

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Almeida AM, Murakami Y, Baker A, et al. Targeted therapy for inherited GPI deficiency. *N Engl J Med* 2007;356:1641-7.

## **Supplementary methods**

**Flow-cytometry and RQ- PCR.** These were performed as previously described.<sup>6</sup>

**Reporter assays.** Constructs and their transfection to HeLa cells have been described.<sup>6</sup> Sodium butyrate (3mM; Sigma) or mithramycin (100nM; Sigma) were added to the culture immediately following transfection. Forty-eight hours post-transfection, the luciferase activities of cell lysates were measured using Dual-Luciferase Reporter Assay System (Promega).

**Statistical analysis.** Point estimates and confidence intervals for in vitro and in vivo percentage or fold changes were calculated from log transformed ratios that were averaged and exponentiated. For Figure 2B, changes between baseline and day 90 were derived from linear regression analyses allowing point estimates to be calculated as above.

**Supplementary Table 1. Progression of neurological symptoms**

<b>Age (years)</b>	<b>Neurological History</b>	<b>Investigations</b>	<b>Therapy</b>
4	First absence seizure		
5	Hepatic encephalopathy with ensuing learning difficulties; first tonic clonic seizure		Sodium valproate
9	<i>Status epilepticus</i>	Cerebral MRI, MRA, MRV <sup>1</sup> normal Normal serum electrolytes and renal function Mildly deranged hepatic function (bilirubin 22, AST <sup>2</sup> 39) Normal amino acids and short chain fatty acids Negative thrombophilia screen apart from partial GPI deficiency	Sodium valproate Lamotrigine
12	Frequency of tonic-clonic seizures increased from monthly to weekly		Sodium valproate stopped Lamotrigine Levetiracetam
13	Tonic-clonic seizures daily		Lamotrigine Levetiracetam Topimarat
14 (pre-SPB <sup>3</sup> )	Seizures: More than 5 tonic-clonic seizures per day; frequent absence seizures Motor function: wheelchair-bound Higher cerebral function: unresponsive, no speech, unable to feed herself	EEG <sup>4</sup> : <i>status epilepticus</i> <i>PIGM</i> promoter mutation Reduced <i>PIGM</i> expression	Lamotrigine Levetiracetam Topimarat Clobazam
14 (post-SPB)	Seizures: none Motor function: mobilizing independently; global hypotonia; Higher cerebral function: alert, playing/interacting with others, feeding herself, residual developmental delay with limited vocabulary, learning difficulties	Increased <i>PIGM</i> expression in PBMN <sup>5</sup> cells Partial restoration of GPI expression on blood cells	SPB (30mg/kg) Sodium valproate Topimarat

<sup>1</sup> MRI, MRA and MRV: magnetic resonance imaging, arteriography and venography respectively

<sup>2</sup> AST: aspartate aminotransferase

<sup>3</sup> SPB: Sodium Phenyl Butyrate

<sup>4</sup> EEG: electroencephalogram

<sup>5</sup> PBMN: peripheral blood mononuclear