda,H}(c)$ is an increasing function in c , we conclude

$$E_H(a, b) = E_H(\lambda c, (1 - \lambda)c) = f_{\lambda,H}(c) \geq f_{\lambda,H}(\tilde{c}) = E_H(\tilde{a}, \tilde{b}) = x = E_L(a, b).$$

In addition, (i) TallaridaLB is by definition lower than TallaridaUB and (ii) the HSA model predicts systematically lower effects than all other null models, for instance

$$E_{\text{Bliss}}(a, b) = f_A(a) + \underbrace{f_B(b) \cdot (1 - f_A(a))}_{\geq 0} = f_B(b) + \underbrace{f_A(a) \cdot (1 - f_B(b))}_{\geq 0} \geq \max\{f_A(a), f_B(b)\}.$$

In summary, the following systematic inequalities hold for the reference effect values obtained by the different null models for any combination drug C_λ and combination drug concentration c :

- HAS < Loewe < Hand
- HSA < Bliss
- HSA < TallaridaLB < TallaridaUB

Comparison of null models using experimental data. To assess the characteristic features of the different null models in practice, we considered the drug screening data collected by O'Neil *et al.*³⁸. The dataset reports the effects of 39 cancer cell lines to 38 drug individual drugs and 583 drug combinations of these drugs. Each combination was measured on a 4 by 4 dosing regime. The proliferation compared to the untreated condition is taken as the readout of the effect.

For the given data, we compared the reference effects of the Loewe, the Tallarida and the Hand model, as well as the Bliss model and the HSA model. As the Tallarida model does not provide a unique solution (see the section on the Tallarida model), we evaluated both cases. The Tallarida models predicting lower and higher effects for a particular drug combination are as before denoted as the TallaridaLB and the TallaridaUB model, respectively. For the Loewe, the Tallarida and the Hand model, Hill curves and a zero-effect curves were fitted to the

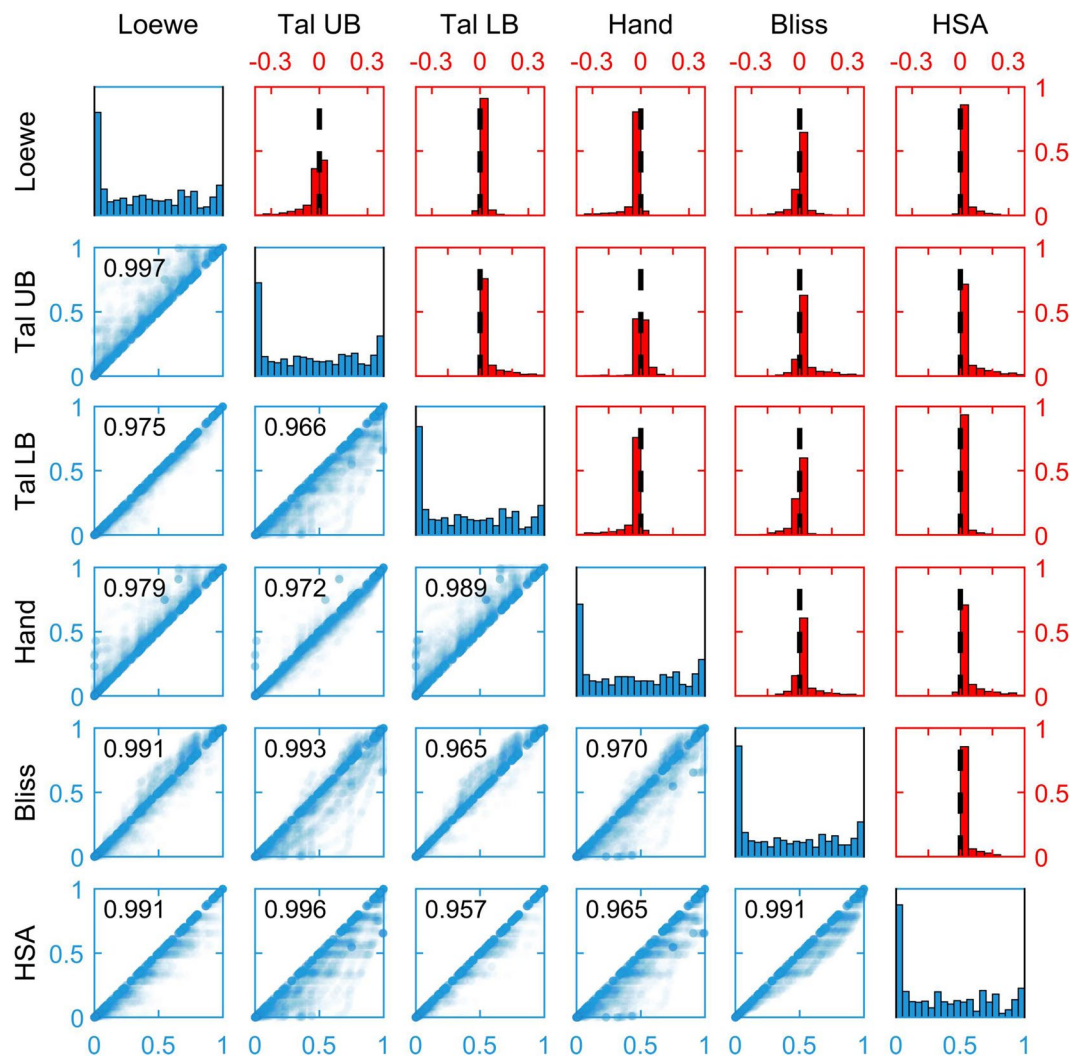


Figure 7. Distributions of reference effects. Below the diagonal a scatter plot of the null model reference effects for all drug doses tested for cell line A375 is shown. On the diagonal the distribution of the reference effects are shown. Above the diagonal a histogram of the differences of the references are given. Zero is marked by a dashed line.

measurement data for the single drug treatments. The Bayesian Information Criterion (BIC)³⁹ was used to select the more suited model (Supplementary Information S6).

For a comparison of the null models, we considered the predictions of all tested dose pairs of all tested drug combinations. For the cell line A375, the predictions are visualized in Fig. 7. Besides the systematic inequalities which have been proven in the corresponding section, we found:

- Reference effects for different null models were highly correlated.
- The TallaridaLB model tended to bound the references of all other models besides HSA from below, while the TallaridaUB model tended to bound the references of all other models besides the Hand model from above.
- The TallaridaLB and the TallaridaUB are potentially quite different. This can lead to problems if the researcher is not aware of the ambiguity of the Tallarida model. The analysis then gives a random choice between the lower and upper prediction and no consistent and meaningful result will be obtained.
- The Hand model provided relatively high reference effects, as suggested by the theory (see the section on the systematic inequalities), and lied close to the TallaridaUB model.
- The Bliss model neither systematically provided lower nor higher reference effects than the Loewe, the TallaridaUB and the Hand model (Fig. 7).

Discussion

The Loewe model has persisted for long as the first choice to model additive behaviour and its validity is in general accepted in the case of constant potency ratio. Scepticism about the applicability in the case of varying potency ratio case has put forth a large variety of null models. For a full and partial agent the Loewe model is in fact incapable of providing a reference effect in a certain dose range, because the reference relies on a shared effect level of

both agents. If both drugs reach the same maximal effect, a Loewe assignment is possible, even if the shapes of the saturation curves are different. This case required further arguments why to doubt the Loewe model's validity. In this manuscript, we showed that the scepticism can be perpetuated only to some extent, see below.

It has been observed that the principle of dose equivalence and the sham combination principle very well characterize the Loewe model in the constant potency ratio case^{19,23,24}. They seem promising if one attempts to modify the Loewe reference. The coupling of both principles has served as sufficient condition²³, necessary condition¹⁴ and proof of non-validity^{19,24} to the Loewe model all alike. Accordingly, Berenbaum's derivation²³ of the Loewe equation ignored the shapes of dose-effect curves while assuming both principles and Fouquier¹⁴ presents them as a necessity, on which the Loewe additivity rests. We argued that the coupling is not meaningful unless the two agents have a constant potency ratio because only then the two principles are compatible at all. The approach suggested by Tallarida converts doses of different drugs by a rule of three, choosing as intermediate linking quantity an absolute effect level. Two doses which attain this effect level are considered equivalent. This notion of equivalence fails as soon as it is used for adding doses. Since one dose is applied on top of the other, it must thus be interpreted as change in dose of the former. This asymmetry in the roles as initial dose and change in dose leads to the observed non-commutativity. If one tries to resolve the ambiguity by choosing as intermediate linking quantity a change in effect rather than an effect level, one merely shifts the ambiguity. A change in dose cannot be assigned a change in effect uniquely, unless the dose-effect follow a linear relation which is rarely the case.

The above ambiguities have been addressed multiple times in the literature, but Hand's substantial suggestion of a model that resolves them has been mostly ignored up to now. The Hand model interprets the change in effect in an instantaneous sense and allows the instantaneous change to depend on the current effect level, when constructing the combined curve. This dependency of the instantaneous change on the current quantity level is the common motive of mechanistic approaches and is appreciated in biochemical modelling because its specification is of a local instead of a global nature. The local nature of the Hand model's additivity concept makes it biochemically plausible even though its black box approach does not incorporate knowledge on explicit molecular reactions. We showed how these instantaneous changes are best visualized when plotted subject to the effect. The resulting effect-sensitivity curves are added in the Hand model. Here, it is crucial to choose the effect as x-axis. The mechanism of the Hand model can be applied in any case in which two increasing curves must be added. Instead of summing them as functions, their dynamics are added. This not only makes the black box ansatz biochemically plausible but contributes to its versatile usage. If two dose-effect-curves exhibit a piecewise positive derivative and share a zero level, this model is applicable. For instance, this holds in the case, in which ideal data points are linearly interpolated. The extension of the Hand model to multi-drug combinations with more than two components is straightforward, as is the case with the Loewe model.

What model should be preferred? Among the five analysed models, the Loewe, Hand and Tallarida model all satisfy the sham combination principle, which is for instance not the case in Fisher's dosage orthogonality or the Bliss model. The Tallarida model can be used if an asymmetry is justified, e.g., by a temporal delay. The Tallarida model can also be used to identify not only a curve but an area of zero-interaction. We have demonstrated by the common framework for all three models, that there is actually no reason to dismiss Loewe in the varying potency ratio case. The condition, which is provided in form of a partial differential equation and which is biochemically plausible is just as well satisfied to a limited extent by the Loewe model as it is by the Hand model. We suggest to rehabilitate the Loewe model for the case of shared maximal effect, but differing Hill coefficient. However, note that the Hand model can predict an effect for the combination of a full and a partial agent without restrictions, whereas the Loewe model fails to do so for a range of dose pairs. In the model comparison on an experimental dataset, we circumvented this handicap by extending the Loewe model via the Highest Single Agent model. Moreover, by proving the convexity of the Hand model's isoboles we demonstrated that the Hand model guarantees a more conservative prediction of zero-interaction compared to the Loewe model.

In summary, the interest in combination drug therapy is steadily increasing and an assessment requires suitable null models. Here, we have compared several established models and have shown that the Hand model is of most versatile use, for its unique assignment, mathematical plausibility, simplicity, conservative prediction and biochemical interpretability.

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Author Contributions

M.S., C.L. and J.H. conceived the project. M.S. carried out the mathematical analysis of the null models. J.V. implemented the null models and analysed the differences for the considered dataset. All authors contributed to the preparation of the article. All authors read and approved the final manuscript.

Additional Information

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