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Beyond Binding Affinity: Drug–Target Residence Time, Protein Turnover, and Pharmacological Response in Molecular Oncology

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Abstract

Biochemical affinity is an incomplete descriptor of anticancer drug action when drug concentrations, target availability, and signaling states change over time. This critical review examines the conditions under which association and dissociation kinetics improve the interpretation of sustained pharmacological responses and the circumstances in which other processes can produce a similar phenotype. Evidence from biochemical measurements, living-cell engagement assays, covalent-inhibitor studies, oncology models, and pharmacokinetic–pharmacodynamic analyses is evaluated according to the level of response directly measured. In a simple reversible binding model, residence time describes complex persistence; it does not incorporate free-drug exposure, target synthesis, cellular rebinding, or pathway compensation. Covalent modification introduces a distinct dependence on chemical inactivation and protein renewal. Washout experiments are especially informative when residual intracellular drug and assay-specific recovery are independently assessed. Examples from kinase inhibition and KRAS G12C-directed pharmacology show that molecular engagement and tumor-cell response may separate because of state-dependent accessibility and adaptive signaling. A temporal interpretation is therefore most defensible when kinetic parameters are connected to cellular occupancy, target abundance, and downstream recovery through orthogonal experiments. The resulting synthesis outlines how apparently durable inhibition can be attributed without assuming that prolonged binding necessarily predicts clinical efficacy.

Keywords: Target residence time, Cellular target engagement, Protein turnover, Covalent inhibition, Washout Pharmacodynamics, Molecular oncology

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Introduction

Anticancer pharmacology is frequently summarized using a concentration required to inhibit an isolated enzyme or suppress a cellular signal. Such measurements are useful for comparing candidate molecules, yet they compress a dynamic encounter into one value. During treatment, unbound drug concentration changes, proteins are synthesized and degraded, and signaling networks adjust their activity. A compound that remains bound after extracellular concentration falls may consequently behave differently from one with similar equilibrium affinity but faster dissociation. The converse also matters: persistent suppression after washout need not demonstrate unusually slow dissociation. Copeland *et al*. (2006) established residence time as a useful lead-optimization consideration, while Copeland (2016) revisited its opportunities and limitations a decade later. The associated kinetic framework is compelling precisely because biological exposure is rarely constant, not because long residence time is inherently beneficial.

The interpretive difficulty is particularly acute in oncology, where a target's conformation, abundance, and pathway function can change with treatment. Knockenhauer and Copeland (2024) describe why the relationship between complex lifetime and pharmacological effect depends on the relevant system. For kinase-targeted agents, cellular adenosine triphosphate (ATP), compartmental accessibility, and cellular target abundance can alter occupancy relative to a purified-protein assay. An apparent pharmacodynamic delay may reflect retention of active drug in a compartment or rebinding to newly available sites rather than a single stable complex. These alternatives create a practical question: which evidence establishes a kinetic cause of sustained response, and what additional measurements are necessary before extending that conclusion to tumor behavior?

Review Approach and Pharmacological Evidence Assessment

This article uses a targeted critical-review approach rather than an exhaustive systematic review. A question-driven public literature search conducted through 1 November 2026 examined drug–target residence time, binding kinetics, target turnover, washout, cellular engagement, rebinding, and covalent inhibitors in cancer-related settings. Accessible records from PubMed, publisher sites, and full-text repositories were assessed alongside targeted citation chaining. A curated set of 31 peer-reviewed journal publications



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was selected for complementary mechanistic, methodological, modeling, and oncology evidence; that bibliographic allocation is not a claim that 31 papers represent all eligible literature or an exact screening denominator.

Evidence is interpreted at four distinct levels: purified-protein binding; intact-cell target occupancy; signaling or cell-fate response; and tumor or patient outcomes. Primary experiments are privileged for specific observations, while reviews supply conceptual synthesis rather than independent experimental replication. Selection emphasizes studies capable of separating competing temporal explanations, including reports with strong measurement limitations or context-dependent outcomes. Full text was available for many publications, but some were checked only through abstracts or publisher summaries; claims drawn from those records are restricted accordingly. No pooled effect, formal certainty rating, or comprehensive negative-study inventory is implied. This boundary is important because assay choice itself influences what can be inferred: St John-Campbell and Bhalay (2025) catalog distinct engagement technologies, whereas Robers *et al*. (2020) focus on the challenges of interpreting small-molecule occupancy inside living cells.

Binding Kinetics, Exposure, and Target Renewal

The first distinction concerns which clock is being measured. Plasma elimination describes change in systemic concentration; biochemical dissociation concerns the departure of a ligand from a particular molecular complex; and cellular response describes a network that may continue evolving after binding changes. A pharmacological response can outlast measurable circulating drug without any one of these processes being slow in isolation. Gabrielsson and Hjorth (2023) incorporate target renewal into open pharmacological systems, illustrating why exchange with newly produced protein changes interpretation of concentration–response relationships. Temporal reasoning must therefore specify both the drug–target mechanism and the environment in which its parameters were obtained.

Affinity, Association, Dissociation, and Target Engagement

For an idealized, single-step reversible interaction, free drug D and unoccupied target T form a complex DT with association-rate constant k_on and dissociation-rate constant k_off. Under the model's equilibrium and mass-action assumptions, K_d = k_off/k_on. The reciprocal 1/k_off represents the mean complex lifetime only when loss of DT follows a first-order dissociation process without meaningful rebinding or additional kinetic states; it is not equivalent to the dissociation half-life, ln(2)/k_off. Neither quantity alone establishes fractional occupancy under changing free-drug concentration. Knockenhauer and Copeland (2024) emphasize the value of placing these parameters in their time-dependent pharmacological context.

The same affinity can arise from different association and dissociation rates, and kinetics may vary with protein conformation or the assay format. Georgi *et al.* (2018) surveyed binding kinetics across kinase inhibitors, illustrating the heterogeneity hidden by equilibrium comparisons. Berger *et al*. (2021) used structural and kinetic analysis to investigate selectivity between focal adhesion kinase and PYK2; the pertinent lesson is that a kinetic selectivity claim requires its measured structural and experimental conditions, not extrapolation to all kinase contexts. These studies motivate reporting the fitted model, temperature, incubation and competitor conditions alongside rate constants.

Purified-protein affinity may also differ from occupancy in cells. Vasta *et al.* (2018) used a live-cell kinase engagement approach to examine how cellular ATP affects apparent target binding, while Ong *et al.* (2020) connected cellular Bruton's tyrosine kinase engagement measurements to functional readouts in a defined assay system. Such findings support cellular confirmation of candidate mechanisms but do not turn a competitive tracer response into a universal measure of tumor drug action. A live-cell readout may depend on tagged target expression, tracer concentration, drug permeability, and the timing of measurement. Cellular target engagement identifies whether the compound reaches and interacts with the tested target under those conditions; it does not, by itself, quantify apoptosis, durable growth arrest, or clinical response.

Protein Turnover, Rebinding, and Covalent Interactions

A target can lose occupancy because an inhibitor dissociates, because a modified protein is degraded and replaced, or because active drug is no longer available to occupy newly synthesized molecules. These mechanisms require different parameters. For covalent inhibitors, chemical bond formation is often represented by a reversible recognition step followed by inactivation, characterized under stated model assumptions by K_I and k_inact. Unlike the first-order dissociation of an ordinary reversible complex, recovery from effectively irreversible occupancy may require target renewal or another verified reversal process. Ali and Tonge (2026) discuss why this distinction becomes central when complex persistence exceeds changing drug exposure. Bradshaw *et al*. (2015) additionally demonstrate that reversible covalent strategies create a separate class whose residence behavior should not be collapsed into permanently irreversible binding.

Target turnover introduces its own timescale. Gabrielsson and Hjorth (2023) show how renewal can change predicted in vivo potency, while Farhan *et al*. (2026) developed a preclinical modeling framework connecting exposure, covalent occupancy, signaling, and tumor growth. The latter does not establish transferable patient-level predictions: model parameters and validation remain tied to the compounds and experimental systems investigated. Newly synthesized target can restore signaling even while older molecules remain inhibited, and changes in protein abundance can shift the fraction of free target without a change in intrinsic inhibitor chemistry.

A further complication is that released drug may remain near its target. Li and Mitchison (2026) investigated cellular drug retention with a two-drug assay and found that repeated binding to abundant target sites can markedly delay apparent washout. Local rebinding does not mean that the same original molecular complex persists; it means the population of occupied sites can remain high through many binding events. Membrane transport and compartmental retention provide competing routes to the same prolonged occupancy profile. For irreversible compounds, proteome-scale

kinetic profiling can help assess reaction rates and selectivity across accessible proteins, as illustrated by Lin *et al*. (2025). Zaro *et al*. (2016) further show why metabolic lability can affect kinetic selectivity; chemical exposure history and off-target reactivity must be considered alongside a nominal irreversible mechanism.

Table 1 organizes these observables by the assumptions required to interpret them. Its purpose is to prevent measurements from different physical levels being treated as interchangeable evidence of a single drug–target lifetime.

Table 1. Temporal pharmacology measurements, their assumptions, and defensible interpretations

Measurement or assay	What it directly informs	Key conditions or unresolved confounders	Inference permitted and evidence
Reversible k_on, k_off and K_d	Association, dissociation and equilibrium within a fitted binding model	Single-step model; assay temperature; conformational states; rebinding	Complex lifetime is 1/k_off only under first-order non-rebinding assumptions (Knockenhauer & Copeland, 2024).
Intact-cell competitive engagement	Relative occupancy or displacement in the tested cell system	Tracer competition; ATP; permeability; tagged or endogenous target	Evidence of cellular access and interaction, not directly of tumor response (Ong *et al*., 2020; Vasta *et al*., 2018).
Covalent k_inact and K_I	Concentration- and time-dependent chemical target modification	Reversible prebinding model; incubation duration; adduct verification	Inactivation efficiency is not reversible residence time (Ali & Tonge, 2026).
Target abundance and turnover	Supply and loss of available target molecules	Tissue, disease state, synthesis and degradation measurements	Recovery can reflect new protein even while existing target remains modified (Gabrielsson & Hjorth, 2023).
Post-washout occupancy or drug retention	Persistence of measured cellular association after exposure change	Residual free drug; compartmental sequestration; rebinding; assay recovery	Slow washout alone cannot identify molecular dissociation (Li & Mitchison, 2026).
Proteome-wide covalent reactivity	Measured time-dependent target and off-target modification within an assay	Site coverage; chemical concentration; reaction versus abundance	Helps compare biochemical selectivity without establishing clinical durability (Lin *et al*., 2025).

Note. This table is an interpretive synthesis of the cited sources, not a set of pooled measurements. K_d, equilibrium dissociation constant; K_I, inhibitor concentration parameter in the stated covalent model; k_inact, maximal inactivation rate constant; k_on, association-rate constant; k_off, dissociation-rate constant.

From Target Engagement to Tumor-Cell Response

A drug can display persuasive binding kinetics yet fail to produce a persistent downstream response when a pathway is buffered, bypassed, or reactivated. Conversely, cellular inhibition can persist even after direct engagement has substantially recovered if downstream processes resolve more slowly. The evidentiary sequence therefore matters: binding and occupancy are proximal molecular observations; phosphorylation and pathway flux are functional intermediates; proliferation, survival, and tumor growth are increasingly integrated outcomes. Robers *et al*. (2015) established a living-cell bioluminescence resonance energy transfer (BRET) strategy for observing engagement and residence behavior, but their technology does not make these distinct outcome levels equivalent.

Washout Assays, Target Recovery, and Measurement Artifacts

A conventional washout replaces extracellular medium and measures recovery over time. It establishes a change in external exposure, not the instantaneous removal of active intracellular compound. Berges *et al*. (2023) examined MET inhibitors in lung-cancer cell models and identified prolonged engagement and lysosomal retention as explanations for sustained cellular effects. This example demonstrates why persistence after washout must be decomposed into drug-location and binding mechanisms before being assigned to a biochemical off-rate. A delayed signal could also reflect the turnover of phosphorylated pathway intermediates or a downstream response with its own relaxation time.

Method selection can reduce, but not eliminate, this ambiguity. Robers *et al*. (2015) tracked target engagement within intact cells using competitive BRET, with a particular application to an HDAC inhibitor prodrug. For covalent KRAS G12C targeting, Kantae *et al*. (2022) describe endogenous engagement and in-cell kinetic assessment, while Meng *et al*. (2022) report a highly sensitive mass-spectrometric approach for evaluating target engagement in tumor biopsy material. These methods address different analytical questions: engineered live-cell reporters provide time-resolved proxy signals; direct analysis of endogenous proteins can assess modified target in the sampled material; neither independently resolves all free-drug, tissue heterogeneity, or downstream pathway questions. The cited abstracts do not warrant inventing cross-platform equivalence or unreported sensitivity thresholds.

An interpretable washout experiment should measure extracellular and intracellular unbound drug where technically feasible, confirm target engagement separately from downstream activity, and evaluate target abundance during recovery. A second inhibitor competing for the same site can help assess rebinding if its assumptions are satisfied, as shown by Li and Mitchison (2026). Orthogonal controls are essential because a tracer can perturb occupancy and a tissue homogenate can erase spatial segregation. As St John-Campbell and Bhalay (2025) emphasize, an assay's

physical readout must be matched to the question being asked rather than treated as an interchangeable proxy for drug response.

Pathway Adaptation and Translation across Biological Settings

Sustained occupancy is only one input into oncology response. KRAS G12C inhibitors illustrate the importance of target state and network adaptation because many bind a particular conformational or nucleotide state. The mechanistic synthesis by Ostrem and Shokat (2022) explains why accessibility of the relevant target state shapes inhibitor action. Huynh *et al*. (2022) further characterize kinetic and redox influences on KRAS G12C covalent inhibition, showing that chemical reactivity cannot be assumed constant across every biological environment. The binding assay, oxidation state, and pathway context must therefore travel with any claim of persistence.

At the cellular scale, Xue *et al*. (2020) reported non-uniform adaptation to conformation-specific KRAS G12C inhibition, including a role for newly synthesized target in escaping suppression. Ryan *et al*. (2020) examined adaptive pathway reactivation and the rationale for combined pathway inhibition. These observations do not refute the value of kinetic measurements; they show that a long-lived drug–target interaction cannot automatically prevent a cell from activating signaling through newly available protein or parallel circuitry. Differences among cell populations further mean that average target occupancy may obscure a subpopulation whose signaling or proliferative behavior diverges.

The translational boundary is equally important. Canon *et al*. (2019) reported antitumor activity and immune effects of a KRAS G12C inhibitor in particular experimental models; those findings cannot identify the clinical contribution of residence time alone. Linking a microscopic rate constant to tumor control requires evidence for local exposure, target-state availability, the kinetics of recovery, and the biological setting. The next section therefore develops a temporal validation strategy designed to discriminate these rival explanations without presuming that any one measured clock is dominant.

A Temporal Evidence and Validation Synthesis

Sustained suppression should first be described as an observation with an explicit endpoint and clock: after extracellular washout, does the assay detect bound compound, inhibition of a molecular target, a pathway change, or continued loss of tumor-cell viability? These outcomes cannot be interchanged. Berges *et al*. (2023) connected prolonged cellular effects of MET inhibitors with both target engagement and lysosomal retention, illustrating the danger of using washout persistence as a direct estimate of biochemical residence time. Conversely, Li and Mitchison (2026) experimentally explored how abundant cellular targets can promote drug rebinding. Neither mechanism should be assumed to operate at the same magnitude in an unrelated tumor model.

A temporal comparison must establish a common exposure origin, then distinguish four parallel measurements: available drug, occupied target, total competent target, and downstream pathway response. For a reversible compound, occupancy may decline as drug dissociates, but continued intracellular exposure or rebinding can replenish complexes. For an effectively irreversible covalent inhibitor, removal of free drug does not necessarily restore an already modified target; recovery can instead reflect replacement of that protein. Adaptation provides a fourth trajectory: a pathway may regain activity despite continuing engagement of the originally inhibited target. The synchronized qualitative lanes in **Figure 1** represent these alternative interpretations, not measured time-series or universal kinetic ordering.

The temporal origin also requires careful definition. A nominal media exchange can mark washout in a cell experiment, whereas a declining plasma concentration marks only a systemic exposure transition in vivo. If target abundance changes during that interval, an apparent fall in percentage engagement can coexist with stable absolute amounts of modified protein. Conversely, absolute engagement may remain substantial while a newly synthesized signaling-competent fraction drives recovery. The denominator should therefore be specified whenever occupancy is compared across conditions. Lin *et al*. (2025) illustrate how covalent inhibition kinetics can be profiled across a proteome, but such comparative chemistry is not by itself a longitudinal measurement of newly synthesized target in a tumor.

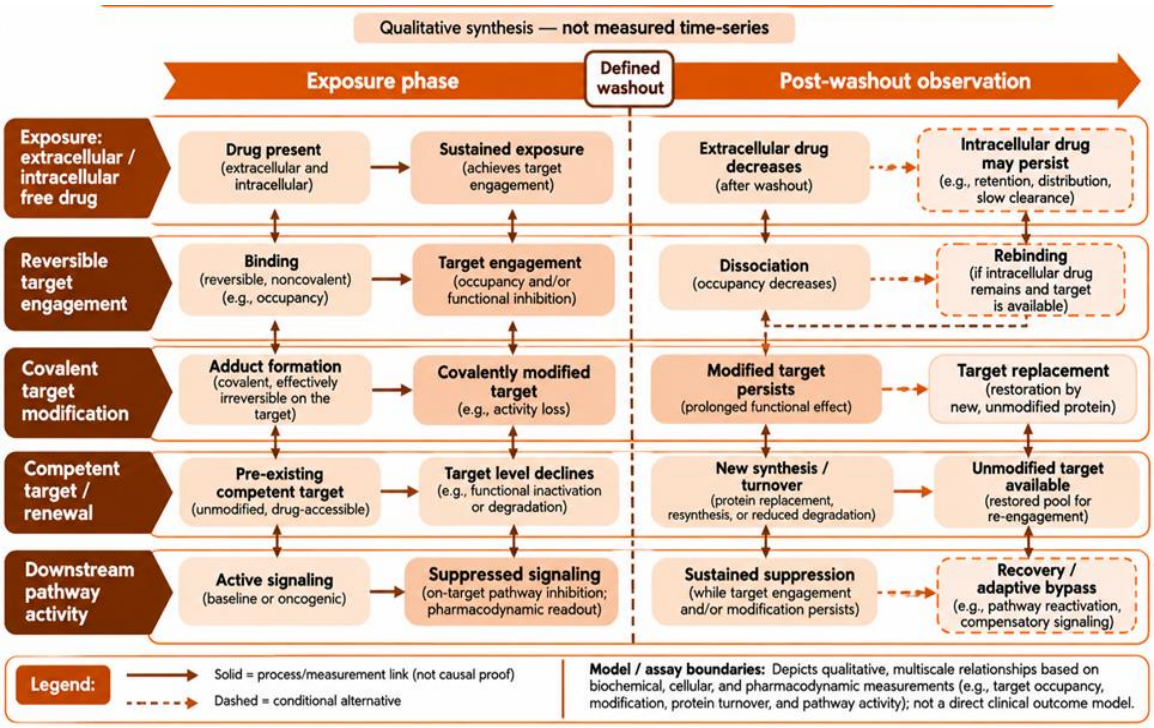


Figure 1. Distinguishing the temporal clocks of persistent anticancer drug action.

Note. Conceptual synthesis, not an empirical time-course. Read left to right across exposure, molecular engagement, target renewal, and cellular signaling lanes. Solid connectors indicate the interpretive sequence of separately measurable processes, not proven causality. Dashed connectors mark conditional alternative routes, including residual intracellular drug, rebinding, covalent persistence, and adaptive bypass. "Washout" denotes an experimentally defined perturbation, not verified elimination of intracellular drug. Evidence informing the distinctions includes Berges *et al*. (2023), Ryan *et al*. (2020), and Xue *et al*. (2020).

A discriminating experiment would pair a measured extracellular washout with time-resolved intracellular exposure, orthogonal occupancy assays, protein-abundance measurements, and downstream signaling. It would then perturb the suspected explanation rather than infer mechanism from similar curves. Zecha *et al*. (2023) demonstrate the value of resolving drug-responsive protein modifications over dose and time; their proteomic readouts characterize signaling consequences, not ligand residence directly. **Table 2** organizes feasible comparisons as proposed validation logic rather than presenting new experimental outcomes.

Table 2. Rival causes of apparent pharmacological persistence and discriminating experiments.

Candidate explanation	Pattern that might prompt investigation	Proposed orthogonal test and interpretive limit
Slow reversible dissociation	Occupancy persists after extracellular drug falls	Measure biochemical off-rate under an explicit model; verify intracellular free drug and rebinding controls (Knockenhauer & Copeland, 2024).
Intracellular retention or rebinding	Washout response outlasts extracellular exposure	Quantify intracellular drug or use competition designed to test rebinding; avoid equating total cellular drug with free drug (Berges *et al*., 2023; Li & Mitchison, 2026).
Persistent covalent modification	Occupancy remains when unbound drug is low	Assay adduct abundance and newly synthesized unmodified target separately; a covalent label does not identify its recovery mechanism (Ali & Tonge, 2026).
Target renewal	Engagement falls as competent target appears	Follow target abundance, turnover, and occupancy together; perturb synthesis only with toxicity controls (Gabrielsson & Hjorth, 2023).
Adaptive or bypass signaling	Pathway output returns despite continued target engagement	Co-measure occupancy and pathway nodes, then test a causal feedback intervention in the same model (Ryan *et al*., 2020; Xue *et al*., 2020).

Note. The experimental tests are this review's prospective synthesis, not a claim that every cited study performed every proposed control. Each row describes a hypothesis to evaluate, not a diagnostic fingerprint.

A particularly informative test is whether the proposed mechanism survives a perturbation designed to remove that mechanism. A retention explanation becomes weaker if persistence remains after intracellular available drug is demonstrably depleted; a rebinding explanation is weakened if a properly validated competitor cannot alter post-washout occupancy. For covalent compounds, observed

recovery should be interpreted alongside synthesis and degradation rather than fitted automatically to a reversible off-rate. These are conditional contrasts, not universal pass/fail rules: any added competitor, protein-synthesis intervention, or cell-handling step can itself change the biological system. A rigorous report should include controls for the perturbation's independent effect and retain inconclusive results.

Results and Discussion

Kinetic data add the greatest interpretive value when competing molecules are compared under defined protein states and when binding measurements are connected to cellular measurements without assuming that the assays are equivalent. Bravo *et al*. (2025) show that structural interactions can be used to probe changes in Bruton's tyrosine kinase binding kinetics, whereas Corrionero *et al*. (2025) compare enzyme inhibition kinetics, selectivity, and activity across BTK inhibitors in specific in vitro settings. Together these reports justify mechanistic profiling, not a claim that one off-rate predicts response across all B-cell malignancies. In another kinase context, Corrionero *et al*. (2026) characterize distinct kinetic signatures across KIT activation states; their mechanistic and clinical interpretation must remain bounded by the kinase conformations and evidence available in that report.

Clinical translation requires still more independent information. Janes *et al*. (2018) established pharmacological feasibility of covalent KRAS G12C targeting in defined preclinical models, and Hallin *et al*. (2020) described responses to MRTX849 across experimental systems and early patient observations. Neither study isolates dissociation rate as the determinant of individual therapeutic benefit. Exposure distribution, target-state access, molecular adaptation, and tumor heterogeneity remain alternative explanations. Pharmacokinetic–pharmacodynamic frameworks can link occupancy to efficacy only to the extent that their target renewal terms, exposure inputs, and response measurements are actually constrained by data, as illustrated by Farhan *et al*. (2026).

The useful next step is thus not to rank anticancer agents by residence time alone but to design comparisons where an apparent mechanistic advantage could be falsified. Align sampling times and drug exposure; demonstrate that a kinetic difference persists in intact cells; test the recovery mechanism; and establish whether measured pathway changes explain a tumor endpoint. Such work can identify when kinetic selectivity offers an advantage while still allowing negative findings or a more parsimonious explanation based on retention, turnover, or network plasticity.

Two kinds of discordance are especially instructive. If biochemical dissociation is slow but cellular occupancy rapidly disappears, the next question concerns cell context: target state, ATP competition, efflux, assay interference, and target replacement should be examined before discarding the biochemical finding. If occupancy remains high while downstream signaling rebounds, the priority shifts to bypass routes, compensatory target activation, and heterogeneity rather than to further optimization of an off-rate already shown to be durable. This decision sequence focuses experiments on the point where inference breaks down. It also makes a null kinetic association useful: an adequate occupancy assay can demonstrate that a hypothesized residence-time advantage fails to propagate into the tested biological endpoint.

Limitations

This targeted review was purposefully selected, not exhaustively screened, and does not estimate pooled effects. Several publications were accessible only through abstracts or publisher summaries, restricting interpretation of method-specific controls and unreported negative results. Purified-protein kinetic constants are sensitive to model fitting, assay temperature, target conformation, and ligand depletion; cellular occupancy assays can depend on reporter abundance or competition artifacts. Intratumoral free exposure and spatial heterogeneity are often less directly measured than plasma concentrations. Finally, conceptual links in **Figure 1** and proposed controls in **Table 2** are a validation agenda, not data demonstrating clinical causality. Future comparisons should preserve unfavorable and context-dependent results instead of reporting only durable responders.

No single assay architecture establishes all four temporal quantities, and cross-platform comparisons may introduce additional calibration error. The perspective also centers small-molecule kinase and KRAS systems; it should not be read as evidence that the same recovery mechanisms dominate across biologics, immunotherapies, or targets with different stoichiometry. Detailed assessment of adverse effects, tumor evolution over repeated treatment cycles, and patient-level outcomes lies outside the available mechanistic evidence mapped here.

Conclusion

Residence time helps explain persistent cancer-drug action when a defined binding mechanism can be connected to changing exposure, cellular target occupancy, target renewal, and pathway recovery. It is insufficient when drug retention, rebinding, irreversible modification, adaptive signaling, or assay artifacts remain unresolved. Mechanism-matched temporal measurements and causal perturbations offer a stronger basis for interpretation than equilibrium affinity or washout persistence alone. The resulting conclusions should remain specific to the molecule, target state, experimental model, and outcome measured.

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