# SUPPLEMENTAL MATERIAL

**Dose-dependent impact of enzyme replacement therapy on anti-drug antibody titers and clinical outcome in patients with Fabry disease**

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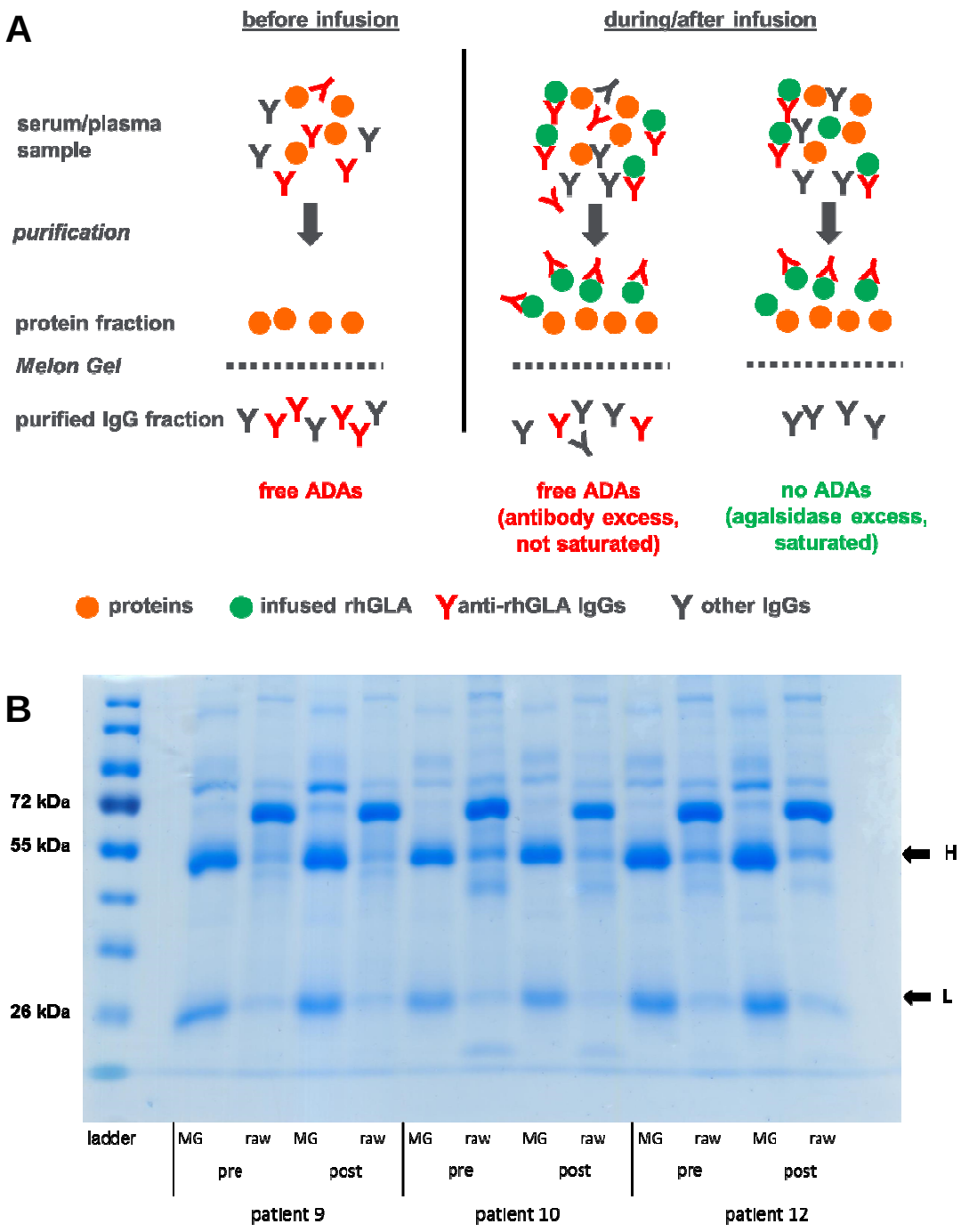
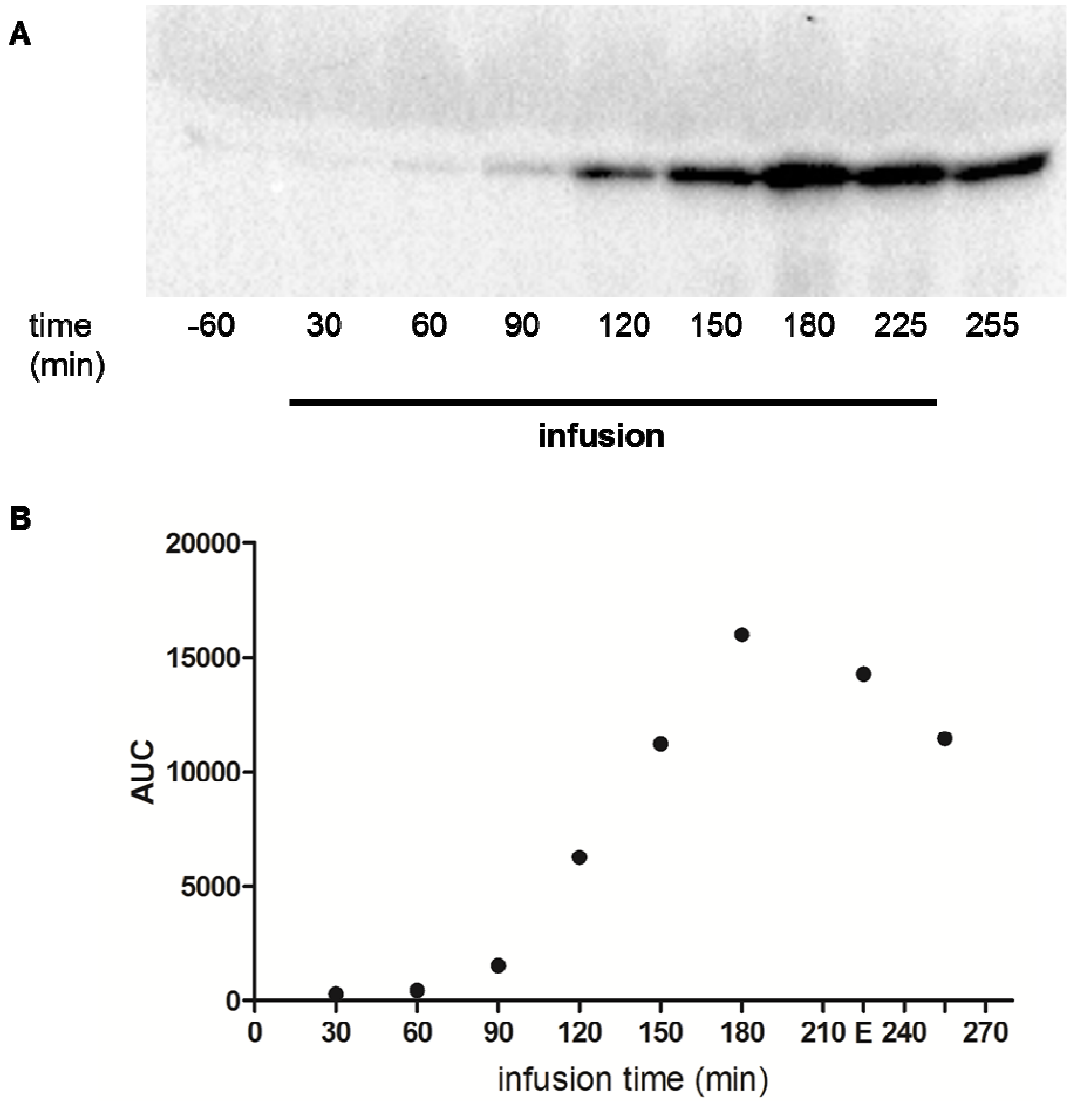
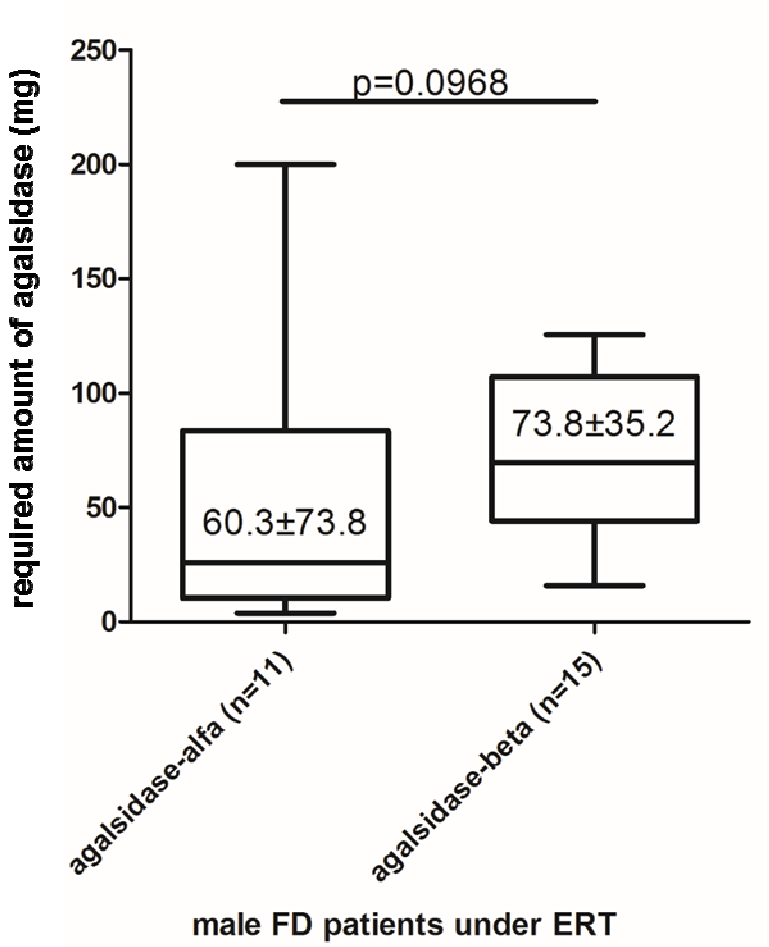
## Contents:




**Supplemental Figure 1: Isolation of human total IgG antibodies from patients with FD.** A) Schematic overview of the purification of IgGs from human serum/plasma samples before and after infusions. B) Representative Coomassie blue staining from pure serum (raw) samples and melon gel-purified IgG (MG) fractions (each 5 µg) before (pre) and after (post) infusions show typical pattern of heavy (H) and light (L) Fc chains in purified IgG samples.




**Supplemental Figure 2: Detection of infused enzyme in patient 9.** A) Western-blot analysis of serum samples during infusions. Blood samples were drawn every 30 minutes or as indicated during infusion. Each lane was loaded with 1 µl serum. B) Densitometry analysis of western-blots. AUC: area under the curve. E: end of infusion.




**Supplemental Figure 3: Comparison of required amount of enzyme due to ADA titers between patients receiving agalsidase-alfa or agalsidase-beta.** Data are presented as median with range. Numbers present mean±SD. ERT: enzyme replacement therapy. FD: Fabry disease. n: number of patients.

**Supplemental Table 1: Genetic and biochemical data.**

| Patient | GLA mutation (position) | Enzymatic activity (%) |
|---|---|---|
| 1 | c.902G>C [p.R301Q] | 67* |
| 2 | c.739T>C [p.S247P] | <5* |
| 3 | c.1090_1103del [p.Y365CfsX5] | <24 |
| 4 | c.881T>C [p.L294S] | 12 |
| 5 | c.658C>T [p.R220X] | 28 |
| 6 | c.658C>T [p.R220X] | 12 |
| 7 | c.762ins282bp | <5* |
| 8 | c.281G>C [p.C94S] | 12 |
| 9 | c.723dupT [p.Ile242fs*8] | <5 |
| 10 | c.679 C>T [p.R227X] | 0 |
| 11 | c.679C>T [p.R227X] | <5 |
| 12 | c.702…709 del8bp | 9 |
| 13 | c.IVS2-2A>G | 4 |
| 14 | c.658C>T [p.R220X] | 16 |
| 15 | c.2T>C [p.M1T] | 2* |
| 16 | c.679 C>T [p.R227X] | 11* |
| 17 | c.453C>A [p.Y151X] | 12 |
| 18 | c.519C>G [p.Y173X] | 1 |
| 19 | deletion in exon 7 | <5* |
| 20 | c.1047G>A [p.W349X] | 2 |
| 21 | c.658C>T [p.R220X] | 3 |
| 22 | c.605G>A [p.C202Y] | 8 |
| 23 | c.679C>T [p.R227X] | 4 |
| 24 | c.272T>C [p.I91T] | <5 |
| 25 | c.1208delAAG | <1 |
| 26 | c.718_719delAA | 5 |
| **total** | | |
| **26** | **6 missense; 20 nonsense** | **16 <5%** |

*Only plasma GLA activities available. Nonsense mutations have been defined as nucleotide exchanges/insertions/deletions resulting in stop codons, frame shifts, large and small deletions or splice site mutations.

**Supplemental Table 2: Outcomes of antibody measurements and titrations.**

| patient and measures | time under ERT (months) | treatment, agalsidase -alfa (α) or -beta (β) | amount of infused ERT (mg) | status after infusion (saturated / not saturated) | required amount of agalsidase-alfa (mg) | required amount of agalsidase-beta (mg) |
|---|---|---|---|---|---|---|
| 1 | 63 | α | 17.5 | saturated | 2.1 | 5.7 |
| 2 | 54 | α | 14 | not saturated | >200 | >200 |
| 3 | 46 | α | 14 | saturated | 11.7 | 13.2 |
| 4 | 114 | β | 70 | not saturated | 128.4 | 115.9 |
| 5 | 192 | β | 70 | saturated | 66.5 | 71.3 |
| 6 | 192 | β | 70 | not saturated | 115.4 | 135.7 |
| 7 | 190 | β | 70 | supersaturated | 72.0 | 67.2 |
| 8.1 | 92 | α | 14 | no sample | 21.3 | 22.8 |
| 8.2 | 117 | α | 14 | no sample | 22.1 | 16.6 |
| 8.3 | 133 | β | 70 | no sample | 45.6 | 41.3 |
| 8.4 | 153 | β | 70 | no sample | 51.9 | 57.8 |
| 8.5 | 153 | β | 70 | saturated | 54.6 | 53.9 |
| 9.1 | 7 | β | 70 | saturated | 52.1 | 57.0 |
| 9.2 | 8 | β | 35 | not saturated | 77.9 | 78.0 |
| 9.3 | 11 | β | 70 | not saturated | 82.2 | 92.4 |
| 9.4 | 11 | β | 105 | saturated | 89.9 | 92.4 |
| 9.5 | 12 | β | 70 | not saturated | 84.6 | 95.4 |
| 9.6 | 13 | β | 70 | not saturated | 90.3 | 102.0 |
| 10.1 | 79 | α | 17.5 | no sample | 39.7 | 57.4 |
| 10.2 | 92 | α | 17.5 | no sample | 25.8 | 44.8 |
| 10.3 | 145 | β | 70 | saturated | 47.6 | 40.6 |
| 10.4 | 167 | β | 70 | saturated | 28.3 | 53.2 |
| 11 | 8 | β | 70 | saturated | 44.0 | 39.8 |
| 12 | 43 | α | 14 | saturated | 4.7 | 6.8 |
| 13 | 92 | α | 14 | saturated | 12.0 | 8.6 |
| 14 | 130 | β | 70 | saturated | 18.0 | 13.7 |
| 15.1 | 95 | α | 14 | not saturated | 43.5 | 41.6 |
| 15.2 | 95 | β | 60 | saturated | 35.4 | 37.8 |
| 15.3 | 95 | β | 60 | saturated | 51.8 | 55.2 |
| 15.4 | 96 | β | 60 | not saturated | 92.2 | 92.2 |
| 15.5 | 97 | β | 60 | not saturated | 142.1 | 159.6 |
| 15.6 | 98 | β | 60 | not saturated | 165.3 | 184.8 |
| 16 | 204 | β | 70 | not saturated | 108.9 | 105.4 |
| 17.1 | 13 | α | 14 | not saturated | 76.9 | 90.1 |
| 17.2 | 16 | α | 14 | not saturated | 68.4 | 73.5 |
| 18 | 122 | β | 105 | saturated | 72.9 | 81.9 |
| 19 | 162 | β | 70 | not saturated | 101.8 | 110.5 |
| 20 | 47 | α | 14 | saturated | 10.8 | 11.8 |
| 21.1 | 191 | α | 17.5 | not saturated | 76.8 | 58.9 |
| 21.2 | 200 | β | 35 | not saturated | 66.0 | 61.8 |
| 21.3 | 201 | β | 70 | saturated | 62.5 | 59.2 |
| 22.1 | 13 | β | 55 | saturated | 29.7 | 23.8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 22.2 | 16 | β | 55 | saturated | 14.6 | 11.9 |
| 23 | 24 | β | 80 | not saturated | 114.0 | 119.4 |
| 24 | 12 | α | 14 | not saturated | 24.5 | 27.4 |
| 25 | 181 | β | 70 | not saturated | 73.8 | 78.6 |
| 26 | 46 | α | 14 | not saturated | >200 | >200 |
| **total** | | | | | | |
| **47** | **95 [7-204]** | | **60 [14-105]** | | **62.5 [2.1-200.0]** | **58.9 [5.7-200.0]** |

ERT: enzyme replacement therapy.

**Supplemental Table 3: Comparison of clinical baseline characteristics, neutralizing ADAs and ERT doses between saturated and not saturated patients.**

| measure | saturated (n=14) | not saturated (n=12) | delta/ RR [95%CI] | p-value |
|---|---|---|---|---|
| **age at ERT initiation, years** | 32±13 | 32±11 | -0.6 [95%CI -10.5 to 9.3] | 0.9082 |
| **treatment duration, months** | 89±66 | 107±76 | -18 [95%CI -75 to 39] | 0.5242 |
| **missense mutations, n** | 3 (21.4) | 3 (25.0) | 0.91 [95%CI 0.37 to 2.22] | 0.9999 |
| **initial treatment with agalsidase-alfa, n** | 8 (57.1) | 6 (50.0) | 1.14 [95%CI 0.55 to 2.36] | 0.9999 |
| **current treatment with agalsidase-alfa, n** | 5 (35.7) | 6 (50.0) | 0.76 [95%CI 0.35 to 1.63] | 0.6922 |
| **current total dose per infusion, mg** | 52±31 | 43±30 | 9 [95%CI -16 to 33] | 0.4788 |
| **required amount of enzyme to saturate ADAs, mg** | 36±26 | 106±54 | -71 [95%CI -104 to -37] | **0.0002** |
| **lyso-Gb3 at baseline, ng/ml** | 77±49 | 79±57 | -1.4 [95%CI -44.3 to 41.5] | 0.9461 |
| **eGFR at baseline, ml/min/1.73 m²** | 105±43 | 102±37 | 3.2 [95%CI -29.7 to 36.1] | 0.8439 |
| **IVSd at baseline, mm** | 13±2 | 11±3 | 1.89 [95%CI 0.01 to 3.78] | **0.0489** |

Values are given as mean±SD, or n (%). If no data at ERT-naïve baseline were available, the earliest available (after ERT-initiation) value was assessed. ADA: anti-drug antibody, eGFR: estimated glomerular filtration rate, Gb3: globotriaosylceramide, IVSd: interventricular septum thickness in diastole.