Rare Diseases 360°
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APTEEUS
engaging patients in discovery

TEE786, first-in-class medicine addressing nonsense mutation mediated disorders

From screening to clinic

Nonsense mutation and nonsense-mediated mRNA decay

Nonsense mutations represent 20% of all genetic mutation responsible for monogenetic diseases[1]. Non sense mutations are single-base pair substitution within the coding sequence of a gene that result in a premature termination codon (PTC). The presence of PTC in a coding sequence triggers the activation of the nonsense-mediated mRNA decay (NMD), a cellular surveillance mechanism, that selectively degrades newly synthesized mutant transcripts[2,3]. This leads to the total absence of the corresponding protein and to a total loss-of-function[4] (Figure 1). Most nonsense associated disorders results from insufficient levels of full-length protein[5].

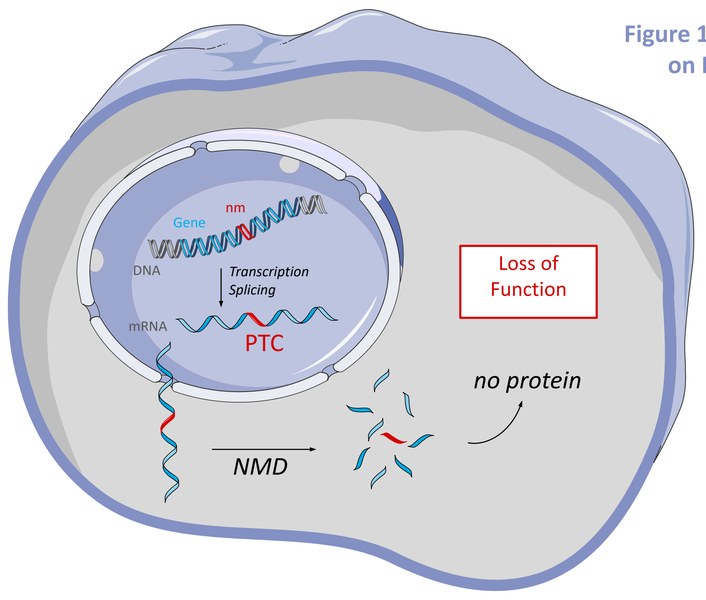
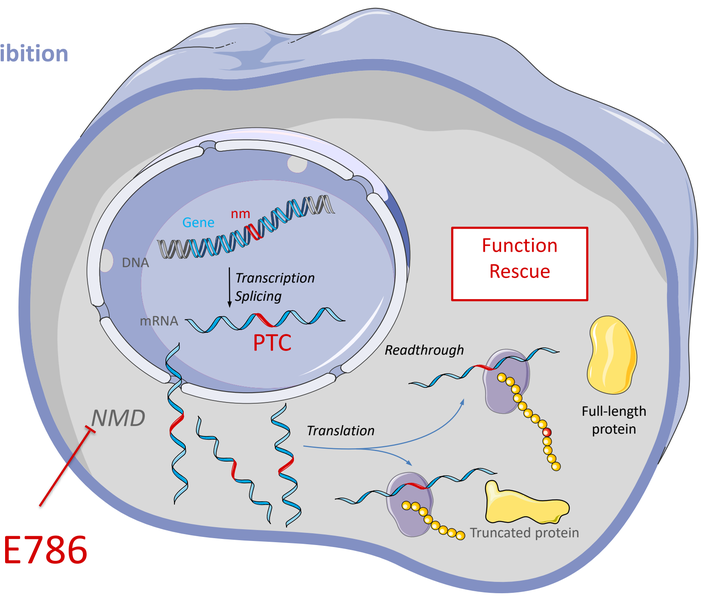


Figure 1: Role of NMD on PTC containing transcripts.

NMD as target for nonsense mutation diseases

One of the strategy used to overcome the presence of nonsense-mutation is based on the incorporation of a random amino-acid at the PTC position through read-through mechanism[3,4,6]. If the PTC is not at a crucial position, a functional full-length protein is synthesized. Nevertheless the efficiency of natural or drug-activated readthrough is limited by the degradation, by the NMD, of the substrates available for readthrough i.e. PTC-containing mRNA. So, inhibiting NMD pathway represents an attractive way to treat nonsense-mutation mediated diseases[5–7] (Figure 2).

Figure 2: NMD inhibition strategy



TEE786, already approved drug discovered by HTS screening

TEE786 was discovered among Apteeus proprietary library of marketed drugs using a screening system dedicated to NMD inhibition[6]. TEE786 is active against several nonsense mutations notably in Duchene muscular dystrophy and cystic fibrosis[6]. Currently two indications is going to be tested in clinic: aspartylglucosaminuria (AGU) and recessive dystrophic epidermolysis bullosa (RDEB).

AGU: Lysosomal disorder[8]

Main collaborators: Dr. M. Schiff, Prof. R. Tikkanen, Dr. Antje Banning

Aspartylglucosaminuria (AGU) is a lysosomal storage disorder caused by mutations in the gene coding for aspartylglucosaminidase (AGA). AGA is involved in glycoprotein degradation in lysosomes.

An AGU patient exhibits two compound heterozygous mutation (S72P/W168X) in the AGA gene. In patient fibroblasts, the AGA mRNA level and AGA activity are significantly reduced (respectively to 50% and 18 % of control, Figure 3A and 4). Treatment with TEE786 induced an increase in mRNA level of 50 % reaching 75% of control (Figure 3B), an increase in AGA polypeptide synthesis and in AGA activity reaching the level of asymptomatic carrier (Figure 4).

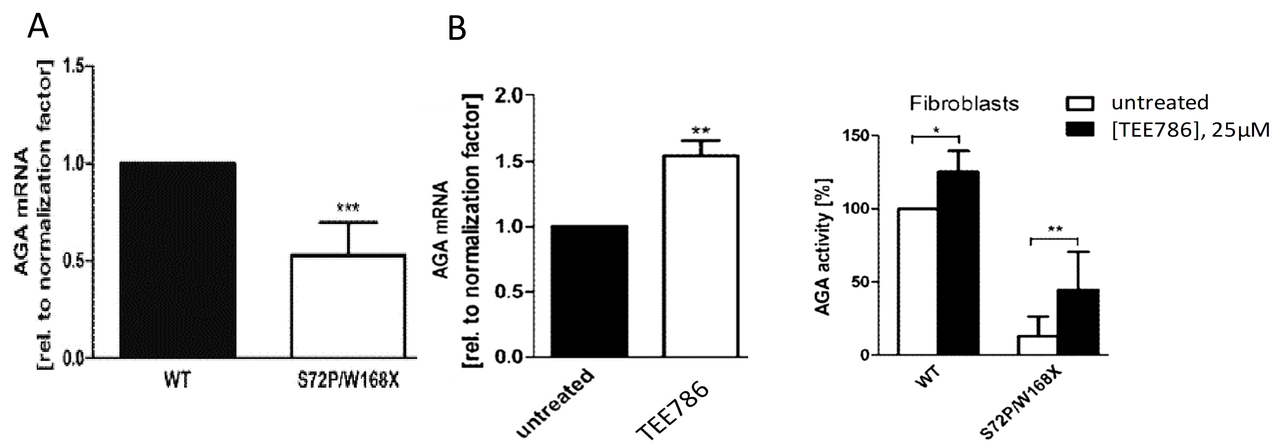
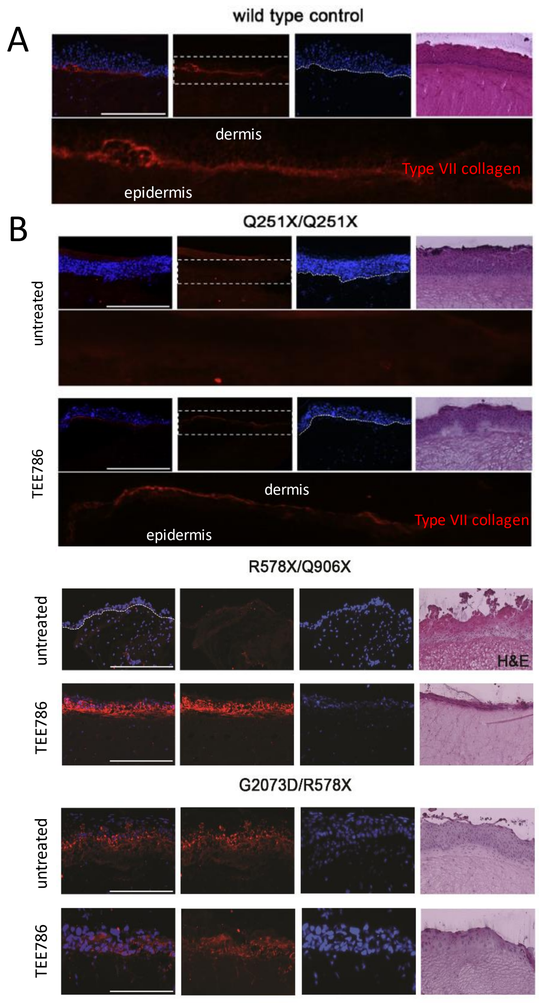


Figure 3: PTC-transcript salvage by TEE786 Quantitative real-time PCR of AGA mRNA analysis with patient fibroblasts carrying S72P/W168X mutations compared to control fibroblast (wild-type) (A) and after treatment with TEE786 (25µM) for 48h.

Figure 4: Enzymatic function rescue AGA activity measured fluorimetrically without and after TEE786 treatment in lysed fibroblast carrying S72P/W168X mutations compared to control.

RDEB: Genodermatosis[9]

Main collaborators: Dr. A. South, Prof. C Bodemer, Dr. S. Hadj Rabia



Recessive dystrophic epidermolysis bullosa (RDEB) is a rare monogenic characterized by cutaneous and mucosal blistering caused by the lack of functional type VII collagen at the dermis epidermis junction (DEJ). Reconstituted skin using primary patient fibroblasts and keratinocytes is the most relevant way to reproduce the causative defect of the symptoms *in vitro*.

TEE786 treatment induces production of a full-length protein (up to 50% relative to normal control) and localization of type VII collagen at the DEJ in organotypic skin culture in 8 of 12 cells from patient with RDEB haboring PTC mutation (Figure 5).

Figure 5: Protein function rescue Organotypic skin cultures prepared using wild-type cells (A) or RDEB cells and treated with TEE786 for 2 weeks (B). Synthesis of type VII collagen at the DEJ was evaluated using immunofluorescence staining (Type VII collagen specific antibody in red). 3 different patients are represented: homozygous Q251X, Compound Heterozygous nonsense R578X/Q906X and compound heterozygous G2073D/R578X.

Compassionate use

Based on these results, a nominative authorization to treat this patient with TEE786 has been given by the French authorities.

Clinic trial

Based on these results, Apteeus is planning a clinical trial in 2019 engaging patients with nonsense RDEB.

1.Mort, M., Ivanov, D., Cooper, D. N. & Chuzhanova, N. A. A meta-analysis of nonsense mutations causing human genetic disease. *Hum. Mutat.* **29,** 1037–1047 (2008). **2**.Kurosaki, T. & Maquat, L. E. Nonsense-mediated mRNA decay in humans at a glance. *J. Cell Sci.* **129,** 461–467 (2016). **3**.Miller, J. N. & Pearce, D. A. Nonsense-Mediated Decay in Genetic Disease: Friend or Foe? *Mutat. Res. Rev. Mutat. Res.* **0,** 52–64 (2014). 4.Bidou, L. & Allamand, V. Nonsense Mutations Causing Inherited Diseases: Therapeutic Approaches. in *eLS* (American Cancer Society, 2010). doi:10.1002/9780470015902.a0022433 **5**.Kuzmiak, H. A. & Maquat, L. E. Applying nonsense-mediated mRNA decay research to the clinic: progress and challenges. *Trends Mol. Med.* **12,** 306–316 (2006). **6.**Gonzalez-Hilarion, S. *et al.* Rescue of nonsense mutations by amlexanox in human cells. *Orphanet J Rare Dis* **7,** 58 (2012). **7**.Keeling, K. M., Du, M. & Bedwell, D. M. *Therapies of Nonsense-Associated Diseases*. (Landes Bioscience, 2013). **8**.Banning, A., Schiff, M. & Tikkanen, R. Amlexanox provides a potential therapy for nonsense mutations in the lysosomal storage disorder Aspartylglucosaminuria. *Biochim. Biophys. Acta* **1864,** 668–675 (2017). **9**.Atanasova, V. S. *et al.* Amlexanox Enhances Premature Termination Codon Read-Through in COL7A1 and Expression of Full Length Type VII Collagen: Potential Therapy for Recessive Dystrophic Epidermolysis Bullosa. *J. Invest. Dermatol.* **137,** 1842–1849 (2017).

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