

Acute Kidney Injury During Therapy With an Antisense Oligonucleotide Directed Against PCSK9

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Antisense oligonucleotides have been explored widely in clinical trials and generally are considered to be nontoxic for the kidney, even at high concentrations. We report a case of toxic acute tubular injury in a healthy 56-year-old female volunteer after a pharmacologically active dose of a locked nucleic acid antisense oligonucleotide was administered. The patient received 3 weekly subcutaneous doses of experimental drug SPC5001, an antisense oligonucleotide directed against PCSK9 (proprotein convertase subtilisin/kexin type 9) that is under investigation as an agent to reduce low-density lipoprotein cholesterol levels. Five days after the last dose, the patient's serum creatinine level increased from 0.81 mg/dL at baseline (corresponding to an estimated glomerular filtration rate [eGFR] of 78 mL/min/1.73 m²) to 2.67 mg/dL (eGFR, 20 mL/min/1.73 m²), and this increase coincided with the presence of white blood cells, granular casts, and minimal hematuria on urine microscopy. The patient's serum creatinine level peaked at 3.81 mg/dL (eGFR, 13 mL/min/1.73 m²) 1 week after the last oligonucleotide dose. Kidney biopsy showed multifocal tubular necrosis and signs of oligonucleotide accumulation. Upon conservative treatment, the patient's serum creatinine level gradually decreased and reached her baseline level 44 days after the last oligonucleotide was administered. The patient recovered fully and kidney function was normal at every follow-up visit.

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INDEX WORDS: Clinical kidney injury; oligonucleotide; PCSK9 (proprotein convertase subtilisin/kexin type 9).

Therapies based on oligonucleotides, such as antisense or RNA interference strategies, have been investigated widely and are considered to be a viable treatment option for a variety of clinical conditions.¹⁻³ Antisense oligonucleotide (ASO)-based therapies are the most advanced and are considered safe.¹ Although clinical experience with oligonucleotides is limited, in general, they have been found to prolong activated partial thromboplastin time, elicit injection-site skin reactions, and accumulate in kidney proximal tubular cells.

SPC5001 is an ASO directed against the messenger RNA of PCSK9 (proprotein convertase subtilisin/kexin type 9). It is a short synthetic DNA oligonucleotide flanked at both ends by conformationally stabilized ("locked") RNA nucleotides, a modification designed to improve sensitivity and specificity of binding. PCSK9, a protease expressed primarily in the

liver, intestine, and kidneys,⁴⁻⁷ is involved in various cellular functions.^{6,8,9} Its role in degradation of the low-density lipoprotein receptor renders it a potential target for treating hypercholesterolemia.^{10,11} We report a case of acute tubular injury in a healthy woman participating in a first-in-human trial of SPC5001. Other participants treated with an equal dose of SPC5001 also showed signs of transient tubular dysfunction, which suggests a causal relationship. We aim to increase awareness of the toxic effects of ASOs on the kidney.

CASE REPORT

A 56-year-old white woman participated in a first-in-human trial of SPC5001. The patient was healthy according to a complete medical screening and did not smoke or use medication. Blood pressure was 126/70 mm Hg, an electrocardiogram was normal, and routine laboratory test results were all within reference ranges.

The participant received 3 subcutaneous injections of SPC5001 (5 mg/kg on days 1, 8, and 15). Apart from developing injection-site reactions (4×4-cm erythematous spots), there were no remarkable findings up to 24 hours after the last dose (day 16). At a scheduled assessment on day 18, the patient was feeling well, but mentioned that she had been nauseous on days 16-17 and had limited her intake of food and fluids. Urine production had been normal and physical examination findings were unremarkable. Serum creatinine level had increased from 0.81 mg/dL at baseline (estimated glomerular filtration rate [eGFR], 78 mL/min/1.73 m² calculated using the 4-variable Modification of Diet in Renal Disease [MDRD] Study equation¹²) to 1.33 mg/dL (eGFR, 44 mL/min/1.73 m²). At day 20, the patient was still well clinically, but serum creatinine level had increased to 2.76 mg/dL (eGFR, 19 mL/min/1.73 m²) and urine microscopy showed white blood cells,

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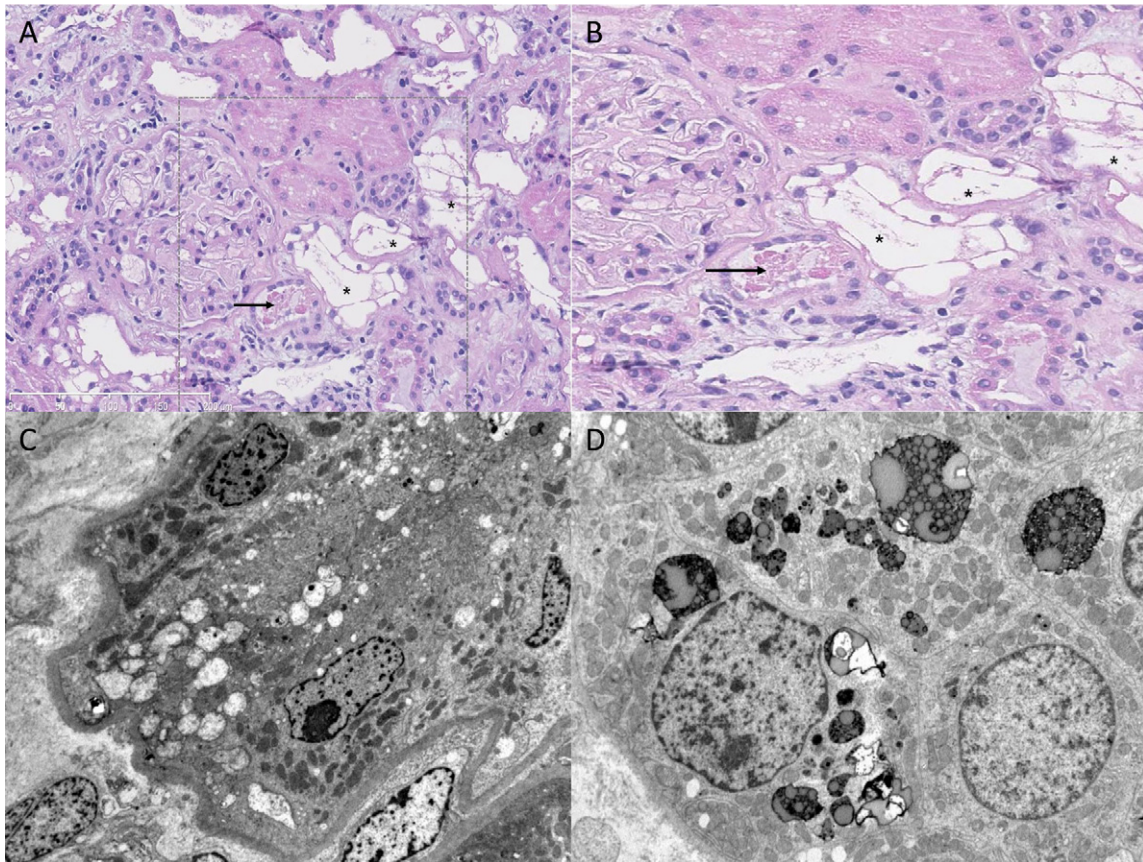


Figure 1. Light micrograph of the renal cortex at original magnification (A) $\times 40$ and (B) $\times 80$ and (C, D) electron microscopy detail of proximal tubule cell. (A-B) Approximately 1 cm of kidney cortex with 21 glomeruli was viewed by light microscopy; $\sim 20\%$ of the tubules showed severe tubular necrosis. (A) One glomerulus in the renal cortex is displayed showing several foci of severe tubular necrosis (*) with total denudation, vacuolization with loss of cytoplasm, nuclear apoptosis, and eosinophilic epithelial degeneration with shedding into tubular lumina, which contained debris with granular material (arrow). The tubular basal membrane was mostly intact. Glomeruli and vascular architecture showed no abnormalities, and interstitial tissue showed some edema and patchy lymphocytic infiltrates. (B) The tubular necrosis in greater detail. Electron microscopy of tubular cells shows (C) increased endosomes and (D) clear nuclear condensation with increased lysosomes between swollen mitochondria.

granular casts, and minimal hematuria. The patient was admitted and intravenous isotonic saline solution treatment was started. On day 21, the patient's serum creatinine level had increased to 3.59 mg/dL (eGFR, 14 mL/min/1.73 m²), and a kidney biopsy was performed.

Histopathologic examination showed several foci of severe tubular injury with total denudation, nuclear apoptosis, and eosinophilic epithelial degeneration with shedding into tubular lumen, which also contained debris with granular material (Fig 1). The tubular basal membrane was mostly intact. Intact tubules showed high epithelium levels and a brush border. Glomeruli and vessels were normal; the interstitium showed focal edema and lymphocytic infiltrates. Results of immunofluorescent staining ruled out deposition of immunoglobulin or complement. Electron microscopy showed cell necrosis, vacuolization, and collapse of the cytoskeleton, a picture commonly seen in toxic tubular damage. Electron microscopy also showed an abundance of phagolysosomes and endosomes in the affected tubular epithelial cells, which reflected antisense accumulation.^{13,14}

Without therapeutic intervention, serum creatinine level decreased from its peak at 3.81 mg/dL (eGFR, 13 mL/min/1.73 m²) at day 22, and the patient was discharged at day 24 with 2 weekly return visits scheduled. The patient's serum creatinine level continued to decrease, reaching her baseline level of 0.88 mg/dL (eGFR,

71 mL/min/1.73 m²) on day 59. Results of urine dipstick and microscopy were normal during this follow-up period, except for a single observation of trace (0–0.3 g/L) protein on dipstick analysis on day 44. At her final visit 8 months after the event, the patient was well and routine laboratory parameters were within reference ranges.

A post hoc analysis of biobanked spot urine samples, which had been collected before each dose of study medication was administered, was performed to assess the kidney injury markers β_2 -microglobulin, α -glutathione *S*-transferase (α -GST), kidney injury molecule 1 (KIM-1), and *N*-acetyl- β -D-glucosaminidase (NAG). NAG levels were unchanged, but urinary β_2 -microglobulin levels increased 4-fold, α -GST levels increased 24-fold, and KIM-1 levels increased 60-fold upon administration of SPC5001 (Fig 2). Importantly, these markers preceded the increase in serum creatinine level, having increased already after the first administration of SPC5001. These observations suggest that SPC5001 adversely affects proximal tubular function.^{15,16}

DISCUSSION

We report a case of acute tubular injury in a healthy woman exposed to the locked nucleic acid (LNA) oligonucleotide SPC5001. Kidney toxicity became

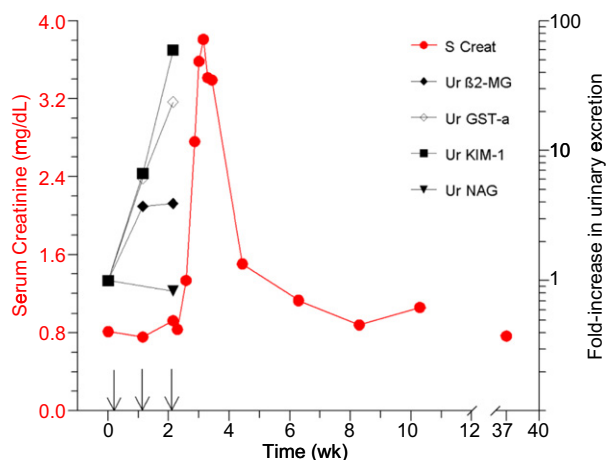


Figure 2. Time course of serum creatinine (S Creat) and urinary kidney damage marker levels. Arrows denote administration of SPC5001 on study days 1, 8, and 15. Conversion factor for S Creat in mg/dL to $\mu\text{mol/L}$, $\times 88.4$. Abbreviations: Ur β_2 -MG, urinary β_2 -microglobulin; Ur GST-a, urinary α -glutathione S-transferase; Ur KIM-1, urinary kidney injury molecule 1; Ur NAG, urinary *N*-acetyl- β -D-glucosaminidase.

apparent within a week after the third and final dose, which suggests a causal relationship. The toxicity first was noted by increases in serum creatinine levels, but in hindsight, it was preceded by increases in α -GST, KIM-1, and β_2 -microglobulin levels after the first dose, which implies that proximal tubular dysfunction was already present before the clinical manifestation or the increase in routine laboratory measurements. Causality is supported by observations in other healthy volunteers who received the same SPC5001 dosage: transient but less pronounced ($\sim 15\%$) increases in levels of serum creatinine, urinary damage markers, and casts were noted in 3 of 5 participants in the week after the final dose.

Antisense compounds generally behave similarly with regard to kidney accumulation. Among the different chemistries of ASOs, 2'-O-methoxyethyl-modified phosphorothioate moieties and 2'-4'-O-methylene LNAs are the most researched and have been shown in animals to accumulate without causing functional impairment, even during dehydration and at dosages higher than those required for pharmacologic activity.¹⁷ Similar to other oligonucleotides,¹⁸ SPC5001 uptake is most prominent in the proximal tubule, but no adverse biochemical or histologic kidney effects were noted in a study of nonhuman primates after a loading dose of 20 mg/kg followed by 4 weekly doses of 5 mg/kg.¹⁹ Dosages of this magnitude with other oligonucleotides have been reported not to induce cell toxicity and loss of kidney function.^{20,21} However, it has been reported that how ASOs affect tubular cells¹⁷ may depend on concentration and that repeated exposure to high ASO doses

may result in kidney toxicity.^{20,22-24} This could reflect lysosomal stress and downstream apoptosis due to oligonucleotide accumulation in proximal tubule cells that are highly metabolically active to maintain integrity and function.²⁵

Acute tubular necrosis associated with long-term methoxyethyl-ASO treatment (74 weekly doses of 10 mg/kg) has been described in a patient with cancer,²⁶ but our case demonstrates that even 3 weekly doses of an LNA-ASO also may have caused acute kidney injury in healthy participants. In both the methoxyethyl-ASO and LNA-ASO clinical cases, the target of the oligonucleotide was expressed in the kidney. Differences include the general condition of the patients (healthy vs metastatic melanoma), cumulative dose (1,050 vs 55,500 mg), time course of recovery (1.5 vs 4 months), and time of the biopsy (1 week vs 4 months after the final dose).

The precise mechanism by which SPC5001 could cause kidney injury may be target related because PCSK9 is expressed in the kidney^{27,28} and upregulation of renal PCSK9 messenger RNA occurs during inflammation,²⁹ probably as a mechanism that mitigates kidney injury, as has been observed in a mouse model of drug-induced kidney toxicity.³⁰ However, the idea that PCSK9 inhibition directly affects the kidneys seems at odds with the data that show that an interfering RNA reduced PCSK9 expression by 60% without an effect on kidney function³¹ and that loss-of-function mutations seem to be unassociated with reduced kidney function.³² The oligonucleotide exposure in our patient is in line with the exposure levels of previously studied ASOs,^{33,34} and her urinary excretion was comparable to that of the other volunteers, which makes it unlikely that the kidney toxicity was due to excessively high local SPC5001 exposure.

Thus, all observations indicate that renal oligonucleotide accumulation may have untoward effects in susceptible people. It is conceivable that susceptibility to developing toxic kidney disease varies among individuals. It may have been that our patient was predisposed to develop overt tubulotoxicity, which is consistent with previously described genetic differences affecting intracellular transport proteins and drug efflux transporters.³⁵

Although the exact cause of the observed kidney toxicity remains uncertain and there may be differences between ASOs based on their chemistry, on- or off-target pharmacology, and possibly other factors,¹ we advocate monitoring kidney function carefully in the process of clinically developing and using ASOs. This should include assessing not only serum creatinine or serum urea nitrogen levels, but also performing urine microscopy³⁶ and measuring specific kidney damage markers. With this approach, earlier detection

and treatment cessation are possible without relying solely on traditional kidney function markers such as serum creatinine, serum urea nitrogen, and electrolytes, particularly in individuals with uncompromised kidney function in whom early signs of toxic kidney disease might be masked.³⁷ These markers also may provide important mechanistic insight into the nature of potential kidney damage.

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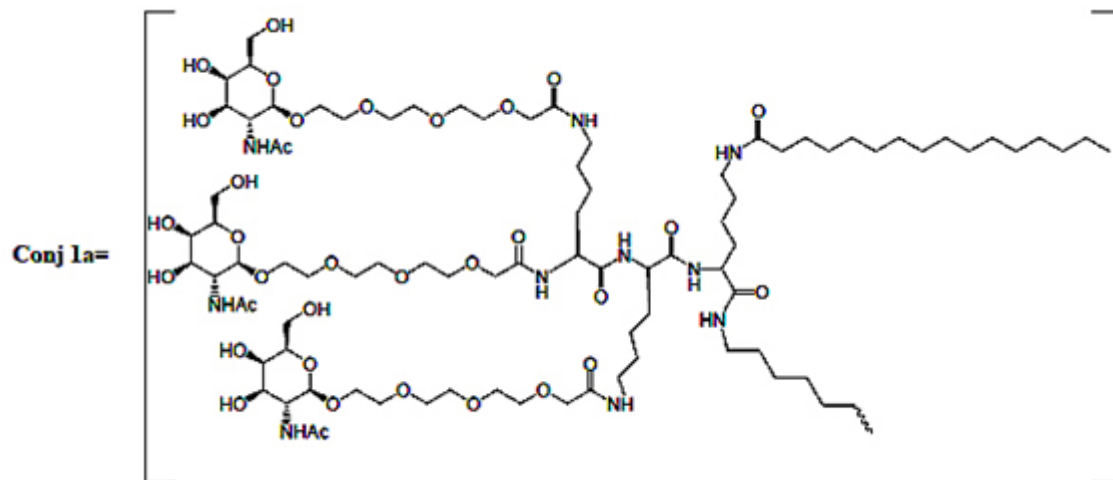
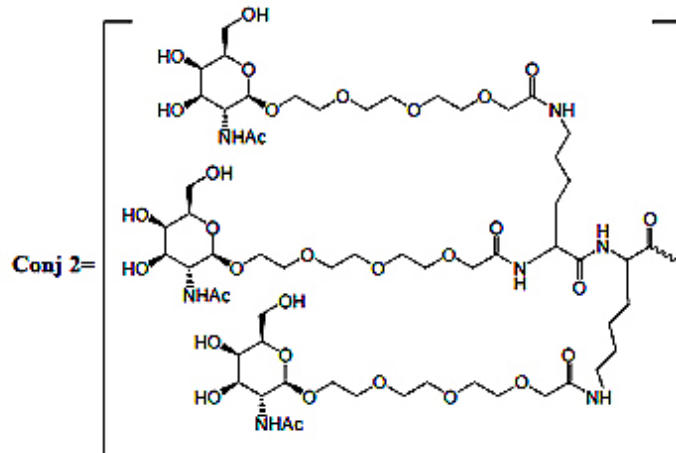
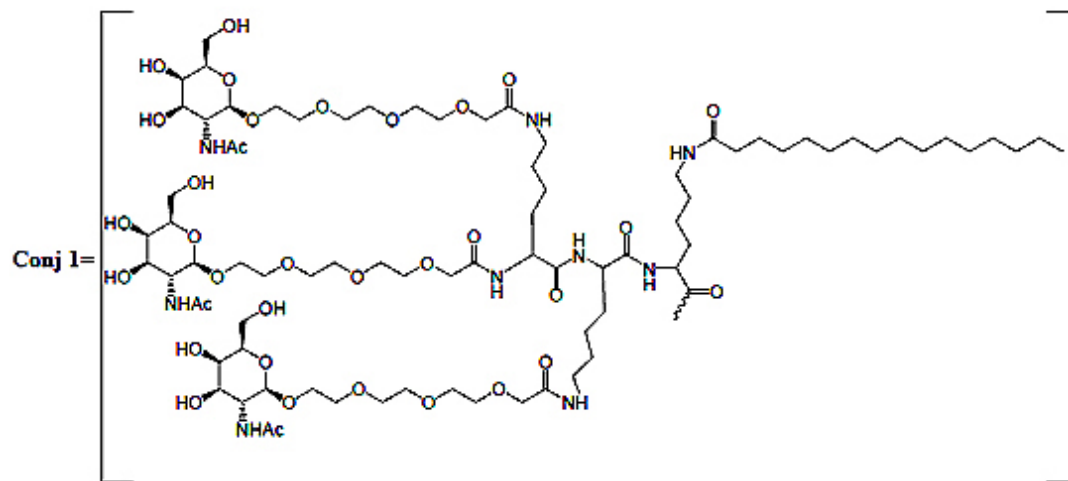
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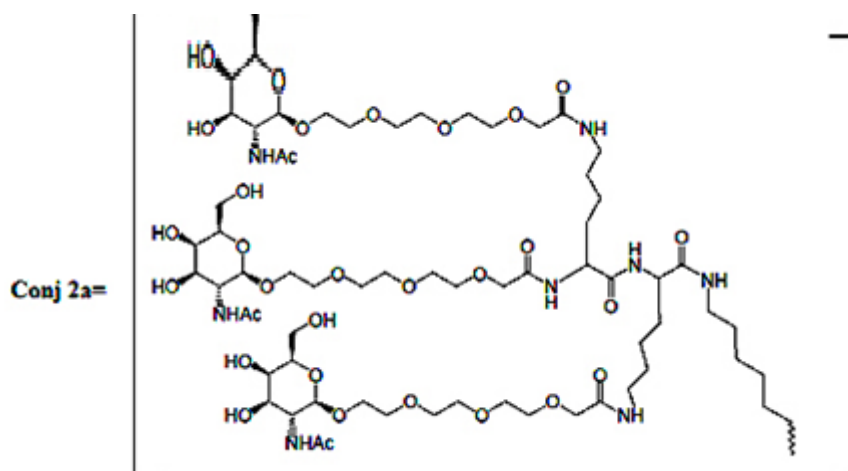
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REIVINDICACIONES

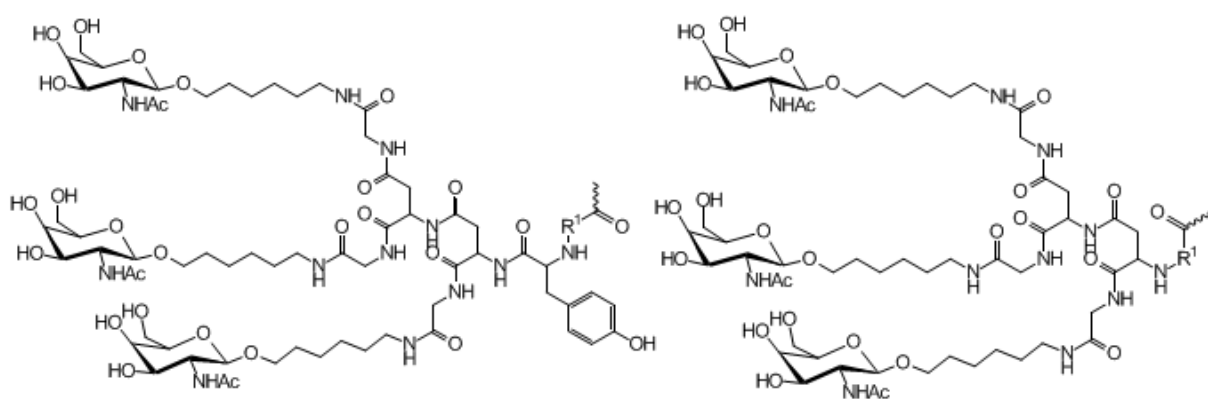
1. Conjugado de oligonucleótido antisentido que comprende:
 - a. un oligómero antisentido (A) de una longitud de entre 16 y 22 nucleótidos, que comprende una secuencia contigua de 16 nucleótidos que son complementarios a un tramo correspondiente de SEC ID NO 31, y
 - b. por lo menos una fracción de conjugado (C) unida covalentemente a dicho oligómero (A).
2. Conjugado de oligonucleótido según la reivindicación 1, en el que el oligómero antisentido comprende la secuencia contigua SEC ID NO 26.
3. Conjugado de oligonucleótido según cualquiera de las reivindicaciones 1 o 2, en el que el oligómero antisentido comprende la secuencia contigua seleccionada del grupo SEQ ID NO 2 y 3.
4. Conjugado de oligonucleótido según cualquiera de las reivindicaciones 1 a 3, en el que la fracción de conjugado (C) comprende N-acetylgalactosamine (GalNAc), como una GalNAc monovalente, divalente, trivalente o tetravalente.
5. Conjugado de oligonucleótido antisentido según cualquiera de las reivindicaciones 1 a 4, en el que la fracción de conjugado (C) comprende un GalNAc trivalente.
6. Conjugado de oligonucleótido antisentido según la reivindicación 5, en el que las tres fracciones GalNAc se unen a un grupo de punto de ramificación dilisina mediante espaciadores de PEG, tal como los mostrados como Conj. 1, 2, 1a o 2a.



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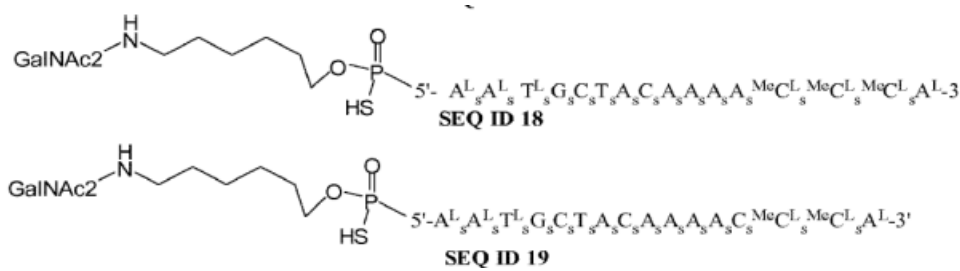
7. Conjugado de oligonucleótido antisentido según cualquiera de las reivindicaciones 1 a 6, que se selecciona de entre el grupo que consiste SEC ID NO 18 y 19.
8. Conjugado de oligonucleótido antisentido según cualquiera de las reivindicaciones 1 a 7, en el que el oligómero antisentido (A) se conjuga con la fracción de conjugado (C) mediante una región conectora situada entre la secuencia contigua del oligómero y la fracción conjugada (B y/o Y), donde la región conectora es cualquiera de:



Donde R1 es un biradical seleccionado preferiblemente de $-C_2H_4-$, $-C_3H_6-$, $-C_4H_8-$, $-C_5H_{10}-$, $-C_6H_{12}-$, 1,4-cyclohexyl ($-C_6H_{10}-$), 1,4-phenyl ($-C_6H_4-$), $-C_2H_4OC_2H_4-$, $-C_2H_4(OC_2H_4)_2-$ or $-C_2H_4(OC_2H_4)_3-$, $C(O)CH_2-$, $-C(O)C_2H_4-$, $-C(O)C_3H_6-$, $-C(O)C_4H_8-$, $-C(O)C_5H_{10}-$, $-C(O)C_6H_{12}-$, 1,4-cyclohexyl ($-C(O)C_6H_{10}-$), 1,4-phenyl ($-C(O)C_6H_4-$), $-C(O)C_2H_4OC_2H_4-$, $-C(O)C_2H_4(OC_2H_4)_2-$ or $-C(O)C_2H_4(OC_2H_4)_3-$.

9. Conjugado de oligonucleótido antisentido según la reivindicación 8, en el que el conector se selecciona de entre los grupos de aminoalquilo C6 a C12 o un conector biocortable fosfato de nucleótidos que comprende entre 1 y 6 nucleótidos, que está unido covalentemente al nucleótido 5' o 3' de la primera región, tal como a través de un grupo de enlace internucleosídico tal como un enlace fosfodiéster, en el que cualquiera de:
- a. el enlace internucleosídico entre la primera y la segunda región es un enlace fosfodiéster y el nucleósido de la segunda región es adyacente a la primera región es ADN o ARN; y/o

- b. al menos 1 nucleósido de la segunda región es un nucleósido de ADN o ARN unido a fosfodiéster.
10. Conjugado de oligonucleótido anti sentido según cualquiera de las reivindicaciones 1 a 9, donde la secuencia contigua comprende, análogos de nucleótidos potenciadores de la afinidad.
11. Conjugado de oligonucleótido anti sentido según la reivindicación 10, donde dichos análogos de nucleótidos son nucleótidos modificados con azúcar independiente o dependientemente seleccionados del grupo que consiste en Unidades de Ácido Nucleico Bloqueado (ANB), unidades 2'-Oalkil-RNA, unidades 2'-OMe-RNA, unidades 2'-amino-DNA, y unidades 2'-fluoro-DNA.
12. Conjugado de oligonucleótido anti sentido según cualquiera de las reivindicaciones 1 a 11, donde el oligomero comprende uno o más enlaces nucleosidos seleccionados del grupo consistente de fosforotioato, fosforoditioato y boranofosfato.
13. Conjugado de oligonucleótido anti sentido según la reivindicación 1, donde el conjugado de oligonucleótido consiste de SEQ ID NO:18 o SEQ ID NO:19



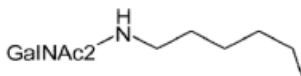
donde

Un superíndice ^L identifica una unidad ANB beta-D-oxi,

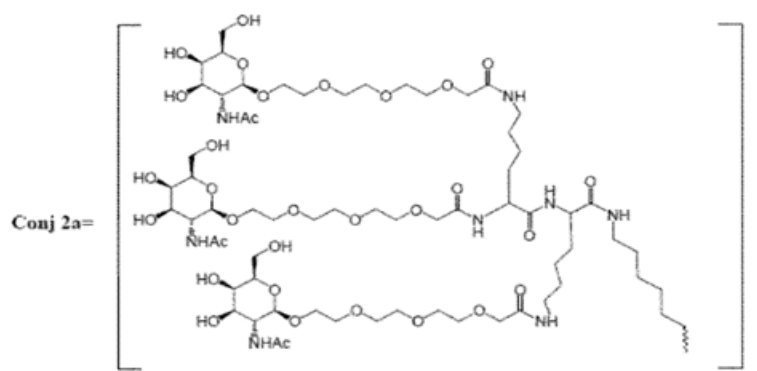
un ^{Me}C identifica un 5-metilcitosina,

un subíndice _s identifica un enlace internucleosídico fosforotioato,

y donde



Es un resto conjugado Conj 2a que se dirige al receptor asialoglicoproteína



14. Oligómero de entre 16 a 20 nucleótidos de longitud, que cualquiera comprende una secuencia contigua de por lo menos 16 nucleótidos que son complementarios a un tramo correspondiente de SEC ID NO 31, donde la secuencia contigua comprende análogos de nucleótidos potenciadores de la afinidad seleccionados independientemente o dependientemente del grupo que consiste en unidades de Ácido Nucleico Bloqueado (ANB), unidades de 2'-O-alkyl-ARN, unidades de 2'-OMe-RNA, 2'-amino-DNA unidades y unidades de 2'-fluoro-DNA.
15. Oligómero según la reivindicación 14, que comprende una secuencia de 16 nucleótidos correspondiente a la SEC ID NO 26.
16. Oligómero según cualquiera de las reivindicaciones 14 a 15, que es un gápmo, en donde el gápmo comprende un ala en cada lado (5' y 3') de 2 a 4 análogos de nucleótidos, preferiblemente análogos de ANB.
17. Oligómero según cualquiera de las reivindicaciones 14 a 16, en el que el oligómero comprende uno o más enlaces de nucleósido seleccionados de entre el grupo que consiste de fosforotioato, fosforoditioato y boranofosfato, en el que los enlaces de nucleósidos unen cada monómero al monómero adyacente 3' del oligómero.
18. Oligómero según la reivindicación 13 o un oligómero según cualquiera de las reivindicaciones 14 a 17, que comprende una secuencia contigua de SEC ID NO 2 o 3.

- 19.** Composición farmacéutica que comprende el oligómero o conjugado de oligonucleótido antisentido según cualquiera de las reivindicaciones 1 a 18 y un diluyente, portador, sal o adyuvante farmacéuticamente aceptable.
- 20.** Método in vitro para la inhibición de PCSK9 en una célula que expresa PCSK9, comprendiendo dicho método la administración de un oligómero o conjugado de oligonucleótido antisentido o composición farmacéutica según cualquiera de las reivindicaciones 1 a 19 en dicha célula.

Trámite: 11
Evento: 378
Actuación: 444

Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

DIVISIÓN DE NUEVAS CREACIONES

E. _____ S. _____ D. _____

Re: PI.-ROCHE INNOVATION CENTER COPENHAGEN A/S.-Solicitud de Patente de Invención en Fase Nacional PCT para "OLIGÓMEROS ANTISENTIDO Y CONJUGADOS CON DIANA EN PCSK9".-Clase C12N 15/113.-Expediente número 15-307.175.-

1. Me refiero al auto dictado por ese Despacho mediante notificación por fijación en línea de Junio 23 de 2017 y a mi solicitud de plazo de Agosto 23 de 2017.

2. Con el fin de responder las objeciones expuestas por la Señora Examinadora en su Concepto Técnico correspondiente al Oficio N° **9238** en relación con la Claridad de las Reivindicaciones, Unidad de Invención y Nivel inventivo, me permito dirigir su atención a las siguientes modificaciones y argumentos técnicos por medio de los cuales no sólo se superan completamente dichas objeciones sino que además se establece de manera incontrovertible la patentabilidad de la solicitud en estudio.

3. **CAPÍTULO REIVINDICATORIO.**

3.1. **Modificaciones al Capítulo Reivindicatorio.**

Inicialmente, me permito manifestar que es deseo del solicitante presentar un **capítulo reivindicatorio modificado**, que remplace al actualmente presentado, con el propósito de superar las objeciones presentadas como parte del concepto técnico realizado por ese Despacho.

En conexión con el mencionado **capítulo reivindicatorio modificado**, me permito resaltar que éste no constituye de ninguna manera una ampliación al alcance de la solicitud, tal como fue originalmente presentada, toda vez que define oligómeros antisentido dirigidos contra secuencias de PCSK9 humanas, que es el objeto de la presente invención de acuerdo con las enseñanzas de la descripción. En este sentido, dichas modificaciones se exponen a continuación:

- ✓ **Nuevas reivindicaciones 1 y 2:** corresponden a las reivindicaciones 1 y 2 actualmente presentadas, en donde se ha limitado el número de secuencias reclamadas.
- ✓ **Nueva reivindicación 3:** corresponden a una nueva reivindicación que tiene sustento en la reivindicación 40 originalmente presenta.
- ✓ **Nueva reivindicación 4:** corresponde a la reivindicación 5 actualmente presentada, en donde se ha modificado la redacción para darle claridad a dicha reivindicación.
- ✓ **Nuevas reivindicaciones 5 y 6:** corresponden a las reivindicaciones 6 y 7 actualmente presentadas.
- ✓ **Nueva reivindicación 7:** corresponde a la reivindicación 9 actualmente presentada, en donde se han limitado el número de secuencias.
- ✓ **Nuevas reivindicaciones 8 y 9:** corresponden a las reivindicaciones 10 y 11 actualmente presentadas, en donde se han incluido las definiciones de la región conectora para dar claridad a la reivindicación. Tales modificaciones tienen sustento en las páginas 43 y 53.
- ✓ **Nuevas reivindicaciones 10 a 13:** corresponden a nuevas reivindicaciones, que tienen sustento en la descripción originalmente presentada en la página 8, página 65 ítem 12 y figuras 1 y 5.
- ✓ **Nuevas reivindicaciones 14:** corresponde a la reivindicación 12 actualmente presentada, en donde se ha incluido la definición de la secuencia contigua.
- ✓ **Nueva reivindicación 15:** corresponde a la reivindicación 13 actualmente presentada en la cual se ha modificado su dependencia.
- ✓ **Nuevas reivindicaciones 16 y 15:** corresponden a las reivindicaciones 15 y 16 actualmente presentadas, en donde se han definido el gápmro y los enlaces de nucleósidos de acuerdo a lo requerido por la examinadora.
- ✓ **Nuevas reivindicaciones 16 a 19:** corresponden a las reivindicaciones 15 a 18 actualmente presentadas.
- ✓ **Nueva reivindicación 20:** corresponde a una nueva reivindicación que tiene pleno sustento en la descripción en la página 69 ítem 45.

De acuerdo a lo anterior, me permito señalar que las reivindicaciones **3, 4, 8 y 14** del Capítulo Reivindicatorio actualmente presentado fueron **eliminadas** del documento modificado.

De esta forma, **el capítulo reivindicatorio modificado queda constituido por 20 reivindicaciones**, las cuales se refieren de manera más clara y concisa a las características técnicas esenciales de los oligómeros antisentido dirigidos contra secuencias de PCSK9 humanas, que son objeto de la presente solicitud, y que cuentan con pleno sustento en las enseñanzas de la descripción.

3.2. Claridad del capítulo reivindicatorio.

Con respecto a la claridad del capítulo reivindicatorio, la Señora Examinadora afirma lo siguiente:

- ✓ *"La reivindicación 1 no es concisa porque:*
- Se refiere a una gran variedad de posibles secuencias de los oligómeros antisentido, toda vez que únicamente se menciona como característica la longitud máxima y mínima de la cadena (10-16 nucleótidos) en base a una variedad de secuencias de longitud variable (14-16 nucleótidos), de esta forma, la reivindicación incluye un sinnúmero de posibilidades que no hacen parte del objeto de la solicitud. Se sugiere restringir la materia reclamada a una razonable generalización de la materia que ha sido producida y ensayada, es decir que se encuentra adecuadamente sustentada.*
 - El término "por lo menos" no es limitativo, por lo que no permiten comprender los alcances de la materia reclamada. Se sugiere reemplazarlo por un rango concreto.*
 - No se establece la estructura del grupo GalNAc presente en el conjugado, toda vez que no se establece su estructura ni tampoco su sitio de unión al oligómero antisentido. Se sugiere incluir las características de dicho componente estructural. Lo mismo es aplicable a las reivindicaciones 5 y 6."*

Respecto a las mencionadas objeciones, me permito llamar la atención de la examinadora al nuevo pliego reivindicatorio, en el cual se ha modificado la reivindicación 1 para especificar que el oligómero antisentido (a) tiene entre 16-22 nucleótidos de longitud, y comprende una secuencia contigua de 16 nucleótidos que son complementarias a una longitud correspondiente de SEQ ID NO 31. En vista de tal enmienda, la nueva reivindicación corresponde a una generalización razonable que tiene pleno sustento en la descripción.

Respecto al término "por lo menos", me permito aclarar que el mismo es limitante y permite la comprensión del alcance de la materia reclamada, ya que claramente se refiere a "uno o más", y por tanto debe considerarse claro. Ahora bien, un indicio importante de su claridad es el hecho que no se han presentado objeciones de este tipo en las solicitudes asociadas presentadas en otras jurisdicciones, y que, por ejemplo, la solicitud de los Estados Unidos, el Japón y la Unión Africana ya están procediendo a la concesión.

Finalmente, respecto a la objeción de la examinadora respecto a la necesidad de especificar la estructura del GalNAc, me permito señalar que para el experto en la materia sería claro que la invención, y que de otra parte, dicha estructura se encuentra incluida en las reivindicaciones dependientes 10 a 13 incluidas en el nuevo pliego reivindicatorio.

Teniendo en cuenta los anteriores argumentos, solicito amablemente que dichas objeciones sean reconsideradas.

- ✓ *Las reivindicaciones 5 y 6 no son claras porque la expresión "GalNAc monovalente, divalente, trivalente o tetravalente" no es claro, toda vez que no se comprende si se refiere al número de sitios de unión por residuo de azúcar o a la cantidad de unidades de acetilgalactosamida por conjugado. Se sugiere indicar la cantidad de dichos residuos de azúcar por oligonucleótido y la forma en que estos se unen al mismo.*

Respecto a la citada objeción, me permito señalar que la persona normalmente versada en la materia entendería que "GalNAc monovalente, divalente o tetravalente" se refiere al número/cantidad de unidades de acetilgalactosamida por conjugado, de manera que dicha terminología que es ampliamente conocida en el campo técnico es completamente clara.

Teniendo en cuenta los anteriores argumentos, solicito amablemente que dichas objeciones sean reconsideradas.

- ✓ *Las reivindicaciones 10-11 no son claras porque las expresiones "región conectora", "conector biocortable fosfato" son ambiguas ya que se refieren únicamente a la función de un elemento, mas no definen sus características técnicas estructurales, por lo tanto, no permiten comprender el alcance de la materia reclamada. Se sugiere definir de manera específica la estructura de dicha región conectora e incluir la posición de la secuencia en la cual se realiza la unión a la misma.*

Respecto a la citada objeción, me permito señalar que las reivindicaciones actuales 10 y 11 (nuevas reivindicaciones 8 y 9) han sido modificadas para incluir las definiciones de la región conectora para dar claridad a la reivindicación. De manera que se ha atendido la objeción del examinador, solicito amablemente que dicha objeción sea dada por superada.

- ✓ *La reivindicación 14 no es clara porque define un oligómero de nucleótidos únicamente en términos de la secuencia a la que puede unirse (diana), de esta forma se define por medio de resultados a alcanzar, que no es una característica técnica esencial del mismo. Se sugiere caracterizar los oligómeros en términos de sus características técnicas esenciales, como su secuencia de nucleótidos y posiciones en las que se realizan modificaciones de la misma, por ejemplo, unión a conjugados.*

Al respecto, se aclara que dicha reivindicación fue eliminada del nuevo pliego reivindicatorio.

Teniendo en cuenta lo anterior, solicito amablemente que dicha objeción sea retirada.

- ✓ *La reivindicación 15 no es clara porque el término "análogos de nucleótido potenciadores de la afinidad", se refiere a un elemento estructural en términos exclusivos de su actividad o función, por lo cual no limitan la materia reclamada de manera concreta. Se sugiere especificar la posición o posiciones de los análogos de nucleótidos y su la estructura de los mismos.*

Al respecto, se aclara que dicha reivindicación fue eliminada del nuevo pliego reivindicatorio.

Teniendo en cuenta lo anterior, solicito amablemente que dicha objeción sea retirada.

- ✓ *La reivindicación 16 no es clara porque el término "gápmero", no limita de manera adecuada el objeto reclamado. Se sugiere al solicitante que caracterizar las moléculas reclamada de acuerdo a las secuencias completas de nucleótidos que las conforman.*

Al respecto, me permito señalar que dicha reivindicación ahora incluye la definición de gápmero, de manera que se ha atendido a la objeción del examinador.

En vista de lo anterior atentamente solicito a ese Despacho que la objeción realizada a éste respecto se reconsiderada.

- ✓ *La reivindicación 17 no es clara porque los términos "fosforotioato", "fosforoditioato" y "boranofosfato" no limitan de forma adecuada la materia reclamada. Se sugiere definir la posición de dicha unión en la secuencia de nucleótidos que conforma las moléculas reclamadas*

El respecto, me permito señalar que dicha reivindicación se ha modificado para definir los correspondientes enlaces nucleósidos, de manera que se han atendido las objeciones del examinador.

Por tanto, amablemente solicito que la Señora Examinadora de por superadas dichas objeciones.

- ✓ *La reivindicación 19 no es clara porque no se definen las características técnicas esenciales de una composición, como son la identidad de sus componentes por medio de su nombre IUPAC, denominación común internacional o estructura y las proporciones o concentraciones en las que estos se encuentran. Se sugiere que se definan dichas características para la composición reclamada.*

Al respecto, me permito señalar que la citada reivindicación es dependiente de las reivindicaciones 1 a 18, de manera que comprende las características estructurales allí divulgadas para el conjugado característico de la composición reclamada, de manera que la objeción de la examinadora carece de fundamento, por lo que amablemente requiero que sea retirada.

- ✓ *La reivindicación 20 no es clara porque no se definen las condiciones necesarias para llevar a cabo el método reclamado. Lo anterior teniendo en cuenta que únicamente se menciona las etapas de (a) administración de un oligómero o conjugado a una célula, sin especificar las condiciones bajo las cuales se da dicha administración, así como tampoco las condiciones de cultivo celular, la concentración del oligómero o conjugado en el medio adicionado, temperatura, pH y tiempo de exposición. Se sugiere realizar las aclaraciones correspondientes.*

Al respecto me permito manifestar respetuosamente mi desacuerdo con la objeción de la examinadora, toda vez que el método se encuentra caracterizado a partir de su única etapa, y la estructura de los compuestos necesarios para el mismo se encuentra divulgada en las reivindicaciones dependiente, de manera que resulta claro para la persona normalmente versada en la materia. Por tanto, amablemente requiero que dicha objeción sea retirada.

En vista de todo lo anterior, me permito resaltar que el nuevo capítulo reivindicatorio es totalmente claro y conciso al reclamar oligómeros antisentido dirigidos contra secuencias de PCSK9 humanas, que son objeto de la presente solicitud. Asimismo, dicho capítulo modificado supera completamente las objeciones de claridad presentadas por la Señora Examinadora y, por lo tanto, cumple a cabalidad con lo estipulado por el Artículo 30 de la Decisión Andina 486 de 2000.

4. **UNIDAD DE INVENCION.**

La Señora Examinadora objeta la unidad de invención de la presente solicitud, señalando que existen los siguientes 3 grupos inventivos en la materia reclamada:

- ✓ Grupo 1: Oligonucleótidos antisentido y conjugados de los mismos dirigidos a PCSK9 que comprenden las secuencias SEC ID No. 1-3, 17-19, 25 y 26, las cuales son complementarias a las secuencias SEC ID No. 30 o 31, enseñados en el documento WO2011009697 (Reivindicaciones 1-11, 19 y 20 parcialmente, 12-18 totalmente).
- ✓ Grupo 2: Oligonucleótidos antisentido y conjugados de los mismos dirigidos a PCSK9 que comprenden las secuencias SEC ID No. 4-6, 20-22, 27 y 28, las cuales son complementarias a las secuencias SEC ID No. 32 o 33, enseñados en el documento WO2012058693 (Reivindicaciones 1-11, 19 y 20 parcialmente).
- ✓ Grupo 3: Oligonucleótidos antisentido y conjugados de los mismos dirigidos a PCSK9 que comprenden las secuencias SEC ID No. 7, 8, 23, 24 y 29, las cuales son complementarias a la secuencia SEC ID No. 34 (Reivindicaciones 1-11, 19 y 20 parcialmente).
- ✓ Grupo 4: Oligonucleótidos antisentido y conjugados de los mismos dirigidos PCSK9 que comprenden la secuencia SEC ID No. 44 que es complementaria a la secuencia SEC ID No. 45 (Reivindicaciones 1-3, 5-8, 10, 11, 19 y 20 parcialmente).

Teniendo en cuenta lo anterior y con base en las modificaciones mencionadas en el numeral 3.1 del presente memorial, me permito resaltar que la materia reclamada se dirige exclusivamente al grupo 1 establecido por la examinadora. Por ende, **la materia aquí reclamada se dirige al primer grupo inventivo, el cual se caracteriza por presentar un único concepto inventivo** y define los oligómeros antisentido dirigidos contra secuencias de PCSK9 humanas nuevos e inventivos de la invención.

Por lo tanto, el nuevo capítulo reivindicatorio cumple a cabalidad con el requerimiento de unidad de invención.

5. NIVEL INVENTIVO.

Finalmente, la Señora Examinadora considera que la materia reclamada respecto al Grupo 1 establecido en las reivindicaciones 1 a 6 y 9 a 20 actualmente presentadas carecerían de altura inventiva con respecto a las enseñanzas de los documentos **WO2011009697 (D1)** y **WO2012058693 (D2)**, así como la materia referente al grupo 1 de las reivindicaciones 7 y 8 actualmente presentadas carecerían de altura inventiva con respecto a las enseñanzas de los documentos **WO2011009697 (D1)** y **WO2012083046 (D4)**.

Adicionalmente, señala que la materia reclamada respecto al Grupo 2 establecido en las reivindicaciones 1 a 6, 9 a 11, 19 y 20 actualmente presentadas carecerían de altura inventiva con respecto a las enseñanzas de los documentos **WO2011009697 (D1)** y **WO2009073809 (D3)**.

En conexión con lo anterior, la Señora Examinadora establece que el Documento D1 es el más cercano a la invención. Sin embargo, es preciso aclarar que las nuevas reivindicaciones han sido drásticamente restringidas, y que las mismas no resultan obvias a partir del documento D1, ni de su combinación con cualquiera de los otros documentos citados D2 a D4.

En primer lugar, es preciso señalar que D1 describe una posición de direccionamiento del oligonucleótido antisentido de 14mer ANB (Unidades de Ácido Nucleico Bloqueado) (SEQ ID NO: 4 de D1) 3643 a 3656 en el mRNA de PCSK-9 (véase la presente solicitud, Tabla 1 en la página 9, SEQ ID NO 1). Es así que, D1 describe que el oligómero reduce la expresión de PCSK9, y propone que el oligómero se puede usar en el tratamiento de trastornos médicos, tales como hipercolesterolemia y trastornos relacionados (D1, Resumen). Ahora bien, el oligonucleótido antisentido 14NA de ANB corresponde a la SEQ ID NO: 1 de la presente solicitud, el cual también se denomina SPC5001 (véase la presente solicitud, en la página 11).

Ahora bien, el oligonucleótido antisentido de la reivindicación 1 presenta varias diferencias estructurales importantes respecto a lo divulgado en D1. En primer lugar, difiere porque comprende una secuencia contigua de 16 nucleótidos, que son complementarias a una longitud correspondiente de SEQ ID NO 31. De esta manera, me permito señalar que la región diana (SEC ID NO 31) del presente oligonucleótido/oligómero antisentido comprende la región objetivo del oligómero de LNA 14mer de D1 (SEC ID NO 30 de la presente solicitud) y difiere de la última en otros dos nucleótidos del extremo 3'.

- *Solución a un problema técnico persistente en el estado de la técnica*

A continuación, demostraré que el problema técnico resuelto por la presente invención fue identificado en el estado del arte, y su solución no había sido propuesta hasta la presente invención.

Poelgeest et al. (Am J Kidney Dis., 2013), publicado en línea el 5 de abril de 2013, es decir, antes de la presentación de la solicitud instantánea, divulga casos de lesión tubular aguda tóxica, de acuerdo con los datos recopilados sobre los sujetos participantes en el ensayo, que desarrollaron necrosis tubular aguda.

Es así como la recopilación de datos de Poelgeest et al. incluye el examen histopatológico y el análisis de biomarcadores urinarios para el daño renal, incluido KIM-1 (página 798, figura 1). Adicionalmente, informa que el aumento en KIM-1 (60 veces) implica que la disfunción tubular proximal estaba presente incluso antes de la manifestación clínica de la lesión (página 798, columna 1). Así mismo, divulga además que otros participantes en el ensayo que recibieron la misma dosis de SPC5001 también mostraron signos de disfunción tubular transitoria (página 796, columna 2), incluido el aumento de los biomarcadores urinarios por daño renal (Página 798, columna 1). Por lo tanto, dicho documento sugiere que existe una causalidad entre la administración de SPC5001 y la lesión tubular observada en el estudio (página 798, columna 1).

De la misma manera, Poelgeest et al. (2013) divulga que los oligonucleótidos antisentido generalmente se comportan de manera similar con respecto a la acumulación en el riñón y además que las modificaciones químicas de los oligonucleótidos antisentido se consideran seguras (página 798, columna 1). Dicho documento además especula sobre cuales características del oligomero pueden causar la lesión sin llegar a una solución al problema. En vista de los datos informados del estudio de prueba en SPC5001, Poelgeest et al. (2013) recomienda aumentar la conciencia del efecto tóxico potencial de los oligonucleótidos antisentido en los riñones (página 796, columna 2).

Adicionalmente, me permito señalar que en 2015 se publicó un primer estudio en humanos sobre el oligómero de 14a ANB de D1 en British Journal of Clinical Pharmacology (Poelgeest et al., 2015), que confirmó la lesión tubular aguda tóxica previamente descrita en Poelgeest et al. en 2013 (Am J Kidney Dis., 2013). Ahora bien, Poelgeest et al. (2015) enseña que el tratamiento con el oligómero de 14a ANB (referido como SCP5001, véase van Poelgeest et al., Página 1351, 1ª columna, último párrafo) se asoció con toxicidad tubular renal (van Poelgeest et al., Página 1350, resumen), donde se observó un aumento de la concentración de creatinina sérica en cuatro de seis sujetos. Uno de los sujetos incluidos en el ensayo desarrolló necrosis tubular aguda 5 días después de la última administración de SCP5001 (van Poelgeest et al., Página 1354,

1ª columna). De esta manera, se canceló el desarrollo clínico adicional de SCP5001 debido al efecto adverso observado en el primer estudio en humanos.

Dados los antecedentes expuestos, me permito destacar que la presente solicitud divulga datos experimentales sobre la evaluación de toxicidad hepática y renal potencial en ratas (Ejemplo 5 de la presente solicitud). Es así que los datos de la expresión de KIM-1 se describen en la Figura 15, y el estudio demuestra el aumento de KIM-1 en animales administrados (en los días 1 y 8) con el oligómero de 14a LNA de D1 (SEQ ID NO 1 de la Figura 15) durante un período de 7 y 14 días. Ahora bien, dichos resultados demuestran sorprendentemente que la extensión del oligómero con 2 nucleótidos reduce significativamente la expresión de KIM-1 (SEQ ID NO 2 y 3 de la Figura 15), donde KIM-1 es un biomarcador urinario para el daño renal (Poelgeest et al., 2013).

En consecuencia, me permito manifestar que la persona normalmente versada en la materia no llegaría de manera evidente a la presente invención en el momento en que se presentó la solicitud.

Por favor note que la persona normalmente versada en la materia al estudiar los informes sobre el ensayo clínico en SCP5001 (Poelgeest et al., 2013), no encontraría ninguna explicación relacionada a la causa de los efectos adversos de SCP5001, tampoco encontraría ningún tipo de motivación para enseñar o sugerir como cómo modificar SCP5001 para superar el efecto adverso observado, y mucho menos si es posible en absoluto superarlo.

De hecho, a partir de la lectura de los antecedentes divulgados en el momento de la invención la persona normalmente versada en la materia, se vería motivada a abandonar SCP5001 como candidato, así como a no usar la región diana correspondiente en el mRNA de PCSK-9 (SEQ ID NO 30 de la presente solicitud) y buscaría dianas alternativas. De hecho, la persona normalmente versada hubiera considerado buscar un ARNm diana completamente diferente que el ARNm PCSK-9.

- *Comparación con el estado de la técnica citado por la examinadora*

Ahora bien, D1 no proporciona ninguna información sobre el efecto adverso de SCP5001 y, por lo tanto, la persona experta no encontraría la solución a dicho problema en D1. De hecho, no hay motivación en D1 que impida que la persona capacitada desista del modelo SCP5001 y la región diana correspondiente en el mRNA de PCSK-9 en vista de los resultados negativos del estudio de prueba.

Incluso si D1 se combina con cualquiera de los documentos citados D3 y D4, en dichos documento no existe ninguna motivación para que la persona normalmente versada en la materia obtenga a la presente invención a la vista de lo anterior. Por lo tanto, debe considerarse que la presente invención es un desarrollo inventivo dadas las enseñanzas de D1.

En efecto, los documentos citados no pueden motivar a la persona normalmente versada en la técnica para modificar el oligonucleótido antisentido 14a de ANB (SCP5001) extendiendo el oligómero con uno o más nucleótidos. En vista de la nefrotoxicidad informada, la persona experta abandonaría SCP5001 como candidato, así como la región diana correspondiente en el mRNA de PCSK-9 (SEQ ID NO 30 de la presente solicitud) y buscaría una región objetivo alternativa. Por favor note que dicha extensión del oligomero resulta en un efecto sorprendente, a saber, la toxicidad se puede reducir significativamente.

Respecto a las objeciones presentadas por la examinadora para el grupo inventivo II, me permito señalar que el mismo no hace parte del capítulo reivindicatorio modificado.

En consecuencia, atentamente me permito manifestar ante ese Despacho que la materia del nuevo capítulo reivindicatorio presenta un inequívoco carácter inventivo frente a las enseñanzas de los documentos del arte anterior, cumpliendo por lo tanto a cabalidad con los requisitos de patentabilidad establecidos en el artículo 18 de la Decisión 486 de 2000.

6. **CONCLUSIÓN.**

Para finalizar, me permito reiterar que el capítulo reivindicatorio modificado presentado junto con este memorial es totalmente claro y conciso al reclamar los oligómeros antisentido dirigidos contra secuencias de PCSK9 que están plenamente soportados dentro del texto de la descripción, cumpliendo así con lo exigido por la mencionada Decisión Andina 486 de 2000.

Por otro lado, en virtud de los argumentos discutidos con respecto a los documentos citados, se estableció de manera incontrovertible que los oligómeros antisentido dirigidos contra secuencias de PCSK9 reclamados por la presente invención de ninguna manera resultarían evidentes para la persona versada en la materia a partir de las enseñanzas de los documentos citados, demostrando irrefutablemente la patentabilidad de la presente solicitud.

7. **SOLICITUD DE NUEVO EXAMEN DE PATENTABILIDAD.**

Finalmente, me permito manifestar que es deseo del solicitante requerir a ese Despacho la expedición de un nuevo examen de patentabilidad, lo anterior en caso de que el Señor Examinador no considere suficientes los argumentos y las modificaciones al capítulo reivindicatorio presentadas en respuesta al Oficio N° 9238.

8. Anexo al presente memorial, me permito presentar copia de las páginas **84 a 89** que corresponden al **capítulo reivindicatorio modificado**, tal como se discutió en el presente memorial, constituido por **20 reivindicaciones**.

9. Adicionalmente, informo que el respectivo **nuevo pago de Examen de Patentabilidad**, se llevó a cabo mediante la plataforma SIPI.

10. En vista de lo anterior, ruego a usted proceder a estudiar nuevamente la solicitud a la luz de las modificaciones y los argumentos presentados y conceder el privilegio de patente solicitado.

Señor Superintendente,



ALICIA LLORED A RICAURTE

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Antisense-mediated reduction of proprotein convertase subtilisin/kexin type 9 (PCSK9): a first-in-human randomized, placebo-controlled trial

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Pharmacological inhibition of proprotein convertase subtilisin/kexin type 9 (PCSK9) by monoclonal antibodies and small molecules seems to be safe and effective in treating hypercholesterolaemia.
- The safety and efficacy of locked nucleic acid antisense oligonucleotides targeting PCSK9 has not been studied before.

WHAT THIS STUDY ADDS

- In this first-in-human study, locked nucleic acid antisense oligonucleotide SPC5001 dose-dependently reduced LDL-C and PCSK9, but treatment was associated with injection site reactions and transient renal tubular toxicity at the highest dose tested.
- Our findings provide detailed insight into the side effects of one oligonucleotide treatment and underline the need for better understanding of the molecular mechanisms behind the side effects of such compounds.

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AIMS

LDL-receptor expression is inhibited by the protease proprotein convertase subtilisin/kexin type 9 (PCSK9), which is considered a pharmacological target to reduce LDL-C concentrations in hypercholesterolaemic patients. We performed a first-in-human trial with SPC5001, a locked nucleic acid antisense inhibitor of PCSK9.

METHODS

In this randomized, placebo-controlled trial, 24 healthy volunteers received three weekly subcutaneous administrations of SPC5001 (0.5, 1.5 or 5 mg kg⁻¹) or placebo (SPC5001 : placebo ratio 6 : 2). End points were safety/tolerability, pharmacokinetics and efficacy of SPC5001.

RESULTS

SPC5001 plasma exposure (AUC_(0,24 h)) increased more than dose-proportionally. At 5 mg kg⁻¹, SPC5001 decreased target protein PCSK9 (day 15 to day 35: -49% vs. placebo, $P < 0.0001$), resulting in a reduction in LDL-C concentrations (maximal estimated difference at day 28 compared with placebo -0.72 mmol l⁻¹, 95% confidence interval -1.24, -0.16 mmol l⁻¹; $P < 0.01$). SPC5001 treatment (5 mg kg⁻¹) also decreased ApoB ($P = 0.04$) and increased ApoA1 ($P = 0.05$). SPC5001 administration dose-dependently induced mild to moderate injection site reactions in 44% of the subjects, and transient increases in serum creatinine of $\geq 20 \mu\text{mol l}^{-1}$ (15%) over baseline with signs of renal tubular toxicity in four out of six subjects at the highest dose level. One subject developed biopsy-proven acute tubular necrosis.

CONCLUSIONS

SPC5001 treatment dose-dependently inhibited PCSK9 and decreased LDL-C concentrations, demonstrating human proof-of-pharmacology.

- Our findings demonstrate the need for sensitive and tailored renal toxicity monitoring in future oligonucleotide studies.

However, SPC5001 caused mild to moderate injection site reactions and renal tubular toxicity, and clinical development of SPC5001 was terminated. Our findings underline the need for better understanding of the molecular mechanisms behind the side effects of compounds such as SPC5001, and for sensitive and relevant renal toxicity monitoring in future oligonucleotide studies.

Introduction

Statin therapy is one of the best-proven interventions in patients at high risk for cardiovascular disease. However, target low density lipoprotein cholesterol (LDL-C) goal concentrations are not always reached. Increasing statin dose to achieve lower LDL-C concentrations may cause adverse events such as skeletal muscle aches and increases in liver enzymes and, very rarely, rhabdomyolysis [1]. Hence, novel treatments to reduce LDL-C with a different mechanism of action could be of value. Modulation of LDL-receptor (LDL-R) expression is an attractive mechanism as LDL-C concentrations depend largely on expression and activity of the hepatic LDL-R [2]. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a protease that mediates endosomal/lysosomal degradation of the LDL-R by prolonging its retention in endosomes, resulting in reduced LDL-R recycling to the cell surface [3]. Gain-of-function mutations in PCSK9 are associated with a severe phenotype of autosomal dominant familial hypercholesterolaemia [4], whereas loss-of-function mutations can result in a 30% lowering of LDL-C [5] and a 47–88% reduction in cardiovascular disease risk [6] in the absence of any other apparent phenotypic change in humans [7]. Preclinical data have shown that inhibition of PCSK9 results in reduction of serum LDL-C concentrations [8, 9], and recent clinical studies have demonstrated PCSK9-antibody mediated reductions in LDL-C concentrations and cardiovascular events [10, 11].

SPC5001 is a 14-mer oligonucleotide with locked nucleic acid (LNA) modifications. The oligonucleotide contains β -D-oxy-LNA (three locked nucleotides in both *termini*), and eight deoxynucleotides, arranged in the sequence 5'-TGmCtaaaaaacmCmCA-3' (where upper case letters are LNAs and mC stands for 5-methyl-LNA-cytidine). Each of the internucleotide linkages is modified with phosphorothioate rather than the native phosphodiester. The LNA modified nucleotides increase the binding affinity for the target and increase nuclease resistance, thereby improving the drug-like properties [12]. SPC5001 is complementary to human PCSK9 mRNA, acts as an antisense inhibitor, and subsequently reduces intra- and extracellular PCSK9 protein levels [8]. Subcutaneously (s.c.) administered SPC5001 for 13 weeks in

mice (maximal dose 24 mg kg⁻¹ week⁻¹) and non-human primates (NHP) (maximal maintenance dose 20 mg kg⁻¹ week⁻¹) demonstrated no rate-limiting toxicity on liver and kidney function, only minimal s.c. injection site reactions and no effect on coagulation parameters (Santaris Pharma A/S preclinical package, data not shown). As with other antisense oligonucleotide (AON) compounds, renal histopathology showed tubular basophilic granules suggestive of oligonucleotide drug accumulation at SPC5001 dose levels up to 20/24 mg kg⁻¹, without signs of functional nephrotoxicity. The maximum safe starting dose for clinical testing of SPC5001 was 2.0 mg kg⁻¹ week⁻¹, based on the absence of adverse effects at the highest dose tested in a 13 week NHP study (20 mg kg⁻¹). Administration of four SPC5001 doses of 6 mg kg⁻¹ resulted in reductions of plasma PCSK9 protein concentrations (37%), hepatic PCSK9 mRNA levels (40%), and serum lipids (32%) in healthy NHPs. A lower dose of 1.5 mg kg⁻¹, administered once every 5 days, also induced significant reductions in LDL-C (16%), but not in plasma PCSK9. Based on these observations, three weekly SPC5001 injections of 0.5 mg kg⁻¹ were considered to be an appropriate starting dose for a first-in-human (FIH) trial, on which we report here. Ascending doses of SPC5001 were administered to healthy volunteers with slightly increased fasting LDL-C concentrations (exceeding 2.59 mmol l⁻¹ or 100 mg dl⁻¹), with a view to enable assessment of the human pharmacology of SPC5001 at the earliest clinical stage. Plasma PCSK9 concentrations and key lipid parameters were evaluated, together with standard safety end points and pharmacokinetic profiling.

Methods

Study design

The study was randomized, ascending dose, double-blind and placebo-controlled, with an SPC5001 : placebo ratio of 6 : 2 per cohort conducted at the foundation Centre for Human Drug Research, Leiden, The Netherlands. Given the exploratory character of the study, no formal power calculations were performed to assess sample size. Drug was administered subcutaneously in the abdominal region as three weekly doses of 0.5, 1.5 or

5 mg kg⁻¹ on study days 1, 8 and 15 (150 mg ml⁻¹ SPC5001 dissolved in water for injection; injection volumes ≤3 ml administered in a single injection and volumes >3 ml in two injections). The starting dose of 0.5 mg kg⁻¹ week⁻¹ was 4-fold lower than the maximum recommended starting dose of 2.0 mg kg⁻¹ week⁻¹, based on a NOAEL of 20 mg kg⁻¹ per dose in NHPs and a human equivalent dose (HED) of 20 mg kg⁻¹ per dose, with an applied safety factor of 10. After the first and last SPC5001 or placebo (0.9% saline) administration, the subjects were confined to the clinical research unit for 24 h. After the second administration the subjects were monitored for 4 h. The last follow-up visit was conducted on study day 78.

Study participants

Thirty-two healthy volunteers, aged 18 to 65 years with a fasting LDL-C of ≥2.59 mmol l⁻¹ (≥100 mg dl⁻¹) and triglycerides ≤4.5 mmol l⁻¹ (≤398 mg dl⁻¹), a body mass index (BMI) of 18–33 kg m⁻², without ultrasonographic signs of liver steatosis and not using concomitant medication, were planned to be enrolled in four subsequent cohorts. The study was approved by the Central Committee on Research involving Human Subjects of The Netherlands (Centrale Commissie Mensgebonden Onderzoek; CCMO, EudraCT number of the study is 2011–000 489–36), and conducted according to the principles of the International Conference on Harmonization and Good Clinical Practice and the Helsinki Declaration. The volunteers gave written informed consent prior to screening.

Safety and tolerability

Safety monitoring was performed by adverse event monitoring, physical examination, assessment of ECG and vital signs, and laboratory evaluations (routine haematology, chemistry including C-reactive protein and gamma globulins, coagulation, complement factors, cytokines, and semi quantitative dipstick urinalysis). In case of clinically significant findings in dipstick analysis, a microscopic investigation of the urine was performed. Hepatic ultrasonography (Siemens P50) was performed at screening and the last follow-up visit for exclusion of hepatic steatosis. Dosing for each subsequent cohort commenced only after satisfactory review of 16 day safety data from the preceding cohort.

Post hoc analysis of exploratory kidney injury biomarkers was performed for urinary β₂-microglobulin (Immulite® 2000, a solid-phase two-side chemiluminescent immunometric assay), α-GST (Argutus Medical Alpha GST EIA enzyme immunoassay), NAG (Diazyme Europe GmbH) and KIM-1 (Quantikine® human TIM-1/KIM-1/HAVCR immunoassay). Samples for these biomarkers were collected on study days 1, 8 and 15 and stored at –80°C until analysis. Additional blood

and urine samples were collected upon renal safety concerns that appeared during study conduct.

Pharmacodynamics

Throughout the study, pharmacodynamic effects of SPC5001 were assessed in fasting blood samples by measurement of PCSK9, TC, HDL-C, TG, ApoA1, ApoB and VLDL-C. Total (LDL-bound and -unbound) PCSK9 was measured using the CircuLex human PCSK9 ELISA kit. The sensitivity was 0.154 ng l⁻¹ and the coefficient of variation was ~3%. LDL-C was calculated according to the Friedewald formula: LDL-C = TC – HDL-C – (0.456*TG). VLDL-C was calculated as TC – HDL-C – LDL-C.

Pharmacokinetics

For the quantification of SPC5001, plasma samples (collected frequently on dosing days 1 and 15, pre-dose on day 8 and during follow-up visits) were analyzed by a validated hybridization-dependent ELISA method (Santaris Pharma A/S, Technical Report), with a lower limit of quantitation (LOQ) of 0.4 ng ml⁻¹. The overall coefficient of variation was ~9%. In addition, urine samples collected on dosing days 1 and 15 (pre-dose and 0–4, 4–8 and 8–24 h post-dose) were analyzed by a comparable qualified method. SPC5001 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}) and the area under the plasma concentration–time curve from dosing to 24 h after dosing (AUC(0,24h)) using WinNonLin (version 5.3, Pharsight Corporation, USA).

Urine was collected prior to dose and during 24 h after the first and the third doses (on days 1 and 15, respectively) from all subjects (except one subject dosed at 1.5 mg kg⁻¹ per dose on day 15). The concentration of SPC5001 in the urine was quantified by the hybridization-dependent ELISA method and the amount of the compound in relation to the dose was calculated.

Statistical methods and analysis

Descriptive statistics were used to summarize demographic and baseline characteristics. Statistical analysis was performed for all pharmacodynamic parameters. To correct for the expected log-normal distribution, PCSK9, HDL-C, TG and VLDL-C were log-transformed prior to analysis. All repeatedly measured pharmacodynamic parameters were analyzed with a mixed model of variance with fixed factors treatment, time and treatment by time and random factor subject, and with the baseline measurement on day 1 as covariate. Contrasts between each SPC5001 dose level and placebo were calculated for a study period up to and including day 49 (the time span in which SPC5001 exerted the intended effects, at least at the highest dose level tested), unless otherwise

indicated. All analyses were performed using SAS for Windows Version 9.1.3 (SAS Institute, Inc., Cary, NC, USA).

Results

Participant characteristics

Twenty-four subjects were enrolled in the study. Demographics are summarized in Table 1. One subject in the 1.5 mg kg⁻¹ dose group was withdrawn after administration of two SPC5001 doses due to non-compliance with the study lifestyle rules. Therefore, 23 subjects completed the study, receiving three doses of SPC5001 or placebo.

Safety summary

One patient dosed at the highest dose (5 mg kg⁻¹ per dose) experienced acute tubular necrosis [11]. Therefore, follow-up was intensified for this subject and all other

participants still under follow-up. Upon review of elevated serum creatinine values together with urine sediment analyses, the Safety Review Committee decided to stop further dose escalation. For this reason, only 24 subjects were enrolled in the study and not the anticipated 32. In addition, a more comprehensive set of kidney injury biomarkers was retrospectively analyzed. The most frequent occurring adverse events were injection site reactions (ISR), observed in 44% of the SPC5001-treated subjects and in none of the placebo-treated subjects.

Renal effects

SPC5001 dose-dependently increased serum creatinine. Whereas in the 0.5 and 1.5 mg kg⁻¹ per dose groups no clinically relevant effects on serum creatinine levels were observed, SPC5001 treatment at 5 mg kg⁻¹ per dose induced a transient increase in serum creatinine (Figure 1,

Table 1

Subject baseline demographics, average (SD)

	0.5 mg kg ⁻¹ (n = 6)	SPC5001 (n = 18) 1.5 mg kg ⁻¹ (n = 6)	5 mg kg ⁻¹ (n = 6)	Placebo (n = 6)
Gender (M : F)	5 : 1	4 : 2	2 : 4	1 : 5
Age (years)	36 (14)	52 (14)	53 (15)	50 (15)
Weight (kg)	82 (11)	73 (10)	74 (16)	70 (13)
BMI (kg m ⁻²)	25 (2)	24 (3)	25 (4)	25 (3)
LDL-C (mmol l ⁻¹)	3.02 (0.56)	4.00 (0.92)	4.26 (0.73)	3.82 (0.44)
Creatinine (μmol l ⁻¹)	78 (14)	84 (11)	80 (14)	70 (8)

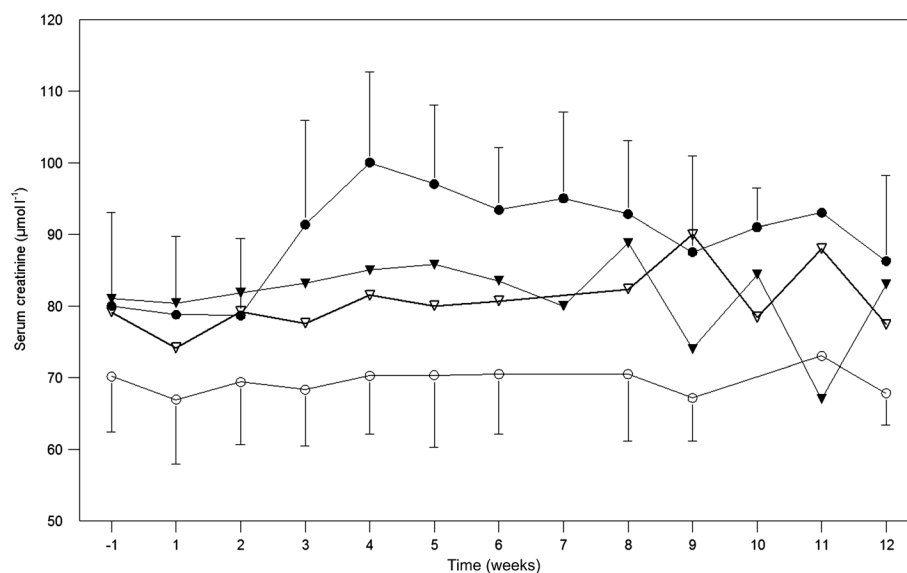


Figure 1

Average serum creatinine concentrations (μmol l⁻¹), with SD bars. SPC5001 administration was on study days 1, 8 and 15. Averages include both scheduled and unscheduled samples, grouped by week. (—○— placebo, —▽— 0.5 mg kg⁻¹, —▼— 1.5 mg kg⁻¹, —●— 5 mg kg⁻¹)

Table 2

Serum creatinine and urinary kidney injury markers. Estimated differences between active treatment and placebo, calculated for scheduled samples over the complete study period of 78 days, expressed as % change, presented with 95% confidence interval and corresponding *P* value. *n*: number of samples analyzed, *N*: number of subjects

	0.5 mg kg ⁻¹ SPC5001 vs. placebo <i>N</i> = 6	1.5 mg kg ⁻¹ SPC5001 vs. placebo <i>N</i> = 6	5 mg kg ⁻¹ SPC5001 vs. placebo <i>N</i> = 6	Placebo Average (SD) <i>N</i> = 6
Serum creatinine (μmol l⁻¹)	7.0% (0.5%, 14.0%) <i>P</i> = 0.04, <i>n</i> = 109	7.5% (0.6%, 14.8%) <i>P</i> = 0.03, <i>n</i> = 105	8.6% (1.6%, 16.2%) <i>P</i> = 0.02, <i>n</i> = 136	70 (8) <i>n</i> = 115
Urinary β₂-microglobulin (μg l⁻¹)	41.5% (-35.4%, 210.2%) <i>P</i> = 0.4, <i>n</i> = 25	114.4% (-0.3%, 361.0%) <i>P</i> = 0.05, <i>n</i> = 34	197.2% (32.4%, 566.8%) <i>P</i> = 0.01, <i>n</i> = 68	46 (35) <i>n</i> = 38
Urinary KIM-1 (ng ml⁻¹)	-20.6% (-59.2%, 54.4%) <i>P</i> = 0.5, <i>n</i> = 24	25.5% (-36.7%, 148.9%) <i>P</i> = 0.5, <i>n</i> = 28	55.3% (-21.5%, 207.1%) <i>P</i> = 0.2, <i>n</i> = 57	0.85 (0.66) <i>n</i> = 35
Urinary α-GST* (μg l⁻¹)	77.8% (-0.19%, 0.70%) <i>P</i> = 0.2, <i>n</i> = 21	29.0% (-0.62%, 0.16%) <i>P</i> = 0.6, <i>n</i> = 21	280.6% (-0.55%, 0.22%) <i>P</i> = 0.004, <i>n</i> = 20	9 (6) <i>n</i> = 20
Urinary NAG (IU l⁻¹)	16.1% (-14.8%, 2.1%) <i>P</i> = 0.5, <i>n</i> = 23	52.4% (-7.2%, 9.8%) <i>P</i> = 0.09, <i>n</i> = 26	8.6% (-6.1%, 11.1%) <i>P</i> = 0.7, <i>n</i> = 50	5.92 (3.82) <i>n</i> = 28

*Urinary α-GST not measured in additional samples for technical reasons.

Table 2, *P* = 0.02), which was observed in four out of six subjects. Average serum creatinine concentrations in that group started to increase after the last SPC5001 administration and peaked approximately 10 days after the final dose (from 84 ± 12 to 106 ± 15 μmol l⁻¹; reference ranges are 64–104 μmol l⁻¹ for males and 49–90 μmol l⁻¹ for females). Subsequently, serum creatinine gradually declined to baseline levels. The rise in serum creatinine coincided with the appearance of urinary granular casts. In addition, one of the subjects developed acute tubular necrosis 5 days after the last SPC5001 administration, which has been described in detail in a case report [13]. Upon appearance of these renal effects, additional blood and urinary samples were collected in all volunteers who were still under follow-up. Both the scheduled samples (collected pre-dose, and in weeks 1, 2 and 11) and the additional samples were analyzed for serum creatinine, urinary β₂-microglobulin, α-glutathione S-transferase (α-GST), kidney injury molecule-1 (KIM-1) and N-acetyl-β-D-glucosaminidase (NAG). Increases in serum creatinine (9%), urinary β₂-microglobulin (200%), and urinary KIM-1 (55%) were observed at the highest SPC5001 dose level tested (5 mg kg⁻¹ week⁻¹ for 3 weeks) (Table 2), with serum creatinine and urinary KIM-1 reaching peak concentrations at the fourth week (Figures 1 and 2). However, the collection of these data was not balanced between the treatment groups.

In the 5 mg kg⁻¹ dosing group, the elevations in serum creatinine, β₂-microglobulin and α-GST reached statistical significance (Table 2). It should be noted that the increases in β₂-microglobulin were isolated, transient and highly variable in timing between subjects and did

not correlate with the observed changes in other tubular markers. No SPC5001-related changes were observed in NAG (Table 2).

Injection site reactions

Injection site reactions (ISRs) developed dose-dependently in 8/18 (44%) of the SPC5001-treated subjects (0/6, 3/6 and 5/6 subjects in the 0.5, 1.5 and 5 mg kg⁻¹ dosing groups, respectively). These skin reactions presented hours to days after the s.c. injections as painless erythema at the site of the injection (approximately 5 cm by 5 cm) with or without transient pruritus and/or swelling. Most skin lesions became clearly visible 1 week after the last SPC5001 administration. Subjects either had skin lesions at all three locations of the abdomen where SPC5001 had been administered, or they had no skin lesions at all. The ISRs did not worsen with each subsequent injection, and all skin lesions within a subject were of similar severity. The ISRs were of mild to moderate severity and caused significant discomfort to a subset of the volunteers. In one female, a maculopapular rash developed 1 week after the final dose. The patient was referred to a dermatologist who treated the patient with topical steroids resulting in resolution of the rash. In general, the ISRs persisted for several days to weeks and then diminished in intensity. However, in one female, subcutaneous skin atrophy developed, which was present at the final visit 2.5 months after dosing. Also, in six out of eight SPC5001-treated subjects (three subjects from 1.5 mg kg⁻¹ group and three subjects from 5 mg kg⁻¹ group) skin hyperpigmentation was present at the last follow-up visit at 2.5 months.

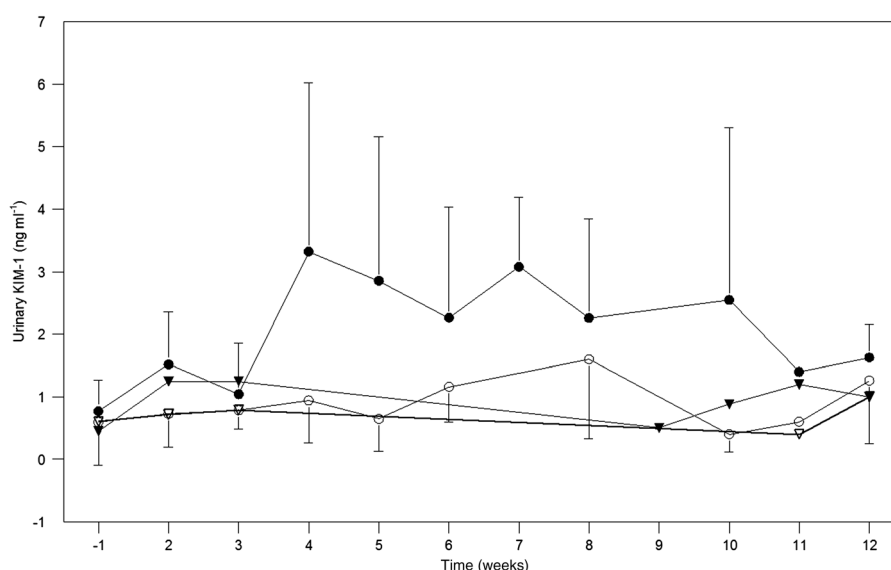


Figure 2

Average urinary kidney injury molecule-1 concentrations (ng ml^{-1}), with SD bars. SPC5001/placebo administration was on study days 1, 8 and 15. Averages include both scheduled and unscheduled samples, grouped by week. (—○— placebo, —▽— 0.5 mg kg^{-1} , —▼— 1.5 mg kg^{-1} , —●— 5 mg kg^{-1})

Other safety assessments

SPC5001 treatment did not result in clinically relevant changes in vital signs, ECG parameters, or coagulation, or in (trends to) increases in complement factors, cytokines or CRP, neither in subjects free of ISRs nor in subjects developing ISRs. Also, no clinically relevant changes in liver biochemistry parameters were observed, and none of the treated subjects had ultrasonographic signs of steatosis of the liver parenchyma at the last follow-up visit. Adverse events that were observed more frequently in SPC5001-treated subjects than in placebo-treated subjects were mild headache (61 vs. 33%) and tiredness (56 vs. 17%), occurring not dose dependently throughout the complete study period with a higher incidence within the first 24 h after SPC5001/placebo administration, and generally spontaneously resolving within hours to days.

Pharmacokinetics

Maximal plasma concentrations were reached at 1.7 ± 0.5 , 1.2 ± 0.4 , and 2.5 ± 2.7 h post-dose for 0.5, 1.5 and 5 mg kg^{-1} SPC5001, respectively (mean $\pm$ SD). The maximal plasma concentrations increased dose-proportionally (281 ± 43 , 757 ± 32 , and $2424 \pm 692 \text{ ng ml}^{-1}$ for 0.5, 1.5 and 5 mg kg^{-1} , respectively), while AUC(0,24 h) increased more than dose-proportionally (1.78 ± 0.13 , 5.01 ± 0.46 and $23.0 \pm 3.8 \mu\text{g ml}^{-1} \text{ h}$ for 0.5, 1.5 and 5 mg kg^{-1} , respectively). The rate constants of the terminal phases describing the decline in SPC5001 plasma concentration were not formally calculated, but the half-life of the final phase was estimated to be 7 days.

SPC5001 excreted in urine was determined in samples collected during 0–24 h after dosing on days 1 and 15. The total amount of SPC5001 in urine increased more than dose-proportionally (Figure 3).

Pharmacodynamics

SPC5001 treatment resulted in a decrease in PCSK9 plasma concentration, reaching a level of significance when compared with placebo at the highest dose levels tested (Figure 4, Table 3). The maximal decrease in PCSK9 concentration was reached 1 week after the last SPC5001 administration (Figure 4; from $302 \pm 80 \text{ ng ml}^{-1}$ at baseline to $156 \pm 85 \text{ ng ml}^{-1}$ on day 21, at 5 mg kg^{-1} SPC5001), remaining decreased until at least the last measurement point on day 35 (Figure 4). SPC5001 also induced a dose-dependent decrease in LDL-C concentrations, with a maximal effect reached 2 weeks after the last SPC5001 administration (Figure 5, from $3.8 \pm 0.8 \text{ mmol l}^{-1}$ at baseline to $2.9 \pm 1.1 \text{ mmol l}^{-1}$ on day 29, for 5 mg kg^{-1} SPC5001). Although the observed effect was not significant for the full time profile (Table 3), the contrast between 5 mg kg^{-1} SPC5001 and placebo was statistically significant at day 28 (estimated difference $-0.72 \text{ mmol l}^{-1}$, 95% confidence interval: $-1.24, -0.16 \text{ mmol l}^{-1}$; $P < 0.01$). LDL-C concentrations returned to baseline 9 weeks after administration of the last SPC5001 dose. Furthermore, 5 mg kg^{-1} SPC5001 decreased apolipoprotein B (ApoB) (Table 3, $P = 0.05$ vs. placebo), with a maximal average decrease from baseline of approximately 15% (0.17 g l^{-1}) observed 1 week after the last administration (data not shown), and increased apolipoprotein A1 (ApoA1) (Table 3, $P = 0.04$ vs. placebo), with a mean maximal average increase over baseline of

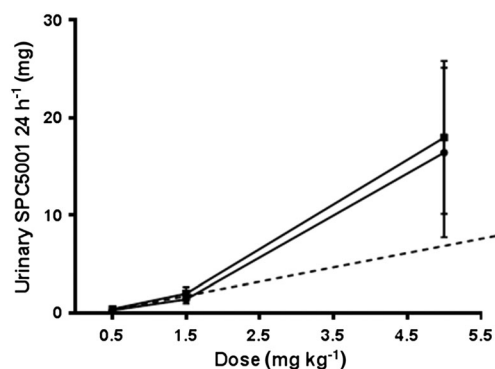


Figure 3

Average SPC5001 urinary excretion over 24 h (mg) after dosing, with SD bars. $n = 6$ subjects/dose level, except for 1.5 mg kg^{-1} ($n = 5$). (○) day 1 (0–24 h), (■) day 15 (0–24 h)

8% (0.13 g l^{-1}) observed at study day 14 (data not shown). High-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and very low density lipoprotein cholesterol (VLDL-C) concentrations were unaffected by SPC5001 treatment (Table 3).

Discussion

This study explored the pharmacokinetics and LDL-C lowering effects of SPC5001, an LNA-based PCSK9-targeted antisense oligonucleotide, in healthy volunteers with moderately elevated LDL-C concentrations. Proof-of-pharmacology for SPC5001 was achieved. SPC5001 administration resulted in a

reduction of plasma PCSK9 and a lowering of LDL-C and ApoB. However, the top dose (5 mg/kg) not only resulted in a maximal decrease in PCSK9 concentration of approximately 50% and a reduction in LDL-C of maximally 25% compared with baseline, but also in renal tubular effects. It is unknown whether the more than dose-proportional increase in SPC5001 plasma exposure ($\text{AUC}_{0-24\text{hr}}$) and urinary excretion, possibly representing a limitation in SPC5001's hepatic uptake/accumulation, relates to the observed nephrotoxicity.

Healthy cynomolgus monkeys dosed at similar or higher levels displayed the intended pharmacology (i.e. reduction in LDL-C) but no indications of renal toxicity (Santaris Pharma A/S, unpublished observations). In these animals, SPC5001 dosing (loading dose of 20 mg kg^{-1} , followed by four weekly doses of 5 mg kg^{-1}) reduced hepatic PCSK9 messenger RNA and plasma PCSK9 protein concentrations by up to 85%, and circulating LDL-C by up to 50% without affecting routine renal markers (urea and creatinine) [9]. Also in a 13-week toxicity study with a loading phase of up to 20 mg kg^{-1} per dose every 5th day for 16 days followed by a maintenance phase of $20 \text{ mg kg}^{-1} \text{ week}^{-1}$, neither clinical chemistry nor histopathology pointed to a tubular risk (Santaris Pharma A/S, unpublished GLP study report). However, interspecies differences in drug sensitivity are not unusual, hence the safety margins conventionally applied from animals to humans.

PCSK9 inhibition *per se* is unlikely to be the cause for the observed renal tubular toxicity observed in our study. Other PCSK9-inhibiting modalities tested in clinical studies have not resulted in renal signals. Inhibition of PCSK9

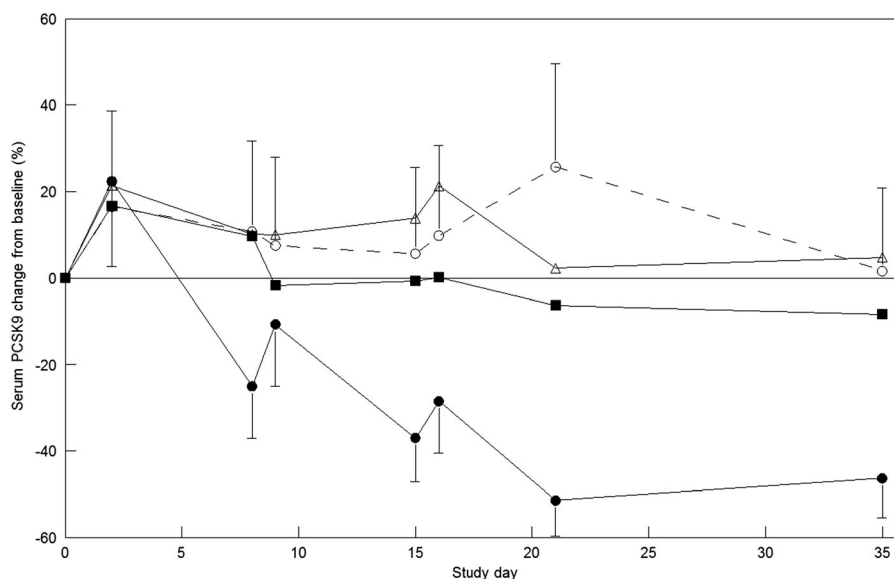


Figure 4

Average estimated PCSK9 concentrations (ng ml^{-1}), expressed as % change from baseline with 95% confidence interval bars. (○) placebo, (△) 0.5 mg kg^{-1} , (■) 1.5 mg kg^{-1} , (●) 5 mg kg^{-1}

Table 3

Effects of SPC5001 treatment on lipid parameters. Estimated differences between active treatment and placebo, calculated over a period from baseline up to and including day 49, expressed as absolute change with corresponding unit or as percentage for log transformed parameters (PCSK9, HDL-C, VLDL-C and triglycerides), presented with 95% confidence interval and corresponding *P* value. Estimated difference for PCSK9 was calculated from baseline up to and including day 35

	0.5 mg kg ⁻¹ SPC5001 vs. placebo	1.5 mg kg ⁻¹ SPC5001 vs. placebo	5 mg kg ⁻¹ SPC5001 vs. placebo
PCSK9 (%)	-2.5 (-20.7, 20.0) <i>P</i> = 0.8	-15.0 (-31.0, 4.7) <i>P</i> = 0.1	-49.0 (-58.5, -37.2) <i>P</i> < 0.0001
ApoA1 (g l⁻¹)	0.04 (-0.07, 0.15) <i>P</i> = 0.5	0.01 (-0.09, 0.12) <i>P</i> = 0.8	0.11 (0.01, 0.21) <i>P</i> = 0.04
ApoB (g l⁻¹)	-0.02 (-0.11, 0.07) <i>P</i> = 0.6	0.01 (-0.08, 0.09) <i>P</i> = 0.9	-0.09 (-0.18, 0.00) <i>P</i> = 0.05
Total cholesterol (mmol l⁻¹)	0.24 (-0.20, 0.69) <i>P</i> = 0.3	-0.22 (-0.60, 0.17) <i>P</i> = 0.3	-0.19 (-0.57, 0.20) <i>P</i> = 0.3
HDL-C (%)	-6.9 (-15.0, 1.9) <i>P</i> = 0.1	0.7 (-7.5, 9.6) <i>P</i> = 0.9	2.8 (-5.5, 11.8) <i>P</i> = 0.5
LDL-C (mmol l⁻¹) *	0.06 (-0.37, 0.50) <i>P</i> = 0.8	-0.18 (-0.57, 0.21) <i>P</i> = 0.4	-0.36 (-0.75, 0.03) <i>P</i> = 0.07
VLDL-C (%) †	34.7 (-2.3, 85.6) <i>P</i> = 0.06	2.9 (-25.8, 42.5) <i>P</i> = 0.9	22.8 (-11.7, 70.8) <i>P</i> = 0.2
Triglycerides (%)	34.5 (-2.2, 84.6) <i>P</i> = 0.07	3.2 (-25.3, 42.5) <i>P</i> = 0.8	23.0 (-11.3, 70.6) <i>P</i> = 0.2

*calculated with Friedewald formula: LDL-C = (TC) - (HDL-C) - (0.456*Triglycerides). †calculated as: (total cholesterol) - (HDL-cholesterol) - (indirect LDL-cholesterol)

synthesis by a single dose of silencing RNA was demonstrated to be a potentially safe and effective strategy, with a mean 70% reduction in circulating PCSK9 plasma protein (*P* < 0.0001) and a mean 40% reduction in LDL cholesterol from baseline relative to placebo (*P* < 0.0001) [14]. Furthermore, no renal toxicity has been reported for plasma PCSK9-directed antibodies, resulting in decreases in plasma PCSK9 concentrations up to 100% and reductions in LDL-C between 60 and 80% in phase 1 trials [15, 16]. Finally, there are no reports to our knowledge of functional renal changes in people with loss of function PCSK9 mutations [17].

The renal effects of SPC5001 included a transient increase in serum creatinine, with first onset after the last SPC5001 administration and peaking approximately 10 days after the final dose. This coincided with the appearance of urinary granular casts, and elevations of urinary kidney damage markers. One subject in the highest dose group developed acute tubular necrosis (ATN), which resolved spontaneously within 8 weeks. The observation of ATN is uncommon for unmodified oligodeoxynucleotides, 2'-MOE modified and LNA modified oligonucleotides, which have all been successfully

administered to humans without causing clinically meaningful renal functional changes [18–20]. The target-unrelated toxicity of individual oligonucleotides is diverse and probably driven by a range of factors including backbone and nucleoside chemistry, sequence and length. The mechanism behind renal toxicity associated with some oligonucleotides is currently unknown. It may relate to accumulation-related degenerative effects on the proximal tubule although in animal models molecules that accumulate most are not necessarily the most toxic. For instance, miravirsin accumulated to a 8-fold higher degree than SPC5001 in NHP kidney cortex and showed no renal toxicity in humans (Santaris Pharma A/S, Technical Reports and [19]). Other factors such as individual susceptibility of the patient may be involved as well. It is important to note that adverse renal effects may also occur in AONs with chemistries other than LNA. There are reports on second generation AON-induced proteinuria in patients with Duchenne's muscular dystrophy [21] and ATN in a metastatic cancer patient [22]. It has also been previously stated in the literature that shorter oligonucleotides have lower plasma protein binding capability and, therefore, a larger proportion of

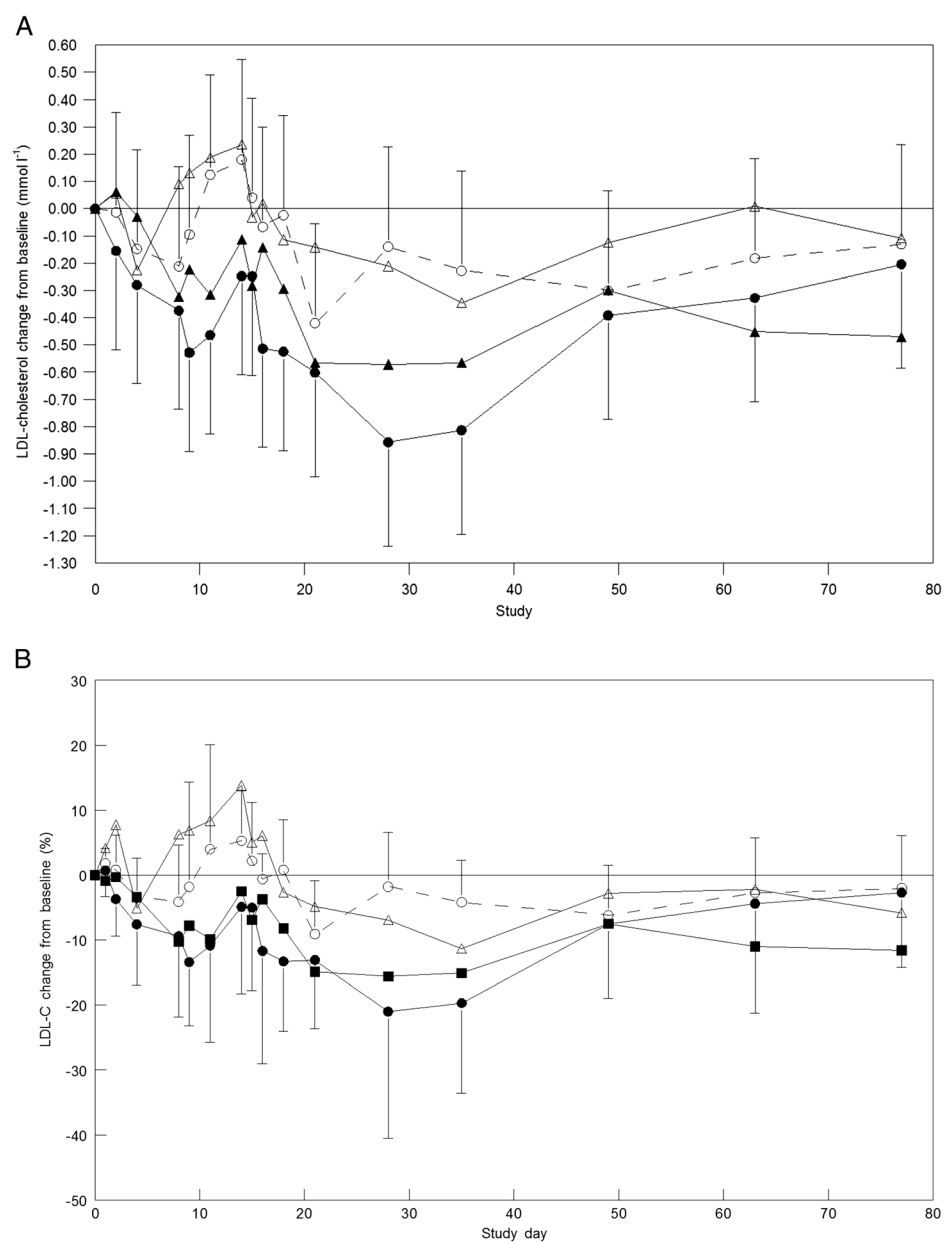


Figure 5

Average estimated LDL-cholesterol concentrations in mmol l⁻¹ (A) and in percentage (B), expressed as change from baseline with 95% confidence interval bars. (A) ○ – placebo, △ – 0.5 mg kg⁻¹, ▲ – 1.5 mg kg⁻¹, ● – 5 mg kg⁻¹; (B) ○ – placebo, △ – 0.5 mg kg⁻¹, ■ – 1.5 mg kg⁻¹, ● – 5 mg kg⁻¹

the dose may pass the glomerulus and accumulate in the proximal tubular cells [23]. However there is no relation, to our knowledge, between the rate of filtration and the level of tubular accumulation. Moreover, SPC5001 is a relatively short molecule (14-mer) but shows a high binding (of >95%) to human serum albumin (Santaris Pharma A/S, internal data). In the current trial, only 1–3% of the dose was excreted in urine during the first 24 h after administration (approximately corresponding to the unbound fraction), which is low compared with a 24 h urinary dose recovery of 8–10% for miravirsin, a 15-mer oligonucleotide that did not induce tubulotoxicity in early clinical trials [24, 25].

Because oligonucleotides accumulate in the proximal tubules to varying degrees [26], renal monitoring is performed for investigational compounds of this drug class. Not all clinical adverse events can be predicted pre-clinically and it is therefore suggested that extensive renal safety monitoring for AONs should not be limited to routine measures such as serum creatinine and urinalysis, but should also include regular urine microscopy. In addition, measurement of the urinary excretion of specific tubular damage markers such as KIM-1, β_2 -microglobulin, α -GST, and possibly NAG may also be informative. Thorough assessment of renal damage markers may allow early detection of impending renal damage and possibly provide

mechanistic insight [27], which is crucial for the understanding of why some, but not all, oligonucleotides have been demonstrated to induce unintended renal effects. These proposed urinary biomarkers are not fully clinically validated, and while their use is encouraged, they may not yet be ready to support real-time decision-making.

Since the observed renal effects of SPC5001 in this clinical study, the stringency of renal safety screening of LNA oligonucleotides has increased. A recent rat-based standard study including both routine and advanced biomarkers reproduced the tubulotoxicity of SPC5001 [28]. Further mechanistic studies are in progress to understand the factors causing this type of toxicity.

SPC5001 treatment dose-dependently induced mild to moderate ISRs. The occurrence of ISR suggests that SPC5001, like other charged phosphorothioate oligonucleotides [29], has the potential to induce local (subcutaneous) inflammatory changes. In the present study, there was no evidence for systemic inflammation as indicated by the absence of elevations in C-reactive protein and gamma globulins, circulating cytokines or complement activation (data not shown).

It has been reported that ISRs do occur in rodents and to a lesser extent in primates but also that animal models poorly predict this risk in humans [30]. Subcutaneous administration of SPC5001 in preclinical models resulted in only minimal injection site effects, so the toxicology studies did not predict the magnitude of ISRs upon SPC5001 treatment in humans. However, the occurrence of AON-induced ISRs in humans has been reported before. During the clinical development of mipomersen (2'-MOE phosphorothioate AON), 84% of all mipomersen-treated patients displayed ISRs compared with 33% of all placebo-treated patients [31]. Furthermore, PRO051 (a fully modified 2'-O-methyl phosphorothioate AON) administered to patients with Duchenne's muscular dystrophy resulted in erythema and inflammation at the injection site in 9 out of 12 treated patients [21]. This shows that phosphorothioate AONs have the potential to induce injection site reactions. It is currently unknown how sequence and structure of AON compounds relates to the nature of skin lesions and dose, concentration and volume are factors that may modulate these effects. However, a wide range of ISR-inducing potencies has been observed across development compounds (Santaris Pharma A/S, unpublished observations). The increased potency and duration of action of next-generation antisense oligonucleotides (and related less frequent dosing regimens and smaller injection volumes) may reduce the risk of ISR development [32–34]. Data need to be generated more systematically and for that purpose it is essential that injection site reactions are documented in more detail, preferably following a standardized (AON-specific) scoring system. Furthermore, the underlying pathophysiology of ISRs remains poorly understood.

In conclusion, SPC5001 administered to healthy volunteers with mildly elevated LDL-C resulted in dose-dependent reductions in LDL-C and PCSK9. However, subcutaneous injections of SPC5001 were associated with ISRs and transient renal tubular toxicity at 5 mg/kg. It is now recognized that within the LNA chemical class, these toxicities vary greatly across compound sequences and further screens have been or are being phased in to reduce those risks at the discovery stage [28]. The ultimate goal is to obtain insights into the molecular mechanisms behind the side effects of compounds such as SPC5001, with a view to improve the quality of compound libraries. In the same spirit, sensitive and relevant monitoring strategies for renal toxicity should be implemented for future oligonucleotide therapies.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare this clinical trial (ClinicalTrials.gov Identifier NCT01350960) was sponsored by Santaris Pharma A/S with no other relationships or activities that could appear to have influenced the submitted work. MH, YT, AL, RP, ML, KD and HØ were employees of previously Santaris Pharma A/S, now Roche Pharmaceutical Research and Early Development, Roche Innovation Center Copenhagen, Hørsholm, Denmark. AL had financial relationships with miRagen Therapeutics Inc, Boulder, Colorado, USA and Avidity NanoMedicines, La Jolla, California, USA in the previous 3 years. The drug was supplied by KLIFO A/S, Copenhagen Science Park Symbion, Fruebjergvej 3, 2100 Copenhagen, Denmark.

Contributors

EvP performed research, analyzed data and wrote the manuscript. MM designed research, analyzed data and reviewed the text. M.H. designed research, analyzed data and reviewed the text. YT designed research, analyzed data and wrote the manuscript. AL designed research, analyzed data and reviewed the text. RP analyzed data and reviewed the text. KDE reviewed the text. ML designed research and reviewed the text. HØ designed research and reviewed the text. AC designed research, analyzed data and reviewed the text. JB performed research, analyzed data and wrote the manuscript.

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