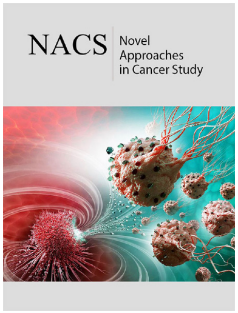
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Three Gates, One Bottleneck: An Occupancy-Based Framework for Translating Phytochemicals into Cancer Therapeutics

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Abstract

Plant secondary metabolites supply a substantial share of approved anticancer drugs, yet the dietary phytochemicals that dominate the experimental literature have produced none. The discrepancy is usually attributed to weak potency or to insufficient investment. We argue that both are symptoms of a single error of interpretation: phytochemicals are weak, multi-node ligands, and a field that evaluates them with the conceptual instruments of single-target pharmacology will misjudge every stage of their development. From this proposition we derive a connected set of instruments rather than a catalogue. First, a serial three-gate framework exposure, engagement, evidence in which each gate constrains what the next can mean, and in which most published work stops before the first while claiming to have passed the second. Second, two quantitative diagnostics: the therapeutic exposure gap (TEG), comparing the free intratumoral concentration achievable at tolerable doses with the concentration required for pathway-level engagement, and the occupancy dose (OD), the time integral of aggregate fractional occupancy, which predicts that schedules delivering equal OD are equivalent even when their dose profiles differ. Third, a threshold-differential account of selectivity: because a weak multi-node ligand cannot discriminate between targets by affinity, its selectivity must arise from differences in network redundancy between tumor and normal tissue, which prescribes node selection by topology rather than by potency. Fourth, a translational taxonomy that assigns each molecule to one of four development routes from two measurable quantities, potency and achievable exposure, converting a literature of compounds into a queue of decisions. Fifth, we apply the completed framework to Sirtuin 1 (SIRT1), the anti-aging deacetylase that curcumin, resveratrol and berberine are most often said to activate, and show that the claim is not so much wrong as unmeasured: reported activation sits two to four orders of magnitude above attainable free exposure, the opposing roles of SIRT1 in normal and tumor tissue follow from threshold-differential selectivity rather than from contradictory data, and concomitant SIRT1-inhibiting medication is a computable antagonism that no current trial records. We state three falsifiable hypotheses, specify the experiments that would refute them, define the minimum evidence without which a clinical claim is uninterpretable, and propose bioactivity-based rather than marker-based standardization of botanical material.

Keywords: Phytochemicals; Sirtuin 1; Network pharmacology; Fractional occupancy; Therapeutic exposure gap; Occupancy dose; Threshold-differential selectivity; Target deconvolution; Anti-body-drug conjugates; Botanical standardization

Introduction: An Imbalance That Is Usually Misdiagnosed

The experimental literature on plant-derived anticancer agents is enormous, and its clinical yield is close to zero. Thousands of papers describe curcumin, resveratrol, epigallocatechin-3-gallate or berberine inhibiting proliferation, migration or survival in cancer cell lines, yet no unmodified dietary phytochemical is approved as an anticancer drug. Because natural products as a class remain highly productive roughly one third of small-molecule drugs approved be-tween 1981 and 2019 are natural products or direct derivatives, and the fraction is higher in oncology [1,2] - the failure cannot be attributed to the chemical class as such [3].

Two explanations are usually offered and neither survives inspection. The first is insufficient potency: phytochemicals are said to be too weak to compete with synthetic cytotoxins. But potency is only meaningful relative to the exposure a patient can tolerate and the field has rarely measured that exposure, so the comparison on which the judgement rests has generally not been made. The second is insufficient investment: phytochemicals are said to be unpatentable and therefore under-developed. But investment follows plausibility, and the plausibility of a compound class that has been studied for four decades should by now rest on something other than further cell-culture papers.

We propose a third explanation with more explanatory power: the field evaluates weak, multi-node ligands using the conceptual instruments of single-target pharmacology, and therefore misjudges every stage of development. Three consequences are observable. Mechanisms are described at concentrations that no patient can reach, so the resulting literature is internally consistent and externally inapplicable [4-6]. The same compounds are rediscovered indefinitely, because no result is ever decisive enough to close a question. And the field has lost predictive capacity: nothing in the literature allows a reader to say in advance which molecule deserves a clinical program.

One node recurs throughout this literature and deserves separate treatment. Sirtuin 1 (SIRT1) is a NAD+-dependent deacetylase that the field invokes more often than any other as the mechanistic bridge between dietary phytochemicals, delayed senescence and cancer prevention and curcumin, resveratrol and berberine - the three compounds named in the previous paragraph are all reported to activate it. We devote Section 7 to this case because it concentrates every problem the framework addresses: an anti-aging target whose role in cancer reverses between normal and tumor tissue, activators whose reported potency exceeds their attainable exposure by orders of magnitude and a patient population in which SIRT1-inhibiting medication is common and unrecorded.

This article develops the consequences of a single proposition that phytochemicals are weak, multi-node ligands and should be evaluated as such into four interlocking instruments. Section 2 replaces the single-target model with a network-occupancy account and shows that, for a multi-node ligand, raising exposure is quantitatively a better lever than raising affinity. Section 3 states the serial three-gate framework and a translational taxonomy of four development routes. Sections 4 to 6 examine each gate and supply, in order, two diagnostics (TEG and OD), a threshold-differential theory of selectivity with a potency criterion for chemical reformatting, and an evidence standard defined by the minimum set of measurements that makes a clinical claim interpretable. Section 7 applies the completed framework to a single node, Sirtuin 1, whose plant activators are the most cited and the least measured compounds in the field. Section 8 proposes three decisive experiments, one for each hypothesis and Section 9 assembles the design rules and the closed-loop workflow that follow.

Why the Single-Target Model Misreads Phytochemicals

Three assumptions that fail

Single-target pharmacology rests on three assumptions: that affinity determines potency, that the dose-response relationship is monotonic, and that the identity of the target explains the phenotype. Each fails in a specific and diagnosable way for phytochemicals. Affinity does not determine potency when several nodes contribute to the same phenotype, because what matters is the fraction of the pathway occupied rather than the fraction of one protein. Monotonicity fails when a compound occupies different nodes at different concentrations, so that the phenotype changes as occupancy spreads. And target identity does not explain phenotype when the compound binds a dozen proteins at similar concentrations, which is the rule rather than the exception for polyphenols: their active concentrations are typically tens of micromolar, their selectivity is low, and their cellular responses are frequently non-monotonic — curcumin, for example, activates Nrf2-dependent antioxidant programs in normal cells while exacerbating oxidative stress in tumor cells [4,7,8]. None of these failures is evidence that the compounds are inactive. They are evidence that the model is inappropriate.

Aggregate occupancy: why exposure outranks affinity

Network pharmacology provides the alternative. Hopkins argued that most effective drugs act on several nodes of a disease network and that partial perturbation of multiple nodes can be more robust than complete inhibition of one [9-11]. For a ligand occupying several nodes of a pathway, the relevant quantity is the aggregate fractional occupancy, obtained by summing the term $[L]/([L] + K)$ over those nodes, where $[L]$ is the free ligand concentration and K the dissociation constant at each node. Two consequences follow.

The first is a quantitative argument that is rarely stated explicitly, although it follows directly from the occupancy formulation. At a single node, doubling $[L]$ and halving K produce the same change in occupancy, so exposure and affinity are interchangeable. At several nodes they are not. Halving K at one node improves occupancy at that node only, whereas doubling $[L]$ improves occupancy at every node simultaneously. For a ligand with n comparable nodes, the aggregate gain from a tenfold increase in exposure is therefore of order n times the gain from a tenfold improvement in affinity at a single target. This asymmetry, rather than any intrinsic superiority of formulation over medicinal chemistry, is why pharmacokinetic optimization has repeatedly outperformed structural optimization for this compound class and why the piperine-curcumin result, a twentyfold increase in systemic exposure produced by inhibiting glucuronidation [5,12], is a more informative experiment than another analogue series.

The second consequence is a threshold. Phenotypes change when occupancy exceeds a network-dependent value, not continuously with concentration. The effect-exposure relation-ship should therefore be sigmoidal: a shallow foot at concentrations

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where only the highest-affinity nodes are occupied, a steep transition as occupancy spreads, and a plateau once the responding module is saturated. Hypothesis 1 states that for weakly binding phytochemicals this transition is the pharmacologically relevant region, that the plateau lies well below the concentrations used in most published work, and that the transition can be located experimentally by measuring a pathway-level marker rather than a cytotoxic endpoint. The hypothesis is falsified if, for a compound with independently established target engagement, the pathway response continues to increase in proportion to exposure beyond the point at which occupancy at those targets is saturated.

Dose-stratified pleiotropy

The threshold account generates a further prediction that resolves a persistent confusion. If a compound binds nodes with different affinities, then increasing exposure does not simply amplify a fixed effect: it recruits new nodes. The pharmacological identity of the molecule there-fore changes with dose, a phenomenon we term dose-stratified pleiotropy. Low exposure engages only the tightest nodes; intermediate exposure recruits the next tier; high exposure approaches the non-selective occupancy that produces toxicity in proliferating normal tissue.

This has three uses. It explains why the same polyphenol is reported as chemo-preventive at dietary concentrations and as cytotoxic at culture concentrations: these are different pharmacological objects, not inconsistent findings. It predicts the existence of a pathway-selective window, an exposure range in which a desired module is occupied while others are not, and im-plies that this window should be found by titration against pathway markers rather than by maximum tolerated dose. And it reframes the customary complaint that phytochemicals have too many targets: the number of targets is not the problem, the absence of a defined occupancy range is [11].

The Framework: Three Gates and Four Routes

We propose that a phytochemical be evaluated as a candidate that must pass three serial gates. Gate 1, exposure, asks whether a free concentration sufficient for pathway-level engagement can be sustained in tumor tissue at a tolerable dose. Gate 2, engagement, asks whether at that concentration the compound actually binds the intended nodes and produces a pathway-level rather than a downstream-only response. Gate 3, evidence, asks whether a coherent exposure-response relationship, and ultimately clinical benefit, can be demonstrated in a defined population. The gates are serial, not parallel: failure at Gate 1 cannot be compensated by elegance at Gate 2, and a mechanism established at supraphysiological concentrations carries no information about Gate 3. Table 1 gives the operating criterion, the measurement that establishes it and the failure mode characteristic of each gate.

Table 1: Operating criteria for the three gates.

Gate	Operational Criterion	How it is Established	Characteristic Failure
1. Exposure	Free tumor concentration at least equal to the pathway-effective concentration, sustained for the required duration	Plasma and tumor pharmacokinetics; physiologically based modelling	Activity reported only at unattainable concentrations
2. Engagement	Direct binding and pathway-level response at Gate-1-attainable concentrations	Chemical proteomics; thermal shift; phosphor-proteomics; functional rescue	Mechanism inferred from downstream readouts
3. Evidence	Coherent exposure-response, then benefit in a defined population	Pharmacokinetic-guided window trials; randomized trials with prespecified selection	Efficacy claimed from small uncontrolled studies

The gates say where a compound is lost; they do not say what should be done with it. For that we propose a translational taxonomy based on two measurable quantities, intrinsic potency and achievable exposure, which together define four development routes (Table 2). Class I molecules are potent enough that clinically attainable exposure exceeds their effective concentration; they require no pharmacological invention and should proceed through ordinary development, as paclitaxel did [13,14]. Class II molecules are active only at micromolar concentrations but are chemically tractable and can plausibly be brought into range by formulation, pro-drug design or absorption enhancement; curcumin, resveratrol and berberine belong here, and the first experiment for each is a pharmacokinetic one [4-6,8,15-17]. All three are also the most frequently cited plant SIRT1 activators, a coincidence that Section 7 treats as a case study rather than as an endorsement.

Table 2: A translational taxonomy of phytochemicals.

Class	Potency and Exposure	Default Route	First Action
I. Directly druggable	Effective concentration below attainable free exposure	Conventional development	Standard phase I with exposure monitoring
II. Exposure-limited	Micromolar potency, chemically tractable	Formulation, prodrug, absorption enhancement	Measure TEG; if negative, fix pharmacokinetics before any mechanism work
III. Reformattable	Sub-nanomolar, or made so by semi-synthesis	Conjugate payload or degrader warhead	Establish potency window and linker compatibility
IV. Not currently translatable	High micromolar, poor tractability	Chemical-biology probe or chemoprevention	Do not initiate a therapeutic programme

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Class III molecules are potent but exposure-limited in their native form, or can be made po-tent by semi-synthesis; their natural habitat is the conjugate or degrader, not the tablet. May-tansine and the camptothecins are the historical examples, and their derivatives now constitute the payloads of approved antibody-drug conjugates [18-20]. Class IV molecules are neither potent enough nor tractable enough for any of these routes; they are not failures of research but failures of destination, and their legitimate uses are as chemical-biology probes or as chemo-preventive agents studied at dietary exposures. The taxonomy is a statement about the present state of chemistry and formulation, not about the molecule: a Class IV compound becomes Class II if a prodrug strategy succeeds, which is precisely why the first action listed for each class matters more than the label.

The taxonomy can be checked against the historical record, and it survives the check. Paclitaxel sits in Class I: nanomolar potency, attainable exposure, ordinary development [13]. Artemisinin derivatives sit at the boundary of Classes I and II: modest potency in cancer cells, but selective activation by labile iron, which raises the effective concentration inside tumor tissue and widens the window [21,22]. Cucurbitacin B sits in Class II: sub-micromolar activity against JAK2/STAT3-dependent models, constrained by a narrow margin between cytotoxic and tolerable doses a Gate-1 problem in the language of this framework, not a target problem [23]. Most terpenoids studied for metabolic modulation fall into Class II or IV depending on whether formulation can close their exposure gap [24]. No member of the class occupies a route that the taxonomy forbids, which is a modest but genuine validation.

Gate 1. Exposure: Two Diagnostics and One Design Specification

The therapeutic exposure gap

Exposure can be made quantitative with a single index. We define the therapeutic exposure gap as the base-10 logarithm of the ratio between the free tumor concentration achievable at the maximum tolerated dose and the concentration required for pathway-level engagement, both in the same units. A TEG of zero means achievable exposure just reaches the requirement; a TEG of minus one means exposure must improve tenfold before any mechanism study can be interpreted; a positive TEG indicates headroom. The index is deliberately crude, and its purpose is to disqualify rather than to predict: it answers only the question of whether a proposed experiment could ever be performed in a patient.

Applied to the most studied phytochemicals the answer is unambiguous. Oral curcumin at 2g produces free plasma concentrations in the low nanomolar range, two to three orders of magnitude below the concentrations at which most of its mechanisms have been described [4,5]. Co-administration of piperine raises systemic exposure roughly twentyfold by inhibiting intestinal and hepatic glucuronidation [5,12] — a large pharmacokinetic effect that still leaves the TEG negative, which is the single most informative number in the curcumin literature. Berberine is absorbed so poorly that systemic exposure to the parent compound is unlikely to account for its reported in vivo effects [8,15,17] and resveratrol is subject to rapid and extensive phase II metabolism [6,16]. These are order-of-magnitude gaps, not marginal shortfalls.

From concentration to time: the occupancy dose

TEG has a deliberate blind spot: it compares concentrations and ignores duration. For a weak ligand the omission is not innocent. A ligand with a micromolar dissociation constant dissociates within seconds to minutes, so occupancy tracks the free concentration almost instantaneously and falls as soon as plasma levels decline; efficacy therefore depends on the integral of occupancy, not on its peak. We define the occupancy dose as the time integral over the dosing interval of the aggregate fractional occupancy, expressed in occupancy-hours. The quantity is measurable from existing pharmacokinetic data combined with a dissociation constant and a target-turnover model, and it converts a schedule into a single number that can be compared across administration routes.

Hypothesis 2 states that two schedules delivering equal occupancy dose produce equal pathway-level effect, irrespective of whether the same total dose is given as a bolus or as a continuous infusion, provided that no adaptive or saturable process intervenes. The hypothesis has a sharp corollary: for weak binders, continuous or frequent low-level exposure should outperform intermittent high-peak dosing at matched area under the curve, whereas for tight binders the reverse holds because occupancy persists after clearance. If this is correct, much of the dose-schedule variability in the phytochemical literature is not noise but an uninterpreted signal. The hypothesis is falsified if matched-occupancy schedules diverge systematically and the di-vergence correlates with peak concentration rather than with the integral, which would indicate that a threshold or adaptive mechanism rather than occupancy governs the response.

Reverse dosimetry as a design specification

The same arithmetic can be run backwards, and this is its most useful application. If achievable free tumor concentration is fixed by pharmacology, then it dictates the potency a molecule must have: the effective concentration must fall below the attainable concentration, not the reverse. Stated as a specification, a compound intended for oral use must reach an effective concentration roughly one order of magnitude below the free plasma concentration that a tolerable dose can sustain. For a compound like curcumin, whose attainable free concentration is nanomolar while its cellular effects require micromolar concentrations, the specification demands a thousandfold improvement in potency. Conventional structural optimization typically delivers one to two orders of magnitude. The conclusion is not that such molecules are worth-less but that they cannot be developed as oral systemic agents, and must be routed to Class III or Class IV in the taxonomy of Table 2. This is a decision that can be taken in a week, before any animal work.

Three interventions, one common error

Three families of intervention can close an exposure gap, and they are not interchangeable. Formulation amorphous solid

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dispersions, co-crystals, prodrugs, lipid and polymeric carriers increase dissolved and circulating drug, and nanoparticle systems can additionally alter bio-distribution and release kinetics [25,26]. Metabolic strategies, exemplified by piperine, reduce first-pass loss [12]. Targeting strategies, such as ligand decoration or stimulus responsive release, redistribute an existing exposure towards tumor tissue [25,27,28]. Only the first two create exposure; the third redistributes it. A targeting strategy applied to a compound with a negative TEG therefore concentrates an insufficient dose more precisely, which is a real achievement in imaging and a common failure in therapy. The error is not technical but logical, and it accounts for a substantial fraction of the nanomedicine literature on phytochemicals.

Gate 2. Engagement: An Evidence Ladder and a Theory of Selectivity

The evidence ladder

Claims of engagement in the phytochemical literature are heterogeneous to the point of being incommensurable. We propose that they be graded on a four-level ladder. Level E0 is a downstream phenotypic change, such as loss of viability or inhibition of a reporter, with no evidence of direct interaction; it is the default in the field and carries no information about target. Level E1 is direct binding demonstrated in cells at Gate-1-attainable concentrations, by chemical proteomics, thermal shift or photoaffinity labelling [29-31]. Level E2 adds a pathway-level response measured as a change in the phosphorylation or transcriptional state of the module the target belongs to, together with reversal by a genetic or pharmacological rescue. Level E3 is dependency: the response is lost when the target is depleted or mutated, in vivo. We suggest that Gate 2 be considered passed only at E2 or above, and that reviews report the level of each cited claim, because a literature that mixes E0 and E3 without distinction cannot be synthesized.

Target deconvolution, not additional screening, is the field's principal methodological deficit [32]. The techniques for E1 and E3 now exist and are routine in other areas of pharmacology; what is missing is the expectation that they be applied before a mechanism is asserted. The practical consequence is that most published mechanisms could be correct, and none of them can be used for anything, because a mechanism that cannot be located on the ladder cannot guide dosing, combination or patient selection.

Threshold-differential selectivity

A weak multi-node ligand cannot achieve selectivity through affinity, because its dissociation constants at the relevant nodes differ by less than the concentrations of ligand it can attain. If selectivity is nevertheless observed and it is, in some compounds and some models it must come from elsewhere. We propose that it comes from differences in network redundancy. A tumor cell frequently depends on a single dominant survival module, whereas the corresponding normal cell buffers the same module through parallel pathways and feedback. At a given occupancy, therefore, the tumor module may cross its threshold while the normal module does not. Selectivity is then a property of the two networks, not of the ligand, and can be written as the difference between the two thresholds rather than as a ratio of affinities.

Hypothesis 3 states that for weak multi-node ligands the selectivity index measured across matched tumor and normal organoid pairs is predicted by the difference in pathway redundancy, and is uncorrelated with the ratio of binding affinities. The hypothesis is falsified if selectivity tracks affinity ratios across a series of analogues at constant network context. Its practical value is that it changes the criterion for target selection: the question becomes not which node is bound most tightly but which node is buffered in normal tissue and unbuffered in tumor a question answerable with expression, dependency and perturbation data before any compound is tested. It also supplies a principled reason to prefer nodes with feedback control, such as NF-κB, STAT3 and Nrf2, and chromatin enzymes that convert transient signals into stable pro-grammes [4,33,34]. Sirtuin 1, discussed in Section 7, is the clearest instance: a chromatin enzyme that is protective in normal epithelium and permissive in tumor, and therefore a node that must be selected by context rather than by affinity.

The same logic explains an apparent contradiction that the single-target model cannot accommodate: a compound may be chemo-preventive in one context and tumor-promoting in another because the two contexts differ in which modules are rate-limiting, not because the compound has changed. Context dependence is a prediction of the framework, not an anomaly within it.

Reformatting: potency decides the route

The taxonomy of Section 3 implies a criterion for what we call chemical reformatting converting a phytochemical into an antibody-drug conjugate payload or into the target-binding module of a degrader. The two formats impose opposite constraints. A conjugate delivers a small amount of payload to antigen-positive cells and must kill them before linker hydrolysis in circulation releases bystander toxin, so the payload must be active at sub-nanomolar concentrations; the payloads that have succeeded are extraordinarily potent, and derive from maytansine, the auristatins and camptothecin [18,19]. A polyphenol active at tens of micromolar cannot satisfy that requirement at any achievable drug-to-antibody ratio, which is a structural rather than a strategic limitation [20]. Degraders are the mirror image: because they act catalytically, sub-stoichiometric occupancy suffices, and a ligand with micromolar affinity can in principle drive efficient degradation, provided it is cell-permeable and directs the target to an available E3 ligase [35-38]. Counter-intuitively, the weaker phytochemical ligand may be the better degrader warhead and the worse conjugate payload and this inversion is testable with existing chemistry.

Two mechanisms deserve separate mention because they convert modest occupancy into durable effects, and therefore behave disproportionately well within this framework. Immunogenic cell death converts a local cytotoxic event into systemic immune priming through calreticulin exposure, ATP release and HMGB1 secretion, so that a small direct kill can yield a larger immunological effect [39-41]. Epigenetic inhibition converts transient enzyme occupancy into heritable changes in gene expression, so that a

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weak and brief exposure can produce a persistent phenotype [33,34]. In both cases the pharmacodynamics are integrative rather than proportional, which is the behaviour predicted for threshold-based network effects and both argue for schedule design rather than dose maximization. Two further contexts amplify the same logic: remodeling of the tumor microenvironment and of the gut microbiota, both of which convert a local pharmacological event into a systemic one, and ferroptosis, an iron-dependent death modality that several phytochemicals engage at concentrations below those required for apoptosis [42-44].

Gate 3. Evidence: Equivalence, Sufficiency and Trial Design

The standardization paradox and biological equivalence

Botanical standardization seeks batch-to-batch consistency by specifying the content of one or more marker compounds. The markers are chosen for analytical convenience, not for activity and tightening their specification can reduce bioactivity if the active constituents are minor or unknown, while loosening it makes regulatory acceptance implausible. We propose to re-solve the paradox by analogy with biosimilars: define a reference batch, measure a multivariate bioactivity profile across a fixed panel of isogenic reporter lines capturing the pathways the product is claimed to engage and accept a batch as equivalent if its profile falls within a pre-specified equivalence margin. This converts batch control from a chemical question into a pharmacological one, gives regulators a quantitative criterion [45] and allows the active constituents to remain unidentified which, for most botanical extracts, they will.

The minimum interpretable evidence set

Gate 3 also needs a definition of sufficiency, because the field currently treats any positive clinical report as progress. We propose a minimum interpretable evidence set of four items, without which a clinical claim about a phytochemical cannot be evaluated: a measured human exposure at the dose used; an estimate of tumor or target-tissue concentration, direct or model-based; a pathway-level pharmacodynamic marker measured before and after dosing; and a demonstration that the biological effect varies with exposure across the sampled range. A study lacking any of the four can report safety or tolerability, but not activity. Applied retrospectively, this standard explains why so much of the clinical literature is uninterpretable rather than negative and it is the reason we prefer the phrase under-exposed over inactive when describing compounds such as curcumin [5,46].

Window trials as the cheapest decisive test

Trial design follows from the same logic. For compounds with a narrow, threshold-located window, maximum tolerated dose escalation samples the wrong part of the curve. A window trial short preoperative dosing with paired tumor biopsies, measuring intratumoral concentration alongside a pathway-level pharmacodynamic marker establishes within weeks whether Gates 1 and 2 are passed in humans, at a fraction of the cost of an efficacy trial, and produces the exposure-response data without which a later trial cannot be designed. That such trials remain rare in phytochemical research is itself diagnostic: the field has generally proceeded from cell culture to efficacy testing with no human evidence of either exposure or engagement and then interpreted the resulting null result as a property of the molecule.

Sirtuin 1: One Node That Tests the Entire Framework

Sirtuin 1 is the gene on which the claims of this field and its evidence diverge most widely, and it is therefore the best single test of the framework. It encodes an NAD+-dependent deacetylase that removes acetyl groups from histones and from a large set of transcription fac-tors and repair proteins, and it is the gene most consistently described as anti-aging: its activity sustains circadian and metabolic programmes, holds the senescence programme in check and restrains mitochondrial apoptosis in tissue exposed to stress [47]. The complementary claim that inactivation of this one gene is sufficient to generate the metabolic and multi-organ phenotype of chronic disease is unusually strong for a single locus and it is an unusually strong reason to take the target seriously rather than as a slogan [48]. Nutritional and phytochemical exposure are among the levers reported to act on this axis, in part through the hepatic and xenobiotic metabolism that sets the exposure ceiling for any dietary molecule [49].

A node whose role reverses between prevention and therapy

Two literatures describe SIRT1 in cancer and they do not agree. In the prevention literature the gene is protective: by deacetylating p53, FOXO3a and Ku70 it raises the threshold for apoptosis and senescence in cells under genotoxic or oxidative stress, supports base-excision and homologous recombination repair, and restrains the NF-κB-dependent inflammatory programme that builds a pro-tumorigenic microenvironment. On this account, sustaining SIRT1 activity in normal tissue keeps damaged cells from accumulating, and from secreting the senescence associated phenotype that recruits immune cells and remodels the stroma which is what chemoprevention means when the target is the tissue rather than the tumor [47,48]. In the tumor literature the same activity is a liability: deacetylation of p53 and FOXO3a is a survival mechanism for cells that should have died, SIRT1 is overexpressed in several tumor types and has been associated with chemoresistance, and SIRT1 inhibition has been pursued as an anti-cancer strategy in its own right.

Section 5.2 predicts this disagreement rather than being embarrassed by it. A node whose rate-limiting role differs between two network contexts will reverse the direction of its therapeutic effect when the context changes, and SIRT1 is such a node: in a normal epithelium buffered by parallel survival pathways, raising occupancy moves the tissue away from its senescence threshold without transforming it, whereas in a tumor cell that depends on p53-dependent apoptosis as its only restraint, the same occupancy removes that restraint. The practical implication is narrow and

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worth stating in a form that can be acted on. SIRT1 activation is a defensible objective in chemoprevention and in the protection of normal tissue during cytotoxic therapy; it is an unattractive objective as tumor-directed monotherapy. A paper that reports SIRT1 activation in a cancer cell line without saying which of the two contexts it models has not reported a result that a clinician can use, and the same objection applies to the reviews that cite such papers as support for treating patients.

The plant activators are Class II ligands, and the label has not been earned

The compounds named as plant SIRT1 activators are, with few exceptions, the compounds that dominate the rest of this literature: curcumin, resveratrol and berberine first, then epigallo-catechin-3-gallate, quercetin, fisetin and pterostilbene. The label rests almost entirely on cell-culture work at concentrations of roughly one to fifty micromolar, read out as increased deacetylation of a SIRT1 substrate, increased SIRT1 protein, or protection in a stress model. Measured against Gate 1 the position is the one Section 4.1 describes. Oral curcumin at 2 to 8 g yields free plasma concentrations in the low nanomolar range [5]. Resveratrol at 5 g yields a total plasma concentration near 2μM, of which one to two per cent is free [6,16]. Berberine is absorbed so poorly that exposure to the parent compound is unlikely to exceed low nanomolar concentrations [15,17]. The resulting therapeutic exposure gap lies between minus two and minus four for all three (Table 3), which places them in Class II: exposure must be fixed before the mechanism can be interpreted, and no SIRT1-directed experiment performed at unattainable concentrations can be rescued by a better assay.

Table 3: Plant SIRT1 activators assessed against Gate 1.

Compound	Reported Concentration for SIRT1 Engagement	Free Exposure at a Tolerable Oral Dose; TEG	Route; Next Decisive Measurement
Curcumin	10-50μM; deacetylation of p53 and FOXO3a in cells	2–8 g: free plasma in the low nanomolar range; TEG ≈ -3 to -4	Class II; SIRT1 occupancy by CETSA at achieved exposure
Resveratrol	5-50μM; the founding assay was partly reporter-dependent [50]	5 g: total plasma ≈ 2μM, 1-2% free; TEG ≈ -2 to -3	Class II; repeat with a reporter-free assay
Berberine	1–10μM, inferred mostly from SIRT1 expression and substrate acetylation	Poor absorption; parent drug in the low nanomolar range; TEG ≈ −3	Class II; parent drug or microbial metabolite?

The resveratrol case adds a second lesson that is methodological rather than pharmacological. The founding demonstration of SIRT1 activation used a fluorophore-tagged peptide substrate, and the magnitude of the activation was subsequently shown to depend on the reporter chemistry as much as on the enzyme [50]. For a decade the result was cited as engagement. The lesson is not that resveratrol does not act on SIRT1 at high enough concentrations it does, as do many polyphenols but that for a weak ligand a claim of engagement is worth exactly as much as the assay's freedom from artefact and the concentration's proximity to what a patient can reach. Neither condition was met, and the citation chain that followed could not detect the failure, because no link in it was required to report a concentration alongside its exposure gap.

For SIRT1, as for the compounds of Section 4, the decisive measurement is occupancy at attainable exposure: not SIRT1 expression, which is a transcriptional response and not the acetylation of a substrate, which integrates a dozen acetyltransferases and deacetylases. Cellular thermal shift analysis or thermal proteome profiling in paired tumor and adjacent normal tis-sue, performed at concentrations measured in the same patients, would place every one of these compounds on the evidence ladder within a single study [30-32]. We would run that study before any further cell-culture work on plant SIRT1 activators, because it is the only experiment that can change the status of the claim.

SIRT1 inhibitors are an unmeasured antagonism in the same patients

A problem specific to this target is absent from every trial of these compounds in cancer patients. A large number of patients take, or are given, drugs that lower SIRT1 activity. Nicotinamide is sold over the counter and inhibits SIRT1 at concentrations reachable by mouth, while also serving as an NAD+ precursor, so its net effect on the axis is dose- and tissue-dependent. Synthetic inhibitors EX-527 (selisistat), sirtinol, cambinol and tenovin are in preclinical or clinical development, some of them as anticancer agents and several cytotoxics deplete NAD+ and therefore reduce SIRT1 activity without binding the enzyme.

When a patient receiving a SIRT1 inhibitor for another indication, or for the tumor itself, is also given a plant SIRT1 activator as supportive care, the two interventions cancel in occupancy terms, and the framework makes the cancellation computable. The net occupancy dose of Section 4.2 is the integral of the activator's occupancy minus that of the inhibitor's; if the inhibitor's integral is the larger, the supportive intervention is not neutral but negative, and its effect will appear in the trial as unexplained heterogeneity rather than as an interaction. None of the clinical literature on curcumin, resveratrol or berberine reports concomitant SIRT1-inhibiting medication, circulating NAD+ or target occupancy, so the interaction is invisible in the literature rather than absent from the clinic. Three additional variables comedication with SIRT1 inhibitors or NAD+-depleting agents, a NAD+ proxy, and occupancy in a paired biopsy would convert an uninterpretable combination into a testable one, at negligible marginal cost to a window trial of the kind described in Section 6.3.

What the case implies for discovery

Whether plant SIRT1 activators contribute to cancer prevention therefore remains open, but it is now a question with a shape and

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the shape has consequences for discovery. First, a screen for new plant SIRT1 activators should begin from exposure rather than from activity: candidates should be ranked by the free concentration that a tolerated oral dose can sustain before any deacetylase assay is performed. Applied to the polyphenol literature this criterion removes most of the entries and promotes the few dietary constituents with genuine systemic bioavailability, which is why we would search among compounds already known to reach plasma in the high nanomolar or micromolar range rather than among the most studied ones. Second, activation must be demonstrated with a reporter-free assay and located on the evidence ladder, because the assay artefact described above cost the field a decade. Third, the endpoint of inter-est is occupancy in normal tissue relative to tumor, not activity in a cell line, because the node protects in one compartment and permits in the other. A plant SIRT1 activator satisfying these three conditions would be the first member of its class to be a candidate rather than a citation, and the programme that produces it is the exposure-anchored loop of Section 9.

Three Decisive Experiments

A framework that cannot be refuted is a description. We therefore specify, for each hypothesis, the experiment that would settle it and note that all three are feasible with current methods and current compounds.

Experiment 1, for Hypothesis 1 (threshold-shaped response). Take a compound with an established, E2-level target interaction and a measurable pathway marker. Administer it at six or more doses spanning two orders of magnitude and measure both the pathway marker and aggregate occupancy at the target. The hypothesis predicts a sigmoidal marker response that saturates while occupancy is still rising, and a plateau well below the concentrations used in the original cell-culture reports. A linear marker-occupancy relationship would refute the threshold account and restore the single-target model for that compound. SIRT1 is the natural first subject for this experiment: the compounds are available, the readout exists, and the result would settle a claim that has been repeated for two decades without being measured.

Experiment 2, for Hypothesis 2 (occupancy-dose equivalence). Match total exposure across two schedules, continuous infusion and once-daily bolus, in a tumor-bearing model and compare pathway response and tumor growth. The hypothesis predicts equivalence when the occupancy integral is equal and superiority of the continuous schedule when it is larger, with divergence correlated to the integral rather than to peak concentration. Divergence correlated with peak would indicate a threshold or adaptive mechanism and refute the equivalence claim.

Experiment 3, for Hypothesis 3 (threshold-differential selectivity). Assemble matched tumor and normal organoid pairs from the same tissue, measure dose-response for a series of analogues whose affinities differ by more than an order of magnitude, and compute selectivity indices. The hypothesis predicts that selectivity is predicted by measured network redundancy and is uncorrelated with the affinity ratio across the series. A significant affinity-selectivity correlation would refute the theory and return the problem to medicinal chemistry.

Synthesis: A Closed Loop and Seven Design Rules

The gates and the taxonomy together imply a workflow rather than a list. Computational triage proposes candidate compounds and target hypotheses from chemical and transcriptomic data [51,52]; patient derived organoids test whether a response is selective across genetically defined tumors rather than merely present in one line [53-55]; chemical proteomics places each claimed mechanism on the evidence ladder; formulation and prodrug design close the exposure gap; and a window trial returns human exposure response data to the beginning of the loop. Every element exists. What is missing is their connection into a sequence in which the output of one stage constrains the design of the next, and in which a compound can be removed from the queue at the earliest gate that it fails.

Seven rules follow, each addressing a documented failure mode rather than a hypothetical one. R1: measure exposure first and report every in vitro concentration together with its TEG. R2: describe exposure as an occupancy dose, not as a dose, so that schedules become comparable. R3: select nodes by network topology buffered in normal tissue, unbuffered in tumor rather than by binding affinity. R4: reformat only within the potency window, conjugating sub-nanomolar molecules and degrading with weaker ligands. R5: standardize botanical material by biological equivalence to a reference batch. R6: publish negative exposure data and negative trials, because they are the only results that stop a compound from being rediscovered in-definitely. R7: for SIRT1 and other survival nodes, report occupancy in tumor and normal tissue rather than expression, and record SIRT1-inhibiting and NAD+-modulating comedication, because an unmeasured antagonist makes an activator trial uninterpretable.

The framework has a cost that should be stated plainly. It implies that a large fraction of the compounds currently under investigation should not be developed, and that much of the mechanistic literature should be re-read as chemical biology rather than as pharmacology. That conclusion is uncomfortable, but it is also the only one that converts four decades of work into usable knowledge, because a queue of decisions is more valuable than a catalogue of possibilities.

Conclusion

The chemical wealth of plants is not in doubt; the yield of that wealth in oncology is. We have argued that the discrepancy follows from evaluating weak, multi-node ligands with the instruments of single-target pharmacology, and have derived from that single proposition a set of interlocking instruments: a serial three-gate framework, two diagnostics that make exposure and duration measurable and comparable, a taxonomy that assigns molecules to development routes rather than to chemical classes, a threshold-differential theory of selectivity, an evidence ladder, and a minimum standard for clinical interpretability. Three hypotheses and three experiments make the argument vulnerable to data, which is the

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only condition under which a framework of this kind is worth adopting. For SIRT1, the node this literature invokes most and measures least, the verdict is narrower than either its advocates or its critics would like: the tar-get is worth engaging, the compounds named as its activators have not been shown to engage it at concentrations a patient can reach, and the trials that would settle the question have not been run. If the framework is broadly right, the future of phytochemical oncology lies less in discovering new molecules than in deciding, earlier and more honestly, what each existing molecule is for.

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