Peer Review File

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Reviewer #1:

This is a very well written overview of enzyme replacement therapy, chaperone therapy, and also new drug developments, including substrate reduction and gene therapy. This article nicely summarizes the current state-of-the-art in targeted Fabry-therapy and provides also future perspectives. The most relevant data on treatment strategies and outcomes are critically discussed in this excellent and up-to-date review. Furthermore, cardiovascular aspects of cardiologic patient management are included in a detailed section. The references given are comprehensive; the table is very informative and useful. In conclusion, this is a very relevant article, particularly valuable for cardiologists following Fabry-patients who have cardiac comorbidities and complications.

Reply: Thank you very much for the positive feedback. We highly appreciate your comments.

Reviewer #2:

Comment 1: As the only comment, the strength of enzyme replacement therapy (ERT) is the long experience with its use. At present, there are series published with follow-up of more than 15 years. However, no comment is made on the long-term results of ERT use in the review article. I think it is important to include a section on long-term results of the use of ERT at the cardiac and renal level, both of agalsidase alfa and beta.

Reply: Thank you very much for this important and valuable comment aiming to improve the quality our manuscript. Indeed, you are very right about the literature reporting results over 15 years of therapy. We have added additional data and literature in the section *"Long-term results on first-generation ERT"*.

Comment 2: Finally, as the authors indicate, in June 2009, viral contamination of agalsidase beta production facilities resulted into a worldwide drug shortage implying dose reduction or product switch in most patients with FD on agalsidase beta. The review states "Recent studies have shown that a dose change or therapy switch of patients previously stable on ERT bears the risk to result in deterioration of renal function and worse outcome".

In this work, other articles that show the opposite have not been taken into account. Pisani et al (Genetics in Medicine 2016;19:275) carry out a meta-analysis on the topic and I think should be included in the review

Reply: Thank you very much for your comment and the valuable recommendation to more critically discuss this part in our manuscript. We have added respective discussion:

"While some studies indicate kidney function deterioration following therapy alterations in patients who had previously been stable on ERT (40,43,44), others discuss a neutral or clinically non-relevant impact of dose change, respectively therapy switch in patients with

FD. Even though short-term observation by Smid et al. did not reveal an increase in adverse events after dose reduction to 0.5 mg/kg, an increase of lyso-Gb3 levels still indicated a rising disease activity.(17) However, the latter has not been confirmed by other studies. (29,45,46) In 2017, Pisani et al. performed a meta-analysis on seven studies focusing on the effects of therapy switch and in fact did not observe any significant differences in renal function during follow-up.(47) In part contradictory results were observed in regard of cardiac organ manifestations and function deterioration, which however overall also remained stable with no clinically relevant disease progression being triggered by therapy alterations. (45-47)

In conclusion, individual response, therapy success and disease-progression might significantly vary on a case-to-case basis with personal risk factors, such as genotype, age and gender, time of ERT-initiation, the patients' personal disease activity, and phenotype expression potentially serving as disease-modifying factors indirectly influencing therapeutic success."

Reviewer #3:

Comment 1: p5 - Could the authors please give a source for their statement, that Lyso-Gb3 is a "valid indicator of therapy success or failure"?

Reply: We have altered our sentence and provided respective references indicating the value of lyso-Gb3 as a biomarker of disease course and thus also indirectly therapy success in FD.

"As plasma lyso-Gb3 has been shown correlating with clinically feasible severity of FD, it seems to be a reliable biomarker besides of Gb3 deposition clearance on a histological level and thus presumably a valid indicator of therapy success, respectively failure.(25,26)"

Comment 2: p5 - All sources the authors are citing to prove the risk of dose change or therapy switch of patients previously stable on ERT esp. on renal functions come from the same group and describe the same cohort with somewhat confusing and marginal results. Are there any additional sources avaliable to underline the evidence?

Reply: Thank you for this important request. We have added further information and discussion in the section *"Long-term results on first-generation ERT":*

Comment 3: p6 - typo (migalastat)

Reply: Thank you for the hint. We have checked for typos.

Comment 4: p11 - This is my most important concern. Where do the authors see the relation between the ability to cross the blood-brain barrier (BBB) and the ability to prevent cerebrovascular events (CVE)? CVE are a pre-, not a post-BBB problem. Stroke of any origin is the result of impaired blood supply to a certain brain region by micro- or macroangiopathy (autochthonic-thrombotic or embolic). The ability of a therapeutic compound to cross the BBB would be of interest for disorders going along with neurotoxic or neurodegenerative processes as known from other lysosomal storage disorders (e.g. Gaucher disease type 2+3). Although Gb3 deposition in certain brain regions has been demonstrated also in FD by neuropathological examination, no

clinical symptoms correlating with this result are obvious so far. To prevent CVE a specific compound must show beneficial effects on vascular pathology not necessarily the potential to cross the BBB.

Reply: Indeed, we totally agree with you, that especially the therapeutic point of attack remains a challenging problem for cerebrovascular events in FD. As already stated by you, CVE are assumedly a pre-BBB problem, which maybe could not be solved by the sole introduction of a new FD-specific drug able to cross the BBB. We suspect a multifactorial pathophysiology potentially including e.g. inflammatory processes at an early stage as has already been observed for the development of cardiomyopathy in FD. However, unfortunately we are not able to provide a satisfactory response to your question, as the eventual pathophysiological mechanisms of stroke remains poorly understood in FD.