



Type VII collagen correction by RNA *trans-splicing*

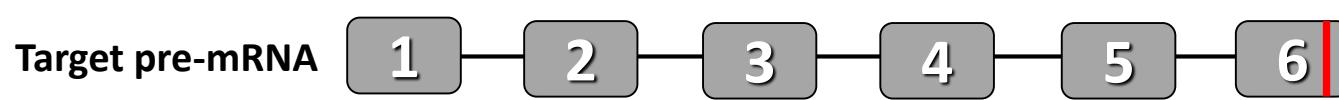
(ERA-NET E-RARE-2)

Patricia Peking

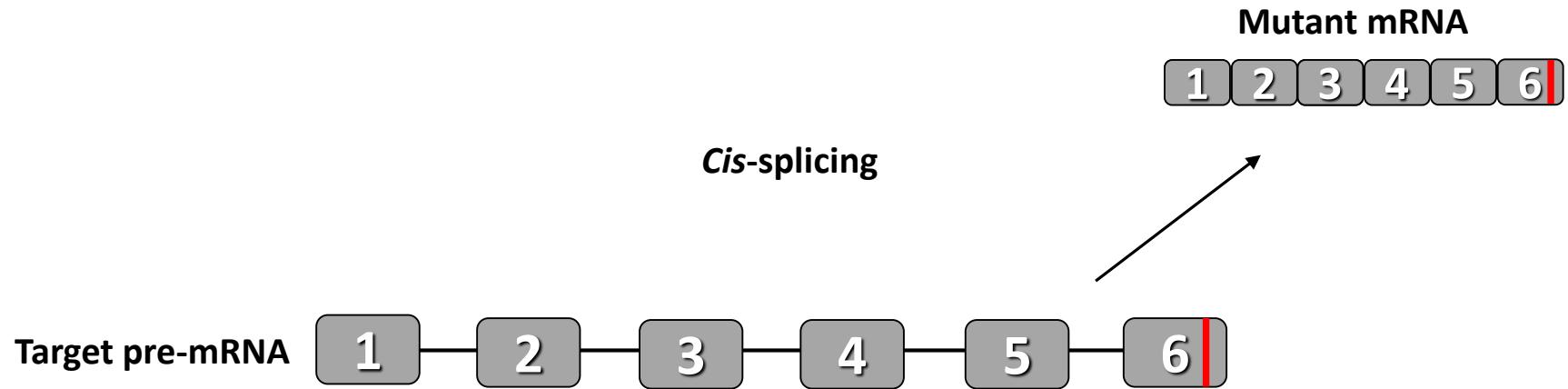
Outline

- 3' RNA *trans*-splicing technology (SMaRT) to correct *COL7A1* mutations
- 3' *trans*-splicing in a cell culture model system
- *Ex vivo* approach in a xenograft mouse model
- Conclusion

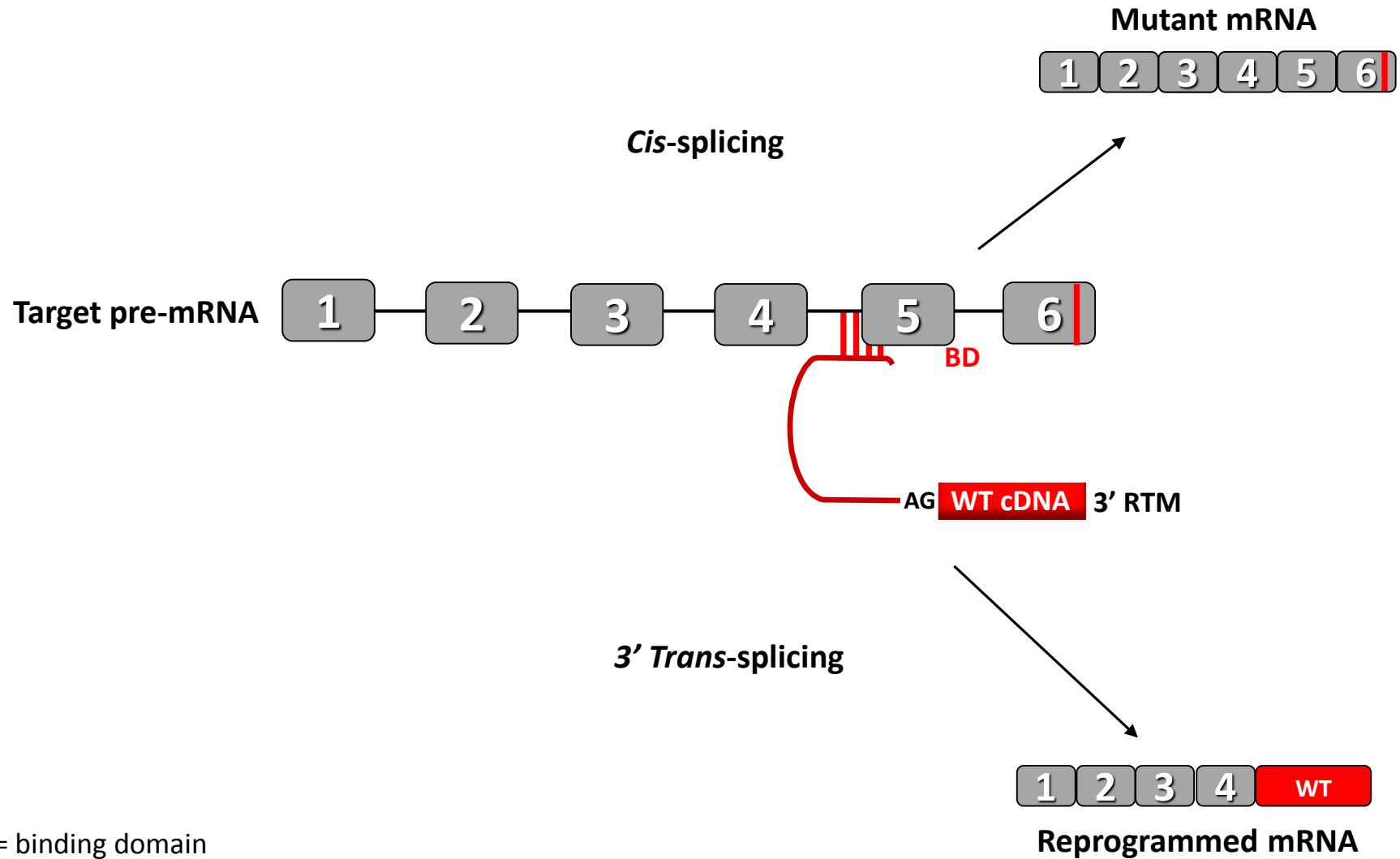
SMaRT: Spliceosome-mediated RNA *trans*-splicing



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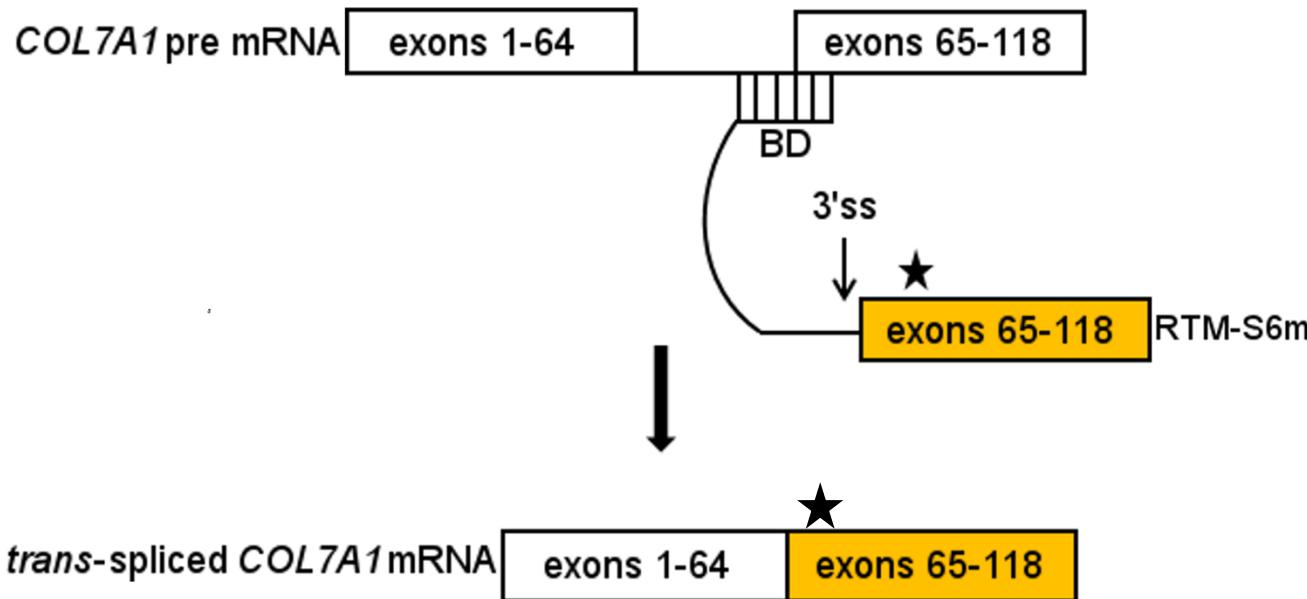
SMaRT: Spliceosome-mediated RNA *trans*-splicing



SMaRT Advantages

- Preservation of the endogenous regulation
- Small transgene size
- One molecule > correction of many *COL7A1* mutations possible
- Constructed to correct mutations located 5', 3' or internal
- Intervention at RNA level

Establishment of stably corrected keratinocytes

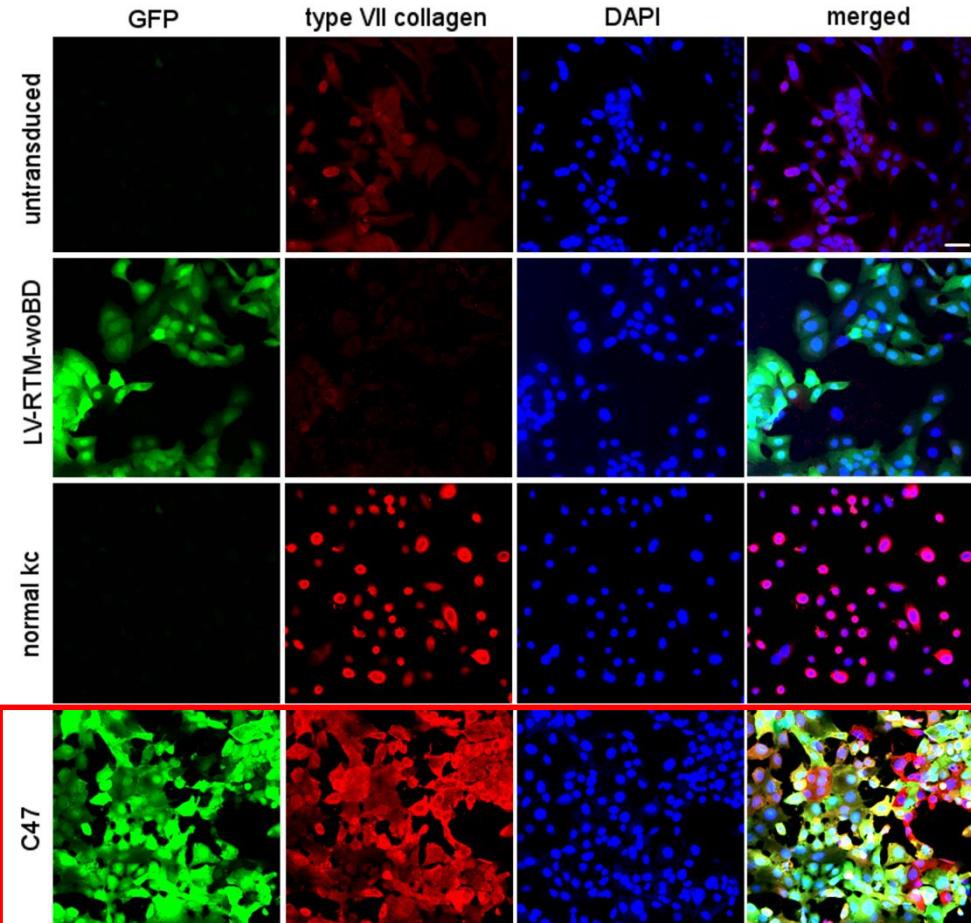


- Covering ~ 40 % of all DEB causing mutations
- SIN lentiviral vector (LV-RTM-S6m)
- RDEB keratinocyte line: c.6527dupC in exon 80 in *COL7A1*

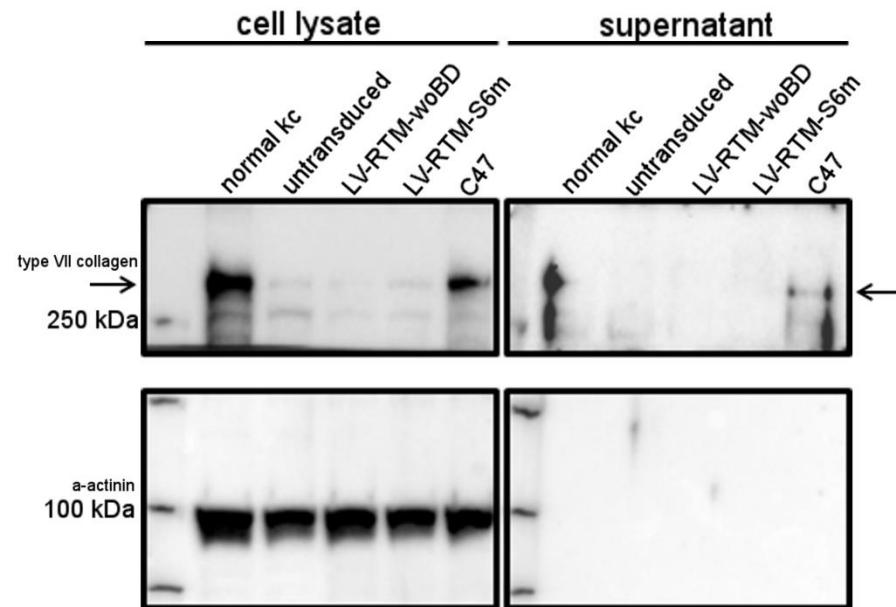
Murauer *et al.* (2011). *JID*
Peking *et al.* (2017). *NAR*

Analysis of single cell clones

IF: anti human type VII collagen:



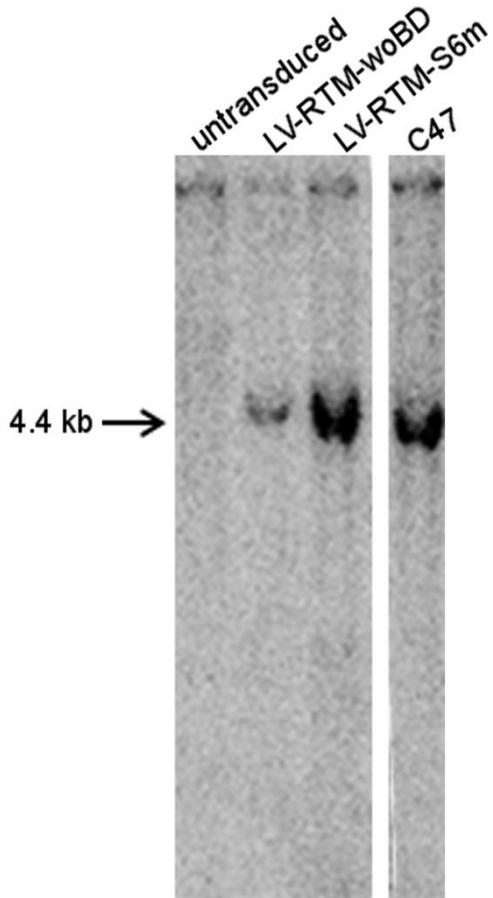
Western blot: anti human type VII collagen



Peking *et al.* (2017). NAR

Safety profiling

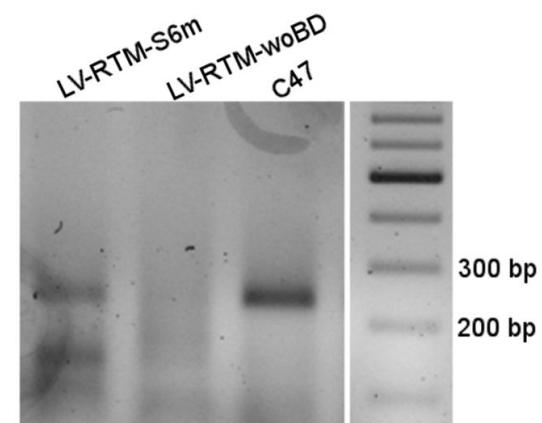
RTM integration: Southern blot



Vector copy number: sqPCR

Sequence	VCN
normal kc	0.03
LV-RTM-S6	1.34
LV-RTM-woBD	1.35
C47	1.25

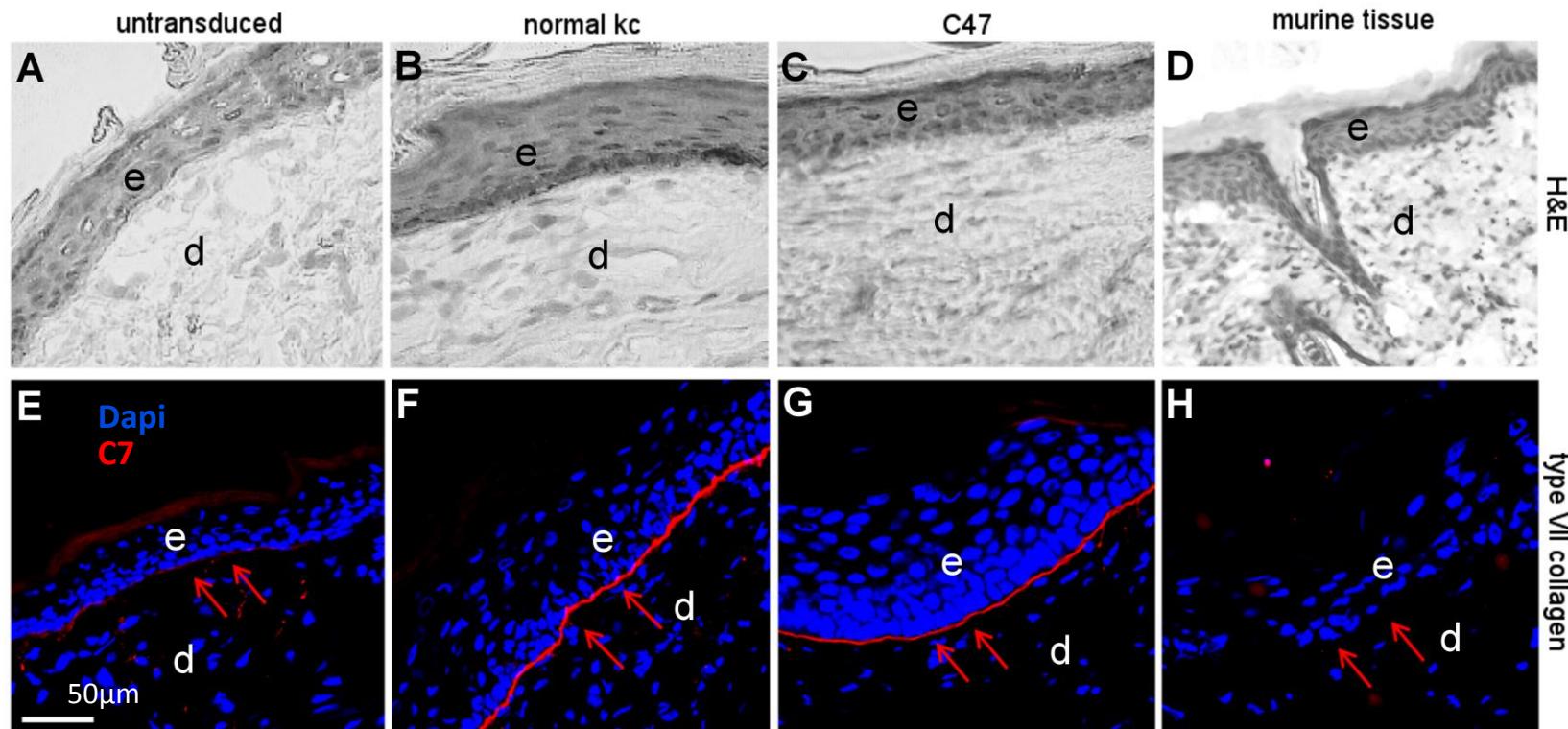
Trans-splicing COL7A1: PCR



Peking *et al.* (2017). NAR

Transplantations onto immuno-deficient mice

- Transplantation of skin sheets derived from RTM S6 corrected keratinocytes
- Analysis 12 weeks after transplantation



Peking et al. (2017). NAR

Conclusion

- Establishment of *trans*-splicing corrected RDEB keratinocytes using a safe SIN lentiviral vector
- Long term type VII collagen expression at basement membrane zone in xenograft mouse model

An RNA-targeted therapy for dystrophic epidermolysis bullosa

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