PART I

RISK-BASED STRATEGIES

1

PRINCIPLES OF RISK ASSESSMENT AND MONITORING OF ANTIBODY RESPONSES TO BIOPHARMACEUTICALS

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1.1. RISK ASSESSMENT

The first step in establishing a risk-based strategy for detection and characterization of antibodies to biopharmaceuticals is risk assessment. Ideally, risk assessment comprises an objective evaluation of risk factors that are clearly defined and understood. Assessing the risk of antibody response to biopharmaceuticals can be difficult because multiple factors contribute to the immunogenicity of therapeutic proteins: for example, structural properties; impurities and contaminants; formulation; the route and frequency of administration; dose level and duration of treatment; and patient health and genetic background.

Nevertheless, industry-wide experience with biopharmaceuticals over the last 25 years provides the basis for the design and validation of anti-drug antibody (ADA) methods [1–3] as well as rational, risk-based strategies for ADA monitoring during development and postapproval administration of biopharmaceuticals [4]. Clinical sequelae caused by antibodies directed against biopharmaceuticals can range from no apparent or mild side effects to

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diminished efficacy, immune complex mediated symptoms, allergic reactions, or even autoimmune syndromes.

The first, and probably most important, step in risk assessment of a biopharmaceutical is to consider its type and its mechanism of action. If the biopharmaceutical is a molecule that has a nonredundant endogenous counterpart responsible for a vital physiological function, a great deal of caution should be taken. It has been shown that autoimmune-like syndromes can develop in patients with ADAs capable of neutralizing the biological activity of both the drug and its endogenous counterpart [5, 6]. These autoimmune-like syndromes induced by biopharmaceuticals can be life threatening and could persist for prolonged periods of time [7], as a continuous cycle of antibody production is maintained by the endogenous molecule even after treatment with the biopharmaceutical is stopped. In addition to the molecular nature of a biopharmaceutical and its mechanism of action, there are other important factors that should be considered during the risk assessment, such as the route and frequency of drug administration as well as the patient's immune status. It is generally accepted, and has been demonstrated in some cases, that in head-to-head clinical comparison the subcutaneous route is more immunogenic than the intramuscular or intravenous route [8].

1.2. HIGH-RISK BIOPHARMACEUTICALS

One of the most illustrative examples of a high-risk biopharmaceutical is recombinant human thrombopoietin (rhTPO). According to the criteria outlined in Section 1.1, thrombopoietin belongs to the category of high-risk molecules because it represents a nonredundant, endogenous growth factor that regulates a vital physiological function. In one development program, an antibody monitoring strategy as well as dosing regimen was designed on the basis of a rigorous risk assessment. During clinical development, administration of rhTPO was limited to immunocompromised cancer patients. In several clinical studies, rhTPO was dosed exclusively via the intravenous route. This dosing scheme was also accompanied by extensive antibody monitoring, utilizing three different assays: (a) a screening enzyme-linked immunosorbent assay (ELISA) for antibodies that bound to the full-length rhTPO molecule; (b) an ELISA for antibodies binding to the amino-terminal (bioactive) portion of the molecule; and (c) an ELISA for antibodies that inhibit binding of rhTPO to its receptor. These assays were used sequentially in a contingent fashion. If positive in assay (a), the sample was analyzed in assay (b); then if positive in assay (b), the sample was tested in assay (c). In addition, samples positive in all three ELISAs were analyzed in a human megakaryocyte proliferation assay for antibodies capable of neutralizing rhTPO's biological activity. These assays were carried out in real time (weekly) to provide clinicians with timely information on the occurrence and type of antibodies so that rhTPO administration could be stopped if the presence of neutralizing antibodies was detected. No

neutralizing antibody–related adverse effects were observed in any of the multiple clinical studies utilizing intravenous rhTPO administration [9].

In another development program, a truncated form of recombinant thrombopoietin (TPO), megakaryocyte growth and development factor (MGDF), was subcutaneously administered to immunocompromised cancer patients and to healthy human subjects. Neutralizing anti-MGDF antibodies were detected in 0.6% and 4% of these subjects, respectively. Long-lasting severe thrombocytopenia was associated with anti-Tpo neutralizing antibodies in some subjects [7, 10]. Differences in clinical outcomes observed in these two thrombopoietin development programs underscore the importance of a careful risk assessment combined with a comprehensive ADA monitoring strategy and an appropriate dosing strategy.

Another example of a high-risk biopharmaceutical is recombinant human erythropoietin (rhEPO). It has been shown that neutralizing antibodies directed against rhEPO can induce pure red cell aplasia (PRCA) [6, 11]. In a number of kidney dialysis patients who were treated with rhEPO and who developed PRCA, ADA response was characterized by the surface plasmon resonance (SPR) method combined with a cell-based bioassay. The SPR assay allowed for determination of the overall ADA level, isotypes, and affinity. High levels of the high-affinity immunoglobulins IgG1 and IgG4 were found. The same samples also inhibited *in vitro* cell proliferation induced by the rhEpo, demonstrating the presence of neutralizing antibodies [12]. In contrast, low-affinity, non-neutralizing IgM ADAs have been detected occasionally in patients with no signs of PRCA (Amgen, data on file). These data indicate that the high levels of high-affinity ADAs with neutralizing activity are more likely to induce PRCA.

1.3. LOW- TO MODERATE-RISK BIOPHARMACEUTICALS

Therapeutic humanized and human antibodies represent lower risk because ADAs directed against these biopharmaceuticals are not likely to cross-react with endogenous molecules and cause autoimmune syndromes. The risks of ADAs to therapeutic antibodies have been associated primarily with loss of efficacy. For example, in patients with Crohn's disease treated with infliximab, the chimeric monoclonal antibody to tumor necrosis factor alpha (TNF-α), the median therapeutic response to this drug was significantly shorter in patients with higher ADA levels [13]. Diminished response to infliximab was also observed in ADA-positive patients with rheumatoid arthritis (RA) and ankylosing spondylitis [14–16]. Adalimumab, the completely human anti-TNF-α antibody, can also induce ADAs, which in some cases are associated with reduced efficacy in patients with RA [17, 18]. Antibodies to natalizumab, the humanized anti–very late activation antigen-4 (anti-VLA4), can cause loss of efficacy as well [19]. The precise mechanisms by which the efficacy of these therapeutic antibodies is diminished remain unclear without more detailed

ADA characterization. Some clinical observations suggest that binding of ADA to the drug increases its clearance. However, it is conceivable that some ADAs could neutralize efficacy by blocking a drug's complementarity-determining regions (CDRs) or adjacent epitopes. Monitoring such neutralizing ADAs with appropriate assays might be necessary if there is a loss of efficacy even in the absence of clearing antibodies. With the more widespread and more complex use of therapeutic antibodies, new types of risks have emerged. For example, RA patients who developed ADAs during treatment with infliximab more frequently developed ADAs to adalimumab in a subsequent "switch-over" treatment with adalimumab [20]. Furthermore, ADA responses may be influenced by allotypic differences between the therapeutic antibody and a patient's own immunoglobulins [21]. For example, the ADA response against adalimumab is higher in a Japanese population than in Caucasians due to allotypic differences [22]. These and other observations [23–25] suggest that genetic factors may play a role in the ADA responses, which should not be overlooked during clinical development. Nevertheless, clinical experience so far supports the view that humanized or human therapeutic antibodies generally fall into the category of biopharmaceuticals with lower to moderate risk.

Multicomponent biopharmaceuticals may add another level of risk because even the antibodies directed to the nonactive component of a drug could result in adverse clinical outcomes. For example, in patients treated with pegylated asparaginase, antibodies to polyethylene glycol (PEG) have been associated with changes in pharmacokinetics (PK) and loss of product efficacy [26]. Anti-PEG antibodies also have been associated with changes in PK and infusion reactions even in the absence of antibodies to the active protein component. It is, therefore, important to develop and implement separate assays specific to different components of a multicomponent biopharmaceutical.

Special consideration should be given to biopharmaceuticals that are used in treatment of genetic deficiencies such as lysosomal storage diseases or hemophilia. Given the inherent lack of immune tolerance toward missing proteins in these conditions, the high frequency of ADAs (80–100%) directed against the replacement biopharmaceuticals is not surprising. Obviously, ADAs cannot cross-react with missing endogenous counterparts and the danger of autoimmune syndromes does not exist. However, because of the serious nature of the aforementioned diseases, the loss of efficacy due to ADAs could have adverse clinical consequences. Monitoring of total and neutralizing ADAs using appropriate assays is highly recommended because such data can be useful in adjusting the therapeutic regimen if necessary. For example, in the case of mucopolysaccharidosis VI (MPS VI), recombinant human arylsulfatase B (Naglazyme, BioMarin, Novato, CA) is used to diminish lysosomal accumulation of dermatan sulfate. The strategy for monitoring ADAs in this treatment included total as well as neutralizing ADA assays designed on the basis of Naglazyme's two-step mechanism of action. One assay is used to detect ADA-mediated inhibition of Nagalazyme's binding to its mannose-6-phosphate

receptor and the other to determine potential inhibitory effect on Naglazyme's catabolic activity [27]. The data available to date from the Naglazyme Phase IV Clinical Surveillance Program (NCT00214773) show a high incidence of seroconversion (92%). However, no significant correlation has been established between antibody levels and changes in urinary glycosaminoglycan (GAG), used as a pharmacodynamic efficacy marker [28]. One explanation of this observation could be that a sufficient quantity of free Naglazyme can be taken up by cells even in the presence of circulating ADAs and that the internalized drug does reach the lysosomal compartment and degrades accumulated GAG. Another explanation could be that the drug–ADA complexes dissociate within the lysosomal compartment due to the low pH and/or proteolytic degradation of antibodies, allowing the enzyme to exert its natural function. Similarly, preservation of drug efficacy despite the high incidence of seroconversion has been observed in other storage diseases treated with replacement enzymes [29, 30]. In contrast, those replacement proteins that act outside cells, such as recombinant factors VIII and IX, are much more vulnerable to inhibitory effects of ADAs [31, 32].

1.4. DRUG–ADA COMPLEXES

Formation of immune complexes between biopharmaceuticals and ADAs can pose significant risks as well. A case has been reported of a patient with Pompe disease who developed reversible nephrotic syndrome during prolonged, high-dose enzyme replacement therapy with recombinant human acid–alpha glucosidase (rhGAA; Myozyme). Due to the development of ADAs to rhGAA and concomitant clinical decline, escalating doses of rhGAA were administered as part of an experimental immune tolerance regimen. Histological evaluation of kidney tissue revealed glomerular deposition of immune complexes containing rhGAA and IgG ADAs in a pattern of membranous nephropathy [33].

Nephrotic syndrome also has been observed in patients with hemophilia B undergoing immune tolerance therapy to eliminate ADAs directed against recombinant factor IX. Although the incidence of ADAs in patients with hemophilia B is generally lower (30–40%), high levels of ADA and ongoing high-dose drug treatment can lead to the reversible nephrotic syndrome [34–36]. Therefore, it appears that the presence of high levels of both drug and ADAs increases the risk of immune complex formation and that kidneys are particularly vulnerable to deposition of immune complexes. It was also found that antibody responses to rhGAA depend on the presence or absence of cross-reactive immunological material (CRIM). Patients with deleterious GAA mutations who are completely unable to form native enzyme are CRIM-negative, whereas patients with some residual, functioning or nonfunctioning enzyme present are CRIM positive. Kishnani and colleagues showed that IgG antibodies to rhGAA developed earlier and titers were higher and more

sustained in the CRIM-negative patients. CRIM-negative status predicted poorer clinical outcomes and reduced overall survival in infants with Pompe disease who were treated with rhGAA [37]. The effect of CRIM status on therapeutic outcome appears to be mediated by antibody responses to the exogenous protein.

All therapeutic proteins pose the risk of IgE ADA response that could lead to a variety of allergic reactions. Anaphylactic shock is certainly the most serious reaction and can be life threatening if not immediately treated, usually with antihistamines, epinephrine, and cortisone. It is prudent to develop and implement assays such as drug-specific IgE and serum tryptase whenever clinical symptoms indicate potential allergic reactions. Detection of drug-specific IgE provides the rationale and justifies antihistamine pretreatment in subsequent drug administrations.

1.5. CONCLUSION

In conclusion, a risk-based strategy (see Fig. 1.1) should include comprehensive evaluation of the molecular characteristics of the biopharmaceutical, its target and mechanism of action, and therapeutic indication(s) as well as the intended patient population. In addition, route of administration, formulation,

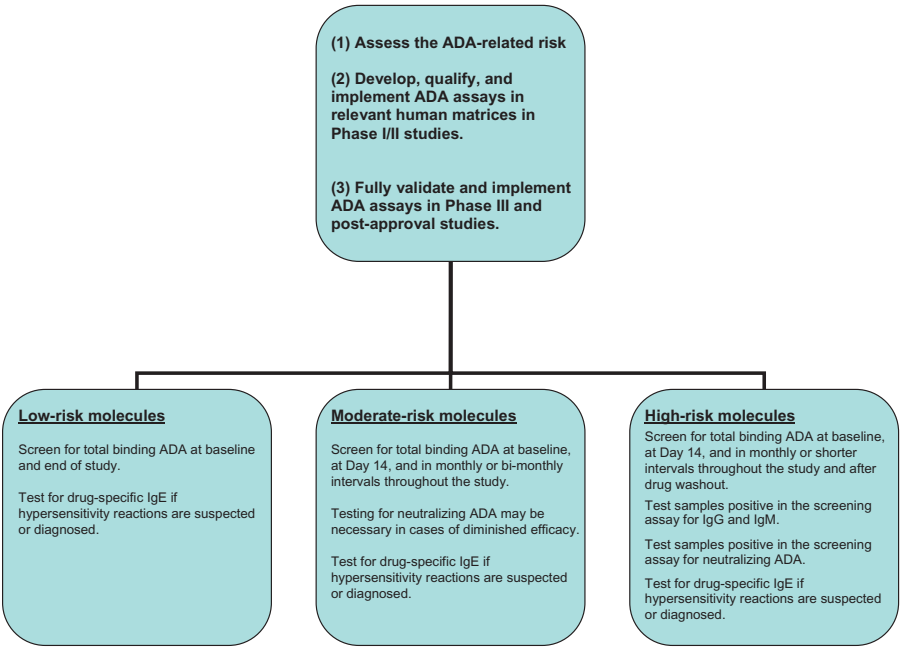

Fig. 1.1 Flow diagram of recommended risk-based strategy for monitoring antibody responses to biopharmaceuticals during clinical development and after approval.

frequency of administration, dose levels, and concomitant immunomodulatory medications should be taken into account. Because of its complexity, the risk assessment should be carried out in a collaborative fashion between clinicians, toxicologists, pharmacokineticists, and assay experts. Timely consultations with regulatory agencies and/or clinical safety monitoring boards may be necessary as well. The identification of the risk level will affect the antibody testing scheme in terms of timing and frequency of sampling, neutralizing activity assessment, and qualitative, semiquantitative, or quantitative measurement, as well as isotype and affinity characterization. The greater the assessed risk, the more extensive and more frequent the ADA testing and characterization, along with cautious dosing regimens, that should be applied. It should be kept in mind that risk-based antibody monitoring will not make any biopharmaceutical product less immunogenic than it really is, but it will certainly help to minimize or even avoid antibody-related adverse effects. As the use and complexity of biopharmaceuticals have evolved, the implementation of a risk-based strategy has become increasingly important. An overall outline of risk-based strategy for ADA monitoring during clinical development is illustrated in Figure 1.1.

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