Towards Enzyme Substitution Therapy
of Transglutaminase 1 deficient
Lamellar Ichthyosis

Obstacles to translation

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Transglutaminase 1 deficient Lamellar Ichthyosis

- Congenital recessive skin disorder characterized by generalized scaling
- Apparent at birth and persisting throughout life
- Prevalence 1:200,000
  2,000 patients in Europe
  1,300 in USA, 700 in Japan (estimates)
- Deplorable therapeutic situation
- Emollients, urea, lactic acid and retinoids


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Lamellar Ichthyosis – animal model

TGM1-knock-out mouse
(Matzuki et al., PNAS, 95: 1044-1049, 1998; Steinert et al. US 2003/0072795)

- 1000 times reduced barrier function
- trans epidermal water loss 100 times higher than wild-type
- mice die 4 - 5 h after birth
  - Difficulties to provide definite proof of concept in a “lethal mouse model”
Transglutaminases have key functions in the corneocyte formation process by cross linking the proteins of the cornified envelope.

Transglutaminase 1 (TG1) has a key function in the catalysis of the first steps of cornified envelope formation and in the covalent fixation of the lipid envelope to the cornified envelope.
Transglutaminase 1

• Synthesized as a 817 residue membrane-anchored precursor
• Ca\(^{2+}\) dependent
• Intracellular
• Expressed in the upper differentiated layers of the epidermis
• Attachment of long chain omega hydroxyceramides to involucrin e.g.
• In situ enzyme test established - shows pericellular distribution and most marked activity in stratum granulosum
Transglutaminase 1 in the skin

According to Steinert et al., 1996

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Cloning of TGase 1 constructs

Plasma membrane

FL hTGk His

catalytic region

His-tag

67/33 hTGk His

93R 94G

His-tag
The Gateway Technology

- Universal cloning method
- provides rapid and highly efficient way to move DNA sequences into multiple vector systems
- based on site-specific recombination of bacteriophage lambda
**Baculovirus-Expressionsystem**

- Cloning of TGase 1 cDNA into the entry-vector pENTR 3C
- LR-reaction with linear BaculoDirect DNA

**Diagram:**

1. Cloning of TGase 1 cDNA into the entry-vector pENTR 3C
2. LR-reaction with linear BaculoDirect DNA
3. Insertion of TGase 1 cDNA
4. Infection of Sf9 insect cells
5. Virus amplification
6. Direct transformation of Sf9 cells with recombinant BaculoDirect DNA and selection with ganciclovir
7. Infektion of HighFive insect cells in liquid culture
8. TGase 1 Expression

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Interaction between neighbouring residues in the 6x His-tag and Ni-NTA matrix.
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Activity of recombinant TGase 1

Fluorescence Spectroscopy

Specific Activity [U/mg]

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Transglutaminase 1 activity in normal skin and lamellar ichthyosis

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Obstacles to translation (1)

- No definite proof of concept yet (TG1 deficient mice die shortly after birth....)
  - Keratinocyte-cultures of TGase 1-deficient patients / treatment with recombinant TGase 1 constructs
  - inducible mouse model
  - skin equivalents
- Stabilization and formulation
  - liposome or micelle formulation needed and specialists for this
- Upscale production under GMP conditions needed
  - requires investor / classical problem of orphan drugs
- Preclinical toxicity studies (ten dogs, ten mice...)
- Clinical studies to prove benefit over emollients and retinoids
Obstacles to translation (2)
Lamellar Ichthyosis Enzyme replacement Therapy
Consortium LIERTHEC

A novel approach to treat Lamellar Ichthyosis

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- It is intended to make use of a recombinant transglutaminase construct of N-Zyme BioTec, a leading company in transglutaminase technology

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Economical Consideration

Cost estimation: 6.0 Mio Euro
- Production of GMP-grade enzyme (CMO)
- Formulation and Toxicology
- Clinical trials / approval (orphan drug - status)
- Project coordination and consulting

Market estimation
- Prevalence 1:200,000; about 2,000 patients in Europe, 1,300 in the US and 700 in Japan
- Treatment costs per year and patient 50,000 EURO
- Market penetration of 25% (peak sales) 50 Mio EURO

reference e.g. Cerezyme (Genzyme)
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