DISCLOSURE

The authors declare no conflict of interest.

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Response to de Vries *et al.*

To the Editor: In their paper "Pompe disease in adulthood: effects of antibody formation on enzyme replacement therapy",[1] de Vries et al. describe their study of a cohort of 73 adult Pompe disease patients who received enzyme replacement therapy (ERT) with alglucosidase alfa for a median duration of 35 months. Patients were classified into high, intermediate and none/low titer groups based on their highest antibody titer. Using muscle strength, pulmonary function and in vitro neutralization assays, the authors concluded that antibody titers had limited interference with ERT efficacy. It was proposed that patients with the IVS1/delex18 *GAA* genotype may have an attenuated antibody response. We commend the authors' efforts as this study adds to the existing knowledge about clinical course and outcomes in late-onset Pompe disease (LOPD). However, we feel that some conclusions need to be drawn with caution as there are limitations of this study, namely: (i) the classification of patients based on a single peak antibody titer; (ii) the clinical endpoints used for outcome assessment; (iii) the role of neutralizing versus non-neutralizing antibodies in predicting clinical outcome; and (iv) genotype correlation with immune response.

The approval of alglucosidase alfa in 2006 was a breakthrough in the treatment of Pompe disease. However, this therapeutic protein has faced challenges of immunogenicity, with the development of high-sustained antibody titers at one extreme and no/low titers at the other. Recently, there has been an increase in the understanding of the impact of sustained intermediate titers on ERT efficacy. The Myozyme package insert documents that patients with antibody titers greater than or equal to 12,800 had a 50% increase in enzyme clearance from week 1 to week 12 of treatment. The negative impact of high-sustained antibody titers and sustained intermediate titers is well established in patients with infantile Pompe disease (IPD), who have lower ventilator-free and overall survival and show deterioration in other measures, such as left ventricular mass index, gross motor development and urinary glucose tetrasaccharide when compared to IPD patients with low antibody titers.[1] We have seen that some of our IPD patients with overall low antibody titers may have a single high titer value, but eventually have good clinical outcomes compared with those who have high-sustained antibody titers or sustained intermediate titers. Our experience shows that it is the persistence and trend of the immune response over time that is closely related to treatment outcome, rather than a single peak antibody titer. Therefore, it is important to reclassify patients in this published study as "high sustained" instead of "high" titer, and, "sustained intermediate" instead of "intermediate" titer, as the current classification based on a single, maximal value may lead to lack of clarity of the role of antibody titers.

Neutralizing antibodies inhibit enzyme activity by at least two mechanisms: inhibiting enzyme uptake by cells and/or inhibiting catalytic activity. It is noteworthy that even in cross-reactive immunologic material (CRIM)-negative IPD patients with high-sustained antibody titers, only a subset demonstrated neutralizing antibodies in in-vitro assays.[2,3] Even among those who had neutralizing antibodies, some demonstrated antibodies only to the catalytic domain and not to the uptake domain. However, irrespective of the presence/type of neutralizing activity, they all experienced poor clinical outcomes. The role of non-neutralizing antibodies should not be overlooked because, when present in high titers, they can reduce the efficacy of ERT by altering its biodistribution; for instance, into Fc receptor-expressing cells and hepatic uptake of complement-bound soluble immune complexes. Consequently, the inability to detect neutralizing activity by in vitro assays should not lead to conclusions about availability of enzyme activity in-vivo since in-vitro measurement does not always reflect the true in-vivo situation.

Another factor contributing to patient classification is the performance of the assays, since assay variability may affect how patients were grouped. The reported percentage coefficient of variation appears to be associated with control reagents with no specific information on the reproducibility of titering patient samples. Neutralizing antibody was detected by measuring enzyme activity both in the medium directly after enzyme addition and before cell harvest, while activity incorporated was measured in cell homogenates. Standardization of this type of measure and the variability that could be encountered by a cellular matrix is not addressed. Overall, repeat measures over time, such as looking at the persistence of antibody titers and including this temporal aspect, could better align the way patients are categorized based on antibody responses.

The study detected a very limited association between antibody titers and clinical outcomes. In IPD, with its dramatic presentation, clinical endpoints (such as left

ventricular mass/left ventricular mass index, ventilator-free survival or overall survival) are easy to measure and observe. Clinical outcome measures used to assess ERT response were the Medical Research Council scale and forced vital capacity. In a slowly progressive disease such as LOPD, such distinct end points take years to be evident and these measures may not be sufficiently sensitive to capture the gradual change. Moreover, these metrics may not be able to differentiate between the impact of natural disease progression versus the age-related decline in function. The Medical Research Council scale has long been criticized for its limitations, and there have been numerous attempts to improve its accuracy.

The authors speculate that the IVS1/delex18 genotype may protect against developing high antibody titers. It is known that genotype alone does not predict ERT response in Pompe disease. A number of factors, such as major histocompatibility complex class II polymorphisms, human leukocyte antigen haplotypes, the extent of non-endogenous epitopes relative to ERT, and epitope spreading (which may lead to high titers), may play a role in the treatment response. In our experience, a small fraction of CRIM-negative IPD patients do not develop high antibody titers and respond favorably to ERT, which suggests that genotype alone is not responsible for the immune response.[4] Thus, the observation of genotype association in LOPD should be stated with caution.

In summary, we believe that the persistence of elevated titers over time, rather than the absolute values at a single time point, is a key predictor of clinical outcomes. It remains to be examined whether a complete elimination of antibody formation from the time of ERT initiation would change the outcome. Outcome measures that have the ability to capture small changes in LOPD need to be developed.

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Response to Herbert *et al.*

To the Editor: We thank Herbert et al.[1] for their interest in our work.[2] Their laboratory has shown to be instrumental in studying the effects of enzyme replacement therapy (ERT) in infants with Pompe disease. However, there are some misunderstandings about our study on adult Pompe patients and antibody formation. Below we explain these in detail.

Herbert et al.[1] suggest that our patients be reclassified based on sustained titers rather than peak titers, because "the current classification based on a single, maximal value may lead to a lack of clarity on the role of antibody titers". We agree that the duration of high neutralizing antibody titers is important to consider. This is why we measured titers at multiple time points over a period of 3 years (Figures 1 and 3, and Supplementary Figure 1A–C in de Vries et al.[2]). We observed two trends: (i) a decline of peak titers over this period at a group level and (ii) relatively few patients with high (≥31,250) peak antibody titers (16 of 73; 22%). Nine (12%) of these had high sustained antibody titers. Eight patients (11%) had very high (≥156,250) peak titers, and these classified for all but one patient as sustained high. This shows that no matter how the groups are generated, in all of these cases group sizes are very small. The statistical power to analyze potential effects on clinical outcome is limited. Therefore, we have also analyzed the eight patients with a very high peak titer and seven patients with a high sustained titer on an individual basis, and we concluded that antibodies were likely to have interfered with the effect of ERT in only one patient. We previously reported on the counteracting effect of high sustained antibodies in this particular patient.[3] Herbert and colleagues may have missed the fact that only a few adult patients develop high sustained antibodies, which is in contrast to the situation in classic infantile patients. A recent study by Masat et al.[4] on behalf of the French Pompe Registry Study Group also concluded that antibodies are not a major concern in adults with Pompe disease.[4]