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Correspondence

The migalastat GLP-HEK assay is the gold standard for determining amenability in patients with Fabry disease

Dear Editor,

The pharmacological chaperone migalastat is indicated for the treatment of Fabry disease in patients with an amenable *GLA* variant. Amenability is determined by an in vitro, good laboratory practice (GLP)-validated assay using HEK293 cells (GLP-HEK assay) performed at a single, highly experienced, GLP-certified laboratory using rigorous standards and extensive analytical validation to limit inter-assay variability [1]. The recent report by Oommen et al. entitled "Inter-assay variability influences migalastat amenability assessments among Fabry disease variants" showed [2], despite technical differences between a non-GLP-validated assay and the GLP-HEK assay, 53 out of the 59 *GLA* variants tested in the non-GLP assay matched the GLP-HEK amenability classification [1]. Considering the non-GLP assay was done without identical procedures and validated quality standards as in the GLP-HEK assay, differences in results are expected. We noted at least two deviations from the GLP-HEK assay that likely account for the discrepancies reported for 6 variants (Table 1). First, the GLP-HEK assay uses qPCR to directly measure the amount of transfected plasmid DNA for transfection efficiency control [1]. The method employed by Oommen et al., an indirect measurement of co-transfected, secreted embryonic alkaline phosphatase (SEAP), may be inaccurate because overexpression of mutant α-galactosidase A (α-Gal A) can affect trafficking and secretion of SEAP [2]. Second, Oommen et al. used the relative activity (% of wild type) instead of absolute activity (nmol/mg/h) to calculate the fold-increase in α-Gal A activity in response to migalastat, causing values for 4 variants to narrowly miss the amenability criteria (Table 1).

In conclusion, the concern over assay variability seems unfounded, since amenability to migalastat is determined in a single GLP-certified laboratory. We believe physicians can have a high level of confidence in the approved GLP-HEK assay, which identifies *GLA* variants with the potential to respond to migalastat. Of course, individual response will need to be assessed clinically.

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Table 1
Comparison of discrepant amenability assay results from Benjamin et al. and Oommen et al.

Variant	Benjamin et al [1] (GLP-HEK) data								Oommen et al [2] data						
	Baseline α-Gal A		α-Gal A activity with 10 μM migalastat		Mann-Whitney U *P* value (one-tail)	Absolute increase (% WT)	Relative increase (fold)	Relative increase by % WT activity	Amenable? (Yes/No)	Baseline α-Gal A		α-Gal A activity with 10 μM migalastat		Mann-Whitney U *P* value (one-tail)	Absolute increase (% WT)	Relative increase (fold)	Amenable? (Yes/No)
	(nmol/mg/h)	(% WT)	(nmol/mg/h)	(% WT)						(nmol/mg/h)	(% WT)	(nmol/mg/h)	(% WT)				
A108T	20,760	57.1	29,391	80.8	0.0002	23.7	1.42	1.41	Yes	14,287	73.3	16,476	84.5	NA	11.2	1.15	No
S126G	34,476	83.7	46,491	113.9	0.0001	30.2	1.35	1.36	Yes	36,722	143.7	43,598	170.6	NA	26.9	1.19	No
D175E	15,726	44.3	18,946	53.4	0.0206	9.1	1.20	1.21	Yes	23,110	57.9	26,931	67.4	NA	9.6	1.17	No
S304 N	30,563	94.1	39,629	121.8	0.0001	27.7	1.30	1.29	Yes	19,488	77.8	22,622	90.4	NA	12.5	1.16	No
D264A	BLD	BLD	BLD	BLD	NA	NA	NA	NC	No	1020	4.4	1840	7.9	NA	3.5	1.80	Yes
S276G	BLD	BLD	694	2.0	0.0001	2.0	NC	NC	No	1252	3.0	8764	21.0	NA	18.0	7.00	Yes

The amenability criteria for the GLP-HEK assay are ≥ 1.20-fold over baseline with an absolute increase of ≥3.0% wild-type α-Gal A activity in the presence of 10 μM migalastat.
α-Gal A = α-galactosidase A; BLD = below level of detection; GLP-HEK = good laboratory practice-validated HEK293 cell assay; NA = not applicable; NC = not calculated; WT = wild-type.

References

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