

# 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



Oji V. and Traupe H., Ichthyoses:  
Differential diagnosis and molecular genetics,  
European Journal of Dermatology, in press, 2006



# Lamellar Ichthyosis – animal model

## TGM1-knock-out mouse

(Matzki *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 in the Epidermis

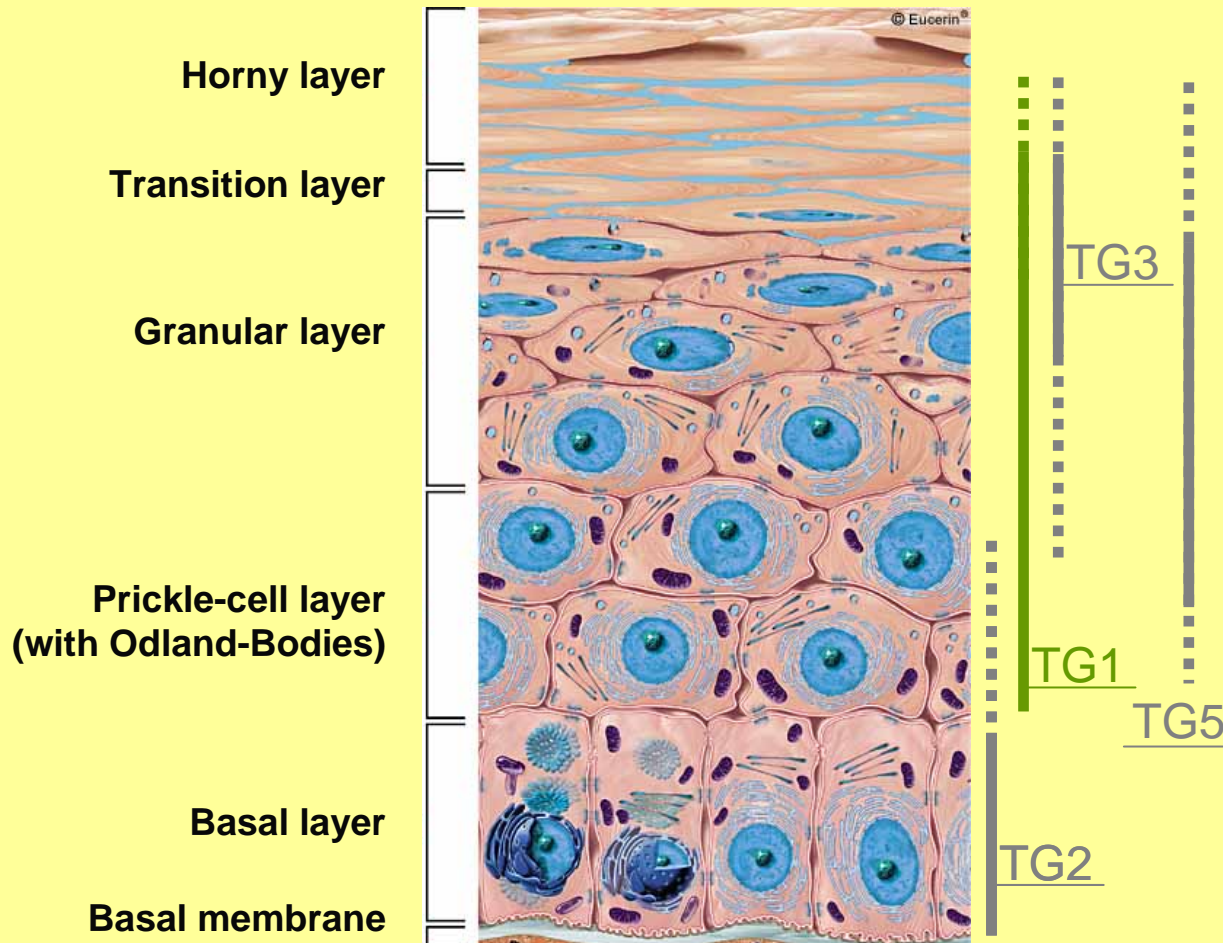
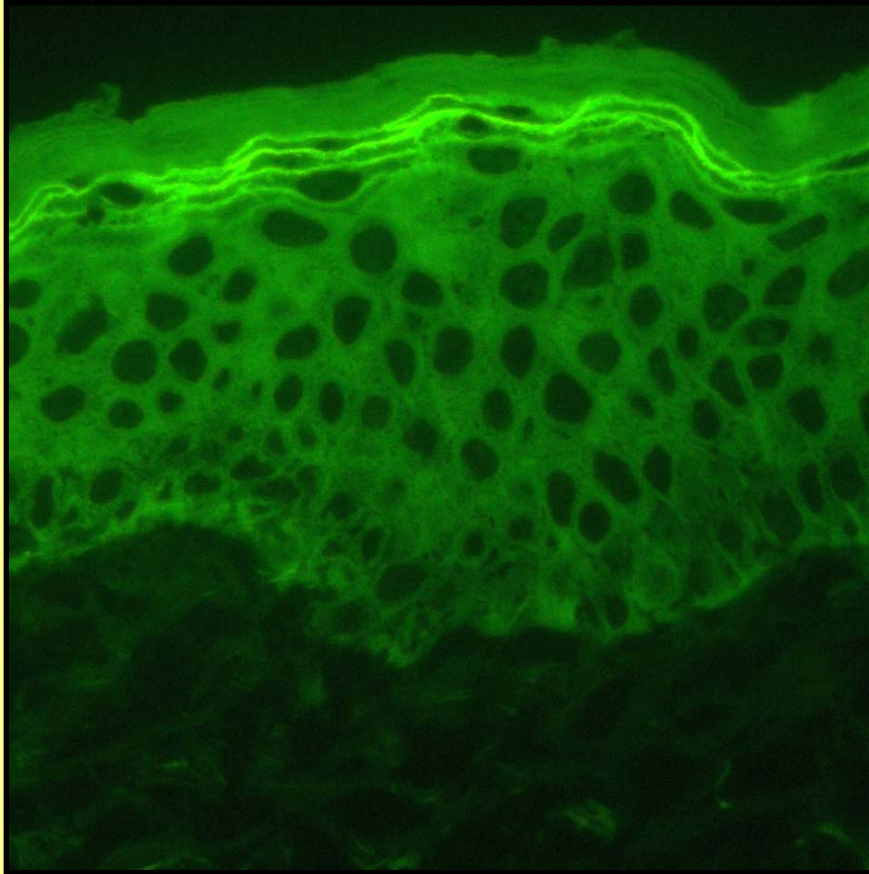


image source: Beiersdorf

Transglutaminases have key functions in the corneocyte formation process by cross linking the proteins of the cornified envelope.

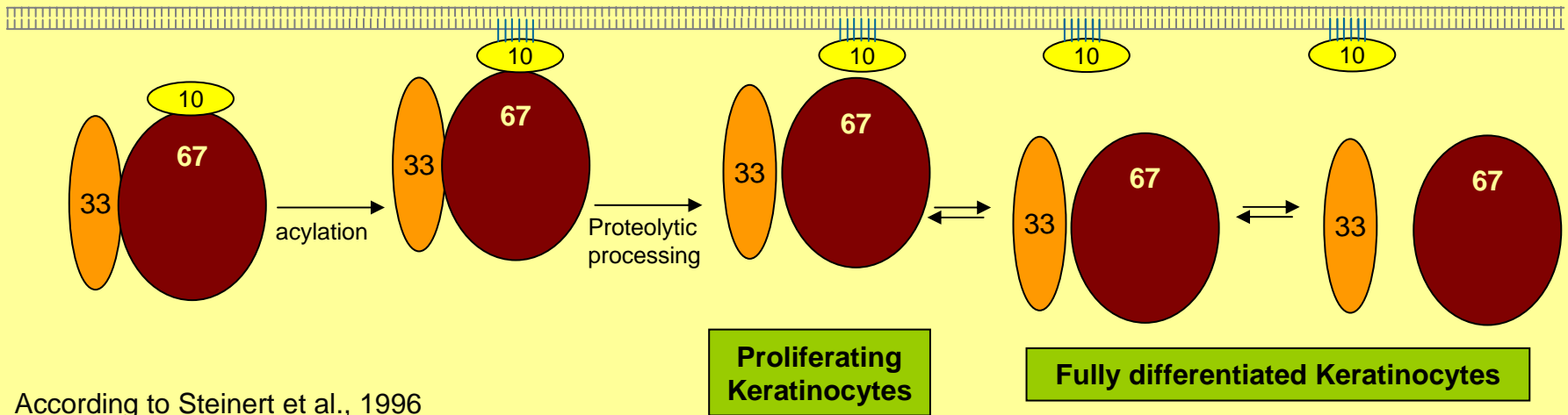
Transglutaminase 1 (TG1) has a key function in the catalysis of the first steps of cornified envelope formation and in the covalent fixation of the lipid envelope to the cornified envelope

# Transglutaminase 1



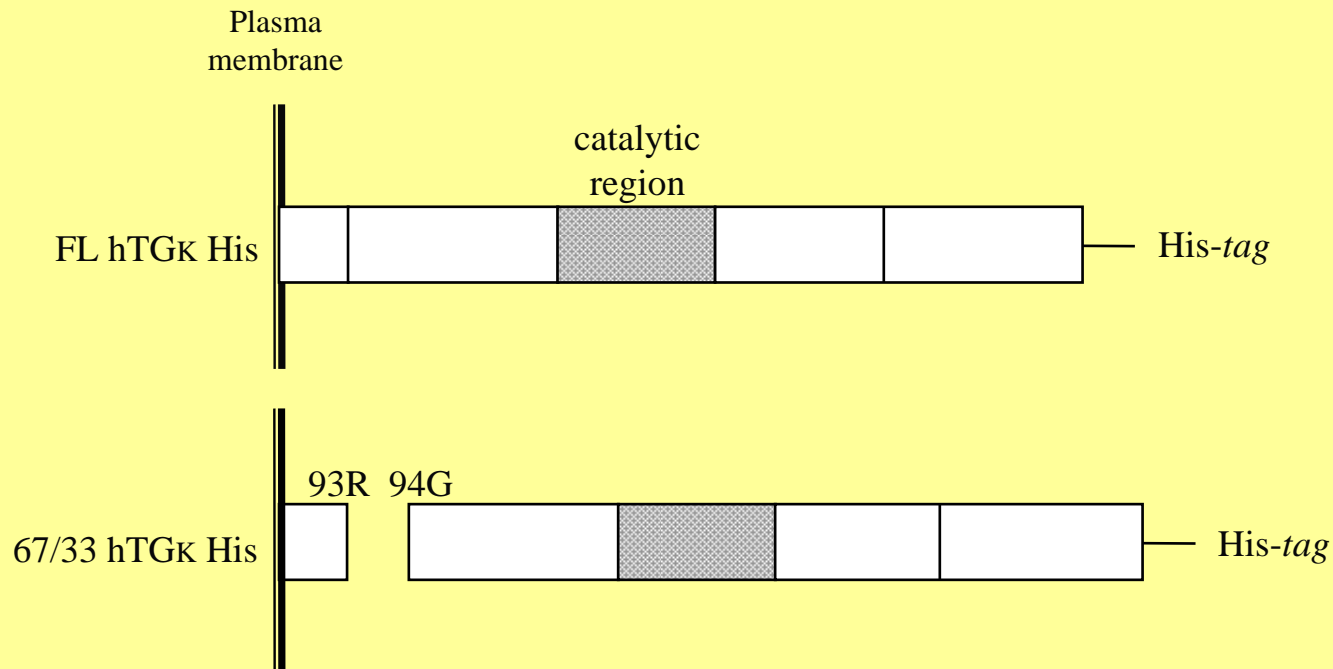
- Synthesized as a 817 residue membrane-anchored precursor
- $\text{Ca}^{2+}$  dependent
- Intracellular
- Expressed in the upper differentiated layers of the epidermis
- Attachment of long chain omega hydroxyceramides to involucrin e.g.
- In situ enzyme test established - shows pericellular distribution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

# Transglutaminase 1 in the skin



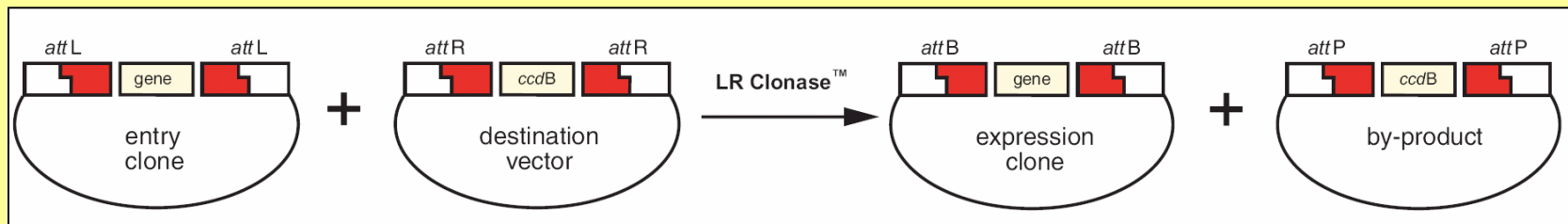
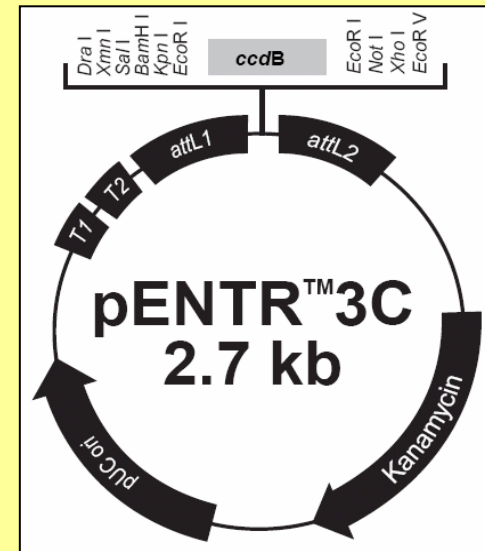
According to Steinert et al., 1996

# Cloning of TGase 1 constructs



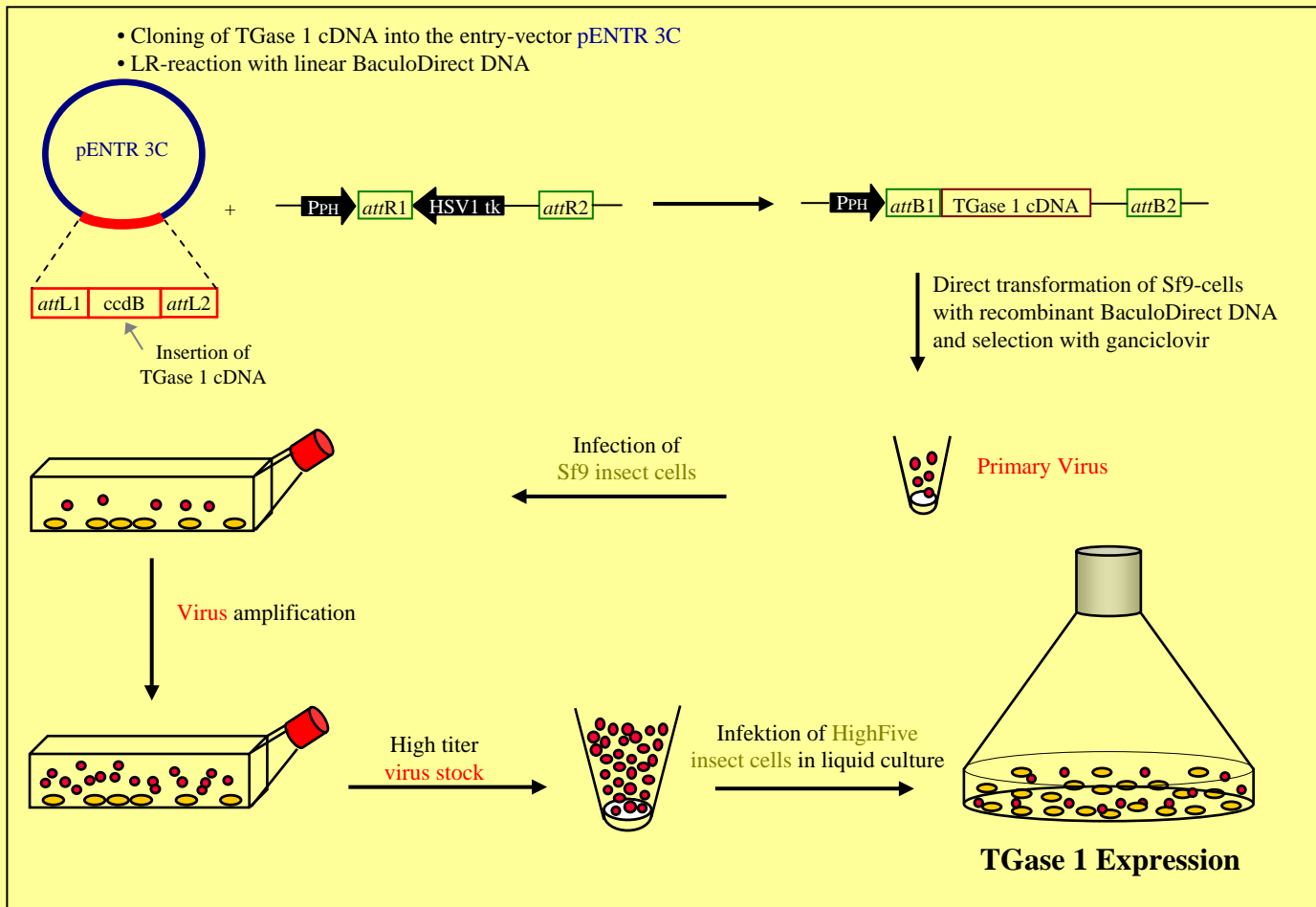
# The Gateway Technology

- 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

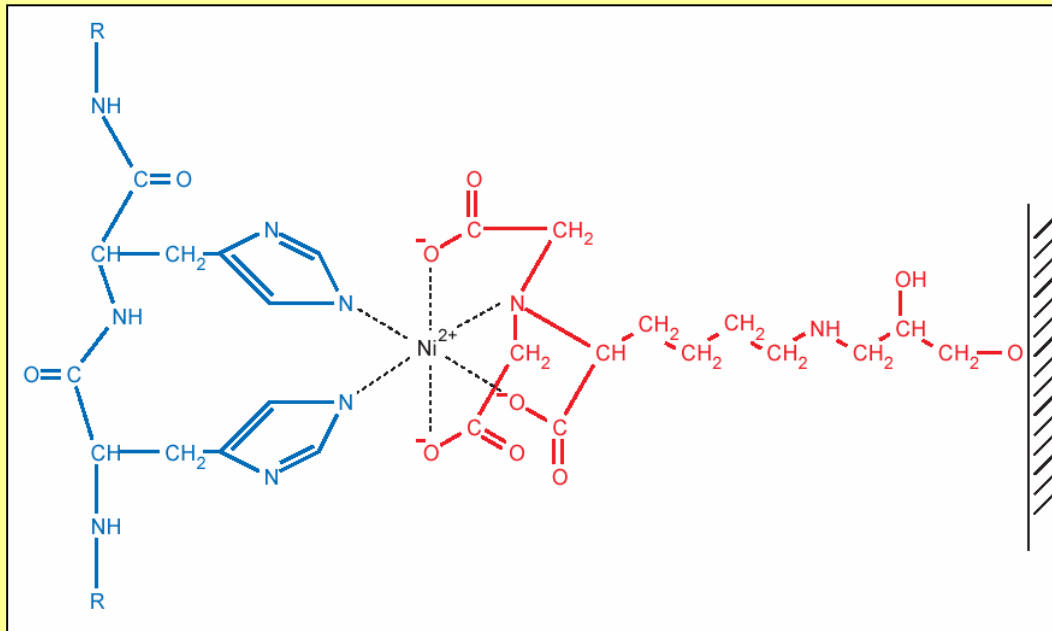




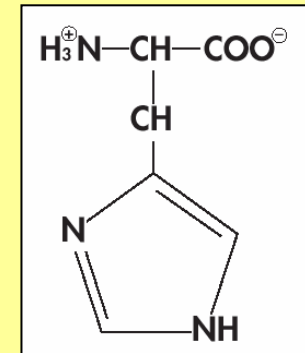
# Baculovirus-Expressionsystem



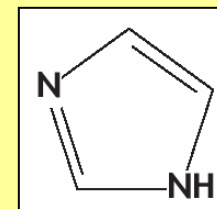
# Purification: Ni-NTA-affinity-chromatography



Interaction between neighbouring residues in the 6x His-tag and Ni-NTA matrix.

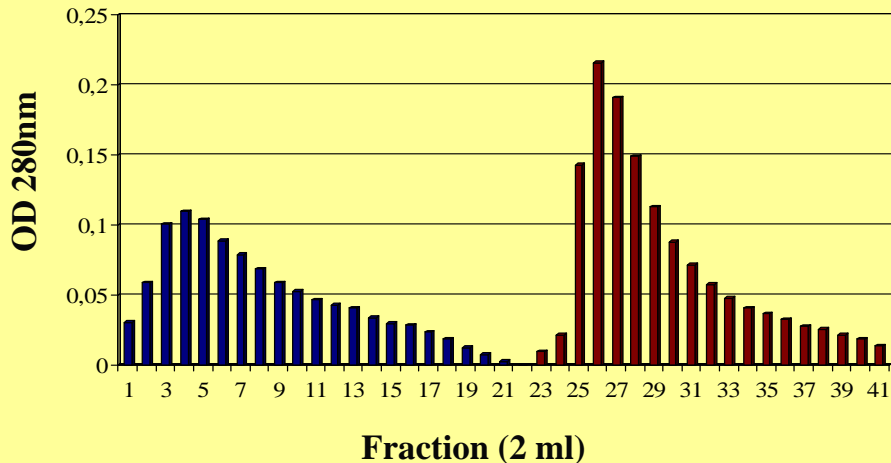


Histidin

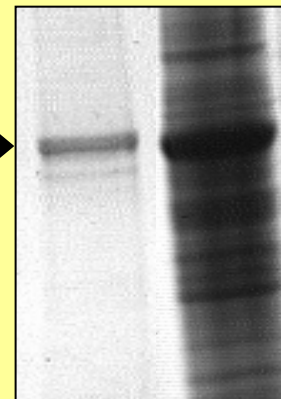


Imidazol

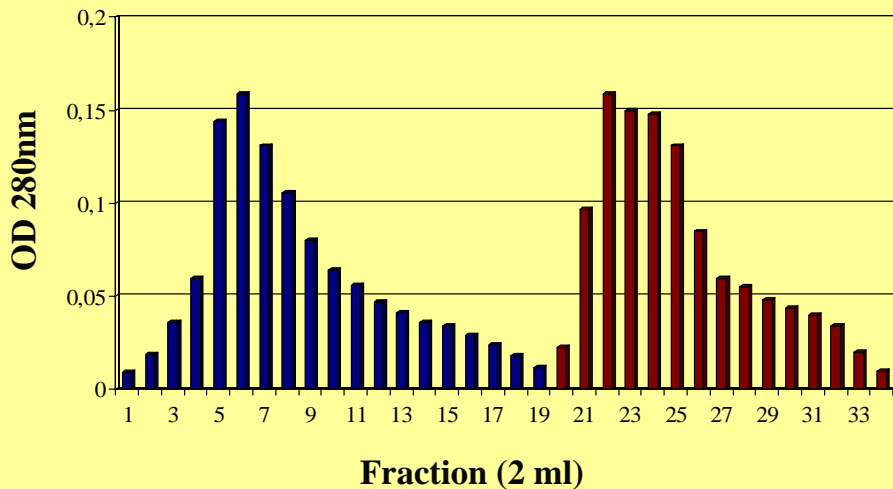
### FL hTG<sub>K</sub> His



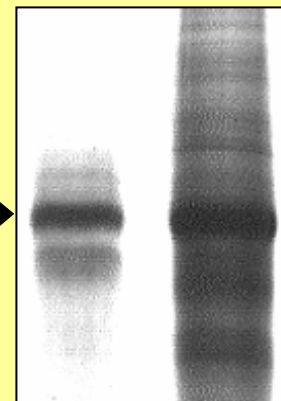
FL hTG<sub>K</sub> His  
(92 kDa)



### 67/33 hTG<sub>K</sub> His



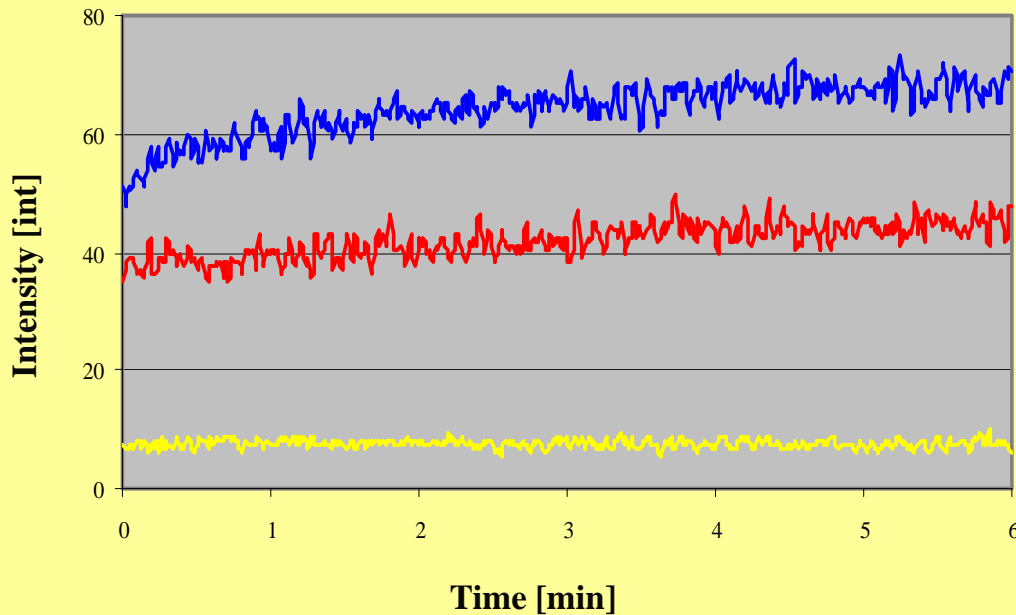
67/33 hTG<sub>K</sub> His  
(80 kDa)



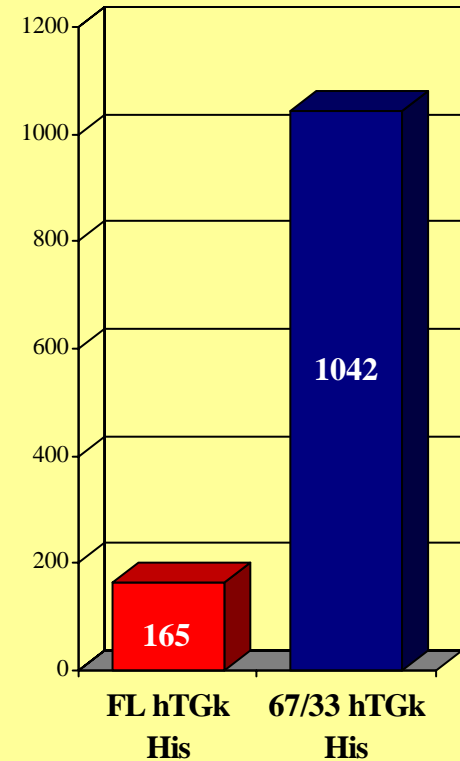
50 mM imidazol 100 mM imidazol

# Activity of recombinant TGase 1

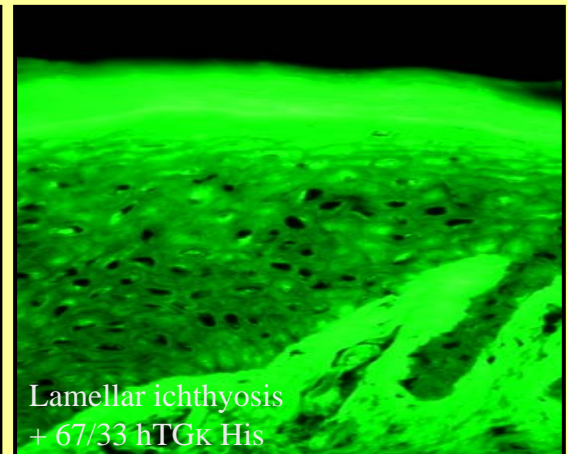
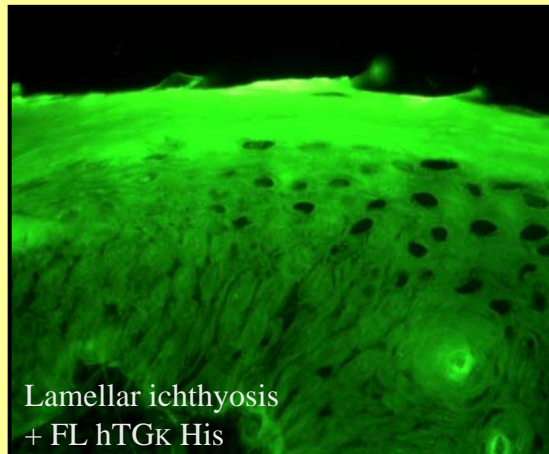
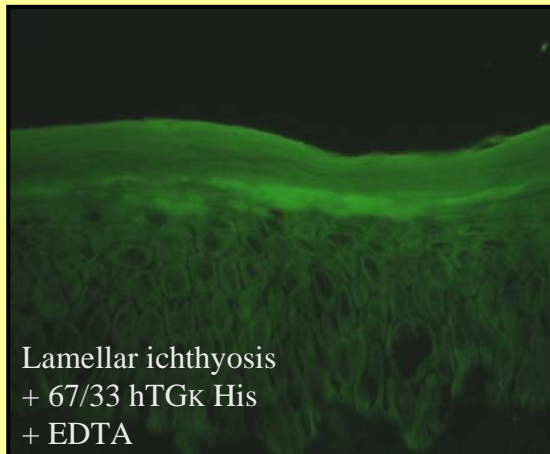
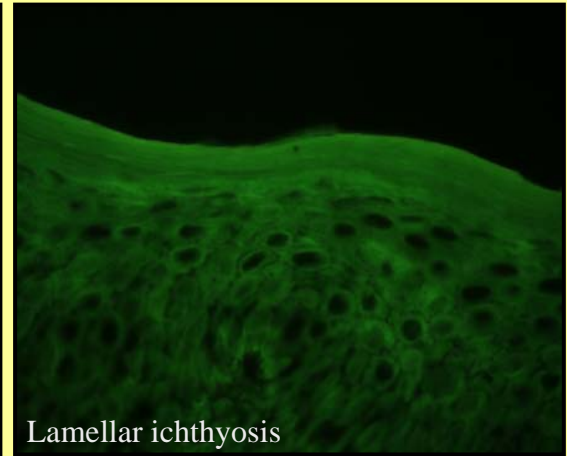
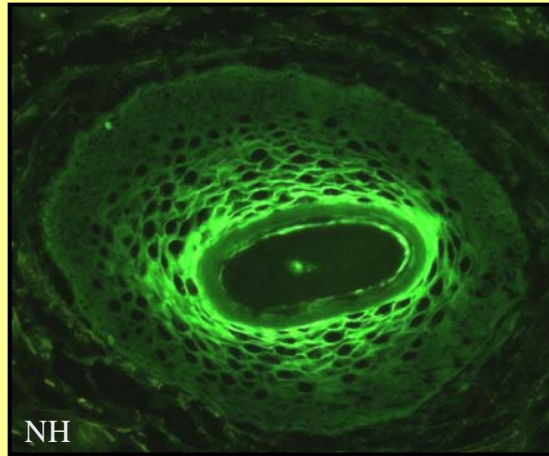
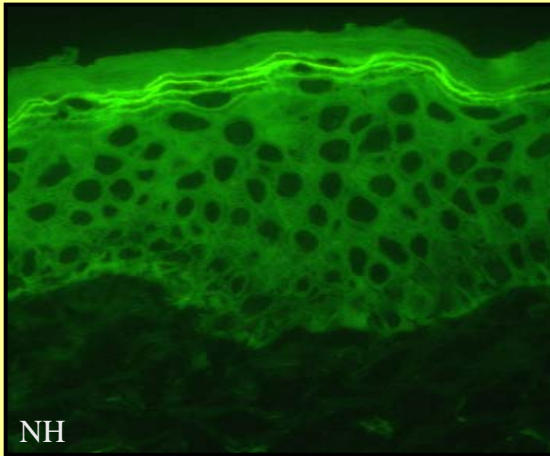
## Fluorescence Spectroscopy



## Specific Activity [U/mg]



# Transglutaminase 1 activity in normal skin and lamellar ichthyosis



# 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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#### N-Zyme BioTec GmbH

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#### Hans C. Korting

Klinik und Poliklinik  
für Dermatologie und Allergologie  
Ludwig-Maximilians-Universität München

#### Pharma Company

N. N.

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



# 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 **4,000 patients**
- Treatment costs per year and patient **50,000 EURO**
- Market penetration of 25 % (peak sales) **50 Mio EURO**

reference e.g. Cerezyme (Genzyme)





# Acknowledgements

## Münster

- Karin Aufenvenne
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Bundesministerium  
für Bildung  
und Forschung



Network for Ichthyosis  
and related  
keratinisation disorders

