Current Landscape of Enzyme Replacement Therapy and Considerations for GAMT Deficiency

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Background

Enzyme replacement therapy (ERT) is the delivery of exogenous enzymes to individuals with dysfunctional enzyme disorders. It has been in use since 1991 when Ceredase was approved for treatment of Gaucher disease. There are currently over 15 FDA-approved ERTs (see table below). Most ERTs are approved for treating lysosomal storage disorders, as this is where most recombinant proteins easily localize.

ERTs are a lifelong treatment, requiring dosing at least every two weeks. This is commonly done in a hospital, due to the risk of immune-related adverse events, such as hypersensitivity and infusion-associated reactions. Pretreatment with antihistamines and/or antipyretics is sometimes used to mitigate potential reactions. However, some ERTs are safe enough to be approved for at home administration.

Current Approved Enzyme Replacement Therapies

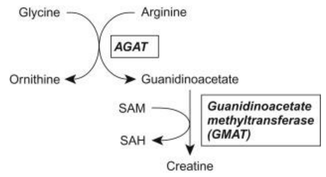
Drug (Generic Name)	Disease	Dosing	Administration Technique*
Cerezyme (Imiglucerase)	Gaucher Disease Type 1	Ranges from 2.5 units/kg 3 times a week to 60 units/kg once every 2 weeks	IV
Strensiq (Asfotase alfa)	Hypophosphatasia	1 mg/kg 6 times a week or 2 mg/kg 3 times a week	SC
Revcovi (Elapegademase)	Adenosine deaminase severe combined immune deficiency	0.2 mg/kg once a week or 0.2 mg/kg twice a week	IM
Nexviazyme (Avalglucosidase alfa-ngpt)	Late-onset Pompe disease	40 mg/kg every 2 weeks or 20 mg/kg every 2 weeks	IV
Agalsidase alfa**	Fabry disease	0.2 mg/kg body weight every other week	IV
Lamzede (Velmanase alfa)	Alpha-mannosidosis	1 mg/kg once a week	IV
Xenpozyme (Olipudase alfa)	Acid sphingomyelinase deficiency	Inital: 0.1 mg/kg for adult patients and 0.03 mg/kg for pediatric patients Maintenance: 3 mg/kg	IV
Elfabrio (Pegunigalsidase alfa)	Fabry disease (adult)	1 mg/kg every 2 weeks	IV
Pombiliti (Cipaglucosidase alfa)	Pompe disease	20 mg/kg every other week	IV
Palynziq (Pegvaliase-pqpz)	Phenylketonuria	Ranges from 20, 40, or 60 mg daily	SC
Elelyso (Taliglucerase alfa)	Gaucher disease	60 U/kg every 2 weeks	IV
VPRIV (Velaglucerase alfa)	Gaucher disease	60 U/kg every other week	IV
Fabrazyme (Agalsidase beta)	Fabry disease	1 mg/kg every two weeks	IV
Lumizyme (Alglucosidase alfa)	Pompe disease	20 mg per kg every 2 weeks	IV
Brineura (cerliponase alfa)	TPP1 deficiency	300 mg every 2 weeks	ICV
Myozyme (Algulcosidase alfa)	Pompe disease	20 mg/kg every 2 weeks	IV
Aldurazyme (Laronidase)	Hurler, MPS I	0.58 mg/kg once a week	IV
Elaprase (Idursulfase)	Hunter, MPS II	0.5 mg/kg once a week	IV
Vimizim (Elosulfase alfa)	Morquio Snydrome A, MPS IVA	2 mg/kg once a week	IV
Naglazyme (Galsulfase)	Maroteaux-Lamy, MPS VI	1 mg/kg once a week	IV
Mepsevii (Vestronidase alfa-vjjbk)	MPS VII	4 mg/kg every 2 weeks	IV
Kanuma (Sebelipase alfa)	Wolman, LAL Deficiency	1, 3, or 5 mg/kg once a week	IV

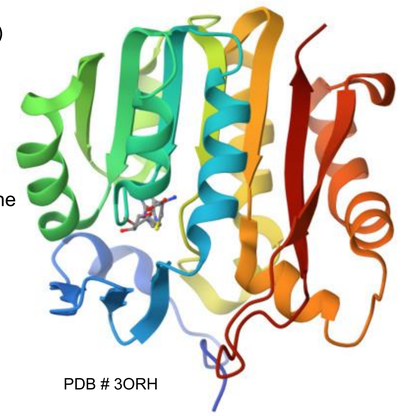
*IV = intravenous, SC = subcutaneous injection, IM = intramuscular injection, ICV = Intracerebroventricular
**Approved in EU, not US

- Current approved therapies are almost all for lysosomal storage disorders. These enzymes act within the lysosome or extracellular space, rather than inside the cytosol of cells
- Most are dosed at least every two weeks, some much more frequently
- Most common administration technique is IV

GAMT Enzyme Characteristics

- Monomeric (active form is one subunit)
- Small size (~26 kDa)
- Theoretical pI 5.74
- Predicted T_m 50 °C
- No cofactors
- Cytosolic enzyme
- Mostly produced in liver
- Catalyzes conversion of GAA to creatine





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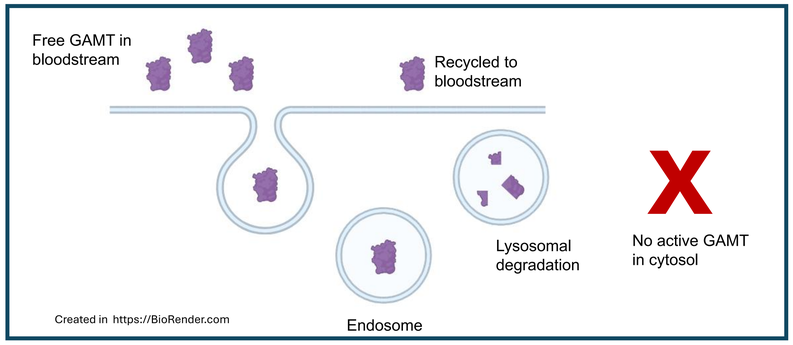
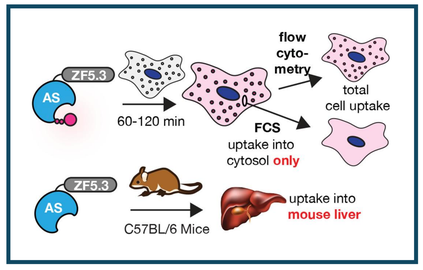
GAMT Modifications for Enzyme Replacement Therapy

Engineering Endosomal Escape

At least four companies currently sell research-grade recombinant GAMT enzyme. Why won't one of these work for enzyme replacement therapy?
When enzymes are introduced into the bloodstream they are endocytosed into cells, where they are recycled back into the bloodstream or broken down within lysosomes. Enzymes do not easily make it to the cytosol.



- Several strategies have been developed for endosomal escape, with varying levels of success.
- Cell penetrating peptides disrupt endosomes via pH-dependent conformational changes, pore formation, or membrane lysis
  - E.g. HA2, GALA, KALA, Tat
  - However, generally unclear how much material eventually localizes to cytosol
  - Unclear whether material is intact upon cytosolic localization

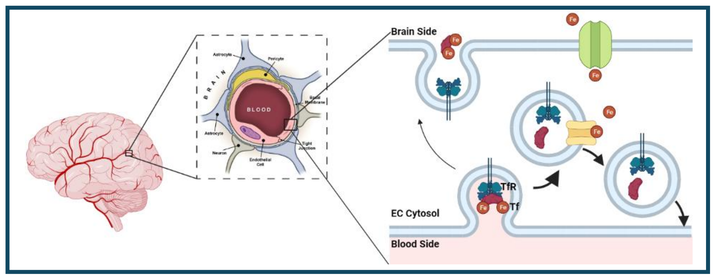


- Emerging technology being applied to GAMT: ZF5.3 is a cell-permeant miniature protein with demonstrated cytosolic delivery capabilities
  - Can reach 500 nM or higher cytosolic concentration
  - Liver delivery demonstrated in mice
  - Endosomal escape is dependent on:
    - Intact HOPS complex
    - Disordered cargo protein or amenability to unfolding at low T_m
    - Small cargo protein size

Crossing the Blood-Brain Barrier

Whether GAMT delivery to the brain is required for an effective treatment is still a matter of debate. However, most recombinant proteins, including GAMT, cannot pass the blood-brain barrier. This is a major hurdle for developing drugs to treat diseases that affect the central nervous system. Modifying GAMT with a moiety that can cross the blood-brain barrier is a strategy for delivery to the brain.

- Transferrin-receptor mediated delivery
  - Transferrin receptor is highly expressed in brain endothelial cells and normally transports transferrin to maintain iron homeostasis
  - Attaching a transferrin receptor antibody or ligand to GAMT that binds the receptor may be a way to cross the blood-brain barrier



Manufacturing Considerations

An excellent enzyme can still fail to be an effective drug if it isn't stable or can't be made in large enough quantities for human doses.

Expression system selection
- Bacterial – cheaper, higher yield
  - GAMT possibly toxic to bacteria?
- Mammalian – more expensive, more likely to preserve native glycosylation

Purification strategy
- How to separate small enzyme (GAMT) from endogenous proteins in expression system?
- Fc fusion

Stability over time
- Determine optimal buffering system, excipients, pH for liquid formulation
- Lyophilized formulation

Conclusions

- Enzyme replacement therapy has been shown to be safe and effective for numerous lysosomal storage disorders
- ERT for GAMT deficiency is in very early stages of development
- Making an effective ERT for GAMT deficiency may require modifying the enzyme
  - Adding tag for endosomal escape or brain delivery, altering native stability, adding FC tag for purification and improved circulation time

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