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When Parent-Drug Exposure Misrepresents Pharmacological Burden: A Clinical Pharmacokinetic–Pharmacodynamic Framework for Active Metabolites, Opposing Activities, and Organ-Specific Toxicity

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ABSTRACT

Parent-drug plasma exposure is frequently used to interpret efficacy, toxicity, and interaction risk, although biotransformation can generate active products with different kinetics, tissue access, and pharmacodynamic actions. The same parent-drug area under the concentration–time curve can therefore correspond to distinct biologically effective exposure histories. To specify when parent-only inference becomes unreliable and develop testable conditions for integrating active-metabolite contributions without treating circulating chemical abundance as proof of clinical effect. This nonempirical, mechanism-based clinical pharmacokinetic–pharmacodynamic analysis draws on published parent–metabolite studies, exposure–response applications, tissue-distribution investigations, and physiological modeling. It separates biotransformation and elimination, protein binding, organ penetration, target engagement, and endpoint-specific response. Pharmacological burden is defined as a time-dependent, organ- and endpoint-indexed effect attribution problem. Parent and metabolite concentrations enter a proposed effect calculation only after chemical identity, accessible unbound exposure, local transport and binding, potency, direction of action, and temporal response have been justified. Additivity is a testable special case, not a universal property. Parent–metabolite discordance may reflect altered formation, metabolite clearance, tissue partition, delayed response, competing receptor actions, or errors in matrix conversion. These explanations are not interchangeable and require discriminating data. Metabolite-inclusive assessment is scientifically warranted when it changes an identifiable pharmacological prediction relative to a parent-only comparator; applicability to patient outcomes and organ toxicity requires prospective, endpoint-specific validation.

Key words: *Active metabolites, Parent–metabolite pharmacokinetics, Unbound drug exposure, Target-site distribution, Pharmacodynamic antagonism, Organ-specific toxicity*

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INTRODUCTION

Systemic parent-drug exposure is attractive as a common currency in clinical pharmacology. An area under the plasma concentration–time curve (AUC) compresses repeated measurements into a reproducible summary useful for comparing regimens, patient groups, and interactions. But the inference from parent AUC to effect implicitly assumes that active products, accessible target-site concentrations, and receptor responses either follow the parent closely or add no independent information. That assumption can fail without an obvious change in parent AUC. A mechanistic analysis of CYP probe studies cautions that changes in plasma clearance under pathophysiological

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conditions need not isolate changes in intrinsic enzyme activity [1]. This distinction matters when the measured analyte is only one branch of a network of formation, distribution, and elimination.

Precision dosing illustrates why drug-specific evidence is more informative than a universal exposure surrogate. Contemporary analyses of cytotoxic therapy emphasize clinical context, exposure–response uncertainty, and the practical conditions under which concentration-guided adjustment is informative [2]. Meanwhile, methodologies for modeling target-site exposure demonstrate that an observation in blood and a concentration at a site of action are different objects, connected by explicit assumptions about transport, exchange, and sampling [3]. The question is thus not whether parent exposure is useful—it often is—but when its information is sufficient for a specified therapeutic or adverse endpoint.

Here, pharmacological burden denotes a hypothesized, endpoint-specific history of biologically effective parent and metabolite activity at a relevant organ, not a measured unit, summed plasma AUC, or established clinical risk score. For some drugs, a parent-only relationship will remain adequate; for others, chemically identified active metabolites may materially change predictions. Mechanism-based theory is suitable because competing routes can produce the same plasma pattern yet imply different interventions. The analysis does not report an original cohort, trial, simulation, or validated estimator. Its distinctive contribution is to state the causal distinctions and observations required before any metabolite-inclusive quantity can claim clinical interpretation.

The limits of parent-drug exposure as a pharmacological indicator

An AUC is a property of an analyte, matrix, time window, and sampling procedure. Parent total-plasma AUC, parent unbound-plasma AUC, and parent whole-blood exposure are not interchangeable. In thoracic organ recipients treated with tacrolimus, models based on contemporaneous whole-blood, total-plasma, and unbound-plasma observations identified prominent erythrocyte binding and hematocrit-related changes in their relationship [4]. Tacrolimus is primarily an illustration of matrix-dependent parent exposure, rather than proof that an active metabolite drives toxicity. Its relevance is logical: an analytically accurate number may still capture the wrong pharmacologically accessible pool. For clozapine, inflammation-associated alpha-1-acid glycoprotein was linked with changes in the unbound fraction, making protein-binding state an additional condition for interpreting total concentrations [5]. Neither observation independently establishes the causal tissue concentration responsible for a clinical event.

A second limitation lies in causal attribution. Therapeutic drug monitoring and pharmacogenetic testing answer different questions: circulating drug levels describe net realized exposure, while a genotype identifies one contributor to metabolism under qualifying conditions. A quantitative comparison of these strategies emphasizes their complementary information and constraints [6]. Endogenous markers proposed for renal-transporter drug–drug interaction assessment likewise indicate transporter behavior in a validated context, not necessarily the concentration or clinical activity of every transported parent and metabolite [7]. Variability in hepatic extraction, transporter flux, plasma binding, dose history, and organ function may leave parent AUC unchanged while shifting the amount of a metabolite delivered to a vulnerable compartment.

These considerations motivate four non-equivalent observables: administered dose, total chemical concentration, unbound concentration, and pharmacological response. The third is closer to a plausible mobile or target-accessible fraction, but free plasma concentration still need not equal free intracellular or receptor-site concentration. The fourth depends on concentration–effect relationships and biological susceptibility; it cannot be recovered solely by multiplying chemical exposure by an assumed potency coefficient. Consequently, an adequate parent-only model must be judged against its intended endpoint and prediction interval, not dismissed simply because a metabolite can be detected. An added metabolite measurement deserves clinical weight only where identification, uncertainty, and incremental explanatory value can be demonstrated.

Current evidence on active metabolites and drug–metabolite pharmacodynamics

The relevance of an active metabolite depends jointly on formation, systemic persistence, binding, potency, and endpoint. Filgotinib forms GS-829845, which shares its preference for Janus kinase 1 yet has lower activity in a whole-blood assay and higher circulating exposure than the parent [8]. That example defeats the simple ranking of compounds by either AUC or in-vitro potency alone. Tamoxifen offers a different clinical rationale: endoxifen therapeutic drug monitoring has been used to adjust dose toward a prespecified concentration target in hormone-sensitive breast cancer [9]. Achieving a monitored target demonstrates feasibility of exposure management; it does not, on its own, prove a causal reduction in recurrence for each individual or justify exporting that target to unrelated metabolites.

Parent and product may also have different temporal roles. Joint PK–PD modeling of allopurinol, oxypurinol, and purine-pathway biomarkers in neonates links a formation product with an observed biochemical chain rather than relying only on parent disappearance [10]. CYP2D6 phenotype-dependent PBPK models of aripiprazole and dehydro-aripiprazole illustrate how metabolite formation and persistence can be modeled explicitly while retaining uncertainty about patient-level therapeutic response [11]. A useful mechanistic distinction emerges: an apparent change in combined active exposure could arise from altered formation, slower product removal, altered binding, or changes in parent kinetics; a single concentration ratio cannot uniquely assign the cause.

Interactions make this distinction clinically consequential. Under moderate CYP3A4 inhibition, cariprazine and its active metabolites desmethyl-cariprazine and didesmethyl-cariprazine contribute to a measured total active-moiety profile; the published interaction analysis does not establish that every component has identical kinetics or endpoint potency [12]. By contrast, the ribociclib development literature demonstrates how exposure–efficacy and exposure–safety reasoning can be drug-specific and tied to measurable adverse endpoints, even without making metabolite accumulation the primary explanatory variable [13]. This is an important negative comparator: introducing a metabolite term is unnecessary when it does not outperform a sufficiently supported parent-based account.

The limits of analogy become sharper across age and physiology. Pediatric morphine population-PK studies differ in how metabolic routes, maturation, and sampling are represented; reviewing those models is informative but cannot substitute for a demonstrated brain effect of morphine glucuronides [14]. Pediatric oxcarbazepine models explicitly consider conversion to its pharmacologically active 10-monohydroxy derivative under obesity-related covariates [15]. Yet changes in predicted circulating derivative concentrations remain separate from evidence of seizure control or toxicity in a given child. Taken together, these examples identify chemically and clinically different drug–product relationships that must be considered separately. They also show why a metabolite may be important for one therapeutic endpoint while remaining a weak explanation for another.

The comparison in **Table 1** distinguishes metabolite observations that directly support a particular inference from additional pharmacological claims that remain conditional. Its unit of analysis is the specific parent–product pair under a stated measurement and effect context; an entry is not a transferable potency ranking.

Table 1. Parent–product systems that demand different clinical pharmacokinetic–pharmacodynamic interpretations

Parent → identified active product	Exposure or formation evidence	Pharmacodynamic evidence or measured correlate	Conditional interpretation and unresolved claim
Filgotinib → GS-829845	Product formation and higher plasma exposure reported	Similar JAK1 selectivity, lower whole-blood assay activity	High metabolite AUC need not mean dominant organ effect; target-site contribution remains context dependent.
Tamoxifen → endoxifen	Monitored endoxifen concentration can guide dose adjustment	Prespecified concentration target used in an intervention study	Target attainment is demonstrated; individual recurrence benefit from adjustment was not established by that design.
Allopurinol → oxypurinol	Joint parent–product PK described in neonates	Hypoxanthine, xanthine and uric acid biomarkers modeled	Biochemical response is more specific than product AUC; neuroprotection cannot be inferred from the concentration model alone.
Aripiprazole → dehydro-aripiprazole	CYP2D6-sensitive parent–product disposition modeled	Active-moiety interpretation supported pharmacologically	Model-based dose adjustment cannot itself verify receptor occupancy or clinical benefit.
Cariprazine → desmethyl- and didesmethyl-cariprazine	CYP3A4-inhibitor interaction assessed across three analytes	Active moiety assembled from pharmacologically active species	A summed exposure metric requires endpoint-relevant weighting; equal contribution should not be assumed.
Oxcarbazepine → 10-monohydroxy derivative	Pediatric obesity-sensitive parent–product population PK	Derivative is pharmacologically active	Circulating exposure may inform dosing, but seizure and toxicity prediction need outcome-linked validation.

Table note. Entries distinguish published molecular/PK observations from interpretation proposed here. No study is taken to validate a universal additive burden score. JAK1, Janus kinase 1; CYP, cytochrome P450; PK, pharmacokinetics; PD, pharmacodynamics; AUC, area under the concentration–time curve.

Unresolved questions in tissue exposure and pharmacological effect interpretation

Even chemically resolved metabolite profiles may leave effect-site exposure unidentified. Remdesivir exemplifies the problem of a parent/precursor with multiple downstream chemical species and intracellular activation: systemic disposition and antiviral target engagement occupy different compartments and may have different clocks [16]. Population modeling of remdesivir and the circulating product GS-441524 in hospitalized patients provides valuable plasma information, but that product is not interchangeable with intracellular active nucleotide concentrations [17]. The analytical issue is one of state identity. A chemical name, a mass-spectrometric signal, and a pharmacologically active intracellular moiety cannot be collapsed into a single measurement without a validated transformation relationship.

Drug distribution within diseased organs introduces further conditionality. Minimal physiologically based modeling of bedaquiline and pretomanid penetration into tuberculosis lung lesions makes target attainment a tissue-dependent prediction that must inherit the assumptions of the lesion model [18]. A clinical microdosing investigation of ciprofloxacin assessed feasibility of gathering plasma and tissue pharmacokinetics but cannot be treated as universal proof that therapeutic-dose tissue partition is invariant [19]. Both settings demonstrate ways to ask more discriminating questions: which concentration is observed, where is activity needed, how does the tissue exchange rate vary, and what independent measurement could invalidate the assumed connection? Tissue total drug may include sequestered or bound material; it is not automatically the freely accessible concentration at an organism, enzyme, or receptor.

Physiology can shift this relation over time. Measurements of penicillin/beta-lactamase-inhibitor exposure in plasma and soft tissue after cardiac arrest under temperature control illustrate that physiological state can complicate extrapolation between blood and peripheral sites [20]. Saliva offers a potentially convenient matrix, but systematic examination of drug excretion into saliva indicates that its usefulness depends on compound-specific physical properties and sampling conditions [21]. Salivary recovery should therefore be evaluated as a sampling question, not promoted to a general readout of organ toxicity or efficacy. These cases support a hierarchy of *sampled matrix → chemically defined circulating species → inferred tissue availability → measured local engagement → clinical effect* in which each transition requires independent justification.

Figure 1 separates the disposition mechanisms from the measurement paths that might reveal them. This arrangement matters because a metabolite with increased plasma AUC may still have restricted tissue entry, or may accumulate at a site where the corresponding receptor or susceptibility state is absent. The diagram also preserves a reverse possibility: an altered tissue response can reflect changes in organ sensitivity despite stable parent and product concentrations. Each route must be evaluated on its own terms before increased chemical exposure can be read as increased pharmacological burden.

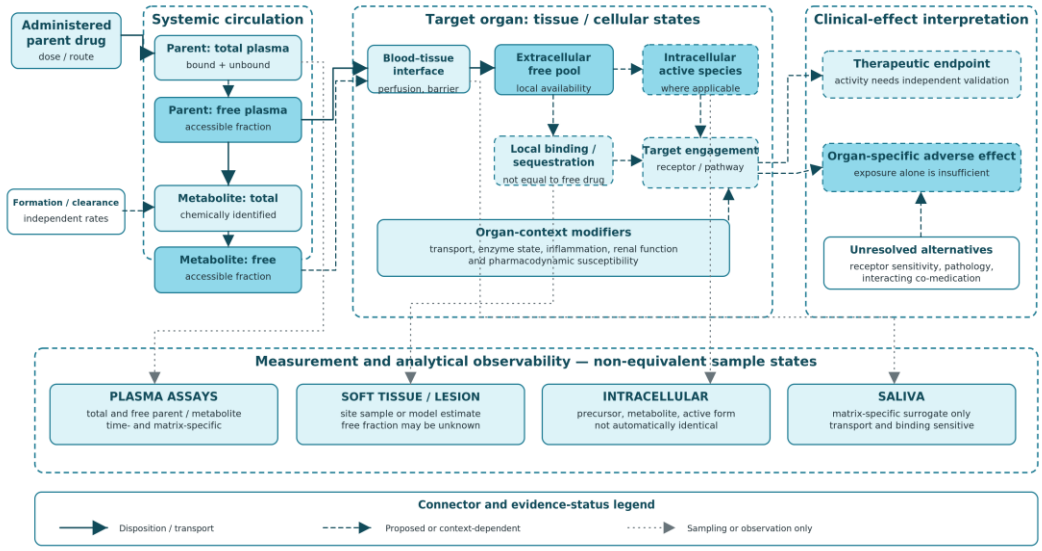


Figure 1. Parent and metabolite concentrations acquire organ-specific meaning through separable transport, activation, and observation paths

Figure 1 legend. Multicompartment map read principally from left to right. Parent input enters a circulating unbound parent pool and a distinct formation/clearance route to circulating metabolites. Blood binding and

analytical matrix sampling are represented separately from organ transport; organ compartments distinguish extracellular availability, intracellular processing where applicable, and local target engagement. Solid directional connections represent established classes of disposition processes; dashed connections represent compound- and context-dependent mechanisms needing drug-specific demonstration; dotted observation paths connect a chemical state to a candidate measurement and do not establish a causal pharmacodynamic effect. A bounded clinical-effect region depends on receptor activity and organ susceptibility; it is not the inevitable endpoint of detected tissue drug. Plasma, tissue, lesion, saliva, and intracellular assays provide non-equivalent evidence. Tissue arrows are conceptual, not measured flow rates. Parent, metabolite, unbound fraction, transporter influence, and target engagement should be assigned only when supported for the actual drug. The displayed cross-compartment paths are an original comparative synthesis and do not constitute a fitted PK–PD model.

Figure 1 alt text. Wide, asymmetric compartment map with a left-side parent administration and plasma region, a separate lower branch for metabolite formation and clearance, and center-right organ and tissue territories linked by distinct transport connectors. Sampling paths emerge toward measurement boxes for plasma, saliva, lesion/soft tissue and intracellular material, while separate conditional links connect free local concentrations to receptor engagement and two diverging possible clinical endpoints. A dashed return path from organ physiological change indicates altered susceptibility or transport independent of blood analytes. The legend distinguishes disposition, proposed context-dependent links, and observational measurement arrows; no quantitative effect values are shown.

Competing mechanisms of therapeutic and toxic pharmacological burden

A concentration discordance becomes informative only after rival generating processes are separated. Reduced parent clearance, increased metabolite formation, and reduced metabolite clearance can yield different product trajectories even when a total plasma profile appears comparable. In patients with cirrhosis, investigations of UDP-glucuronosyltransferase activity document how impaired metabolic capacity can modify pathways under disease-specific conditions [22]. This does not imply a fixed proportional reduction across all substrates or organs. In tuberculosis therapy, simultaneous rifampicin and 25-deacetylrifampicin modeling identified time-dependent autoinduction with declining exposure of both compounds [23]. Here an active metabolite is real, yet its observed contribution is constrained; a parent-only index may be relatively serviceable for some purposes and insufficient for resolving the change mechanism. Duration of treatment and sampling occasion are causal variables, not merely nuisance covariates.

Interaction studies offer a particularly strong contrast. Enzalutamide lowers oxycodone exposure in men with prostate cancer, creating a parent-availability explanation for a potential change in analgesic effect; clinical symptoms still need assessment [24]. A different interaction study found similar morphine exposure with and without enzalutamide, alongside increased systemic morphine-6-glucuronide exposure [25]. The authors highlighted uncertainty about the metabolite's potency and access to the brain, and did not establish clinically greater analgesia or toxicity. This is the manuscript's critical discriminating case: a metabolite-inclusive chemical observation succeeds in detecting a change overlooked by parent AUC, but may still fail to provide an accurate clinical effect forecast. Potential rival explanations include altered renal metabolite elimination, transporter-mediated distribution, incomplete assay coverage of other products, and altered target sensitivity. These explanations predict different responses to direct renal-clearance assessment, transport measurement, or independent analgesia and sedation endpoints.

Finally, quantitative mechanism assignment is model dependent. A middle-out PBPK analysis of voriconazole metabolism uses in-vitro and clinical information to address multiple metabolic contributions and the uncertainty of extrapolation [26]. This supports mechanistic investigation, not an assumption that every parent–product discrepancy has a unique answer from routine plasma samples. Opposing parent/metabolite pharmacological activities require particular caution: biochemical antagonism, receptor competition, partial agonism, or receptor-independent tissue injury would have to be established experimentally in the *same* drug system and relevant concentration range before a signed effect term is defensible. An increase in metabolite AUC is consistent with an increased, unchanged, or diminished measured endpoint if local potency, access, and interaction differ. Without those observations, claiming that an active metabolite causes organ-specific toxicity crosses from a plausible mechanism into unsupported attribution.

The competing accounts in **Table 2** make the discrimination explicit: each discordance pattern has at least one alternative route whose predicted measurement differs. None is treated as verified for an unstudied organ or population.

Table 2. Mechanism-discriminating observations when parent exposure and clinical effects diverge

Observed discordance or trigger	Plausible formation/disposition route	Closest non-equivalent rival	Measurement needed to discriminate	Interpretation limit
Stable parent AUC; increased product AUC	Lower product clearance or higher product formation	Altered protein binding or tissue transport	Paired parent/product free profiles; metabolite formation or excretion evidence	Does not prove larger receptor effect or clinical toxicity.
Parent and product exposures decline over successive doses	Time-dependent enzyme/transporter induction	Changed adherence, sampling time or clinical state	Serial dose histories, timed analyte profiles and mechanism-linked markers	Time trend alone cannot identify the responsible pathway.
Lower parent AUC during co-medication	Accelerated parent elimination or reduced bioavailability	Formulation, adherence or altered distribution	Route-specific kinetic sampling and relevant interaction assessment	Clinical loss of efficacy needs independent outcome evidence.
Normal plasma exposure; unexpected organ response	Local penetration, accumulation, or susceptibility shift	Receptor sensitivity, pathology, co-medication or endpoint misclassification	Tissue-relevant free exposure plus independent target/organ biomarkers	Parent/product plasma cannot settle organ causality.
Product activity opposes parent effect in an assay	Competitive or functionally opposed target engagement	Off-target assay interference or different exposure timing	Same-system receptor assays across free concentrations and relevant time courses	Opposing **clinical** burden remains hypothetical until validated.

Table note. Mechanistic distinctions are proposals for discriminating studies; examples are informed by published interaction, hepatic-metabolism, and joint parent–product investigations. No row reports newly measured results or universal clinical decision thresholds. AUC, area under the concentration–time curve.

Development of the proposed integrated parent–metabolite PK–PD framework

The proposed pharmacological burden is indexed to organ *o*, endpoint *e*, and time *t*. Its inputs are chemically identified parent and active-metabolite free concentrations accessible to the relevant target; its output is a predicted endpoint-specific response, never an inferred mass of drug. Parent and metabolite state histories require separate formation, elimination, binding, and distribution descriptions. Static versus dynamic CYP interaction models show why a single exposure ratio may misrepresent time-dependent disposition [27]. P-glycoprotein interactions are substrate-dependent; include transport only when evidence supports its direction [28].

Operationally, define the candidate burden B(o,e,t) as an effect operator H acting on the histories of free parent and metabolite target-site concentrations, target potency and efficacy, receptor competition, lag, and organ susceptibility. H is estimated separately for therapeutic and adverse endpoints. A parent-only H is a nested comparator; a simple potency-weighted sum is permissible only if independence and approximately linear, same-direction responses hold in the evaluated range. Competition can reduce net activity; distinct injury pathways may not admit a common effect scalar. Physiological ageing changes PK and PD heterogeneously [29], illustrating why susceptibility cannot be silently absorbed into an exposure multiplier. Vericiguat PK–PD illustrates drug-specific concentration–response linkage, without implying additivity across species [30].

The inference sequence follows three discriminating questions: did formation or elimination alter *free exposure*; did transport or local conversion alter *site exposure*; and did receptor or tissue sensitivity alter *effect given exposure*? Identical parent AUC can then yield different predictions. **Figure 2** separates their observational and causal content while retaining the parent-only prediction as a competing explanation. Each conditional link requires drug-, organ-, and endpoint-specific confirmation.

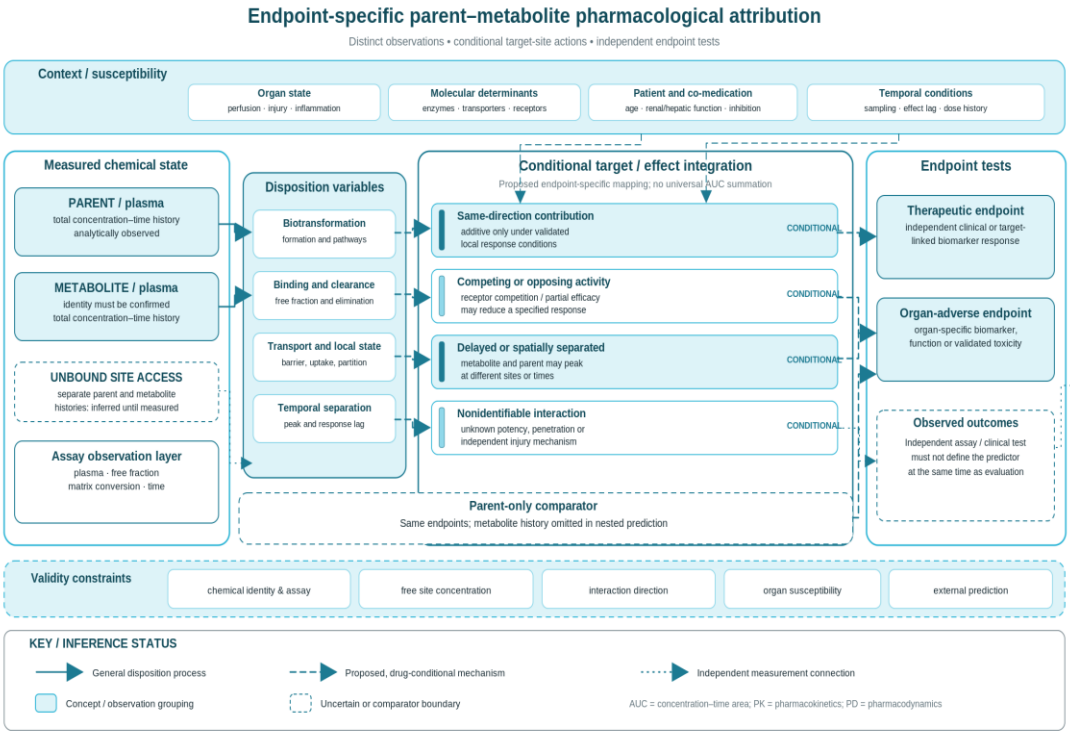


Figure 2. Endpoint-specific pharmacological attribution when parent and metabolite actions may combine, compete, or decouple

Figure 2 caption and legend. The conditional effect-integration field reads from distinct measured plasma parent and metabolite time courses (left), through inferred accessible unbound target-site histories and pharmacodynamic interaction classes (center), to separately judged therapeutic and organ-adverse endpoints (right). A parallel parent-only prediction provides the comparison against which incremental metabolite information must be evaluated. Solid arrows indicate established classes of chemical disposition or independent observation; dashed arrows indicate proposed or drug-dependent site-access, interaction, and effect links; thin dotted lines identify assay observations without assigning causality; returning dashed lines represent possible changes in susceptibility or transporter activity. A conditional interaction may be same-direction, competitive/opposing, delayed, or nonidentifiable. The title and branches describe an original synthesis, not an empirically fitted effect function. AUC, area under the concentration–time curve; PK, pharmacokinetics; PD, pharmacodynamics.

Figure 2 alt text. Wide conditional-effect map with two unequal left input lanes for parent and chemically verified metabolite concentration histories. Distinct measurement and unbound-exposure boundaries lead into a central nested interaction territory, within which same-direction action, competing activity, time lag, and unknown effect direction are separated. An independent parent-only comparator passes beneath this territory. The right side divides therapeutic from organ-adverse effect predictions, with a dotted observation track and a dashed susceptibility feedback path. A bottom legend identifies solid disposition arrows, dashed conditional hypotheses, dotted measurement paths, and boxed interpretation limits; color never carries meaning alone.

Implications for clinical pharmacology and therapeutic decision-making

A metabolite-inclusive prediction becomes clinically usable only when a decision changes for a stated endpoint. For an agent with a characterized effect time course, such as remimazolam, the proper comparator is the existing drug-specific PK–PD prediction and measured sedation endpoint, not an abstract metabolite score [31]. Paired neonatal allopurinol–oxypurinol sampling around cardiopulmonary bypass illustrates time-resolved assessment, not adult toxicity prediction [32]. Brigatinib pharmacology likewise ties dosing to the studied molecular system [33].

A defensible decision rule has three levels. First, identify discordant parent and product trajectories; second, ask whether the product improves an *independent* biomarker or organ-endpoint prediction; third, act only if the prediction improves a clinically relevant choice within an acceptable uncertainty range. For routine monitoring,

assay feasibility and actionable uncertainty may outweigh mechanistic refinement. The theory supplies criteria for testing decisions, not a new universal dose-adjustment algorithm.

Evidence requirements and testable framework predictions

Attribution begins with identity. Pharmacometabolomic work detecting previously unreported metoprolol urinary products shows that additional detectable chemical species cannot be assumed pharmacologically active or present at a target site [34]. Paired total and unbound assays should quantify relevant parent and confirmed products with sampling sufficient to distinguish formation from elimination. Matrix conversion requires calibration in the actual population: clinical venetoclax microsampling validation documents why whole-blood–plasma conversion needs empirical assessment [35]. Measure independent endpoints without using them to define the predictor.

A decisive study should prospectively compare a parent-only model with a predeclared metabolite-inclusive alternative on held-out patients or sampling periods. Physiologically based prediction research stresses the role of verification outside model construction [36]. Three falsifiers matter: no reproducible improvement in prediction after adding a chemically verified active metabolite; a predicted reversal or delay that disappears when effect-site measurements are obtained; or a change attributed to metabolite action that is instead explained by renal clearance, co-medication, assay bias or illness severity. **Table 3** identifies the minimum contrast needed for each proposed inference.

Table 3. Measurement contrasts required to test metabolite-attributed pharmacological effects

Discriminating test	Paired observations needed	Comparator that could defeat metabolite attribution	Endpoint required to establish relevance
Chemical identity and active status	Parent and product identification; validated free concentration assays; direct potency data	Product is inactive, misidentified, or present below relevant free concentrations	Mechanism-linked biomarker, independently measured
Formation versus product clearance	Serial paired free profiles, dose history, renal/hepatic indices	Product change follows clearance without greater target-site activity	Target-relevant exposure or response across time
Plasma-to-site extrapolation	Paired plasma and justified site or validated surrogate measurement	Transport or binding disrupts plasma-to-site mapping	Site-proximal measure with stated anatomical scope
Opposing or delayed PD contribution	Same-system concentration–effect/time-course assays	Apparent opposition reflects assay interference or time lag	Predeclared therapeutic and adverse endpoints
Added prediction value	Held-out prediction with parent-only and metabolite-inclusive models	Equal or better calibration/discrimination from parent-only model	Clinically meaningful decision or validated outcome

Table note. These are proposed falsification contrasts, not completed study findings or numerical acceptance thresholds. PD, pharmacodynamics.

Discussion, clinical transferability, and future research

Transfer is possible at the level of *questions*—molecular identification, accessible free exposure, effect interaction and endpoint validation—more readily than at the level of a numerical weighting rule. A systematic review of salivary antiviral monitoring found limited and inconsistent paired evidence, showing that even low-burden sampling matrices need drug-specific confirmation [37]. The caution extends to lesion, soft-tissue and metabolite observations [18, 20, 25]. Sampling convenience cannot substitute for a validated relation to action at the therapeutic or toxic site.

Research should therefore prioritize pharmacologically contrasting parent–product pairs, paired effect-site or surrogate measurements, longitudinal renal/hepatic perturbations and prespecified rival models. Negative findings are essential: where the parent-only comparator predicts the endpoint equally well, a more elaborate representation increases complexity without demonstrated benefit. Organ toxicity remains particularly difficult because susceptibility, concomitant illness and drug exposure can change together. Cross-population claims should specify which formation, transport, binding and effect parameters are transported and which must be re-estimated.

Limitations and model assumptions

This work proposes mechanisms without new patient observations, prospective validation or quantified clinical benefit. Free plasma concentrations can still be poor proxies for intracellular or receptor-site exposure; a measurable metabolite is not necessarily pharmacologically important [3]. Pediatric model heterogeneity, uncertainty about intracellular activation, and matrix-specific assay bias limit identifiability [14, 17]. The formulation permits nonlinear, delayed and opposing actions but does not estimate their strength. For the enzalutamide–morphine comparison, elevated morphine-6-glucuronide exposure cannot itself establish altered analgesia or organ harm [25]. Measurement conversion assumptions also need population-specific validation [35]. Multi-metabolite pathways may remain indistinguishable even with dense plasma sampling.

CONCLUSION

Parent-drug plasma AUC can misrepresent pharmacological burden when chemically active metabolites contribute differently across time, organs or endpoints. The warranted inference is conditional: separately establish free parent and metabolite exposure, target access, pharmacodynamic interaction and an independently observed effect. Opposing activity, toxicity and net clinical value cannot be read from metabolite abundance alone. A parent-only model remains preferable whenever metabolite inclusion fails to improve a prespecified, externally tested clinical prediction.

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REFERENCES

1. de Jong LM, van de Kreeke M, Ahmadi M, Swen JJ, Knibbe CAJ, van Hasselt JGC, et al. Changes in plasma clearance of CYP450 probe drugs may not be specific for altered in vivo enzyme activity under (patho)physiological conditions: How to interpret findings of probe cocktail studies. Clin Pharmacokinet. 2024;63(11):1585-95. doi:10.1007/s40262-024-01426-8
2. Kicken MP, Deenen MJ, van der Wekken AJ, van den Borne BEEM, van den Heuvel MM, ter Heine R. Opportunities for precision dosing of cytotoxic drugs in non-small cell lung cancer: Bridging the gap in precision medicine. Clin Pharmacokinet. 2025;64(4):511-31. doi:10.1007/s40262-025-01492-6
3. Mehciz M, Rietveld PCS, Koch BCP, Preijers T. Methodologies for population pharmacokinetic modeling of target-site drug exposure: A narrative review of current strategies. Clin Pharmacokinet. 2026;65(4):497-513. doi:10.1007/s40262-026-01620-w
4. Sikma MA, van Maarseveen EM, Hunault CC, Moreno JM, van de Graaf EA, Kirkels JH, et al. Unbound plasma, total plasma, and whole-blood tacrolimus pharmacokinetics early after thoracic organ transplantation. Clin Pharmacokinet. 2020;59(6):771-80. doi:10.1007/s40262-019-00854-1
5. Man WH, Wilting I, Heerdink ER, Hugenholtz GWK, Bognár T, ten Berg MJ, et al. Unbound fraction of clozapine significantly decreases with elevated plasma concentrations of the inflammatory acute-phase protein alpha-1-acid glycoprotein. Clin Pharmacokinet. 2019;58(8):1069-75. doi:10.1007/s40262-019-00744-6
6. Centanni M, Reijnhout N, Thijs A, Karlsson MO, Friberg LE. Pharmacogenetic testing or therapeutic drug monitoring: A quantitative framework. Clin Pharmacokinet. 2024;63(6):871-84. doi:10.1007/s40262-024-01382-3
7. Choi HJ, Madari S, Huang F. Utilising endogenous biomarkers in drug development to streamline the assessment of drug–drug interactions mediated by renal transporters: A pharmaceutical industry perspective. Clin Pharmacokinet. 2024;63(6):735-49. doi:10.1007/s40262-024-01385-0
8. Namour F, Anderson K, Nelson C, Tasset C. Filgotinib: A clinical pharmacology review. Clin Pharmacokinet. 2022;61(6):819-32. doi:10.1007/s40262-022-01129-y

9. Braal CL, Jager A, Oomen-de Hoop E, Westenberg JD, Lommen KMWT, de Bruijn P, et al. Therapeutic drug monitoring of endoxifen for tamoxifen precision dosing: Feasible in patients with hormone-sensitive breast cancer. Clin Pharmacokinet. 2022;61(4):527-37. doi:10.1007/s40262-021-01077-z
10. Chu WY, Annink KV, Nijstad AL, Maiwald CA, Schroth M, el Bakkali L, et al. Pharmacokinetic/pharmacodynamic modelling of allopurinol, its active metabolite oxypurinol, and biomarkers hypoxanthine, xanthine and uric acid in hypoxic-ischemic encephalopathy neonates. Clin Pharmacokinet. 2022;61(2):321-33. doi:10.1007/s40262-021-01068-0
11. Kneller LA, Zubiaur P, Koller D, Abad-Santos F, Hempel G. Influence of CYP2D6 phenotypes on the pharmacokinetics of aripiprazole and dehydro-aripiprazole using a physiologically based pharmacokinetic approach. Clin Pharmacokinet. 2021;60(12):1569-82. doi:10.1007/s40262-021-01041-x
12. Szabó M, Hujber Z, Harsányi J, Szatmári B, Dombi ZB, Magyar G, et al. Coadministration of cariprazine with a moderate CYP3A4 inhibitor in patients with schizophrenia: Implications for dose adjustment and safety monitoring. Clin Pharmacokinet. 2024;63(10):1501-10. doi:10.1007/s40262-024-01431-x
13. Ji Y, Schiller H, Yang S, Quinlan M, Darstein C, Huth F, et al. Use of pharmacokinetic and pharmacodynamic data to develop the CDK4/6 inhibitor ribociclib for patients with advanced breast cancer. Clin Pharmacokinet. 2024;63(2):155-70. doi:10.1007/s40262-023-01338-z
14. Stokes MA, Kamel NA, Festa MS, Sandaradura I, Stocker SL. Scoping review of paediatric population pharmacokinetic models of morphine. Clin Pharmacokinet. 2025;64(6):791-813. doi:10.1007/s40262-025-01477-5
15. Sinha J, Zimmerman K, Balevic SJ, Hornik C, Muller WJ, Rathore M, et al. Population pharmacokinetic modeling of oxcarbazepine and its active metabolite 10-monohydroxy derivative to inform dosing in children with obesity. Clin Pharmacokinet. 2026;65(2):329-44. doi:10.1007/s40262-025-01579-0
16. Humeniuk R, Mathias A, Kirby BJ, Lutz JD, Cao H, Osinusi A, et al. Pharmacokinetic, pharmacodynamic, and drug-interaction profile of remdesivir, a SARS-CoV-2 replication inhibitor. Clin Pharmacokinet. 2021;60(5):569-83. doi:10.1007/s40262-021-00984-5
17. Roberts DM, Liu X, Parker SL, Burke A, Peek J, Carland JE, et al. Population pharmacokinetic modelling of remdesivir and its metabolite GS-441524 in hospitalised patients with COVID-19. Clin Pharmacokinet. 2025;64(5):743-56. doi:10.1007/s40262-025-01496-2
18. Mehta K, Guo T, van der Graaf PH, van Hasselt JGC. Predictions of bedaquiline and pretomanid target attainment in lung lesions of tuberculosis patients using translational minimal physiologically based pharmacokinetic modeling. Clin Pharmacokinet. 2023;62(3):519-32. doi:10.1007/s40262-023-01217-7
19. Oesterreicher Z, Eberl S, Wulkersdorfer B, Matzneller P, Eder C, van Duijn E, et al. Microdosing as a potential tool to enhance clinical development of novel antibiotics: A tissue and plasma PK feasibility study with ciprofloxacin. Clin Pharmacokinet. 2022;61(5):697-707. doi:10.1007/s40262-021-01091-1
20. Stommel AM, Matzneller P, al Jalali V, Wulkersdorfer B, Lackner E, Mueller M, et al. Impact of hypothermic temperature control on plasma and soft tissue pharmacokinetics of penicillin/beta-lactamase inhibitor combinations in patients resuscitated after cardiac arrest. Clin Pharmacokinet. 2025;64(5):691-701. doi:10.1007/s40262-025-01497-1
21. Nguyen TA, Chen RH, Hawkins BA, Hibbs DE, Kim HY, Wheate NJ, et al. Can we predict drug excretion into saliva? A systematic review and analysis of physicochemical properties. Clin Pharmacokinet. 2024;63(8):1067-87. doi:10.1007/s40262-024-01398-9
22. Duthaler U, Bachmann F, Ozbey AC, Umehara K, Parrott N, Fowler S, et al. The activity of members of the UDP-glucuronosyltransferase subfamilies UGT1A and UGT2B is impaired in patients with liver cirrhosis. Clin Pharmacokinet. 2023;62(8):1141-55. doi:10.1007/s40262-023-01261-3
23. Goutelle S, Bahuaud O, Genestet C, Millet A, Parant F, Dumitrescu O, et al. Exposure to rifampicin and its metabolite 25-deacetylrifampicin rapidly decreases during tuberculosis therapy. Clin Pharmacokinet. 2025;64(3):387-96. doi:10.1007/s40262-025-01479-3
24. Detert Oude Weme SEH, Hulskotte LMG, Vervenne WL, Imholz ALT, Cremers RGHM, Taxis K, et al. Enzalutamide reduces oxycodone exposure in men with prostate cancer. Clin Pharmacokinet. 2023;62(7):989-96. doi:10.1007/s40262-023-01255-1
25. Op 't Hoog CJP, Mehra N, van der Weij B, Olofsen E, Somford DM, van Oort IM, et al. Effect of enzalutamide on morphine exposure in patients with prostate cancer. Clin Pharmacokinet. 2026;65(5):763-72. doi:10.1007/s40262-026-01640-6

26. Saleh A, Schulz J, Schlender JF, Aulin LBS, Konrad AP, Kluwe F, et al. Understanding voriconazole metabolism: A middle-out physiologically-based pharmacokinetic modelling framework integrating in vitro and clinical insights. Clin Pharmacokinet. 2024;63(11):1609-30. doi:10.1007/s40262-024-01434-8
27. Tiryannik I, Heikkinen AT, Gardner I, Onasanwo A, Jamei M, Polasek TM, et al. Static versus dynamic model predictions of competitive inhibitory metabolic drug–drug interactions via cytochromes P450: One step forward and two steps backwards. Clin Pharmacokinet. 2025;64(1):155-70. doi:10.1007/s40262-024-01457-1
28. Coumau C, Csajka C. A systematic review and classification of the effects of P-glycoprotein inhibitors and inducers in humans, using digoxin, fexofenadine, and dabigatran as probe drugs. Clin Pharmacokinet. 2025;64(6):849-63. doi:10.1007/s40262-025-01514-3
29. Ngcobo NN. Influence of ageing on the pharmacodynamics and pharmacokinetics of chronically administered medicines in geriatric patients: A review. Clin Pharmacokinet. 2025;64(3):335-67. doi:10.1007/s40262-024-01466-0
30. Fritsch A, Meyer M, Blaustein RO, Trujillo ME, Kauh E, Roessig L, et al. Clinical pharmacokinetic and pharmacodynamic profile of vericiguat. Clin Pharmacokinet. 2024;63(6):751-71. doi:10.1007/s40262-024-01384-1
31. de Jong BT, Eleveld DJ, Mason KP, Struys MMRF. Clinical pharmacokinetics and pharmacodynamics of remimazolam. Clin Pharmacokinet. 2025;64(9):1263-82. doi:10.1007/s40262-025-01548-7
32. Chu WY, Nijman M, Stegeman R, Breur JMPJ, Jansen NJG, Nijman J, et al. Population pharmacokinetics and target attainment of allopurinol and oxypurinol before, during, and after cardiac surgery with cardiopulmonary bypass in neonates with critical congenital heart disease. Clin Pharmacokinet. 2024;63(8):1205-20. doi:10.1007/s40262-024-01401-3
33. Gupta N, Hanley MJ, Griffin RJ, Zhang P, Venkatakrishnan K, Sinha V. Clinical pharmacology of brigatinib: A next-generation anaplastic lymphoma kinase inhibitor. Clin Pharmacokinet. 2023;62(8):1063-79. doi:10.1007/s40262-023-01284-w
34. Heddema WA, Hof MAJ, Sosnowski P, Bakker SJL, Hopfgartner G, Klont F, et al. Pharmacometabolomics detects various unreported metoprolol metabolites in urine of (potential) living kidney donors and kidney transplant recipients. Clin Pharmacokinet. 2025;64(5):779-89. doi:10.1007/s40262-025-01502-7
35. Levens AD, Rijstenbil J, Metscher E, von dem Borne P, Albersen A, Swen JJ, et al. Clinical validation of venetoclax volumetric microsampling in patients with leukemia with assessment of whole-blood-to-plasma conversion strategies and self-microsampling feasibility. Clin Pharmacokinet. 2026;65(6):863-82. doi:10.1007/s40262-026-01635-3
36. Jackson C, Graves RH, Tran TT, Miller NA. Impact of preclinical verification in physiologically based pharmacokinetic modelling for first-in-human predictions: Application of a published model building strategy for five compounds. Clin Pharmacokinet. 2026;65(7):1043-59. doi:10.1007/s40262-026-01655-z
37. Lai CWY, Wenker SAM, Groundwater PW, Beardsley J, Sutrave G, Chen RH, et al. How feasible is the use of saliva for antiviral therapeutic drug monitoring? A systematic review and analysis. Clin Pharmacokinet. 2026;65(8):1155-72. doi:10.1007/s40262-026-01675-9