Antibodies against recombinant enzyme in the treatment of Fabry disease: Now you see them, now you don’t

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In their article published in this issue of Molecular Therapy – Methods & Clinical Development,¹ Lenders and co-workers study the affinity of pre-existing anti-drug antibodies (ADAs), which developed in patients with Fabry disease (FD) after treatment with recombinant alpha-galactosidase-A (αGALA), to a new PEGylated form of the enzyme (Pegunigalsidase-α, PRX-102). For the majority of patients, pre-existing ADAs showed less affinity to PRX-102 compared with the unPEGylated compounds. Since the amino acid sequence of the unPEGylated and PEGylated enzyme is identical, these results suggest an altered epitope availability.

FD is a rare lysosomal storage disease caused by pathogenic variants in the gene coding for the enzyme αGALA. Treatment most often consists of enzyme replacement therapy (ERT) with recombinant αGALA (agalactosidase-α or agalsidase-β), so-called biologicals. Biologicals are drugs produced in living organisms (e.g., enzymes, hormones, monoclonal antibodies), and their development has greatly changed the outlook for many diseases. The downside of biologicals is their immunogenicity and, specifically, their ability to induce anti-drug-antibody (ADA) formation. ADAs can impact pharmacokinetics, inhibit the biological function of the proteins, and/or cause severe anaphylactic and anaphylactoid reactions. In particular, the inhibiting/neutralizing ADAs (iADAs) have been proven to cause therapy failure in many diseases, including Pompe disease and hemophilia. In these disorders, the impaired efficacy results in direct observable treatment failure. In FD, it has taken decades before the deleterious effect of iADAs on treatment efficacy was also recognized.² This is partially explained by the disease heterogeneity and slow progression (requiring long follow-up of a large patient cohort to detect the effect) but probably also by insufficient access to ADA assays and independent disease registries.

Now that the negative effect of ADAs against αGALA on treatment effectiveness is finally recognized, several approaches are being tried by different specialized centers to lower existing ADA titers or prevent ADA formation in newly treated patients. In other metabolic diseases treated with ERT, immunomodulatory protocols using immunosuppressant drugs were either unsuccessful and/or required very high doses of immunosuppressants.²⁻⁵ Immunomodulation protocols focusing on a more constant drug exposure, for example those used in hemophilia, look more promising.⁶ With this in mind, our group is currently testing an alternative treatment initiation protocol in patients at high risk for developing ADAs,⁷ aiming to induce tolerance by giving more frequent infusions with gradually increasing doses of ERT. However, the necessity of more frequent infusions can be a burden for patients and can theoretically be solved by increasing the half-life of the drug.

PEGylated αGALA (PRX-102) has a plasma half-life of several days as opposed to minutes for the non-PEGylated enzymes. Additionally, epitope exposure may be different for PRX-102 compared with the currently used recombinant enzymes since polyclonal rabbit-anti-αGALA reacted stronger to normal recombinant-GLA compared to PRX-102.⁸

In the paper from Lenders et al., a set of sophisticated experiments were performed, showing that ADAs that developed in patients with FD after exposure to agalsidase-α and/or -β indeed tend to bind with less affinity to the PEGylated enzyme PRX-102. The results from the pull-down experiment and cross-ELISAs suggest that PRX-102 has a reduced exposition of some of the immunogenic epitopes. However, the conclusion that this results in reduced immunogenicity needs to be confirmed in future clinical studies. The fact that a small subset of patients have ADAs that bind to PRX-102 with more affinity suggests that the PEGylated compound displays different epitopes but not necessarily less immunogenic ones. And, as all patients were immunized with either agalsidase-α or -β, it makes sense that most of their ADAs react more strongly to those compounds when there is a difference in epitope availability between the enzymes. The only way to test this would be to do the reversed experiment with patients immunized with PRX-102. Nonetheless, the idea of switching treatment in patients with ADAs with a favorable in vitro response to PRX-102 seems appealing, provided that the ADA response after switch is closely monitored.

The latter sounds a lot easier than it is in practice. As the authors accurately described in the discussion, measuring ADAs when the immunogenic compound is still in the circulation is very difficult since the ADAs will remain bound and currently used assays only measure free ADAs (Figure 1). ADA

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levels in patients treated with PEGylated enzymes can therefore not be compared with levels in patients treated with agalsidase-α or β. The authors’ suggestion to measure ADA-drug complexes or use assays that dissociate the complexes first also come with pitfalls: the pH alterations needed to break ADA-drug interactions can potentially alter ADA binding capacity, and the unbound drug needs to be removed to prevent rebinding. Additionally, as the epitopes differ between compounds, we need assays using PRX-102 as antigen instead of the currently available assays that use the unPEGylated enzyme. Finally, the clinical effect of ADAs in patients may be different for different compounds, especially if the compounds greatly differ in half-life (Figure 2).

In the phase 1/2 study of PRX-102, 3 out of 11 male patients had measurable ADA levels, and increasing ADA titers resulted in a vastly reduced half-life of the drug. Interestingly, when ADA titers went down, the half-life of the drug recovered. Pharmacokinetic measurements may therefore be a good (though time-consuming) way to monitor the effect of ADAs in patients after switch (Figure 3). In addition to measuring the effect of PRX-102 on ADA titers, it should be confirmed that the potential lower immunogenicity of the drug leads to a measurable improvement of biochemical (plasma lysosomal concentrations), and ultimately clinical, therapeutic effectiveness.

In conclusion, the potential for a less immunogenic therapy for the treatment of male

Figure 1. Interference of residual drug antigens in patient plasma with ADA measurements using ELISAs

(A) For patients treated with agalsidase-α or -β, drug concentrations in plasma are unmeasurable within hours after infusion ended. Circulating ADAs are unbound, and titers can be adequately measured using conventional ELISAs. (B) For patients treated with PEGunigalsidase-α (PRX-102), the drug is continuously present in patient plasma, and an unknown amount of ADAs will remain bound to PRX-102 and will thus not be detected, resulting in falsely low titers or false negative results.

Figure 2. Potential detrimental effects of ADAs against biologicals

(A) ADAs against biologicals can impair effectiveness of treatment by directly neutralizing the drug, rendering it incapable to perform its function. Neutralizing antibodies work immediately and are most strongly linked to reduced treatment efficacy. (B) Non-neutralizing antibodies can impact the pharmacokinetic profile of a drug by increasing clearance from the circulation, though its effect on treatment efficacy is more variable. It is presumed that with increased circulatory half-life, the relative importance of these non-neutralizing ADAs increases. (C) In addition to the effect on treatment efficacy, ADAs can induce infusion reactions. Classical infusion reactions are common in male patients with classical FD and are characterized by a sudden onset of fever, cold chills, nausea, and general malaise, which recover quickly after stopping the infusion. (D) Finally, it has been described for biologicals other than ERT for Fabry disease that ADA-drug aggregates can accumulate in the vascular wall or in the kidney, causing vasculitis, thrombosis, and renal failure. Although rare, the occurrence of this complication should be monitored for every new biological.
As ADA titers against PRX-102 cannot simply be compared with those against agalsidase-α and -β, it may be considered to also monitor the pharmacokinetic profile in ADA-positive patients after switch. One could measure the total concentration of PRX-102 in plasma (yellow), to assess total drug clearance, as well as enzyme activity in plasma (pink), to assess in vivo enzyme inhibition by ADAs. After switching patients whose ADAs have low in vitro cross-reactivity to PRX-102 from agalsidase-β to PRX-102, there are three possible scenarios, described as follows. (A) No response: PK curve is normal or slightly lower than normal. PK slope remains unchanged, suggesting no changes in the ADA response (titer and affinity). (B) Most favorable response: PK curves improve to fully follow. (A) No response: PK curve is normal or slightly lower than normal. PK slope remains unchanged, suggesting that constant exposure induced further tolerization (ADAs disappear). (C) Least favorable response: PK curves worsen over time, suggesting new ADAs develop (higher titers) with specific affinity to normal, suggesting that constant exposure induced further tolerization (ADAs disappear). (C) Least favorable response: PK curves improve to fully follow. (B) Most favorable response: PK curves worsen over time, suggesting new ADAs develop (higher titers) with specific affinity to normal, suggesting that constant exposure induced further tolerization (ADAs disappear). (C) Least favorable response: PK curves improve to fully follow.

patients with classical FD is promising, and the idea to switch treatment in patients whose pre-existing ADAs show a significant lower affinity to PRX-102 is justifiable. However, there is a need for international consensus on how best to monitor ADAs and their effect on treatment after switch to PRX-102 to be able to confirm the benefit in a timely manner.

DECLARATION OF INTERESTS
M.L. is involved in pre-marketing studies with Genzyme, Protalix, and Idorsia. S.J.vdV. was involved in a pre-marketing study with Protalix. All financial arrangements are made through AMC Research BV. No fees, travel support, or grants have been obtained (directly or indirectly) from the pharmaceutical industry.

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