



Review Article

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Immunogenicity Assessment in Biosimilars from Molecular Difference to Clinical Relevance

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ABSTRACT

Immunogenicity assessment in biosimilar development spans analytically distinct questions. Molecular or product differences may create theoretical immunogenicity concerns, anti-drug antibody assays detect immune responses under method-dependent conditions, and only a subset of detected responses has demonstrable pharmacokinetic, pharmacodynamic, efficacy, or safety consequences. Treating these observations as interchangeable can exaggerate the significance of analytical differences or antibody positivity. This scoping review maps how biosimilar immunogenicity evidence moves across product characteristics, patient and treatment conditions, assay and sampling design, anti-drug antibody characteristics, clinical consequences, and switching contexts, with emphasis on the boundaries between observation and clinically relevant inference. Peer-reviewed literature published from 2017 through 2024 was scoped using a Population–Concept–Context framework. Evidence was charted according to product and analytical attributes, population and treatment context, immunogenicity-assay characteristics, antibody phenotype, pharmacological consequences, clinical outcomes, and switching exposure. Evidence derived from analytical characterization, comparative clinical studies, methodological scholarship, and broader immunogenicity or switching syntheses was retained in analytically distinct categories. The mapped evidence indicates that biosimilar immunogenicity cannot be interpreted from a single evidentiary layer. Analytical differences require functional and risk-context interpretation; anti-drug antibody incidence is sensitive to assay and sampling conditions; antibody positivity does not necessarily imply neutralization or altered exposure; and clinically relevant immunogenicity depends on convergence with pharmacological, efficacy, or safety evidence. Patient state, treatment history, concomitant therapy, repeated exposure, and expectations surrounding switching can further alter the interpretation of observed outcomes. Immunogenicity assessment is best treated as a bounded inference problem rather than a linear progression from molecular difference to clinical harm. The review supports separation of product-level concern, measured immune response, functional consequence, and clinical relevance while identifying the evidence required to justify movement between these levels.

Keywords: Biosimilars, Immunogenicity, Anti-drug antibodies, Analytical similarity, Neutralizing antibodies, Switching

INTRODUCTION

Biosimilarity is established through a comparative totality of evidence in which extensive structural and functional characterization reduces residual uncertainty before clinical comparisons are interpreted. Contemporary regulatory scholarship consequently places increasing emphasis on sensitive analytical and pharmacological

evidence rather than requiring clinical studies to reproduce knowledge already established for the reference product [1–3]. Immunogenicity complicates this logic because immune responses are simultaneously product-dependent, host-dependent, treatment-dependent, and measurement-dependent.

The difficulty is not simply whether anti-drug antibodies (ADAs) occur. Comparative trials across inflammatory diseases have demonstrated substantial variation in ADA incidence between biological products and have also exposed differences in assay procedures and reporting practices [4]. Moreover, clinically significant antibodies are distinguished not by detection alone but by evidence that the response materially affects pharmacokinetics (PK), pharmacodynamics (PD), efficacy, or safety [5]. An ADA result therefore occupies an intermediate evidentiary position: it documents a measured immune response under defined analytical conditions but does not, without additional evidence, establish functional neutralization or clinical harm.

Biosimilar development adds another inferential boundary. Product differences identified through increasingly sensitive physicochemical methods may be real yet remain functionally inconsequential; conversely, a theoretically relevant attribute can justify additional investigation without proving that an altered clinical immune response will occur. Experience with the first natalizumab biosimilar illustrates how analytical, functional, PK, clinical, and immunogenicity evidence can be assembled as product-specific components of biosimilarity rather than as substitutes for one another [6].

This review therefore examines immunogenicity as an evidence-translation problem. Its central proposed distinction separates product-level immunogenicity concern, measured immune response, functional or exposure consequence, and clinical relevance. The purpose is not to establish a universal predictive hierarchy. It is to identify what observations permit movement between these levels, what evidence leaves that movement uncertain, and where molecule, assay, population, treatment, temporal, or switching context limits extrapolation.

MATERIALS AND METHODS

The review was designed as a scoping review because the relevant literature spans heterogeneous evidence classes rather than a common intervention–outcome structure suitable for pooled estimation. Reporting and evidence-charting decisions were informed by PRISMA-ScR principles, particularly explicit definition of the review question, eligibility boundaries, evidence-source selection, and transparent mapping of included evidence [7].

Evidence was charted at the level required by the claim being assessed. Analytical comparability findings were kept distinct from clinical immunogenicity observations; ADA detection was separated from neutralizing-antibody assessment; and PK, PD, efficacy, and safety consequences were charted independently. Comparative immunogenicity methodology was evaluated in light of biosimilar-specific assay-development considerations [8], while broader bioanalytical literature was used to retain matrix, drug-interference, target-interference, and platform-dependent measurement boundaries where relevant [9].

No meta-analysis was planned because study populations, products, assay platforms, sampling schedules, definitions of treatment-emergent ADA, and clinical endpoints were not sufficiently uniform to support a single pooled immunogenicity parameter.

Review question and PCC framework

The review asked: How do product-related differences, patient and treatment determinants, assay and sampling conditions, ADA characteristics, and switching exposure contribute to the interpretation of biosimilar immunogenicity, and what evidence is required before an observed immune difference can be considered clinically relevant?

The Population component included healthy volunteers and patients receiving reference biologics, biosimilars, or comparative treatment sequences. The Concept comprised product-related immunogenicity determinants, ADA and neutralizing-antibody measurement, assay variability, PK/PD consequences, clinical relevance, and switching. The Context encompassed comparative biosimilar development and clinical use across therapeutic areas. Evidence transfer between therapeutic proteins was permitted only for methodological or mechanistic principles; product-specific conclusions were not generalized without direct support.

Search strategy

Search logic combined terms for biosimilar, immunogenicity, anti-drug antibody, neutralizing antibody, analytical similarity, quality attribute, assay, sampling, pharmacokinetics, pharmacodynamics, clinical consequence, switching, and interchangeability. The publication window was restricted to 2017–2024.

Search concepts were organized into complementary blocks rather than one highly restrictive string. One block targeted comparative immunogenicity and ADA/NAb assessment; another targeted molecular and product attributes potentially relevant to immune recognition; additional blocks addressed assay performance, patient and treatment determinants, clinical consequences, and reference-to-biosimilar or biosimilar-to-biosimilar switching. Citation chaining was used to identify methodological foundations and longitudinal reports connected with eligible comparative evidence.

Search outputs were deduplicated before title/abstract screening. Reports judged potentially relevant were assessed against the PCC framework and evidence-class requirements. Selection counts, retrieval failures, exclusion reasons, and final inclusion arithmetic were maintained in the review-selection ledger rather than inferred from the manuscript reference list.

Eligibility and evidence charting

Eligible evidence had to inform at least one inferential step between a product characteristic and an immunogenicity interpretation. This prevented purely descriptive biosimilar reports from being treated as immunogenicity evidence when they contained no relevant analytical, ADA, PK/PD, clinical, or switching information. Analytical studies were eligible when they characterized attributes with plausible relevance to product comparability or demonstrated how an observed difference was functionally interrogated. Clinical studies were charted only to the extent that they reported immunogenicity, exposure, efficacy, safety, or switching information relevant to the review question.

Evidence was charted without forcing different observations into a single “immunogenicity” variable. The principal fields distinguished product attribute, analytical or functional observation, biological or clinical setting, assay platform and sampling design where reported, ADA status and characteristics, NAb status, PK/PD association, clinical association, treatment sequence, and interpretive limitation. Methodological publications were charted separately from product-specific empirical evidence so that assay recommendations could not be mistaken for validation of a clinical relationship.

The resulting evidence states required different discriminating information. In particular, an analytical difference with preserved function, an ADA signal without demonstrated neutralization, and an ADA-associated clinical event represent fundamentally different inference problems. The distinctions needed to keep those states separate are condensed in **Table 1**.

Table 1. Evidence states that determine when an immunogenicity observation can support a stronger biosimilar inference

Proposed evidence-charting state	What is directly observed	Inference that remains bounded	Principal evidence gap	Evidence that would discriminate competing interpretations	Unresolved biosimilar-assessment question
Attribute discordance	A measurable physicochemical or product-quality difference	The difference may warrant risk assessment; immunogenicity impact is not established	Biological relevance of the attribute	Orthogonal structural analysis, functional testing, stability/process context and, where justified, immune-response evidence	Is the difference biologically relevant enough to alter the immunogenicity assessment?
Functional concordance despite analytical difference	Sensitive assays detect a difference while relevant biological function remains comparable	Functional concordance reduces one source of concern but does not prove identical immune behavior	Relationship between the altered attribute and immune recognition	Product-specific mechanistic or clinical evidence directed at the residual concern	Does residual analytical uncertainty require additional immunogenicity evidence?
Measured ADA without functional consequence	Treatment-emergent or boosted binding antibodies are detected	Immune recognition occurred under the assay conditions; clinical significance is undetermined	Persistence, specificity, neutralization and exposure consequence	Titer/persistence characterization, NAb testing and ADA-stratified PK/PD evaluation	Does the detected response alter biological activity or exposure?

ADA with pharmacological perturbation	Antibody response accompanies altered exposure or biological activity	A functional relationship becomes plausible but clinical importance still requires context	Link to efficacy or safety and alternative causes of altered PK/PD	Temporal concordance, neutralization data and clinically interpretable outcomes	Does the pharmacological effect cross the boundary into clinically relevant immunogenicity?
Clinical event with uncertain immune attribution	Loss of effect, hypersensitivity or another clinical event occurs in an ADA-positive context	Co-occurrence cannot alone establish antibody causation	Competing disease, treatment, adherence and assay explanations	Temporal analysis, antibody phenotype, exposure data and appropriate comparator evidence	Is the clinical event attributable to the immune response rather than another treatment-context factor?

Note: The evidence-state classification is a proposed synthesis of this review. It is not an empirically validated scoring system and does not assign numerical weights or regulatory thresholds.

The review-selection process itself must remain visually distinct from this evidence-state classification because screening arithmetic answers which evidence entered the map, whereas **Table 1** addresses what different included observations can legitimately mean. **Figure 1** therefore preserves source retrieval, deduplication, screening, eligibility, exclusion, and evidence-use boundaries rather than converting reference-list size into a study-selection count.

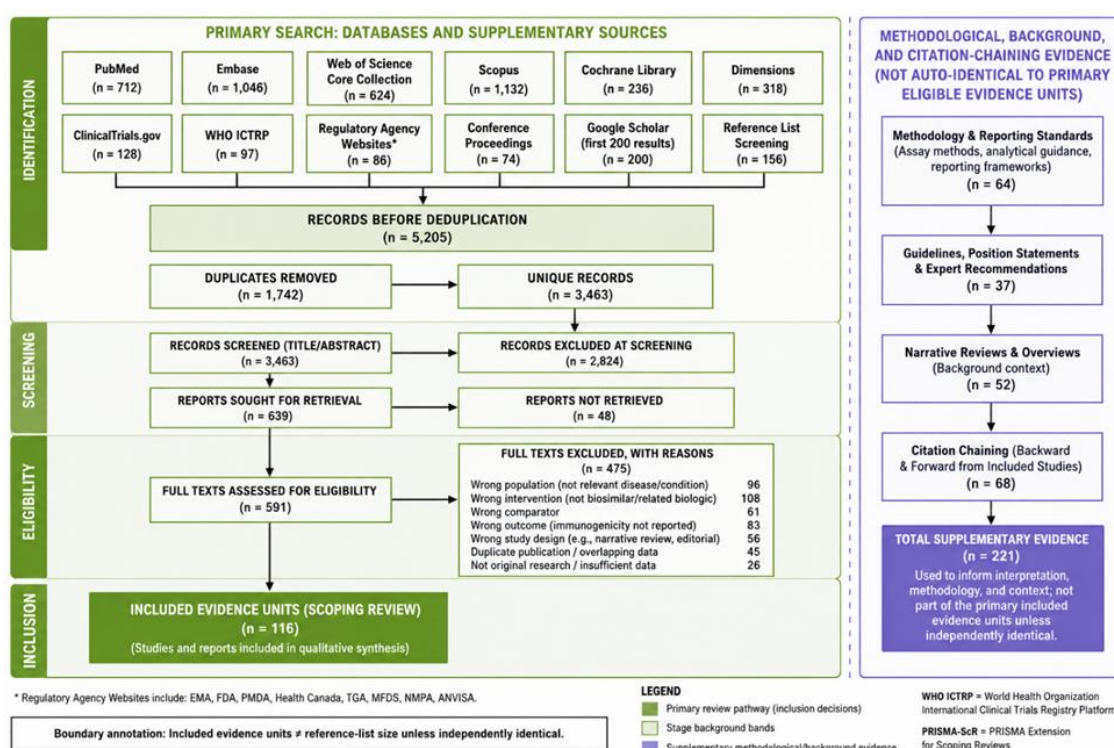


Figure 1. Source-resolved selection logic separating eligible immunogenicity evidence from contextual citation use

RESULTS AND DISCUSSION

The evidence map was heterogeneous in both evidentiary level and therapeutic context. Analytical comparability studies provided dense characterization of structure, variants, purity, and biological function, whereas clinical studies more often contributed ADA incidence, NAb results, PK, efficacy, safety, or switching observations. These evidence classes were therefore synthesized by inferential function rather than treated as interchangeable measures of immunogenicity.

Analytical characterization of SB12 versus reference eculizumab illustrates the first level of this map: extensive orthogonal physicochemical and functional testing can define similarity and identify residual differences, but the analytical dataset itself is not a clinical immunogenicity outcome [10]. Across the mapped literature, this

distinction recurred. Product differences were informative when linked to biological function or a plausible immunogenicity concern; ADA findings became more informative when accompanied by antibody phenotype or PK/PD assessment; and clinical events required assessment of competing treatment- and disease-related explanations.

The evidence accordingly supported a layered descriptive synthesis rather than a single immunogenicity frequency. The principal mapped layers were product-related determinants, patient and treatment determinants, measurement conditions, ADA/NAb phenotype, pharmacological consequences, clinical outcomes, and switching context.

Product-related immunogenicity determinants

Product-related immunogenicity begins with molecular and process characteristics but cannot be inferred from the mere detectability of a difference. Modern analytical platforms can resolve charge variants, size variants, glycosylation patterns, higher-order structure, aggregation-related species, impurities, and functional properties at a sensitivity that may exceed the clinical relevance of the detected variation. The appropriate question is therefore not whether two independently manufactured biological products are analytically identical, but whether residual differences alter a function or create a plausible immunogenicity concern.

BAT1806/BIIB800 and reference tocilizumab provide a useful example. Minor physicochemical differences were observed within an otherwise broad demonstration of physicochemical and functional similarity; those differences were investigated in relation to functional performance rather than automatically interpreted as altered immunogenicity [11]. This supports an important boundary: analytical discordance is evidence that requires explanation, not evidence of an immune consequence.

Clinical pharmacology can add another layer. Comparative evaluation of HLX11 against regionally sourced pertuzumab reference products integrated PK, safety, and immunogenicity observations, showing how comparative clinical evidence can test whether residual product uncertainty manifests under controlled exposure conditions [12]. Similarly, a single-dose comparison of BAT2206 and ustekinumab in healthy participants combined PK similarity with immunogenicity and safety assessment [13]. Such studies are sensitive for comparative exposure but remain bounded by population, duration, dosing, and disease-context differences.

Patient studies extend the assessment into therapeutic settings. In plaque psoriasis, CT-P43 and originator ustekinumab were compared for efficacy, safety, and immunogenicity, allowing product-level similarity to be interpreted alongside repeated clinical exposure [14]. Insulin aspart biosimilarity likewise requires molecule-specific interpretation because pre-existing or treatment-emergent insulin antibodies occur within a distinctive therapeutic and endogenous-protein context; comparative MYL-1601D evidence therefore cannot simply be generalized to monoclonal antibodies [15].

A bevacizumab biosimilar comparison in metastatic or recurrent non-small-cell lung cancer further demonstrates that immunogenicity evidence is embedded within disease, concomitant treatment, and oncology-specific clinical conditions [16]. In contrast, pegfilgrastim-cbqv was evaluated across randomized studies in healthy participants with tiered ADA characterization. Small differences in detected antibody incidence were attributed to low-titer, non-neutralizing polyethylene-glycol-reactive antibodies, with no apparent effect on PK, PD, or safety [17]. This case is especially informative because it demonstrates why antibody quality and consequence, rather than positivity alone, determine interpretation.

Taken together, product evidence supports a proposed two-stage discipline: first determine whether an analytical difference has plausible biological relevance; then determine whether any observed immune response has functional or clinical consequences. It does not support a universal molecular threshold above which immunogenicity can be predicted. Comparative bevacizumab evidence likewise reinforces the need to interpret immunogenicity within the total clinical comparison rather than as an isolated binary endpoint [18].

Patient and treatment determinants

Immunogenicity is produced in a host-product-treatment system. Disease activity, immune competence, previous exposure, treatment duration, route, dose pattern, concomitant immunomodulation, and baseline antibody status can alter either the probability of detecting an immune response or its consequences. These determinants limit direct comparison of ADA percentages obtained from healthy-volunteer PK studies, chronic inflammatory disease trials, oncology studies, and routine clinical cohorts.

Treatment interpretation is further complicated during non-medical switching. In an inflammatory bowel disease switching program, temporary deterioration in patient-reported disease control occurred without parallel

deterioration in objective clinical or biomarker assessments, illustrating a potential placebo contribution to outcomes after switching [19]. Such findings must not be reclassified as immunogenicity unless immune evidence supports that attribution.

Patient understanding and expectations constitute a different evidence layer. A systematic review of patient perceptions identified variable knowledge and concerns surrounding biosimilars [20]. These perceptions may affect acceptance, adherence, persistence, and reporting, but they are not molecular mechanisms of ADA formation. Their relevance is interpretive: subjective treatment deterioration after a switch cannot automatically be used as evidence that the biosimilar generated a different immune response.

The cross-scale problem is therefore to preserve each determinant at the level where it is observed while asking what additional evidence is needed before it can support an immunogenicity conclusion. **Table 2** makes that transfer boundary explicit.

Table 2. Cross-scale interpretation of patient and treatment conditions that can modify biosimilar immunogenicity assessment

Treatment-context case	Immediate functional relevance	Possible PK/PD consequence	Immunogenicity relevance	Clinical linkage that can be assessed	Residual uncertainty	Comparability interpretation	Proposed framework endpoint
Single exposure in a healthy-volunteer comparison	Sensitive comparison of exposure under controlled conditions	PK divergence can be detected with limited disease-related variability	Early ADA detection is possible but chronic maturation may be underrepresented	Short-term safety and tolerability	Generalization to repeated dosing and diseased populations	Strong for comparative exposure; bounded for long-term immune consequence	Exposure-conditioned immune observation
Repeated treatment in chronic inflammatory disease	Captures sustained target engagement in the intended clinical context	Persistent ADA may alter drug concentrations in susceptible products	Allows treatment-emergent, persistent and potentially neutralizing responses to emerge	Disease control, loss of response and safety can be related temporally	Confounding by disease activity, concomitant therapy and treatment history	Clinically informative when product groups are otherwise comparable	Contextualized immune response
Concomitant immunomodulatory treatment	Alters host immune activation independently of product similarity	May indirectly modify exposure by reducing clinically relevant ADA formation	ADA frequency cannot be interpreted independently of co-therapy	Treatment persistence and efficacy may differ by regimen	Degree of confounding varies by molecule and disease	Comparability is strongest within matched treatment regimens	Host-treatment boundary
Prior biologic exposure or pre-existing reactivity	Baseline immune state precedes study treatment	Baseline antibodies may complicate exposure interpretation	Apparent post-dose positivity may not represent a newly induced response	Clinical importance depends on treatment-emergent change and consequence	Cross-reactivity and assay definitions	Requires baseline-adjusted comparative interpretation	Pre-existing-state boundary
Patient-reported deterioration after switching without objective concordance	May influence adherence and persistence	No PK/PD effect can be assumed	Does not establish increased ADA formation	Subjective outcomes may diverge from biomarkers or disease activity	Expectation, attribution and other placebo-related effects	Must remain separate from biological immunogenicity evidence	Clinical-attribution boundary

Note: The final column is a proposed synthesis of this review, intended to identify the inferential endpoint supported by each evidence pattern rather than rank patient or treatment factors.

Assay and sampling variability

An ADA result is generated jointly by biology and measurement. Assay sensitivity, specificity, cut-point strategy, drug tolerance, antigen format, matrix interference, confirmatory procedures, titer determination, and neutralizing-antibody methods all influence what is classified as positive. Sampling time adds another dimension because transient responses, persistent responses, treatment-boosted responses, and antibodies masked by circulating drug may be detected differently across schedules.

This is particularly important for biosimilars because comparative immunogenicity requires the biosimilar and reference product to be assessed without introducing assay-related asymmetry. Direct comparison using a suitably validated assay can reduce analytical bias, but the assay must still demonstrate that relevant antibodies to either product can be detected appropriately. Comparative assay methodology therefore concerns measurement comparability, not proof that a detected ADA is clinically consequential.

Signal magnitude may add information beyond binary positivity when interpreted within a validated analytical context. In an adalimumab biosimilar switching analysis, ADA signal-to-noise ratios were used to examine whether repeated switching produced evidence of increased immunogenicity relative to continuous treatment [21]. The approach illustrates how quantitative assay signal can interrogate a specific comparative question, but its predefined analytical interpretation should not be converted into a universal clinical threshold applicable to other assays or products.

The separation between detection and consequence is also evident in ranibizumab biosimilar evidence. Comparative SB11 analyses related immunogenicity findings to efficacy, safety, and PK rather than treating antibody incidence as an autonomous clinical endpoint [22]. This is the necessary direction of inference: assay results identify immune reactivity; their significance depends on antibody phenotype, exposure effects, biological activity, clinical outcomes, and temporal coherence.

Sampling design can therefore alter apparent comparability even when underlying immunobiology is unchanged. A schedule concentrated early after dosing may characterize emerging responses differently from long-term repeated sampling; high circulating drug concentrations may impair detection in insufficiently drug-tolerant assays; and different definitions of treatment-emergent or treatment-boosted ADA can change reported incidence. Cross-study ADA percentages should consequently not be ranked as though they arose from a common measurement system.

The proposed synthesis used in this review treats assay performance as an interpretive gate rather than another biological determinant. Before an apparent biosimilar–reference difference is moved toward clinical interpretation, the analysis should establish that the difference is not adequately explained by assay performance, sampling, baseline reactivity, drug interference, or classification rules. Only after that gate is crossed should antibody specificity, persistence, neutralizing activity, PK/PD changes, efficacy, and safety determine whether the response has functional or clinical relevance.

Anti-drug antibodies and clinical consequences

ADA positivity is an analytical observation, whereas clinically relevant immunogenicity is an inference requiring additional evidence. The distinction matters because binding antibodies can differ in titer, persistence, epitope specificity, cross-reactivity, neutralizing capacity, and relationship to circulating drug. A treatment-emergent signal may therefore represent detectable immune recognition without materially changing exposure or biological activity. Conversely, persistent or neutralizing antibodies may become clinically important when they alter PK, reduce target engagement, contribute to loss of response, or accompany an immune-mediated safety event.

Clinical attribution is particularly difficult for adverse events that are biologically compatible with immunogenicity but have alternative causes. Population-level evidence comparing biosimilar and originator rituximab found no increased risk of hospitalization for severe hypersensitivity reactions among biosimilar initiators or patients switching from originator therapy [23]. Such evidence is informative for comparative safety but should not be inverted into a claim that hypersensitivity cannot be immune mediated in an individual patient. It instead demonstrates that occurrence of a clinically compatible event does not by itself establish a product-specific immunogenicity difference.

The central interpretive problem can therefore be expressed as a series of failure modes. An assay can detect antibodies whose functional importance is unknown; altered exposure can occur without sufficient evidence that

ADA caused it; a clinical deterioration can coincide with antibody positivity without satisfying causal attribution; and absence of a clinical difference can coexist with low-frequency immune responses that are insufficiently powered for product-level comparison. **Table 3** separates these failure patterns according to the evidence needed before a stronger inference is justified.

Table 3. Failure modes that can convert an anti-drug antibody observation into an unsupported clinical immunogenicity claim

Misleading evidence pattern	Likely origin of uncertainty	Observable signature	Competing explanation requiring exclusion	Corrective evidence or test	Decision consequence	Lifecycle trigger for reassessment	Proposed article-specific endpoint
Binary ADA positivity treated as clinical harm	Detection is substituted for consequence	Binding ADA without demonstrated functional change	Transient or low-level non-neutralizing response	Persistence, titer, specificity, NAb and PK/PD assessment	Retain the signal as immune evidence without assigning clinical importance	New assay, altered formulation or changed exposure conditions	Measured response only
Reduced exposure attributed directly to ADA	Temporal association is treated as mechanism	ADA positivity and lower drug concentration coexist	Disease state, clearance variability, adherence, dosing or target-mediated disposition	Temporal ADA–PK analysis and functional antibody characterization	Clinical interpretation remains conditional	Unexpected PK divergence during development or surveillance	Pharmacological consequence requiring attribution
Loss of response assigned to immunogenicity	Clinical deterioration and ADA coexist	Reduced efficacy after previous disease control	Disease progression, background therapy change or subjective/objective effects	Exposure, NAb, objective disease measures and treatment-history assessment	Immune attribution requires concordant evidence	Treatment failure or post-switch deterioration	Clinically contextualized immune effect
Hypersensitivity assumed to prove product-specific immune difference	Compatible adverse event is overinterpreted	Acute or delayed hypersensitivity event	Background biologic risk, infusion factors or non-ADA mechanisms	Comparative event patterns plus patient-level immune investigation where feasible	Event remains a safety signal unless comparative attribution is supported	Initiation, switch or manufacturing/formulation change	Safety attribution boundary
Similar group outcomes interpreted as zero immunogenicity risk	Lack of detected difference is equated with absence	Comparable aggregate ADA, efficacy or safety findings	Rare, delayed or subgroup-specific immune effects	Adequate follow-up, sensitive assays and context-appropriate surveillance	Supports comparability within tested conditions, not universal absence of risk	Expanded populations, prolonged use or repeated switching	Bounded comparability inference

Note: The endpoint terminology is a proposed synthesis of this review. The states are not regulatory categories, numerical grades, or validated predictors.

These distinctions are integrated visually because clinical relevance is established by convergence, not by a single unidirectional chain. The central boundary in **Figure 2** separates evidence that an immune response exists from evidence that the response matters pharmacologically or clinically.

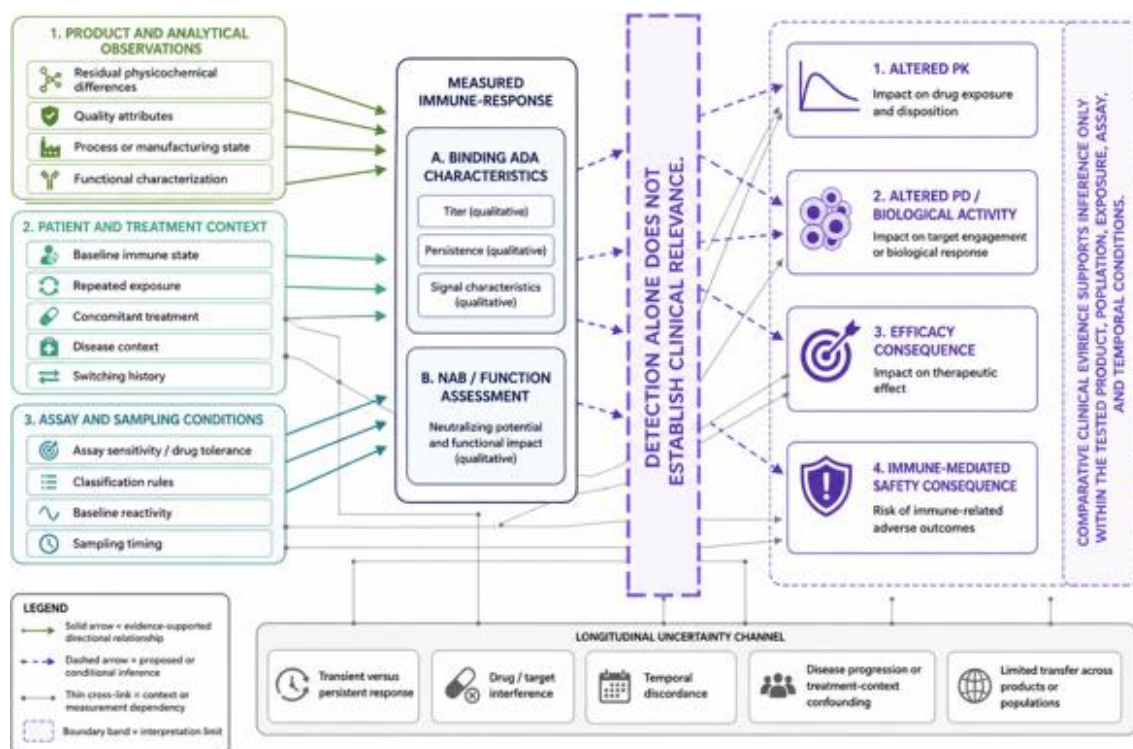


Figure 2. The clinical-relevance boundary that separates detected immune reactivity from consequential biosimilar immunogenicity

Switching evidence

Switching poses a specific immunogenicity question: whether changing between highly similar versions of the same biological therapy introduces an immune perturbation beyond that expected during continued treatment. The NOR-SWITCH randomized trial found that switching stable patients from originator infliximab to CT-P13 was not inferior to continued originator treatment across the prespecified overall analysis, while the study was not powered to establish noninferiority separately within each disease [24]. Its open-label extension likewise reported comparable ADA frequency and clinical outcomes between patients maintained on CT-P13 and those switched later [25].

Multiple-switch designs provide a different test. In plaque psoriasis, repeated switching between GP2017 and reference adalimumab did not reveal a clinically meaningful deterioration in efficacy, safety, or immunogenicity under the trial conditions [26]. Comparative SB5 development first established similar efficacy, PK, safety, and ADA responses to reference adalimumab [27], after which the transition phase showed no important new immunogenicity signal among patients switched from reference adalimumab to SB5 [28]. AVT02 studies similarly assessed continued and switched treatment in psoriasis [29], while VOLTAIRE-X directly evaluated repeated switching between BI 695501 and reference adalimumab using PK together with safety and immunogenicity outcomes [30]. The AVT02 interchangeability study further illustrates the use of repeated switching and PK as a sensitive clinical-pharmacology test of possible treatment-sequence effects [31].

Real-world data widen the context but introduce greater confounding. Successive infliximab switches from originator to CT-P13 and subsequently to SB2 were not associated with an apparent increase in ADA development attributable to the number of biosimilars received [32]. In inflammatory bowel disease, originator-to-CT-P13 switching likewise showed stable trough concentrations and no clear increase in immunogenicity, although transient subjective deterioration illustrates why post-switch symptoms and immune mechanisms should not be conflated [33].

Broader syntheses are generally reassuring while retaining important design boundaries. Evidence on biosimilar-to-biosimilar switching has expanded, but remains disproportionately concentrated in selected molecules and observational settings; broader switching reviews likewise emphasize heterogeneity in study design, duration, and sensitivity to rare outcomes [34–36]. These data support a bounded conclusion that switching has not generally produced an additional immunogenicity signal in the studied settings. They do not establish that every switching sequence, product pair, patient population, or future manufacturing state is immunologically interchangeable.

The switching scenarios therefore differ in what they actually test. **Table 4** separates direct sequence evidence from interpretations that would require stronger or different evidence.

Table 4. Switching scenarios that require different tests before treatment sequence can be excluded as an immunogenicity explanation

Switching condition	Directly observable evidence	Inference that can be made	Competing explanation	Discriminating test	Decision consequence	Lifecycle trigger	Proposed article-level test
Single reference-to-biosimilar transition	ADA/NAb, PK, efficacy and safety before and after one switch	Whether one studied transition produces a detectable comparative signal	Natural disease variation or background immunogenicity	Concurrent comparator or well-characterized longitudinal baseline	Supports the specific transition when concordant	Formulary or routine clinical switch	Sequence-neutrality test
Repeated reference-biosimilar switching	Exposure and immune outcomes across alternating administrations	Whether repeated alternation measurably perturbs PK or immune response	Time-on-treatment effects	Randomized switching versus continuous-reference comparison	Relevant to repeated-switch conditions actually studied	Interchangeability-focused development	Repeated-exposure perturbation test
Biosimilar-to-biosimilar transition	Outcomes after movement between products sharing one reference biologic	Whether a studied biosimilar pair shows new clinical or immune problems	Previous reference exposure and accumulated treatment history	Longitudinal ADA/PK and clinical comparison across defined sequences	Informative but often observational	Procurement or formulary change	Shared-reference transfer test
Subjective deterioration without objective immune or disease concordance	Patient-reported worsening following transition	A treatment-transition effect may exist; immune attribution is unsupported	Nocebo effect, expectation, adherence or natural fluctuation	Objective disease measures, PK and ADA characterization	Avoids misclassifying subjective deterioration as immunogenicity	Non-medical switching program	Attribution-separation test
Rare or delayed event after switching	Temporally post-switch safety event	Temporal association only	Background event rate or non-immune mechanism	Adequate comparative surveillance and individual immune characterization	Prevents universal inference from isolated events	Extended use or pharmacovigilance signal	Rare-event boundary test

Note: The final column is a proposed synthesis and does not constitute a validated interchangeability or regulatory algorithm.

Evidence gaps

Several uncertainties remain despite increasingly reassuring comparative evidence. First, cross-study ADA incidence remains difficult to compare when assay sensitivity, drug tolerance, sampling schedules, and positivity definitions differ. Second, long-duration data are uneven across therapeutic proteins, particularly for repeated or

biosimilar-to-biosimilar switching. Third, rare immune-mediated events may require surveillance populations substantially larger than pre-approval trials.

Evidence transfer also remains product dependent. Findings from anti-TNF agents cannot establish identical behavior for molecules with different structure, mechanism, route, endogenous counterparts, immunogenicity background, or therapeutic context. Finally, clinical practice recommendations remain heterogeneous across specialties and organizations, demonstrating that evidence interpretation and implementation are not synonymous [37].

Future work should therefore prioritize standardized reporting of ADA phenotype and assay conditions, longitudinal linkage between ADA and exposure, explicit documentation of treatment sequence, and product-specific investigation when analytical or clinical discordance creates a plausible residual immunogenicity concern.

The evidence mapped in this review supports a restrained interpretation of biosimilar immunogenicity. Analytical characterization identifies differences or residual uncertainty; immunogenicity assays establish whether immune reactivity is detected; functional and PK/PD studies test whether that reactivity alters drug behavior; and clinical evidence determines whether any alteration matters for efficacy or safety. These layers are related but not interchangeable.

The principal contribution of this review is a proposed clinical-relevance boundary. Movement across that boundary should require convergent evidence rather than the presence of any single marker. This approach explains why a minor analytical difference can be clinically unimportant, why ADA positivity can coexist with unchanged PK and clinical performance, and why post-switch symptoms can occur without evidence of altered immunogenicity.

The framework also prevents the opposite overreach. Absence of a detected comparative difference under one assay, dose, population, or follow-up period does not prove universal absence of immunogenicity risk. Biosimilarity assessment remains product specific, and the evidential value of a finding depends on whether the measurement system and clinical context were capable of detecting the consequence being considered.

Implications for biosimilar assessment

A proportionate assessment should begin by asking what uncertainty actually remains after analytical and functional comparison. If an attribute differs, the next step is not automatically a larger clinical trial; it is identification of the biological question created by that difference and selection of the most discriminating evidence.

Likewise, a comparative ADA difference should trigger evaluation of assay validity, sampling, baseline status, titer or persistence, neutralizing activity, and PK/PD before being labelled clinically meaningful. Clinical significance becomes more credible when immune, pharmacological, temporal, and outcome evidence converge and plausible non-immune explanations are insufficient.

For switching, the relevant question is sequence specific. Evidence from a single transition, repeated switching, and biosimilar-to-biosimilar substitution should remain distinguishable. The proposed architecture therefore functions as an interpretation discipline rather than a regulatory scoring instrument: every stronger inference must cross an explicit evidence boundary.

CONCLUSION

Immunogenicity assessment in biosimilars is not a linear journey in which molecular difference inevitably produces antibodies and antibodies inevitably produce clinical harm. Molecular and product observations, patient and treatment context, assay conditions, antibody phenotype, pharmacological effects, and clinical outcomes occupy distinct evidentiary levels.

The most defensible interpretation is therefore one of conditional convergence. Analytical differences identify questions; assays identify immune responses; PK, PD, efficacy, and safety evidence determine whether those responses matter. Switching evidence is broadly reassuring within studied conditions but remains product-, sequence-, population-, and method-bounded. Preserving these boundaries allows immunogenicity evidence to contribute proportionately to biosimilar assessment without either exaggerating detectable differences or overlooking clinically consequential immune responses.

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