Available online **www.ijpras.com**

International Journal of Pharmaceutical Research & Allied Sciences, 2026, 15(3):134-145
https://doi.org/10.51847/K5na4jHe17

Original Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Biopharmaceutical Quality Attributes Should Be Ranked by Clinical Consequence Rather Than Analytical Detectability

Nokuthula Dlamini[1]*, Sipho Zulu[1], Thabo Nkosi[2], Lerato Molefe[3]

[1]Department of Biopharmaceutical Quality and Clinical Consequence, Faculty of Pharmacy, University of KwaZulu-Natal, Durban, South Africa.
[2]Department of Analytical Detectability and Product Safety, Faculty of Pharmacy, University of Cape Town, Cape Town, South Africa.
[3]Department of Quality Attribute Ranking, Faculty of Pharmaceutical Sciences, University of the Witwatersrand, Johannesburg, South Africa.

***Email:** dlaminin@ukzn.ac.za*

ABSTRACT

Biopharmaceutical characterization can now resolve molecular heterogeneity at a level that was inaccessible during earlier generations of therapeutic-protein development. This analytical progress is essential for process understanding, comparability and biosimilarity, but it creates a recurring interpretive problem: an attribute can be measured reproducibly without being equally important to product performance or patient outcome. Treating detectability as a surrogate for criticality risks over-prioritizing analytically conspicuous changes while underweighting less visible attributes with stronger links to potency, exposure, immunogenicity or safety. This Current Opinion develops a risk framework in which quality attributes are interpreted through consequence rather than analytical prominence. Structural differences, glycosylation, charge variants, aggregation, particles and functional attributes are considered as distinct evidence problems rather than interchangeable signals of product quality. Particular emphasis is placed on discordant cases in which measurable differences do not produce corresponding functional or clinical effects, or in which apparently modest analytical differences acquire importance because they alter mechanism-relevant activity, pharmacokinetics or immunogenicity. The proposed approach does not replace analytical similarity assessment. Instead, it positions analytical observation as the entry point to a sequence of consequence-oriented validation steps spanning molecular mechanism, function, exposure and clinical relevance. Ranking therefore becomes product-, mechanism-, lifecycle- and context-dependent rather than a fixed hierarchy based on assay sensitivity.

Keywords: *Biopharmaceutical quality attributes, Critical quality attributes, Biosimilarity, Analytical comparability, Immunogenicity, Clinical consequence*

Received: 13 July 2026; **Revised:** 24 September 2026; **Accepted:** 28 September 2026

INTRODUCTION

Analytical characterization has become one of the strongest components of contemporary biosimilar and biopharmaceutical development. Successful comparative programs increasingly combine high-resolution structural methods with orthogonal functional assays so that residual uncertainty can be reduced before reliance is placed on less sensitive clinical comparisons [1, 2]. This evolution is scientifically justified: many structural and biochemical attributes can now be characterized with far greater precision than was possible when conventional clinical comparability paradigms were established.

© 2026 The International Journal of Pharmaceutical Research and Allied Sciences (IJPRAS). **Open Access** - This article is under the **CC BY NC SA** license (https://creativecommons.org/licenses/by-nc-sa/4.0).

The difficulty is that analytical sensitivity and clinical importance answer different questions. Criticality has historically been framed around the potential impact of an attribute on safety or efficacy together with the uncertainty surrounding that impact, with analytical tiering applied only after that assessment [3]. This distinction remains fundamental. The ability to separate two products analytically does not itself specify whether the observed difference alters molecular function, pharmacokinetics, immunogenicity, efficacy or safety.

Statistical treatment creates a second boundary. Analytical similarity methods can determine whether an observed difference is compatible with a predefined reference distribution or risk-sensitive acceptance framework, but the resulting statistical classification is not a direct measure of patient consequence [4]. A small but mechanism-critical change may matter despite apparently favorable statistical behavior, whereas a highly significant analytical difference may be clinically unimportant if it does not affect function or exposure.

This article therefore argues that quality attributes should be ranked primarily by the strength and severity of their plausible or demonstrated clinical consequences rather than by how readily modern assays detect them. The argument is not that analytical sensitivity is undesirable. It is that detectability should initiate investigation rather than complete interpretation. The proposed framework separates analytical observation, mechanistic plausibility, functional consequence, exposure consequence and clinical consequence so that evidence from different biological and pharmaceutical levels is not collapsed into a single notion of "difference."

Detectability is not clinical importance

Modern analytical platforms can reveal multiple dimensions of product heterogeneity simultaneously. Multi-attribute LC-MS approaches, for example, can produce quality information that corresponds with conventional chromatographic measurements, while direct binding assays can test whether perturbations in structural attributes alter interaction with a biologically relevant target [5, 6]. These two capabilities should not be treated as equivalent. The first establishes that a molecular state can be measured consistently and related to other analytical readouts; the second begins to test whether that state changes a function linked to product activity.

The distinction becomes especially important as analytical resolution improves. Near-native capillary electrophoresis–mass spectrometry can separate and identify acidic and basic monoclonal-antibody variants, post-translational modifications and glycoform-associated heterogeneity with considerable molecular detail [7]. Such resolution is valuable for characterization and process control, but resolution alone does not establish the clinical criticality of every resolved species.

A consequence-oriented interpretation therefore requires at least two questions after an analytical difference is observed. First, is there a biologically credible pathway by which the attribute could alter product behavior? Second, is that pathway supported by functional, exposure or clinical evidence at a level appropriate to the decision being made? An analytically visible difference with neither mechanistic plausibility nor functional effect should not automatically receive the same priority as an attribute repeatedly linked to potency, clearance or immunogenicity.

This separation also protects against the opposite error. Low analytical abundance or modest deviation does not imply low importance when the affected attribute controls a sensitive biological function. Detectability is thus best considered an evidence-entry condition. Criticality depends on what the measured difference does, under which product and patient conditions, and with what degree of evidentiary confidence.

Structural attributes

Structural characterization is often the first location in which the distinction between difference and consequence becomes visible. Comparative evaluation of trastuzumab products has shown that biosimilars can exhibit differences in size variants, charge-related attributes or glycosylation while remaining broadly similar across multiple functional measurements [8]. The appropriate interpretation is not that structural differences are unimportant, but that structural observations require a structure–function assessment before they can be translated into a consequence claim.

A second complication is that the reference product itself is not analytically static. Longitudinal evaluation of Herceptin lots demonstrated changes in Fc-related quality characteristics over time, followed by later recovery toward earlier patterns [9]. Such lifecycle behavior can alter the reference range against which a candidate product is assessed. A detected deviation may therefore represent a true product difference, temporal reference variability, manufacturing evolution, or some combination of these factors.

Post-approval characterization reinforces the need for contextual interpretation. Multilot analyses of marketed reference products and biosimilars have identified product-specific attributes that can fall outside observed

reference ranges even when no clear overall pattern of continuing drift is evident [10]. An out-of-range result should consequently be treated as an investigation trigger rather than as an automatic declaration of clinical non-similarity.

For structural attributes, the proposed consequence logic is therefore sequential but not rigidly linear. Analytical deviation establishes that products differ at a measured molecular level. Mechanistic knowledge then determines whether that difference could affect folding, stability, receptor interaction, Fc-mediated activity, clearance or another relevant pathway. Functional testing evaluates the most plausible pathways, and higher-level evidence determines whether remaining uncertainty warrants additional development, control or clinical investigation.

Glycosylation and charge variants

Glycosylation provides a strong example of why broad attribute categories should not be assigned uniform criticality. Regulatory analyses of therapeutic antibodies show that glycan composition can influence properties relevant to efficacy, safety and pharmacokinetics, but the significance of individual glycans depends on antibody architecture and mechanism of action [11]. A glycan involved in Fc-mediated effector activity may be highly consequential for one molecule while being much less important for another whose principal therapeutic effect is driven by Fab-mediated neutralization.

Analytical glycan differences therefore require biological interpretation. Glycosylation is intrinsically heterogeneous, and available methods can characterize that heterogeneity with increasing precision, but analytical divergence does not translate automatically into altered clinical performance [12]. The relevant question is whether the observed glycan state modifies a mechanism that contributes materially to the product's therapeutic or safety profile.

Charge variants present a related but distinct problem. Stress studies of bevacizumab and trastuzumab demonstrate that charge states can differ in degradation and aggregation propensity and that the direction and magnitude of these differences depend on the molecule and imposed stress [13]. Charge heterogeneity may therefore matter because it signals a stability pathway, a chemical modification or a functionally relevant molecular state rather than because "charge difference" is itself uniformly critical.

At the same time, modern methods can resolve charge variants even in analytically demanding co-formulations containing multiple monoclonal antibodies [14]. Such capability is important for process monitoring and stability assessment, yet strong precision, specificity or resolution remains a statement about assay performance. It does not determine by itself whether each detected species requires the same level of control or clinical concern.

A consequence-based interpretation of glycosylation and charge therefore avoids category-level ranking. It asks which molecular species are present, what process or degradation state generated them, which biologically relevant functions they could modify, whether those functions are affected at the observed magnitude, and whether any effect persists at the level of exposure or clinical outcome.

Aggregation and particles

Aggregation is often treated as intrinsically high risk because protein aggregates can reflect instability, loss of native structure and potential immune stimulation. The evidence, however, supports a more conditional interpretation. Thermal stress, air–liquid interfaces and mechanical agitation can generate heterogeneous aggregate populations, and process variables such as stirring conditions can materially alter submicron and subvisible particle formation; yet animal studies also show that the amount of aggregation alone may not determine the magnitude of an immune response under a particular route and formulation context [15-17]. Aggregation therefore cannot be reduced to a binary distinction between "detected" and "clinically dangerous."

The physical state and burden of particles matter. Translational work comparing aggregate exposure across in vitro, animal and clinical settings indicates that pronounced immune effects can require substantially greater aggregate burdens than those encountered under representative administration-associated conditions [18]. This finding should not be converted into a universal numerical safety threshold. Rather, it demonstrates that particle number, aggregate state, route of administration and biological context modify consequence.

The method by which aggregates are generated also changes interpretation. Process-relevant or spontaneously occurring aggregates have produced limited donor-dependent innate immune responses in human-cell systems without the adaptive responses observed or anticipated from more extreme stressed preparations [19]. Such results caution against extrapolating from artificially harsh stress models directly to routine manufacturing or administration conditions.

The practical consequence is that an aggregation signal should be decomposed into a lifecycle decision case: what was detected; how the aggregate or particle was generated; what physical or structural state it occupies; whether it alters potency or another mechanism-relevant function; what exposure the patient is likely to experience; and whether immune or clinical effects are demonstrated, plausible or merely theoretical.

The distinctions required for this interpretation are easier to compare explicitly than in prose alone. **Table 1** therefore separates analytically observed particle states from the unresolved inferences and validation evidence that determine whether escalation in quality concern is warranted.

Table 1. Discriminating aggregation and particle signals that warrant escalation in biopharmaceutical quality assessment

Scientific configuration	Observable support	Mechanistic test and consequence
What remains unresolved / Comparability interpretation	**What is analytically observed**	**Lifecycle decision case / Evidence that would discriminate competing interpretations / Clinical-consequence synthesis**
What remains unresolved: Whether the signal reflects reversible association, irreversible structural damage, or functionally consequential species Comparability interpretation: A difference merits investigation, but stress sensitivity alone does not establish clinical non-comparability	Increased aggregate or particle signal after defined stress	**Lifecycle decision case:** Thermal or interfacial stress produces additional aggregate species **Evidence that would discriminate competing interpretations:** Structural characterization plus mechanism-relevant functional testing under matched stress conditions **Clinical-consequence synthesis:** Proposed: rank increases when the aggregate state is linked to loss of function, clinically relevant exposure, or biologically plausible safety consequence
What remains unresolved: Whether the detected particles are representative of routine manufacturing and administration exposure Comparability interpretation: Process dependence should trigger control and root-cause assessment	Particle burden changes with process or handling state	**Lifecycle decision case:** Mechanical or handling conditions increase subvisible or submicron particles **Evidence that would discriminate competing interpretations:** Process mapping, orthogonal particle characterization and realistic-use exposure assessment **Clinical-consequence synthesis:** Proposed: consequence rank depends on patient exposure and particle state, not particle detectability alone
What remains unresolved: Whether current assays lack sensitivity or the particles are genuinely low consequence Comparability interpretation: Analytical discordance remains real but should not automatically be translated into clinical risk	Analytical difference with weak or absent biological response	**Lifecycle decision case:** Detectable particles occur without a corresponding immune or functional signal **Evidence that would discriminate competing interpretations:** Orthogonal functional, innate-immune and exposure-relevant testing **Clinical-consequence synthesis:** Proposed: retain uncertainty while avoiding automatic high-criticality classification
What remains unresolved: Whether extreme stress models represent the product states encountered in practice Comparability interpretation: Stress-model findings should not be transferred uncritically to routine product conditions	Distinct aggregate populations generated by different conditions	**Lifecycle decision case:** Process-relevant aggregates differ from highly stressed preparations **Evidence that would discriminate competing interpretations:** Structural comparison of aggregate states and matched biological testing **Clinical-consequence synthesis:** Proposed: evidence from realistic lifecycle states receives greater decision weight than hazard signals produced only under remote stress conditions
What remains unresolved: Whether route, formulation or tissue exposure is driving the observed immune response Comparability interpretation: Product attribute and administration context must be evaluated together	Similar product identity but different local or systemic exposure context	**Lifecycle decision case:** Aggregate burden differs across route or formulation conditions **Evidence that would discriminate competing interpretations:** Route-specific exposure and immune-response assessment **Clinical-consequence synthesis:** Proposed: criticality is context dependent when the same analytical state produces different biological consequences under different exposure conditions

Aggregation therefore provides the clearest demonstration of why quality-attribute ranking needs cross-scale validation rather than a single analytical threshold. **Figure 1** translates the section's evidence into a consequence map in which analytical detection, physical state, function, exposure and immune or clinical consequence remain

separate, while uncertainty can return the assessment to earlier assumptions rather than forcing every signal forward.

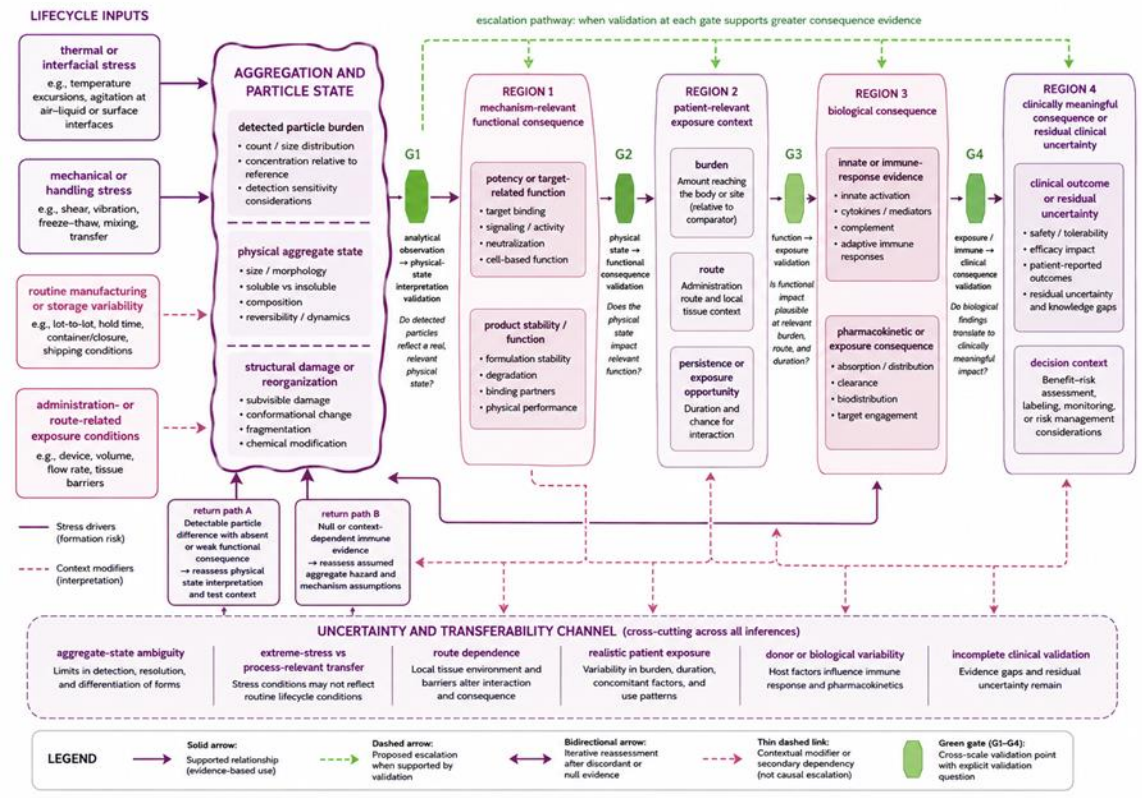


Figure 1. Aggregation-related quality risk is determined by cross-scale validation rather than particle detection alone

Potency and functional attributes

Functional characterization provides the most direct test of whether an analytical difference affects the mechanism by which a biopharmaceutical is expected to work. For monoclonal antibodies, relevant assays may include target binding, receptor competition, neutralization, Fc-receptor engagement, cell-based activity or other mechanism-specific readouts. No single method is universally sufficient, because different assays interrogate different biological steps and possess different sensitivity and variability characteristics [20].

The importance of function is illustrated by cases in which analytically measurable differences do not translate into altered biological activity. Comparative structure–activity characterization of BAT1806/BIIB800 and reference tocilizumab identified differences in several molecular attributes, yet targeted experiments did not demonstrate corresponding impairment of the tested target-binding or potency functions [21]. This is not evidence that analytical differences can be ignored. It is evidence that direct functional interrogation can downgrade an initial concern when the suspected consequence pathway is not supported.

A consequence-based ranking should therefore privilege functionally validated pathways over analytical magnitude alone. An attribute with modest analytical deviation but a demonstrated effect on a mechanism central to efficacy may deserve high priority. Conversely, a conspicuous chromatographic or mass-spectrometric difference may warrant lower consequence ranking if appropriately sensitive functional studies repeatedly fail to demonstrate an effect and no alternative mechanism of concern remains credible.

Functional evidence itself remains bounded. Cell-based potency or receptor-binding measurements do not automatically establish clinical equivalence, and complex therapeutics may have multiple mechanisms that cannot be reduced to one assay. The purpose of the functional layer is to determine whether molecular heterogeneity has crossed from analytical difference into biologically meaningful product behavior.

PK and immunogenicity consequences

Clinical consequence frequently becomes clearer only when quality attributes are considered through exposure. Immunogenicity is an important example because anti-drug antibody detection is an intermediate biological event rather than a clinical outcome. The significance of an immune response depends on its magnitude, persistence and

relationship to drug concentration, clearance, pharmacodynamic effect, efficacy or safety [22]. A positive ADA result therefore has greater consequence when it is accompanied by altered exposure or clinically relevant response than when it occurs without measurable pharmacological effect.

Even immunogenicity measurements themselves have context-dependent interpretation. Analysis of two adalimumab clinical datasets found a strong overall relationship between signal-to-noise ratio and ADA titer, but the relationship changed with time and circulating drug concentration [23]. A measurement that appears interchangeable at one level of analysis may therefore behave differently after pharmacokinetic or temporal stratification.

Regulatory experience with monoclonal-antibody biosimilars similarly supports a totality-of-evidence interpretation. FDA review analyses show that ADA incidence, neutralizing-antibody findings, pharmacokinetics, switching information and broader clinical context are considered together rather than treating a nominal immunogenicity difference as independently decisive [24]. This approach is compatible with consequence-based CQA ranking because it asks whether a product difference propagates into exposure or clinical behavior.

Recent ustekinumab biosimilar experience provides an additional cross-scale example. Comparative analytical characterization and single-dose pharmacokinetics were sufficiently sensitive in that product context to support conclusions that aligned with comparative immunogenicity evidence, although absolute ADA rates across different development programs remained difficult to compare because assay methods differed [25]. The lesson is not that pharmacokinetic studies or analytical characterization universally replace comparative clinical studies. It is that lower-level evidence becomes more decision-relevant when it is demonstrably connected to a clinically meaningful pathway.

For quality-attribute ranking, PK and immunogenicity should therefore be treated as consequence amplifiers rather than as independent checkboxes. An attribute moves upward in clinical priority when a credible molecular or functional effect is accompanied by altered exposure, persistent immune response, reduced pharmacological activity or another plausible patient-level consequence. When analytical or immunogenicity differences do not propagate through those levels, uncertainty should be retained, but criticality should not be inflated solely because a sensitive assay can detect the difference.

Evidence linking attributes to outcomes

The evidentiary problem is not simply whether a quality attribute can be measured, but how far its consequence can be followed across biological and pharmaceutical levels. Cell-based assays that detect innate immune-response-modulating impurities illustrate an intermediate evidentiary layer: they move beyond chemical detection by asking whether a measured product difference elicits a biological response [26]. Such assays strengthen causal plausibility, but they remain in vitro models and cannot by themselves establish human immunogenicity or clinical harm.

The strongest evidence chains therefore contain linked observations rather than isolated assay signals. A molecular difference becomes more compelling when a mechanism-relevant function changes; a functional difference becomes more consequential when exposure is altered; and a pharmacokinetic or immunogenicity effect gains clinical importance when it affects efficacy, safety or another patient-relevant endpoint. At each step, however, transfer between evidence levels must be demonstrated rather than assumed.

Product evidence also shows why discordance should be preserved rather than normalized away. Post-approval analytical characterization has identified attributes outside reference ranges in some products without demonstrating a corresponding universal clinical consequence [10]. In a separate product system, analytically detectable differences in a tocilizumab biosimilar were investigated directly and did not impair the tested binding or potency functions [21]. Ustekinumab experience, by contrast, illustrates how analytical characterization and pharmacokinetic evidence can converge with comparative immunogenicity conclusions in a particular molecule and development context [25].

These examples define three distinct evidentiary states. In the first, a measured difference remains unresolved because appropriate downstream testing is incomplete. In the second, downstream evidence reduces concern because a plausible consequence pathway is not supported. In the third, multiple evidence layers align and increase confidence that an attribute does or does not matter clinically. The article therefore treats evidence linking as directional but reversible: failure to observe a downstream effect can legitimately lower consequence rank, provided that the relevant assay, exposure state, population and mechanism have been interrogated adequately.

This distinction is especially important for attributes that are analytically prominent but biologically remote from the therapeutic mechanism. Their control may remain necessary for manufacturing consistency or lifecycle

monitoring, yet their clinical-consequence rank should not be inflated merely because the analytical platform detects them reproducibly.

Clinical-consequence ranking

Analytical similarity and clinical-consequence ranking should be treated as different decision spaces. Statistical methods can determine whether a candidate product falls within a predefined analytical similarity construct, but the answer depends partly on the statistical definition, reference variability and acceptance logic used [27]. A similarity conclusion is therefore a statement about the observed analytical comparison under a specified method; it is not a direct estimate of patient risk.

More sophisticated analytical statistics improve the reliability of that comparison without changing its conceptual scope. Bootstrap-based methods can address operating characteristics and error control in analytical similarity testing, but even a statistically well-behaved decision rule does not determine whether a measured attribute affects safety, efficacy or exposure [28]. Statistical validity should therefore be considered a reliability layer beneath, rather than a substitute for, clinical-consequence interpretation.

The ranking proposed here begins with the severity and plausibility of the consequence pathway, not with the magnitude of the analytical signal. Existing CQA frameworks already support incorporating potential clinical impact and uncertainty into criticality assessment [3]. The present contribution extends that principle across evidence levels by separating five questions: whether an attribute is analytically different; whether the difference has a credible mechanistic consequence; whether the relevant function is altered; whether patient exposure or immune response is affected; and whether a clinically meaningful outcome is demonstrated or strongly supported.

Aggregation evidence shows why this layered approach matters. Particle burden and biological context can substantially modify immune consequence, so an analytical observation cannot be converted into a fixed risk category without considering the state and exposure of the particles [18]. Immunogenicity creates the same problem at a different level: ADA detection may be important, but clinical interpretation depends on its relationship to pharmacokinetics, persistence and response [22]. Statistical analytical similarity likewise remains method-dependent and cannot substitute for these downstream layers [27].

The resulting hierarchy is deliberately non-numerical. No universal weighting is proposed, because the importance of a glycan, charge variant, aggregate, potency change or immune response depends on product architecture, mechanism of action, route, dose, treatment duration, patient population and lifecycle state. The article therefore proposes clinical-consequence rank as an ordinal reasoning construct rather than a score. Higher rank requires stronger evidence that an attribute can propagate through biologically relevant levels toward patient consequence; lower rank reflects either weak mechanistic plausibility, negative downstream testing, or evidence that the measured difference remains clinically remote.

Table 2 translates that reasoning into a design-space constraint map for recurrent quality-attribute discordance cases. It is intended to show where escalation is justified, where uncertainty should be retained, and where analytical difference should not be mistaken for demonstrated clinical importance.

Table 2. Design-space constraints for ranking discordant biopharmaceutical quality attributes by clinical consequence

Observed configuration	Mechanism and competing diagnosis	Corrective discrimination	
Evidence gap that prevents confident ranking / Unresolved article question	**Lifecycle trigger / Product attribute state**	**Clinical-consequence inference scenario / Evidence layer that carries the decision**	**Non-negotiable decision boundary**
Evidence gap that prevents confident ranking: Alternative function or exposure pathway not yet excluded **Unresolved article question: Proposed: when is negative functional evidence sufficient to reduce consequence rank?**	**Lifecycle trigger:** New process, comparability exercise, post-approval drift signal **Product attribute state:** Clearly detectable structural or compositional difference	**Clinical-consequence inference scenario:** Large analytical deviation with no demonstrated functional effect **Evidence layer that carries the decision:** Mechanism-relevant functional testing	Analytical magnitude alone cannot establish clinical criticality

Evidence gap that prevents confident ranking: Clinical exposure consequence may remain unmeasured **Unresolved article question: Proposed: how much mechanistic evidence is enough to escalate before clinical confirmation?**	**Lifecycle trigger:** Minor manufacturing or formulation shift **Product attribute state:** Modest change in a high-leverage attribute	**Clinical-consequence inference scenario:** Small analytical deviation affecting a mechanism-critical function **Evidence layer that carries the decision:** Direct functional evidence	Small magnitude does not justify low rank when the affected mechanism is central
Evidence gap that prevents confident ranking: Transfer from stress model to patient exposure remains uncertain **Unresolved article question: Proposed: which lifecycle evidence should dominate when analytical and biological signals disagree?**	**Lifecycle trigger:** Handling, shipping, device, storage or administration change **Product attribute state:** Increased particle burden	**Clinical-consequence inference scenario:** Particle or aggregate increase without corresponding biological response **Evidence layer that carries the decision:** Particle state plus realistic exposure and biological testing	Extreme-stress hazard cannot be transferred automatically to routine-use conditions
Evidence gap that prevents confident ranking: Direct clinical outcome may still be absent **Unresolved article question: Proposed: when does altered exposure become sufficient evidence of clinically relevant criticality?**	**Lifecycle trigger:** Process or product change with exposure shift **Product attribute state:** Attribute difference with plausible clearance or distribution pathway	**Clinical-consequence inference scenario:** Analytical difference accompanied by altered pharmacokinetics **Evidence layer that carries the decision:** PK/exposure evidence	Exposure consequence carries more decision weight than detectability alone
Evidence gap that prevents confident ranking: Persistence and patient-level consequence unresolved **Unresolved article question: Proposed: should consequence rank remain intermediate until exposure or outcome changes are demonstrated?**	**Lifecycle trigger:** New assay, formulation, switching or post-marketing observation **Product attribute state:** Immune-response difference	**Clinical-consequence inference scenario:** Immunogenicity signal without PK, efficacy or safety consequence **Evidence layer that carries the decision:** Integrated ADA, PK and clinical context	ADA detection cannot be equated automatically with clinical harm
Evidence gap that prevents confident ranking: Whether range shift represents benign variability or untested biological change **Unresolved article question: Proposed: when should lifecycle evidence justify re-ranking an established CQA?**	**Lifecycle trigger:** Reference-product drift or lifecycle manufacturing evolution **Product attribute state:** Range discordance	**Clinical-consequence inference scenario:** Attribute outside historical reference range but within functional performance expectations **Evidence layer that carries the decision:** Longitudinal product and functional evidence	Historical range exceedance is an investigation trigger, not a clinical conclusion

A risk-based CQA prioritization framework

The proposed framework starts by reversing a common analytical instinct. Instead of asking first which attributes are easiest to separate statistically or which generate the largest analytical differences, it asks which plausible consequences would matter most if they occurred. The initial stage therefore identifies the product function, exposure pathway or safety mechanism that could be affected. Only then is the analytical observation interpreted in relation to that pathway.

This ordering is consistent with the established principle that CQA criticality should reflect potential clinical impact and uncertainty [3]. Statistical reliability remains essential because a consequence framework built on an unstable measurement is not defensible [4]. Direct functional evidence can then strengthen or weaken the

proposed link between the measured attribute and a mechanism-relevant effect [6]. When analytical uncertainty itself remains substantial, error-controlled similarity methods help determine whether the observed difference is sufficiently credible to warrant further escalation [28].

The second stage is discordance resolution. When analytical and downstream evidence disagree, the response should not be to privilege automatically the most sensitive assay. Aggregation data show that detectable particles may not yield proportional immune effects under realistic exposure conditions [18]. Structure–activity experiments show that measurable molecular differences can fail to alter tested biological functions [21]. Immunogenicity measurements demonstrate that biological response becomes clinically meaningful only when its temporal and pharmacokinetic context is considered [22]. Functional immune assays can provide an additional intermediate layer when chemical detection alone does not reveal biological consequence [26].

Accordingly, discordance can produce three outcomes. Concern may increase when independent evidence converges across molecular, functional and exposure levels. Concern may decrease when sufficiently sensitive downstream testing fails to support the proposed consequence pathway. Or uncertainty may remain unresolved when the relevant assay, exposure state or population has not been tested adequately. The last outcome should not be forced into either "critical" or "noncritical." Uncertainty is itself a legitimate decision state.

The third stage is lifecycle re-evaluation. CQA prioritization should not be assumed to be permanently fixed at development. Manufacturing changes, new reference-product history, post-approval analytical data, improved functional assays and accumulating clinical experience can alter the amount or quality of evidence linking an attribute to patient consequence. Re-ranking should therefore be possible when the underlying evidence changes, while preserving established controls until sufficient evidence supports a different interpretation.

This framework is deliberately proposed as a reasoning architecture rather than a validated scoring system. It does not provide numerical weights, universal thresholds or product-independent rules. Its purpose is narrower: to prevent analytical detectability from becoming an unexamined surrogate for clinical importance and to make explicit which evidence is required before a quality difference is escalated or downgraded.

Development and regulatory use

The framework is most useful where development programs must decide how to allocate analytical, functional, pharmacokinetic and clinical effort. Contemporary biosimilar policy discussions increasingly emphasize regulatory convergence and the possibility of streamlining development when robust lower-level evidence resolves meaningful uncertainty [29]. These discussions do not establish a universal rule, but they reinforce the value of distinguishing evidence that changes a clinical decision from evidence that merely increases analytical description.

For development teams, consequence-based prioritization could guide assay selection, comparability investigations and change-control strategy. A highly detectable attribute with repeatedly negative mechanism-relevant testing may remain part of routine control without dominating the residual-uncertainty assessment. Conversely, an attribute that is difficult to quantify but strongly linked to potency, clearance or immunogenicity may justify disproportionate analytical and functional attention.

Lifecycle context is also essential. Regulatory experience shows that analytical packages are already central to successful biosimilar evaluations [1]. Longitudinal reference-product data demonstrate that quality profiles can change over time [9]. Post-approval characterization shows that product-specific distributions may not remain confined to narrow historical ranges [10]. Ustekinumab experience further demonstrates how analytical and pharmacokinetic evidence can become more persuasive when they converge with immunogenicity findings in a defined product context [25].

A consequence-based approach would therefore not reduce regulatory rigor. Its purpose is to focus rigor where the evidence suggests that patient consequence is plausible or unresolved. It also provides a transparent basis for explaining why two analytically similar differences may receive different levels of concern in different products, mechanisms, routes or lifecycle states.

Regulatory adoption of any formal ranking algorithm would require prospective evaluation, cross-product testing and agreement on how uncertainty should influence escalation. The present framework should therefore be viewed as a decision-organizing proposal rather than as an accepted regulatory method. Its principal value is to separate analytical capability from the evidentiary burden required to claim clinical importance.

CONCLUSION

Biopharmaceutical characterization is now capable of detecting molecular differences at a resolution that exceeds the clinical interpretability of many individual signals. This is an analytical success, but it creates a responsibility to distinguish what can be measured from what matters to patients.

Quality attributes should therefore be interpreted through the consequences they plausibly or demonstrably produce. Structural variation, glycosylation, charge heterogeneity, aggregation, particles and potency changes do not carry fixed clinical meaning independent of product architecture, mechanism, exposure, lifecycle state and patient context. Analytical deviation initiates assessment; it does not complete it.

The proposed consequence-based framework preserves analytical rigor while requiring progressively stronger evidence before an attribute is assigned greater clinical importance. Mechanistic plausibility, function, pharmacokinetics, immunogenicity and clinical outcome represent distinct evidentiary levels, and discordance among them should remain visible rather than being compressed into a single criticality label.

Such an approach does not make every analytically detectable difference less important. It makes the basis of importance more explicit. The central decision becomes not whether an assay can detect an attribute, but whether the attribute can alter product behavior in a way that is credible, exposure-relevant and consequential for the patient.

ACKNOWLEDGMENTS: None

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

ETHICS STATEMENT: None

REFERENCES

1. Aithal R, Majedi OM, McCarthy D, Atouf F, Peckham N. Review of quality attributes and analytical methods used for comparative analytical assessment of monoclonal antibodies as part of successful biosimilar approvals in the United States and European Union. BioDrugs. 2026;40(4):713-32. doi:10.1007/s40259-026-00786-w
2. Rathore AS, Suryanarayanan R, Nejadnik R, Gurvich VJ, Singh SK, Sarin D. Best practices for performing analytical and functional biosimilarity assessment of recombinant monoclonal antibody biosimilars. AAPS J. 2026;28(2):62. doi:10.1208/s12248-026-01206-1
3. Vandekerckhove K, Seidl A, Gutka H, Kumar M, Gratzl G, Keire D, et al. Rational selection, criticality assessment, and tiering of quality attributes and test methods for analytical similarity evaluation of biosimilars. AAPS J. 2018;20(4):68. doi:10.1208/s12248-018-0230-9
4. Burdick R, Coffey T, Gutka H, Gratzl G, Conlon HD, Huang CT, et al. Statistical approaches to assess biosimilarity from analytical data. AAPS J. 2017;19(1):4-14. doi:10.1208/s12248-016-9968-0
5. Jiang T, Kwofie F, Attanasio N, Haas M, Higgins J, Kosanam H. Exploring the correlation between LC-MS multi-attribute method and conventional chromatographic product quality assays through multivariate data analysis. AAPS J. 2025;27(1):5. doi:10.1208/s12248-024-00973-z
6. Wang S, Wang Y, Li Z, Hong Y, Wang Z, Fan J, et al. Early determination of potential critical quality attributes of therapeutic antibodies in developability studies through surface plasmon resonance-based relative binding activity assessment. MAbs. 2024;16(1):2374607. doi:10.1080/19420862.2024.2374607
7. van der Zon AAM, Höchsmann A, Bos TS, Neusüß C, Somsen GW, Jooß K, et al. Characterization of monoclonal antibody charge variants under near-native separation conditions using nanoflow sheath liquid capillary electrophoresis-mass spectrometry. Anal Chim Acta. 2024;1331:343287. doi:10.1016/j.aca.2024.343287
8. Joshi S, Rathore AS. Assessment of structural and functional comparability of biosimilar products: Trastuzumab as a case study. BioDrugs. 2020;34(2):209-23. doi:10.1007/s40259-020-00404-3
9. Lee JH, Paek K, Moon JH, Ham S, Song J, Kim S. Biological characterization of SB3, a trastuzumab biosimilar, and the influence of changes in reference product characteristics on the similarity assessment. BioDrugs. 2019;33(4):411-22. doi:10.1007/s40259-019-00362-5

10. Shibata H, Harazono A, Kiyoshi M, Saito Y, Ishii-Watabe A. Characterization of biosimilar monoclonal antibodies and their reference products approved in Japan to reveal the quality characteristics in post-approval phase. BioDrugs. 2025;39(4):645-67. doi:10.1007/s40259-025-00722-4
11. Luo S, Hess K, Rogstad S, Zhang B. Glycan profiles of FDA-approved therapeutic antibodies: Insights from regulatory submissions. AAPS J. 2026;28(2):53. doi:10.1208/s12248-026-01207-0
12. Duivelshof BL, Jiskoot W, Beck A, Veuthey JL, Guillarme D, D'Atri V. Glycosylation of biosimilars: Recent advances in analytical characterization and clinical implications. Anal Chim Acta. 2019;1089:1-18. doi:10.1016/j.aca.2019.08.044
13. Malani H, Shrivastava A, Nupur N, Rathore AS. LC-MS characterization and stability assessment elucidate correlation between charge variant composition and degradation of monoclonal antibody therapeutics. AAPS J. 2024;26(3):42. doi:10.1208/s12248-024-00915-9
14. Liu A, Liang T, Wu W, Weng J, Wu H, Zhou F, et al. Protein concentration and analyzing charge variants in a co-formulation comprising three monoclonal antibodies: A cation-exchange chromatography approach. Int J Pharm. 2025;670:125138. doi:10.1016/j.ijpharm.2024.125138
15. Sreenivasan S, Schöneich C, Rathore AS. Aggregation of therapeutic monoclonal antibodies due to thermal and air/liquid interfacial agitation stress: Occurrence, stability assessment strategies, aggregation mechanism, influencing factors, and ways to enhance stability. Int J Pharm. 2024;666:124735. doi:10.1016/j.ijpharm.2024.124735
16. Tathe U, Khopkar S, Rasam P, Kancherla A, Dandekar P, Jain R. Impact of stirring material on formation of submicron and subvisible aggregates in mAbs by quantitative laser diffraction, dynamic light scattering and background membrane imaging. Int J Pharm. 2024;660:124321. doi:10.1016/j.ijpharm.2024.124321
17. Mahri S, Cassiers C, Gracin S, Tyteca D, Uwambayinema F, Huaux F, et al. Impact of protein aggregation on the immunogenicity of a human monoclonal antibody following pulmonary administration in mice. Int J Pharm. 2024;667(Pt B):124880. doi:10.1016/j.ijpharm.2024.124880
18. Cohen JR, Brych SR, Prabhu S, Bi V, Elbaradei A, Tokuda JM, et al. A high threshold of biotherapeutic aggregate numbers is needed to induce an immunogenic response in vitro, in vivo, and in the clinic. Pharm Res. 2024;41(4):651-72. doi:10.1007/s11095-024-03678-2
19. Swanson MD, Rios S, Mittal S, Soder G, Jawa V. Immunogenicity risk assessment of spontaneously occurring therapeutic monoclonal antibody aggregates. Front Immunol. 2022;13:915412. doi:10.3389/fimmu.2022.915412
20. Dash R, Singh SK, Chirmule N, Rathore AS. Assessment of functional characterization and comparability of biotherapeutics: A review. AAPS J. 2022;24(1):15. doi:10.1208/s12248-021-00671-0
21. Liu Y, Xie J, Li Z, Mei X, Cao D, Li S, et al. Comparative structure activity relationship characterization of the biosimilar BAT1806/BIIB800 to reference tocilizumab. BioDrugs. 2025;39(2):307-20. doi:10.1007/s40259-024-00698-7
22. Williams JH, Liao KH, Yin D, Meng X. Implications of immunogenicity testing for therapeutic monoclonal antibodies: A quantitative pharmacology framework. AAPS J. 2024;26(2):31. doi:10.1208/s12248-024-00901-1
23. Jang D, Kim J, Jo Y, Lee H, Go A, Kim J, et al. Possibilities and limitations in substituting anti-drug antibody titers with signal-to-noise ratios: A comprehensive comparison using two clinical trial datasets of adalimumab. AAPS J. 2025;27(1):3. doi:10.1208/s12248-024-00991-x
24. Cheng CA, Jiang AL, Liu YR, Chang LC. Investigation of immunogenicity assessment of biosimilar monoclonal antibodies in the United States. Clin Pharmacol Ther. 2023;114(6):1274-84. doi:10.1002/cpt.3033
25. Schiestl M, Roy N, Trieb M, Park JP, Guillen E, Woollett G, et al. Analytical data and single-dose PK are sufficient to conclude comparable immunogenicity for biosimilars: An ustekinumab case study. BioDrugs. 2025;39(5):769-76. doi:10.1007/s40259-025-00733-1
26. Her C, Thacker S, Balsamo J, Baker LK, Ireland DDC, Pang E, et al. Cell-based assays to detect innate immune response modulating impurities: Application to biosimilar insulin. AAPS J. 2025;27(1):20. doi:10.1208/s12248-024-00983-x
27. Mielke J, Innerbichler F, Schiestl M, Ballarini NM, Jones B. The assessment of quality attributes for biosimilars: A statistical perspective on current practice and a proposal. AAPS J. 2019;21(1):7. doi:10.1208/s12248-018-0275-9

28. Zahel T. A novel bootstrapping test for analytical biosimilarity. AAPS J. 2022;24(6):112. doi:10.1208/s12248-022-00749-3
29. Car E, Barbier L, Schwarzenberger I, Huys I, Simoens S, Vulto AG. Toward regulatory convergence and streamlined biosimilar development: Recommendations from an international qualitative study. BioDrugs. 2025;39(6):943-55. doi:10.1007/s40259-025-00746-w