The Molecular Mechanisms That Underlie the Immune Biology of Anti-drug Antibody Formation Following Treatment With Monoclonal Antibodies

Anna Vaisman-Mentesh1, Matias Gutierrez-Gonzalez2, Brandon J. DeKosky2,3 and Yariv Wine1*

1 George S. Wise Faculty of Life Sciences, School of Molecular Cell Biology and Biotechnology, Tel Aviv University, Tel Aviv, Israel, 2 Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS, United States, 3 Department of Chemical and Petroleum Engineering, The University of Kansas, Lawrence, KS, United States

Monoclonal antibodies (mAbs) are a crucial asset for human health and modern medicine, however, the repeated administration of mAbs can be highly immunogenic. Drug immunogenicity manifests in the generation of anti-drug antibodies (ADAs), and some mAbs show immunogenicity in up to 70% of patients. ADAs can alter a drug’s pharmacokinetic and pharmacodynamic properties, reducing drug efficacy. In more severe cases, ADAs can neutralize the drug’s therapeutic effects or cause severe adverse events to the patient. While some contributing factors to ADA formation are known, the molecular mechanisms of how therapeutic mAbs elicit ADAs are not completely clear. Accurate ADA detection is necessary to provide clinicians with sufficient information for patient monitoring and clinical intervention. However, ADA assays present unique challenges because both the analyte and antigen are antibodies, so most assays are cumbersome, costly, time consuming, and lack standardization. This review will discuss aspects related to ADA formation following mAb drug administration. First, we will provide an overview of the prevalence of ADA formation and the available diagnostic tools for their detection. Next, we will review studies that support possible molecular mechanisms causing the formation of ADA. Finally, we will summarize recent approaches used to decrease the propensity of mAbs to induce ADAs.

Keywords: monoclonal antibodies, anti-drug antibodies, immune response, immunogenicity, neutralizing antibodies

INTRODUCTION

In the last three decades, the pharmaceutical industry experienced a massive shift toward the use of protein drugs, often referred to as “biologics.” Biologics offer higher specificity and better characterized mechanisms of action compared to small molecule drugs, and their use has revolutionized the treatment of a wide range of diseases and disorders. In general, monoclonal antibodies (mAbs) are the most widely used class of biologics (1).
Monoclonal antibodies account for a growing number of blockbuster drugs with their US sales reaching over $24 billion (2), and will maintain a dominant position in the pharmaceutical market that exceeds $125 billion by the end of 2020 (3).

To date, over 73 mAbs have been approved by the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Hundreds more mAbs are in different stages of clinical development. mAbs are used for various clinical indications including cancer, chronic autoimmune and inflammatory diseases, allergies, infections, transplantations, and cardiovascular diseases (4).

The mechanism of action (MOA) of mAbs can vary across different use cases. For example, the anti-CD20 rituximab induces cell death by binding to surface receptors, resulting in a signaling cascade that leads to apoptosis (5). Other mAbs, including the anti-HER-2 trastuzumab, block receptor-ligand interactions to achieve a desired effect, either by blocking the receptor domain to inhibit an activation signal by removing a soluble ligand entirely from circulation (6). mAbs can also induce fragment crystallizable (Fc)-dependent effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), which are important for the anti-CD20 drug obinutuzumab that is used for the treatment of lymphoproliferative disorders (7). Other mAbs target specific proteins involved in pathogenesis of disease, such as anti-TNFα mAbs infliximab and adalimumab that are used to treat inflammatory bowel disease (IBD) and rheumatoid arthritis (RA) (8). Other mAbs in this category are omalizumab, an anti-IgE mAb that is used to treat patients with allergic asthma (9), palivizumab which targets an epitope in the A antigenic site of the F protein of the respiratory syncytial virus (RSV) (10), and bezlotoxumab which binds and neutralizes Clostridium difficile toxin B (11). Some mAbs, such as cetuximab and panitumumab (12), target the epidermal growth factor receptor (EGFR) which is overexpressed in a number of cancers. In recent years, checkpoint inhibitor mAbs were also developed to manipulate anti-tumor T-cell responses, like the anti-PD-1 nivolumab that is used to treat melanoma and non-small cell lung cancer (13).

The tremendous progress in mAb discovery began in 1975, when Köhler and Milstein reported in vitro screening and production of murine mAbs from hybridomas (14). In the late 1980s, murine mAbs were in rapid clinical development, but had significant drawbacks as they were often induced allergic reactions and the formation of human anti-mouse antibodies (HAMA). Examples include T101 used to treat chronic lymphocytic leukemia (CLL) and cutaneous T cell lymphoma (CTCL), and 9.2.27 to treat melanoma (15). Additionally, murine mAbs exhibited a relatively short half-life in humans, possibly due to low affinity toward the human neonatal Fc receptor (FcRn) (16), and were relatively poor recruiters of effector functions, crucial for some mAb efficacy (17).

To overcome the immunogenicity and reduced effector function of murine mAbs, chimeric antibodies (mouse–human) were next developed by fusing the antigen-specific variable domain of a murine mAb with the constant domains of a human mAb. This resulted in chimeric mAbs of approximately 65% human origin by amino acid content (18). Human gene sequences were mostly taken from the κ light chain and the IgG1 heavy chain, as IgG1 has the highest efficiency in activating complement and cytotoxic effector cells, and the κ light chain is more common in human serum antibodies (19, 20). The development of chimeric mAbs indeed reduced immunogenicity and increased efficacy. For example, metastatic colorectal carcinoma patients who received the chimeric mAb 17-1A did not show any toxic or allergic reactions, and the chimeric antibody was significantly less immunogenic than its parental murine antibody (21).

Chimeric mAbs exhibited an extended half-life and reduced immunogenicity, but they still presented a considerably high propensity for ADA induction (22). Aiming to further reduce mAb immunogenicity, humanized mAbs were developed by grafting the murine complementarity determining regions (CDR) onto framework regions (FR) of the human mAb heavy and light chain variable domains (VH and VL, respectively), for mAbs that are approximately 95% human (23). mAb humanization often significantly reduces immunogenicity and ADA formation (24).

 Technological advances of phage display technology (25, 26) based on human single chain Fv (scFv) libraries (27) next enabled the discovery of antibodies comprised entirely of human genes. These human mAbs were additionally aided by the more recent development of transgenic mouse strains expressing human antibody variable domains (28–30).

While both humanized and fully human mAbs reduce immunogenic potential and show properties similar to human endogenous IgGs, they fail to completely eliminate mAb immunogenicity and ADA formation (31). Table 1 summarizes mAbs that are currently approved in the US and EU, along with their reported immunogenicity rates.

In the past decade, next-generation sequencing (NGS) technologies enabled a rapid increase in the capacity to sequence human and animal genomes (32). Like many other areas of modern biology, NGS is now frequently used in basic and applied immunology. NGS is often applied for sequencing the VH and VL antibody domains (33–36), as well as T-cell receptors (37, 38) and antibody derivative [e.g., scFv, F(ab)] libraries screened using display systems (39–41). NGS analysis of B cells can elucidate the features of antibody immune responses at a molecular level, and has been further exploited for advanced mAb discovery and engineering (42–44).

In addition to NGS of bulk populations, single-cell sequencing comprises an important group of technologies for antibody discovery, as single cell data is necessary to reveal the native VH and VL antibody domains (33–36), as well as T-cell receptors (37, 38) and antibody derivative [e.g., scFv, F(ab)] libraries screened using display systems (39–41). NGS analysis of B cells can elucidate the features of antibody immune responses at a molecular level, and has been further exploited for advanced mAb discovery and engineering (42–44).

A recently introduced technology combines proteomic analyses of antibodies in blood or secretions with NGS analysis of antibody-encoding B cells. Proteomics thus provides invaluable information about the molecular, monoclonal properties of human serum antibodies in health and disease (46–48). All of the above recently developed technologies have expedited mAb discovery and revolutionized our understanding about the nature of monoclonal antibodies.
| International non-proprietary name | Brand name | Target | Format | Indication first approved or reviewed | First EU/US approval year | %ADA | %ntADA | References |
|-----------------------------------|------------|--------|--------|---------------------------------------|--------------------------|------|--------|------------|
| Adalimumab                        | Humira     | TNFa   | Human IgG1 | Rheumatoid arthritis                  | 2003/2002                | 28%  | Not reported | (139, 140, 141) |
| Alemtuzumab                       | Lemtrada; MabCampath, Campath-1H | CD52 | Humanized IgG1 | Multiple sclerosis; chronic myeloid leukemia# | 2013; 2001/2014;2001# | 67.1–75.4% | Not reported | (102, 103) |
| Alirocumab                        | Praluent | PCSK9 | Human IgG1 | High cholesterol                      | 2015/2015                | 5.1% | 1.30% | (142) |
| Atezolizumab                      | Tecentriq | PD-L1 | Humanized IgG1 | Bladder cancer                         | 2017/2016                | 30–48% | Not reported | |
| Avelumab                          | Bavencio | PD-L1 | Human IgG1 | Merkel cell carcinoma                  | 2017/2017                | 4.10% | Not reported | |
| Basiliximab                       | Simulect | IL-2R | Chimeric IgG1 | Prevention of kidney transplant rejection | 1998/1998                | 1.17% | Not reported | |
| Belimumab                         | Benlysta  | BLyS  | Human IgG1 | Systemic lupus erythematosus           | 2011/2011                | 0–4.8% | Not reported | (74) |
| Benralizumab                      | Fasenra   | IL-5R α | Humanized IgG1 | Asthma                                 | 2018/2017                | 15.62% | Not reported | (143) |
| Bevacizumab                       | Avastin   | VEGF  | Humanized IgG1 | Colorectal cancer                      | 2005/2004                | 0% | 0% | (144) |
| Bezlotoxumab                      | Zinplava  | Clostridium difficile enterotoxin B | Human IgG1 | Prevention of Clostridium difficile infection recurrence | 2017/2016 | 0% | 0% | |
| Brodalumab                        | Siliq, LUMICEF | IL-17R | Human IgG2 | Plaque psoriasis                       | 2017/2017                | 2.70% | 0% | (145) |
| Burosumab                         | Crysvita  | FGF23 | Human IgG1 | X-linked hypophosphatemia              | 2018/2018                | 0% | 0% | https://www.ultragenyx.com/file.cfm/29/docs/Crysvita_Full_Prescribing_Information.pdf |
| Canakinumab                       | Ilaris    | IL-1β | Human IgG1 | Muckle-Wells syndrome                  | 2009/2009                | <1% | 0% | https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/125319Orig1s085,086,087MedR.pdf |
| Cemiplimab                        | Libtayo   | PD-1  | Human mAb | Cutaneous squamous cell carcinoma      | 2019/2018                | 1.30% | Not reported | |
| Cetuximab                         | Erbitux   | EGFR  | Chimeric IgG1 | Colorectal cancer                      | 2004/2004                | 22.36% | Not reported | |
| Crizanlizumab                     | Adakveo   | CD62 (aka P-selectin) | Humanized IgG2 | Sickle cell disease                     | In review/2019 | 0–1.6% | 0% | |
| Daratumumab                       | Darzalex  | CD38  | Human IgG1 | Multiple myeloma                       | 2016/2015                | 0.70% | Not reported | |

(Continued)
| International non-proprietary name | Brand name | Target | Format | Indication first approved or reviewed | First EU/US approval year | %ADA | %ntADA | References |
|-----------------------------------|------------|--------|--------|--------------------------------------|---------------------------|------|--------|------------|
| Denosumab                         | Prolia     | RANK-L | Human IgG2 | Bone Loss                           | 2010/2010                  | 0%   | 0%     | (146)      |
| Dinutuximab                       | Unituxin   | GD2    | Chimeric IgG1 | Neuroblastoma                        | 2015/2015                   | 28%  | Not reported | (147)     |
| Durvalumab                        | IMFINZI    | PD-L1  | Human IgG1 | Bladder cancer                       | 2018/2017                   | 2.90% | Not reported | https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/761069s002bl.pdf |
| Eculizumab                        | Soliris    | C5     | Humanized IgG2/4 | Paroxysmal nocturnal hemoglobinuria | 2007/2007                   | 0%   | 0%     | (148)      |
| Elotuzumab                        | Empliciti  | SLAMF7 | Humanized IgG1 | Multiple myeloma                     | 2016/2015                   | 33.30% | Not reported | (149)      |
| Emapalumab, emapalumab-tszg       | Gamifant   | IFNγ   | Human IgG1 | Primary hemophagocytic lymphohistiocytosis | In review/2018             | 5%   | 1.60%  | https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/761107Orig1s000MultidisciplineR.pdf |
| Erenumab                          | Aimovig    | CGRP   | Human IgG2 | Migraine prevention                  | 2018/2018                   | 8.90% | 0%     | https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/761077Orig1s000SumR.pdf |
| Evolocumab                        | Repatha    | PCSK9  | Human IgG2 | High cholesterol                     | 2015/2015                   | 0.16% | 0%     | (150)      |
| Evolocumab                        | Dupixent   | IL-4Rα | Human IgG4 | Atopic dermatitis                    | 2017/2017                   | 2–6%  | 4–9%   | https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/761055s007lbl.pdf |
| Frenzumezumab                     | Ajovy      | CGRP   | Humanized IgG2 | Migraine prevention                  | 2019/2018                   | 0.4–1.6% | 0.06–0.9% | https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/761089s000lbl.pdf |
| Galcanezumab                      | Emsgality  | CGRP   | Humanized IgG4 | Migraine prevention                  | 2018/2018                   | 12.50% | Most ADA were mADA | https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/761063Orig1s000ClinPharmR.pdf |
| Golimumab                         | Simponi    | TNFa   | Human IgG1 | Rheumatoid and psoriatic arthritis, ankylosing spondylitis | 2009/2009                   | 31.70% | Not reported | (151)      |
| Gusekumab                         | TREMFYA    | IL-23 p19 | Human IgG1 | Plaque psoriasis                     | 2017/2017                   | 5.50%  | 0.40%  | https://www.accessdata.fda.gov/drugsatfda_docs/nda/2017/761061Orig1s000MultidisciplineR.pdf |
| Ibalizumab, ibalizumab-uyk        | Trogarzo   | CD4    | Humanized IgG4 | HIV infection                        | 2019/2018                   | 0.83%  | 0.83%  | https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/761065Orig1s000ClinPharmR.pdf |
| Infliximab                        | Remicade   | TNF    | Chimeric IgG1 | Crohn’s disease                      | 1999/1998                   | 66.70% | Not reported | (139, 152) |
| Iplilumab                         | Yervoy     | CTLA-4 | Human IgG1 | Metastatic melanoma                  | 2011/2011                   | 26%, 1.1–5.4% | Not reported, 0% | (153), United States Product Information 2018 |
| Ixekizumab                        | Taltz      | IL-17α | Humanized IgG4 | Psoriasis                            | 2016/2016                   | 9%    | Not reported | (154)      |
| Lanadelumab                       | Takhzryo   | Plasma kallikrein | Human IgG1 | Hereditary angioedema attacks         | 2018/2018                   | 12%   | Not reported | https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/761090s000bl.pdf |
(Continued)
| International non-proprietary name | Brand name | Target | Format | Indication first approved or reviewed | First EU/US approval year | %ADA | %ntADA | References |
|------------------------------------|------------|--------|--------|--------------------------------------|---------------------------|------|--------|------------|
| Mepolizumab                        | Nucala     | IL-5   | Humanized IgG1                      | Severe eosinophilic asthma | 2015/2015                 | 3%   | <1%    | (155)      |
| Mogamulizumab                      | Poteligeo  | CCR4   | Humanized IgG1                      | Mycosis fungoides or Sézary syndrome | 2018/2018                 | 3.90% | 0%     | https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/761051Orig1s000MultidisciplineR.pdf |
| Natalizumab                        | Tysabri    | a4 integrin | Humanized IgG4                  | Multiple sclerosis            | 2006/2004                 | 8–9% | Not reported | (156)      |
| Necitumumab                        | Portrazza  | EGFR   | Human IgG1                        | Non-small cell lung cancer   | 2015/2015                 | 4.10% | 1.40%  | https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/125547s000lbl.pdf |
| Nivolumab                          | Opdivo     | PD1    | Human IgG4                        | Melanoma, non-small cell lung cancer | 2015/2014                 | 12.7%, 4.1–37.8% | 0.8%, 0–4.6% | (157) https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/125554s070lbl.pdf |
| Obiltoxaximab                      | Anthim     | B. anthracis PA | Chimeric IgG1    | Prevention of inhalational anthrax | In review/2016               | 0%   | 0%     | (158)      |
| Obinutuzumab                       | Gazyva, Gazyvaro | CD20 | Humanized IgG1                  | Chronic lymphocytic leukemia | 2014/2013                 | 7%    | Not reported | https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125486s017s018lbl.pdf |
| Ocrelizumab                        | OCREVUS    | CD20   | Humanized IgG1                   | Multiple sclerosis             | 2018/2017                 | 0.9%, 0.2–0.5% | 0.15%, 0–0.2% | https://www.accessdata.fda.gov/drugsatfda_docs/nda/2017/761063Orig1s000ClnPharmR.pdf, (159) |
| Ofatumumab                         | Arzerra    | CD20   | Human IgG1                       | Chronic lymphocytic leukemia | 2010/2009                 | <1%   | Not reported | https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/125326s062lbl.pdf |
| Olaratumab                         | Lartruvo   | PDGFRa | Human IgG1                       | Soft tissue sarcoma           | 2016/2016                 | 3.50% | 3.50%  | https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/761038Orig1s000MultiDisciplineR.pdf |
| Omalizumab                         | Xolair     | IgE    | Humanized IgG1                  | Asthma                        | 2005/2003                 | 0%    | 0%     | (160)      |
| Palivizumab                        | Synagis    | RSV    | Humanized IgG1                   | Prevention of respiratory syncytial virus infection | 1999/1998                 | 1.80% | 0%     | (161)      |
| Panitumumab                        | Vectibix   | EGFR   | Human IgG2                       | Colorectal cancer             | 2007/2006                 | 4.60% | 1.60%  | https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/125147s080lbl.pdf |
| Pembrolizumab                      | Keytruda   | PD1    | Humanized IgG4                   | Melanoma                      | 2015/2014                 | 1.80% | 0.50%  | (162)      |
| Pertuzumab                         | Perjeta    | HER2   | Humanized IgG1                   | Breast Cancer                  | 2013/2012                 | 0.60% | Not reported | (163)      |

(Continued)
| International non-proprietary name | Brand name | Target | Format | Indication first approved or reviewed | First EU/US approval year | %ADA | %ntADA | References |
|-----------------------------------|------------|--------|--------|--------------------------------------|---------------------------|------|--------|------------|
| Ramucirumab | Cyramza | VEGFR2 | Human IgG1 | Gastric cancer | 2014/2014 | 3.80% | 0.18% | https://www.accessdata.fda.gov/drugsatfda_docs/nda/2014/125477Orig1s000MedR.pdf |
| Ravulizumab (ALXN1210) | Ultomiris | C5 | Humanized IgG2/4 | Paroxysmal nocturnal hemoglobinuria | 2019/2018 | >0.5% | 0% | https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/761108Orig1s000MultidisciplineR.pdf |
| Raxibacumab | (Pending) | B. anthracis PA | Human IgG1 | Anthrax infection | NA/2012 | 0% | 0% | https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/125349s000lbl.pdf |
| Reslizumab | Cinqaero, Cinqair | IL-5 | Humanized IgG4 | Asthma | 2016/2016 | 4.8–5.4%, 5% | Not reported, 0% | (164, 165) |
| Risankizumab | Skyrizi | IL-23 p19 | Humanized IgG1 | Plaque psoriasis | 2019/2019 | 24% | 14% | https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/761105Orig1s000MultidisciplineR.pdf |
| Rituximab | MabThera, Rituxan | CD20 | Chimeric IgG1 | Non-Hodgkin lymphoma | 1998/1997 | 26–37%, 12.5% | Not reported | (73, 144) |
| Romosozumab | Eventy | Sclerostin | Humanized IgG2 | Osteoporosis in postmenopausal women at increased risk of fracture | NA/2019 | 18.10% | 4.60% | https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/761062Orig1s000MultidisciplineR.pdf |
| Sarilumab | Kevzara | IL-6R | Human IgG1 | Rheumatoid arthritis | 2017/2017 | 14–19.3% | 1.8–3.3% | https://www.accessdata.fda.gov/drugsatfda_docs/nda/2017/761037Orig1s000ChemR.pdf |
| Secukinumab | Cosentyx | IL-17a | Human IgG1 | Psoriasis | 2015/2015 | 0.41% | 0.20% | (166) |
| Situximab | Sylvant | IL-6 | Chimeric IgG1 | Psoriasis | 2014/2014 | 0.20% | 0% | (167) |
| Tildrakizumab | Ilumya | IL-23 p19 | Humanized IgG1 | Psoriasis | 2018/2018 | 6.8–8.8%, 4.1–8.2% | 2.7–3.34%, 0.6–3.2% | https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/761067Orig1s000MultidisciplineR.pdf |
| Tocilizumab | RoActemra, Actemra | IL-6R | Humanized IgG1 | Rheumatoid arthritis | 2009/2010 | 5 | Not reported | (169) |
| Trastuzumab | Herceptin | HER2 | Humanized IgG1 | Breast cancer | 2000/1998 | 16.30% | Not reported | (144) |
| Ustekinumab | Stelara | IL-12/23 | Humanized IgG1 | Psoriasis | 2009/2009 | 6.50% | Not reported | (170) |
| Vedolizumab | Entyvio | a4β7 integrin | Humanized IgG1 | Ulcerative colitis, Crohn’s disease | 2014/2014 | 17% | Not reported | (171) |
of the immune responses, including in the formation of ADAs following immunization and administration of mAbs.

Monoclonal antibodies immunogenicity is mainly manifested in ADA generation (49). The formation of ADAs alters a drug’s bioavailability and pharmacokinetic and pharmacodynamic properties, and most often reduces drug efficacy (50, 51). ADAs have a significant impact on mAb drug safety, as they can lead to serious adverse immune reactions in the clinic (52). Patients with ADAs can be stratified by their effect on the clinical treatment course. Patients are designated as having primary loss of response (LOR) when the administrated mAb fails to show any efficacy within several weeks following treatment initiation, or secondary LOR when patients show significant side effects or the drug loses effectiveness over time despite an initial therapeutic response (53–55).

For multiple decades, many studies focused on possible mechanisms that govern ADA formation, development of improved assays for ADA detection, and advancement of tools for immunogenicity and prediction of ADA formation. This review provides an overview on these topics, underlining the challenges and potential solutions for this important research field. While this review focuses on ADA as an important outcome of mAb immunogenicity, there are other immunogenicity outcomes such as allergic reactions, cytopenia, and anaphylaxis that are widely reviewed elsewhere (56).

THE MOLECULAR MECHANISMS THAT LEAD TO ADA FORMATION

Anti-drug antibodies can be generated by a T-cell dependent or independent B cell activation pathway. In the T-cell dependent pathway, mAbs act as antigens and are internalized by antigen presenting cells (APCs), processed, and presented to T cells via the cognate interaction between the MHC class II molecules and T-cell receptor. Depending on the cytokine milieu during this interaction, several different immune responses can occur (57). In the T-cell dependent pathway, ADAs are generated when a T helper cell (Th) differentiates into a Th1 or Th2 phenotype and, following their cognate interactions with B cells, induces the proliferation of plasma cells (PC) that secrete ADAs. Previous studies showed that a Th2 response mostly induces the production of IgG4 isotype, in comparison to the Th1 response, that in the case of anti-factor VIII elicits the generation of IgG1 and IgG2 ADA (58, 59).

For example, infliximab-specific Th2 cells can be detected in circulation after infliximab infusion, and these cells were correlated with the presence of infliximab-specific ADA (60). Interestingly, this cellular response was observed mostly in patients with hypersensitivity reactions, rather than in the LOR group. In another study, T cell epitopes of infliximab and rituximab were identified by isolating antibody-specific T cells after repeated rounds of antibody-loaded dendritic cells (DCs) in co-culture (61). These T cells were specific to peptides derived from V_H and V_L and encompassed CDRs and FRs, reflecting the immunogenicity of the chimeric part of these antibodies. Importantly, these peptides were also eluted from antibody-loaded DCs, highlighting the importance of MHC Class II antigen presentation in the ADA formation process.

In contrast, for the T cell independent pathway mAbs with multiple epitopes can crosslink B cell receptors (BCRs) and stimulate B cells to differentiate into PC to produce ADAs (62–66). It was previously demonstrated that impurities and aggregates of the mAbs may increase the number of adjacent epitopes on the mAb, potentially steering the immune response toward a T-cell independent pathway by B cell crosslinking (67–70).

DRUG AND PATIENT CHARACTERISTICS CONTRIBUTING TO ADA FORMATION

Anti-drug antibodies formation depends on the interplay between several factors, which can be patient-related or drug-related. Possible causes for ADA formation are summarized in Figure 1.

Patient-Related Factors

The study of why and how ADAs are generated is complicated by the fact that some patients develop ADAs and some, with the same clinical indication and receiving the same therapeutic mAb, do not. The extent of immunogenicity thus differs among patients receiving the same mAb, which could be related to the immune pathways underlying the pathogenesis of the disease (71). For example, RA patients have a higher likelihood of developing ADAs toward a mAb drug than spondyloarthritis patients (57). When examining a specific disease or immune target, different mAbs may have a varying effect on the induction of ADAs. RA patients develop higher ADA levels when treated with two different mAbs (72). In multiple sclerosis (MS) patients, treatment with rituximab (chimeric anti-CD20 mAb) generated an unwanted immune response in up to 37% of patients (73). On the contrary, belimumab (a fully human anti B-cell activating factor (BAFF) mAb), which is used to treat systemic lupus erythematosus (SLE) patients, showed low rates induction ADA (74). Of note, in autoimmune diseases the hyperactivation of both the innate and adaptive immune responses may further complicate the study of mAb immunogenicity (57, 75). On the other hand, when administering mAbs to cancer patients, ADA formation often depends on the stage of the cancer. ADA levels tend to be higher in early stages of the disease than in later stages (76).

Much of the variability in the propensity of administrated mAb to induce ADA formation may result from different immune contexts; Principally, disease status and HLA alleles, which could promote or inhibit an ADA response. The idea that ADA formation is often derived from a T-dependent response has recently led to studies focusing on how ADA formation correlates with HLA polymorphism in the population. Although limited by sample size, Benucci et al. showed that patients with the HLA-DRβ-11, HLA-DQ-03, and HLA-DQ-05 alleles were at a higher risk to develop ADA responses after treatment with an anti-TNF mAb (5 different mAbs were included in this study).
Another report revealed that a G1m1 allotype in the IgG1 created a protease cleavage site in the CH3 domain of the antibody Fc and enabled presentation of a CH3_{15–29} peptide epitope (78). The CH3_{15–29} peptide epitope was tolerated in patients with a G1m1 allotype. However, donors homozygous for nG1m1 did not natively display the G1m1 MHC-II peptide and developed T cell CD4+ responses against antibody therapeutics containing the G1m1 allotype sequence; these ADA were also correlated with HLA-DRB1*07 allele. Some therapeutic mAbs (including trastuzumab) do not harbor this allotype, which could partially explain differences in immunogenicity across different mAb drugs (78, 79). This allotype difference could impact future development of antibody products, since ~40% of the Caucasian population is homozygous for nG1m1, and thus may be at a greater risk for ADA generation (80). In two recent studies, ADA formation against infliximab and adalimumab was correlated with the HLADQA1*05A > G genotype in IBD patients (81, 82). One detailed recent study examined the immune response to natalizumab, a humanized monoclonal IgG4 antibody to α4 integrins that is used to treat patients with MS, and that induces ADA formation in ~6% of the patients. The immune response was found to be polyclonal and targeted different epitopes of the natalizumab idiotype, with a single immunodominant T cell epitope spanning the FR2-CDR2 region of the VL (83).

Generation of a T cell-dependent ADA response is also a multifactorial process, depending not only on the existence of a potential MHC-II peptide epitope in the mAb, but also on the ability of that epitope to be processed, presented and recognized by T cells. The influence of HLA allotypes on the probability of ADA responses should be considered during the design of immunogenicity studies and clinical trials for mAb development. Conclusions from studies that rely on smaller cohorts might not have general applicability for ADA predictions if the study population has substantially different MHC-II gene backgrounds from a larger treatment population.

**Drug-Related Factors**

The molecular mechanisms that lead to induction of ADAs were initially related to the murine origin of the first mAbs, which were recognized as "non-self" by the human immune system. Unfortunately, even the use of complete human antibody genes has not completely eliminated immunogenicity and the associated induction of ADA (84). Fully human mAbs contain new epitopes in the CDRs that can steer the immune response through an idiotype/anti-idiotype interaction (85, 86). As discussed above, mAb-derived peptides presented by MHC-II are necessary for T cell-dependent ADA formation. Efforts to remove T cell epitopes during mAb engineering are used consistently, but the high genetic variability of human populations greatly complicates efforts to remove all MHC-II-binding peptides from human mAbs (87, 88). Changes in Fc glycosylation may also affect ADA induction. The removal of N-linked glycosylation of the Fc was shown to reduce immunogenicity (89). Fully human mAbs lacking Fc functions were also shown to be immunogenic and have direct effects on the ability to recruit macrophages and activate complement. For example, galactose-α-1,3-galactose, which is a foreign glycan not found in humans, is present on the antigen-binding (Fab) portion of the cetuximab V_H (a chimeric mAb used in cancer therapy targeting the EGF receptor). This glycan was shown to induce ADA formation of the IgE isotype, and was responsible for anaphylactic reactions in patients (90, 91). On the other hand, immunogenicity is sometimes linked to impurities in the formulation process, and not necessarily due to glycosylation differences. A review of the differences between 18 biosimilars and mAbs originators concluded that the differences between
them are mainly in glycosylation patterns, and do not impact immunogenicity (92).

Other drug-related factors that play a role in mAb immunogenicity are "danger signals" that are released by tissues undergoing stress, damage or abnormal death. The danger model was first suggested in 1994, where it was first postulated that the immune system responds to substances that cause damage, rather than to those that are simply foreign (93, 94). In the case of therapeutic antibodies, process-related impurities (such as aggregates and residual DNA or proteins from the mAb expression system) can influence immunogenicity (95).

The mAb target may also have high importance for the MOA of ADA formation. We recently found that repeated administration of infliximab (a TNFα antagonist) results in a vaccine-like response, where ADA formation is governed by the extrafollicular T cell-independent immune response (96). The administration of infliximab blocks TNFα and shifts the immune response toward the marginal zone (MZ) instead of the germinal center (GC), as observed in TNFα knockout mice (97). Another possible explanation is that a strong T cell-independent immune response in the MZ may be induced by a drug/ADA/TNFα immunocomplex (IC). As a trimer, TNFα may form “super complexes” upon engagement with TNFα antagonist antibodies (98–100).

Another example of mAb target importance is alemtuzumab, a mAb specific to the CD52 lymphocyte cell surface glycoprotein. Alemtuzumab is used to treat MS (101) and induces ADAs in about 85% of patients, of which around 92% develop neutralizing ADAs (102). Alemtuzumab’s high frequency of ADA induction may be related to CD52 expression patterns. Alemtuzumab targets APCs, which include DCs, monocytes, and memory B cells, based on their CD52 expression. When monocytes repopulate, they encounter the circulating mAb that rapidly presents antigen to the antigen-specific lymphocytes (103, 104). Memory B cells often exhibit homeostatic expansion following treatment with alemtuzumab (105), which could complement ADA generation.

mAb dosage and schedule are other possible factors influencing ADA formation rates. Increased numbers of injections and higher mAb doses are associated with higher ADA risk, although some cases of chronic treatment and higher doses have lower immunogenicity (92, 106). For example, rituximab, a chimeric mAb anti-CD20, targets surface antigens on pre-B cells and B cells before their differentiation into PCs. As rituximab selectively depletes CD20 positive B cells, it does not affect mature PCs and does not have a propensity to elicit ADAs (107).

ASSAYS FOR IMMUNOGENICITY ASSESSMENT AND TOOLS FOR IMMUNOGENICITY REDUCTION

Pre-clinical Setting
Due to the growing importance of mAb immunogenicity, there has been a growing need for tools to assess immunogenicity and reduce the propensity of mAbs to induce ADAs. Great efforts in tools such as in silico prediction algorithms and cell-based experimental assays are facilitating immunogenicity assessment, especially during the initial development phases of the mAb (108).

In silico CD4+ T cell epitope prediction models are often used to identify potentially immunogenic MHC-II peptide epitopes. These algorithms are based on the affinity of mAb-derived peptides to MHC-II (109–111).

With recent advances in proteomics and sequencing, several MHC-II peptide epitope databases have been constructed that provide a library of MHC-II binding data to enable immunogenicity prediction (112). Most algorithms that predict the immunogenic sequences recognized by T cells are later confirmed by assessing peptide binding to MHC molecules (88, 113). For example, a strong correlation was found between in silico evaluation of T cell epitopes from a recombinant Fc fusion protein, and the immunogenicity rate when administered to patients in a clinical trial (114). While such predictive algorithms are common used, they capture only a fraction of the system’s complexity. Most CD4+ T cell epitope prediction algorithms are based on binding affinity and stability to MHC-II molecules (88, 110), but fail to consider other essential factors in the recognition of T cell epitopes. Among these factors are protease cleavage sites (115), T cell precursor frequency (116), and peptide and T cell competition (117).

Experimental tools are also used to make pre-clinical predictions about mAb immunogenicity risk. These include HLA binding assays, DC related assays, T cell stimulation assays, peripheral blood mononuclear cell (PBMC) stimulation assays, and various animal models (115). HLA binding and DC antigen presentation assays can evaluate potential T cell epitopes derived from the mAb, while T cell and PBMC stimulation assays examine whether a mAb can activate immune cells in vitro and ex vivo in terms of cell proliferation and cytokine release. For example, T cell epitopes in the variable regions of infliximab and rituximab were able to stimulate peripheral blood mononuclear cells (PBMCs) to secrete a variety of cytokines (61). In another study, the immunogenicity of secukinumab, an anti-interleukin-17A mAb used to treat plaque psoriasis, was assessed by examining T-cell proliferation (118).

Each of these experimental tools has limitations in assessing and predicting immunogenicity. While considered reliable and straightforward, most of the experimental assays are labor intensive and are impractical to implement with a large number of mAb candidates. These assays are often performed with cells derived from a naïve population, where the frequency of antigen-specific cells is relatively low and precludes a clear positive result due to low signal-to-noise ratios (88).

Other advancements are being made in the development of mAbs to which patients will be more tolerant. A previous study identified a set of naturally occurring human regulatory T cell epitopes ("Tregitopes"), present in the Fc and Fab domains of IgG, that induce tolerance when co-administered with other proteins (119). When incubated with PBMCs in vitro, Tregitopes activated CD4+ T cells and increased expression of regulatory cytokines, chemokines, and CD25/Foxp3. When were administered in vivo with protein antigens, Tregitopes inhibited
T cell proliferation, reduced effector cytokine expression, and induced antigen-specific adaptive tolerance. Co-administration of Tregitopes along with mAbs may be a useful tool for tolerization of mAbs.

Clinical Settings
Early and accurate ADA detection is extremely important for patients treated with biologics, especially for mAbs (120). ADA detection is required to provide the clinicians with sufficient information to monitor treatment and determine optimal intervention strategies (121). Detection of ADA against therapeutic mAbs is highly challenging since both the drug and the analyte are antibodies. Moreover, immunoassays are prone to biases due to the presence of the drug and immune-complexes in patients’ serum. Historically, studies of the response following mAb administration and ADA prevalence have been inconsistent, partly due to the various assay formats used to monitor immunogenicity in clinical trials (122). Each available format has its limitations that can reduce the assay’s utility in clinical and research settings, and also complicate interpretation of the data. Some assays have poor dynamic range and may generate false-negative results because of interfering interactions with the active drug, or false-positive results due to other antibodies like rheumatoid factor (123). Figure 2 shows the competing factors which affect accurate measurement of ADAs.

An ELISA-based bridging assay is one of the most commonly used assays for ADA screening, where the mAb drug is used to first capture ADA present in the patient sera, and the latter are detected by adding additional labeled mAb as a secondary probe. Bridging ELISA assays are used for ADA detection of a large variety of mAbs, and some include an acidic step to dissociate ADA from the mAb. The excess mAb is then captured or removed, and free ADA can be detected. These assays often have significantly higher background and suffer from low sensitivity due to the disassociation of antibodies. Bridging assays can also result in false-negatives, as they are more likely to “miss” low affinity IgM ADAs present in early stages of the immune response (124). Most ELISA-based bridging assays are also sensitive to the mAbs’ trough levels (levels of circulating mAb at sampling time). ADA and mAbs tend to form high molecular weight immune-complexes, making ADA detection more challenging (125). To overcome this challenge, several drug-tolerant assays have been developed to measure ADA levels in the presence of high mAb concentrations (126). Most of these assays also use an acidic treatment step. Several other techniques have been reported to evaluate serum ADA levels. These assays include radio-immunoassays (127), Biotin-drug Extraction with Acid Dissociation (BEAD) (128), Precipitation and Acid dissociation (PANDA) (129), Affinity Capture Elution ELISA (ACE) (130), and Homogenous Mobility Shift Assay (HMSA) (131); these assays have been reviewed in detail elsewhere (126). While these assays presumably detect all serum ADA, they primarily provide qualitative measures to assist healthcare providers deciding on appropriate patient interventions, and many (if not all) studies underestimate actual ADA levels. These assays also lack standardization that could enable comparisons of ADA levels across health centers. The great diversity in these assays poses tremendous difficulty in studying ADA levels between different mAbs, across studies of the same mAb, and across different assays.

In a clinical context, it important both to assess ADA levels in patient serum, and also to assess the presence of neutralizing antibodies that interfere with biological and clinical activity of the mAb. The neutralizing effect of ADAs can be assayed by testing whether ADAs in serum inhibit binding of the mAb to its target (132). Several cell-based assays were developed to detect ntADA in patients’ serum. One of these assays is a functional ADA cell-based assay that was developed to quantify the activity of TNFα antagonists. This assay assesses both drug activity and ntADA levels (133), but correlations between the clinical outcome and assay results were not thoroughly tested. Another assay developed for ntADA detection is the reporter gene assay, which is based on excretion of IL8 by HT29 cells due to TNFα stimulation (77). When the assay was applied to sera samples with low-level ADA, it detected ntADA even prior to clinical LOR to the mAb, which allows the prediction of clinical LOR with high probability.

While these assays are accurate and sensitive, they require an active cell line, which complicates assay implementation. We recently reported on a newly developed quantitative bio-immunoassay for quantifying ADA specific to TNFα antagonists. The bio-immunoassay was further modified to easily assess the neutralization capacity of ADA using an in vitro assay (96). This assay can be readily used in a clinical setting that performs routine ADA measurements.

Other clinical approaches to reduce immunogenicity include active interference of the T cell responses to mAbs, thereby inducing individual tolerance of the immune system (“tolerization”).

![Figure 2](image-url)
For example, administration of methotrexate (MTX) with infliximab reduced ADA formation in RA patients (134). MTX also reversed high ADA levels in infantile Pompe disease patients treated with rituximab, when administered alongside bortezomib, a proteasome activity inhibitor that leads to cell death (135). Azathioprine is also an immunosuppressive drug that can be given in combination with infliximab or adalimumab to improve treatment and reduce immunogenicity and ADA formation (136–138). However, such non-specific immunosuppressive approaches have potentially harmful side effects that must be balanced with the patient's overall treatment plan.

CONCLUDING REMARKS

Monoclonal antibodies have the potential to treat a wide range of diseases and disorders, but they can be highly immunogenic and induce undesirable ADA responses. ADAs can reduce mAb drug efficacy by altering its bioavailability and/or accelerating clearance from circulation. While the molecular mechanisms of ADA generation are not fully understood, it is dependent on both patient and drug characteristics. While early ADAs were related to the murine origin of the first mAb therapeutics, ADAs also occur against fully human mAbs. Indeed, complete humanization cannot completely abrogate mAb immunogenicity and ADA formation. The questions of why and how ADA are generated also depend on variability of the reported immunogenicity rates, which emphasizes the need for standardized clinical assays for ADA detection. Understanding the mechanisms of ADA generation and the major factors that influence immunogenicity of mAbs will help us design safer mAbs with lower drug rejection rates. Recent and ongoing efforts to study mAb immunogenicity at the molecular level is augmenting our understanding of these mechanisms that lead to ADA formation, which may help provide new guidelines to improve the safety and efficacy of mAb therapeutics.

AUTHOR CONTRIBUTIONS

AV-M, MG-G, BD, and YW wrote the sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

Funding for this work was provided by the United States-Israel Binational Science Foundation #2017359, and by US NIH grants R21AI143407, R21AI144408, and DP5OD023118.

ACKNOWLEDGMENTS

We thank Colette Worcester for assistance with manuscript preparation.
20. Reff ME, Carner K, Chambers KS, Chinn PC, Leonard JE, Raab R, et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood. (1994) 83:435–45. doi: 10.1182/blood.v83.2.435. bloodjournals.org/doi/10.1182/bloodjournals.org-2014-9324

21. LoBuglio AF, Wheeler RH, Trang J, Haynes A, Rogers K, Harvey EB, et al. Mouse/human chimeric monoclonal antibody in man: kinetics and immune response. Proc Natl Acad Sci USA. (1989) 86:4220–4. doi: 10.1073/pnas.86.11.4220

22. Presta LG. Engineering of therapeutic antibodies to minimize immunogenicity and optimize function. Adv Drug Deliv Rev. (2006) 58:640–56. doi: 10.1016/j.addr.2006.01.026

23. Jones PT, Dear PH, Foote J, Neuberger MS, Winter G. Replacing the

24. Hwang WY, Foote J. Immunogenicity of engineered antibodies. Methods. (2005) 36:3–10. doi: 10.1016/j.ymeth.2005.01.001

25. Winter G, Griffiths AD, Hawkins RE, Hoogenboom HR. Making antibodies by phage display technology. Annu Rev Immunol. (1994) 12:333–55. doi: 10.1146/annurev.iy.12.040194.002245

26. Vaughan TJ, Williams AJ, Pritchard K, Osbourn JK, Pope AR, Earnshaw JC, et al. Next generation deep sequencing and vaccine therapeutics: the key causes, consequences and challenges. Self Nonself. (2010) 1:314–22. doi: 10.4161/self.1.1.13904

27. Luciani F, Bull RA, Lloyd AR. Next generation deep sequencing and vaccine design: today and tomorrow. Trends Biotechnol. (2012) 30:443–52. doi: 10.1016/j.tibtech.2012.05.005

28. DeKosky BJ, Ippolito GC, Deschner RP, Lavinder JJ, Wine Y, Rawlings BM, et al. High-throughput sequencing of the paired human immunoglobulin heavy and light chain repertoire. Nat Biotechnol. (2013) 31:166–9. doi: 10.1038/nbt.2492

29. DeKosky BJ, Kojima T, Rodin A, Charab W, Ippolito GC, Ellington AD, et al. In-depth determination and analysis of the human paired heavy- and light-chain antibody repertoire. Nat Med. (2015) 21:86–91. doi: 10.1038/nmm.3743

30. Doria-Rose NA, Schramm CA, Gorman J, Moore PL, Bhiman JN, DeKosky BJ, et al. Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. Nature. (2014) 509:55–62. doi: 10.1038/nature13230

31. Wu X, Zhou T, Zhu J, Zhang B, Georgiev I, Wang C, et al. Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. Science. (2011) 333:1593–602.

32. Robins HS, Camppreger PV, Srivastava SK, Wacher A, Turtle CJ, Khosai O, et al. Comprehensive assessment of T-cell receptor beta-chain diversity in the human T-cell receptor repertoire.血

33. Glanville J, Zhai W, Berka J, Telman D, Huerta G, Mehta GR, et al. Precise determination of the diversity of a combinatorial antibody library gives insight into the human immunoglobulin repertoire. Proc Natl Acad Sci USA. (2009) 106:20216–21. doi: 10.1073/pnas.0907757106

34. Vaisman-Mentesh A, Wine Y. Monitoring phage biopanning by next-generation sequencing. Methods Mol Biol. (2018) 1701:463–73. doi: 10.1007/978-1-4939-7447-4_26

35. Wang B, DeKosky BJ, Timm MR, Lee J, Normandin E, Misasi J, et al. Functional interrogation and mining of native paired human VH/VL antibody repertoires. Nat Biotechnol. (2018) 36:152–5. doi: 10.1038/nb.4052

36. Georgiou G, Ippolito GC, Beausang J, Beausang B, Carver J, Quake SR. The promise and challenge of high-throughput sequencing of the antibody repertoire. Nat Biotechnol. (2014) 32:158–68. doi: 10.1038/nb.2782

37. Reddy ST, Ge X, Mikkos AE, Hughes RA, Kang SH, Hoi KH, et al. Monoclonal antibodies isolated without screening by analyzing the variable gene repertoire of plasma cells. Nat Biotechnol. (2010) 28:965–9. doi: 10.1038/nbt.1673

38. Rosati E, Dowds CM, Liaskou E, Henriksen EKK, Karlsen TH, Franke A. Antigen-specific human monoclonal antibodies from mice engineered with human Ig heavy and light chain YACs. Nat Genet. (1994) 7:13–21. doi: 10.1038/ng0594-13

39. Cohenaram M, Saif MW, Panitumumab the first fully human monoclonal antibody: from the bench to the clinic. Ancticancer Drugs. (2007) 18:7–15. doi: 10.1097/cad.0b013e32800fecc8

40. Rau R. Adalimumab (a fully human anti-tumour necrosis factor alpha antibody) in the treatment of active rheumatoid arthritis: the initial results of five trials. Ann Rheum Dis. (2002) 61(Suppl. 2):i170–3.

41. Baker MP, Reynolds HM, Lumicisci B, Bryson CJ. Immunogenicity of protein therapeutics: the key causes, consequences and challenges. J Mol Biol. (2014) 217604.

42. Georgiou G, Ippolito GC, Beausang J, Busse CE, Wardemann H, Quake SR. Molecular-level analysis of the serum antibody repertoire in young adults before and after seasonal influenza vaccination. Nat Med. (2016) 22:1456–64. doi: 10.1038/nm.4224

43. Lavinder JJ, Wine Y, Giesecke C, Ippolito GC, Horton AP, Lungu OI, et al. Identification and characterization of the constituent human serum antibody elicited by vaccination. Proc Natl Acad Sci USA. (2014) 111:2239–64. doi: 10.1073/pnas.131779311

44. Bentz DR, Horton AP, Wine Y, Lavinder JJ, Georgiou G, Marcotte EM. Proteomic identification of monoclonal antibodies from serum. Ann Chem. (2014) 86:4758–66. doi: 10.1021/ac4037679

45. van Schouwenburg PA, Rispens T, Wolbink GJ. Immunogenicity of anti-TNF biologic therapies for rheumatoid arthritis. Nat Rev Rheumatol. (2013) 9:164–72. doi: 10.1038/nrrheum.2013.4

46. Atzeni F, Talotta R, Salaffi F, Cazzinotti A, Vargas E, Battilino M, et al. Immunogenicity and autoimmunity during anti-TNF therapy. Autoimmun Rev. (2013) 12:703–8. doi: 10.1016/j.autrev.2012.10.021

47. De Groot AS, Scott DW. Immunogenicity of protein therapeutics. Trends Immunol. (2007) 28:482–90. doi: 10.1016/j.it.2007.07.011

48. Hansel TT, Kropshofer H, Singer T, Mitchell JA, George AJ. The safety and side effects of monoclonal antibodies. Nat Rev Drug Discov. (2009) 9:325–38. doi: 10.1038/nrd3003

49. de Vries MK, Wolbink GJ, Stapel SO, de Groot ER, Dijkmans RA, Aarden LA, et al. Inefficacy of infliximab in ankylosing spondylitis is correlated with antibody formation. Ann Rheum Dis. (2007) 66:133–4. doi: 10.1136/ard.2006.075774

50. Yanai H, Hanauer SB. Assessing response and loss of response to biological therapeutics in IBD. Am J Gastroenterol. (2011) 106:685–98. doi: 10.1038/ajg.2011.103

51. Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn's disease. Aliment Pharmacol Ther. (2011) 33:987–95. doi: 10.1111/j.1365-2636.2011.04612.x

52. Baldo BA. Adverse events to monoclonal antibodies used for cancer therapy. J Mol Biol. (2017) 471:1761. doi: 10.1016/j.jmb.2017.01.053

53. Vultaggio A, Petroni G, Pratesi S, Nencini F, Cammelli D, Milla M, et al. Circulating T cells to infliximab are detectable mainly in treated patients.
developing anti-drug antibodies and hypersensitivity reactions. *Clin Exp Immunol.* (2016) 186:364–72. doi: 10.1111/cei.12858

61. Hamze M, Meunier S, Karle A, Gdoura A, Goudet A, Szely N, et al. Characterization of CD4 T cell epitopes of infliximab and rituximab identified from healthy donors. *Front Immunol.* (2017) 8:500. doi: 10.3389/fimmu.2017.00500

62. Vos Q, Lees A, Wu ZQ, Snapper CM, Mond JJ. B-cell activation by T-cell-independent type 2 antigens as an integral part of the humoral immune response to pathogenic microorganisms. *Immunol. Rev.* (2000) 176:154–70. doi: 10.1034/j.1600-065x.2000.00607.x

63. Alugupalli KR, Leong JM, Woodland RT, Muramatsu M, Honjo T, Gerstein RM. Bb lymphocytes confer T-cell-independent long-lasting immunity. *Immunity.* (2004) 21:379–90. doi: 10.1016/j.immuni.2004.06.019

64. Obukhanych TV, Nussenzweig MC. T-independent type II immune responses generate memory B cells. *J Exp Med.* (2006) 203:365–10. doi: 10.1084/jem.20052036

65. El Shikh ME, El Sayed RM, Szakal AK, Tew JG. T-independent antibody responses to T-dependent antigens: a novel follicular dendritic cell-dependent activity. *J Immunol.* (2009) 182:3482–91. doi: 10.4049/jimmunol.0802317

66. Taillardet M, Hafler DA, Mondié P, Asensio MJ, Gheit H, Burdin N, et al. The identification of long-lived plasma cells in blood depends on the activation status of follicular dendritic cells. *J Exp Med.* (2015) 295:118–26. doi: 10.1084/jem.2015.03.002

67. Batista FD, Harwood NE. The who, how and where of antigen presentation to B cells. *Nat Rev Immunol.* (2009) 9:15–27. doi: 10.1038/nri2454

68. Kumar S, Singh SK, Wang X, Rup B, Gill D. Coupling of aggregation and immunogenicity in biotherapeutics: T- and B-cell immune epitopes may contain aggregation-prone regions. *Pharm Res.* (2015) 28:949–61. doi: 10.1007/s11095-011-1414-9

69. Yin L, Chen X, Vicini P, Rup B, Hickling TP. Therapeutic outcomes, assessments, risk factors and mitigation efforts of immunogenicity of therapeutic protein products. *Cell Immunol.* (2015) 295:118–26. doi: 10.1016/j.cellimm.2015.03.002

70. Fehr T, Bachmann MF, Bucher E, Kalinke U, Di Padova FE, Lang AB, et al. The mechanistic impact of N-glycosylation on stability, pharmacokinetics, and immunogenicity of therapeutic proteins. *Nat RevDrug Saf.* (2015) 14:534–55. doi: 10.1038/jjcmd.2013.09.006

71. Zhou Q, Qu H. The mechanistic impact of N-glycosylation on stability, pharmacokinetics, and immunogenicity of therapeutic proteins. *J Pharm Sci.* (2019) 108:1366–77. doi: 10.1016/j.xphs.2018.11.029

72. Pasparakis M, Alexopoulou L, Episkopou V, Kollias G. Immune and inflammatory responses generate memory B cells. *Nat Rev Immunol.* (2009) 9(Suppl. 1):27–34. doi: 10.1038/nri2454

73. Johnson MJ, de Lange G, Cavailler-Sforza LL. Ig gamma restriction fragment length polymorphisms indicate an ancient separation of Caucasian haplotypes. *Ann Hum Genet.* (1986) 38:817–40.

74. Zalonovs A, Kennedy NA, Moutsianas L, Heap GA, Rice DL, Reppell M, et al. HLA-DQA1*05 carriage associated with development of anti-drug antibodies to infliximab and adalimumab in patients with Crohn's disease. *Gastroenterology.* (2020) 158:189–99.

75. Wilson A, Peel C, Wang Q, Pananos AD, Kim RB. HLA-DQA1*05 genotype predicts anti-drug antibody formation and loss of response during infliximab therapy for inflammatory bowel disease. *Aliment Pharmacol Ther.* (2020) 51:356–63. doi: 10.1111/apt.15563

76. Cassotta A, Mikol V, Bertrand T, Pouzieux S, Le Parc J, Ferrari P, et al. A single T cell epitope drives the neutralizing anti-drug antibody response to natalizumab in multiple sclerosis patients. *Nat Med.* (2019) 25:1402–7. doi: 10.1038/s41591-019-0568-2

77. Vaisman-Mentesh A, Rosenstein S, Yavzori M, Dror Y, Fudim E, Ungar B, et al. Molecular landscape of anti-drug antibodies reveals the mechanism of the immune response following treatment with TNFα inhibitors in autoimmune inflammatory disease. *A systematic review and meta-analysis. Autoimmun Rev.* (2017) 16:564–75. doi: 10.1016/j.autrev.2017.04.002

78. Harding FA, Stickler MM, Razo J, DuBridge RB. The immunogenicity of humanized and fully human antibodies: residual immunogenicity resides in the CDR regions. *MAbs.* (2010) 2:256–65. doi: 10.4161/mabs.2.3.11641

79. Hardin S, Zalonovs A, Kennedy NA, Moutsianas L, Heap GA, Rice DL, Reppell M, et al. The mechanistic impact of N-glycosylation on stability, pharmacokinetics, and immunogenicity of therapeutic proteins. *J Pharm Sci.* (2019) 108:1366–77. doi: 10.1016/j.xphs.2018.11.029

80. Pasparakis M, Alexopoulou L, Episkopou V, Kollias G. Immune and inflammatory responses generate memory B cells. *Nat Rev Immunol.* (2009) 9(Suppl. 1):27–34. doi: 10.1038/nri2454

81. Johnson MJ, de Lange G, Cavailler-Sforza LL. Ig gamma restriction fragment length polymorphisms indicate an ancient separation of Caucasian haplotypes. *Ann Hum Genet.* (1986) 38:817–40.

82. Zalonovs A, Kennedy NA, Moutsianas L, Heap GA, Rice DL, Reppell M, et al. HLA-DQA1*05 carriage associated with development of anti-drug antibodies to infliximab and adalimumab in patients with Crohn's disease. *Gastroenterology.* (2020) 158:189–99.

83. Wilson A, Peel C, Wang Q, Pananos AD, Kim RB. HLA-DQA1*05 genotype predicts anti-drug antibody formation and loss of response during infliximab therapy for inflammatory bowel disease. *Aliment Pharmacol Ther.* (2020) 51:356–63. doi: 10.1111/apt.15563

84. Cassotta A, Mikol V, Bertrand T, Pouzieux S, Le Parc J, Ferrari P, et al. A single T cell epitope drives the neutralizing anti-drug antibody response to natalizumab in multiple sclerosis patients. *Nat Med.* (2019) 25:1402–7. doi: 10.1038/s41591-019-0568-2

85. Vaisman-Mentesh A, Rosenstein S, Yavzori M, Dror Y, Fudim E, Ungar B, et al. Molecular landscape of anti-drug antibodies reveals the mechanism of the immune response following treatment with TNFα inhibitors in autoimmune inflammatory disease. *A systematic review and meta-analysis. Autoimmun Rev.* (2017) 16:564–75. doi: 10.1016/j.autrev.2017.04.002

86. Harding FA, Stickler MM, Razo J, DuBridge RB. The immunogenicity of humanized and fully human antibodies: residual immunogenicity resides in the CDR regions. *MAbs.* (2010) 2:256–65. doi: 10.4161/mabs.2.3.11641

87. Hardin S, Zalonovs A, Kennedy NA, Moutsianas L, Heap GA, Rice DL, Reppell M, et al. The mechanistic impact of N-glycosylation on stability, pharmacokinetics, and immunogenicity of therapeutic proteins. *J Pharm Sci.* (2019) 108:1366–77. doi: 10.1016/j.xphs.2018.11.029

88. Pasparakis M, Alexopoulou L, Episkopou V, Kollias G. Immune and inflammatory responses generate memory B cells. *Nat Rev Immunol.* (2009) 9(Suppl. 1):27–34. doi: 10.1038/nri2454

89. Johnson MJ, de Lange G, Cavailler-Sforza LL. Ig gamma restriction fragment length polymorphisms indicate an ancient separation of Caucasian haplotypes. *Ann Hum Genet.* (1986) 38:817–40.

90. Zalonovs A, Kennedy NA, Moutsianas L, Heap GA, Rice DL, Reppell M, et al. HLA-DQA1*05 carriage associated with development of anti-drug antibodies to infliximab and adalimumab in patients with Crohn's disease. *Gastroenterology.* (2020) 158:189–99.

91. Johnson MJ, de Lange G, Cavailler-Sforza LL. Ig gamma restriction fragment length polymorphisms indicate an ancient separation of Caucasian haplotypes. *Ann Hum Genet.* (1986) 38:817–40.
113. Fleri W, Paul S, Dhanda SK, Mahajan S, Xu X, Peters B, et al. The immune
111. Sacks D, Baxter B, Campbell BCV, Carpenter JS, Cognard C, Dippel D, et al.
102. Baker D, Herrod SS, Alvarez-Gonzalez C, Giovannoni G, Schmierer K.
117. Kedl RM, Kappler JW, Marrack P. Epitope dominance, competition and T
116. Harrington CJ, Paez A, Hunkapiller T, Mannikko V, Brabb T, Ahearn M,
115. Manoury B, Mazzeo D, Fugger L, Viner N, Ponsford M, Streeter H, et al.
110. Yin L, Calvo-Calle JM, Dominguez-Amorocho O, Stern LJ, HLA-Dm
109. Messitt TJ, Terry F, Moise L, Martin W, De Groot AS. A comparison
106. Herskovitz J, Ryman J, Thway T, Lee S, Chirnule N, et al. Immune
107. Cohen SB, Emery P, Greenwald MW, Dougados M, Furie RA, Genovese
104. Thomas K, Eisele J, Rodriguez-Leal FA, Hainke U, Ziemssen T. Acute
103. Collet-Brose J, Coulbe P, Dechan MR, Nelson RJ, Felin WG, Lory S. Evaluation of multiple immunoassay technology platforms to select the anti-drug antibody assay exhibiting the most appropriate drug and target tolerance. J Immunol Res. (2016) 2016:5069678.
102. Baker D, Herrod SS, Alvarez-Gonzalez C, Giovannoni G, Schmierer K. Interpreting lymphocyte reconstitution data from the pivotal phase 3 trials of alemtuzumab. JAMA Neurol. (2017) 74:961–9. doi: 10.1001/jamaneurol.2017.0676
101. Cohen JA, Coles AJ, Arnold DL, Confavreux C, Fox EJ, Hartung HP, et al. Alemtuzumab versus interferon beta 1a as first-line treatment for patients with relapsing-remitting multiple sclerosis: a randomised controlled phase 3 trial. Lancet. (2012) 380:1819–28.
100. Bar-Yoseph H, Pressman S, Blatt A, Vainberg SG, Mainon N, Starosvetsky E, et al. Inflliximab-tumor necrosis factor complex elicits formation of anti-drug antibodies. Gastroenterology. (2019) 157:1338–51.e8.
99. De Groot AS, Moore L, McMurry JA, Wambre E, Van Overtveldt L, Moinogeon P, et al. Activation of natural regulatory T cells by IgG Fc-derived peptide “Tregitopes”. Blood. (2008) 112:3303–11. doi: 10.1182/blood-2008-02-138073
98. Kriekkaert C, Rispens T, Wolkink G. Immunogenicity of biological therapeutics: from assay to patient. Carr Opin Rheumatol. (2012) 24:306–11. doi: 10.1097/bor.0b013e3283521e4e
97. Hart MH, de Vrieze H, Wouters D, Wolkink GJ, Killestein J, de Groot ER, et al. Differential effect of drug interference in immunogenicity assays. J Immunol Methods. (2011) 372:196–203. doi: 10.1016/j.jim.2011.07.019

methylene in rheumatoid arthritis. *Arthritis Rheum.* (1998) 41:1552–63. doi: 10.1002/1529-0131(199809)41:9<1552::AID-ARTS>3.0.CO;2-W

135. Banugaria SG, Prater SN, McGann JK, Feldman JD, Tannenbaum JA, Bailey C, et al. Bortezomib in the rapid reduction of high sustained antibody titers in disorders treated with therapeutic protein: lessons learned from Pompe disease. *Genet Med.* (2013) 15:123–31. doi: 10.1038/gim.2012.110

136. Ruffolo CR, Scarpa M, Bassi N. Infliximab, azathioprine, or combination therapy for Crohn’s disease. *N Engl J Med.* (2010) 363:1086–7; author reply 1087–8.

137. Anderson PJ. Tumor necrosis factor inhibitors: clinical implications of their different immunogenicity profiles. *Semin Arthritis Rheum.* (2005) 34:19–22. doi: 10.1016/j.semarthrit.2005.01.005

138. Garces S, Demengeot J, Benito-Garcia E. The immunogenicity of anti-TNF therapy in immune-mediated inflammatory diseases: a systematic review of the literature with a meta-analysis. *Ann Rheum Dis.* (2013) 72:1947–55. doi: 10.1136/annrheumdis-2012-202220

139. Bartelds GM, Krieckaert CL, Nurmohamed MT, van Schouwenburg PA, Paul S, Moreau AC, Del Tedesco E, et al. Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up. JAMA. (2011) 305:1460–8. doi: 10.1001/jama.2011.406

140. Paul S, Moreau AC, Del Tedesco E, Rinaudo M, Genin C, et al. Pharmacokinetics of adalimumab in inflammatory bowel diseases: a systematic review and meta-analysis. *Inflamm Bowel Dis.* (2014) 20:1288–95. doi: 10.1097/MIB.0000000000000377

141. Deisseroth A, Ko CW, Nie L, Zhao L, Bullock J, et al. Pharmacokinetic and exposure-response analysis of pertuzumab in patients with HER2-positive metastatic gastric or gastroesophageal junction cancer. *Cancer Chemother Pharmacol.* (2019) 84:339–50. doi: 10.1007/s00280-019-03871-w

142. Bagel J, Lebwohl M, Israel RJ, Jacobson A. Immunogenicity and skin clearance recapture in clinical studies of brodalumab. *J Am Acad Dermatol.* (2020) 82:344–51. doi: 10.1016/j.jaad.2019.05.094

143. Chen Q, Hu C, Liu Y, Song R, Zhu W, Hao H, et al. Pharmacokinetics, pharmacodynamics, safety, and tolerability of single-dose denosumab in healthy Chinese volunteers: a randomized, single-blind, placebo-controlled study. *PLoS One.* (2013) 8:e101794. doi: 10.1371/journal.pone.0101794

144. Ozkaynak MF, Gilmam AL, London WB, Naranjo A, Diccianni MB, Tenney SC, et al. Trial of chimeric antibody 14.18 with GM-CSF, IL-2, and future prospects. *Front Immunol.* (2018) 9:164983.

145. Bagel J, Lebwohl M, Israel RJ, Jacobson A. Immunogenicity and skin clearance recapture in clinical studies of brodalumab. *J Am Acad Dermatol.* (2020) 82:344–51. doi: 10.1016/j.jaad.2019.05.094

146. Chen Q, Hu C, Liu Y, Song R, Zhu W, Hao H, et al. Pharmacokinetics, pharmacodynamics, safety, and tolerability of single-dose denosumab in healthy Chinese volunteers: a randomized, single-blind, placebo-controlled study. *PLoS One.* (2013) 8:e101794. doi: 10.1371/journal.pone.0101794

147. Oztaynay MF, Gilmam AL, London WB, Naranjo A, Diccianni MB, Tenney SC, et al. Trial of chimeric antibody 14.18 with GM-CSF, IL-2, and future prospects. *Front Immunol.* (2018) 9:164983.

148. Passey C, Mora J, Dodge R, Gibiansky L, Sheng J, Roy A, et al. An integrated assessment of the effects of immunogenicity on the pharmacokinetics, safety, and efficacy of elotuzumab. *AAPS J.* (2017) 19:557–67. doi: 10.1208/s12248-016-0033-9

149. Koren MJ, Sabatine MS, Giugliano RP, Langslet G, Wiviott SD, Ruza A, et al. Long-term efficacy and safety of evolocumab in patients with hypercholesterolemia. *J Am Coll Cardiol.* (2019) 74:2132–46. doi: 10.1016/j.jacc.2019.08.1024

150. Leu JH, Adedokun OJ, Gargano C, Hsia EC, Xu Z, Shankar G. Immunogenicity of golimumab and its clinical relevance in patients with rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis. *Rheumatology.* (2019) 58:441–6. doi: 10.1093/rheumatology/key309

151. Ben-Horin S, Yavzori M, Katz L, Kopylov U, Picard O, Fudim E, et al. The immunogenic part of infliximab is the F(ab')2, but measuring antibodies to the intact infliximab molecule is more clinically useful. *Gut.* (2010) 60:41–8. doi: 10.1136/gut.2009.195153

152. Quistrebert I, Hassler S, Bachelet D, Mbogning C, Musters A, Tak PP, et al. Incidence and risk factors foradalimumab and infliximab anti-drug antibodies in rheumatoid arthritis: a European retrospective multicohort analysis. *Semin Arthritis Rheum* (2019) 48:967–75. doi: 10.1016/j.semarthrit.2019.10.006

153. Van L, Wang B, Ciai YL, Roskos LC. Population pharmacokinetic modeling of benralizumab in adult and adolescent patients with asthma. *Clin Pharmacokinet.* (2019) 58:943–58. doi: 10.1007/s40262-019-00738-4

154. Saffari F, Jahzardeh A, Kalantari Khandani B, Saffari F, Soleimanyoli S, Mohammadi M. Immunogenicity of rituximab, trastuzumab, and bevacizumab monoclonal antibodies in patients with malignant diseases. *Int J Cancer Manag.* (2018) 11:e64983.

155. Bagel J, Lebwohl M, Israel RJ, Jacobson A. Immunogenicity and skin clearance recapture in clinical studies of brodalumab. *J Am Acad Dermatol.* (2020) 82:344–51. doi: 10.1016/j.jaad.2019.05.094

156. Chen Q, Hu C, Liu Y, Song R, Zhu W, Hao H, et al. Pharmacokinetics, pharmacodynamics, safety, and tolerability of single-dose denosumab in healthy Chinese volunteers: a randomized, single-blind, placebo-controlled study. *PLoS One.* (2013) 8:e101794. doi: 10.1371/journal.pone.0101794

157. Oztaynay MF, Gilmam AL, London WB, Naranjo A, Diccianni MB, Tenney SC, et al. Trial of chimeric antibody 14.18 with GM-CSF, IL-2, and future prospects. *Front Immunol.* (2018) 9:164983.
169. Burmester GR, Choy E, Kivitz A, Ogata A, Bao M, Nomura A, et al. Low immunogenicity of tocilizumab in patients with rheumatoid arthritis. *Ann Rheum Dis*. (2017) 76:1078–85. doi: 10.1136/annrheumdis-2016-210297

170. Chiu H-Y, Chu TW, Cheng Y-F, Tsai T-F. The association between clinical response to ustekinumab and immunogenicity to ustekinumab and prior adalimumab. *PLoS One*. (2015) 10:e0142930. doi: 10.1371/journal.pone.0142930

171. Ungar B, Kopylov U, Yavzori M, Fudim E, Picard O, Lahat A, et al. Association of vedolizumab level, anti-drug antibodies, and alpha4beta7 occupancy with response in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol*. (2018) 16:697–705.e7. doi: 10.1016/j.cgh.2017.11.050

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

*Copyright © 2020 Vaisman-Mentesh, Gutierrez-Gonzalez, DeKosky and Wine. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.*