Review

Immunogenicity of Therapeutic Antibodies: Mechanisms, Prediction, and Mitigation Strategies in the Era of Personalized Biologics

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Posted Date: 27 March 2026

doi: 10.20944/preprints202603.2174.v1

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 doi:10.20944/preprints202603.2174.v1

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Immunogenicity of Therapeutic Antibodies: Mechanisms, Prediction, and Mitigation Strategies in the Era of Personalized Biologics

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Abstract

Therapeutic monoclonal antibodies have transformed treatment across cancer, autoimmune disorders, and infectious diseases, yet their clinical utility remains challenged by immunogenicity and the resulting formation of anti-drug antibodies (ADAs), which can alter pharmacokinetics, neutralise therapeutic activity, and cause serious adverse events. This review synthesises current evidence on the mechanisms, prediction frameworks, and mitigation strategies relevant to therapeutic antibody immunogenicity, with particular focus on identifying underexplored risk dimensions not addressed by existing models. We conducted a comprehensive narrative review of peer-reviewed literature covering T-cell-dependent and T-cell-independent ADA formation pathways, multifactorial determinants of immunogenic risk, in silico prediction tools including NetMHCIIpan, SITA, DeepImmuno, and PRIME, in vitro assays including MAPPs and DC-T cell co-culture systems, and engineering and clinical mitigation strategies. Persistent challenges across the field include systematic overprediction, inadequate modelling of conformational B-cell epitopes, HLA diversity, and lack of data standardisation. Critically, molecular mimicry — structural similarity between therapeutic epitopes and pathogen-derived or self-peptides — emerges as a mechanistically distinct and currently invisible risk axis that may explain a subset of inter-patient variability in ADA incidence unaccounted for by existing sequence-based frameworks. Next-generation immunogenicity prediction requires multimodal approaches integrating structural epitope validation, patient HLA profiling, infection history, and machine learning to achieve biologically complete risk stratification.

Keywords: monoclonal antibodies; immunogenicity; anti-drug antibodies; pharmacokinetics; in silico prediction; HLA diversity; personalized medicine; predictive modeling; molecular mimicry

1. Introduction

The treatment of certain diseases, including cancer, autoimmune disorders, and infectious diseases, has been completely transformed by therapeutic antibodies, often known as monoclonal antibodies (mAbs) [67]. These are a brand-new class of biopharmaceutical medications that is quickly becoming a significant market [52]. As of 2022, the U.S. Food and Drug Administration (FDA) had approved over 100 therapeutic antibody medications, demonstrating the increasing acceptance of these medications and their therapeutic value [49].

Monoclonal antibodies are bivalent entities with specific molecules and are considered to be of the IgG subclass [67]. The fragment crystallizable (Fc) domain is crucial for stability, Fc-mediated recycling, effector functions (like antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC)), and a prolonged half-life in the blood [62]. The fragment antigen-binding (Fab) region, also known as a paratope, is involved in antigenic specificity of binding or recognition of cell receptors and activation of cytokines [48]. But exceptions to this general standard exist; for example, certolizumab pegol does not have an Fc region [61].

 doi:10.20944/preprints202603.2174.v1

A major barrier to the creation and application of therapeutic antibodies is immunogenicity, or a substance's ability to trigger a humoral or cell-mediated immune response [52,67]. Most therapeutic antibodies were previously produced in mice or other model animals. When patients were given these non-human sequences, their immune systems would get triggered. Consequently, the given therapeutic protein may become the target of the production of anti-drug antibodies (ADAs) [2]. And the production of these anti-drug antibodies can reduce the efficacy of the therapeutic antibody and complicate patient care that can sometimes even prove to be life-threatening [68,69].

ADAs have the ability to attach to the therapeutic mAb and change its biodistribution and pharmacokinetics (PK). This often increases the drug's clearance from the host, decreasing its concentration in circulation or tissues [51,54].

ADAs can lead to hypersensitivity reactions, severe infusion reactions, serum sickness, Arthus reaction, bronchoconstriction, or thromboembolic events, and in some cases, even anaphylaxis, and their development represents a significant limitation in biologic therapy, contributing to primary or secondary drug failure [29,66].

Immunogenicity prediction is one of several developability assessments used to screen antibody candidates in order to identify and minimize the triggering of ADA formation, alongside aggregation propensity, expression yield, and manufacturability [53,58].

2. Immunogenicity and Impact of Immunogenicity and ADAs:

Immunogenicity frequently leads to the generation of anti-drug antibodies (ADAs) against the delivered protein medication in the setting of antibody therapies [57,66]. The effectiveness and safety of these medicines are seriously threatened by ADA triggering [52,68]. It has even contributed to the discontinuation of multiple antibody drug programs, making early prediction and mitigation essential for de-risking clinical development [7].

The presence of ADAs can have various clinical impacts, ranging from no effect to severe toxicity, and remains a major challenge in mAb development [1,29]. In rare cases, they can interfere with the function of endogenous proteins through cross-reactivity [66]. Their development has led to the discontinuation of clinical trials and the non-approval or withdrawal of drugs after approval, and regulatory agencies like the FDA and EMA require drug developers to report immunogenicity incidents and their clinical impact on patients [49,63].

ADA-induced therapeutic failure not only compromises clinical outcomes but also imposes a heavy financial burden. Development costs for biologics can exceed $1–2 billion USD, and ADA-related failure in late-phase trials or post-market withdrawal results in substantial losses for developers and healthcare systems alike [53].

2.1. Pharmacokinetic and Efficacy Loss

ADAs frequently speed up drug clearance from the host and lower drug concentrations in tissues or circulation by having a substantial impact on the pharmacokinetics and biodistribution of therapeutic proteins [51,54]. Rarely, ADAs may make people more exposed to drugs [69].

Reduced therapeutic efficacy may result from decreased medication levels brought on by ADA-mediated clearance. Especially in patients with elevated ADA titers, this is a significant cause of therapy failure [52,66]. By attaching to the protein therapeutic's active site or other vital functional areas, neutralizing ADAs (nADAs – a subset of ADAs that directly inhibit drug function by binding to the active site or CDR region, distinct from non-neutralizing binding ADAs) immediately reduces its biological action [2]. Target binding is hampered by the majority of ADAs generated in response to therapeutic monoclonal antibodies (mAbs) because they target the antigen-binding region (Fab), most especially the complementarity-determining domains (CDRs).

Neutralizing antibodies can directly impair treatment efficacy, and the creation of immune complexes can cause tissue damage or hypersensitivity, even though not all ADAs cause adverse effects [2,29]. Hypersensitivity reactions, including fever, chills, headaches, serum sickness, Arthus reaction, bronchoconstriction, and even potentially fatal anaphylaxis, are among the risks [69].

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Additionally, ADA-therapeutic mAb immune complexes might accumulate in tissues, causing inflammation and injury [66]. For instance, human anti-murine antibodies (HAMA) and severe immunogenic reactions led to the 1986 approval of the completely murine antibody OKT3, which was later taken off the market [1]. Also, Cetuximab caused severe anaphylaxis in certain patient populations due to mouse-derived glycans, highlighting the importance of PTM control in reducing immunogenicity [4]. Along this pattern, Abciximab, Rituximab, and Daclizumab have also shown varying degrees of immunogenicity [7,66].

2.2. Mechanisms of ADA Formation:

ADAs are formed when the therapeutic antibody is recognized completely or partially as foreign by the host immune system [2,66]. There are two primary pathways by which this can happen: the T-cell-dependent pathway and the T-cell-independent pathway [5,59].

T cell-dependent mechanism as shown in Figure 1 (A) is mostly taken up by long-lasting and high-affinity IgG isotype ADAs. These involve the antigen-presenting cells (APCs) like dendritic cells that internalize the therapeutic mAb and expose naïve CD4+ T cells to linear peptide epitopes in the context of MHC-II [6]. Together with the MHC–T-cell receptor interaction, APCs also generate cytokines that induce naïve T cells to develop into CD4+ helper T cells [52]. After stimulating B cells to develop into plasma cells, activated helper T cells produce IgG antibodies against the treatment, which also have a high affinity and are long-lasting (Table 1) [55].

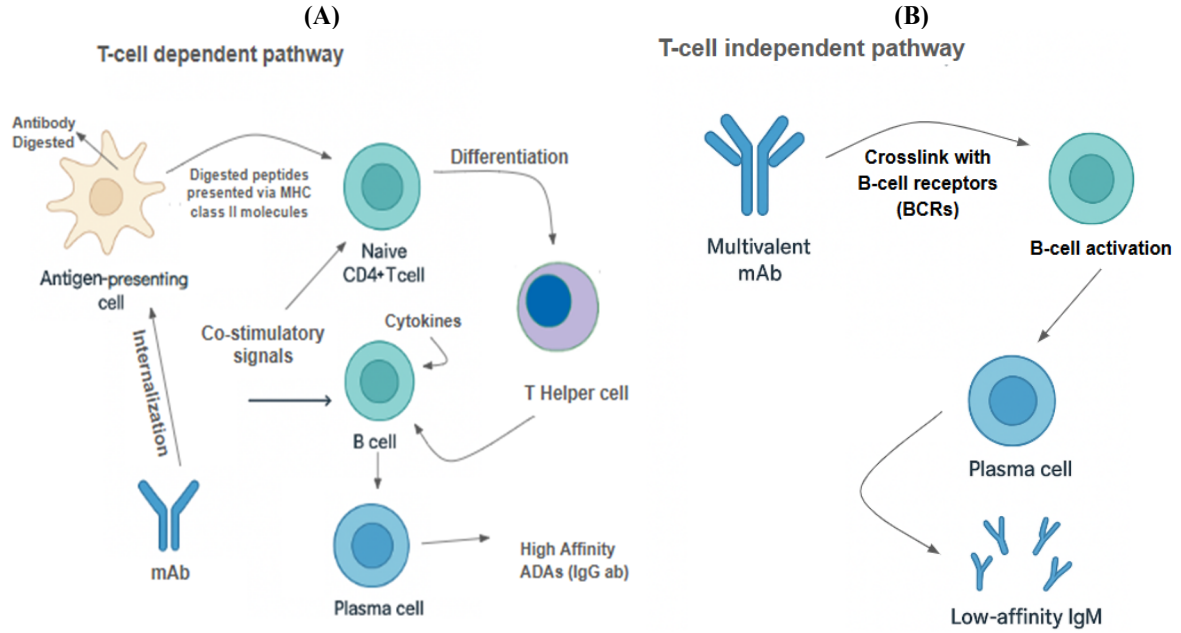

Figure 1. Mechanisms of Anti-Drug Antibody (ADA) Formation. (A) T-cell-dependent pathway: Antigen presenting cells (APCs) internalize the therapeutic monoclonal antibodies (mAbs), then process them into linear peptide fragments, and then present these epitopes via MHC class II molecules to naïve CD4+ T cells. In the presence of co-stimulatory signals and APC-derived cytokines, CD4+ T cells differentiate into helper T cells. These helper T cells then secrete cytokines that stimulate B cells to undergo differentiation into plasma cells, ultimately producing high-affinity, class-switched ADAs which are primarily IgG antibodies. (B) T-cell-independent pathway: Multivalent mAbs with repetitive epitopes can directly crosslink B-cell receptors (BCRs), leading to B cell activation and to the production of primarily low-affinity IgM antibodies without T cell help.

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Table 1. Mechanisms of ADA Formation.

Pathway	Key Immune Components	ADA Type	Features
T-cell-dependent	APCs, CD4+ T cells, B cells	IgG (high affinity)	Long-lasting, undergoes affinity maturation, major contributor to immunogenicity
T-cell-independent	B cells (BCR cross-linking)	IgM (low affinity)	Short-lived, does not require T cell help, triggered by protein aggregates

T-cell-independent pathway typically produces IgM isotype ADAs (Figure 1 (B)), which have a shorter half-life and lower affinity due to not undergoing affinity maturation [59]. Therapeutic mAbs with multiple epitopes can directly crosslink B-cell receptors (BCRs), stimulating the secretion of drug-specific IgM antibodies. B-cell epitopes (BCEs) are specific epitopes on antigens recognized by B cells, usually conformational in nature and restricted to the surface of the therapeutic protein [52].

2.3. Factors Influencing ADA Development

ADA formation is a complex, multifactorial process influenced by drug-related, patient-related, and regimen-related factors [57,66].

2.3.1. Drug-Related Factors:

Antibodies derived from animals, such as mice, are seen as foreign and frequently trigger powerful immunological reactions, like human anti-murine antibody (HAMA) reactions [1,67]. The humanization techniques, which involve replacing non-human sequences with human counterparts, aim to limit immunogenicity. However, even humanized and fully human antibodies can still induce ADAs, suggesting that human sequence alone does not eliminate immunogenic risk [52]. Residual immunogenicity often comes from the CDRs [55].

Immunogenicity is influenced by the mAb's three-dimensional structure and main amino acid sequence [2]. Post-translational modifications (PTMs) also play a role in immunogenicity. Glycans on bioprocess-produced mAbs that do not match human glycosylation patterns can be immunogenic. For example, Cetuximab, with mouse glycans, caused hypersensitivity reactions due to pre-existing IgE antibodies [4]. Other PTMs such as deamination, oxidation, and isomerization can also increase immunogenicity.

mAb aggregation, caused by production or storage, can lead to clustering of multiple B-cell epitopes, cross-linking BCRs, and triggering both T-cell-independent and T-cell-dependent ADA responses [58,64]. Aggregates have been shown to enhance immunogenicity and neutralizing antibody production [2,64].

mAbs with a higher pI (more positive charge) tend to be more immunogenic. This is potentially due to stronger interactions with negatively charged cell surfaces, leading to increased uptake and antigen presentation by APCs.

The mAb's immunogenicity may be influenced by its target or mechanism of action [51]. Accordingly, immune-activating drugs like checkpoint inhibitors and bispecific anti-CD3 antibodies that trigger an immune response against themselves may be the source of elevated ADA incidences [66].

APCs may be more equipped to absorb the antibody-antigen complex when mAbs that target cell-surface proteins are present, thereby enhancing the immune response [51]. For instance,

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alemtuzumab is highly immunogenic because it targets CD52 on APCs [7]. Conversely, immunogenicity is less likely to result from mAbs that inhibit the immune system (such as anti-CD19/CD20 and Rituximab, which decrease B cells) [69].

The presence of both T-cell epitopes (TCEs) and B-cell epitopes (BCEs) contributes to ADA development [52,66]. BCEs are usually conformational, requiring prediction tools that can model protein structures in 3D space. TCEs are linear fragments presented by MHC-II [10]

2.3.2. Patient-Related Factors:

Whether an ADA immune response is elicited depends on certain haplotypes of the human leukocyte antigen (HLA). HLA-II haplotypes dictate whether an antigen will bind and be presented on APC surfaces, affecting ADA formation [24]. Correlations between specific HLA-II haplotypes and ADA responses have been observed for drugs like Adalimumab, which has been linked to specific HLA class II alleles (e.g., HLA-DRB1*04) [66]. Additionally, Rituximab and Atezolizumab are also examples of drugs triggering ADA responses [68]. The patient's immunological status and underlying medical condition also has a significant impact on immunogenicity [29]. ADA response can be accelerated by active immunity and cytokines present in the underlying inflammatory milieu of some patients suffering from autoimmune or inflammatory illnesses, as in the case of systemic lupus erythematosus and rheumatoid arthritis [69]. Rituximab can be taken as an example of this, which is used to treat non-Hodgkin lymphoma and rheumatoid arthritis, and it presents different results for each [69]. ADA rates may be lower in immunocompromised patients than in immune-competent individuals [51].

Patients with lower baseline levels of the drug target may be more likely to develop an ADA response, as unbound mAb is more likely to trigger an immune response [24].

2.3.3. Regimen-Related Factors:

ADA formation may be impacted by the administration route (Table 2). Infliximab may be less immunogenic when administered subcutaneously, according to some research, whereas Tocilizumab and Trastuzumab may be more immunogenic when administered subcutaneously as opposed to intravenously [53].

Table 2. Summary of Factors Influencing ADA Development.

Category	Key Factors
Drug-Related	Origin/humanization, sequence & structure, PTMs, aggregation, isoelectric point, MOA, epitopes
Patient-Related	HLA haplotypes, immune status (autoimmunity vs. immunosuppressed), endogenous protein levels
Regimen-Related	Route of administration, dose/frequency, concomitant medications (e.g., methotrexate)

Consistently high doses or increasing doses can induce a state of immune tolerance. Chronic exposure to therapeutic proteins might induce T-regulatory cells, contributing to peripheral tolerance [55].

Reduction of the overall immunological response can be done by the combined use of immunosuppressive drugs (such as azathioprine, methotrexate, and glucocorticoids), which can

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prevent the synthesis of ADA [69]. On the other side, treatments that boost immunological responses (such as when used with immune checkpoint inhibitors) may hasten the creation of ADA.

2.4. Assessment and Prediction of Immunogenicity

Assessing and predicting immunogenicity are critical early in drug development to identify and mitigate risks [17,53].

2.4.1. In Silico Approaches (Computational Tools):

For the task of **Epitope Prediction**, tools need to predict potential immunogenic threats within a protein's sequence.

MHC-II binding prediction uses machine learning to predict if a drug candidate contains epitopes that can be presented by various human MHC-II alleles. Tools like *NetMHCIIpan* integrate binding affinity and mass-spectrometry-eluted ligand data for improved performance [10]. However, the sole use of T-cell epitope presence often overpredicts clinical immunogenicity [24].

The Immune Epitope Database (*IEDB*) is a vast resource containing known T and B cell epitopes and providing access to prediction tools [11].

Site-specific Immunogenicity for Therapeutic Antibody (SITA) is a novel computational framework that predicts B-cell immunogenicity scores for both the overall antibody and individual residues, based on physicochemical and spatial features of antibody structures [35]. SITA can differentiate immunogenicity levels of whole human, therapeutic, and non-human-derived antibodies. It also showed potential in guiding antibody humanization by identifying modification sites.

Trained on human antibody data, **Protein Language Models** models can suggest humanizing mutations to bring down immunogenicity risk while maintaining therapeutic properties [57].

However some challenges that come with In Silico methods may be that they can overpredict, potentially eliminating good candidates. They often focus on linear T-cell epitopes, neglecting the influence of tertiary structure, PTMs, and individual patient factors [58]. But while computational approaches provide a valuable first-pass prediction, experimental validation remains essential [53].

2.4.2. In Vitro Approaches (Laboratory Assays):

Dendritic Cell (DC) Internalization Assay – co-incubates the drug candidate with DCs and measures if the drug is internalized; a correlation between internalization rate and clinical immunogenicity has been observed [66].

MHC-Associated Peptide Proteomics (MAPPs) Assay – involves using the therapeutic protein to culture APCs, separate MHC-II-peptide complexes, then use mass spectrometry to identify bound peptides [24].

T-cell Activation/Proliferation Assays – use PBMCs from healthy donors, incubated with overlapping peptides from the mAb sequence, to monitor CD4+ T-cell proliferation [10].

Polyspecificity and Hydrophobicity Assays – high-throughput in vitro assays that predict clinical progression more effectively than their In Silico counterparts [17].

2.4.3. Clinical Factors in Prediction:

Clinical studies demonstrate that factors beyond epitopes (like drug MOA, comedication, patient disease, and route of administration) significantly contribute to ADA responses [51]. Machine-learning models combining In Silico epitope prediction with these clinical factors have shown improved accuracy in predicting immunogenicity risk [53]. Regulatory bodies require thorough immunogenicity testing since ADAs might jeopardize safety and effectiveness, especially when developing biosimilars [57].

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2.5. Mitigation Strategies

Improving the clinical results of mAb treatments requires strategies to stop or lessen the production of ADA which we will discuss in this section. The financial burden of therapy development is largely caused by ADA-related immunogenicity problems, which go beyond clinical setbacks. Late-stage clinical trial failures can cost a biologic candidate anywhere from hundreds of millions to over a billion dollars [24,52]. Some of these strategies are listed below and shown in Figure 2.

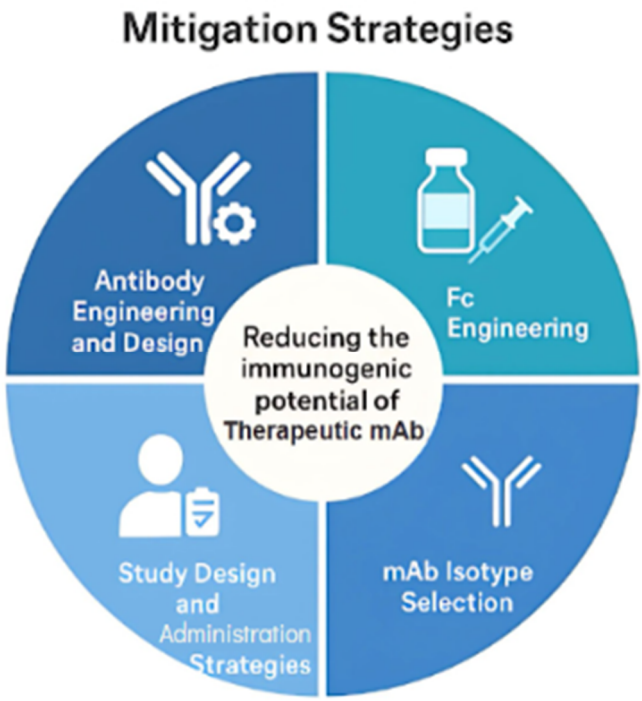

Figure 2. Strategies for Mitigating Anti-Drug Antibody (ADA) Formation. Summary of engineering, formulation, and administration strategies designed to reduce the immunogenic potential of therapeutic monoclonal antibodies. Visual depictions of each mitigation category are arranged as segments of a wheel to illustrate their complementary roles.

2.5.1. Antibody Engineering and Design:

Reducing non-human content is a primary strategy. However, *deimmunization*—removing or modifying immunogenic epitopes (both T-cell and B-cell) from the protein sequence and structure, ideally without disrupting function—is also a logical strategy [62,66]. Computational tools aid in identifying problematic antigens [53]. In parallel, rational design and directed evolution are key engineering strategies. These help develop antibodies with reduced immunogenicity and improved antigen binding [17,58]. They can identify and modify immunodominant epitopes and create libraries of mutated mAb variants using high-throughput screening to select variants with desirable properties, such as reduced immunogenicity and increased antigen binding [53].

2.5.2. Fc Engineering

By modifying the Fc region, effector functions can be enhanced (e.g., Antibody-Dependent Cellular Cytotoxicity (ADCC), Antibody-Dependent Cellular Phagocytosis (ADCP), Complement-Dependent Cytotoxicity (CDC)), and potentially immunogenicity may also be influenced [23,45,62].

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2.5.3. mAb Isotype Selection

Limited research suggests IgG2 mAbs might elicit lower quantities of ADAs compared to IgG1 counterparts, despite IgG1 being the most widely used isotype in mAb therapy [3,67].

2.5.4. Mutations to Reduce Aggregation and Epitope Shielding:

This involves disguising antigenic fragments from the immune system, particularly when epitope removal would impair functionality [2,48]. One way is modifying glycosylation patterns—engineering mAb glycans to be more similar to human patterns can control immunogenicity [39,41]. Another way is PEGylation, which involves attaching polyethylene glycol (PEG) polymers to mAbs to shield surface epitopes. This can potentially lead to longer circulatory half-lives, improved solubility, and reduced immunogenicity [19,61]. Certolizumab pegol is the only FDA-approved Fab version of a TNF-alpha inhibitor that uses PEGylation [69]. As a modification of PEGylation, more recently developed methods such as PASylation and XTENylation offer less immunogenic alternatives by attaching specific polypeptide sequences to the therapeutic protein [18,47].

Modified treatment approaches like dose ramp-up protocols, using mAbs with lower immunogenic profiles, or co-administration with immunosuppressive drugs may prove beneficial for patients who have been classified as high-risk for ADA development based on genetic and immunological profiling [29,57]. Additionally, since host cell proteins (HCPs) can increase immunogenicity, reducing HCPs and controlling aggregation minimizes contaminants during production, making this pattern crucial to understand [58]. It is also essential to optimize production procedures to reduce the creation of protein aggregates [2].

2.5.5. Study Design and Administration Strategies:

Immune tolerance can be induced by regularly giving high doses of mAb treatment, which suppresses ADA synthesis [63]. By preventing T-cell activation signals, co-administration of therapeutic mAbs with immunosuppressants (such as methotrexate, anti-CD20 mAbs like rituximab, and CTLA-4-Ig fusion proteins) can limit ADA production and lower total immunological responses [24,68]. Dose and dosing regimen adjustments also play a role. Higher doses may induce immune tolerance by maintaining sufficient free drug levels to neutralize ADAs or by inducing regulatory T cells [51,54].

3. Novel Delivery Approaches for Reducing Immunogenicity:

Encoding therapeutic immunoglobulin heavy and light chains in viral vectors (e.g., adeno-associated virus (AAV), which can deliver mAb genes for endogenous production—with some serotypes targeting the liver—has also been shown to decrease immunogenicity) or nucleic acids (mRNA in lipid nanoparticles, which deliver mRNA encoding therapeutic mAbs, leading to endogenous translation) allows for production of mAbs within the patient's body, which is hypothesized to reduce immunogenicity [21]. This approach can be simple, cost-effective, provide immediate protection, and has shown no reported ADA development in some trials. However, viral vectors themselves introduce foreign components that might increase immunogenicity [24].

Another strategy to stop ADA development is *oral tolerance*, which is frequently investigated in relation to protein replacement treatments [22].

In efforts to prevent the hazards imposed by immunogenicity and ultimately ADAs, detection of potential side effects during early stages is essential for successful clinical development in the larger framework of antibody therapeutics [52]. It is crucial to ensure that developing therapeutic antibodies are less likely to cause adverse drug reactions and are safer and more effective [57]. To achieve this, computational and experimental approaches are constantly being improved [53,58].

The engineering, formulation, and administration strategies described in Section 4 represent the primary approaches currently employed to mitigate ADA-related immunogenicity risk, summarised in Figure 2.

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4. Immunogenicity Prediction and Assessment:

To minimise unintended immune reactions against therapeutic proteins, immunogenicity prediction and evaluation are essential tools to develop antibody therapies [16]. Because the host immune system can identify therapeutic monoclonal antibodies (mAbs) as foreign and produce anti-drug antibodies (ADAs), it is essential to bypass that mechanism for treatments to properly work, and therefore these efforts are essential [3].

4.1. Importance of Immunogenicity Prediction and Assessment

For ensuring the safety and efficacy of therapeutic antibodies all through the drug development, it is imperative to accurately forecast and assess immunogenicity due to the risks [50]. Developers can select the best candidates, modify medication candidates as necessary, and make decisions about dosage and administration when immunogenicity risk is detected early [36]. It can also cut down on the time and money spent on possibly immunogenic medications [38].

4.2. Methods for Immunogenicity Prediction and Assessment

In the development of therapeutic proteins, regulatory bodies like the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) require the comprehensive evaluation and reporting of immunogenicity [49,56]. Developers must monitor the occurrence of ADA, characterize neutralizing versus non-neutralizing reactions, and evaluate their clinical importance in order to conduct preclinical and clinical studies [63]. Therefore, the immunogenicity profiles of biosimilars must resemble those of the reference product in order for regulators to approve them for market [25]. The FDA's 2019 recommendations on immunogenicity testing and the EMA's guidelines commonly emphasize the importance of tiered testing methodology, established assay techniques, and post-marketing surveillance strategies in guaranteeing patient safety and treatment consistency [49,56]. Hence, a combination of in vitro, in vivo, and in silico techniques are used to assess immunogenicity risk [49].

4.2.1. In Silico Prediction Tools

Computational approaches leverage algorithms and databases to predict potential immunogenic threats early in the drug development pipeline [6].

T-cell Epitopes (TCEs) are linear sequences, typically around 15 amino acids, that are presented by MHC-II (also known as HLA-II) to T cells [8]. Their presence is a key indicator, but their sole use can lead to overprediction [9]. Algorithms like NetMHCIIpan (v3.0, 3.2, 4.0, 4.2) and ISPRI are trained on extensive datasets of peptide–HLA-II binding affinities and/or mass spectrometry–HLA-II eluted-ligand data [10,28]. These tools predict peptide interaction or presentation on specific HLA-II alleles, identifying peptides presented by multiple HLA-II [46].

The Immune Epitope Database (IEDB) is a widely used resource that contains a large library of pre-established B- and T-cell epitopes and hosts various algorithms for predicting highly immunogenic sequences [11].

B-cell Epitope (BCE) prediction is more challenging because over 90% of B-cell epitopes are conformation-dependent, requiring tools to predict protein 3D structures. These tools apply machine learning and structural bioinformatics methods to predict BCEs based on characteristics like hydrophilicity, flexibility, accessibility, and propensity to form secondary structures [27]. Examples include BepiPred, ElliPro, and DiscoTope [12,13].

Humanness Analysis and Sequence Similarity on the other hand include tools like BLAST (Basic Local Alignment Search Tool), which can be used to predict potential immunogenicity by assessing the amount of sequence divergence from the human germline antibody sequence, as this is positively correlated with the magnitude of an ADA response [43]. The "humanness score" concept evaluates the humanization degree of engineered antibodies based on sequence similarity to human antibodies. Metrics include the Z-score, H-score and G-score, and T20 score [43].

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AbNatiV is a deep learning tool that assesses the likelihood an antibody's variable fragment sequence is similar to native human antibodies, providing a score to predict immunogenicity and offering humanization capabilities [43].

OASis percentile (Observed Antibody Space) is a metric well correlated with ADA response on clinical antibody data, computed by overlapping 9-mer amino acid sequences within an antibody compared to human 9-mers in the OAS database [30].

SITA (Site-specific Immunogenicity for Therapeutic Antibody) models conformational epitopes from the perspective of B-cell immunogenicity based on antibody structures, rather than solely "humanness sequence similarity" [35]. It was successfully used to infer overall and site-specific immunogenicity for 25 therapeutic antibodies. SITA has been reportedly used in IND-enabling studies to rationally reduce B-cell epitope loads in therapeutic antibody leads [35].

DeepImmuno is a deep learning approach (CNN, GAN) for predicting and generating immunogenic peptides for T-cell immunity [32]. It aims to improve immunogenicity predictions beyond MHC binding by weighting pMHC complexes based on experimental evidence [32].

PRIME (Predictor of Immunogenic Epitopes) captures molecular properties of both antigen presentation and TCR recognition, aiming to improve prioritization of neo-epitopes and correlating with T-cell potency [42]. It trains HLA-specific models as amino acid enrichment patterns differ between immunogenic peptides presented by different HLAs. PRIME's predictive capability has been considered during personalized neoantigen-based vaccine design strategies [42].

DiffRBM is a sequence-based approach using transfer learning and Restricted Boltzmann Machines to predict antigen immunogenicity and specificity, particularly for HLA-specific peptides [33]. It can predict peptide sites in contact with the TCR and classify immunogenic from non-immunogenic peptides [33].

Generative Humanization reframes humanization as a conditional generative modeling task, where humanizing mutations are sampled from a language model trained on human antibody data [31]. It can incorporate models of therapeutic attributes to obtain candidates with diminished immunogenicity risk and maintained/improved therapeutic properties [31].

Structural Modeling Tools like SWISS-MODEL, ABodyBuilder2, AlphaFold, and Phyre2 can predict 3D and secondary structures of mAbs, which is crucial for identifying conformational epitopes [14,15,34].

New models of Integrated Machine Learning Models which combine computational T-cell epitope prediction with clinical factors such as drug's mechanism of action (MOA), comedications, routes of administration, disease indications, and dose intervals have shown to significantly improve prediction accuracy of clinical ADA incidences [54].

Table 4. Comparison of Immunogenicity Prediction Tools.

Tool	Prediction Focus	Input Type	Output	Notable Feature	Strengths
NetMHCIIpan	T-cell epitopes (MHC-II)	Peptide sequences	Binding affinity scores (IC50)	Integrates mass spec and binding affinity datasets [10]	Trained on binding + eluted ligand data [10]
SITA	B-cell immunogenicity	Antibody structures	Residue-level scores	Structure-based; site-specific prediction [35]	Structure-aware, residue-level precision [35]

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DeepImmuno	Immunogenic peptide generation	Peptide sequences	Immunogenicity probability	Deep learning-based with CNNs and GANs [32]	Accounts for TCR contact probability [32]
PRIME	TCR recognition + presentation	Peptide + HLA context	Immunogenic epitope rank	Models both T-cell recognition and MHC binding [42]	Combines MHC binding + TCR recognition [42]
AbNatiV	Humanness of mAb sequences	Antibody sequences	Humanness likelihood scores	Sequence similarity to native human antibodies [43]	Useful for humanization and de-risking [43]

4.2.2.. In Vitro Assays

These methods evaluate immunogenicity at different stages of immune activation, providing early indications for deimmunization planning or ranking candidate drugs. In addition to laboratory-based evaluations, clinical considerations also provide vital insight into ADA risk [37].

T-cell Proliferation Assays like CD4+ T cell proliferation assays, as discussed before, use peripheral blood mononuclear cells (PBMCs) from healthy donors, incubated with overlapping peptides from the mAb sequence. Proliferation of CD4+ helper T cells indicates immunogenic epitopes.

DC-T-cell coculture assays measure CD4+ helper T-cell proliferation after exposure to donor PBMC-derived dendritic cells (DCs) [10].

Dendritic Cell (DC) Internalization Assays, as mentioned before, with strategies including direct fluorescent labeling or indirect detection, with pHrodo dye and FRET-based assays offer robust signals for internalization and processing [24].

MHC-associated Peptide Proteomics (MAPPs) Assays, helps in identifying processed and presented epitopes, providing information about the immunogenic potential and antigenic portions [24]. MAPPs identify known immunogenic epitopes in infliximab and rituximab, correlating with clinical ADA frequency [24].

Other **Biophysical Assays** like high-throughput in vitro assays measure properties like polyspecificity and hydrophobicity, which are more predictive of clinical progression than their in silico counterparts. Aggregation rate and self-interaction measurements are also assessed.

5. Challenges and Limitations in Prediction and Assessment

Even with improvements, there are still a number of obstacles to correctly predicting and evaluating immunogenicity. The discrepancy between expected epitope immunogenicity and observed ADA incidence in patients—which is impacted by variables like HLA distribution, immunological condition, and medication co-administration—is a significant translational challenge [1,51].

5.1. Biological and Patient-Specific Complexity:

Developing a generic method for predicting immunogenicity is difficult due to the intricacy of the in vivo immune system (*[5]*). T-cell receptor (TCR) recognition, antigen presentation, and the interaction of various immune cells are some of the contributing factors.

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A particularly significant source of this biological complexity is HLA diversity, which directly shapes whether a given epitope triggers an ADA response in any individual patient. As discussed earlier in section 2.3.2.1, specific HLA haplotypes (MHC-II), a patient's immune system status, and endogenous baseline level of a drug can influence whether an ADA immune response is triggered [24]. Emerging machine learning frameworks incorporate clinical metadata—such as disease state, immune status, and patient-specific HLA alleles—to enhance immunogenicity prediction, moving toward a more individualized risk model.

5.2. Data Quality, Standardisation, and Reproducibility:

Small training datasets for many methods, which struggle with limited available data on immunogenic antibody–antibody conjugates, limit the scope of progress for prediction.

Data heterogeneity from ADA data that stems from different clinical trials are collected heterogeneously using various assays and sampling schedules, making it difficult to compare ADA rates across protein therapeutics [16]. The lack of standardization in terminology and methodologies further complicates harmonizing clinical ADA data [63]. This makes direct comparison and validation challenging.

Further, lack of "true negatives"—where a "negative" T-cell assay doesn't definitively mean a peptide is non-immunogenic; it might just mean a cognate T-cell was absent in that experiment.

Prediction accuracy for current algorithms often lacks precision and can overpredict immunogenicity, potentially discarding viable drug candidates [6]. They primarily rely on linear T-cell epitopes, often neglecting the influence of tertiary structure, post-translational modifications (PTMs), and individual patient factors.

These data limitations are compounded by a broader lack of assay harmonisation across the field, which further undermines the comparability of immunogenicity data. A major limitation in the field of immunogenicity assessment is the lack of assay harmonization and reproducible ADA datasets. Multi-tiered testing techniques, such as screening ELISA or bridge assays followed by confirmatory and neutralization assays, are frequently used for ADA detection [63]. However, assay formats, drug tolerance levels, positive cutoffs, and validation criteria vary greatly between sponsors and laboratories.

It is therefore challenging to assess the prevalence of ADA and determine the clinical importance of immunological responses among studies because of these discrepancies [16]. Cross-trial studies are further hampered by the absence of uniform consistency in the language used to characterize ADA data (such as "binding ADA," "neutralizing ADA," and "persistent ADA"). Even medications that target the same antigen may have disparate immunogenicity profiles due to the lack of standardized ADA definitions and thresholds [63].

Additionally, the majority of ADA data from clinical studies are either unpublished or only available in summary form. When data are provided, they frequently lack detail about immunogenic response length, cohort split, or assay methods [63]. Consequently, immunogenicity prediction models trained on such datasets may not generalize, especially across mAbs with diverse mechanisms or clinical settings.

The validation of computational tools for immunogenicity prediction is also restricted. Limited access to well-characterized datasets that link epitope sequences to confirmed ADA outcomes is a major barrier. Model performance evaluation is variable and frequently non-reproducible in the absence of benchmark criteria.

The FDA and EMA are among the regulatory bodies that recognize these limits [49,56]. The FDA's 2019 guidance gives sponsors considerable freedom by recommending a tiered approach to ADA testing without imposing a fixed assay format. While assay innovation is encouraged, reproducibility among biologic programs is decreased due to variations in data collection and interpretation. As the FDA notes: "Due to limitations in assay sensitivity and drug tolerance, negative ADA results do not necessarily rule out immunogenicity."

 doi:10.20944/preprints202603.2174.v1

Notably, the FDA's 2019 guidance explicitly cautions against direct cross-product comparisons of ADA incidence rates, acknowledging that differences in assay sensitivity, drug tolerance levels, and patient populations make such comparisons methodologically unreliable [49].

5.3. Limitations of Current Predictive Models:

Some models only focus on B-cell immunity without considering T-cell epitopes, or vice versa [55]. Many traditional in silico tools also primarily focus on MHC presentation, which is necessary but not sufficient for T-cell immunogenicity [55]. Predicting ADA incidence alone is not sufficient; future models need to predict the clinical impact of ADAs (e.g., on pharmacokinetics, safety, efficacy), which is challenging due to heterogeneous efficacy measures and safety concerns across different disease areas. Differences in homology modeling, reliance on complex reagents for in vitro assays, and data curation contribute to reproducibility challenges [63]. The lack of standardization across ADA detection assays and reporting methods poses a significant barrier to comparative immunogenicity evaluation across biologics [63]. Beyond structural and modeling limitations, immunogenicity prediction must also account for disease-specific immunobiology [55].

Beyond architectural limitations in current models, a further challenge lies in the fundamental differences between pathogen-driven and cancer-associated immunogenicity, which existing frameworks do not adequately distinguish. Evidence suggests that different features and parameter thresholds may be required to predict immunogenic peptides in pathogen versus cancer settings [40]. This is due to fundamental differences in T-cell responses and self-dissimilarity. Most existing models are primarily trained on pathogenic epitopes, which may convolute neoantigen prediction [44].

5.4. Molecular Mimicry as an Underexplored Dimension of Therapeutic Antibody Immunogenicity:

The immunogenicity of therapeutic antibodies has traditionally been framed as a product-specific problem — an unintended consequence of foreign sequence recognition by the host immune system. However, an emerging body of evidence suggests that a deeper molecular mechanism may contribute to immunogenic cross-reactivity in ways that current prediction frameworks do not adequately capture: molecular mimicry between therapeutic antibody epitopes and endogenous pathogen-derived or self-peptides.

Molecular mimicry refers to the structural or sequence similarity between foreign peptides and host proteins sufficient to trigger cross-reactive immune responses [60]. While this phenomenon has been studied extensively in the context of infection-triggered autoimmunity — where pathogen peptides structurally resembling host proteins activate autoreactive T and B cells — its relevance to therapeutic antibody immunogenicity has received comparatively little attention.

The mechanistic connection is direct. Therapeutic monoclonal antibodies are administered to patients with pre-existing immune histories shaped by prior infections. If a therapeutic antibody contains epitopes that structurally resemble peptides from pathogens previously encountered by the patient, pre-existing memory T and B cells could be reactivated upon drug administration, accelerating ADA formation through a molecular mimicry-driven mechanism rather than through classical de novo immunisation. This would manifest clinically as unexpectedly rapid or severe immunogenic responses in patients with specific infection histories, potentially explaining a subset of the inter-patient variability in ADA incidence that current models attribute to HLA diversity and immune status alone.

Recent computational evidence supports this hypothesis. Wrabl et al. [65] demonstrated that antibody cross-reactivity across the human proteome correlates with conformational ensemble compatibility rather than sequence or structural similarity, introducing the concept of ensemble molecular mimicry — wherein antibodies raised against one protein can bind structurally dissimilar proteins that share thermodynamic conformational properties. Applied to therapeutic antibody immunogenicity, this suggests that even fully humanised antibodies could trigger cross-reactive responses if their conformational ensembles overlap with self or pathogen-derived proteins present in the patient's immune memory, a risk invisible to current sequence-based screening tools.

 doi:10.20944/preprints202603.2174.v1

Furthermore, the limitations of sequence-based immunogenicity prediction documented throughout this review — the overprediction of T-cell epitopes, the neglect of conformational B-cell epitopes, the failure to account for patient infection history — are precisely the limitations that would allow mimicry-driven immunogenicity to go undetected. Structural validation of therapeutic epitope similarity to pathogen-derived and self-peptides represents a logical and currently underutilised layer of immunogenicity risk assessment.

Integrating molecular mimicry screening into early-stage immunogenicity pipelines — using tools such as TM-align for atomic structural comparison of candidate epitopes against curated pathogen-host peptide databases — could identify high-risk candidates whose immunogenicity stems not from their foreignness but from their structural resemblance to immunologically primed targets. This represents a mechanistically distinct and complementary risk axis to existing T-cell epitope and humanness-based approaches, and warrants dedicated investigation as the field moves toward more comprehensive immunogenicity prediction frameworks.

5.5. Empirical Support: Preliminary Evidence from a Structural Mimicry Dataset:

To provide preliminary empirical grounding for the hypothesis that sequence-based metrics are insufficient discriminators of structural mimicry, we present key findings from an ongoing computational study involving a curated dataset of 399 pathogen-host peptide pairs spanning 32 rheumatoid arthritis-associated organisms, covering both MHC class I and II epitopes. Peptide pairs were validated using TM-align structural comparison, with RMSD < 2.0 Å used as the threshold for confirming structural mimicry, yielding 262 confirmed mimics and 137 non-mimics.

Sequence identity showed near-zero correlation with structural RMSD across the full dataset ($r = -0.127$), and Mann-Whitney U tests confirmed non-significant differences between confirmed mimic and non-mimic classes for all sequence-derived features including BLOSUM80 score ($p = 0.193$), identity percentage ($p = 0.082$), and alignment coverage ($p = 0.102$). These findings indicate that no individual sequence feature reliably discriminates structurally validated mimics from non-mimics, consistent with the central argument of this review.

A Random Forest classifier trained exclusively on sequence and immunological features — explicitly excluding RMSD and TM-score to prevent label leakage — achieved AUC 0.954 with 100% sensitivity for confirmed mimics, suggesting that while a multivariate sequence signal exists, it is insufficient as a standalone discriminator and does not substitute for structural validation. BLOSUM80 per residue ranked as the top predictive feature (importance = 0.257), yet the non-significant univariate statistics confirm that this signal only becomes detectable through feature combination rather than individual metrics.

Full methodology, complete dataset, and reproducible analysis pipeline are available at github.com/minbaku/molecular-mimicry-RA-pipeline. A detailed research paper presenting the complete pipeline and findings is in preparation.

 doi:10.20944/preprints202603.2174.v1

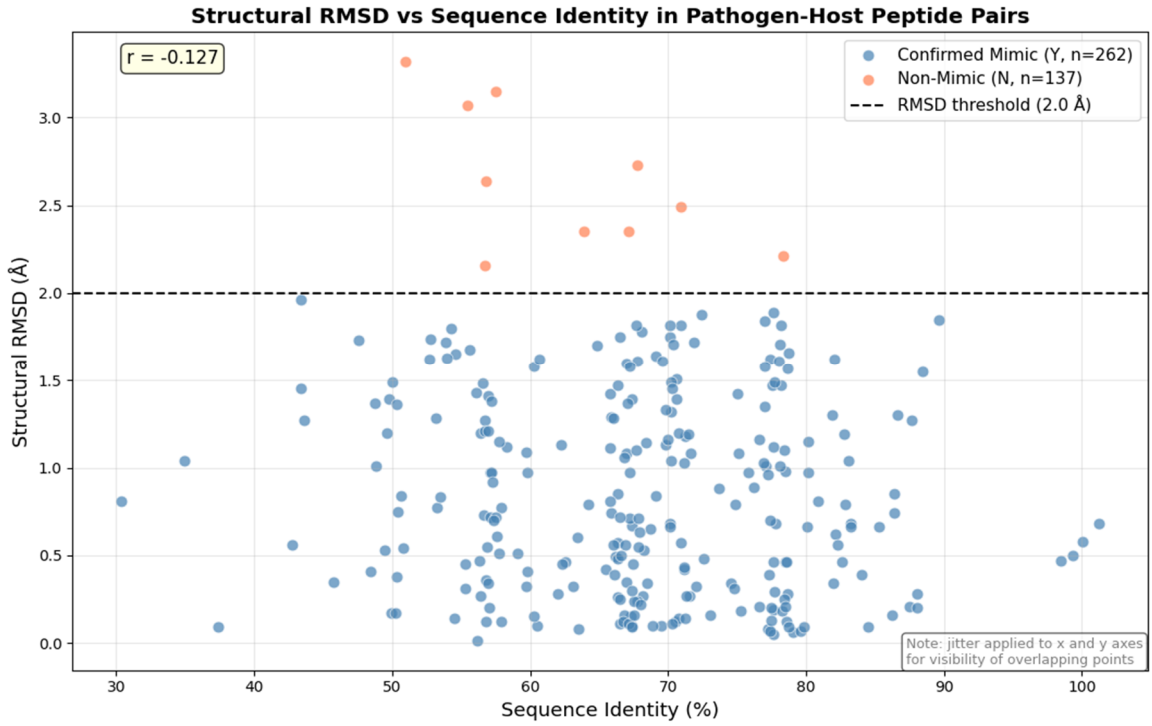

Figure 3. Structural RMSD versus sequence identity across 399 pathogen-host peptide pairs. Confirmed mimics (RMSD < 2.0 Å, n = 262, blue) and non-mimics (RMSD ≥ 2.0 Å, n = 137, coral) show no clear separation by sequence identity (r = -0.127), supporting the conclusion that sequence similarity alone is insufficient for structural mimicry identification. Jitter applied to x-axis for visibility of overlapping points.

6. Future Directions

Future efforts focus on improved models for developing more accurate immunogenicity prediction models that integrate various features, including T-cell and B-cell epitopes, structural properties, and clinical factors [55,63]. The need for more extensive and consistently curated datasets of immunogenic and non-immunogenic peptides, along with standardized assay procedures and reporting, is crucial for better model training and validation [63]. Further research is needed to clarify the link between HLA binding affinity and overall immunogenicity [20]. To find patients who are more likely to develop ADA, HLA genotyping can be used as a pre-treatment screening method [55]. Proactive immunogenicity reduction and stratified therapy decisions may be made possible by integrating patient HLA data with in silico prediction algorithms. HLA-informed deep learning techniques have been investigated in recent research to predict ADA susceptibility, underscoring the increasing viability of customized immunogenicity dashboards in clinical settings.

Subsequent research will investigate the integration of T-cell epitope prediction into models that presently only concentrate on B-cell immunity [55]. As a fundamental component of precision immunotherapy, routine immunogenicity screening using patient-specific risk ratings may also direct antibody selection, dosage, and mitigation tactics [55].

Additionally, the development of personalized medicine, genetic profiles of patients—particularly HLA variants—are essential for identifying risk factors and implementing personalized treatment strategies to enhance clinical outcomes, which are essential to be worked upon [26]. Guidelines from agencies such as the FDA and EMA underscore the need for robust, multi-tiered immunogenicity assessment — encompassing predictive, in vitro, and clinical methods — as part of therapeutic antibody development [49,56].

 doi:10.20944/preprints202603.2174.v1

7. Conclusion:

In the development and clinical application of monoclonal antibody treatments, immunogenicity continues to be one of the most difficult obstacles. Although early detection and reduction of anti-drug antibody dangers have been made possible by computational and experimental improvements, overprediction, patient response variability, and a lack of clinical data integration continue to impede real-world applicability [63]. Emerging evidence also implicates molecular mimicry as an underexplored contributor to inter-patient variability in ADA incidence, representing a mechanistically distinct risk axis that current prediction frameworks do not address and that warrants dedicated investigation.

The future of therapeutic antibody development depends on the concept of personalized medicine. This would combine immunological profiling, HLA type, and machine learning to develop unique immunogenicity risk models [26]. Also, standardizing the ADA assay and larger datasets would be essential to improve the accuracy of the prediction process and unify international regulatory reviews [63]. Developing next-generation biologics that are both powerful and low-immunogenic — thereby optimizing therapeutic effect and lowering patient risk — is becoming more feasible with ongoing research into antibody engineering, delivery improvements, and immunological tolerance induction.

Author Contributions: Conceptualization, M.I.; methodology, M.I.; formal analysis, M.I.; investigation, M.I.; data curation, M.I.; writing — original draft preparation, M.I.; writing — review and editing, M.I. The author has read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The dataset and reproducible analysis pipeline supporting Section 5.5 are publicly available at https://github.com/minbaku/molecular-mimicry-RA-pipeline.

Conflicts of Interest, Declaration and Acknowledgments: The authors declare that there is no conflict of interests regarding the publication of this paper. The author also acknowledges the support and guidance provided by supervisor, **Sayan Chatterjee** (Assistant Professor, GGSIPU) and associated professors. During the preparation of this work, the author used Grammarly and Google Gemini in order to proofread the grammar and vocabulary used for this document. After using these tools, the author reviewed and edited the content as needed and takes full responsibility for the content of the published article. Computational analysis supporting Section 5.5 was performed using Python 3.12 with pandas, scikit-learn, scipy, and matplotlib libraries. The dataset and analysis notebooks are publicly available at github.com/minbaku/molecular-mimicry-RA-pipeline.

Abbreviations

mAbs	**Monoclonal antibodies**
ADAs	**Anti-drug antibodies**
nADAs	**Neutralizing anti-drug antibodies**
MHC	**Major histocompatibility complex**
HLA	**Human leukocyte antigen**
TCE	**T-cell epitope**

 doi:10.20944/preprints202603.2174.v1

BCE	**B-cell epitope**
PK	**Pharmacokinetics**
ADCC	**Antibody-dependent cellular cytotoxicity**
CDC	**Complement-dependent cytotoxicity**
Fc	**Fragment crystallizable**
Fab	**Fragment antigen-binding**
RMSD	**Root mean square deviation**
AUC	**Area under the curve**
MAPPs	**MHC-associated peptide proteomics**
PBMCs	**Peripheral blood mononuclear cells**
CDRs	**Complementarity-determining regions**

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