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Immunogenicity for antisense oligonucleotides: a risk-based assessment

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“given currently favored routes of administration, the disease indications being treated and mechanism of action of ASOs, the risk for ASO immunogenicity is generally quite low”

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Antisense oligonucleotides (ASOs) are single-stranded synthetic oligonucleotides usually between 15 and 25 nucleotides in length [1] that typically include modifications to improve stability and pharmacokinetic properties [2–6]. They are rapidly internalized via endocytotic uptake mechanisms [7] and are able to target RNA transcripts resulting in reduced or modified protein expression [8]. The specificity and application of ASOs have been broadly demonstrated in both animal models and humans for various disease targets [9], resulting in the approval of several drugs, including Spinraza[™] (Biogen, MA, USA), Vitravene[™] (CIBA Vision[®] A Novartis Company, GA, USA) and Macugen[™] (OSI Pharmaceuticals, NY, USA).

ASOs are a unique class of compounds that share characteristics of both large and small molecules. They are like large molecule drugs in that they are characterized by a relatively high molecular weight, long half-life (2–4 weeks) [10] and possess excellent target specificity. However, they also share many characteristics of small molecules including synthetic production, relatively simple chemical structure, and the inclusion of chemical modifications to improve performance characteristics, such as potency and resistance to nucleases. This ambiguous identity has created debate regarding their relative immunogenicity risk. It may be argued that immunogenicity to ASOs is comparable to that for antagonistic monoclonal antibodies, where only the engineered complementarity-determining regions (CDRs) or the residual nonhuman sequences are considered immunogenic, since the body is tolerant to endogenous/humanized antibodies. In the same way, the modified sugar backbone of ASOs comprises the highly engineered/non-native portion of the molecule. Although these modifications are potentially immunogenic in the periphery, to date they have shown minimal impact on safety when delivered to sites that are not readily accessed by peripherally generated or delivered antibodies and are therefore immunologically restricted, such as the ocular space or the CNS.

From a regulatory perspective there is also some ambiguity. Although the US FDA considers ASOs as drugs and not biologics insofar as these molecules are filed as new drug applications, which would typically not include immunogenicity evaluation, there have been instances where immunogenicity data have been expected and therefore provided as part of the new drug application filing.

Immunogenicity risk assessments process

Generally, the immunogenicity risk assessment is based on following two considerations:

- How likely is it that the drug will elicit an immune response?
- What is the potential impact of such a response to patient safety and drug efficacy?

Key to understanding the probability of immunogenicity is to consider whether the immune system is likely to see the drug as self and be tolerant to it or not. Other aspects impacting the likelihood of immunogenicity include

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the route of administration and the specific disease treated. Some routes, such as oral administration, tend to generate immunogenic tolerance while others, such as subcutaneous injection, tend to be more immunogenic. Disease states such as systemic lupus erythematosus (SLE) may present a higher risk of immunogenicity, due to a heightened autoreactive immune system. In this specific case, nucleosomes released from apoptotic cells result in the accumulation of DNA, histones and nucleosomes in both circulation and tissues [11,12]. These DNA–protein complexes are thought to give rise to anti-double stranded DNA antibodies, a hallmark of SLE and other autoimmune diseases, such as rheumatoid arthritis, Sjögren's syndrome, multiple sclerosis and some forms of cancer [13–16].

When assessing the potential impact of an immunogenicity response, it is important to do so in terms of both safety and efficacy. For molecules with endogenous counterparts, safety is a key concern depending on how similar the drug is to its endogenous equivalent (i.e., are there modifications or is it completely native). In cases of high similarity, it is likely that any immunogenicity generated against the drug will spread to the endogenous counterpart and potentially exacerbate, rather than ameliorate, the disease. This has been the case for therapeutics, such as recombinant human erythropoietin (anemia), α -glucosidase (Pompe's), α -galactosidase (Fabry's) or glucocerebrosidase (Gaucher's) [17].

Conversely, for antagonistic molecules with no endogenous counterpart, the impact of immunogenicity risk is primarily loss of efficacy of the drug due to reduced exposure. In these cases, the risk to patients is considered low since exposure can easily be monitored. In all cases, a thoughtful and robust risk assessment is critical for determining the most appropriate immunogenicity-monitoring strategy, including when and how the presence and impact of neutralizing activity should be monitored.

ASO risk assessment

Likelihood of immunogenicity

Structure

When compared with most typical biologics, such as monoclonal antibodies, ASOs are relatively small and as such contain fewer potential epitopes than their larger protein counterparts and thereby are less likely to generate immunogenicity. Furthermore, DNA is generally thought to be relatively nonimmunogenic. Although anti-double stranded DNA antibodies are found in multiple autoimmune diseases, to date these antibodies have not been demonstrated to be sequence specific, and the mechanism by which they are generated is not completely understood [18]. Low immunoreactivity of ASOs is consistent with our own experiences in generating anti-ASO positive controls for immunogenicity assays. In all cases, we have resorted to conjugating the ASO to an immunogenic carrier protein in order to elicit a robust immune response, a standard and common practice for poorly or non-immunogenic molecules.

Route of administration

ASOs can be delivered via multiple routes of administration (intravenous, intrathecal, intra-ocular, pulmonary, oral and topical). This is a key factor to be considered when determining the likelihood that an ASO will elicit an immune response. To date, all the approved ASO therapeutics have been delivered to immune restricted sites (i.e., Vitrevec, intravitreal injection or Spinraza, intrathecal injection). A similar approach is currently being taken for the development of a significant number of new ASOs targeting neuromuscular diseases, for which direct delivery into the CNS has been proposed.

Diseases treated

Currently, ASO therapeutics are primarily delivered to subjects affected by genetic diseases (e.g., spinal muscular atrophy, amyotrophic lateral sclerosis) which do not represent populations with a heightened immunoreactive state the way that immunogenic diseases, such as SLE do. Thus, the diseases currently being treated have a lower likelihood of generating immunogenicity.

Impact of immunogenicity

From the perspective of safety, not only is the likelihood of developing immunogenicity to ASOs considered low, but the impact of any immunogenicity is also expected to be benign. This is based on two main considerations:

- The level of immunogenicity anticipated to reach the site of action;

- The mechanism of action of ASOs, which involves intracellular uptake.

Although one might expect ASOs delivered to immune restricted sites to remain locally confined, in our experience they do reach the periphery, albeit at reduced levels [19], where they can interact with the immune system. However, any peripherally generated immunogenicity would need to traffic back to the site of action in sufficient quantities to impact the ASO concentrations locally. In the case of CNS targets, this would require either unusually high levels of peripherally circulating anti-drug antibody (ADA) or a breakdown in the integrity of the blood–brain barrier [20]. Since ASO therapeutics distribute to intracellular sites of action (systemically or in the CNS) within just a few hours following administration [7], even if an immune response occurred proximal to the site of action, the majority of the targeted drug would be inaccessible to the ADA. However, the formal possibility remains that there could be impact of immunogenicity on efficacy/exposure if the response to the ASO were of great enough magnitude to effectively impact levels of drug reaching the site of action and thereby reduce exposure. Such cases can be mitigated by careful monitoring of exposure.

Conclusion

Overall, given currently favored routes of administration, the disease indications being treated and mechanism of action of ASOs, the risk for ASO immunogenicity is generally quite low. Moreover, for ASOs delivered locally to immune restricted sites, such as the CNS or the eye, the risk is deemed even lower in terms of both safety and efficacy.

We propose that as for biologic therapeutics, immunogenicity risk assessments for ASOs be performed early in the course of drug development. The decisions regarding if, and when, immunogenicity should be monitored during clinical development should be driven by the risk assessment. It is anticipated that in many cases, where the risk is low, the best course of action will be to collect and bank samples in early phases of development and investigate immunogenicity as dictated by emerging clinical and exposure data. Together with early interactions with regulators, this would inform how to proceed in late stage/pivotal studies.

This approach is consistent with our commitment to evidence-based, science-driven strategies that focus efforts where they will add genuine value to patients and ultimately enable us to deliver more and better therapies that will improve the lives of greater numbers of patients.

Financial & competing interests disclosure

M Petrillo and CC Stebbins are the full-time employees of and hold stock/stock options in Biogen. LF Stevenson is a full-time employee of Immunologix Laboratories and holds stock/stock options in Biogen. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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