Trends in Cancer

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Review

Drug independence and the curability of cancer by combination chemotherapy

Amy E. Pomeroy,[1] Emmett V. Schmidt,[2] Peter K. Sorger,[3] and Adam C. Palmer[1,*]

Combination chemotherapy can cure certain leukemias and lymphomas, but most solid cancers are only curable at early stages. We review quantitative principles that explain the benefits of combining independently active cancer therapies in both settings. Understanding the mechanistic principles underlying curative treatments, including those developed many decades ago, is valuable for improving future combination therapies. We discuss contemporary evidence for long-established but currently neglected ideas of how combination therapy overcomes tumor heterogeneity. We show that a unified model of interpatient and intratumor heterogeneity describes historical progress in the treatment of pediatric acute lymphocytic leukemia (ALL), in which increasingly intensive combination regimens ultimately achieved high cure rates. We also describe three distinct aspects of drug independence that apply at different biological scales. The ability of these principles to quantitatively explain curative regimens suggests that supra-additive (synergistic) drug interactions are not required for successful combination therapy.

Highlights

Describes and distinguishes between three noncompeting aspects of drug independence:

- Bliss' independence model describes how combination therapies decrease the fraction of cells that survive killing by one or more agents.
- The Law independence model describes how combination therapies can overcome subpopulations of drug-resistant cells in a cancer.
- The Frei independence model describes how combination therapies can increase the probability of tumor response in a population of patients without any drug additivity or synergy.

A unified model of these drug independence principles quantitatively describes historical progress in curing pediatric acute lymphocytic leukemia.

Combination cancer therapy

Several types of cancer are routinely cured by combination chemotherapy, and many incurable cancers can be controlled longer by combination therapy than by monotherapy. Why is combination therapy superior in these respects? In this review we consider this question from the perspective of three historical concepts used to explain the clinical benefits of combination therapy, and by reviewing the quantitative basis for the curability of childhood ALL. Our purpose is to examine these foundational principles of oncology in the light of contemporary evidence, and to better understand how more effective drug combinations might now be developed, including for more difficult-to-treat solid cancers that are only infrequently cured at present.

The creators of the first curative combination regimens postulated that the probability of resistance to multiple mechanistically distinct drugs is lower than the probability of resistance to a single drug, and therefore combining multiple, individually effective chemotherapeutic mechanisms could overcome tumor heterogeneity, producing longer-lasting remissions – and perhaps even cures – in more patients [1–4]. Modern research has also demonstrated the relevance of this idea to combinations including targeted therapies, while also emphasizing the challenge presented by mechanisms of multidrug resistance [4–6]. Pragmatic guidelines have defined much of the historical development of combination therapies: (i) each single agent should have antitumor activity, (ii) agents should have distinct mechanisms of action and thus distinct mechanisms of drug resistance, and (iii) the combination should be tolerable with few compromises in dosage. These guidelines led to combination regimens able to cure some hematological cancers and a few solid cancers, as well as to adjuvant and neoadjuvant combination therapies that improve cure rates in surgically resectable cancers. Despite the development of these successful combination regimens, many of which have been used to treat patients for decades, the

[1]Department of Pharmacology, Computational Medicine Program, UNC Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
[2]Oncology Early Development, Merck & Co., Inc., Kenilworth, NJ 07033, USA
[3]Harvard Ludwig Center and the Harvard Laboratory of Systems Pharmacology, Department of Systems Biology, Harvard Medical School, Boston, MA, USA

*Correspondence: palmer@unc.edu (A.C. Palmer).

mechanistic basis of treatment with curative intent remains incompletely understood. Relatedly, it is an open question why some of these regimens cure only a fraction of patients, and why many cancers still cannot be cured by combinations built according to these guidelines.

A major complication in the treatment of cancer is that tumor heterogeneity and drug resistance are phenomena that manifest in multiple ways. At the most basic level, we can distinguish between intratumor heterogeneity (differences between cancer cells in one patient) and intertumor or interpatient heterogeneity (differences between the essential characteristics of cancers in different patients). Drug resistance can be present prior to the onset of therapy – whether in most cancer cells or in a rare subpopulation – or it can be acquired over time. Consequently, there are multiple ways in which tumor heterogeneity can limit therapeutic efficacy, and multiple ways in which combination therapy might overcome the challenges posed by tumor heterogeneity.

The first of three quantitative principles which describes the probability of death when a cell (or organism) is treated with multiple toxins was developed by Chester Bliss in 1939 [7]. The Bliss independence model is applied to combination therapy research in many diseases, and substantially overlaps with Loewe's model of dose additivity [8,9]. The two models are exactly concordant when dose response functions are exponential, which is common for chemotherapies [10], since $e^{-A} \times e^{-B}$ (Bliss model) = $e^{-(A+B)}$ (Loewe model). The second principle describes a cancer cell's probability of acquiring heritable multidrug resistance and was described by Lloyd Law in 1952 [11]. The third principle describes a patient's probability of response when treated with multiple therapies, and was described by Emil Frei III *et al.* in 1961 [12], and then updated for progression-free survival data by Palmer and Sorger in 2017 [13,14]. Although these principles have similar mathematical structures, they describe biologically distinct phenomena, and their analysis requires different types of data. However, the three principles are mutually compatible and can all apply to a particular combination therapy, although their relative importance will depend on context.

The curability of childhood leukemia by multidrug chemotherapy

The development of combination regimens to cure the majority of children with ALL is among the greatest successes of cancer research. This success required solutions to both therapeutic and toxicological challenges [2]. In this review we focus on understanding the cancer-killing effect of combination therapy. With ALL, it was observed that increasingly intensive combination regimens improved clinical outcomes from low rates of remission and no cures to a high rate of cure. Remarkably, calculations to estimate the clinical efficacy of combination therapy were used in the 1960s by Emil Freireich and Emil Frei, as part of their development of the four-drug regimen VAMP – vincristine, doxorubicin hydrochloride (Adriamycin), methotrexate and prednisone – which they correctly predicted would provide the first cures of ALL. Based on clinical and experimental data, they described two calculations, one concerning interpatient heterogeneity and chance of remission [12], and one concerning intratumor heterogeneity and duration of remission [3].

Their first calculation suggested that drug combinations would provide patients with multiple chances of complete response, since single drugs induced responses in a limited fraction of patients (where 'complete response' means no detectable amount of leukemia). When more than one drug was used, the fraction of patients whose leukemia responds to treatment would therefore increase even if no single patient benefited from more than one drug [2,12]. This was sufficient to explain the higher remission rates observed with two-drug combinations [15]. However, increasing the chance of a response to one drug was not a sufficient basis for cure.

Their second calculation concerned duration of remission and the prospects of complete eradication of leukemic cells within a single patient [3]. Skipper *et al.* [16] had demonstrated in

mouse models of leukemia that the duration of drug-induced remission was proportional to the logarithmic reduction in the number of leukemia cells ('log-kills'). For example, killing 99.99% of cancer cells (4 log-kills) should produce a remission approximately twice as long as killing 99% (2 log-kills). Freireich and Frei applied this principle to clinically measured durations of remission from various chemotherapies. They sought to estimate the fraction of leukemic cells killed (and the fraction left alive) when a single drug produces a complete response. Next, informed by Lloyd Law's experiments on combination chemotherapy in mouse models of leukemia [11], they hypothesized that the fraction of cancer cells surviving multiple drugs would be the product of the fractions that survive exposure to each of the drugs used individually. For example, if each of two drugs produced 3 log-kills (one in 10^3 cancer cells survive), then the combination of drugs is expected to produce 6 log-kills (one in 10^6 cancer cells survive). The disease burden in human leukemia was estimated to be ~10^{12} cells, based on counting the numbers of cancer cells in different organs. These calculations suggested that at least 12 log-kills would be required for treatment to eradicate cancer cells and achieve a cure [3].

Based on these calculations, Freireich and Frei estimated that whereas two and three drug combinations were incapable of curing ALL, the four-drug VAMP regimen should be able to cure some patients. The VAMP regimen was famously controversial because of the near-lethality of a regimen involving simultaneous treatment of children with four toxic agents, but cures were ultimately observed in a fraction of patients with ALL [17], although many experienced relapse, often in the central nervous system. The landmark achievement of cures by VAMP had radical implications and raised the question of whether even more intensive combination regimens (including treatment of the brain) might deliver a higher cure rate. Motivated by the same logic as Frei and Freireich [18], this idea was pursued by Donald Pinkel with an approach that he named 'total therapy' [19]:

> We said, "Let's put it all together. Let's attack the disease from different directions, all at once." My hypothesis was that there were some leukemia cells that were sensitive to one drug and other cells that were sensitive to another. But if we use all these drugs at once and hit them along different pathways, we would permanently inhibit the development of resistant cells.

Pinkel's approach was so successful that his results were at first considered unbelievable by many oncologists. Ultimately, 'total therapy' became an ever-improving series of regimens which raised the 10-year survival for pediatric ALL patients from ~10% to over 90%, while also reducing toxicity to tolerable levels [20].

The quantitative principles developed by Bliss, Frei, Freireich, Law, and others provides a remarkably accurate description of trial results in pediatric ALL over the years 1948–1988, during which time patient outcomes improved from infrequent remissions to high rates of cure (defined as 10-year disease-free survival). What follows is a revival of Frei and Freireich's calculations [3], using the power of modern computation to describe response distributions in patient populations. This computation involves two parts. First, the fractions of cancer cells that resist multiple independently acting drugs is modeled as the product of the fraction of cells that resist each single drug; this is equivalent to addition of the log-kills achieved by each drug. Second, patients experience different magnitudes of response to individual drugs, due to intrinsic drug resistance; this can be described by drawing samples from single empirically determined drug response distributions. Such distributions can be obtained from early trials by the Acute Leukemia Group B (ALGB) that measured the distribution of remission times achieved by various single chemotherapies (Figure 1A). As demonstrated by Skipper *et al.* [16], relapse occurs from the growth of cancer cells

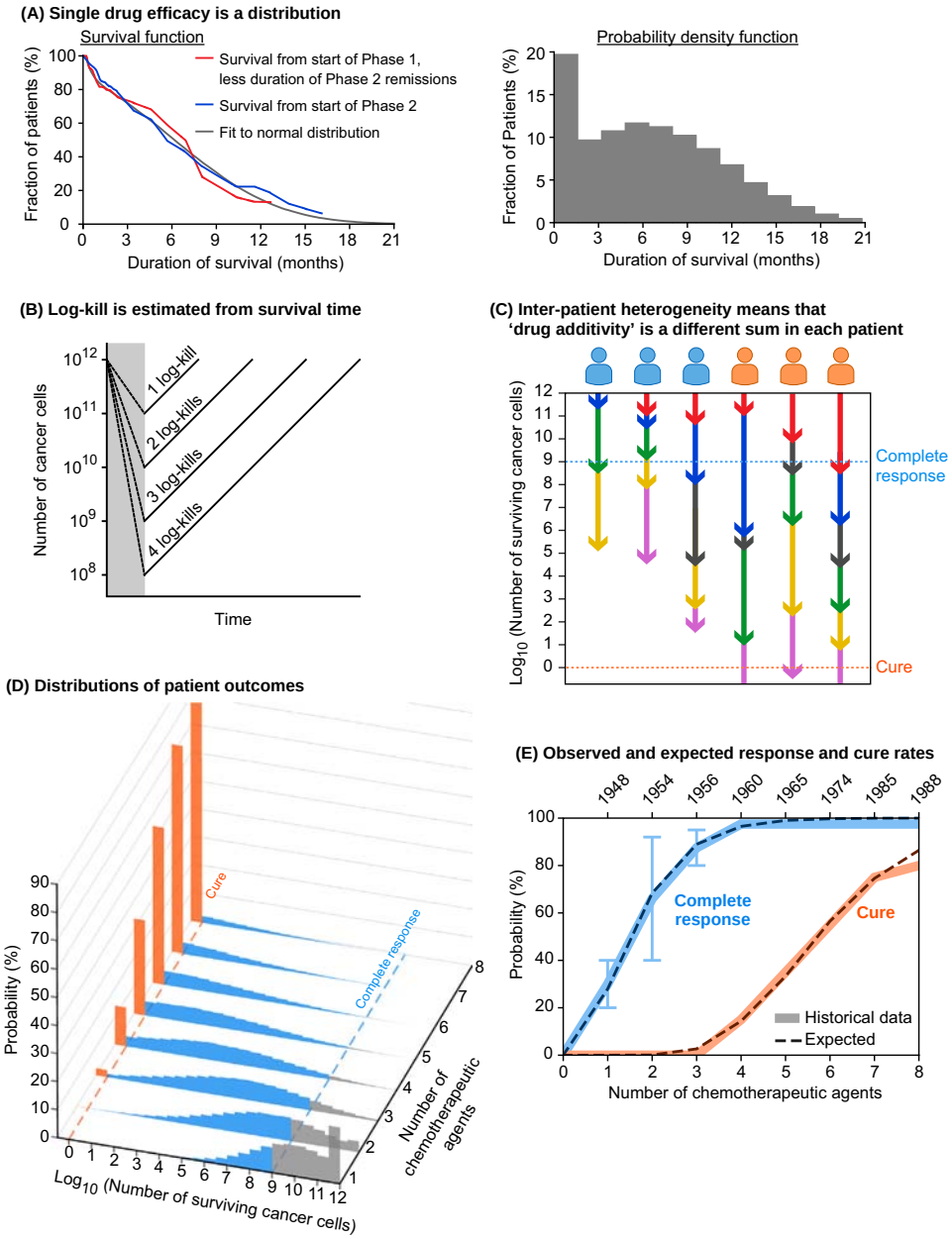

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Figure 1. Conceptual model of how multiagent chemotherapy overcomes intertumor and intratumor heterogeneity to cure childhood acute lymphocytic leukemia (ALL). (A) The Acute Leukemia Group B (ALGB) protocol 2 showed that single-agent chemotherapy for childhood ALL (methotrexate or 6-mercaptopurine) produces a survival distribution that is approximately normal, though truncated at zero (left); this is visualized by the probability density function of survival (right). (B) Response to chemotherapy can be quantified as 'log-kills' (the reduction in the logarithm of the number of cancer cells). Log-kills can be estimated from therapy-induced increase in survival time, because when fewer cancer cells remain they take longer to grow back [16]. (C) Independently acting drugs are expected to produce additive log-kills, but in a heterogeneous human population where patients have different responsiveness to different chemotherapies (as in panel A), 'drug additivity' involves a different sum of effects in each patient. Here patient responses are illustrated for six independently acting chemotherapies: for each drug in each patient, the number of log-kills is randomly sampled from the single-drug distribution (panel A), and the net effect of the combination therapy is additive.

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that survived treatment. Thus, response durations in ALL can be approximately modeled as being proportional to the log-kills achieved by a given course of therapy (Figure 1B) [3]; note this idea is not universally true, as evidenced by the general failure of overall response rates to predict survival times in solid tumors. From this principle we can infer that the monotherapies used in early ALL regimens produced log-kill distributions that are approximately normal, having a median of ≈2 log-kills and a standard deviation of ≈2 log-kills. (Most survival distributions in oncology are close to log-normal [21].) The ALGB's 'Protocol 2' trial tested sequential monotherapy and observed no correlation between a patient's response to their first and second therapies (Figure 1A); in our previously described formulation of independent action this corresponds to a correlation of zero [12]. Thus, a patient treated with *n* independently active chemotherapies can be modeled by drawing *n* responses from the observed distribution of log-kills, and adding them up (Figure 1C). This simple model lacks kinetic detail, instead approaching the problem by counting the proportion of leukemic cells that survive the full course of therapy. As described in the original calculations of Frei and Freireich, cure is presumed to require 12 or more log-kills, and a 'complete response' >3 log-kills (99.9% of leukemia cells killed) [3].

When this model is used to estimate the proportion of pediatric ALL patients experiencing complete response or a cure when treated with different numbers of chemotherapies used in combination (Figure 1D), we find close agreement with clinically observed rates over a 40-year period (1948–1988) (Figure 1E) [1]. This analysis spans early trials of sequential monotherapy and two-drug combinations, the four-drug VAMP regimen that achieved the first cures, and subsequent progress to higher cure rates with Pinkel's total therapy regimens. The match between model and data is striking given that the calculations omit many biologically and therapeutically important details, such as intrathecal chemotherapy and radiation to treat the brain and spinal cord, and the use of different drugs not all at once, but distributed across multiple phases of therapy (remission induction, consolidation, and maintenance). Thus, at a high level, these calculations provide a quantitative illustration of the value of classical concepts about the role of multiagent chemotherapy in addressing interpatient and intratumor heterogeneity. More specifically, the simulation's workings provide two key insights. First, in a patient population exhibiting a typical level of heterogeneity in responses to monotherapy, 'drug additivity' represents a different sum of effects in every patient, such that the most efficacious drug differs by patient (Figure 1C). Second, synergistic drug interaction, or supra-additive activity in general, is not required to overcome tumor heterogeneity. Moreover, with reference to the diagram in Figure 1C, supra-additive activity – if it can be achieved in some cases – is likely to be an enhancement of only one of the multiple arrows contributing to overall activity. Indeed, supra-additive efficacy was never a rationale for the design of these regimens, and their clinical efficacy does not demonstrate it. Instead, the simulation shows that the additive effect of individually potent drugs is a quantitatively sufficient basis for the progressive development of curative therapies.

These ideas have conceptual and practical value in the design of contemporary combination therapies and clinical trials [22]. In particular, interpatient variation in response to drugs within combinations has significance for precision oncology, as reviewed in Plana *et al.* [23]. In solid tumors, responsiveness to single agents is generally less frequent than in liquid tumors, and

Half of the illustrated patients achieve over 12 log-kills from combination therapy, which cures a cancer with initial population 10^{12} (patients represented in orange), and half have a complete response but are not cured (blue). (D) Combinations of one to eight chemotherapies were simulated by the principles in panel C. Distributions of patient outcomes are plotted for each number of chemotherapies, where orange represents cured patients and blue represents patients with a complete remission. (E) The historically measured rates of complete remission (blue band) and/or cure (orange band) achieved by increasing numbers of chemotherapies are compared to the rates expected according to independent drug action (dashed blue and orange lines).

drug additivity within a patient is less impactful. Instead, combination therapy in these cases appears to rely on what we refer to here as Frei independence. Thus, patients with solid tumors are likely to receive one or more drugs that are ineffective against their tumors. Were reliable pretreatment biomarkers of sensitivity or on-treatment pharmacodynamic assays available for more drugs, then inactive agents could be omitted for specific patients to reduce toxicity without compromising therapeutic efficacy. A tangible demonstration of this principle was recently provided for combination chemoimmunotherapy for gastric cancer [24].

Laws of probability and combination chemotherapy

The case study of pediatric ALL demonstrates that three different manifestations of independent drug action are relevant to thinking about combination chemotherapy; these originate with Bliss, Law, and Frei. They are not competing theories (in contrast to Loewe additivity versus Bliss independence [25]), but rather they describe distinct phenomena occurring at different biological scales that can be applied simultaneously to understanding the impact of tumor heterogeneity on drug response. These principles share a common mathematical basis in the addition law of probability (Figure 2A), which is that the probability that either of two events (A and B) will occur is:

$$P_{\text{A or B}} = P_{\text{A}} + P_{\text{B}} - P_{\text{A and B}} \quad [1]$$

This simply states that the probability that either event A or event B occurs (which includes A and B both happening) is the sum of their individual probabilities, minus the probability that both events occur (otherwise this would be counted twice). If events A and B are uncorrelated, $P_{\text{A and B}} = P_{\text{A}} \times P_{\text{B}}$ and the equation can be rearranged to read:

$$P_{A\ or\ B} = P_A + P_B\ (1 - P_A) \quad [2]$$

However, if events A and B are correlated, $P_{\text{A and B}}$ is greater than $P_{\text{A}} \times P_{\text{B}}$ and the overall benefit is therefore less. In the case of chemotherapy, correlations in response arise from partial or complete cross-resistance between drugs, and it is therefore logical that the benefit of combining drugs will be less.

Bliss independence: the probability of death from toxin

The Bliss independence model applies to the probability of cell death and describes how combinations of therapies kill a larger fraction of cancer cells. This applies even before explicit consideration of heritable intratumor heterogeneity, which is the purview of Law's independence model.

Chester Bliss's research on combinations of chemical agents predated cancer chemotherapy and concerned insecticides. Bliss used the addition law to analyze the proportion of animals killed by multiple toxins, and his versatile theory has been widely applied to analyzing the proportion of cancer cells killed by cytotoxic drugs or bacteria killed by antibiotics (Figure 2B). Bliss's 1939 article [7] actually described three different models of 'joint action' of which the widely used concept of 'Bliss independence' is only one. The 'independent joint action' model postulated that, when two toxins cause death in distinct ways, and have no correlation in susceptibility (thus $P(\text{A and B}) = P_{\text{A}} \times P_{\text{B}}$), the proportion of individuals killed by the combination of toxins is:

$$P_{Combination} = P_A + P_B\ (1 - P_A) \quad [3]$$

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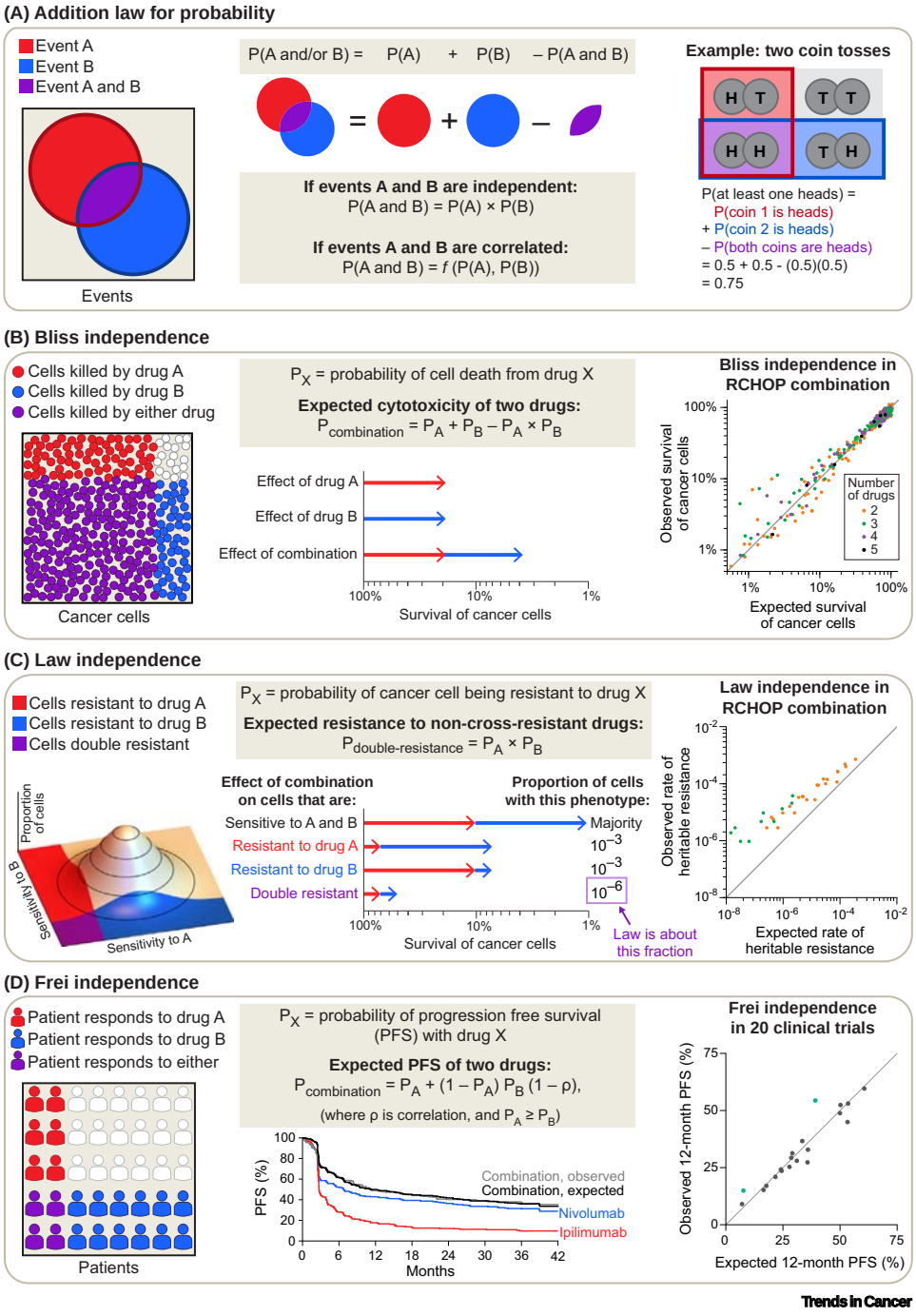

Figure 2. The addition law for probability and its application to three distinct meanings of drug independence. (A) The addition law for probability states that the probability of one or both of two events A and B occurring is the sum of the probability of event A and event B minus the probability of both events occurring. (B) Bliss independence applies the addition law to the toxicity of drug combinations. In the case of cancer chemotherapy, this is equivalent to adding log-kills. The Bliss independence model was experimentally observed to describe the cytotoxicity of multiple drugs (two, three, four, or five) from the RCHOP combination – rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine (Oncovin), and prednisone – in diffuse large B-cell lymphoma (DLBCL) cultures (replotted from [28]). (C) Law independence describes the expected

(Figure legend continued at the bottom of the next page.)

where P_A and P_B are the proportion of individuals killed by single therapies (this is the simplest case of the addition law). Bliss also considered the case of correlated susceptibility, such that some individuals are more resistant to both agents, and some more sensitive to both. With a correlation in drug sensitivity equal to ρ, the expected combination effect is:

$$P_{Combination} = P_A + (1 - P_A) \times P_B \times (1 - \rho) \quad [4]$$

where P_A is the larger of the two probabilities (A is more cytotoxic).

When applied to killing cancer cells, Bliss independence is one generally accepted definition of 'drug additivity' [25,26] such that 90% killing by drug A plus 90% killing by drug B = 99% killing by the combination. When cytotoxicity is quantified as log-kills, or reduction in the logarithm of tumor cell number, Bliss independence corresponds to the addition of effects (e.g., 1 + 1 log-kills = 2 log-kills). If cytotoxicity is greater than expected by Bliss independence, this satisfies a quantitative definition of synergistic drug interaction, and is evidence that one or more drugs has become more effective in combination [25].

Quantitative evidence of synergy – an effect that is 'more than the sum of its parts' – implies some mechanism of positive drug–drug interaction. Mechanisms of synergistic drug interaction are so diverse as to defy categorization, but the general outcome is that one drug enhances the effect of another [25]. However, synergy, in the rigorous meaning of positive drug–drug interaction, is not synonymous with efficacy, nor alone sufficient to make a clinically effective regimen. A consequence of Bliss independence is that combining 'weak' drugs (small log-kills) is expected to provide small benefit, and combining 'strong' drugs is expected to provide large benefit. For example, if two uncorrelated therapies individually produce 50% kill, their combination is expected to produce 75% kill (the surviving fraction drops from 50% to 25%, so tumor reduction is twofold greater than monotherapy). If the combination exceeded an additive effect and achieved 90% kill it would be classified as synergistic (the surviving fraction drops from 50% to 10%, so tumor reduction is fivefold greater than monotherapy). However, consider a different pair of more active therapies that individually produce 99% kill; an additive combination effect would result in 99.99% kill (from 1% to 0.01% survival is a 100-fold greater tumor reduction than monotherapy). This illustrates that a combination of strong drugs that is 'merely' additive can be far more efficacious than a synergistic combination of weaker drugs. This matches clinical experience that the most effective drug combinations in oncology are those that combine individually active rather than inactive single agents, usually with distinct mechanisms so as to minimize cross-resistance [27].

Preclinical evidence that Bliss independence rather than synergistic interaction can explain curative therapeutic regimens was recently obtained in a study of the five-drug RCHOP –rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine (Oncovin), and prednisone – regimen for diffuse large B-cell lymphoma (DLBCL) [28]. Single and combined dose–response measurements

fraction of cells in a tumor that are resistant to two or more drugs. Whereas Bliss independence describes the net toxicity of multiple drugs (how the 'arrows' add up), Law independence concerns how many cells belong to the subpopulation with multidrug resistance (the group in which both 'arrows' are smaller). Law independence was tested by comparing the observed and expected number of DLBCL clones with resistance to multiple drugs in RCHOP (replotted from [28]); a higher observed rate of resistance indicated a modest degree of cross-resistance. (D) Frei independence applies the addition law to the probability that a patient with cancer will respond to a drug combination; it can also be applied to progression-free survival (PFS) versus time. The Frei independence model was tested for 20 clinical trials of combinations of cancer therapies; two had greater PFS than expected at 12 months (green points) (data replotted from [13,31]).

in cultured DLBCL cells showed that many pairs of the drugs comprising RCHOP exhibited additivity, some were non-additive (including antagonistic), but the full combination was as effective as predicted by Bliss independence (Figure 2B). This regimen combines mechanistically independent therapies that are each highly active in the treatment of lymphoma and are tolerable in combination. Thus, the ability of RCHOP to provide a fractional cell kill far surpassing monotherapy is both an expected and observed consequence of Bliss independence.

Law independence: the probability that a cell has drug resistance

The Law independence model applies to heritable intratumor heterogeneity, and describes how combination therapy lowers the probability that a tumor cell will possess resistance mechanisms applicable to every drug in a combination.

Lloyd Law studied combination chemotherapy using mouse models of leukemia, and was conceptually inspired by the ability of multidrug combinations to cure tuberculosis. Law used the following example to explain how combination therapy decreases the probability of resistance: if the frequency of resistance to drug A is 10^{-6}, and the frequency of resistance to drug B is 10^{-5}, then doubly resistant mutants will occur at a frequency of 10^{-11} if the drugs are not cross-resistant [29]. More generally, if P_X is the probability of resistance to treatment X, then in the absence of cross-resistance:

$$P_{Combination} = P_A \times P_B \quad [5]$$

This has the same mathematical structure as Bliss independence (counting resistance instead of death), but it describes a different biological phenomenon: whereas Bliss is concerned with how many cells die at a given combination of doses, Law is concerned with the proportion of cells having heritable multidrug resistance.

A simple example illustrates how Bliss and Law independence both apply to drug response in a tumor where rare subpopulations have resistance to single or multiple drugs. Suppose most cells are drug-sensitive and are killed at a rate of 90% by either drug A or drug B. The 10% of surviving cells do not necessarily all have heritable resistance: even genetically identical cells have stochastic fluctuations in state that cause a fraction of cells to survive therapy [30]. By Bliss independence, combination therapy A + B is expected to kill 99% of the drug-sensitive cells ($0.9 + 0.9 - 0.9 \times 0.9$). Next, suppose that subpopulations having heritable resistance to drug A or drug B (for any reason, genetic or epigenetic) are present at a frequency of 10^{-3}, and in these cells the resisted drug elicits only 30% killing. In this population of single-drug-resistant cells, Bliss independence expects 93% killing from combination therapy ($0.9 + 0.3 - 0.9 \times 0.3$). Law's statement is not about magnitude of killing, but instead concerns the abundance of double resistance; in this example it is expected at a frequency of 10^{-6} ($10^{-3} \times 10^{-3}$). Bliss independence concerns the probability of killing without explicitly considering non-uniformity in the population.

Here then, Law independence states that one in 10^6 cells are heritably multidrug-resistant, and Bliss independence states that if the response of these cells to either monotherapy is 30% cell death, then their response to combination therapy is 51% cell death ($0.3 + 0.3 - 0.3 \times 0.3$). Both concepts must be applied to understand the proportion of cells with various drug–response phenotypes that survive combination therapy. For clarity, we propose that this principle of multiplicative resistance frequency be named the Law independence model. We anticipate that, in scenarios involving modest levels of cytotoxicity, stochastic cell survival (Bliss independence) is the more relevant principle, but with increasing cytotoxicity and smaller fractions of surviving cells, heritable drug resistance (Law independence) becomes increasingly relevant.

Cross-resistance arises when resistance to one drug (acquired or primary) also results in resistance to another drug; this is a deviation from Law independence. The equation for correlated probabilities (originally from Bliss) can be adapted to this situation, where the fraction of cells resistant to both drugs A and B is calculated by taking the fraction of cells resistant to drug A (which is P_A), and subtracting the fraction that is resistant to A (P_A) but sensitive to B (which is $1 - P_B$). Thus, if P_X is the probability of resistance to X, and ρ is correlation in resistance:

$$P_{Combination} = P_A - P_A \times (1 - P_B) \times (1 - \rho) \quad [6]$$

where P_A is the smaller of the two probabilities (A-resistance is rarer). This shows that a small amount of cross-resistance can be a major therapeutic limitation. For example, with single-drug resistance frequencies of 10^{-3}, a degree of cross-resistance of ρ = 0.1 results in a double-resistance frequency of 10^{-4}, which is 100-fold larger than the value of 10^{-6} expected for no cross-resistance. Thus, cross-resistance is highly problematic in a combination therapy. Testing for Law independence can in principle be experimentally tested in preclinical experiments by isolating single-drug-resistant clones and measuring what fractions also have resistance to different therapies. Such experiments were historically challenging but are today straightforward using multiplexed clone-tracing and CRISPR-Cas9 libraries, as was applied to study the RCHOP regimen for DLBCL [28]. For RCHOP, the frequency of multidrug-resistant clones declined exponentially with increasing number of drugs, deviating slightly from the theoretical minimum and demonstrating mild cross-resistance (Figure 2C).

Frei independence

The Frei independence model applies to interpatient heterogeneity, and describes how combination therapy can increase the fraction of patients whose cancers respond to treatment.

Emil Frei III led the ALGB during the first studies of ALL. In the group's first comparison of sequential versus combination therapy ('Protocol 2'), they observed that responses to methotrexate and 6-mercaptopurine were uncorrelated, and that the complete remission rate achieved by combination therapy was equal to the calculated expectation of independently acting drugs based on the remission rates achieved by either monotherapy alone [12]. Specifically, if P_X is the fraction of patients with complete remission when treated with X:

$$P_{\text{Combination}} = P_{\text{A}} + (1 - P_{\text{A}}) \times P_{\text{B}} \quad [7]$$

Just like Bliss independence, this is the addition law of probability, but applied to remission rates of patients rather than to death rate of cells. We propose that this be called the Frei independence model. Under Frei independence, the expected response rate is computed as though each patient received only the single drug that was most effective for them (this corresponds to a 'highest single agent' response). Frei independence can produce statistically significant and clinically meaningful benefit to populations because different patients respond best to different single agents (Figure 2D, red or blue patients), allowing for substantially higher response rates at a population level.

Recently we showed that the Frei model can also be applied to progression-free survival (PFS) data from clinical trials, and that it can account for cross-resistance, or correlations in the probability of response [13]. If P_X is the probability that a patient treated with X remains 'progression-free' at time t, then the PFS expected of combination therapy can be estimated by:

$$P_{Combination} = P_A + (1 - P_A) \times P_B \times (1 - \rho) \quad [8]$$

where P_A is the larger probability at time t, and ρ is correlation (cross-resistance) between responses to therapies A and B. A more precise solution that can also account for confidence intervals in trial data was derived by Chen *et al.* [22], and is:

$$P_{Combination} = P_A + P_B - P_A \times P_B - \rho\,[P_A \times (1 - P_A) \times P_B \times (1 - P_B)]^{1/2} \quad [9]$$

These equations should be calculated at a series of times t to construct a Kaplan–Meier plot of PFS versus time. Because treatment failure and progression take time to observe (first radiological scans are commonly 6–8 weeks after commencing therapy), this model is valid for advanced cancers after the first month or two, when there has been an opportunity to observe treatment effects.

The Frei independence model can analyze clinical trial results to assess whether the efficacy of a combination therapy is less than, equal to, or greater than expected from independent drug action. An example of the Frei model is shown in Figure 2D for the Checkmate 067 trial of nivolumab and/or ipilimumab for metastatic melanoma. In this trial, 37% of patients treated with nivolumab remained progression-free at 24 months, and 12% of patients treated with ipilimumab remain progression-free at 24 months, so the PFS expected of nivolumab plus ipilimumab at 24 months is 42% when applying a partial correlation (ρ = 0.3; expected PFS is 45% if ρ = 0). Expected PFS agrees with the clinically observed PFS of 41.5% at 24 months and shows that the independent activity of ipilimumab is sufficient to explain the ~5% improvement in PFS achieved by adding it to nivolumab; the agreement shown for 24 months after treatment initiation is also observed at all times over multiple years of follow-up.

The Frei independence model has so far been applied to 20 clinical trials of combination therapies where PFS results were available for both monotherapies and the combination therapy [13,31]. Sixteen of 20 combinations follow Frei independence, and three combinations are superior, which suggests at least additive effects of multiple drugs (Figure 2D). Notably, approved combinations involving immunotherapies were all found to exhibit Frei independence. Benefit exceeding Frei independence could arise from synergistic interaction, but could also occur from Bliss or Law independence because they are means by which multiple drugs can kill more cancer cells in a patient. A limitation of this model is that it applies to combination therapies involving the same drug doses as in monotherapy trials. Application to drug combinations involving lowered doses would require knowledge of monotherapy efficacy at those doses. Dose–response relationships vary among drugs and are not generally predictable, but empirical data on dose relationships could inform future models that account for dosage. For the practical purpose of trial design, one should anticipate that lower dosage will lower efficacy compared with the predicted effect of a full dose combination (demonstrated by BRAF+MEK+PD-1 inhibition in *BRAF*-mutant melanoma [14,32]); this emphasizes the importance of tolerability.

The finding that many combination therapies for advanced cancers are consistent with Frei independence has the surprising implication that, in many circumstances, few patients benefit from all drugs in the combination (in either an additive or a synergistic manner). This can be understood as a consequence of monotherapies having low response rates in these difficult-to-treat diseases. When strong response to monotherapy is relatively rare, it logically follows that few patients will have overlapping responses to multiple therapies (Figure 2D, purple patients), and thus additivity or synergy will rarely occur. Conversely, an additive effect is expected to be common in chemosensitive blood cancers treated with many highly active drugs [23]. In such scenarios, the principles of Frei, Bliss, and Law independence may all be acting together to produce successful combination therapies. Dramatic clinical benefits can therefore arise without drug–drug interaction. Unfortunately, such success is commonly referred to as 'synergistic' in the

loose sense that it is an improvement on monotherapy; such an inexact statement should not be understood as having a mechanistic basis.

Applications of drug independence to multiple disease scenarios

The principles described in this review can all aid in understanding the efficacy of combination therapies in a variety of contexts. Pediatric ALL is a disease now treated with many highly active therapies with little cross-resistance, which is anticipated to produce additivity and a high level of tumor cell killing, leading to high cure rates (Figure 3A). Notably, before the advent of rituximab,

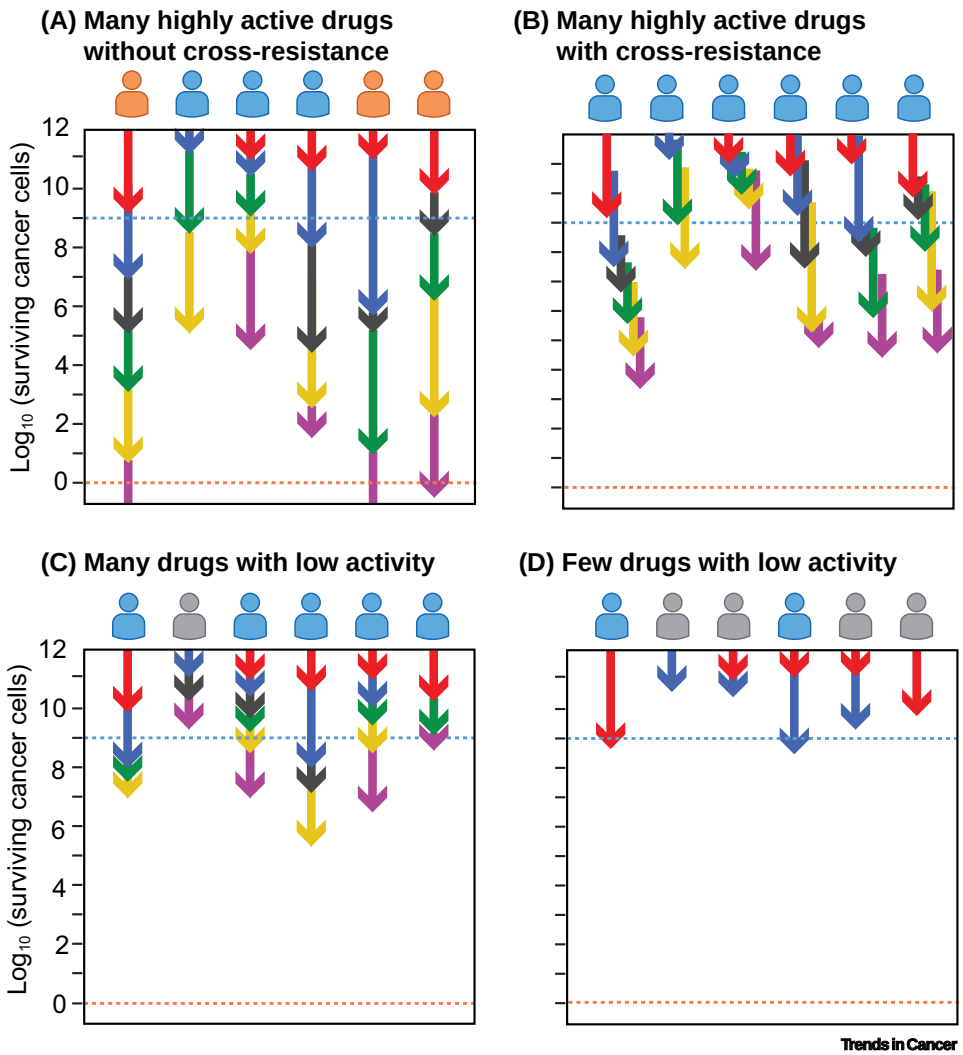

Figure 3. Implications of drug additivity in different scenarios in oncology. (A) In chemosensitive cancers such as pediatric acute lymphocytic leukemia (ALL), the use of many highly active and non-cross-resistant therapies cures some patients. (B) When available drugs have 'cross-resistance', cancer cells that survive one therapy have a greater likelihood of also surviving other therapies; this is schematized as 'less-than-additive' log-kills. Substantial cross-resistance can limit depth of response and could be an obstacle to cure. (C) In a cancer with limited sensitivity to available therapies, combinations of many agents may increase the response rate and median survival time, but depth of response is unlikely to produce cure unless used as adjuvant/neoadjuvant therapy for microscopic disease. (D) In a cancer where few active therapies are available, none of which have a high response rate, the advantage of combination therapy may be to increase the chance that at least one agent is active for a patient.

trials in DLBCL compared the four-drug 'CHOP' regimen with more intensive six- to eight-drug regimens – m-BACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone), MACOP-B (methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin), and ProMACE-CytaBOM (prednisone, methotrexate, doxorubicin, cyclophosphamide and etoposide alternating with cytarabine, bleomycin, vincristine and methotrexate) – but observed no improvement in response or survival [33]. It is likely that lymphoma cells which resisted the drugs in CHOP also resisted the additional chemotherapies, although this has not been studied. As discussed earlier, such cross-resistance is a deviation from Law independence, and can negate the ability of additional therapies to improve tumor cell kill and cure rates (Figure 3B).

Disease type is of course all important in the treatment of cancer, and therapies that are highly effective in one cancer type may be much less active in another. In a malignancy in which available monotherapies each have weak antitumor effects, combinations of many therapies may yield modest benefits because the combination treatment can only achieve the addition of small effects (Figure 3C). Such a scenario is demonstrated by the four-drug FOLFIRINOX regimen (folinic acid, fluorouracil, irinotecan, oxaliplatin) in advanced pancreatic cancer, which extends survival by 3 months relative to gemcitabine monotherapy [34]. Finally, in cancers in which few highly active therapies are available, opportunities to achieve additivity in many patients are limited, and the potential advantages of combination therapy are likely to involve Frei independence (Figure 3D). This currently applies to many solid tumors.

The implication of these principles for hard-to-treat cancers is that developing clinically superior drug combinations is likely to depend on developing (or identifying) therapies that are individually active for the disease and are not cross-resistant. Several decades of precedents suggest that new ways of combining existing weak therapies are unlikely to provide major clinical benefits. Existing examples of synergy – such as BRAF plus epidermal growth factor receptor (EGFR) inhibition in *BRAF*-mutant colorectal cancer – have extended median survival by 3 months [35]. The goal of developing new highly active therapies for solid cancer is not unrealistic: for example, the HER2 antibody trastuzumab improved the efficacy of combination therapies for HER2+ breast cancer [36], and further improvements have been achieved with HER2 antibody–drug conjugates [37]. Generally, ongoing development of new therapies with clinical activity in relapsed/refractory cancers provides opportunities to build combinations of mechanistically distinct agents that each have meaningful single-agent activity. A reanalysis of contemporary response data in terms of the classical theories developed by Bliss, Law, and Frei suggests that greater clinical benefit might be found in a search for new drugs with high levels of clinical activity than in searches for an elusive 'synergy' that is rarely observed clinically.

Concluding remarks

In this article we reviewed three historical principles that describe how combinations of independently active therapies can address the challenge of tumor heterogeneity and kill more cancer cells in more patients. None of these principles requires synergistic drug interaction (meaning supra-additive activity) to improve treatment outcomes, although their substantial clinical benefits are often colloquially called synergistic (meaning good for patients). Thus, the common sentiment that 'to overcome drug resistance we need synergistic drug combinations' is false in the quantitative sense. The multiple meanings of 'synergy' are a long-recognized source of confusion about mechanisms of combination therapy [38], and have caused tumor heterogeneity and drug cross-resistance to be overlooked as key factors in the efficacy of combination therapy.

The independence principles described by Bliss, Law, and Frei are not competing theories but different phenomena that can all apply in one setting, although their contributions vary by clinical

Outstanding questions

Treatment for pediatric ALL allocates many therapies across induction, consolidation, and maintenance phases to make the regimen more tolerable. Which other cancer types and combination therapies could benefit from this strategy?

When a drug in a combination benefits a fraction of patients, how can we identify those individuals, to avoid giving an unhelpful therapy to the other patients?

After therapy with curative intent, could sensitive diagnostics such as liquid biopsies identify patients who need more intensive therapy to achieve cure?

What resistance mechanisms are most relevant to interpatient heterogeneity (some tumors having primary drug resistance) versus intratumor heterogeneity (some cells emerging with acquired resistance)? Are different combination strategies best for overcoming primary versus acquired drug resistance?

What are the clinical patterns of cross-resistance across various chemotherapies, targeted therapies, and immunotherapies? What therapies might overcome multidrug resistance in different disease contexts?

When rare cancer cells survive initial therapy, do they have any unique vulnerabilities that would not have been evident in the initial tumor?

Which clinically effective combination therapies are truly 'more than additive'?

Can drug synergy screens be reanalyzed to assess overall antitumor efficacy at clinically relevant doses?

Could the principles used to cure ALL also improve cure rates of adjuvant or neoadjuvant therapies for early-stage solid cancers?

In currently incurable cancers, how much activity will new therapies need in order to make combinations that can cure? Are there any cancer types where we have recently reached this point?

context. For example, in combinations of two therapies that have relatively low response rates, Frei independence – increasing the chance of a single drug response via bet hedging – is the dominant principle and alone explains many clinical trial results. Conversely, curative combination therapies generally involve many active therapies where all principles apply. Recent experimental, computational, and statistical innovations have expanded our ability to measure and predict the consequence of independent drug action to inform the design of combination therapies [6,13,14,22,39–42]. There are exciting opportunities to apply these principles to develop clinically superior combination therapies (see Outstanding questions), including via newly discovered vulnerabilities in cancer cells (e.g., metabolism) or previously undruggable targets (e.g., RAS), which may render previously incurable cancers curable. All three drug independence principles described here remain highly relevant to contemporary combination cancer therapy. Exploiting them will require rethinking preclinical and early-phase clinical studies, with less emphasis on looking for supra-additive interactions and greater emphasis on combining individually effective agents with non-overlapping resistance to overcome tumor heterogeneity.

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A.C.P. is a consultant for Merck. E.V.S. is an employee and stockholder of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA. P.K.S. is a member of the SAB or BOD of Applied Biomath, RareCyte Inc., and Glencoe Software, which distributes a commercial version of the OMERO database; P.K.S. is also a member of the NanoString SAB and consultant for Merck and Montai Health. In the last 5 years the Sorger lab has received research funding from Novartis and Merck, and the Palmer lab has received research funding from Prelude Therapeutics.

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