



Model the Therapeutic Microenvironment Before Optimizing the Natural Product Lead Across Oxygenation, Matrix Properties, Cellular Crosstalk, Metabolism, and Local Drug Exposure

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ABSTRACT

Natural-product lead optimization commonly treats potency, selectivity, and chemical developability as properties of the candidate while treating the biological environment as background. Yet therapeutic activity is expressed within tissues whose oxygenation, redox state, extracellular matrix, cellular composition, local metabolism, and drug distribution can differ markedly from the conditions used for primary screening. This conceptual framework argues that such differences should be represented as pharmacological state variables when they are capable of changing a lead-selection decision. Evidence from cancer pharmacology shows that hypoxia can alter drug vulnerability, extracellular-matrix mechanics can reshape signaling and resistance, stromal signals can modify target dependence, local metabolic systems can transform therapeutic molecules, and spatially heterogeneous exposure can separate nominal dose from tissue-level pharmacology. The proposed therapeutic-microenvironment framework therefore treats lead performance as conditional on a set of plausible biological states rather than as a single potency value obtained under one reference condition. It further distinguishes evidence-supported environmental mechanisms from proposed integration, measured exposure from inferred target engagement, and biological complexity from model complexity. The intended use is not to maximize experimental realism, but to identify when environmental dimensions are sufficiently plausible, measurable, and decision-relevant to justify inclusion during optimization. The framework remains oncology-dominant, non-validated as an integrated ranking system, and unsuitable for assuming universal microenvironmental effects across natural products, diseases, or treatment mechanisms without direct testing.

Key Words: Natural products, Tumor microenvironment, Hypoxia, Extracellular matrix, Drug exposure, Pharmacological modeling

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INTRODUCTION

A lead compound is never pharmacologically evaluated in an environment-free state. Even a conventional assay specifies oxygen availability, nutrient composition, substrate mechanics, cell density, neighboring-cell absence or presence, extracellular binding opportunities, and a

particular relationship between administered and locally available drug. Cancer pharmacology makes this dependence especially visible because malignant cells coexist with stromal, immune, vascular, and extracellular components that can alter therapeutic response [1]. The relevant conceptual point is broader than the observation that the tumor microenvironment matters: optimization

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performed under one fixed assay condition implicitly treats that condition as the biological state against which potency and selectivity are worth maximizing.

Physical features further challenge the assumption that exposure and response can be represented by nominal medium concentration alone. Solid stress, interstitial pressure, stiffness, abnormal tissue architecture, and vascular dysfunction can influence transport and cellular behavior within tumors [2]. A candidate that appears potent in a well-mixed assay may therefore encounter a substantially different combination of access, signaling, metabolism, and adaptive state in tissue. This does not mean that every environmental variable must be modeled for every lead. It means that the experimental state should be recognized as a pharmacological reference state rather than mistaken for a neutral baseline.

The central proposal of this article is consequently selective rather than maximalist. Microenvironmental complexity should enter lead optimization when three conditions converge: there is credible evidence that the variable can alter the relevant pharmacology; the variable can be represented or measured with sufficient fidelity for the intended decision; and changing that variable could plausibly alter candidate ranking, mechanistic interpretation, or progression. For natural products, this distinction is particularly important because claims of promising activity should not be strengthened merely by adding more elaborate models. The purpose is instead to test whether the activity survives the biological contexts that the candidate is expected to encounter.

Why standard screening conditions create a pharmacological reference state that rarely exists in vivo
 Conventional cell culture is valuable because it is controllable, scalable, and interpretable, but those

advantages arise partly from reducing environmental dimensions. Microfluidic tumor models, scaffold-based three-dimensional systems, and immune-containing tumor-on-chip platforms demonstrate that geometry, matrix structure, transport gradients, and multicellular interactions can be manipulated experimentally rather than treated as uncontrollable background [3-5]. Their significance is not that they constitute uniformly superior replacements for two-dimensional assays. Each platform reproduces selected features while omitting others, and increasing biological complexity can also increase variability, parameter uncertainty, and interpretive ambiguity.

The more useful distinction is therefore between a reference assay state and a challenge state. The reference state provides a reproducible basis for detecting activity and comparing compounds. A challenge state deliberately perturbs one or more environmental dimensions that have a mechanistic or pharmacological reason to matter. Under this proposed interpretation, escalation from simple culture to more complex systems is warranted only when the additional dimension tests a meaningful uncertainty. A matrix-bearing model is useful if matrix context may alter access or signaling; a gradient-controlled system is useful if oxygen, nutrients, or transport are plausible response modifiers; an immune-integrated model is useful when non-malignant-cell signaling is germane to the treatment mechanism.

The consequence is a shift from asking which experimental model is “most physiological” to asking which model can falsify an environmentally fragile lead-selection decision.

Table 1 organizes the resulting experimental states by the pharmacological uncertainty they are capable of exposing rather than by an assumed hierarchy of physiological realism.

Table 1. Experimental reference states for testing microenvironment-sensitive lead activity

Experimental construct	Principal environmental feature represented	Pharmacological question exposed	Interpretive boundary
Standard monolayer reference	Controlled cell-intrinsic response under simplified transport and substrate conditions	Is measurable activity present under a reproducible baseline state?	Does not establish robustness to tissue architecture or multicellular context
Matrix-structured three-dimensional state	Cell–matrix contact, geometry, diffusion path and material properties	Does activity change when access and matrix-dependent signaling are altered?	Matrix composition and mechanics remain model-specific
Gradient-controlled microfluidic state	Spatially controlled transport, oxygenation or nutrient gradients	Does the response persist across defined local environmental gradients?	Represents selected gradients rather than complete tissue physiology
Immune-integrated multicellular state	Tumor–immune or other selected cellular interactions	Is response modified by neighboring-cell signaling or immune context?	Cell composition, source and platform can constrain transferability

Note: The classification is a proposed decision-oriented organization of experimental states. It does not imply that greater model complexity necessarily produces greater predictive validity.

Oxygenation changes metabolism and drug response

Oxygenation is not simply an environmental descriptor; it can reorganize signaling, metabolism, and redox control. Hypoxia and oxidative stress interact through HIF- and NRF2-associated programs that support adaptation to altered oxygen and reactive-species conditions [6]. These programs should therefore not be compressed into a single binary label such as “hypoxic versus normoxic.” Oxygen tension, duration of exposure, redox buffering, and the molecular pathway engaged can produce different pharmacological consequences.

Mechanistic evidence demonstrates why this distinction matters for lead evaluation. HIF-1 α can drive resistance to cuproptosis in defined cancer systems, showing that an oxygen-responsive program can suppress a specific therapeutic vulnerability rather than merely correlate with resistant disease [7]. Such evidence does not justify generalizing hypoxia as a universal resistance state. It establishes a more limited and useful proposition: a candidate's apparent vulnerability can be conditional on oxygen-dependent cellular state.

Natural-product pharmacology provides a direct reason to retain this conditionality. Selected natural products have been discussed as modulators of mechanisms involved in cisplatin resistance within hypoxic tumor settings [8]. That literature does not establish a class effect or clinical reversal of hypoxic resistance. It instead indicates that natural-product activity and combination effects may interact with oxygen-responsive pathways. The proposed optimization consequence is to test oxygen/redox dependence when the candidate mechanism or disease context makes that dependence plausible, and to regard loss of activity under such a state as mechanistic information rather than automatically as assay failure.

Extracellular matrix properties reshape access and signaling

The extracellular matrix is both a transport environment and a signaling environment. Matrix composition, architecture, mechanics, and remodeling influence cell adhesion, mechanotransduction, tissue organization, and therapeutic response, while treatment itself can remodel the matrix [9-11]. A candidate evaluated on a rigid culture substrate and a candidate evaluated within a fibrotic or mechanically conditioned tissue state are therefore not necessarily being tested against the same signaling network or the same physical route to cellular exposure.

Matrix effects also intersect with other microenvironmental domains. In metastatic cancer models, ECM mechanotransduction has been linked through mitochondrial fission to metabolic redox homeostasis and chemotherapy resistance [12]. The significance for

modeling is that matrix stiffness, oxygen/redox state, and metabolic adaptation should not automatically be treated as independent covariates. When evidence supports a cross-domain mechanism, changing one environmental dimension may shift another pharmacologically relevant state.

Human translational evidence also cautions against reducing matrix biology to a generic “stiff versus soft” variable. In HER2-negative breast cancer, high mechanical conditioning of the tumor matrix has been investigated as a predictive biomarker in the context of antifibrotic therapy [13]. Its relevance is specific to that disease and intervention rather than evidence for a universal mechanical threshold. Accordingly, the proposed framework represents matrix context through experimentally meaningful descriptors—such as architecture, mechanics, remodeling state, or transport consequence—chosen for the candidate and disease question. Matrix complexity should be introduced to resolve a decision, not merely to make an assay visually resemble tissue.

Cellular crosstalk alters target dependence

Target dependence is usually attributed to properties of the treated cell, yet neighboring cells can create alternative signaling conditions under which the same target becomes more or less consequential. In urothelial carcinoma models, adipocyte-precursor-derived NRG1 promoted resistance to FGFR inhibition, directly showing that a stromal paracrine signal can modify the response to a nominally tumor-cell-directed therapy [14]. A lead can therefore retain biochemical activity against its target while losing functional activity because the cellular neighborhood changes the network in which that target operates.

Fibroblast-mediated effects provide a second form of this problem. IGF2-driven fibroblast states have been shown to support immunoevasion and resistance to immunotherapy in studied systems [15]. Likewise, in colorectal-cancer liver metastasis, an SPP1-linked interaction involving cancer-associated fibroblast CXCL12 production contributed to an immunotherapy-resistant microenvironment [16]. These mechanisms are treatment- and disease-specific; they do not establish fibroblasts or secreted signals as universal causes of resistance.

For natural-product optimization, the useful inference is therefore conditional. A response measured in a purified tumor-cell system should not automatically be interpreted as a stable property of the candidate if the relevant tissue contains neighboring cells capable of supplying bypass, survival, inflammatory, or immunoregulatory signals. The proposed therapeutic-microenvironment framework consequently treats cellular composition and secreted-

signal context as candidate model variables when evidence suggests that they can alter target dependence. Their inclusion should be driven by a falsifiable pharmacological question: does the lead preserve its intended functional effect when the relevant cellular partners are added, removed, or experimentally perturbed? A change in response then becomes evidence of context dependence that requires mechanistic resolution, not proof that the observed crosstalk is the sole causal explanation.

Local metabolism changes the molecular species present

The pharmacological species present at a target site need not be identical to the molecule initially administered. Tumor and stromal metabolism can reshape local nutrient and metabolite states, creating cell-type- and region-dependent metabolic conditions that are poorly represented by uniform culture medium [17]. For a natural-product lead, this matters in two separable ways: the environment can change the state of the responding cell, and local enzymes or organisms can change the chemical identity of the candidate itself.

The second possibility requires direct evidence rather than inference from microbial presence. Intratumoral bacteria can metabolize gemcitabine and mediate resistance in defined experimental systems; bacteria have also been detected within human tumors with tumor-type-specific distributions, while host and bacterial enzymes can share anticancer-drug transformation pathways [18-20]. Together, these findings support the plausibility of local biotransformation but not a general microbiome effect on natural products. Compound structure, enzyme repertoire, microbial composition, tissue compartment, and exposure all condition whether transformation occurs.

Metabolomic measurements can help characterize these local metabolic interactions, but abundance does not by itself establish flux, cellular origin, or the transformation route responsible for a pharmacological phenotype [21]. The proposed framework therefore separates environmental metabolic state, candidate biotransformation, and measured molecular species. A lead should be modeled as chemically transformed only when analytical or mechanistic evidence supports that transformation.

Diffusion, binding, and local drug exposure

Nominal dose is not synonymous with local exposure. Tissue architecture, diffusion distance, vascular delivery, extracellular binding, cellular uptake, sequestration, and metabolism can create spatial differences between the

amount administered and the amount available at a pharmacological site. Mass spectrometry imaging provides a direct methodological demonstration that drug and metabolite distributions can be spatially resolved in tissue [22]. Its value is precisely that bulk plasma or homogenate measurements may conceal heterogeneous regions of high, low, or chemically altered exposure.

Primary multimodal imaging of gemcitabine in pancreatic ductal adenocarcinoma further shows that parent compound and metabolites can be mapped alongside local tissue features [23]. Such measurements should not be overinterpreted: spatial analytical signal does not automatically equal free intracellular concentration, target engagement, or functional effect. For natural products, where protein binding, membrane partitioning, conjugation, instability, or multiple metabolites may be important, this distinction is essential.

The proposed modeling consequence is to represent exposure at the level required by the decision. A simple concentration may remain appropriate when distribution is demonstrably homogeneous. When tissue heterogeneity is plausible, the relevant variable becomes a local exposure field containing measured or defensibly inferred parent and metabolite availability, with uncertainty explicitly retained between detection, bioavailable concentration, target engagement, and response.

Microenvironment-dependent resistance and sensitivity

Resistance is often encoded as a property of a cell line or genotype, but spatially resolved data show that treatment response can also be associated with multicellular microenvironmental states. Single-cell spatial multiomics has identified response- and resistance-associated tumor-microenvironment features in treated cancers [24]. These associations can nominate candidate mechanisms or vulnerabilities, yet spatial colocalization alone does not establish causal resistance.

Therapy adaptation is also not necessarily binary. Cellular responses can move along a continuum of adaptive states during treatment [25]. This creates a practical distinction between intrinsic inactivity, acquired adaptation, and environmental fragility. The last is proposed here to mean reproducible loss of a candidate's functional activity when a defined, biologically plausible environmental state changes while the candidate and intended pharmacological question remain otherwise comparable.

Table 2 separates these response categories because they require different interpretations and experiments.

Table 2. Distinguishing pharmacological inactivity, adaptation, and proposed environmental fragility

Response pattern	Defining observation	Interpretation	Required follow-up
Intrinsic inactivity	Little activity in the reference state	Candidate lacks useful activity under the tested baseline	Confirm assay validity and exposure
Adaptive loss	Activity declines during treatment-associated state change	Response may evolve under therapeutic pressure	Resolve temporal state transition and mechanism
Environmental fragility — proposed	Activity collapses after a predefined microenvironmental perturbation	Candidate ranking depends on biological context	Reproduce the state shift and test mechanism/exposure
Environmentally robust activity — proposed	Activity remains within an acceptable decision range across relevant states	Candidate is less sensitive to the tested environmental dimension	Validate in orthogonal and more integrated models

The integration is summarized in **Figure 1**: environmental fields alter cell state, target dependence, molecular identity, and local exposure before converging on

resistance or sensitivity, while proposed multi-state ranking sits outside the mechanistic core and remains subject to measurement and validation boundaries.

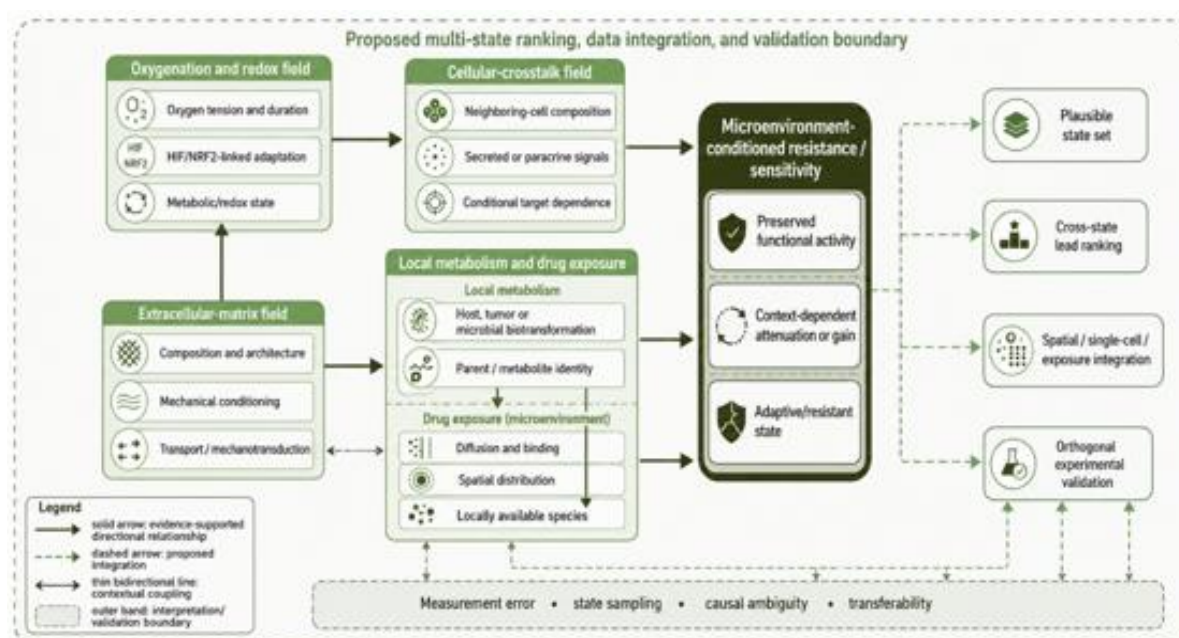


Figure 1. Therapeutic-microenvironment state fields conditioning natural-product lead activity

A therapeutic-microenvironment modeling framework

The proposed therapeutic-microenvironment framework treats lead activity as a conditional function of the candidate, the biological state, and the local exposure state. It does not require every possible environmental variable. Instead, a state description should contain only dimensions that are mechanistically plausible, experimentally representable, and capable of changing the decision being made. Oxygen/redox state, matrix properties, cellular crosstalk, metabolism, and exposure are therefore domains from which candidate-specific variables are selected, not a mandatory universal feature vector.

Single-cell transcriptomic response modeling demonstrates that cell-state-resolved information can contribute to patient-level treatment-response prediction in evaluated cancer datasets [26]. That precedent supports

state-conditioned prediction but does not validate the framework proposed here. Likewise, benchmarking of large single-cell foundation models shows that drug-response performance is task- and dataset-dependent [27]. Model sophistication should therefore not substitute for testing whether the environmental representation itself is relevant and transferable.

A practical implementation would separate three layers. The first is the measured state layer: oxygenation, matrix, cell composition, metabolites, or spatial exposure measured with an appropriate modality. The second is the pharmacological response layer: target-proximal readouts, functional phenotype, or other candidate-relevant outcomes. The third is the decision layer, in which candidates are compared across plausible states. Computational tumor-model scholarship similarly

emphasizes calibration, validation, multiscale discipline, and alignment between complexity and available data [28]. Accordingly, interactions between environmental dimensions should be included only when evidence or designed perturbation supports them.

The framework is a proposed organization for experiments and models, not a validated algorithm. It also requires that model outputs retain their biological meaning. A predicted response conditional on hypoxia, for example, should not be interpreted as evidence for an HIF mechanism unless that pathway was independently measured or perturbed. Likewise, a matrix-associated response should not be attributed to mechanics when altered diffusion could explain the same observation. This separation between state description, mechanism assignment, and decision output is intended to prevent a richer input space from producing stronger causal language than the experiment permits.

Ranking leads across multiple plausible microenvironmental states

A single potency estimate answers how a compound performs in one specified state. Lead optimization often needs a different question: whether the candidate retains useful pharmacology across the set of states it is reasonably expected to encounter. This article therefore proposes cross-state ranking rather than replacement of conventional potency. The state set should be defined before comparing candidates and should be limited to biologically plausible, decision-relevant conditions.

Three quantities are conceptually distinct. Reference-state activity captures conventional potency or functional response. State sensitivity captures how strongly that response changes when a defined environmental dimension is perturbed. Robustness captures whether the resulting response remains acceptable for the intended decision across the tested state set. These are proposed decision concepts, not validated numerical scores, and they should not be collapsed into an arbitrary composite without justification.

Such ranking also needs uncertainty. A candidate should not be penalized as environmentally fragile when the apparent loss of activity can be explained by poor analytical recovery, altered free exposure, platform artifacts, or an inadequately characterized state. Conversely, extreme potency in one reference condition should not dominate selection when the same candidate repeatedly loses activity under well-supported challenge states. The framework thus shifts optimization from maximizing a single assay result toward interrogating the stability of the pharmacological conclusion.

Ranking can also be asymmetric. Failure in a physiologically credible state may be more informative

than success in several weakly relevant states, whereas an extreme challenge condition that is unlikely to occur in the target tissue should not dominate selection. State weighting, if used, must therefore be justified by disease biology, exposure plausibility, and the intended development question rather than tuned post hoc to favor a preferred compound.

Identifying candidates whose activity collapses outside standard culture

Environmental fragility should be assigned only after a prespecified state change produces a reproducible loss of functional activity. Hypoxia-dependent loss of a defined therapeutic vulnerability illustrates the kind of state-conditioned response that motivates such testing [7]. However, the first task after an apparent collapse is not to declare resistance; it is to determine whether biology, chemistry, or measurement changed.

A minimal diagnostic sequence is proposed. First, confirm that the intended environmental perturbation occurred. Second, verify parent compound and, where relevant, active or inactive metabolite exposure. Third, determine whether target-proximal pharmacology persists. Fourth, establish whether the altered phenotype can be rescued or reproduced by manipulating the implicated environmental mechanism. Cross-domain mechanisms deserve particular attention because ECM mechanotransduction can, in specific models, alter metabolic redox state [12].

This sequence distinguishes loss of access from loss of target dependence and both from downstream adaptation. A natural-product lead whose activity collapses only because the tested formulation or analytical method fails should not be classified as biologically fragile. Conversely, reproducible loss after exposure is controlled and a relevant environmental mechanism is perturbed provides a stronger basis for deprioritization or mechanistic redesign.

Integrating spatial, single-cell, and exposure data

Single-cell and spatial omics can resolve cell states, neighborhood structure, and response-associated heterogeneity that bulk measurements obscure [29]. Their role in the proposed framework is to define and localize plausible biological states, not to stand in for pharmacological exposure. Spatial pharmacology supplies a complementary layer by mapping parent compounds and metabolites within tissue [22]. Integration becomes most informative when these measurements can be aligned at compatible spatial and temporal scales.

The critical distinction is between co-location, association, and mechanism. A resistant cellular neighborhood located in a low-exposure region may reflect a delivery problem; the same neighborhood at adequate exposure may instead implicate target dependence or adaptive biology. Spatial

multiomic associations therefore require functional or perturbational validation before being promoted to causal model inputs [24].

Table 3 summarizes how these data layers contribute different information and why they should remain separate until an explicit integration question is defined.

Table 3. Proposed framework dimensions for integrating biological state with local pharmacology

Data dimension	Primary information	Framework role	Main interpretive limit
Spatial/single-cell state	Cell identity, state and neighborhood	Define heterogeneous biological states	Association does not establish causality
Matrix/physical context	Architecture and mechanical environment	Condition transport and signaling	Measurement and model scale may differ
Metabolic identity	Local metabolites and biotransformation products	Distinguish environmental state from chemical conversion	Abundance does not prove flux or activity
Spatial exposure	Parent and metabolite localization	Test whether response occurs under adequate local exposure	Signal does not equal free drug or target engagement
Functional perturbation	Response after controlled state manipulation	Test whether a proposed state variable changes pharmacology	Model-specific effects require external validation

When microenvironment complexity should enter lead optimization

Microenvironment complexity should enter optimization when it can resolve a decision that simpler assays cannot. Controlled microfluidic models show that selected gradients and interactions can be deliberately represented [3], but the ability to add a variable is not itself a reason to add it. The proposed entry rule is therefore based on decision sensitivity: introduce a microenvironmental dimension when credible biology suggests that varying it could change candidate ranking, mechanistic interpretation, or progression criteria.

Complexity should also be constrained by validation capacity. Computational tumor models become difficult to interpret when parameterization, calibration, or external testing cannot support their biological detail [28]. A smaller state model with measurable variables can therefore be more scientifically useful than a comprehensive but weakly identified representation.

Natural-product identity alone is not an escalation criterion. The hypoxia literature shows context-dependent pharmacology for selected natural products [8], not a universal class property. Escalation is better justified by candidate chemistry, mechanism, exposure behavior, disease physiology, or prior evidence of context sensitivity. The objective is to spend complexity where it can falsify an important assumption.

Boundary conditions and experimental validation

The framework is deliberately bounded. First, no in-vitro construct should be interpreted as a complete reproduction of in-vivo physiology; controlled models represent selected environmental features [3]. Second, measured tissue localization should remain separate from free exposure, intracellular availability, target engagement, and causal response. Multimodal drug-distribution studies

show how orthogonal measurements can strengthen spatial interpretation without eliminating these distinctions [23]. Validation should therefore proceed across levels. Environmental-state measurements should verify that the intended perturbation was achieved. Analytical measurements should establish candidate and relevant metabolite identity and exposure. Target-proximal or mechanistic assays should test whether the proposed pathway changes. Functional assays should determine whether the response phenotype follows. Finally, transfer should be assessed in an independent or more integrated system whose added biology is relevant to the same decision.

The evidence supporting this framework is predominantly oncological. Tumors offer unusually strong examples of hypoxia, matrix remodeling, multicellular signaling, local metabolic variation, and heterogeneous drug distribution, but these dimensions cannot be assumed to have the same importance in infectious, inflammatory, neurological, cardiovascular, or other therapeutic settings. Nor does the framework establish prospective superiority over conventional lead optimization. Its value is presently conceptual: to make environmental assumptions explicit and experimentally challengeable.

A further boundary concerns natural-product complexity itself. Extract-derived mixtures, unstable constituents, stereochemical heterogeneity, conjugation, or poorly resolved active species can create chemical uncertainty before microenvironmental effects are considered. In such cases, improving environmental realism cannot compensate for uncertain candidate identity. The proposed framework therefore assumes that the tested pharmacological entity is sufficiently characterized to distinguish environmental modulation from changes in what was actually administered or measured.

CONCLUSION

Lead optimization is always environment-conditioned, whether that conditioning is recognized or hidden inside a standard assay. The proposed therapeutic-microenvironment framework makes this assumption explicit by separating reference-state activity from the biological states and local exposures capable of changing pharmacological interpretation.

Its purpose is not to demand maximal model complexity. It is to identify the smallest set of environmental challenges needed to determine whether a promising natural-product lead remains pharmacologically credible when oxygenation, matrix context, neighboring cells, metabolic transformation, or local exposure change. Cross-state ranking, environmental-fragility testing, and integration of spatial, single-cell, and exposure measurements are proposed tools for that purpose, not validated predictors of therapeutic success.

The central boundary is equally important: an environmental association is not a mechanism, measured distribution is not target engagement, and a complex model is not inherently more predictive. Progress therefore depends on matched perturbation, measurement, and validation. Optimizing the molecule for the biology it will actually encounter means testing whether the pharmacological conclusion survives that biology—not assuming in advance that it will.

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