Local delivery of an antisense oligonucleotide for recessive dystrophic epidermolysis bullosa

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Introduction
Recessive dystrophic epidermolysis bullosa (RDEB) is a genetic blistering disorder caused by mutations in the COL7A1 gene encoding for type VII collagen (C7) protein. We have demonstrated that QR-313, a single stranded antisense oligonucleotide (AON), is efficient in the skipping of exon 73 from COL7A1 in human skin equivalents, thereby removing mutations in exon 73 of COL7A1 and enabling the restoration of C7 protein expression (Figure 1). One of the main hurdles in development of potential medicines based AONs is sufficient delivery to the target organ. It has been shown that systemic administration leads to high AON concentrations in the liver and kidneys, with hardly detectable delivery to the skin. Hydrogels are part of the daily care of RDEB patients, therefore a conventional carrier based hydrogel was developed for QR-313.

Aims
In this study we wanted to assess Cy5-labeled QR-313 delivery in different (wounded) models, including human skin equivalents (HSEs), ex vivo porcine skin and an in vivo wound model using minipigs. Cy5-labeled QR-313 was formulated in PBS or hydrogel was applied. New skin was formed in all models, and new skin was peeled off to fit onto filters to culture at the air liquid interface. Ex vivo porcine skin was observed, when part of the epidermis was removed to mimic the skin of DEB patients at wound sites. It is demonstrated that Cy5-labeled QR-313 delivery in ex vivo human skin after 24 hrs. Cross sections of FFPE skin were counterstained with DAPI and the Cy5 QR-313 signal was visualized by fluorescence microscopy. Scale bar indicates 200µm.

Material and Methods
Full-thickness HSEs were grown from healthy skin donors in an air liquid interface on filters. Ex vivo human skin was obtained, washed and excess dermis was removed manually, next the skin was peeled off to fit onto filters to culture at the air liquid interface. Ex vivo porcine skin was obtained, the skin was peeled to 1 mm thickness using a dermatome and cultured at the air liquid interface. An in vivo minipig model was used, where a foot of a minipig was excised to form a dermabrade. In all models a DEB-like wound was created by removing the epidermis, either with a scalpel or with a dermatome. Next Cy5-labeled QR-313 was applied onto the skin pieces in PBS or formulated in a hydrogel. Cy5 diffusion into the skin models was followed for 24 hrs up to 7 days. All skin sections were processed for fluorescent stained with DAPI or haematoxylin and analyzed using (fluorescent) microscopy.

Results

1. Cy5-labeled QR-313 is delivered in wounded human skin equivalents

Human skin equivalents (HSEs) were used to assess behavior of the AONs in a 3D environment. Part of the epidermis was removed to mimic DEB wounds before application of Cy5-labeled QR-313 formulated into PBS (50µg/ml) or hydrogel (0.5 µg/ml). After 24 hrs no penetration into the epidermis of intact models was observed. In the HSEs where part of the epidermis was removed diffusion into the dermal compartment of the model was observed. When Cy5-labeled QR-313 was formulated into the hydrogel more diffusion also lateral undermeth the epidermis was observed (Figure 2).

Results (continued)

Figure 1. Mode of action of QR-313. Prevention of exon 73 inclusion in the mRNA of COL7A1 to create a slightly shorter C7 protein. Exon 74

2. Cy5-labeled QR-313 is delivered in the dermis of wounded ex vivo porcine and human skin

Application of Cy5-labeled QR-313 for 24 or 48 hrs onto intact and wounded ex vivo porcine skin demonstrated diffusion of Cy5-labeled QR-313 into the dermis, whereas in intact skin no Cy5-labeled QR-313 was taken up. QR-313 formulated in hydrogel showed a deeper penetration of the dermis than QR-313 in solution (PBS) (Figure 3). Similar results were obtained after application of Cy5-labeled QR-313 formulated into hydrogel or PBS onto ex vivo human skin for 24 hrs. Diffusion of the oligo was visible in skin where the epidermis was removed, almost no lateral diffusion was observed at the wound edges (Figure 4).

Discussion
QR-313 is designed to exclude exon 73 from COL7A1 (see poster). In vitro evaluation of QR-313, an anti-sense oligonucleotide designed to skip exon 73 from the COL7A1 mRNA. In order to perform its function, QR-313 should be present in the nucleus. Based on the confocal images of the in vivo delivery in minipig it is reasonable to assume that QR-313 is efficiently delivered to the cell nucleus in vivo.

Conclusion
Here it is demonstrated that Cy5-labeled QR-313 delivery in vitro, ex vivo and in vivo models is successful in wounded skin, but not in intact skin. We conclude that hydrogel is a suitable formulation that enables diffusion of QR-313 into the skin of DEB patients at wound sites.

Literature
2. Bonnet et al., 2016. Functional evaluation of targeted exon deletion reveals prospects for dystrophic epidermolysis bullosa therapy. Molecular Therapy. accepted article preview online 05 May 2016. doi:10.1038/mt.2016.52

Figure 5. Delivery of Cy5-labeled QR-313 formulated into hydrogel on ex vivo human skin after 24 hrs. Cross sections of FFPE skin were counterstained with DAPI and the Cy5 QR-313 signal was visualized by fluorescence microscopy. Scale bar indicates 200µm.

Figure 6. Cy5-labeled QR-313 delivery to wounded skin of minipigs. Cy5-labeled QR-313 was applied 3 times with 48 hrs intervals. 7 days after wound healing tissues were taken and cross sections of FFPE skin were counterstained with Hoechst (blue) and the Cy5 signal (red) was visualized by confocal microscopy.

Figure 7. Delivery of Cy5-labeled QR-313 into FFPE skin after 24 hrs. Cross sections of FFPE skin were counterstained with DAPI and the Cy5 signal was visualized by fluorescence microscopy. Scale bar indicates 200µm.

Figure 8. Delivery of Cy5-labeled QR-313 into FFPE skin after 24 hrs. Cross sections of FFPE skin were counterstained with DAPI and the Cy5 signal was visualized by fluorescence microscopy.

Figure 9. Delivery of Cy5-labeled QR-313 into FFPE skin after 24 hrs. Cross sections of FFPE skin were counterstained with DAPI and the Cy5 signal was visualized by fluorescence microscopy.

Figure 10. Delivery of Cy5-labeled QR-313 into FFPE skin after 24 hrs. Cross sections of FFPE skin were counterstained with DAPI and the Cy5 signal was visualized by fluorescence microscopy.