



Review Article

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Preclinical-to-Clinical Translation of Exposure and Target Engagement

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ABSTRACT

Translation from preclinical pharmacology to human dosing is often described as an exposure-scaling problem, yet systemic pharmacokinetics, tissue exposure, target engagement, downstream pharmacology, and clinical decision utility are not interchangeable evidence layers. A compound can achieve predicted plasma exposure without reaching the relevant tissue compartment, demonstrate measurable target occupancy without sufficient functional inhibition, or produce coherent pharmacodynamic changes without resolving safety or efficacy uncertainty. This state-of-the-art review examines these separations across free-drug and tissue-exposure concepts, target-engagement measurement, species scaling, pharmacokinetic-pharmacodynamic bridging, biomarker translation, and human dose projection. The evidence supports an explicitly conditional view of translation in which each transfer between exposure, access, engagement, function, and dose constitutes an inferential boundary requiring its own validation. We therefore develop a proposed falsification-oriented translational architecture: exposure predictions should be tested against target-site access, engagement should be distinguished from functional consequence, biomarkers should be interpreted according to context of use and timing, and dose hypotheses should remain revisable after human pharmacokinetic, pharmacodynamic, and safety observations. The resulting approach treats translational development as progressive reconciliation of partially independent evidence rather than confirmation of a single preclinical prediction.

Keywords: Target engagement, Tissue exposure, Translational pharmacology, PKPD modelling, Biomarker translation, Human dose projection

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INTRODUCTION

Successful preclinical pharmacology does not translate automatically when systemic exposure is reproduced in humans. Drug development instead depends on a chain of distinct questions: was sufficient active drug available at the relevant site, did it interact with its intended target, did that interaction alter the required biological process, and was the resulting pharmacology compatible with a clinically usable dose? Target-engagement assays have expanded the ability to interrogate one part of this chain, but different assay principles measure different physical or biological manifestations of engagement and therefore impose different interpretive limits [1]. Free-drug concepts similarly provide an essential basis for understanding disposition and pharmacological availability, while remaining conditional on binding, equilibration, transport, and tissue-specific behavior [2].

The practical uncertainty begins before a target-engagement measurement is obtained. Industry approaches to projecting human pharmacokinetics differ substantially in their use of allometry, in vitro–in vivo extrapolation (IVIVE), physiologically based pharmacokinetic (PBPK) modelling, and integrated empirical strategies [3]. Translation is therefore not a single scaling operation followed by clinical confirmation. It is an evidence-reconciliation problem in which errors may arise at different biological scales and may remain hidden when plasma exposure, tissue availability, target occupancy, functional pharmacology, biomarkers, and dose are treated as substitutes for one another.

This review argues that the central translational question is not whether preclinical exposure or engagement is “predictive” in isolation, but whether the evidence chain survives successive opportunities for falsification. The proposed synthesis distinguishes exposure opportunity, target-site accessibility, direct engagement, functional consequence, biomarker context, and human dose as analytically separate states. Its purpose is not to establish a universally validated framework, but to make explicit where assumptions enter, where species or assay transfer occurs, and what evidence is needed before a stronger development claim becomes justified.

Translational exposure concepts

Systemic pharmacokinetics defines the drug available to distribute, not necessarily the drug available to the target. Tissue concentrations can differ from plasma because of permeability, transport, local binding, sequestration, metabolism, and compartment-specific kinetics. Consequently, total tissue concentration, unbound tissue concentration, and pharmacologically accessible concentration need not carry the same meaning. Zang and colleagues emphasized that plasma–tissue concentration asymmetry can materially alter exposure–effect interpretation, particularly when the pharmacological site is not in rapid equilibrium with circulating drug [4]. An observed plasma concentration should therefore be treated as evidence of systemic exposure opportunity rather than direct evidence of target-site exposure.

Mechanistic PK approaches attempt to represent the processes responsible for these differences. The extended-clearance concept and PBPK modelling can integrate hepatic uptake, efflux, metabolism, tissue distribution, and physiological determinants, but the quality of the resulting prediction remains dependent on the completeness and transportability of the underlying inputs [5]. Practical discovery applications of PBPK similarly show that model preverification, empirical scaling, and recognition of knowledge gaps remain necessary even when the modelling architecture is mechanistically detailed [6]. Greater mechanistic description can improve interpretability without eliminating uncertainty.

Temporal structure adds another problem. Exposure–response relationships may change when clearance itself varies with disease state or treatment response. In therapeutic antibodies, time-dependent clearance can confound conventional exposure–response analyses because improving disease may reduce clearance and thereby increase exposure, creating an association partly driven by response rather than exposure causing response [7]. Translational interpretation must therefore preserve directionality and timing. The same concentration can mean different things depending on when it is measured, which biological state produced it, and whether the system is approaching equilibrium, adapting, or recovering.

The proposed translational distinction is consequently hierarchical but not linear: systemic exposure creates an opportunity for tissue access; tissue access creates an opportunity for target interaction; neither proves engagement, and engagement itself does not prove functional consequence. Each transition requires evidence appropriate to its biological scale.

Free drug and tissue exposure

Free-drug reasoning is strongest when binding and distribution approach the assumptions under which unbound concentrations can equilibrate. Translation becomes more difficult when metabolism, active transport, permeability, intracellular sequestration, or tissue-specific binding create persistent asymmetry. IVIVE studies across preclinical species show that hepatic-clearance prediction is dependent on species, compound class, assay system, and the mechanisms contributing to elimination [8]. A scaling factor that performs satisfactorily for one chemical or mechanistic domain should not be presumed to transfer to another.

This problem is not solved simply by replacing mechanistic models with machine learning. In a comparison involving hundreds of compounds, machine-learning and mechanistic IVIVE approaches produced overlapping but model-dependent performance for human intrinsic-clearance prediction [9]. The useful distinction is therefore not “empirical versus mechanistic” but whether a prediction is supported within the chemical, assay, and biological domain from which it was derived.

Mechanism-specific failure is particularly informative. Aldehyde oxidase-mediated clearance remains difficult to extrapolate quantitatively despite multiple *in vitro* systems and accumulated experience [10]. Conversely, experiments that jointly measure hepatic influx, egress, and metabolism illustrate how coupled processes can sometimes be resolved more directly instead of being estimated independently [11]. Even then, broader validation is required before proof-of-concept performance can be generalized.

Model complexity itself is not a guarantee of better translation. Comparisons among well-stirred, parallel-tube, dispersion, and permeability-limited hepatic models show that more elaborate structural assumptions can improve some predictions without producing uniform superiority across chemical classes and conditions [12]. The relevant translational question is therefore whether the chosen model captures the rate-limiting processes for the molecule under development.

For exposure decisions, three quantities should consequently remain distinct: measured systemic exposure, estimated or measured target-site exposure, and the pharmacologically available concentration capable of interacting with the target. Treating them as equivalent risks attributing a failure of access to target biology, or attributing successful systemic PK to pharmacological success.

Target engagement measurement

Target engagement provides the most direct bridge between drug availability and intended molecular interaction, but the meaning of an engagement signal depends on its spatial resolution and physical basis. Single-cell measurements have demonstrated that high average target engagement can coexist with substantial cellular heterogeneity, including subpopulations with comparatively low engagement [13]. A tissue-average or population-average occupancy value can therefore conceal whether the relevant cells experience the exposure required for pharmacology.

Engagement can also diverge from the functional event that motivated target inhibition or activation. Modelling of checkpoint inhibitors has shown that receptor occupancy and inhibition of receptor–ligand interaction can track similarly for one mechanism yet differ substantially for another [14]. Occupancy should consequently not be treated as a universal surrogate for pathway inhibition. The appropriate readout depends on whether biological effect requires simple binding, sustained occupancy, competition with an endogenous ligand, receptor internalization, complex formation, catalytic inhibition, or another mechanism.

Human studies illustrate how engagement evidence becomes stronger when the readout is connected to a defined mechanistic consequence. In a first-in-human study of the CXCR7 antagonist ACT-1004-1239, dose-dependent changes in CXCL12 supported pharmacodynamic engagement while another candidate ligand biomarker, CXCL11, did not show the same response [15]. The contrast is important: a biomarker must earn its mechanistic interpretation rather than acquire it merely through correlation with dose. With the TRPA1 inhibitor GDC-0334, inhibition of allyl-isothiocyanate-induced dermal blood flow provided a functional clinical readout that linked exposure with target-related pharmacology in healthy participants [16]. Neither example establishes therapeutic efficacy, but each shows how fit-for-purpose measurements can reduce a specific uncertainty.

The proposed falsification sequence is therefore: exposure must first support plausible target access; engagement must then be demonstrated using a readout compatible with the target mechanism; and engagement must subsequently be connected to the functional event required by the development hypothesis. Failure at any step should redirect investigation toward access, assay interpretation, mechanism, dose, or timing rather than being averaged into a general claim of “insufficient pharmacology.”

The practical distinctions are condensed below because the value of a target-engagement result depends on what preceded it, what it permits investigators to claim, and which observation would invalidate that claim.

Table 1. Falsification states that determine how a target-engagement result can be carried across the preclinical-to-clinical boundary

Translational state	Precursor evidence	Observable transition	Transfer boundary	Development claim currently justified	Next discriminating validation
Scientific anchor	Precursor evidence	Observable transition	Transfer boundary	Development claim currently justified	Next discriminating validation

Exposure opportunity established	Systemic PK supports relevant circulating exposure	Drug is measurable over the intended interval	Plasma-to-tissue transfer	Target access is plausible, not demonstrated	Resolve tissue or target-proximal exposure where consequential
Target-site access supported	Local exposure is compatible with target interaction	Drug reaches the relevant compartment or cell population	Access-to-binding transfer	Engagement is biologically possible	Measure target interaction with a fit-for-purpose assay
Direct engagement demonstrated	Access and assay suitability are adequate	Binding, occupancy, complex formation, or equivalent target-specific signal is observed	Engagement-to-function transfer	The drug interacts with the intended target under measured conditions	Test the functional event required by the mechanism
Functional engagement supported	Direct engagement coincides with mechanism-relevant biological change	Pathway or functional readout changes in the predicted direction	Preclinical-to-human functional transfer	Target interaction is pharmacologically consequential in that context	Confirm magnitude, timing, reproducibility, and human relevance
Translation remains discordant	One evidence layer is positive while the next is absent or inconsistent	Expected downstream transition fails	Interpretation boundary	The evidence chain is incomplete	Falsify access, assay, mechanism, timing, or dose explanations separately

Note: The state sequence is a proposed synthesis of the evidence reviewed here; it is not a validated scoring system and does not rank assay platforms.

The same logic can be represented visually as a set of cross-scale gates rather than as a pipeline, because evidence can fail, reverse, or feed back to an earlier interpretation.

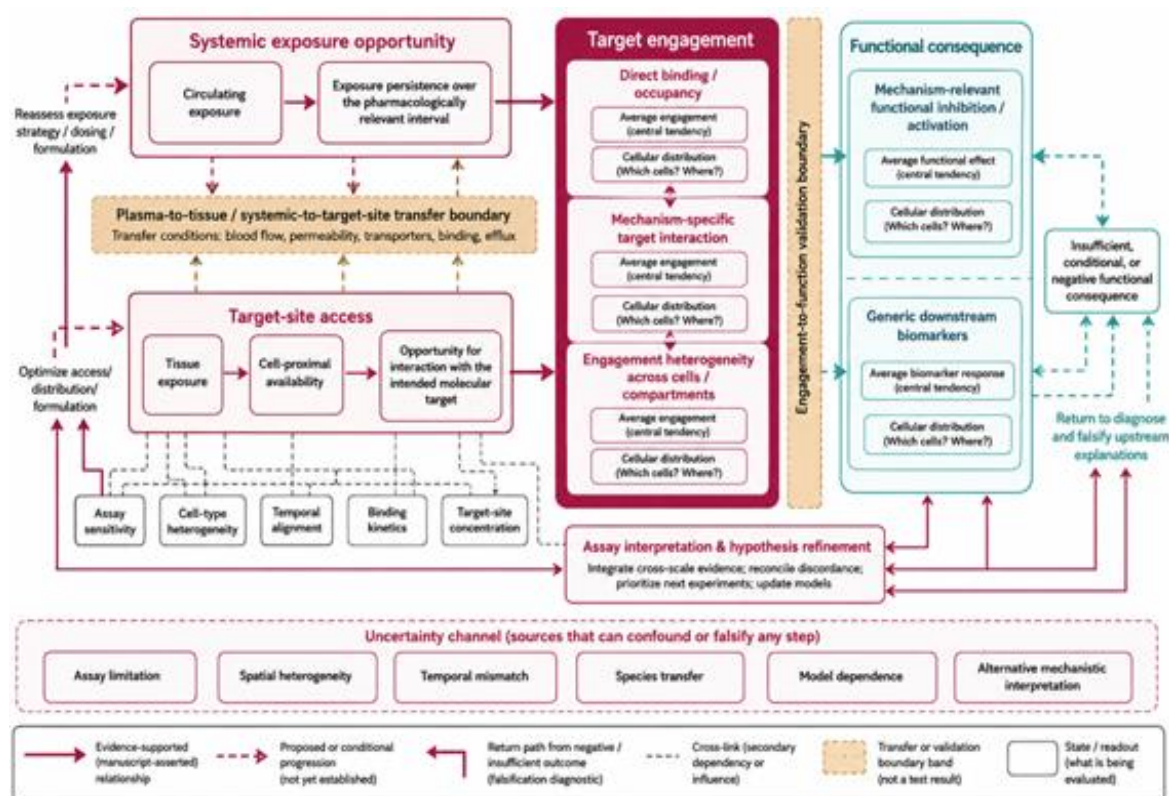


Figure 1. Target-engagement evidence changes meaning at each cross-scale validation boundary

Species scaling

Species scaling becomes difficult when the determinant of human exposure is not conserved quantitatively across animals, assays, or molecular classes. Data-driven approaches face the same problem in another form: their predictions become less secure when a candidate lies outside the chemical neighborhoods represented in training data. Structure-based machine-learning prediction of PK properties has shown that performance depends on the similarity and support of the chemical domain from which the estimate is generated [17]. A prediction therefore requires not only a value but also an explicit statement of where that value is being extrapolated.

Prospective PBPK provides one way to make such assumptions testable. For GDC-2394, preclinical information from multiple species was incorporated into PBPK predictions and subsequently confronted with clinical PK and biomarker observations, allowing the model to be refined rather than treated as a fixed preclinical truth [18]. This learn–predict–test sequence is more informative than retrospective agreement because it exposes which assumptions survive human observation.

Safety scaling creates a separate boundary. Simulation analyses of no-observed-adverse-effect-level-based clinical dose escalation show that species sensitivity and interindividual variability can produce substantial uncertainty even when animal exposure limits appear well characterized [19]. Animal exposure can constrain human dosing, but it cannot by itself establish a safe pharmacological window.

Species translation should therefore preserve at least three independent uncertainties: whether the PK determinants scale, whether target biology and engagement relationships remain comparable, and whether safety sensitivity is conserved. Concordance in one domain does not resolve the others.

PKPD bridging

PKPD bridging is most useful when it connects measurements that arise at different scales without pretending they are equivalent. Translational work with MTBT1466A illustrates this principle: PK, pharmacodynamic biomarkers, and target-engagement information contributed differently to first-in-human reasoning, while disease-model and healthy-animal contexts did not produce interchangeable biological responses [20]. Such discordance is not necessarily a failure of the biomarker; it may reveal that the biological state controlling the biomarker differs between systems.

Mechanistic PKPD models can make these assumptions explicit. Translational modelling for a PD1/IL15 targeted cytokine integrated in vitro and nonhuman-primate information to support a human dose hypothesis [21]. Likewise, modelling of a trifunctional natural-killer-cell engager showed that predicted dose depended strongly on parameters governing tumor growth and killing, making sensitivity analysis part of the translational result rather than a peripheral technical exercise [22]. A useful bridge should identify what drives the dose prediction and what observation would cause it to change.

Orthogonal pharmacodynamic evidence can strengthen pathway interpretation. In the first-in-human evaluation of SAR445088, pharmacokinetics were interpreted together with complementary assays of classical complement pathway activity [23]. By contrast, GDC-2394 demonstrates a critical boundary: a compound can yield interpretable PK and PD while later safety findings invalidate further development. Severe drug-induced liver injury led to termination despite otherwise informative pharmacological characterization [24].

PKPD bridging should therefore be understood as conditional integration rather than a proof of translation. Exposure, engagement, functional response, and safety can support one another when concordant, but each remains capable of independently falsifying the development hypothesis.

Biomarker translation

A biomarker becomes translationally useful only when its measurement properties and biological interpretation match the decision for which it is being used. European regulatory experience with biomarker qualification demonstrates that analytical validation, biomarker characteristics, and context-of-use definition are recurring evidentiary issues rather than administrative details [25]. A reproducible measurement may be scientifically interesting without being suitable for dose selection, enrichment, safety monitoring, or regulatory decision-making.

Formal qualification is also not synonymous with all forms of scientific utility. Work on neurofilament light chain in amyotrophic lateral sclerosis illustrates how a biomarker may have substantial evidence supporting defined prognostic, response, or monitoring roles even while formal qualification questions remain context-specific [26]. The implication is not that qualification is unnecessary, but that biomarker claims must be matched to the exact use being asserted.

Timing further changes biomarker meaning. In neuropsychiatric drug development, pragmatic biomarker frameworks emphasize that the same marker need not serve target validation, early pharmacology, proof of mechanism, patient selection, and outcome prediction equally well [27]. Regulatory perspectives similarly place context of use at the center of the validation burden [28]. A biomarker that successfully demonstrates acute pathway perturbation should not automatically be promoted into a predictor of durable clinical benefit.

The proposed temporal interpretation therefore distinguishes exposure-proximal biomarkers, engagement biomarkers, functional pharmacodynamic biomarkers, and later disease- or outcome-proximal measures. These categories can overlap, but the evidence required to move a biomarker from one decision role to another increases because the inferential distance from the measured event increases.

The temporal dependence of biomarker meaning is summarized below without treating any state as an automatic progression.

Table 2. Temporal biomarker states and the changing evidentiary burden across translational development

Baseline biological context Exposure-proximal response		
Biomarker decision state	Baseline biological context	Exposure-proximal response
Entry condition	Assay is analytically reliable before dosing	Drug exposure precedes measurable marker change
Evidence the state can support	Defines starting pathway or disease state	Supports drug-system interaction
Transition or persistence question	Is baseline variability relevant to later interpretation?	Does the marker track exposure over the intended interval?
Delayed consequence if misclassified	Apparent treatment effects may reflect pre-existing heterogeneity	Exposure correlation may be mistaken for target specificity
Time-dependent validation need	Characterize biological and analytical variability	Establish temporal and dose coherence
Engagement-linked response Functional pharmacodynamic response		
Biomarker decision state	Engagement-linked response	Functional pharmacodynamic response
Entry condition	Marker is mechanistically connected to target interaction	Engagement coincides with pathway-relevant change
Evidence the state can support	Supports target-related pharmacology	Supports biological consequence of engagement
Transition or persistence question	Does the signal require direct engagement rather than parallel biology?	Is the effect sustained, reversible, or adaptive?
Delayed consequence if misclassified	Correlation may be overinterpreted as mechanism	Transient perturbation may be mistaken for durable pharmacology
Time-dependent validation need	Demonstrate mechanistic specificity or orthogonal support	Match sampling to mechanism and recovery kinetics
Development-decision biomarker		
Biomarker decision state	Development-decision biomarker	
Entry condition	Evidence supports a defined context of use	
Evidence the state can support	Informs a specified dose, enrichment, monitoring, or development decision	
Transition or persistence question	Does utility transfer to the next population or stage?	
Delayed consequence if misclassified	Analytical validity may be mistaken for clinical utility	
Time-dependent validation need	Revalidate after population, disease-stage, or decision change	

Note: These temporal states are a proposed synthesis. They do not constitute a regulatory qualification scheme and do not imply that every biomarker should progress through every state.

Human dose projection

Human dose projection is the point at which multiple translational uncertainties are compressed into a single actionable hypothesis. For that reason, dose should not be treated as the mechanical output of an allometric equation or exposure target. Medicinal-chemistry perspectives on dose prediction emphasize that human dose reflects the interaction among pharmacokinetics, potency, dosing interval, and the concentration required to produce the intended effect [29]. The apparent precision of a dose estimate can therefore exceed the precision of the assumptions that generated it.

Point-of-design dose prediction extends this logic earlier into discovery by combining structure-derived PK estimates, cross-species translation, and explicit propagation of uncertainty [30]. Its value is prioritization: it can reveal when a candidate is unlikely to achieve a plausible human dose under reasonable assumptions. It does not remove the need for experimental PK, tissue-access, target-engagement, or safety evidence.

For first-in-human selection, pharmacological constraints can complement toxicological limits. Model-informed development of the agonistic anti-GITR antibody GWN323 used minimum anticipated biological effect level and biologically active dose reasoning to define a starting-dose strategy [31]. Such approaches are most defensible when the underlying pharmacology, receptor biology, potency, and model assumptions are transparent rather than reduced to a single numerical threshold.

Later human evidence can change the preferred dose substantially. For datopotamab deruxtecan, population PK and iterative exposure–response analyses contributed to late-stage dose selection instead of treating maximum tolerated dose as the sole endpoint of escalation [32]. Ubatamab provides a related example at the regimen level: in vitro pharmacology, animal efficacy, nonhuman-primate PK, and emerging clinical observations were successively reconciled to support starting, step-up, and expansion-regimen decisions [33]. These cases reinforce an important principle: dose projection becomes more reliable by being revisable.

The proposed dose-reconciliation rule is therefore deliberately falsification-oriented. A human dose hypothesis should survive four independent questions: whether predicted human exposure is plausible, whether that exposure reaches and engages the target, whether engagement produces the required functional pharmacology, and whether the resulting exposure–response region remains compatible with safety. Failure of any one condition should trigger revision of the dose rationale rather than selective reliance on the evidence layers that remain concordant. The most informative dose scenarios are consequently the discordant ones, because they identify the next experiment or observation capable of distinguishing among competing explanations.

Table 3. Evidence-reconciliation states that determine whether a projected human dose remains scientifically defensible

Governing evidence pattern	Validation	Interpretation	Validation
Scientific anchor	Failure criterion	Inference currently justified Inference not justified Unresolved development question	Next validation step
Predicted human PK is plausible, but target-site access is uncertain	Plasma exposure lacks evidence of relevant local availability	Inference currently justified Systemic exposure target may be achievable Inference not justified Target engagement at that dose Unresolved development question Is dose limited by distribution rather than potency?	Resolve tissue or target-proximal exposure
Exposure and access are supported, but engagement is inadequate	Target-specific interaction remains below the required pharmacological state	Inference currently justified Delivery to the target environment occurs Inference not justified Increasing systemic dose will necessarily solve engagement Unresolved development question Is the bottleneck affinity, accessibility, kinetics, or measurement?	Test binding mechanism, local free concentration, and assay validity

Engagement is present, but functional pharmacology is weak	Mechanism-relevant downstream effect is absent or insufficient	Inference currently justified Target interaction occurs Inference not justified Occupancy is an adequate surrogate for biological effect Unresolved development question Does target binding produce the intended mechanism in humans?	Measure functional inhibition, adaptation, or competing pathway activity
PK, engagement, and PD are concordant	Safety or tolerability becomes unacceptable	Inference currently justified Pharmacological translation is supported within the observed range Inference not justified A wider therapeutic window exists Unresolved development question Is the development constraint pharmacological or safety-driven?	Reconcile exposure with the emergent safety mechanism
Early clinical evidence differs from preclinical projection	Human PK, engagement, PD, or safety falls outside preclinical expectation	Inference currently justified A model assumption has failed or requires updating Inference not justified The original projected dose should be preserved unchanged Unresolved development question Which preclinical transfer assumption failed?	Update parameters and re-evaluate the complete evidence chain

Note: The grid is a proposed evidence-reconciliation device, not a validated dose algorithm. No row supplies a numerical dose, threshold, or probability of success.

The relationship among these conditions is better represented as a dose hypothesis under repeated challenge than as a one-way preclinical-to-clinical calculation.

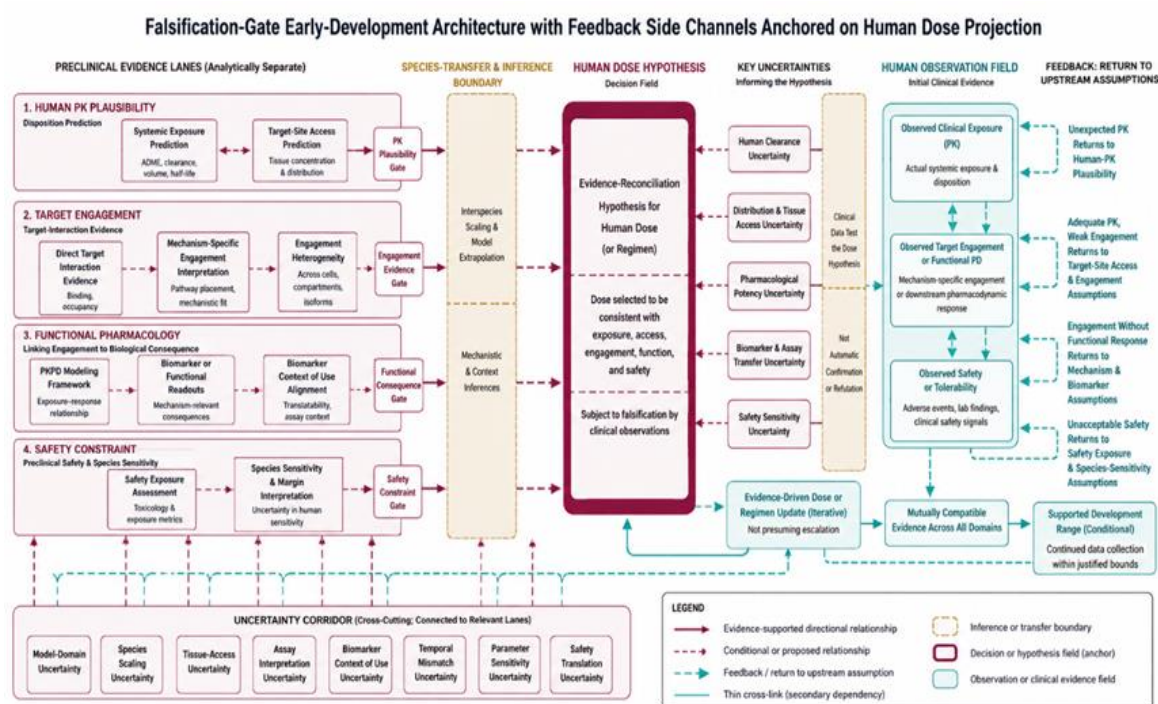


Figure 2. A human dose hypothesis survives only while exposure, engagement, function, and safety remain mutually compatible

Discordance between exposure and engagement

Discordance becomes most informative when apparently adequate systemic exposure fails to produce the expected target-level pharmacology. Tissue asymmetry provides one explanation: plasma concentrations may remain compatible with the intended pharmacological range while local unbound concentrations are insufficient or temporally misaligned with the target compartment [4]. Cellular heterogeneity provides another. Single-cell target-engagement measurements show that apparently high average engagement can coexist with subpopulations

experiencing substantially lower target interaction [13]. A conventional exposure summary can therefore be correct at the population level while being biologically misleading at the level that determines response.

The inverse pattern is also possible. Target binding may be demonstrable while the required functional consequence remains weak or absent. Comparison of checkpoint-inhibitor dose-selection metrics illustrates that receptor occupancy and inhibition of receptor–ligand interaction are not necessarily equivalent [14]. Translational interpretation should therefore resist the temptation to repair discordant evidence by privileging whichever measurement appears most favorable. Adequate plasma PK with poor engagement should redirect attention toward tissue access, local free concentration, binding kinetics, assay suitability, or cellular distribution. Adequate engagement with weak functional response should instead challenge the assumed mechanism, functional threshold, temporal design, or downstream biological competence.

Cross-species discordance must be treated similarly. In the MTBT1466A program, PK, pharmacodynamic, and target-engagement information did not transfer identically across disease-model and healthy-animal contexts [20]. The proposed interpretation is that discordance is a diagnostic state rather than merely a development failure: it identifies the boundary at which the evidence chain has stopped supporting a stronger claim.

Translational failure modes

Translational failure is often attributed retrospectively to “poor predictivity,” but that label hides distinct mechanisms. Failure may originate in species-specific clearance, transporter or enzyme behavior, tissue partitioning, model structure, target biology, biomarker interpretation, or safety sensitivity. Aldehyde-oxidase IVIVE demonstrates how a specific elimination pathway can remain difficult to translate even when the responsible mechanism is known [10]. Comparative liver modelling likewise shows that increasing structural complexity does not guarantee better prediction when the relevant determinants are incompletely identified [12]. A second failure mode is false concordance. Multiple preclinical measurements can agree because they share the same upstream assumption. Agreement among exposure predictions, for example, does not independently establish target-site access. Likewise, pharmacodynamic coherence does not eliminate the possibility of a clinically unacceptable safety signal. The GDC-2394 first-in-human program is instructive because interpretable PK and PD were obtained before severe drug-induced liver injury terminated development [24]. Pharmacological translation and safety translation therefore require separate evidentiary gates.

A third failure mode is model persistence after contradiction. When human observations fall outside preclinical expectations, preserving the original parameterization for the sake of continuity converts a prediction into a defended narrative. Prospective translation instead requires explicit updating of the model, dose rationale, or mechanistic interpretation. Failure becomes scientifically useful when it changes the next experiment.

Integrated development strategies

The evidence reviewed here supports a development strategy based on triangulation rather than accumulation. PBPK can formalize assumptions about systemic exposure and tissue distribution, but prospective human observations should test those assumptions and feed back into the model [18]. Mechanistic PKPD can connect exposure with biological consequence, yet sensitivity analysis should identify which parameters dominate the dose hypothesis [22]. Biomarkers should be selected according to the decision they are intended to inform, rather than because they are technically measurable [25].

The article therefore proposes an integrated falsification architecture with five linked but non-substitutable questions: Is human systemic exposure plausible? Is pharmacologically available drug present at the relevant site? Is the intended target engaged? Does engagement produce the required functional consequence? Is that pharmacological state compatible with safety and the intended clinical context? Each affirmative answer permits a stronger development claim, but none validates the next question automatically.

This architecture also changes the role of modelling. A model should not merely interpolate a preferred answer; it should expose which observation could overturn that answer. The iterative refinement of PBPK predictions with human data [18], the use of biologically active dose and MABEL reasoning for first-in-human selection [31], and the later updating of dose or regimen using emerging patient evidence [32] illustrate different stages of this logic. For complex therapeutics, ubamatamab further demonstrates how in vitro, animal, pharmacokinetic, pharmacodynamic, and clinical evidence can be reconciled progressively rather than forced into a single preclinical prediction [33].

Future directions

Three priorities follow from this synthesis. First, translational models should report domain and parameter uncertainty as part of the scientific result. Human-clearance prediction already shows that machine-learning and mechanistic approaches can fail differently even when aggregate performance appears similar [9]. Structure-based PK models likewise lose reliability outside their chemical support [17]. Prospective translation will benefit from designing experiments specifically to discriminate between competing model explanations rather than merely adding more measurements.

Second, target engagement should increasingly be resolved at the biological scale that controls response. Spatial, cellular, and temporal heterogeneity can make average occupancy an inadequate representation of effective pharmacology [13]. Fit-for-purpose engagement assays should therefore be paired with mechanism-relevant functional readouts whenever the biology allows, while preserving the distinction between target interaction and downstream effect.

Third, biomarker and dose strategies should be explicitly revisable across development stages. Context of use determines what evidence a biomarker must carry [28], while point-of-design human dose estimates remain conditional on subsequent experimental validation [30]. Future translational programs should predefine not only the expected evidence chain but also the observations that would trigger re-estimation of tissue exposure, target engagement, biological activity, or dose.

CONCLUSION

Preclinical-to-clinical translation is strongest when exposure and target engagement are treated as separate but connected evidence layers rather than interchangeable proof of pharmacology. Systemic exposure establishes opportunity, tissue access constrains availability, target engagement demonstrates interaction, functional pharmacology tests mechanistic consequence, and human safety and clinical context determine whether the resulting state is developable. The principal contribution of this review is a proposed falsification-oriented architecture that makes the boundaries between these states explicit and treats discordance as information rather than noise. Its value depends on preserving uncertainty, respecting species and assay limits, and revising models when human observations contradict preclinical expectations. Translation should therefore be judged less by whether an early prediction survives unchanged and more by whether the evidence chain becomes progressively more discriminating as development moves toward patients.

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REFERENCES

1. St John-Campbell S, Bhalay G. Target engagement assays in early drug discovery. *J Med Chem.* 2025;68(12):12331-68. doi:10.1021/acs.jmedchem.4c03115
2. Summerfield SG, Yates JWT, Fairman DA. Free drug theory - no longer just a hypothesis? *Pharm Res.* 2022;39(2):213-22. doi:10.1007/s11095-022-03172-7
3. Petersson C, Zhou X, Berghausen J, Cebrian D, Davies M, DeMent K, et al. Current approaches for predicting human PK for small molecule development candidates: Findings from the IQ Human PK Prediction Working Group Survey. *AAPS J.* 2022;24(5):85. doi:10.1208/s12248-022-00735-9
4. Zang R, Barth A, Wong H, Marik J, Shen J, Lade J, et al. Design and measurement of drug tissue concentration asymmetry and tissue exposure-effect (tissue PK-PD) evaluation. *J Med Chem.* 2022;65(13):8713-34. doi:10.1021/acs.jmedchem.2c00502
5. Sugiyama Y, Aoki Y. A 20-year research overview: Quantitative prediction of hepatic clearance using the in vitro-in vivo extrapolation approach based on physiologically based pharmacokinetic modeling and extended clearance concept. *Drug Metab Dispos.* 2023;51(9):1067-76. doi:10.1124/dmd.123.001344

6. Santos LGA, Jaiswal S, Chen KF, Jones HM, Templeton IE. Real-world application of physiologically based pharmacokinetic models in drug discovery. *Drug Metab Dispos.* 2025;53(1):100015. doi:10.1124/dmd.122.001036
7. Proctor JR, Wong H. Time-dependent clearance can confound exposure-response analysis of therapeutic antibodies: A comprehensive review of the current literature. *Clin Transl Sci.* 2024;17(1). doi:10.1111/cts.13676
8. Tess DA, Ryu S, Di L. In vitro-in vivo extrapolation of hepatic clearance in preclinical species. *Pharm Res.* 2022;39(7):1615-32. doi:10.1007/s11095-022-03205-1
9. Keefer CE, Chang G, Di L, Woody NA, Tess DA, Osgood SM, et al. The comparison of machine learning and mechanistic in vitro-in vivo extrapolation models for the prediction of human intrinsic clearance. *Mol Pharm.* 2023;20(11):5616-30. doi:10.1021/acs.molpharmaceut.3c00502
10. Izat N, Bolleddula J, Abbasi A, Cheruzel L, Jones RS, Moss D, et al. Challenges and opportunities for in vitro-in vivo extrapolation of aldehyde oxidase-mediated clearance: Toward a roadmap for quantitative translation. *Drug Metab Dispos.* 2023;51(12):1591-606. doi:10.1124/dmd.123.001436
11. Schulz Pauly JA, Sande E, Feng M, Wang YT, Stresser DM, Kalvass JC. Proof of concept of an all-in-one system for measuring hepatic influx, egress, and metabolic clearance based on the extended clearance concept. *Drug Metab Dispos.* 2024;52(10):1048-59. doi:10.1124/dmd.124.001768
12. Nagar S, Parise R, Korzekwa K. Predicting clearance with simple and permeability-limited physiologically based pharmacokinetic frameworks: Comparison of well-stirred, dispersion, and parallel-tube liver models. *Drug Metab Dispos.* 2024;52(10):1060-72. doi:10.1124/dmd.124.001782
13. Dubach JM, Kim E, Yang K, Cuccarese M, Giedt RJ, Meimetis LG, et al. Quantitating drug-target engagement in single cells in vitro and in vivo. *Nat Chem Biol.* 2017;13(2):168-73. doi:10.1038/nchembio.2248
14. Head SA, Johnson C, Sarkar S, Matteson A, Marcantonio DH, Hua F, et al. Comparison of dose selection based on target engagement versus inhibition of receptor-ligand interaction for checkpoint inhibitors. *CPT Pharmacometrics Syst Pharmacol.* 2024;13(6):919-25. doi:10.1002/psp4.13152
15. Huynh C, Henrich A, Strasser DS, Boof ML, Al-Ibrahim M, Meyer Zu Schwabedissen HE, et al. A multipurpose first-in-human study with the novel CXCR7 antagonist ACT-1004-1239 using CXCL12 plasma concentrations as target engagement biomarker. *Clin Pharmacol Ther.* 2021;109(6):1648-59. doi:10.1002/cpt.2154
16. Chan P, Ding HT, Liederer BM, Mao J, Belloni P, Chen L, et al. Translational and pharmacokinetic-pharmacodynamic application for the clinical development of GDC-0334, a novel TRPA1 inhibitor. *Clin Transl Sci.* 2021;14(5):1945-54. doi:10.1111/cts.13049
17. Pillai N, Abos A, Teutonico D, Mavroudis PD. Machine learning framework to predict pharmacokinetic profile of small molecule drugs based on chemical structure. *Clin Transl Sci.* 2024;17(5). doi:10.1111/cts.13824
18. Yu J, Tang F, Ma F, Wong S, Wang J, Ly J, et al. Human pharmacokinetic and CYP3A drug-drug interaction prediction of GDC-2394 using physiologically based pharmacokinetic modeling and biomarker assessment. *Drug Metab Dispos.* 2024;52(8):765-74. doi:10.1124/dmd.123.001633
19. Chen C, Lavezzi SM, McDougall D. The estimation and translation uncertainties in applying NOAEL to clinical dose escalation. *Clin Transl Sci.* 2024;17(6). doi:10.1111/cts.13831
20. Yadav R, Sukumaran S, Lutman J, Mitra MS, Halpern W, Sun T, et al. Utilizing PK and PD biomarkers to guide the first-in-human starting dose selection of MTBT1466A: A novel humanized monoclonal anti-TGFβ3 antibody for the treatment of fibrotic diseases. *J Pharm Sci.* 2023;112(11):2910-20. doi:10.1016/j.xphs.2023.07.005
21. Yadav R, Schubert S, Holder PG, Chiang EY, Kiabi N, Bogaert L, et al. Translational PK/PD and the first-in-human dose selection of a PD1/IL15: An engineered recombinant targeted cytokine for cancer immunotherapy. *Front Pharmacol.* 2024;15:1380000. doi:10.3389/fphar.2024.1380000
22. Choi SL, Valente D, Virone-Oddos A, Mauriac C. Developing a mechanistic translational PK/PD model for a trifunctional NK cell engager to predict the first-in-human dose for acute myeloid leukemia. *Clin Transl Sci.* 2024;17(1). doi:10.1111/cts.13689
23. Chow T, Shamszad P, Vinnard C, Yoon E, Belinski J, Karpenko I, et al. First-in-human study with SAR445088: A novel selective classical complement pathway inhibitor. *Clin Transl Sci.* 2023;16(4):673-85. doi:10.1111/cts.13481

24. Tang F, Kunder R, Chu T, Hains A, Nguyen A, McBride JM, et al. First-in-human phase 1 trial evaluating safety, pharmacokinetics, and pharmacodynamics of NLRP3 inflammasome inhibitor, GDC-2394, in healthy volunteers. *Clin Transl Sci.* 2023;16(9):1653-66. doi:10.1111/cts.13576
25. Bakker E, Hendrikse NM, Ehmann F, van der Meer DS, Llinares Garcia J, Vetter T, et al. Biomarker qualification at the European Medicines Agency: A review of biomarker qualification procedures from 2008 to 2020. *Clin Pharmacol Ther.* 2022;112(1):69-80. doi:10.1002/cpt.2554
26. Benatar M, Ostrow LW, Lewcock JW, Bennett F, Shefner J, Bowser R, et al. Biomarker qualification for neurofilament light chain in amyotrophic lateral sclerosis: Theory and practice. *Ann Neurol.* 2024;95(2):211-16. doi:10.1002/ana.26860
27. Umbricht D, Kas MJH, Dawson GR. The role of biomarkers in clinical development of drugs for neuropsychiatric disorders - A pragmatic guide. *Eur Neuropsychopharmacol.* 2024;88:66-77. doi:10.1016/j.euroneuro.2024.08.511
28. Schuck RN, Sekar V. Use of biomarkers in drug development for regulatory purposes. *Clin Transl Sci.* 2025;18(10). doi:10.1111/cts.70377
29. Maurer TS, Smith D, Beaumont K, Di L. Dose predictions for drug design. *J Med Chem.* 2020;63(12):6423-35. doi:10.1021/acs.jmedchem.9b01365
30. Van Rompaey D, Ray Chaudhuri S, Ahmad M, Cisar J, Van Den Bergh A, Ash J, et al. Toward dose prediction at point of design. *J Med Chem.* 2024;67(24):22282-90. doi:10.1021/acs.jmedchem.4c02385
31. Ji Y, Knee D, Chen X, Dang A, Mataraza J, Wolf B, et al. Model-informed drug development for immuno-oncology agonistic anti-GITR antibody GWN323: Dose selection based on MABEL and biologically active dose. *Clin Transl Sci.* 2022;15(9):2218-29. doi:10.1111/cts.13355
32. Lu Y, Liang S, Hong Y, Tajima N, Patel K, Li H, et al. Application of the model-informed drug development paradigm to datopotamab deruxtecan dose selection for late-stage development. *CPT Pharmacometrics Syst Pharmacol.* 2024;13(1):23-28. doi:10.1002/psp4.13058
33. Zhu M, Madia P, Crawford A, Brouwer-Visser J, Krueger P, Haber L, et al. Translational findings support regimen selection for first-in-human study of ubamatamab (MUC16 × CD3 bispecific antibody) in patients with recurrent ovarian cancer. *Clin Transl Sci.* 2024;17(12). doi:10.1111/cts.70082