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

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IV

RENAL EFFECTS OF SGLT2 INHIBITION WITH A NOVEL ANTISENSE OLIGONUCLEOTIDE

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Abstract

The aim of this study was to study the effects of the SGLT2 inhibitor ISIS 388626. ISIS 388626 is an antisense SGLT2 inhibitor, designed to treat type 2 diabetes mellitus. ISIS 388626 was demonstrated to be safe and effective in preclinical trials, reducing renal SGLT2 mRNA expression in rodent and monkeys, translating into effective glucosuria. A randomized, placebo-controlled, dose-escalation Phase 1 study was designed to evaluate the effects of ISIS 388626 in healthy volunteers. Twenty-nine subjects were enrolled sequentially into 1 of 2 dose cohorts at a 3:1 (active/placebo) ratio. Subjects received 13 weekly doses of 100 or 200 mg over 12 weeks. The primary pharmacodynamic endpoint was change in urinary glucose excretion. In addition, biomarkers of kidney toxicity were assessed throughout the dosing period to explore the safety profile. ISIS 388626 increased 24 hour urinary glucose excretion dose-dependently with 508.9 ± 781.45 mg/day in the 100 mg and 1299.8 ± 1833.4 mg/day in the 200 mg cohort, versus 88.7 ± 259.29 mg/day in the placebo group. ISIS 388626 also induced a reversible increase in serum creatinine, with the largest effect after 8 doses of 200 mg ISIS 388626 (0.38 ± 0.089 mg/dL; 44% increase over baseline). Three subjects were discontinued due to creatinine increases. The creatinine increases were accompanied by a rise in the levels of urinary renal damage markers (B2M, total protein, KIM1, AGST, NAG). Other treatment related AEs included mild ISRs, occurring in 8-19% of the subjects. In conclusion, ISIS 388626 treatment induced glucosuria at a dose level of 200 mg/week. This intended pharmacological effect was small, amounting approximately 1% of total amount of filtered glucose. Changes in serum and urinary markers were indicative of transient renal dysfunction, most likely of tubular origin. Whether the glycosuria is caused by specific SGLT2 inhibition or general tubular dysfunction or a combination remains uncertain.

Introduction

The antisense oligonucleotide ISIS 388626 inhibits the synthesis of the renal SGLT2 receptor, which accounts for 90% of the reabsorption of glomerular filtrated glucose [1;2]. SGLT2 inhibition affects renal glucose reabsorption,

resulting in glucosuria and lowered serum glucose levels. The strategy of SGLT2 inhibition for the treatment of type 2 diabetes mellitus is efficacious and has led to the registration and approval of a number of small molecule SGLT2 inhibitors [3-5]. ISIS 388626 treatment was effective and safe in animals, in studies ranging from 6 weeks to 6 months in duration [1;2]. Reductions of 80% and more in renal SGLT2 mRNA expression were observed at doses of 1-3 mg/kg/week (rodents) to 30 mg/kg/week (monkeys), resulting in effective glucosuria [1;2]. The initial animal models did not show signs of ISIS 388626-induced toxicity [2]. In spite of the favorable preclinical data, the first clinical ISIS 388626 studies halted early due to unexpected increases in serum creatinine (*manuscript First-in-man studies with novel SGLT2 inhibitor: antisense compound is associated with unexpected renal effects, L. van Meer et al.*). Discovery of these effects of the oligonucleotide led to analysis of banked serum samples collected at an earlier timepoint in the monkey studies, which revealed transient rises in serum creatinine and proteinuria. These early effects were not observed in the initial (more infrequent) sample analysis. A subsequent preclinical study that focused on the renal effects of ISIS 388626 demonstrated that no rises in serum creatinine and proteinuria occurred at a dose level of 30 mg/kg/week ISIS 388626 when abandoning the earlier applied loading dose regimen (unpublished data, on file). Based on these results, clinical studies were re-initiated, exploring the effects of weekly subcutaneous doses of 50, 100 and 200 mg ISIS 388626 for a period of 12 weeks (13 doses), avoiding the loading dose. Nonetheless, weekly ISIS 388626 treatment at a dose level of 50 mg induced increases in serum creatinine and renal damage markers. Further dose escalation was halted and instead a dedicated clinical experiment was performed to explore whether ISIS 388626 (50 mg, weekly during 12 weeks) affected renal blood flow and/or glomerular filtration. This appeared not to be the case (*manuscript Novel antisense SGLT2 inhibitor causes serum creatinine increases without affecting renal blood flow or glomerular filtration, L. van Meer et al.*). It was judged to be safe and rational to perform additional clinical experiments to study higher doses of ISIS 388626 that may exert the intended pharmacodynamic effects. In this study, healthy volunteers received treatment with 100 and 200 mg ISIS 388626 weekly for a period of 12 weeks. The study design included oral glucose tolerance tests (OGTT) to estimate the intended pharmacodynamic effect (the induction of glucosuria) and close monitoring of renal function and injury, applying strict predefined stopping criteria. This paper reports on the findings from



these clinical experiments. For reference, the results of the earlier cohort of healthy volunteers exposed to 50 mg ISIS 388626 (chapter 3 of this thesis, *L. van Meer et al.*) have been included.

Materials and Methods

SUBJECTS

Adult subjects (18–65 yrs), male or female (post-menopausal or surgically sterile) with a BMI < 30 kg/m² and a fasting plasma glucose and HbA_{1c} below the upper limit of normal could participate in this study. Significant abnormalities in medical history, physical examination, 12-lead electrocardiogram, and clinical laboratory evaluations (including positive protein in urine dipstick analysis and calculated eGFR below 60 ml/min by MDRD equation [6] led to exclusion. The study was conducted in accordance with good clinical practice guidelines, after approval by the national ethics committee.

STUDY DESIGN

This was a double-blind, randomized, placebo-controlled multiple ascending dose study of 12 weeks duration and 5 weeks follow-up, with weekly administration of ISIS 388626 to establish the safety profile and pharmacodynamics of the compound, performed at the Centre for Human Drug Research in the Netherlands. Per cohort, 16 randomly assigned subjects received multiple doses of either ISIS 388626 or placebo (in a 3:1 ratio), administered as subcutaneous injection. An oral glucose tolerance test (OGTT) was performed before the first administration of ISIS 388626 (or matching placebo) and at after the 9th and 13th dose. The OGTT consisted of ingestion of a 75 mg glucose solution, given after an overnight fast. Subsequently blood was drawn regularly during 4 hours for determination of glucose, insulin and c-peptide concentrations.

SAMPLE SIZE

The selection of a total of 12 subjects per treatment group was based on previous data obtained after conducting an OGTT in normal subjects. It is estimated that the standard deviation of change in plasma glucose

AUC_{0-120min} during the OGTT is approximately 85 mmol*min/L. With 6 subjects in the pooled placebo group and 6 subjects in the ISIS 388626 treated group, this would result in at least 80% power to detect a 170 mmol*min/L difference in plasma glucose AUC_{0-120min} at an alpha level of 0.05. To ensure sufficient power of the efficacy analysis also in case of more than expected variation, additional subjects were included.

DOSE RATIONALE

It was anticipated to explore 50, 100 and 200 mg of ISIS 388626. The doses were based on a MABEL approach, taking into account a No Adverse Effect Level estimated to be 10 mg/kg/week (including a loading dose regimen) in monkeys. In preclinical studies across multiple species, the pharmacologically active dose range of ISIS 388626 was 1–3 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 [1;7;8]. Based on this, estimation of the equivalent human effective dose falls in the range of 1–3 mg/kg/week. Experience with other 2'-MOE-modified antisense oligonucleotides, safely administered (intravenously and subcutaneously) in multiple clinical studies at doses 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 [9], further supports the safety of this dose range.

The dose regimen was chosen because the loading dose (3 doses in the first week) resulted in creatinine increases in prior human studies (*manuscript First-in-man studies with novel SGLT2 inhibitor: antisense compound is associated with unexpected renal effects, L. van Meer et al.*) and dedicated experiments in monkeys showed that changes in renal markers occurred only with the loading dose regimen. The treatment duration of 12 weeks (13 doses) was selected, which was expected to be safe and resulting in sufficient steady state tissue concentrations, based on animal studies.

CLINICAL MEASUREMENTS

Safety assessments, performed throughout the study period, included vital signs, electrocardiograms, physical examinations, and clinical



laboratory tests (including clinical chemistry, hematology, coagulation, cytokines, complement tests and urinalysis) as well as registration of adverse events. Adverse events were defined as any new medical occurrence or worsening of a pre-existing condition after administration of the study drug or placebo. Predefined stopping rules regarding renal parameters were defined as changes in serum creatinine change from baseline of more than 0.3 mg/dL or more than 40% on two consecutive weeks, or proteinuria of more than 0.5 g/24hr occurring on two consecutive weeks.

RENAL DAMAGE MARKERS

The biomarkers KIM1, Cystatin C, EGF, NGAL/LCN2, Osteopontin, Uromodulin, agST and NAG were chosen based on their performance on detecting injury to the proximal tubule where SGLT2 is located [10]. Analysis of renal damage markers agST and NAG was performed batch-wise upon study completion by quantitative enzyme immunoassays (Argutus Medical NEPHKITO immunoassay for agST, and Diazyme 70010 Rev. F, colorimetric end point assay for NAG) and KIM1, Cystatin C, EGF, NGAL/LCN2, Osteopontin, Uromodulin by enzyme-linked immunosorbent assay (R&D Systems ELISA).

PHARMACOKINETICS

ISIS 388626 plasma levels were measured in using a validated hybridization enzyme-linked immunosorbent assay (PDD laboratories, Richmond, USA) frequently for a 24 hour profile after the first and 13th ISIS 388626 dose, and predose on weeks 3, 8, 10 during treatment and on 5 weekly follow-up visits. In addition, ISIS 388626 urine levels were measured using a validated Capillary Gel Electrophoresis method (PDD laboratories, Richmond, USA), in 24 hour collections after the first and 13th dose (up to 24 and 48 hours post-dose).

DATA ANALYSIS AND STATISTICAL METHODS

Safety and tolerability evaluation was based on descriptive statistics. ISIS 388626 plasma concentrations were subjected to non-compartmental pharmacokinetic evaluation in order to determine the maximum observed plasma concentration (C_{max}), the time to maximum plasma

concentration (T_{max}), the area under the plasma concentration-time curve from dosing to 24 hours after dosing (AUC_{0-24h}) using WinNonLin (version 5.3, Pharsight Corporation, USA).

Pharmacodynamic evaluation was based on descriptive statistics as well as statistical analysis using ANCOVA with baseline as a covariate. Endpoints were urinary glucose excretion (UGE) and fractional glucose excretion (defined as (UGE / filtered glucose load (GFR*fasted plasma glucose) *100), and plasma glucose, insulin and c-peptide concentrations.

Results

SUBJECTS

Twenty one subjects participated in the 100mg/placebo cohort, of which fifteen subjects completed the study. Another sixteen subjects participated in the 200mg/placebo cohort, of which fourteen subjects completed the study. Subject demographics are presented in table 1.

PHARMACODYNAMICS

ISIS 388626 dose-dependently increased 24 hour urinary glucose excretion (figure 1A, to 575.6 ± 789.5 and 1413.1 ± 1804.6 mg/day glucose excretion at end of treatment in the 100 and 200 mg cohort, versus 172.3 ± 387.0 mg/day in the placebo group). During the five week follow-up period, values returned to baseline, although not completely for the 200mg cohort. Average values of fractional glucose excretion, calculated from 24-hour urine glucose excretion and serum glucose, showed a very similar pattern (data not shown).

The OGTT resulted in an expected strong increase in serum glucose, insulin and c-peptide levels, followed by a rapid decline towards normal values (data not shown). ISIS 388626 treatment did not affect the OGTT-induced increase in serum glucose. ISIS 388626 treatment resulted in a dose-dependent enhancement of insulin and c-peptide release (Figure 1B and C). Urinary glucose excretion during the first four hours of the OGTT was increased in ISIS 388626 treated groups compared to placebo (figure 1D, 347.3 ± 411.4 and 745.6 ± 1122.4 mg/4hr for the 100 and 200 mg cohort, versus 133.9 ± 327.5 mg/4hr in the placebo groups). Increases were statistically different from placebo.



PHARMACOKINETICS

ISIS 388626 rapidly entered the circulation upon SC injection, with maximum plasma concentrations (C_{max}) occurring within the first 2 hours after dosing and rapidly declining concentrations thereafter (figure 2A, B, C and table 2). C_{max} and area under the plasma concentration time curve (AUC_{0-24hr} ; total exposure) showed no accumulation of ISIS 388626 upon repeated administration at dose levels 100 mg. Some accumulation might be present at 200 mg as C_{max} and AUC increased slightly from the 1st to the 13th dose (figure 2C, table 2).

The total amount of ISIS 388626 in urine (0-24 hours) ranged from 3.7 to 18.4 mg over the dose range tested and increased in a dose-proportional manner (figure 2D). Urinary excretion was approximately 2-fold higher after the 13th dose compared to the first dose.

SAFETY

Adverse events (AEs) occurred in all subjects who received multiple doses of ISIS 388626, and in 85% of subjects who received placebo (table 3). All AEs reported were classified as of mild intensity and transient. The most common AE was nasopharyngitis. Other AEs commonly reported in all groups were headache, fatigue, a range of gastrointestinal complaints (such as diarrhea, nausea or abdominal discomfort) and musculoskeletal complaints (such as myalgia and back pain). These AEs occurred in the active treatment groups and placebo groups with a similar incidence, thus considered unlikely to be ISIS 388626-related. Injection site reactions (ISRs) occurred at frequencies of 19% in the 100mg cohort and 8% in the 200mg cohort. ISRs consisted of mild erythema at the site of the SC injection without itch. In three subjects hyperpigmentation was reported after the initial erythema had resolved. In two subjects re-appearance of erythema occurred after initial resolution. The ISRs were not progressive, not accompanied by local lymphadenopathy, and no study discontinuations occurred due to ISRs. All ISRs resolved completely and spontaneously, ranging from within 12 hours to 50 days.

ISIS 388626 treatment did not result in any clinically relevant changes in vital signs (blood pressure, ECG-derived parameters, body temperature) or parameters of hematology and coagulation. No increases in circulating cytokines (IFN α , IL6, MCP-1 and MIP1 α) were observed at any

dose level. Analysis of complement factor C5a and Bb revealed no changes, except for an increase in factor Bb in the 200mg dose group, maximally at 24 hours after the 13th dose (from 0.74 ± 0.336 mg/mL at baseline to 0.93 ± 0.394 mg/mL at 24hrs after dose of week 13).

Treatment with 100 and 200mg ISIS 388626 did not result in clinically significant changes in chemistry parameters, such as hepatic parameters and glucose levels. However, ISIS 388626 dose-dependent changes in serum creatinine occurred (figure 3A). Baseline serum creatinine levels were comparable between all treatment groups. The largest average increase of 0.38 ± 0.089 mg/dL (44% increase) was reached after 8 doses of ISIS 388626 (200 mg). The effect of ISIS 388626 on serum creatinine was highly variable between subjects ranging individually from no increase at all to a 0.49 mg/dL increase over baseline levels. Despite continued dosing, creatinine levels started to decline after ten doses, returning to baseline during the follow up period. However, at the 200 mg dose average creatinine levels remained above baseline levels at week 17. Three subjects met the protocol-specified stopping rule for renal function tests and were discontinued. The elevation in serum creatinine did not coincide with obvious changes in urea levels. At the highest dose level tested ISIS 388626 induced a short transient increase in aldosterone levels, although the inter- and intra-subject variability was significant (figure 3B).

ISIS 388626 treatment did not result in significant changes in urine flow and urinalysis parameters (data not shown), but did increase the levels of urinary renal damage markers (B2M, total protein, KIM1, agST, NAG). ISIS 388626 treatment dose-dependently increased urinary B2M (figure 3C). In both the 100 and 200 mg group, B2M levels increased gradually with repeated ISIS 388626 administration, to decline after treatment stop during the five week follow-up period. Urinary protein also showed a dose-dependent increase, although the inter- and intra-subject variability was large (figure 3D). ISIS 388626 treatment, at all dose levels tested, resulted in an increase in urinary KIM1, assessed after the 8th administration (figure 3E). Also urinary levels of agST and NAG increased upon ISIS 388626 treatment (figure 3F,G).



Discussion

The antisense oligonucleotide ISIS 388626, targeting the renal SGLT2 receptor, did not induce pharmacological effects (i.e. induction of glucosuria) when applying dose levels up to 50 mg, with weekly dosing for 12 weeks. This was not unexpected since in preclinical studies across multiple species, the pharmacologically active doses were doses exceeding 1 mg/kg/week, which translates into 70 mg/kg/week assuming an average weight of 70 kg [1;7;8]. Repeated ISIS 388626 administration with 50 mg did induce transient and mild rises in serum creatinine. To explore whether the observed transient increases in renal markers coincided with functional changes, a new cohort of volunteers exposed to the same ISIS 388626 dose level and regimen, and PAH and sinistrine clearance was assessed to estimate RPF and GFR. This study revealed no indications that the changes can be explained by ISIS 388626-induced changes in GFR and RPF (chapter 3 of this thesis, *L. van Meer et al.*). This enabled dose escalation to elicit possible pharmacological effects.

ISIS 388626 dose-dependently increased urinary glucose excretion. After 13 weekly doses urinary glucose increased, which ceased after therapy cessation. On average, the maximal level of urinary glucose excretion was 1.4 gram per day, observed in the 200 mg ISIS 388626 treatment group after 13 weekly doses, compared to 0.2 gram in the placebo group (7-fold increase). The observations suggest an ISIS 388626-induced increase in glucosuria, but the effect is rather small, since the average amount of glucose that is filtered daily is approximately 144 grams (800 mmol/day [11]). Furthermore, compared to treatment with small molecule SGLT2 inhibitors the observed level of urinary glucose excretion is minimal, as these compounds induce urinary glucose excretion in the range of 50-80 grams per day [12]. In spite of the relatively small effect observed, the findings do suggest that ISIS 388626 doses of 200 mg and beyond exert intended pharmacodynamic activity. Animal data demonstrated that a certain threshold level of SGLT2 mRNA exists maintaining tubular glucose reabsorption and limiting glucosuria, and that once this threshold is exceeded, glucose reabsorption strongly declines resulting in sudden increases in glucosuria: an ISIS 388626-mediated SGLT2 mRNA reduction of nearly 70% did not induce a significant increase in glucosuria, whereas an SGLT2 mRNA reduction of 85% resulted in a 100-fold increase in glucosuria (data on file; monkey treated with 2 mg/kg/week versus 24 mg/kg/

week ISIS 388626). This is supported by the observation that a phenotype of hereditary SGLT2 mutations with 50-60% loss of SGLT2 does not display significant glucosuria [13]. Compensation by an increase in SGLT1 mediated transport is likely to play a role in the maintenance of renal glucose reabsorption, as was demonstrated in animals after genetic and pharmacological SGLT2 inhibition [14].

In line with the mild glucosuria observed in 24 hour urine collections, urinary glucose excretion after the OGTT also increased in the ISIS 388626 treated groups. Furthermore, ISIS 388626-treatment resulted in elevated levels of circulating insulin and c-peptide during the first hours after the glucose load. These increases seem counter-intuitive, as increased urinary glucose loss is expected to result in lower insulin levels needed to compensate for the glucose load. Probably changes in other mechanisms involved in glucose homeostasis occur simultaneously. Treatment with SGLT2 inhibitors dapagliflozin and empagliflozin resulted in similar paradoxical findings of altered glucose homeostasis and increased c-peptide/insulin levels, probably compensating an increased endogenous glucose production [15-18].

Although the observed glucosuria after ISIS 388626 treatment may result from antisense mediated inhibition of SGLT2 in humans, it may also relate to ISIS 388626-induced tubular dysfunction. At the highest dose level, ISIS 388626 treatment resulted in increases in serum creatinine levels of 40-50% over baseline, with concomitant induction of other markers indicative of renal damage or dysfunction. The correlation between induction of renal damage markers and glucosuria was explored (table 4), which demonstrated a correlation between the renal markers serum creatinine, urinary B2M and urinary protein (with coefficients of 0.46, 0.47 and 0.66, table 4), but no correlation with urinary glucose excretion (with coefficients of 0.086, 0.43 and 0.23 table 4). The absence of a correlation between renal side effects and intended pharmacodynamic effect suggests that the observed mild glucosuria probably results from antisense mediated inhibition of SGLT2, and not from tubular dysfunction. Moreover, it makes it unlikely that a general membrane dysfunction occurs as a result of knock-down of the SGLT2 receptor. This is further supported by the absence of signs of membrane dysfunction with other oligonucleotides directed to transmembrane receptors [19;20]. ISIS 388626 is, however, the first antisense oligonucleotide targeted to a renal receptor, and it remains uncertain if knock down interferes with



membrane function. On the other hand, subjects with homozygous SGLT2 mutations are largely asymptomatic and have no signs of renal tubular dysfunction, hypovolemia or electrolyte imbalance [21].

We hypothesize that the changes in serum creatinine and urinary renal markers induced by ISIS 388626 treatment most likely reflect transient tubular dysfunction. Although urinary B2M and protein may also increase in case of glomerular injury due to increased filtration [22], the reversible nature of the changes in our study suggest interference with tubular reabsorption. This is supported by the observation that elevations in KIM1, AGST and NAG occurred, all markers known to increase in response to different tubulo-toxic agents in animal models [22-26]. Accumulation of antisense oligonucleotides occurs in proximal tubular cells as basophilic granules [27;28] and this was indeed also seen in animal studies with ISIS 388626 [1]. In primates, tubular accumulation of oligonucleotides is usually not associated with renal toxicity and tubular functional changes, unless extremely high doses are used [1;27;28]. In accordance, these compounds are not associated with adverse renal effects in humans during subsequent clinical investigations [19;29]. However, it is of note that the 12-mer chemistry of ISIS 388626 enables more selective distribution to the kidney compared to other 18- to 20-mer second generation oligonucleotides [8], resulting from lower plasma protein binding, higher free fraction and increased renal filtration. It is uncertain if this increased selectivity contributes to the observed adverse renal effects, but it should be taken in account that this is a theoretical possibility. No comparable other 12-mer oligonucleotides have been investigated in humans to date. Despite the observed increases in renal markers, no functional loss occurred at weekly doses of 50 mg demonstrated in the dedicated cohort with renal clearance tests (chapter 3 of this thesis, *L. van Meer et al.*) supporting the hypothesis that the observed changes reflect adaptation and possibly regeneration of tubular cells. Also no trend was detected in regression analysis exploring the relation between values of GFR/RPF and renal damage markers (data not shown). Examples of other drugs affecting tubular creatinine secretion without decreasing glomerular filtration rate include cimetidine and pyrimethamine [30]. Finally, the observation that the effect of ISIS 366828 on serum creatinine diminishes after ten doses, despite continued dosing, supports the likelihood that antisense treatment is associated with a process of tubular adaptation.

Treatment with small molecule SGLT2 inhibitors also increases serum creatinine [31;32]. However, these effects were smaller and considered to be secondary to volume depletion due to osmotic diuresis. These mild effects on serum creatinine coincided with a much larger pharmacological effect, therefore this does not provide sufficient explanation for our findings. Renal side effects are commonly observed for clinically tested oligonucleotide compounds, such as PRO051 (developed for Duchenne Muscular Dystrophy, and associated with proteinuria [33]), LY2181308, (developed for treatment of melanoma and caused a case of reversible kidney damage [34]) and SPC5001 (developed for familial hypercholesterolemia, associated with increased renal markers and a case of acute tubular necrosis [35]). Although these observations suggest an unintended class effect of oligonucleotides, several other antisense compounds with chemical characteristics comparable to ISIS 388626 were free of renal side effects in humans, such as mipomersen [36], ISIS 325568 [19], ISIS 2302 and ISIS 104838 [9]. Recently, it has been shown in monkeys that chronic administration of a compound similar in class, drisapersen, results in C3 glomerulopathy most probably due to an immune-based mechanism [37]. This appears to be consistent with the observation that antisense oligonucleotides may activate the alternative pathway of the complement system via transient inhibition of factor H [38].

Conclusion

Taken together, our data suggest that ISIS 388626 exerts its intended pharmacological effect in humans: treatment for a period of 12 weeks at a dose level of weekly 200 mg resulted in a small but significant elevation in urinary glucose. Concomitantly, changes in serum and urinary markers were indicative of transient renal dysfunction, most likely of tubular origin. Theoretically, the ISIS 388626-induced tubular dysfunction may have contributed to the glucosuria, but as the increases in renal markers do not correlate with the glucosuria, the latter is more likely an effect of oligonucleotide-induced SGLT2 inhibition. The pharmacodynamic effect of ISIS 388626 in type 2 diabetes mellitus patients, with elevated SGLT2 expression and a larger renal glucose load, may outweigh the effect observed in healthy volunteers. However, the efficacy is probably insufficient with the dose levels currently tested, considering the glucosuria



observed with existing small molecule SGLT2 inhibitors. Moreover, the mechanisms underlying the transient renal dysfunction warrant more detailed exploration. In the first place, because the aimed patient population, being subjects with diabetes type 2 are known to be particularly prone to renal injury and chronic kidney disease may develop as a result. And secondly, as we know renal effects are also observed for other oligonucleotides, this might be prohibitive of using this promising drug class for chronic conditions that are not directly life threatening.

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Figure 1 Dose-dependent pharmacodynamic effects of 1S1S 3886262. Increased urinary glucose excretion measured in 24-hour urine collections (A). Increased OGTT insulin (B) and c-peptide. The change after the 13th dose in weighted AUC 0-4hour (C) Response, calculated from the change in weighted AUC 0-4hour. Increased glucose excretion during the 4 hour-OGTT, calculated as absolute change from baseline (D). All values expressed as average with SD error bars. P values (tested to placebo). Calculated as absolute change from baseline after the 13th dose. * = < 0.05, *** = < 0.001

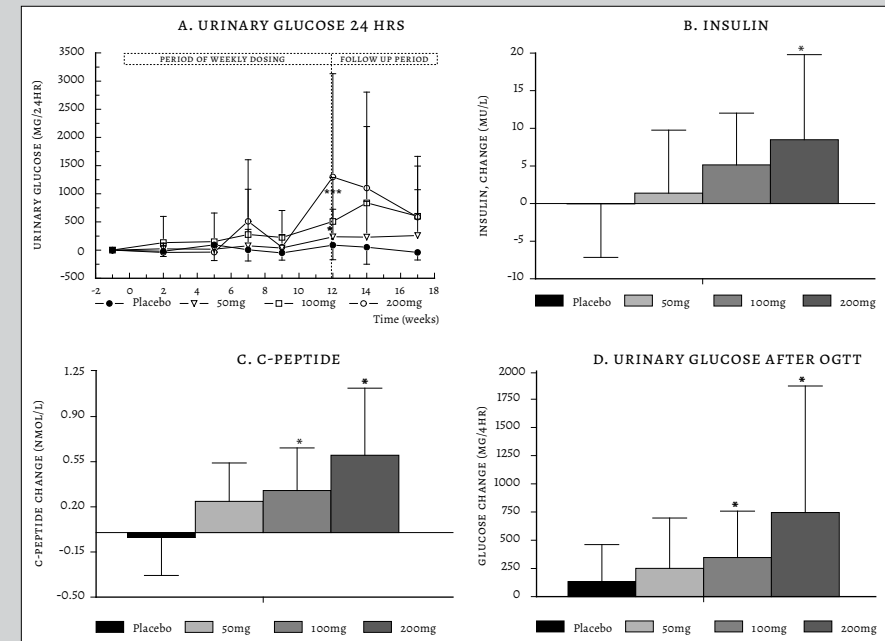


Figure 2 Pharmacokinetic properties of ISIS 388626. The grey line represents the values measured after the 1st dose, the black line after the 13th dose. Average values with SD error bars. Rapid absorption upon injection (C_{max} within 2 hours) and rapidly declining concentrations thereafter at all dose levels (A, B and C). No accumulation at dose levels of 50mg and 100mg, possible accumulation for 200mg, with an increased AUC after 13th dose. 24hr excretion of ISIS 388626 in urine increased dose-proportionally, with an increased excretion after the 13th dose compared to the 1st dose.

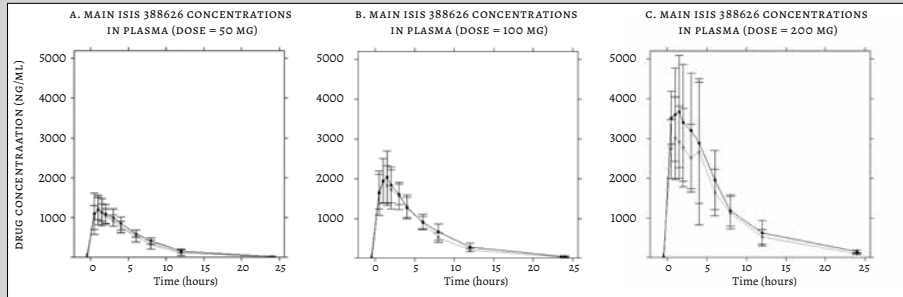


Figure 3 Dose-dependent effects of ISIS 388626 on renal markers and recovery during the follow up period. Average values with SD error bars. Logarithmic Y-axis used for B2M. Serum creatinine increases at all dose levels over time, expressed as change from baseline (A). Increases absolute serum aldosterone for dose levels of 100 and 200mg (B). Increases in urinary B2M (C) and urinary protein (D) over time at all dose levels. Increases in urinary KIM1, expressed as change after the 8th dose compared to baseline (E). Increases in urinary agST (F) and urinary NAG (G) over time. Increases visible at a dose level of 200mg.

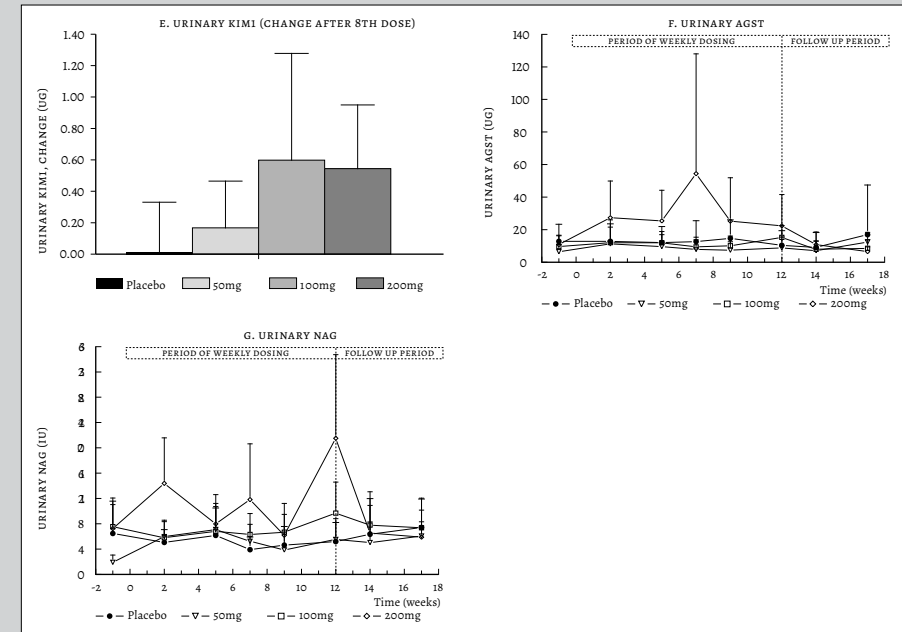
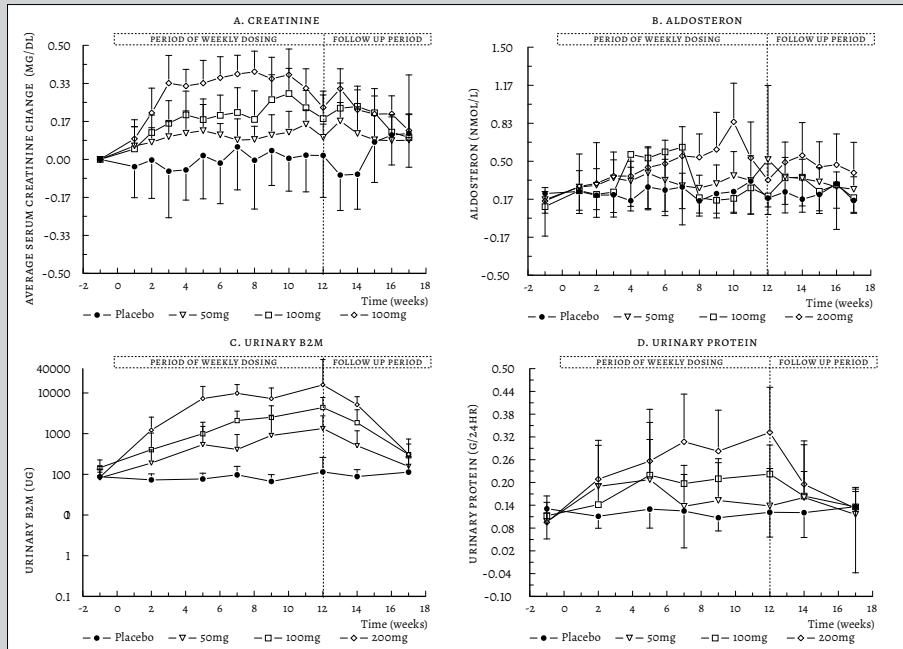


Table 1 Summary of subject demographics

	N	Age (yrs, Std)	BMI (Kg/m ² , Std)	Number female	Early termination of subjects
50 mg ISIS 388626	12	33.9 ± 14.23	23.5 ± 3.13	0	2*
100 mg ISIS 388626	16	37.5 ± 16.18	23.7 ± 3.49	0	5**
200 mg ISIS 388626	12	27.2 ± 12.27	23.7 ± 2.82	1	2***
Pooled Placebo	13	36.1 ± 16.18	23.8 ± 2.87	1	1****

*in one subject dosing was stopped after 5 doses due to increases in serum creatinine and one subject was stopped after 7 doses due to increased liver biochemistry parameters. ** Stopped due to personal reasons (after 1, 2, 4, 5 and 12 doses). Therefore subjects were replaced. *** Stopped (after 7 and 10 doses) due to increases in renal parameters. **** Stopped after 1 dose due to personal reasons. Therefore subject was replaced.

Table 2 Plasma pharmacokinetics after first and 13th dose

	50 mg		100 mg		200 mg	
After dose no	1	13	1	13	1	13
N	12	10	16	12	12	9
AUC 0-24hr (ug*hr/mL)	7627 ± 1202	8626 ± 1037	12650 ± 2085	13970 ± 2041	24250 ± 8796	28320 ± 9875
Cmax (ug/mL)	1240 ± 274.4	1275 ± 470.6	2048 ± 487.5	2185 ± 558.2	3333 ± 1602	4155 ± 1266
Tmax (hr)	1.17 ± 0.324	1.40 ± 0.566	1.38 ± 0.97	1.50 ± 0.88	1.63 ± 1.11	1.56 ± 1.37

Table 3 Frequency overview of adverse events reported in more than one subject (%)

	50mg (n=12)	100mg (n=16)	200mg (n=12)	Placebo (n=13)
Nasopharyngitis	42% (n=5)	38% (n=6)	67% (n=8)	46% (n=6)
Headache	33% (n=4)	56% (n=9)	33% (n=4)	38% (n=5)
Mild gastrointestinal complaints	58% (n=7)	31% (n=5)	42% (n=5)	38% (n=5)
Mild musculoskeletal complaints	17% (n=2)	25% (n=4)	33% (n=4)	38% (n=5)
Fatigue	33% (n=4)	6% (n=1)	8% (n=1)	23% (n=3)
ISRS	42% (n=5)	19% (n=3)	8% (n=1)	0% (n=0)

Table 4 Correlation coefficients of the exploratory analysis of correlations between serum creatinine (s.creat), urinary B2M (u.B2M), urinary protein (u.prot) and urinary glucose (u.gluc), calculated with change from baseline values (CFB)

	CFB s.creat	CFB u.B2M	CFB u.prot
CFB s.creat	X	0.46**	0.47**
CFB u.B2M	0.46**	X	0.66**
CFB u.prot	0.47**	0.66**	X
CFB u.gluc	0.086	0.43	0.23

** indicates a positive correlation that is considered significant.

