Suitability of the minipig as non-rodent animal model to test safety of antisense oligonucleotides

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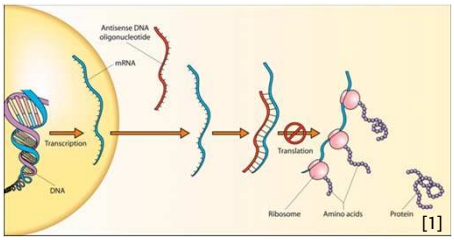
1 – Introduction

Antisense oligonucleotides (AONs) are synthetic nucleotide strands that bind to target mRNA and may catalyze RNase H mediated degradation.



Currently numerous AONs are being evaluated in clinical trials for a variety of therapeutic indications. Traditionally, due to the high sequence homologies, the non-human primate (NHP) has been the non-rodent species used to assess toxicity of AONs and is considered predictive for toxicity in humans.
With the recent sequencing of its genome [2], the minipig may be considered as a potential alternative to NHP in safety assessment of AONs. Very sparse data is available for the use of the minipig in AON safety assessment.

Our aim:
Evaluate the suitability of the minipig as non-rodent animal species for safety assessment of AONs

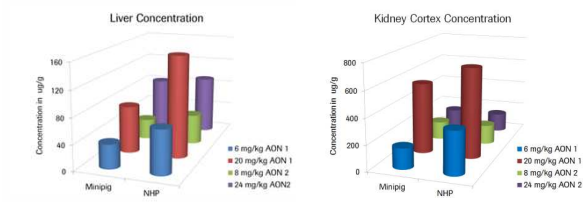
2 – Study design

Two AONs were tested. In each study, the test item was administered subcutaneously on Days 1, 6, 11, and 16 to female Göttingen minipigs allocated to one control group (n= 2 females) and two treated groups of 3 animals each. Animals were sacrificed on Day 17, 24 hours following the last dose. Investigations included standard in-life parameters, exposure assessment, clinical pathology, cytokine measurement, histopathologic examination, immunohistochemistry (IHC), in-situ hybridization (ISH) and electron microscopic examination of the kidney.

The studies were planned and performed using the same test items, treatment regimen and doses as in NHP studies to allow for comparison of the results between species.

3 – Tissue exposure assessment (interspecies comparison)

AONs tend to partition into liver and kidney. Therefore, exposures in these tissues were assessed at the end of the study



Tissue exposure levels in minipigs were within 2-fold of the NHP values

4 – Pharmacology and toxicology (interspecies comparison)

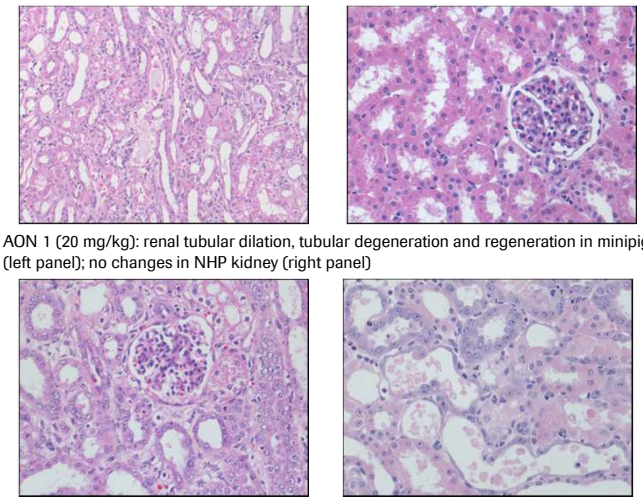
- No effect on cytokines for either AON across species
- For AON 1 similar PD effects across species. For AON 2, PD effect observed in NHP and human but not in minipig likely due to single mismatch in center of oligonucleotide
- Injection site reactions described in human were neither observed in minipig nor in NHP

	AON 1		
Affected organs	**Minipig**	**NHP**	**Human**
Kidney	Creat ↑ (1/3), accumulation in proximal tubular cells; tubular dilation, degeneration/necrosis, regeneration/hypertrophy	Accumulation in proximal tubular cells; non-adverse tubular findings	Creat ↑, acute tubular necrosis in one female at 5 mg/kg (SAE)
Lymph nodes	Vacuolated macrophages	Vacuolated macrophages	Not applicable
Liver	Single cell necrosis in 1/3; PD effects	No changes; PD effects	No changes; PD effects

	AON 2		
Affected organs	**Minipig**	**NHP**	**Human**
Kidney	Creat ↑, accumulation in proximal tubular cells; tubular dilatation, degeneration/necrosis, regeneration/hypertrophy (↑ incidence and severity)	Creat ↑, accumulation in proximal tubular cells, tubular dilation, single cell necrosis and regeneration	Creat ↑ at 1mg/kg
Lymph nodes	Vacuolated macrophages	Vacuolated macrophages	Not applicable
Liver	No changes at terminal sacrifice, no PD effect	Mild ↑ liver enzymes; PD effects; hepatocellular vacuolation, hypertrophy and necrosis (adverse)	Mild ↑ liver enzymes (~1/3 of subjects)

Creat – serum creatinine; - ↑ increased; PD – Pharmacodynamic; SAE – Serious Adverse Event

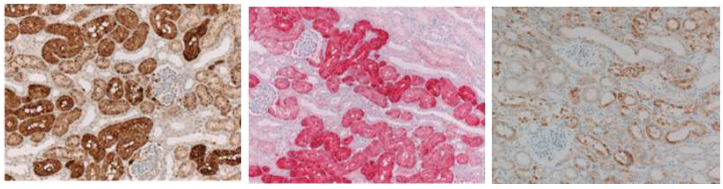
5 – Histopathology



AON 1 (20 mg/kg): renal tubular dilation, tubular degeneration and regeneration in minipig (left panel); no changes in NHP kidney (right panel)

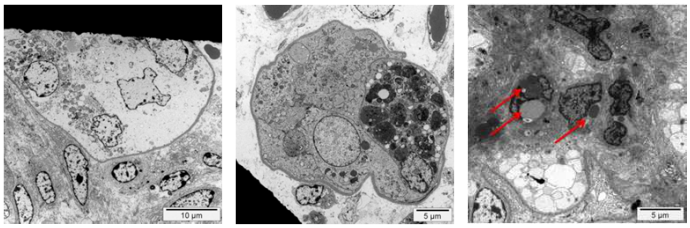
AON 2 (24 mg/kg): renal tubular dilation, tubular degeneration and regeneration in minipig (left panel) and NHP (right panel). Findings in minipig with higher incidence and severity

6 – Immunohistochemistry and electron microscopy in minipig



Immunohistochemistry **In-situ hybridization** **LAMP-2 IHC**

- Confirmation of presence of AON 1 in renal tubular epithelial cells
- Lysosomal involvement in accumulation of AON in proximal renal epithelial cells



Renal tubular damage and necrosis (left panel). Tubule with massive lysosomal storage of AON 2 (central panel). Glomerular basement membrane and podocytes normal, storage in endothelium and / or mesangial cells (right panel, arrows)

7 – Summary and conclusions

- Comparable liver and kidney exposures with same dosing regimen (s.c.) and dose levels for minipigs and NHP for both AONs evaluated
- Observed pharmacodynamic effects where expected based on target homology
- Findings in the kidney of minipig are similar to those described in other animal species including NHPs [3, 4]
- Minipig tends to be more sensitive for human nephrotoxicity than NHP for the two AONs tested
- Vacuolated macrophages, a finding often associated with AON treatment [5] were observed in both minipig and NHP at similar severity
- Injection site reactions described in human were neither observed in minipig nor NHP
- Further studies are ongoing to characterize the minipig as a potential alternative non-rodent species to test the safety of antisense oligonucleotides

References:

1. Vamathevan et al., Tox Appl Phar. 2013
2. Robinson, RNAi Therapeutics: How Likely, How Soon? PLoS Biol, 2004
3. Henry et al., Toxicology, 2012
4. Monteith et al., Toxicol. Pathol., 1999
5. Frazier, Toxicol. Pathology, 2015