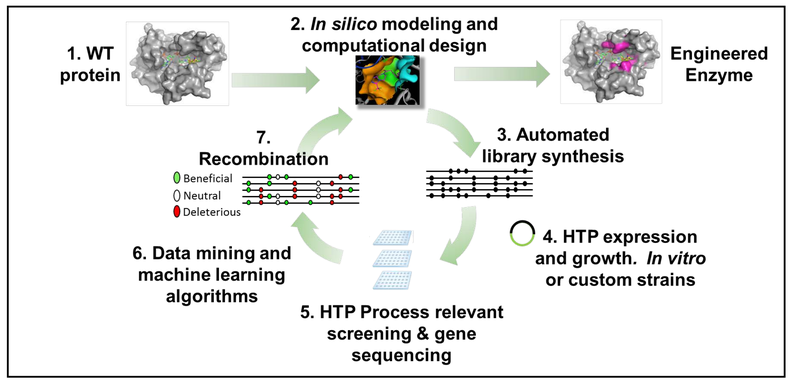
Improving ERTs with CodeEvolver® protein engineering technology to improve protein stability and in vivo half-life, as well as reduce immune response

Abstract

Inborn errors of metabolism (IEM) are inherited disorders characterized by single gene defects that lead to impairment of metabolic pathways and the accumulation of toxic substrates. For some of these disorders, enzyme replacement therapy (ERT) is available. However, issues that commonly compromise the efficacy of ERTs are short *in vivo* half-life and the development of antidrug antibodies. This limits efficacy of treatment, and may cause side-effects. Therefore, development of ERTs with enhanced stability and reduced immunogenicity would be beneficial. To establish proof of concept, we engineered α-galactosidase A (GLA) for such properties as a potentially more effective treatment of Fabry disease. Intravenously administered ERTs for treatment of Fabry disease are known to have a short *in vivo* half-life and induce an immune response in patients. Applying CodeEvolver® protein engineering technology, we looked to improve stability of the enzyme to the conditions in serum and in the lysosome, as well as reduce the predicted immunogenicity of the enzyme. Over eight rounds of enzyme optimization and screening of more than 12,000 variants, new GLA enzymes were discovered that met our objectives of improved stability in both serum and the lysosome while retaining full activity. These improved properties were obtained with the introduction of ~12 mutations. Another 10 mutations removed all predicted immunogenic epitopes. In a mouse model of Fabry disease, such GLA variants showed prolonged half-life in both the serum and lysosome.

Codexis CodeEvolver® Technology

The CodeEvolver® evolution platform is a suite of technologies used for enzyme library design, construction, screening and data analysis.



Evolution Targets for eGLA

- Improved stability in the lysosome
- Improved stability in serum
- Reduced predicted immunogenicity
- Retain key enzyme capabilities

eGLA Variant Key

GLA Variant	# mutations	PTM	Evolution Focus
CDX-6301	0	WT	N/A
CDX-6306	12	+1*	Stability
CDX-6307	11	WT	Stability and PTM
CDX-6308	20	+1*	Stability and Deimmunization

*Predicted glycosylation site (in silico analysis)

1. Serum, pH and thermo stability were improved after bench top challenge with no loss in eGLA specific activity

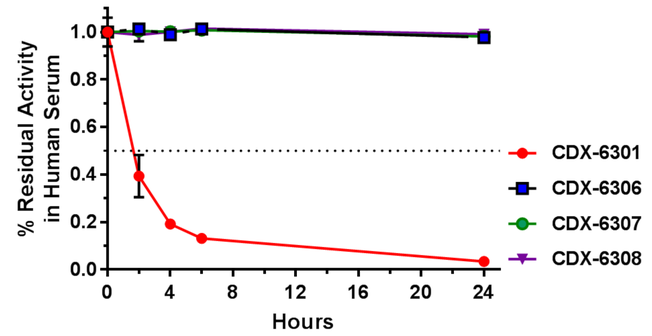
CDX-6301 is identical in sequence to human α-galactosidase A with no difference in stability or enzyme kinetics (data not shown). CDX variants were produced in HEK293T cells.

eGLA variant activity was measured by 4MU method after a variety of bench top challenges. Thermo stability range was improved **(1A)**. eGLA variants retained up to 2-fold greater residual activity after 1h incubation at physiological temperature (37°C). At 50°C, control GLA (CDX-6301) was completely inactivated while CDX variants retained up to 79% activity (CDX-6306). In addition, eGLA variant mutations did not significantly affect specific activity (K_{cat}, **1A**). Residual activity after 24h incubation at both lysosomal and serum pH (data not shown) was not significantly different compared to control (CDX-6301). However, eGLA variants retained >98% activity after incubation at 37°C in human serum **(1B)** over 24h.

A

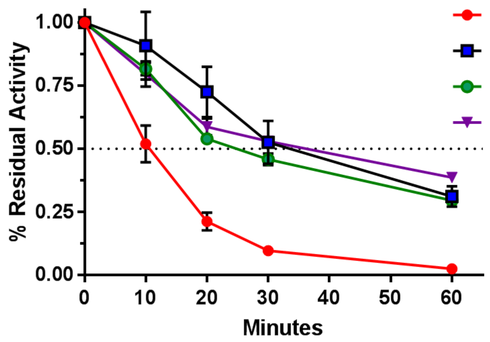
Variant	K_{cat} (μM/μg/min)	% residual activity at 37°C (1h)	% residual activity at 50°C (1h)
CDX-6301	101.1	55%	0%
CDX-6306	107.2	65%	79%
CDX-6307	108.4	78%	52%
CDX-6308	102.9	100%	34%

B



2. Serum stability was increased 4-fold in the Fabry mouse

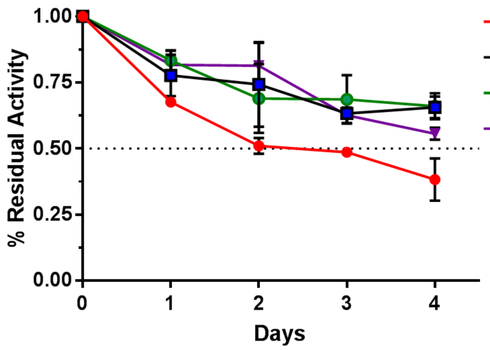
eGLA or Fabrazyme (1mg/kg) was injected into 6 month old male Fabry mice (n=4) via tail vein. GLA activity was measured by 4MU method in serum at the indicated time points. Half-life of CDX variants were extended up to 4-fold compared to Fabrazyme (43.4 min vs. 10.8). By 4 hours all GLA variants returned to baseline (data not shown).



Variant	$t_{1/2}$ (min)
Fabrazyme	10.8
CDX-6306	31.4
CDX-6307	31.5
CDX-6308	43.4

3. Lysosomal stability was increased 2-fold *in vitro*

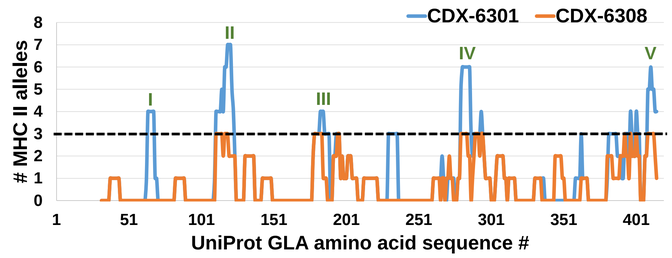
eGLA or Fabrazyme (10μg/mL) was incubated with a Fabry disease endothelial cell line (IMFE1). After 4 hours enzyme-containing media was removed, and cells were incubated with fresh media. Intracellular GLA activity was then measured by 4MU method at 24 hour intervals. Half-life of CDX variants were extended more than 2-fold compared to Fabrazyme (6.8 days vs 3 days).



Variant	$t_{1/2}$ (days)
Fabrazyme	3.07
CDX-6306	6.64
CDX-6307	6.83
CDX-6308	4.86

4. Predicted immunogenicity was reduced in CDX-6308

MHC II predictions were carried out using netMHCIIpan (IEDB software), used to predict likelihoods for 15mer peptides to bind to each of 8 MHC II alleles (95% population coverage). 15mers from each sequence were tested across the entire length of either CDX-6301 or CDX-6308. Each 15mer peptide was considered part of a T cell epitope if it was predicted to bind to 3+ of the 8 MHC II alleles. By this criteria, all five predicted epitopes (I-V) were removed in our deimmunized variant (CDX-6308).



Conclusions

- Improved general stability for all eGLA variants, compared to CDX-6301 / Fabrazyme, with no loss in specific activity.
  - Improvement following serum, temperature, and pH challenge.
- 2-fold increased lysosomal half-life in a Fabry disease endothelial cell line (IMFE1).
- 4-fold increased serum half-life in the Fabry mouse model.
- CDX-6308 was deimmunized by removal of all predicted epitopes (in silico analysis).

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Key Words

- Fabry Disease
- Enzyme Replacement Therapy
- Directed Evolution
- Deimmunization

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