



Universiteit  
Leiden  
The Netherlands

## **Anti sense and sensibility : renal and skin effects of (antisense) oligonucleotides**

Meer, L. van

### **Citation**

Meer, L. van. (2017, January 19). *Anti sense and sensibility : renal and skin effects of (antisense) oligonucleotides*. Retrieved from <https://hdl.handle.net/1887/45389>

Version: Not Applicable (or Unknown)

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/45389>

**Note:** To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/45389> holds various files of this Leiden University dissertation.

**Author:** Meer, L. van

**Title:** Anti sense and sensibility : renal and skin effects of (antisense) oligonucleotides

**Issue Date:** 2017-01-19

# VII

## DISCUSSION AND SUMMARY



## Introduction

This thesis describes the clinical testing of the antisense oligonucleotide ISIS 388626. Oligonucleotides (ONs) are fragments of 12-24 nucleic acids in a target-specific sequence [1]. The sequences of antisense ONs are complementary to part of the mRNA of the targeted protein. The ON binds to the mRNA, prohibits mRNA translation and thereby inhibiting the formation of the targeted protein. The concept of antisense ONs is very promising as it allows highly specific inhibition of any target for which the mRNA sequence is established. ISIS 388626 is complementary to a region of the mRNA of the SGLT2 protein. SGLT2 is a transporter that enables glucose reabsorption in the kidney and inhibition of this transporter is a validated strategy in the treatment of type 2 diabetes [2-4].

### PRECLINICAL DATA

In preclinical studies, ranging from 6 weeks to 6 months in duration, weekly subcutaneous (sc) injection of ISIS 388626 showed to be an effective and safe treatment [5,6]. In normoglycemic animals the reduction in SGLT2 mRNA expression of  $\geq 80\%$  that occurred at doses of 1-3 mg/kg weekly for 13 weeks translated into effective glucosuria (at 3 mg/kg a 60-fold increase in mice and 7-fold increase in monkeys in urine glucose creatinine ratio) [5]. In none of the animal models, signs of toxicity of ISIS 388626 were observed. ISIS 388626 demonstrated a specific and selective renal distribution [7] and no indications for long term changes of general kidney function were noted in studies up to 6 months in duration [5].

### TRANSLATION TO THE CLINIC

The effects of treatment with ISIS 388626 were explored clinical studies in the dose range of 50-200mg. The chosen doses were based on a minimal anticipated biological effect level (MABEL) approach. In preclinical studies across multiple species, the pharmacologically active dose range of ISIS 388626 was 1-30 mg/kg/week. At this exposure, a significant reduction in SGLT2 mRNA occurred (74 to 97% in mice and approximately 30 to 90% in monkeys over the dose range 1-30 mg/kg/week), accompanied by a 25-200 fold increase in urinary glucose excretion [5-7]. Based on this, estimation of the equivalent human effective dose falls in the range of

1-3 mg/kg/week. The No Adverse Effect Level (NOAEL) was estimated to be 10 mg/kg/week in monkeys. The safety of the 1-3 dose range was further supported by previous experience with 2'-MOE-modified antisense oligonucleotides [8-10]. Multiple clinical studies showed that these compounds could be safely administered (intravenously and subcutaneously) at dose levels up to weekly 750 mg (which translates into 10.7 mg/kg/week assuming an average weight of 70 kg), with treatment durations exceeding one year [8-11]. Pharmacokinetic data from preclinical studies showed that a loading dose regimen of 3 doses in the first week ensured rapid achievement of steady state tissue concentrations, therefore in the MAD part of the study this regimen was also applied, followed by a maintenance schedule of weekly dosing for 5 weeks. The results from the preclinical experiments with ISIS 388626 are summarized in table 1.

### TRIAL DESIGN

The initial study design consisted of a double-blind, randomized, placebo-controlled study, starting with a single ascending dose (SAD) study, in which sixteen subjects were assigned a single dose in a 3:1 ratio (50, 100, 200 or 400 mg ISIS 388626 or placebo), followed by a multiple ascending dose (MAD) study, in which subjects were planned to be enrolled in a 12:3 ratio (50, 100, 200 or 400 mg ISIS 388626 or placebo). In the MAD study, the dose levels exceeding 50 mg, the intended pharmacodynamic effects of ISIS 388626 (inhibition of renal urinary glucose reabsorption and lowering of plasma glucose concentrations) were to be estimated by evaluation of glucose handling after an oral glucose tolerance test (OGTT), performed before the first administration of ISIS 388626/placebo and at Week 6. Safety profile was to be continually monitored by weekly evaluation of blinded clinical laboratory assessments and adverse events throughout the cohort. Before escalating the dose the safety profile of the preceding cohort had to be evaluated and considered acceptable.

### FIRST CLINICAL STUDY: UNEXPECTED FINDINGS

The first study started with a single ascending dose study, which was completed without safety problems. However, the following 6 week multiple ascending dose part was halted early because increases in serum creatinine occurred in the subjects participating in the 100 mg multiple



dose cohort. Evaluation of the pharmacodynamic effect was limited due to the small exposure in this study, but there was an indication that glucosuria increased upon active treatment. The pronounced changes in serum creatinine were accompanied by increased urinary excretion of beta-2-Microglobulin (B2M) and KIM1. These findings were unexpected as experiments in animals treated with ISIS 388626 with safety assessments at 6 and 13 weeks had not shown such results. As the possible mechanisms for the changes in serum creatinine were unknown, it was felt more pre-clinical data were needed to justify further clinical investigations.

Therefore bio-banked samples collected earlier than 6 weeks of the previously performed pre-clinical experiments were analyzed. This showed that in animals treated with a loading dose similar transient increases in serum creatinine and urinary excretion of B2M and protein had occurred. Thus, the apparent discrepancy of renal effects of ISIS 388626 treatment between rodent and monkeys on the one hand and humans on the other hand could be explained by the suboptimal timing of the initial assessments [5;7;12]. This prompted a further dedicated experiment in monkeys in which it was explored whether the renal effects by ISIS 388626 were related to the loading dose. Animals were dosed for 13 weeks with either 30 mg/kg every other day or a single dose in the first week, followed by weekly dosing for another 12 weeks. This experiment showed that changes in renal markers in monkeys occurred only with the loading dose regimen. Importantly, in this study it was also shown that abandoning the loading dose did not impair the primary pharmacodynamic effect, as glucosuria still occurred, although onset was delayed.

#### **SECOND CLINICAL STUDY: NO IMPAIRED RENAL FUNCTION**

Based on the clinical findings and the new animal data, clinical studies were restarted with the aim to investigate the effects of SC doses of 50, 100 and 200 mg ISIS 388626, administered without a loading dose as 13 weekly injections. However, omitting the loading dose did not prevent increases in renal markers in humans. Treatment with 50 mg ISIS 388626 induced serum creatinine increases and increases in urinary renal markers. These changes prohibited dose escalation to the 100 mg dose and further explorations were done to investigate whether the transient increases in renal markers in humans could be explained by functional changes in renal blood flow and/or glomerular filtration rate. A new cohort of volunteers

was treated at the 50 mg dose level to explore if the observed transient increases in renal damage markers coincided with functional renal changes. This was assessed with renal clearance tests to evaluate the impact of ISIS 388626 on GFR and renal plasma flow. Weekly ISIS 388626 treatment at a dose level of 50 mg for 13 weeks increased average serum creatinine (with 0.15 mg/dl) and renal damage markers. The changes were relatively mild and fully reversible upon cessation of dosing. The renal clearance test revealed no indications for impairment of glomerular filtration or renal perfusion. No increase in renal glucose excretion was observed at the 50 mg dose level, as was expected based on preclinical data. To induce the intended pharmacological activity, a higher level of drug exposure was required. Exploration of higher ISIS 388626 dose levels in healthy volunteers was considered acceptable using a careful approach with close monitoring of renal function and damage markers.

#### **THIRD CLINICAL STUDY: INTENDED PHARMACOLOGICAL ACTIVITY OR TUBULAR DYSFUNCTION?**

In the continuation of the clinical evaluation of ISIS 388626, repeated doses of 100 and 200 mg were applied. ISIS 388626 induced glucosuria in these studies, with a dose dependent increase in average 24 hour urinary glucose excretion (average increase of 508.9 and 1299.8 mg/day, in the 100 and 200 mg groups respectively, comparing baseline values with end of treatment). As the average amount of glucose that is filtered and reabsorbed amounts to approximately 144 grams per day (800 mmol/day [13]) and the average inhibition of glucose reabsorption that we observed amounted to only 0.8% for the 200 mg dose regimen, it appears that the intended effect is very small indeed. The observed level of urinary glucose excretion is significantly higher upon treatment with small molecule compounds that target SGLT2, which have been reported to induce urinary glucose excretion in the range of 60-70 g/day [14-17]. This robust glucosuria results in an effective glucose-lowering therapy and has led to the registration and approval of a number of small molecule SGLT2 inhibitors [2-4]. In patients with type 2 diabetes addition of an SGLT2 inhibitor to standard therapy resulted in lower risk for cardiovascular events and death [18].

Kidney biomarkers further increased at the 100 and 200 mg dose levels. Maximal average changes in serum creatinine of  $0.28 \pm 0.11$  and  $0.38 \pm 0.09$  mg/dl occurred in for 100 and 200 mg respectively (versus  $0.15 \pm 0.06$



mg/dl after 50 mg). Urinary renal markers of tubular damage, such as B2M, protein and KIM1, increased in parallel in a significant and dose-dependent manner. The only other ISIS 388626 related adverse events were mild injection site reaction (ISRS), occurring in 8-19% of the subjects. Interestingly, an effect on renal function is also reported for small molecule SGLT2 inhibitors [19], suggesting a target-related effect. However, this effect consists solely of a small decline in eGFR and is considered to be secondary to the mild reduction in intravascular volume as a result of the natriuresis that accompanies the glucosuria, although it cannot be excluded that this effect is also caused by diminished tubular secretion of creatinine. However these effects in small molecule SGLT2 inhibitors were mild, not accompanied by increases in other renal markers and coincided with a strong pharmacodynamic effect, therefore this observation shows little similarity to the ISIS 388626-induced effect. Nonetheless, it cannot be excluded that natriuresis adds to the creatinine increase observed with ISIS 388626. Similarity to the effects of small molecule SGLT2 inhibitors on glucose homeostasis is further supported by the significant increases in insulin and C-peptide levels (after OGTT) observed in ISIS 388626 treated subjects, suggesting increased endogenous glucose production, as also observed for dapagliflozin and empagliflozin [20-23].

The low-grade glucosuria, that is considered a pharmacological effect, could also be (partly) caused by tubular dysfunction. The adverse renal effects were unexpected. For some oligonucleotide compounds effects on the kidney have been described [24-26] and in one case an oligonucleotide has caused acute tubular necrosis in a healthy volunteer, however, several others have no renal effects [11;27;28]. The available data to date are too limited to draw conclusions on the relationship between renal adverse effects and compound specific factors, such as size or chemistry. We suggest that comprehensive screening for renal effects using tubular damage markers and functional tests is performed whenever oligonucleotides are administered systemically as animal experiments clearly cannot produce certainty about the absence of these effects in humans.

As the glucosuria observed upon ISIS treatment was minimal, and was accompanied by adaptations of or injury to renal cells due to unclarified mechanisms, the therapeutic window of ISIS 388626 is narrow. It is possible that subjects with type 2 diabetes mellitus (with higher SGLT2 expression and greater renal filtered glucose load) could benefit more from ISIS 388626 compared to the healthy volunteers investigated in these studies. However, the mechanisms underlying the transient renal

dysfunction warrant more detailed exploration before ISIS 388626 could be considered for further clinical development.

#### MONITORING RENAL EFFECTS

The clinical testing of ISIS 388626 resulted in unexpected effects on markers for kidney injury. In preclinical studies, including studies in multiple animal species, no signal was initially detected. This illustrates that monitoring renal function in first in human studies is of great importance, particularly because only 40-60% of animal findings are predictive of toxicities in humans [29]. Generally accepted and routine biomarkers such as serum creatinine and BUN are often used. However they lack sensitivity and early injury responses may be missed [29]. In the relatively long term dosing regimen tested for ISIS 388626 (13 weeks), a clear response of serum creatinine was detected and led to adaptation of the study design and also allowed decisions about dose-escalation. Other potentially useful kidney biomarkers that can be used in clinical trials include KIM1, B2M, aGST and NAG. These urinary biomarkers may outperform serum creatinine, as illustrated by a case of acute tubular necrosis after treatment with an antisense oligonucleotide [26]; KIM1 increases obviously precede serum creatinine increases. However, to catch these early changes frequent urine sampling is required as well as rapid analysis of the samples. The biomarkers KIM1, B2M, aGST and NAG were assessed during the clinical investigations with ISIS 388626 and KIM1 and B2M correlated closely with serum creatinine responses. The markers were not included to create opportunity for early intervention, but were analyzed in a batch at the end of the cohort and were included to gain maximal insight in the nature of the observed effect on serum creatinine.

A serious limitation of novel renal biomarkers is the lack of their validation for clinical use. Due to limited or contradictive information on their clinical performance, choosing the most appropriate biomarker is challenging. Also, when data is obtained, interpretation is complicated by uncertainty on normal ranges and normal variability. It would be helpful if more data on clinical performance and large validation studies on novel biomarkers would be publicly available, to be able to confirm superiority over the more classical choices of serum creatinine and BUN. Also measurement of the markers should become more readily available and lower in cost. The ideal renal biomarker, does not seem to exist or is not yet identified. Nonetheless, some have promising qualities. An



example is KIM1, which suits different purposes and thus often seems to be a right choice. And the field of renal biomarkers is evolving and even newer biomarkers such as TIMP2 and micrornas appear to outperform the biomarkers currently used [30;31]. A marker specific for glomerular injury is yet to be identified, as this is currently lacking. As glomerular involvement often leads to irreversible damage early identification of this type of kidney injury would be of added value.

#### **INJECTION SITE REACTIONS AFTER OLIGONUCLEOTIDE THERAPY**

As mentioned, the subcutaneous (sc) injection of ISIS 388626 induced Injection site reactions (ISRs) in a subset of subjects. At this moment, no oligonucleotides (ONS) are available that are completely devoid of ISRs. Although all ONS that are administered SC result in ISRs, incidence and severity of the observed reactions vary between different ONS. From the relevant literature as well as experience within CHDR, it can be concluded that dose level plays an important role. Higher doses probably result in higher local exposure and more intense reactions. Low-grade ISRs usually consists of erythema and discomfort, but may also progress into more severe manifestations such as induration, ulceration and necrosis. ISRs appear to be common, but are poorly described in literature. The pathophysiology of ISRs remains unclear at this point. Immunological activation is likely to involve recognition of the compounds by innate immune receptors such as TLRs and possibly complement activation. Overcoming the problem of ISRs might add greatly to the potential success of SC administered ONS. Knowledge on these skin reactions in specific and immunostimulatory properties of ONS in general should be increased. Thorough investigation of biopsies from skin lesions after ON therapy with dedicated immunohistochemical staining may shed light on the exact immunological mechanisms involved. Also, to increase knowledge on ISRs, the study design of clinical trials with SC ONS, should include systematic reporting of skin reactions, including photography, scoring of the lesions and proper follow-up.

#### **FUTURE PERSPECTIVES**

The favorable preclinical profile of ISIS 388626 combined with the upcoming target of SGLT2 inhibition to treat diabetes, rendered ISIS 388626 a

promising drug candidate. Small molecule SGLT2 inhibitors are promising agents that are already implemented in the treatment of diabetes. The latter are known to have a limitation of inhibition of less than 50% of the filtered glucose load. This limitation is hypothesized to result from a compensatory increased SGLT1 activity, when SGLT2 is completely blocked. It remains unknown if the maximal pharmacodynamic effect of antisense inhibition of SGLT2 would exceed the maximal effect of SGLT2 inhibition using small molecule compounds. However, the unexpected and incompletely understood effects on the kidney of ISIS 388626, makes further augmentation of the dose unacceptable from a safety perspective. Therefore clinical development of the compound was stopped before a robust pharmacodynamic effect was achieved.

In general antisense oligonucleotides are an attractive class of compounds. The compounds are 'custom-made', highly selective to the specific target, creating inhibition on a whole different level by interfering with the synthesis of the target protein. However, the promise of many drug candidates tested clinically has not rendered many successes. Currently the only approved oligonucleotide is mipomersen used to treat homozygous familial hypercholesterolemia. There are several important challenges to overcome in order to move towards broader implementation of oligonucleotide treatment in clinical practice. Some of these challenges are illustrated in this thesis. Accumulation of the oligonucleotides seems to play an important role in both the renal and cutaneous effects of oligonucleotides. Alternative delivery systems are being investigated. Avoiding ISRs for example could be achieved by oral administration of ONS a concept that is currently being investigated [32]. Renal accumulation is known to be dependent on chemistry [5;33;34], therefore this should be taken into account in future studies with ONS. Strategies to avoid accumulation of ONS also include modification of the distribution properties using oligonucleotide conjugates, such as peptides, proteins, carbohydrates, aptamers and small molecules [35]. The thin line between accumulation of oligonucleotide needed for the effect and the accumulation that induces toxicity should be further explored. Preclinical and early clinical studies should focus on increasing knowledge on how to avoid unintended effects. This has the potential to lead to ONS with a better profile, more suitable for chronic use and applicable for broader patient populations.



REFERENCES

- 1 Dias N, Stein CA: Antisense oligonucleotides: basic concepts and mechanisms. *Mol Cancer Ther* 2002;1:347-355.
- 2 Plosker GL: Dapagliflozin: a review of its use in type 2 diabetes mellitus. *Drugs* 2012;72:2289-2312.
- 3 Sha S, Devineni D, Ghosh A, Polidori D, Hompesch M, Arnolds S, Morrow L, Spitzer H, Demarest K, Rothenberg P: Pharmacodynamic effects of canagliflozin, a sodium glucose co-transporter 2 inhibitor, from a randomized study in patients with type 2 diabetes. *PLoS One* 2014;9:e110069.
- 4 Jahagirdar V, Barnett AH: Empagliflozin for the treatment of type 2 diabetes. *Expert Opin Pharmacother* 2014;15:2429-2441.
- 5 Zanardi TA, Han SC, Jeong EJ, Rime S, Yu RZ, Chakravarty K, Henry SP: Pharmacodynamics and Subchronic Toxicity in Mice and Monkeys of ISIS 388626, a Second Generation Antisense Oligonucleotide that Targets the Human Sodium Glucose Cotransporter 2 (SGLT2). *J Pharmacol Exp Ther* 2012.
- 6 Bhanot Sea: ISIS 388626, an SGLT2 antisense drug, causes robust and sustained glucosuria in multiple species and is safe and well-tolerated.; 2009.
- 7 Wancewicz E: Long term safety and efficacy of ISIS 388626, an optimized SGLT2 antisense inhibitor, in multiple diabetic and euglycemic species.; 2008.
- 8 Akdim F, Stroes ES, Sijbrands EJ, Tribble DL, Trip MD, Jukema JW, Flaim JD, Su J, Yu R, Baker BF, Wedel MK, Kastelein JJ: Efficacy and safety of mipomersen, an antisense inhibitor of apolipoprotein B, in hypercholesterolemic subjects receiving stable statin therapy. *J Am Coll Cardiol* 2010;55:1611-1618.
- 9 Visser ME, Kastelein JJ, Stroes ES: Apolipoprotein B synthesis inhibition: results from clinical trials. *Curr Opin Lipidol* 2010;21:319-323.
- 10 McHutchison JG, Patel K, Pockros P, Nyberg L, Pianko S, Yu RZ, Dorr FA, Kwoh TJ: A phase I trial of an antisense inhibitor of hepatitis C virus (ISIS 14803), administered to chronic hepatitis C patients. *J Hepatol* 2006;44:88-96.
- 11 Kwoh TJ, Crooke ST: *Antisense Drug Technology: Principles, Strategies, and Applications*; An overview of clinical safety experience of first- and second-generation antisense oligonucleotides. Boca Raton, Taylor & Francis, 2007, pp 365-399.
- 12 Bhanot Sea: ISIS 388626, an SGLT2 antisense drug, causes robust and sustained glucosuria in multiple species and is safe and well-tolerated.; 2009.
- 13 Berne R, Levy M, Koeppen B, Stanton B: *Physiology*; 1998, p 699.
- 14 Kasichayanula S, Liu X, Lacreata F, Griffen SC, Boulton DW: Clinical Pharmacokinetics and Pharmacodynamics of Dapagliflozin, a Selective Inhibitor of Sodium-Glucose Co-transporter Type 2. *Clin Pharmacokinet* 2013.
- 15 Sha S, Devineni D, Ghosh A, Polidori D, Chien S, Wexler D, Shalayda K, Demarest K, Rothenberg P: Canagliflozin, a novel inhibitor of sodium glucose co-transporter 2, dose dependently reduces calculated renal threshold for glucose excretion and increases urinary glucose excretion in healthy subjects. *Diabetes Obes Metab* 2011;13:669-672.
- 16 Grempler R, Thomas L, Eckhardt M, Himmelsbach F, Sauer A, Sharp DE, Bakker RA, Mark M, Klein T, Eickelmann P: Empagliflozin, a novel selective sodium glucose cotransporter-2 (SGLT-2) inhibitor: characterisation and comparison with other SGLT-2 inhibitors. *Diabetes Obes Metab* 2012;14:83-90.
- 17 Komoroski B, Vachharajani N, Boulton D, Kornhauser D, Gerald M, Li L, Pfister M: Dapagliflozin, a novel SGLT2 inhibitor, induces dose-dependent glucosuria in healthy subjects. *Clin Pharmacol Ther* 2009;85:520-526.
- 18 Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE: Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *N Engl J Med* 2015;373:2117-2128.
- 19 Ptaszynska A, Johnson KM, Parikh SJ, de Bruin TW, Apanovitch AM, List JF: Safety profile of dapagliflozin for type 2 diabetes: pooled analysis of clinical studies for overall safety and rare events. *Drug Saf* 2014;37:815-829.
- 20 Merovci A, Solis-Herrera C, Daniele G, Eldor R, Fiorentino TV, Tripathy D, Xiong J, Perez Z, Norton L, Abdul-Ghani MA, DeFronzo RA: Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. *J Clin Invest* 2014;124:509-514.
- 21 Scheen AJ, Paquot N: Metabolic effects of SGLT-2 inhibitors beyond increased glucosuria: A review of the clinical evidence. *Diabetes Metab* 2014;40:S4-S11.
- 22 Ferrannini E, Muscelli E, Frascerra S, Baldi S, Mari A, Heise T, Broedl UC, Woerle HJ: Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients. *J Clin Invest* 2014;124:499-508.
- 23 Cefalu WT: Paradoxical insights into whole body metabolic adaptations following SGLT2 inhibition. *J Clin Invest* 2014;124:485-487.
- 24 Goemans NM, Tulinius M, van den Akker JT, Burm BE, Ekhart PF, Heuvelmans N, Holling T, Janson AA, Platenburg GJ, Sipkens JA, Sitsen JM, Aartsma-Rus A, van Ommen GJ, Buysse G, Darin N, Verschuuren JJ, Campion GV, de Kimpe SJ, van Deutekom JC: Systemic administration of PRO051 in Duchenne's muscular dystrophy. *N Engl J Med* 2011;364:1513-1522.
- 25 Herrington WG, Talbot DC, Lahn MM, Brandt JT, Callies S, Nagle R, Winearls CG, Roberts IS: Association of long-term administration of the survivin mRNA-targeted antisense oligonucleotide LY2181308 with reversible kidney injury in a patient with metastatic melanoma. *Am J Kidney Dis* 2011;57:300-303.
- 26 van Poelgeest EP, Swart RM, Betjes MG, Moerland M, Weening JJ, Tessier Y, Hodges MR, Levin AA, Burggraaf J: Acute Kidney Injury During Therapy With an Antisense Oligonucleotide Directed Against PCSK9. *Am J Kidney Dis* 2013.
- 27 Santos RD, Duell PB, East C, Guyton JR, Moriarty PM, Chin W, Mittleman RS: Long-term efficacy and safety of mipomersen in patients with familial hypercholesterolaemia: 2-year interim results of an open-label extension. *Eur Heart J* 2013.
- 28 van Dongen MG, Geerts BF, Morgan ES, Brandt TA, de Kam ML, Romijn JA, Cohen AF, Bhanot S, Burggraaf J: First proof of pharmacology in humans of a novel glucagon receptor antisense drug. *J Clin Pharmacol* 2014.
- 29 van Meer, L, Moerland M, Cohen AF, Burggraaf J: Urinary kidney biomarkers for early detection of nephrotoxicity in clinical drug development. *Br J Clin Pharmacol* 2014;77:947-957.
- 30 Gocze I, Koch M, Renner P, Zeman F, Graf BM, Dahlke MH, Nerlich M, Schlitt HJ, Kellum JA, Bein T: Urinary biomarkers TIMP-2 and IGFBP7 early predict acute kidney injury after major surgery. *PLoS One* 2015;10:e0120863.
- 31 Pavkovic M, Riefke B, Ellinger-Ziegelbauer H: Urinary microRNA profiling for identification of biomarkers after cisplatin-induced kidney injury. *Toxicology* 2014;324:147-157.
- 32 van PM, Young C, van den Berg S, Pronk A, Hulsker M, Karanoukh TG, Vermue R, van Dijk KW, de KS, Aartsma-Rus A: Preclinical studies on intestinal administration of antisense oligonucleotides as a model for oral delivery for treatment of duchenne muscular dystrophy. *Mol Ther Nucleic Acids* 2014;3:e211.
- 33 Monteith DK, Horner MJ, Gillett NA, Butler M, Geary R, Burckin T, Ushiro-Watanabe T, Levin AA: Evaluation of the renal effects of an antisense phosphorothioate oligodeoxynucleotide in monkeys. *Toxicol Pathol* 1999;27:307-317.
- 34 Henry SP, Johnson M, Zanardi TA, Fey R, Auyeung D, Lappin PB, Levin AA: Renal uptake and tolerability of a 2'-O-methoxyethyl modified antisense oligonucleotide (ISIS 113715) in monkey. *Toxicology* 2012;301:13-20.
- 35 Winkler J: Oligonucleotide conjugates for therapeutic applications. *Ther Deliv* 2013;4:791-809.





**Table 1 Summary of the results of preclinical experiments with ISIS 388626**

| Study NR | Description  | Model                      | Route                          | Regimen   | dose (mg/kg)                | PD effect MNRA reduction(%)           | PD effect Urine glucose increase           | Other PD   | Cmax (ug/mL)                | Hypo-glycemia | ISR | Specific Toxicity   |
|----------|--|----------------------------|--------------------------------|---|-----------------------------|---------------------------------------|--|--|-----------------------------|---------------|-----|---|
| APK01    | Pharmacokinetics, Distribution, Metabolism Excretion and Mass Balance of Radioactivity Study in Rats | Rats                       | SC                             | Single Dose   | 3 and 10                    |                                       |  |  | 3                           | NO            | NO  |   |
|          | 4-Week Study in Mice   | CD-1 Mice                  | SC                             | Weekly  | 30                          |                                       |  |  |                             | NO            | NO  |   |
| AS02     | 13-Week Toxicity Study of Mice   | CD-1 Mice                  | SC                             | week 1: 3 wk, week 2-13: 1 wk   | 0<br>1<br>3<br>10<br>30     | no effect<br>75%<br>88%<br>87%<br>94% | no effect<br>14x<br>30x<br>63x<br>125x     |  | 1.52<br>7.14                | NO            | NO  | 10 and 30 mg/kg: kidney and spleen weight increased<br>30 mg/kg: 1.3-1.6 fold increase of ALT and AST   |
|          | 12-Week study in Rats  | Sprague-Dawley Rats        | SC                             |   | 0.8<br>3.2                  | 80%                                   | >50x                                       |  |                             | NO            | NO  |   |
|          | 16-Week Study in Mouse-Model of Diabetes   | db/db Mice                 | SC                             | Weekly  | 2                           | 88%                                   |  | 36% blood glucose reduction<br>43% HbA1c reduction |                             | NO            | NO  |   |
|          | 26-Week Study in Rat-Model of Diabetes   | Zucker Diabetic Fatty Rats | SC                             |   | 1.6                         | 85%                                   |  | 50% blood glucose reduction<br>40% HbA1c reduction |                             | NO            | NO  |   |
| AS03     | 13-Week Toxicity Study in Monkeys  | Cynomolgus-Monkey          | SC                             | week 1: 3 wk, week 2-13: 1 wk   | 0<br>1<br>3<br>10<br>30     | no effect<br>42%<br>65%<br>70%<br>75% | no effect<br>no effect<br>6x<br>16x<br>90x |  | 0.6<br>5.66<br>20.6<br>44.6 | NO            | NO  | 10 and 30 mg/kg: at day 14 creat increase of 15% and 40%, respectively.<br>No pathology findings.   |
|          | 6-Week Pharmacodynamic-Evaluation Monkeys  | Cynomolgus-Monkey          | SC                             | week 1: 3/wk, week 2-6: 1 wk  | 2<br>24                     | 66%<br>85%                            | no effect<br>>100x                         |  |                             | NO            | NO  |   |
| AS06     | 8-Week Repeated Dose Pharmacokinetic and Pharmacodynamic Evaluation in Dogs                          | Beagle Dogs                | SC<br>oral<br>oral<br>SC<br>SC | Daily<br>Daily<br>Daily<br>week 1: 3/wk, week 2-8: 1/wk<br>week 1: 3/wk, week 2-8: 1/wk | 0.5<br>25<br>100<br>0<br>30 |                                       |  |  | 0.42<br><br><br>27.6        | NO            | NO  |   |
|          | 6.5-Week Pharmacodynamic Evaluation in Dogs  | Beagle Dogs                | SC                             | Weekly  | 1<br>10                     | 85%<br>95%                            | 7.5x<br>>250x                              |  |                             | NO            | NO  |   |
| AS09     | 8-Week Study to Assess Renal Function in Monkeys   | Cynomolgus-Monkey          | SC                             | wk 1: 3/wk, wk 2-13: 1/wk<br>Weekly   | 0<br>10<br>30               |                                       |  |  |                             |               |     | 30 mg/kg with loading dose: 1.44% increase creat, and B2M increase 15 fold<br>2. Pathology findings: tubular nephropathy at week 2 in 3/4 animals<br>30 mg/kg without loading dose: no significant findings |