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

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INJECTION SITE REACTIONS AFTER SUBCUTANEOUS OLIGONUCLEOTIDE THERAPY

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Abstract

Oligonucleotides (ONs) are short fragments of nucleic acids, currently being investigated as therapeutic agents. When administered subcutaneously (SC) ONs cause a specific local reaction originating around the injection site, such as erythema, itching, discomfort and pain, including more severe manifestations such as ulceration or necrosis. These injection site reactions (ISRs) are common, but rather poorly described in the literature. With this review, we aim to provide an overview on the extent of the problem of ISRs, based on reported incidence. A structured literature search was performed to identify reported incidence and clinical features of ISRs which yielded 70 manuscripts that contained information regarding ISRs. The data from literature was combined with data on file available at our institution. All SC administered ONs described in literature lead to the occurrence of ISRs. The percentage of trial subjects that developed ISRs differed per ON and ranged from 22 to 100%. The majority of ONs caused ISRs in more than 70% of the trial subjects. Severity of the observed reactions varied between different ONs. Occurrence rate as well as severity of ISRs increases with higher doses. For chemistry and target of the compounds no clear association regarding ISR incidence or severity was identified. All ONs developed to date are associated with ISRs. Overcoming the problem of ISRs might add greatly to the potential success of SC administered ONs. Knowledge of these skin reactions and their specific immunostimulatory properties should be increased in order to obtain ONs that are more suitable for long-term use and clinical applicable in a broader patient population.

Introduction

Oligonucleotides are fragments of 12-24 nucleic acids in a target-specific sequence [1]. These compounds are designed to either inhibit mRNA of the targeted protein using the antisense principle, altering the reading frame by exon-skipping, directly inhibit the targeted protein (antagonist) or bind as an agonist on the receptor (figure 1). The latter ONs are under investigation for their immunostimulatory properties, such as C-phosphate-Guanine ONs (CpGs) [2]. ONs are an attractive class of compounds since the drug synthesis and production has become fully automated, rapid and inexpensive, whereby every desired nucleic acid sequence can be generated. Over the years, different ON subclasses with distinct molecular structures have been generated. Initial ONs, dating from the early 1990s, were unmodified deoxyoligonucleotides. Around the year 2000, a phosphorothioate backbone was added to many ONs. This led to major improvement as phosphorothioate ONs are highly soluble in water, have increased nuclease stability and show excellent biologic activity [1].

Despite the numerous ON drug candidates identified and studied over the last 20 years, up to the highest clinical trial phases, only two ONs achieved marketing approval by the FDA, mipomersen (in 2013) and fomivirsen (in 1998, for the treatment of CMV retinitis). This discrepancy may be explained by frequently untoward effects induced by ONs, including nephrotoxicity, hepatotoxicity, thrombocytopenia and inflammatory responses [3-6]. Subcutaneous (SC) administration of ONs also results in the occurrence of injection site reactions (ISRs), specific local skin reactions originating around the injection site manifesting itself as erythema, induration, itching, discomfort and pain, or more severely as ulceration or necrosis. ON-induced ISRs are considered to be common, nonetheless detailed information in the literature regarding these skin effects is limited.

For example mipomersen (Kynamro®; previously ISIS 301012) the only FDA-approved ON currently available is known to cause ISRs. Mipomersen targets the mRNA for apolipoprotein B to treat homozygous familial hypercholesterolemia. Mipomersen carries a boxed warning for the serious risk of liver toxicity, which is considered to be an off-target effect [7]. Although it is known that in phase 3 trials, 5% of all treated subjects discontinued mipomersen treatment due to ISRs [8], detailed public information on ISR severity, incidence and causal mechanism is scarce. The full prescribing information of mipomersen states that the local reactions typically consist of erythema, pain, tenderness, pruritus and/or local swelling. However, no notification is made that reactions may be more severe, and/or lead to irreversible skin changes. The occurrence of ISRs is unlikely to be a specific feature of mipomersen, but a class effect of oligonucleotides.

With this review we aim to provide a comprehensive and detailed overview of the incidence, severity, clinical manifestations and pathophysiology of ON-induced ISRs after SC administration.

Materials and Methods

A structured literature search was performed to identify reported incidence and clinical features of ISRs in clinical trials up to and including February 2015. The following databases were used: PubMed, MEDLINE, Embase, Embase meeting abstracts, Web of Science, Web of Science meeting abstracts, COCHRANE, CENTRAL, CINAHL, Academic Search Premier (free text), ScienceDirect (free text), Wiley, SAGE (free text), HighWire (free text), LWW (free text). Search terms were injections site reaction or related terms and oligonucleotides or related terms. 514 hits were found, only original trials reporting on phosphorothioate ONs were included. By screening of the manuscript title 255 papers were excluded (animal/preclinical data, oligonucleotide used as adjuvant, no oligonucleotide therapy, compounds with different chemistry or no subcutaneous administration), by abstract screening another 189 results were excluded (review articles e.g. on mipomersen and CpG-structures, and above mentioned reasons for exclusion). Of the 70 results the complete papers (when available) were studied and information regarding ISRs was extracted and reported here. A cross-check was performed by a search for ONs using the Integrity Database [9] (last accessed 18 February 2015). This yielded information on 7 additional ONs clinically tested, not (yet) reported on in the public domain. Further, publicly available documents from manufacturers of ONs and regulatory agencies were screened for relevant information on ISRs.

These data were combined with safety data on file available at the Centre for Human Drug Research in Leiden, the Netherlands, where a total of 204 subjects participated in trials with 4 different ONs. These studies were conducted in accordance with good clinical practice guidelines, after approval by the Central Committee on Research Involving Human Subjects (CCMO) of the Netherlands. Two of these compounds were made anonymous by naming them ON_CHDR to protect intellectual property.

Results

INCIDENCE

The literature search yielded no review papers on ISRs. Twenty-four (24) different SC administered ONs were identified in the papers found by the literature search and the information from the Integrity Database [9]. For 19 compounds reporting was available, and for all these compounds ISRs occurred. For the other 5 compounds identified, no reporting was (yet) available (PRO044, PRO045, PRO053, IsisGCCRRx and IsisTTRRx). The data found in the search was supplemented with CHDR data on file regarding four other ONs for which also ISRs were invariably noted. An overview of incidence of ISRs associated with these ONs is provided in figure 2. The percentage of trial subjects that develops ISRs differs per ON and ranged from 22 to 100%. The majority of ONs causes ISRs in more than 70% of the trial subjects and for two ONs it was reported that all treated individuals developed ISRs. For almost all ONs the incidence of ISRs is clearly dose-dependent. This is illustrated by the incidence for mipomersen [10], ON_CHDR2, IMO-8400 and ISIS325566 (figure 3). For these four ONs higher doses are associated with higher incidence of ISRs. Although the shape of the curve differs, the trend towards higher incidence with higher dose is clear and a plateau at 100% seems to occur from a certain dose level onwards. The only ON that did not show direct dose-dependency was ISIS14803 with a 100% occurrence rate at all dose levels tested, which may reflect that the doses studied were too high to detect dose-dependency.

ISRs following SC injection of ONs are characterized by a symmetrical erythemous skin lesion around the injection site, with a diameter ranging from 4 - 15 cm. The erythema may be accompanied by discomfort, pain, itch, induration and/or ulceration (figure 4), but is usually not accompanied by lymphadenopathy. The erythema generally appears 24-96 hours after the injection. It often reaches a maximal intensity around 48 hours after injection. These data appear to be corroborated by publicly available sources reporting that the most common injection site reactions (incidence between brackets) for mipomersen consisted of erythema (59%), pain (56%), hematoma (32%), pruritus (29%), swelling (18%) and discoloration (17%) [7]. Information on severity and duration of ISRs is not readily available, but it appears that the resolution of a skin lesion differs greatly among individuals. A total of 204 subjects were actively treated with one of 4 different ONs at our centre. ISRs were reported in 122 (60%) of the subjects and complete resolution occurred in this group in over 80% of the cases. The duration to resolution ranged from 14 to 90 days. Approximately 20% of the participants developed a (semi)permanent discoloration of the skin. This manifested itself as persistent mild erythema or hypo/hyperpigmentation of the skin, which was usually smaller

in diameter than the original erythemous lesion (figure 5). Interestingly, the pooled phase III trials with mipomersen report that 7.7% (20/261) of individuals experienced reactions at a previous injection site when subsequent injections were administered at a different site; a so-called injection site recall reaction.

The severity of ISRs is generally described in the literature as 'mild to moderate'. However, in most papers the definitions of the concepts 'mild' and 'moderate' are not specified and usually no information is provided on how many of each were reported. A notable exception was the reporting on the severity of the ISRs occurring for PF3512676 for which a grading system was used (table 2) [11]. The majority of patients were reported to have an ISR of Grade 2 or lower (mild to moderate), up to 10% of patients reported an ISR of Grade 3 or greater which was defined as severe and requiring dose modification. In the combined phase III 6-month trials 5% of all mipomersen-treated individuals discontinued due to ISRs [8]. Other patient drop-outs as a result of ISRs were reported for PF 3512679 and ISIS2302 [12]. The severity profile may differ between compounds and between dose levels, however this is difficult to assess as no grading system is consistently used throughout different studies.

HISTOLOGY

Little is known of the histopathology of ISRs. The largest series currently available is from a dedicated ISR study performed with mipomersen. In this study 32 individuals had post-treatment skin biopsies of injection sites. Histological analyses of these injection sites showed that 9 of the 32 individuals had findings consistent with leukocytoclastic vasculitis e.g. infiltrating neutrophils, prominent nuclear dust, lymphocytes, and eosinophils with local macrophage infiltration [7]. The histology of a biopsy of an erythematous ISR observed in our center showed a non-specific spongiotic appearance with few eosinophils (figure 6A,B,C). The inflammatory influx was mainly perivascular and to small extent also present in pre-existing collagen, and the basal layer of the epidermis. The subcutaneous fat tissue demonstrated necrosis and infiltration with eosinophils (figure 6D).

Discussion

Since detailed information on oligonucleotide-induced ISRs is currently lacking, we conducted a systematic review of all data available in the public domain, and supported the findings with relevant data collected in clinical studies with subcutaneously administered ONs performed at the CHDR. All SC administered ONs described in literature and studied at the CHDR resulted in the induction of ISRs. ON-induced ISRs appear as symmetrical erythematous skin lesions, often accompanied by discomfort, pain, itch, induration and/or ulceration, variable in size, and with variable resolution times between compounds and individuals. This local immune response is in line with the general pro-inflammatory potential of ONs in humans, since flu-like symptoms and elevated CRP are common after SC administration of ONs [7]. Also systemic adverse reactions directly following IV infusion (fever, nausea, malaise) are commonly observed in clinical trials with ONs [13;14]. Based on the data available, it is concluded that ON dose level is an important determinant for the induction of ISRs as occurrence rate and severity increases with higher dose levels. ONs differed mutually with respect to the incidence and severity of the induced ISRs, although no clear association with a specific ON subclass was observed. Although many efforts have been made to design ONs lacking immune-stimulating effects, none of the established chemical modifications did result in the desired effect. The 5-methyl-cytosine substitution is claimed to reduce immune stimulation [7;15]. Locked nucleic acid (LNA) modifications are intended to enhance the antisense binding affinity to the mRNA target and increase its biological half-life, reducing the probability of off-target effects [16]. However, the observed incidence and severity of the skin reactions induced by these newer compounds does not differ from older ON subclasses (table 1). It is remarkable that the ONs with the highest ISR rates, ISIS 14803 and mipomersen, both include a 5-methyl-cytosine substitution. No other potential correlation between ON subclass or length and ISR incidence or severity was observed (table 1). These findings demonstrate that it is at least uncertain that further chemical modification of ONs may result in the desired lack of immune-stimulating activity. Nevertheless, it appears that chemical modifications are pursued. Examples of this approach are to introduce a so-called steric bulk at 5'-position of the sugar-phosphate backbone [17], to conjugate the oligonucleotide with peptides, proteins, carbohydrates,

aptamers and small molecules [18], or introduce receptor-binding molecules such as folate, anisamide or N-acetyl galactosamine (GalNac) or dynamic polyconjugates to the oligonucleotide [19]. An alternative strategy may be to alter the delivery of ONs in a manner which may bypass local immune responses. Altered delivery can be obtained using chelation [20] or the use of nanoparticles or liposomes [21]. Moreover, avoiding ISR s could be achieved by oral administration of ONs, a concept that is currently investigated [22]. Whether these strategies are safe and efficacious in humans remains to be determined.

Our review demonstrates that ISRs after SC administration of ONs are a serious problem. Obviously, severe and long-lasting local inflammatory responses upon SC use of ONs are debilitating for potential future patient populations. In addition, relatively mild discomfort such as itch and cosmetic aspects like erythema and altered pigmentation may jeopardize adherence to therapy, particularly upon chronic use. This is illustrated by the relatively high percentage of participants in phase 3 mipomersen trials discontinuing therapy early due to the occurrence of ISRs [8]. Ultimately, the potential value of SC ON therapy is dependent on the severity of a particular disease and availability of alternative therapies. For example, ISR-inducing SC ON therapy may be acceptable for patients suffering from an otherwise untreatable malignancy or lethal muscular dystrophy, whereas hypercholesterolemic patients would not readily consider the use of such a therapy.

For the future clinical application of SC ONs, detailed understanding of the mechanisms causing the skin reactions is essential. It is unlikely that the occurrence of ISRs can be explained by the ON target or the target distribution. For example, ISIS 14803 and miravirsen both target RNA replication of the Hepatitis C Virus (HCV), however the compounds have the highest and second lowest ISR rates (table 1). It does not seem that the dermal localization of the ON target predisposes to a higher ISR occurrence rate: the target of the ON with the highest incidence is expressed hepatically (ISIS 14803), whereas CPG10101 that has a target in the skin shows a remarkably low incidence in ISRs. This is interesting given the fact CpG ONs are intended to act immunostimulatory. Unlike assumed earlier however, it was shown that TLR9 activation is not confined to the compounds containing unmethylated CpG, but depends on backbone structure [23;24]. In conclusion, the induction of ISRs by SC administration of ONs may be a class effect inherent to the physical-chemical nature of the compounds, which can potentially be circumvented by further chemical modification, although published evidence is currently lacking. Further, we argue that rational development of tailored ON subclasses could benefit from in-depth knowledge on the relationship between chemical modifications and the molecular pathways involved in the immune responses causing the ISRs.

MOLECULAR TARGETS

Which molecular targets may be implied in the observed ON-induced immune responses? The skin functions as a mechanical barrier, and also as a first line immune defense, comprising innate and adaptive immune mechanisms [25]. The skin contains a mixture of immune cells: keratinocytes releasing cytokines and chemokines in response to injury, resident dendritic cells, macrophages, innate, NK- and helper T lymphocytes, and mast cells in the dermis [26]. ON-mediated activation of the immune system is likely to start in the dermis. Unfortunately, relatively limited information is available on the specific pathophysiology of ISRs. In general, drug-induced skin reactions are non-specific hypersensitivity-like responses, characterized by dermal edema with perivascular and interstitial acute and chronic inflammation with involvement of neutrophils, eosinophils and lymphocytes [27-29]. This is compatible with the findings from biopsies performed upon occurrence of ISRs after SC administration of mipomersen [7]. Similar histological responses were observed in a clinical study with a ribozyme construct, chemically resembling an ON [30]. Specific features of ON-induced ISRs include the involvement of immunological memory, as demonstrated by the injection site recall reactions related to previous exposure to the ON [7]. Also, hyper- and hypopigmentation is incidentally observed after administration of mipomersen. They should be considered non-specific post-inflammatory clinical sequellae, also occurring in skin diseases including acne vulgaris, atopic dermatitis, skin infections and allergic reactions [31]. The response is known to result from the activation of melanocytes with overproduction of melanin or an irregular dispersion of pigment, but the exact mechanism underlying post-inflammatory hyperpigmentation is unknown [32]. Some scattered histological information is available on immune responses in ON-induced ISRs, but this information does not tell which specific molecular pathways are driving the initial immune

response. The innate immune system, being the first line of defense, comprises different subsystems for the protection of the body against pathogens. The most likely initial drivers of the ON-induced immune response are innate cytosolic sensors and Toll-like receptors (TLRs) and the complement system, two innate immune pathways that may act synergistically [33]. The TLR system comprises different pattern recognition receptors sensing a variety of pathogens ranging from bacteria to fungi, protozoa, and viruses [34]. More particularly, the cell-membrane bound TLR2 and TLR4 can be activated by viruses and viral components, as are the endosomal TLR3, TLR7/TLR8 and TLR9, which sense double-strand RNA, single-strand RNA, and CpG DNA, respectively. Since ONs are short nucleic acid sequences, which may mimic viral sequences, they could theoretically trigger innate cytosolic sensors such as retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated gene 5 (MDA-5), TLR3, TLR7/8, resulting in MyD88 (TYPE I IFN) and NF-KAPPA-B and the expression of pro-inflammatory cytokines by dendritic cells and macrophages [35-39]. Complement activation is known to play a key role in dermatological inflammatory conditions [40]. Leukocytoclastic vasculitis has been demonstrated in ISR biopsies of mipomersen-treated human subjects [7], which implies the potential involvement of complement since in this type of vasculitis complement complexes and perivascular complement deposits are commonly observed [41]. Leukocytoclastic vasculitis (also known as hypersensitivity vasculitis / angiitis) is commonly confined to the skin and is caused by vascular damage due to nuclear debris from infiltrating neutrophils. The most common cause is secondary to medications. The common clinical observations fit the findings for ON-associated ISRs as the majority of the lesions are acute and show resolution in weeks to months, but 10% turns into a chronic condition characterized by persistent lesions or intermittent recurrence. These chronic lesions eventually develop into morphea (also known as localized scleroderma or circumscribed scleroderma), a condition consisting of patches of hardened skin with no internal organ involvement. Interestingly, the treatment for leukocytoclastic vasculitis is to stop the causative agent and to avoid steroids. The latter would explain why attempts to reduce IISR using steroids have failed. However, based on the data available in the public domain it remains difficult to draw firm conclusions on potential TLR and complement activation in the skin upon SC ON treatment. The occurrence of ISRs appears to be species-dependent, which is not surprising given the large differences in the immune system response between species [42]. For example, monkeys are more sensitive to oligonucleotide-induced complement activation than humans [43;44]. However, dedicated animal studies may shed light on the mechanisms underlying ISRs: in mice and non-human primates immunostimulatory effects of ONs were observed [2;45-47], which demonstrates the potential relevance of these animal models for mechanistic studies. Subcutaneous administration of phosphorothioate ONs to rodents resulted in local swelling and induration at the injection site, with mononuclear cell infiltrates [46;48], lymphoid hyperplasia and multiorgan lymphohistiocytic cell infiltrates [43;49;50]. Administration of CpG-containing ONs to mice resulted in TLR9 activation [35]. Furthermore, experiments in non-human primates have shown that phosphorothioate ONs may activate the alternative complement pathway [51;52], possibly by interaction with Factor H [52;53]. It is uncertain how these preclinical data translate to humans, but when combined with tailored preclinical studies applying human cell cultures or mouse models with a humanized immune system, potentially involved mechanisms in ON-induced ISR development in man may be identified. The involvement of specific TLR pathways and complement in ON-induced ISRs could be systematically explored in a clinical trial with mipomersen, a commercially available oligonucleotide that is considered to be safe, but does induce ISRs. Thorough investigation of biopsies from skin lesions with dedicated immunohistochemical staining may shed light on the exact immunological mechanisms involved. In addition, we would advocate standardized quantitative and qualitative assessments of skin reactions in all future clinical studies with SC administration of ONs including immunohistochemistry and electronmicroscopy. This would provide more insight into the course of the development of the lesions, and allow a more structured comparison between different compounds.

In summary, all ONs tested in clinical studies have been reported to induce ISRs, reflecting a drug class effect. Detailed information on ISRs in the experimental setting is currently lacking. It is recommended to perform a uniform and standardized assessment of the skin reactions for all future studies with ONs, to gain more insight and to allow comparison between different compounds. This assessment should include a standardized way of reporting the clinical features, scoring severity and reporting duration (table 3). Also performing standardized medical photography and

biopsies from affected skin could add greatly to the current knowledge. More recent ON subclasses with specific chemical modifications aiming to avoid immunological skin responses have at present not been successful to completely prevent occurrence of ISRs. The pathophysiology underlying the ISRs and the causative immune pathways remains speculative. The initial immunological activation is likely to be driven by specific TLRs and complement. However, the exact involvement of these pathways has not been studied in detail, or alternatively not reported upon in the public domain. We therefore advocate a systematic approach to elucidate the immune-stimulatory effects of oligonucleotides, by performance of dedicated clinical and preclinical studies. In-depth knowledge on the exact mechanisms underlying these skin reactions will be of importance for the future of all ONs, not only the ones administered subcutaneously. In parallel, strategies to diminish or limit the skin response induced by ONs should be considered. It appears that neither systemic or locally applied corticosteroid treatment prevent development of ISRs [54], but other treatments that have been explored for leukocytoclastic vasculitis may be considered [55]. Further, local ON exposure should be limited by restricting the dose to the minimal level exerting the desired clinical effect, and possibly by spreading an effective total dose over multiple administrations, an approach demonstrated to be effective for mipomersen [56].

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Figure 1 The different modes of actions for therapeutic oligonucleotides. A. activation or inhibition of the target protein to induce or inhibit immune activation B. exon skipping to induce alternative splicing. C. inhibition of mRNA with antisense ON, inhibits production of the protein.

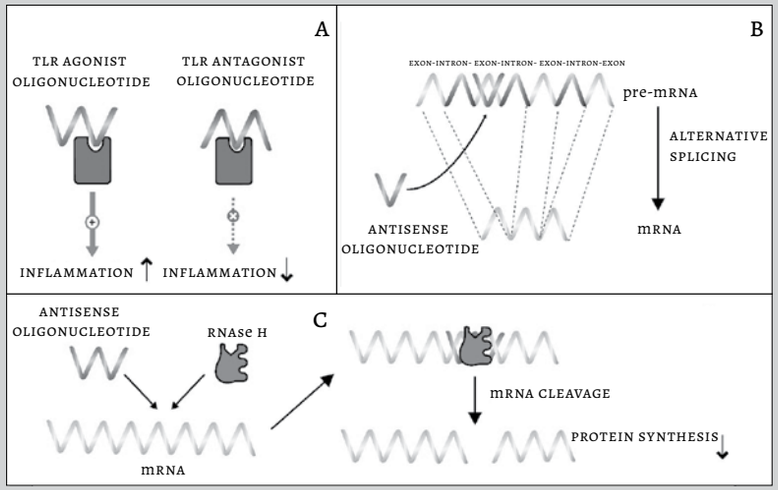


Figure 2 All 21 SC administered ONs resulted in ISRs. Incidence ranged from 22 to 100%. For 4 ONs no incidence numbers were reported, namely ISISAPO(A)RX, ISIS113715, ATL-03 and ISISGCGR-RX. For ONs that were studies at different dose levels and/or multiple trials, an average ISR occurrence was calculated.

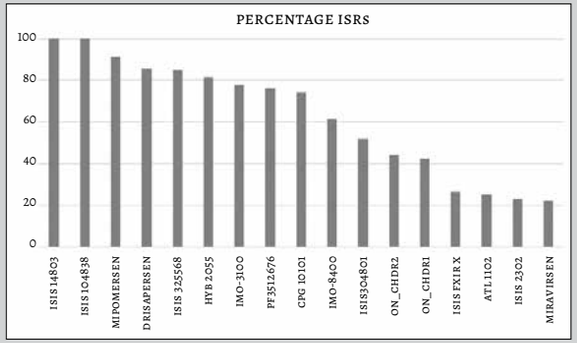
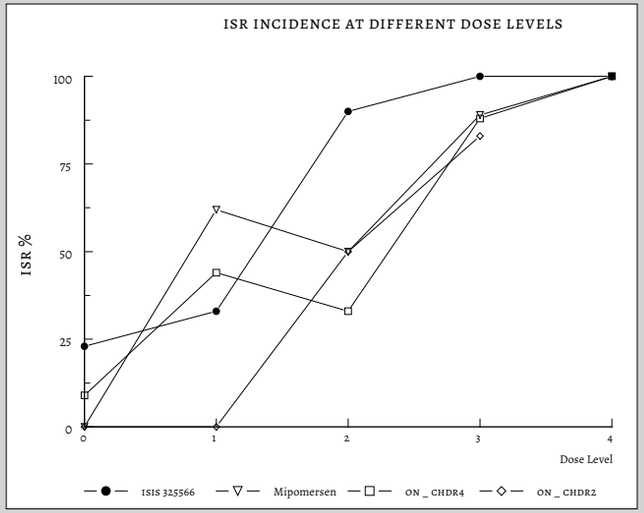


Figure 3 Dose-dependent occurrence of ISRs after administration of four different ONs. Higher dose levels result in increased incidence of ISRs up to 100% at the highest dose level. The dose levels tested for ISIS32566 and Mipomersen [10] were placebo (0), 50, 100, 200 and 400 mg. For IMO-8400 dose levels were: placebo (0), 0.075, 0.15, 0.3 and 0.6 mg/kg, and for ON_CHDR2 dose levels were placebo (0), 0.5, 1.5 and 5 mg/kg.



For figure 4, 5,6: see inside cover.

Table 1 Listing of clinically tested oligonucleotides. PHON = Phosphorothioate Oligonucleotide, LNA = locked nucleid acid structure, 2MOE = 2'-O-MOE structure, 5MCS = 5-methyl-cytosine substitution, CPG = Cytosine triphosphate deoxynucleotide-Guanine triphosphate deoxynucleotide

Name	Structure	Length	MOA	Indication	N*	Ref.	ISR %
ISIS 14803	PHON 5MCS	20 units	Inhibits HCV RNA synthesis	Chronic HCV Infection	1	(19)	100
ISIS 104838	PHON, 2MOE	20 units	Inhibits TNFα	Rheumatoid arthritis, Crohn's disease and psoriasis	1	(56)	100
Mipomersen	PHON, 2MOE 5MCS	20 units	apoB synthesis inhibitor	Hyper-cholesterolemia	19	(13; 55; 57-73)	91.2
Drisapersen	PHON, 2MOE	20 units	Induces Exon 51 skipping in DMD	Duchenne's Muscular Dystrophy	6	(54; 74-78)	85.5
ISIS325568	PHON, 2MOE	20 units	Inhibits GCCR	Diabetes Mellitus Type 2	1	(79)	85
HYB 2055	NR**	NR	Activates TLR 9	Cancer	1	(80; 81)	81.3
IMO-3100	PHON	18 units	Inhibits TLR 7,9 activation	Psoriasis	1	(82)	65
PF 3512676	PHON, CPG	24 units	Activates TLR 9	As adjuvant of vaccination/ chemotherapy	5	(83-87)	76.3
CPG 10101	PHON, CPG	22 units	Activates TLR 9	Hepatitis C (HCV) Infection	3	(88-90)	74.5
ON_CHDR2	PHON, LNA	14 units	undisclosed	undisclosed	-	-	72.2
IMO-8400	PHON, 2MOE	18 units	Inhibits TLR 7, 8, 9 activation	Psoriasis	1	(91)	61.5
ISIS304801	PHON, 2MOE	20 units	Inhibits Apolipo-protein C-III	Dyslipidemia	1	(92; 93)	52
ON_CHDR1	PHON, 2MOE	12 units	undisclosed	undisclosed	-	-	42.7
ISISFIXRX (Isis416858)	PHON, 2MOE	20 units	Reduces human factor XI	Prevention of thrombosis	2	(94; 95)	33.3
ATL1102	PHON, 2MOE	20 units	Inhibits CD49d	Relapsing-remitting multiple sclerosis	1	(96)	25
ISIS 2302	PHON	20 units	Inhibits ICAM-1 expression	Crohn's Disease	1	(16)	23.3
Miravirsen	PHON, LNA	15 units	Inhibits miR-122	HCV Infection	2	(97; 98)	22.2
ISISApo(a) RX	NR	NR	Inhibits apolipo-protein (a) protein	Coronary Artery Disease	1	(99)	NR
ISIS113715	PHON, 2MOE	20 units	Inhibits PTP-1B protein	Diabetes Mellitus Type 2	2	(100; 101)	NR
ATL-03	PHON, 2MOE	20 units	Inhibits GHR Expression	Acromegaly	1	(12; 102)	NR
ISISGCCR-RX	Not reported	NR	Inhibits GCCR	Diabetes Mellitus Type 2	1	(103)	NR

** # Number of Studies found in literature ** Not Reported*

Table 2 An example of an ISR grading system to score the severity used for PF3512676 [11]

Grade 1	mild (does not interfere with daily life)
Grade 2	moderate (interferes with daily life but no dose modification)
Grade 3	severe (requires dose modification)
Grade 4	disabling (requires drug discontinuation)

Table 3 Suggested uniform standardized ISR scoring system. ADL = 'Activities of Daily Living' and are defined as bathing, dressing and undressing, feeding self, using the toilet, taking medications, preparing meals, shopping for groceries or clothes, using the telephone etc.

	0 = No	1 = Mild	2 = Moderate	3 = Severe and undesirable
Injection site reaction	None	Erythema OR Tenderness OR Itching	As 1 and Pain OR Swelling OR Signs of inflammation	Ulceration or necrosis
Maximal diameter ISR	NA	Max 5 cm	Max 10 cm	Max 15 cm OR any diameter and systemic reaction OR flare up previous IS
Duration of symptoms	≤1 day	2-14 days	2-6 weeks, reversible	Permanent
Sequelae	None	Minimal and tolerated by patient	Hardly tolerated OR wish for treatment by patient	Permanent despite treatment OR no treatment options
Likely impact on next dose	None	Injection site can be used in rotation AND no dose adaptation	Injection site should be avoided in rotation OR change dose regimen	Injection site cannot be used anymore OR discontinuation
ADL limitations	None	Minimal	Functional	Self-care limitations