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ORI-1001

Anti-Papilloma Virus Oligonucleotide

20-mer phosphorothioate oligonucleotide whose sequence is: 5'-GTACCTGAATCGTCCGCCAU-3', in which the first fifteen ribonucleotides are 2'-deoxynucleotides and the last five nucleotides are 2'-*O*-methoxy substituted ribonucleotides

EN: 271811

Abstract

The concept of antisense has been applied as a therapeutic strategy in a number of disease areas over the past 15 years. The antisense approach involves the use of a short synthetic oligonucleotide that is designed to complex with and inhibit the translation of target mRNA that encodes the protein. ORI-1001 is a 20-mer hybrid oligonucleotide that has been developed as a novel and potent topically applied antiviral agent against HPV6 and HPV11, which are associated with genital warts. It has demonstrated efficacy in two different animal models of HPV-associated genital warts. ORI-1001 is the first topically applied antisense agent with potential specific antiviral mechanism for the treatment of HPV-associated genital warts.

Introduction

Over the past 15 years, the concept of antisense has been applied as a therapeutic strategy in a number of disease areas. The antisense approach involves the use of a short synthetic oligonucleotide that is designed to complex with and inhibit the translation of target mRNA that encodes the protein. In general, the translation initiation site in an mRNA appears to be most susceptible to the antisense effect and therefore has been the preferred site for targeting. A combination of mechanisms enumerated below may be operative in the antisense-mediated downregulation of mRNA that results in the inhibition of synthesis of the target protein (1-3).

(a) Once inside the cell, the synthetic oligonucleotide can hybridize with the complementary site on the RNA by Watson-Crick base-pairing and produce blockade of translation. This is referred to as "translation arrest".

(b) The oligonucleotide-RNA complex becomes a substrate for intracellular RNase H which degrades the target mRNA.

(c) Binding of oligonucleotides to RNA may inhibit interactions of RNA, or DNA with proteins, and other nucleic acids such as tRNA leading to the inhibition of the function of mRNA in the cell. This is known as "occupancy-only mediated mechanism".

(d) Binding of an oligonucleotide to regions in the pre-mRNA could inhibit splicing and prevent formation of mRNA.

The antisense oligonucleotide is designed to be specific and selective for the target mRNA and is expected to have the following properties: (a) chemical stability and resistance to degradation by nucleases, (b) cellular uptake and intracellular stability, (c) high binding affinity and selectivity for complexation with the target RNA and (d) ability to activate RNase H enzyme that induces the cleavage of RNA.

The choice of a 15- to 20-mer oligonucleotide as an antisense molecule is dictated by a number of considerations: (a) high binding affinity of the oligonucleotide for the target, (b) stability of the complex formed between oligonucleotide and mRNA and (c) selective sequence-specific interaction of the oligonucleotide with the target RNA. The ability to activate RNAse H appears to be associated with the nature of the backbone and substitution in the sugar ring of the oligonucleotide (4). Thus, the negatively charged PS-, and PO-linked deoxyoligoribonucleotides activate, whereas the corresponding ribonucleotides seem to lack this activity.

For the therapeutic development of antisense oligonucleotides, additional considerations are important. Thus, due to their high molecular weight (typically 4500-9000 dalton), with multiple negative charges on the backbone and high water solubility, the absorption, distribution, metabolism and elimination (ADME) attributes of oligonucleotides are quite different from a typical small-molecule drug. For example, it appears that most antisense compounds enter the cells by active transport, unlike most small-molecule drugs that enter cells by passive diffusion. Furthermore, the cellular uptake of phosphorothioate oligonucleotides is concentration- and temperature-dependent, characteristic of an active transport process.

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In vivo, the bioavailability, pharmacokinetics and metabolism of oligonucleotides vary depending on the chemical modifications in the oligonucleotide. Typically, phosphorothioate oligonucleotides are very rapidly distributed (within 15-25 min) into various tissue compartments following their absorption and are eliminated over a prolonged period (>24 h). As expected, the pharmacodynamic properties, defined as the binding affinity of the oligonucleotide for the complementary RNA, is affected by incorporation of chemical modifications and the presence of secondary structure elements in the oligonucleotide (5, 6).

These principles are exemplified by the discovery and development of ORI-1001 as the first topically applied antisense oligonucleotide for the treatment of genital warts. The sections to follow provide a summary of the results obtained both *in vitro* and *in vivo*.

Human Papillomavirus Infections

Human papillomavirus (HPV) is the most common sexually transmitted virus in the world today. HPV is more prevalent in young adults than in older adults, and is most commonly found among those with multiple sex partners and early age of first intercourse. For example, in a recent survey (7), up to 40% of women between 15 and 25 years of age tested positive for the presence of HPV DNA with about 1% of the population being affected by genital warts. Higher rates of cervical infection have been reported in the developing world (8).

More than 220 established and tentative HPV genotypes have been recognized. Each genotype has a particular predilection for certain anatomic sites and disease processes. For example, HPV6 and HPV11 are most frequently associated with condylomata acuminata or genital warts and oral squamous papillomas. HPV6 and HPV11 have been found in laryngeal papillomas and their extensions, nasal papillomas, in lesions such as sebborrheic keratoses, actinic keratoses, keratoacanthomas and epidermoid cysts. HPV11 is also associated with focal epithelial hyperplasia or Heck's disease.

According to the Centers for Disease Control, genital warts account for more than two million annual visits to clinics in the U.S. with 750,000 new cases being diagnosed each year. Diagnosis of anogenital warts is primarily made by the visual inspection of the external female or male genitalia and perianal area. Most treatment modalities for genital warts are based on the ablation or destruction of the lesions by physical or chemical, nonspecific methods such as cold-blade excision, electrosurgery, laser surgery, cryotherapy, topical application of trichloroacetic acid, podofilox (podophyllotoxin) and 5-fluorouracil. Intralesional interferon injections and topical application of imiquimod, an inducer of alpha-interferon and other cytokines, represent alternative approaches. However, all the current therapies are aimed at eliminating the warts (the disease) but not the HPV infection itself and in addition have treatment-associated side effects. Furthermore, a treatment that achieves only the disappearance of HPV lesions does not ensure the lack of disease recurrence and disease transmission. Thus, there remains a significant unmet medical need for the treatment of HPV infection. Therapeutic approaches aimed at inhibiting HPV replication are thus needed not only to prevent recurrence of the disease but also to prevent transmission.

ORF	Function
E1	DNA replication
E2	Transcription regulation and DNA replication
E4	Cytoplasmic protein cytokeratins
E5	Membrane protein tyrosine kinase
E6	Binds to p53 oncoprotein
E7	Binds to Rb oncoprotein
L1	Major caspid protein
L2	Minor caspid protein

Fig. 1. Genomic map of HPV.

Genomic Map of HPV

The genome of all HPV types consists of a circular double-stranded DNA of approximately 7900 base pairs (Fig. 1). The genome can be divided into three distinct functional domains: (i) the upstream regulatory region which contains the origin of replication, as well as binding sites for enhancers and promoters involved in transcription, (ii) the "early" (E) region which encodes genes involved in replication and (iii) the "late" L region that encodes the structural proteins L1 and L2 (9, 10).

The viral proteins are translated from families of alternately spliced mRNAs and have several functions. The viral protein E1 is extremely important in HPV infection, as it is required for episomal DNA replication and is necessary for persistent infection and lesion formation. E1 is ubiquitous in most HPV types, including HPV6 and HPV11. Certain regions of E1 RNA of HPV6 and HPV11 have 100% sequence homology to one another and are highly conserved across the genome. The protein E2 controls transcription and may be involved in the initiation of DNA replication, while E4 interacts with cytokeratins. The proteins E5, E6 and E7 are involved in cell transformation of normal cell to cancer cell.

Downregulation of E1 can inhibit the replication of the viral genome and hence halt the infection of epithelial cells by HPV and therefore prevent the initiation, persistence and growth of warts. Thus, therapeutic strategies aimed at inhibiting the biosynthesis of E1 could lead to the development of potent anti-HPV compounds. Certain

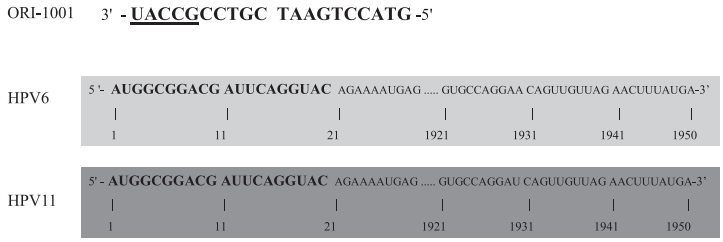

Fig. 2. ORI-1001 is an antisense oligonucleotide complementary to the nucleotide region 1-20 of E1 mRNA in HPV6 and HPV11.

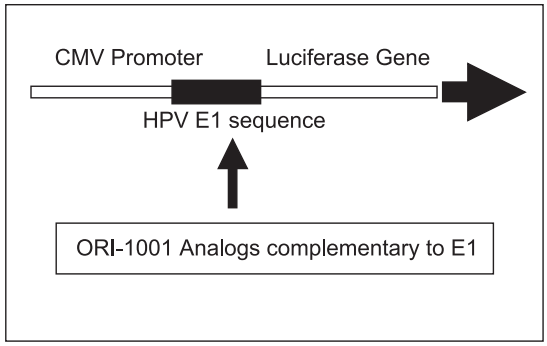

Fig. 3. Discovery strategy employed for ORI 1001. The E1-luciferase fusion construct consisted of 46 nucleotides spanning the translation site of HPV6b E1 gene that is installed between a CMV promoter and luciferase gene in a plasmid. The E1 target and luciferase gene were then subcloned by PCR and inserted into the pcDNA3 vector. These constructs were stably transfected using lipofectamine into CHO-K1 cells. Several geneticin-resistant luciferase-expressing clones were selected at random for each construct. Alternatively, the fusion construct was also employed in a transient transfection assay using CHO cells.

regions of E1 RNA of HPV6 and HPV11 around the AUG initiation site have 100% sequence homology to one another and are, therefore, ideal for targeting using the antisense approach. ORI-1001 is designed as an antisense oligonucleotide that targets the E1 mRNA of HPV6 and HPV11 (Fig. 2) and inhibits translation.

As an antisense oligonucleotide complementary to E1 mRNA, ORI-1001 appears to inhibit translation by binding to the AUG start site of the target E1 mRNA and block the ribosomal assembly and/or activate RNAse H to cleave the E1 mRNA. Being a “hybrid” oligonucleotide with a segment of deoxyribonucleoside phosphorothioate flanked by a segment of 2’-OMe ribonucleoside phosphorothioate at the 3’-end, ORI-1001 is highly resistant to nucleases and shows high affinity for complementary mRNA.

Discovery of ORI-1001

ORI-1001 was discovered after screening a number of oligonucleotides that are complementary to regions of E1 mRNA for their ability to downregulate E1 RNA and the derived protein. Cell-based assays described below were utilized for screening the oligonucleotides.

Development of cell-based assays

The life cycle of papillomavirus is intimately linked to the differentiation state of the keratinocytes. Thus, papillomaviruses cannot be readily propagated in cell culture because it is difficult to mimic the proliferation and differentiation cycle of the epithelium. Consequently, the development of assays aimed at discovering antiviral activity against HPV has remained a challenge. In our approach, a portion of the HPV E1 gene was cloned into a plasmid adjacent to a luciferase reporter gene under the control of a CMV promoter. This construct was transfected into cells and clones were selected using antibiotic resistance. A series of oligonucleoside phosphorothioates targeted to the E1 mRNA were evaluated for their ability to selectively inhibit luciferase expression (Fig. 3). The downregulation of luciferase expression correlated with the inhibitory activity of oligonucleotides on E1 mRNA. Of a number of oligonucleotides screened in this assay, ORI-1001 was among the most potent. ORI-1001 is a hybrid oligonucleotide containing both deoxyribonucleoside phosphorothioate (PS) and 2’-OMe ribonucleoside phosphorothioate segments. Both ORI-1001 and the corresponding PS analog were the most potent analogs with an EC50 of approximately 50 nM (Fig. 4) (11).

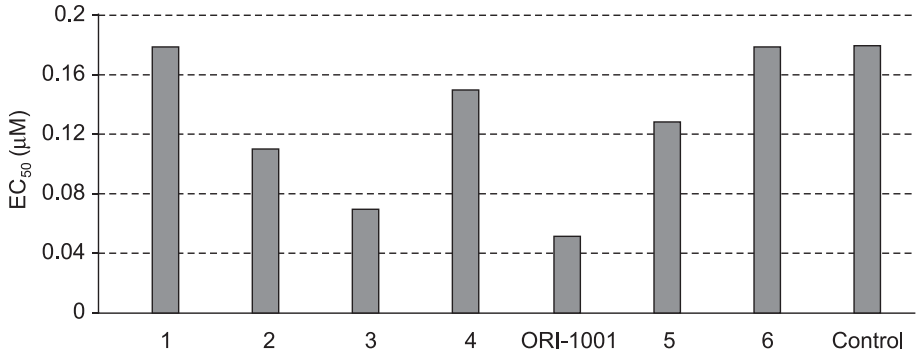

Fig. 4. *In vitro* inhibition of E1 RNA by oligonucleotides using luciferase assay. Stably transfected CHO cells were seeded into 96-well plates. Cellfectin was diluted to a concentration of 4 μg/ml in Optimem serum-free medium and 100 μl dispensed into each 96-well plate. Antisense oligonucleotide 1-6 and ORI-1001, as well as mismatch control were diluted to 5 or 25 μM in 4 μg/ml cellfectin in Optimem and 25 μl used for each well. Four hours after the addition, the wells were aspirated and 100 μl CCM5 medium dispensed into each well. The plates were incubated overnight at 37 °C. The cells were washed twice with PBS and lysed in 50 μl cell-lysis buffer. Luciferase activity was measured in 20-μl lysate using a luminometer. In parallel, cell viability assay was also performed.

Studies in CHO-K1 cells

To confirm the validity of the E1-luciferase enzyme assay, which measured luciferase expression as a surrogate marker for the expression of the actual E1 RNA, CHO cells were stably transfected with the entire reading frame of E1 along with 103 nucleotides of the 5'-untranslated region. These cells were treated with 100 nM antisense, random control (where the sequence is scrambled), as well as mismatch control oligonucleotides. Following incubation, cells were lysed, total RNA isolated and hybridized with labeled probes for either E1 message or actin message and levels of each transcript measured by quantification of label intensity in a phosphorimager. Thus, whereas cells treated with ORI-1001 reduced E1 mRNA by 80%, cells treated with mismatch or randomer oligonucleotide (a compound where the sequence of ORI-1001 is scrambled) did not produce a decrease in the level of E1 mRNA. Furthermore, cellular actin mRNA level was unaffected, indicating that ORI-1001 produced selective downregulation of E1 expression consistent with an antisense mechanism. It is notable that incorporation of one mismatch (G to A) did not noticeably affect the sequence-specific antisense activity, but two or more mismatches abrogated the activity of ORI-1001. Indeed, incorporation of one mismatch (replacement of G by A) also retained the RNase H activity of ORI-1001. Thus, there appears to be a correlation between RNAse H function and antisense activity in the case of ORI-1001 analogs.

These experiments confirmed that antisense activity of ORI-1001 in the E1-luciferase surrogate assay that was employed in routine screening correlated with direct reduction of EI mRNA and protein levels.

Effects of chemical modifications on antisense activity

ORI-1001 is a chemically modified analog derived from the corresponding parent PS analog. A number of chemically modified analogs were therefore evaluated for antisense activity in stably transfected CHO K1 cells and compared to the PS analog. For example, introduction of five 2'-OMe ribonucleoside chemical modifications at the 3'-end (*i.e.*, ORI-1001), or both the 3'- and 5'-ends, increased antisense activity compared to the corresponding PS analog (Fig. 5). However, introduction of methylphosphonate modifications reduced the activity of the compound. Analogs having uniform 2'-OMe RNA PO or PS linkages were also inactive. As is well known, the complex formed between 2'-OMe ribonucleoside oligonucleotide and RNA is not a substrate for RNAse H activity. These studies with modified analogs suggest that there may potentially be a role for RNAse H in the *in vitro* antisense activity of ORI-1001 (Fig. 6). Finally, oligonucleotides of increasing lengths and incorporating different chemical modifications were evaluated to determine whether more potent compounds could be obtained. Although analogs with longer chain length retained antisense activity, there was no significant increase in potency.

In Vivo Studies

Based on these cell culture studies, ORI-1001 was found to be the most potent inhibitor of E1 expression and was therefore selected for further evaluation in two different animal models of HPV infection.

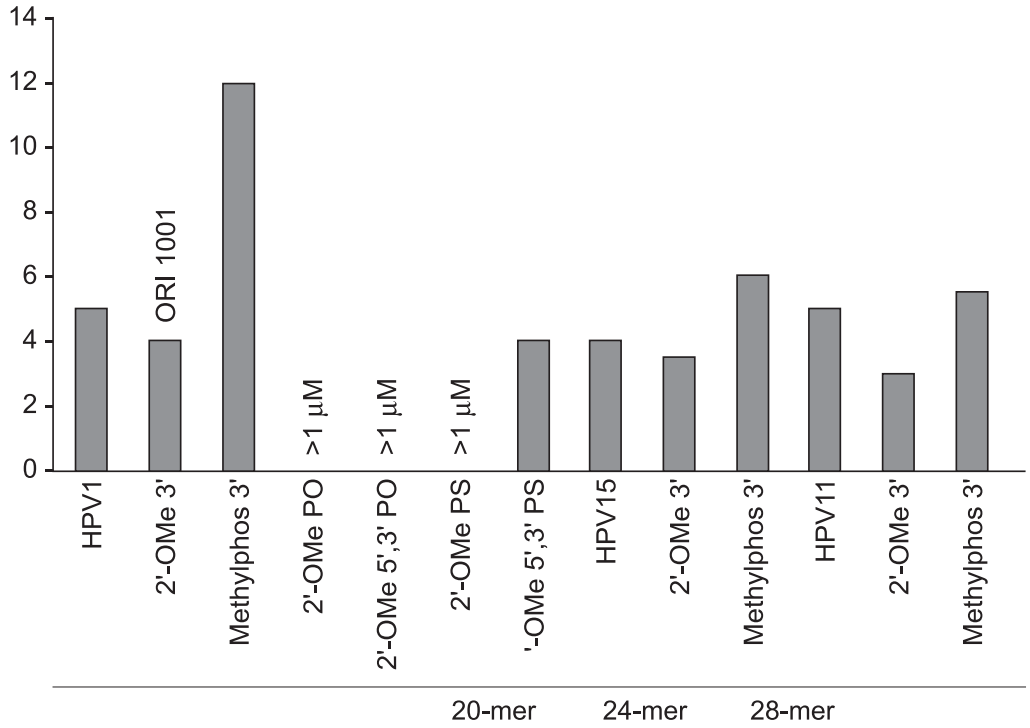

Fig. 5. *In vitro* inhibition of E1-mRNA by oligonucleotides, evaluation of length and chemical modifications. Examples of modified oligonucleotides include those that contained segments of 2'-OMe ribonucleotides and methylphosphonate linkages. Please note that in this figure the designations HPV1, HPV11 and HPV15 refer to compounds and not HPV types.

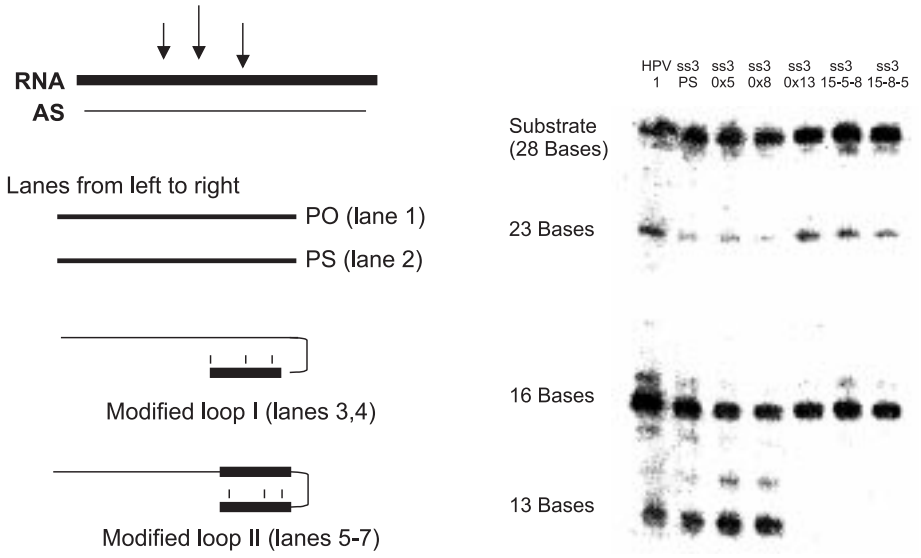

Fig. 6. Evaluating the impact of various chemical modifications in ORI-1001 for RNase H activation, a representative example. Chemical modifications were performed in a looped design corresponding to ORI-1001. The modifications were placed on one or both segments of the loop indicated by the bold lines. A complementary 28-mer RNA template was employed for these studies. The arrow indicates the site of cleavage of the template RNA.

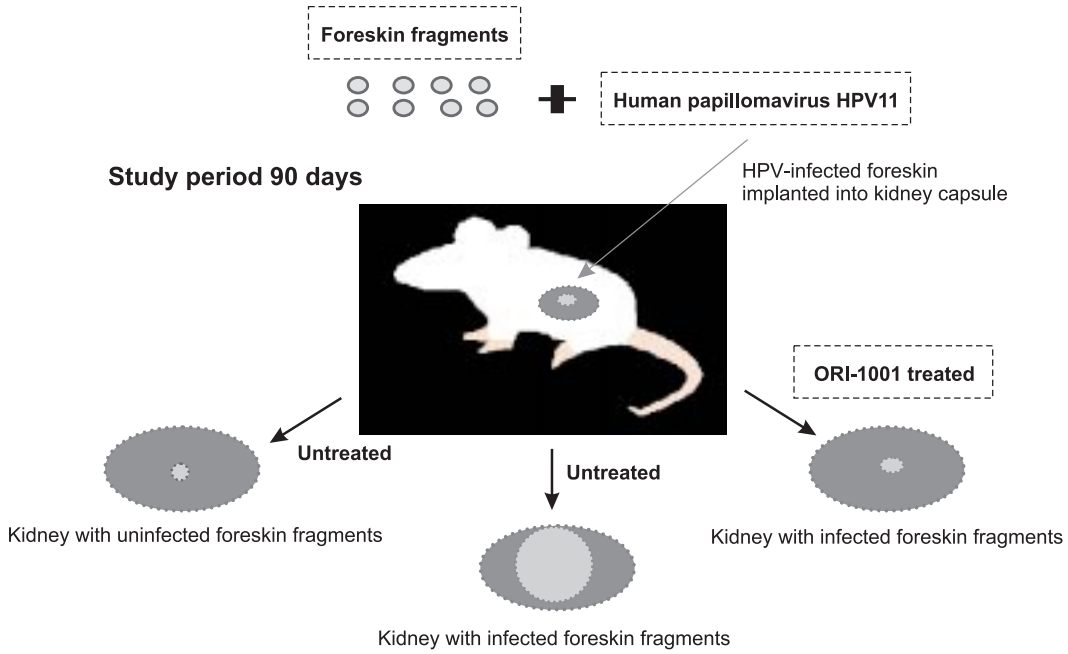

Fig. 7. Kreider model, the internal xenograft model in nude mice. In a typical Kreider experiment, 10 mice each with 2 grafts (1 per kidney) were dosed s.c. with ORI-1001 for a period of 90 days. As controls, 10 mice each with 2 grafts were treated with saline and with mismatch oligonucleotide. Mice were killed by cervical dislocation, kidneys with the cysts were removed and the cyst size measured. The cyst size was expressed as gross mean diameter (GMD) or as cyst volume. After determination of cyst volumes, the kidneys were fixed in neutral buffered formalin, embedded in paraffin, sectioned at 6 microns and stained with hematoxylin and eosin. The cysts were also evaluated for koilocytosis, paraketosis and acanthosis, which are the markers for the presence of HPV.

Kidney capsule implant model

The kidney capsule implant model (Kreider model) is a useful and relevant model for studying HPV-induced warts (12) and was therefore employed for evaluating the in vivo efficacy of ORI-1001. In this model, fragments of human neonatal foreskins infected with HPV6 are implanted subcutaneously under the renal capsule of nude (athymic) mice. This results in the growth of a cyst or wart-like structure due to replication and proliferation of HPV (Fig. 7). ORI-1001 was administered subcutaneously during the course of the experiment. At the end of a 90-day period, anti-HPV activity of ORI-1001 was determined as a measure of reduction in condyloma growth and a reduction in number of koilocytes (peculiar vacuolated cells that are the hallmarks of HPV infection) that was indicative of antiviral effect.

In several repeated studies that included dose-ranging and dosing regimen protocols, ORI-1001 produced a dose-dependent reduction of condyloma development by up to 90-95% when administered by the s.c. route (Fig. 8). ORI-1001 was effective both when administered during the full condyloma development period of 90 days or when administered during the first month of condyloma growth. The mismatch control did not inhibit cyst growth. No significant systemic toxicity was apparent at any of the doses studied. Histology of the cysts revealed that the cysts from vehicle-dosed animals showed a pronounced thickened cyst wall (caused by acanthosis), large vacuoles in the center of the cyst (caused by koilocytosis) and keratinized squames with dark visible nuclei (parakeratosis), whereas those derived from ORI-1001-treated animals showed a thinner basal layer and keratinized squames with few nuclear inclusions. This reduction in virus-induced koilocytosis and acanthosis suggested a reduction in viral loading produced by ORI-1001 administration.

Mechanistic studies

It is interesting to note that the ORI-1001 analog that has methylated cytosine nucleobases adjacent to the guanine also caused a dose-dependent reduction in cyst size in the Kreider experiment. That the observed anti-HPV activity was indeed due to the “intact” C-methylated analog was also confirmed by electrophoretic and mass spectral analysis of oligonucleotide extracted from tissues (Fig. 9). In the capillary electrophoresis profile of the oligonucleotide extracted from various tissues including kidney, there was clear presence of full-length C-methylated oligonucleotide. Also, the mass spectrum of the oligonucleotide extracted from tissues showed the molecular ion corresponding to the methylated analog, thus confirming its structure.

- Each group of 10 balb/mice implanted with HPV 11 infected foreskin (from children under 5 years)
- Doses of ORI-1001. At 2, 5, 10 mg/kg s.c. and doses of mismatch at 2, 5 and 10 mg/kg daily, 90 days
- Control mice (infected) injected with saline
- One control group implanted with noninfected foreskin

- The cyst sizes treated with ORI-1001 are indicated by darks bars and those with mismatch oligonucleotide by lighter bars
- Sequence-specific, dose-dependent reduction in cyst size is seen. Data supports potential antisense mechanism

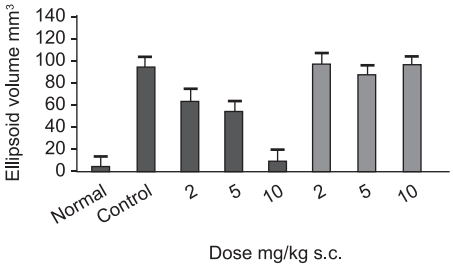

Fig. 8. Efficacy of ORI-1001 in the Kreider mouse xenograft model.

Furthermore, in order to ascertain that the reduction in cyst size in the Kreider studies was not due to a nonspecific antiproliferative effect, ORI-1001 was evaluated in a nude mouse tumor model where the tumor was derived from KB cells. KB cells are non-HPV containing transformed cells derived from human epithelial tumors. In this tumor experiment, s.c. administration of ORI-1001 or a mismatch oligonucleotide did not cause reduction in tumor volume at any doses tested (Fig. 10). Thus, it seems unlikely that ORI-1001-induced reduction in cyst size in the Kreider model is due to a nonspecific antiproliferative mechanism. Indeed, these studies in the Kreider model point to a sequence-specific antisense mechanism for ORI-1001-induced inhibition of HPV6 and HPV11 replication that results in dose-dependent reduction of cyst size.

It is to be noted however, that E1 is conserved across a number of HPV types and therefore certain regions within ORI-1001 sequence may indeed have complete complementarity to short segments of the E1 RNA in some HPV types (such as HPV40) and provoke effective translation arrest of E1 RNA upon hybridization. In fact, it

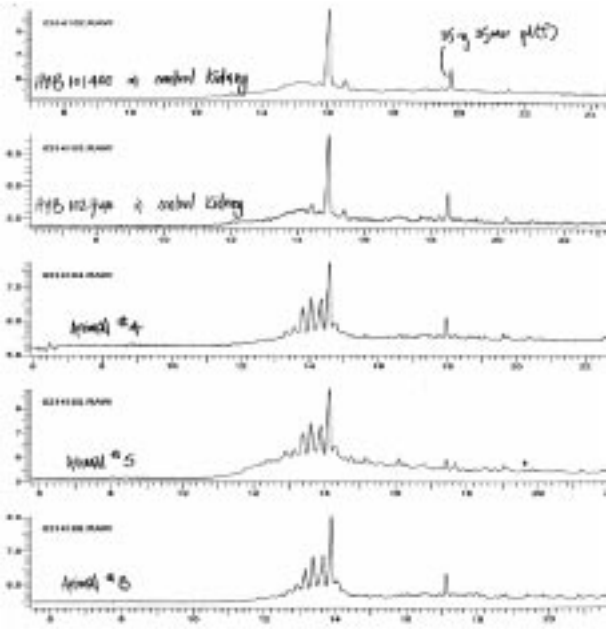

Fig. 9. C-methylated analog of ORI-1001 is active in the Kreider model. This analog is not demethylated *in vivo* and anti-HPV activity is due to the intact molecule.

- 70 female nude MF1 mice injected s.c. with 2 x 10^6 31 KB cells human epithelial tumor
- Cell line; 2 s.c. injections in the rear above hind leg
- Daily s.c. dose at the back of the neck for 14 days; doses 2, 5, and 10 mg/kg. Mismatch same dose. Tumors removed and weighed

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- Dark bars represent ORI-1001-treated tumor weights and open bars represent mismatch oligonucleotide-treated tumor weights
- Conclusion: wart reduction in Kreider model is perhaps related to direct antiviral effect

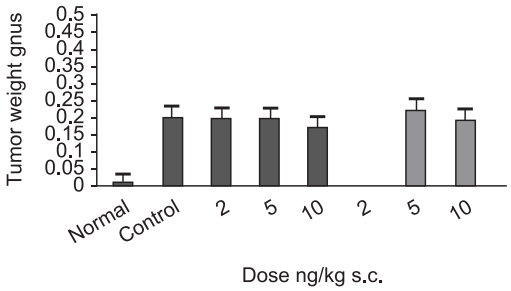

Fig. 10. Evaluation of ORI-1001 in a nude mouse tumor model. Mechanistic studies of ORI-1001 show that ORI-1001 does not have antiproliferative activity in nude mouse tumor model.

has been reported that an oligonucleotide as short as a 7-mer is capable of producing specific and selective antisense inhibition of target mRNA by being able to hybridize to a short complementary linear segment within a highly structured mRNA (13). Furthermore, it has to be mentioned that since ORI-1001 has a polythiophosphate backbone, additional mechanisms could synergistically or additively operate in the observed antiviral effect that is related to the polyanionic nature of the oligonucleotide backbone. For example, some oligonucleoside phosphorothioates and non-nucleoside polyphosphorothioates are reported to be inhibitors of viral polymerases such as reverse transcriptases and other viral enzymes that contain nucleotide-binding domains (14,15). Such compounds are also known to interfere with viral adsorption on cells, thereby blocking viral entry into cells. Consequently, like other PS compounds, ORI-1001 may have nonsequence-specific antiviral activity against certain viruses including other HPV types (16) that may be related to the polyanionic phosphorothioate backbone.

The orthotopic human xenograft model in SCID mice

The orthotopic human xenograft implant model in severe combined immunodeficiency (SCID) mouse is particularly relevant for evaluation of anti-HPV compounds because condylomata acuminata is a dermatological condition. In this model, since the HPV-induced wart develops on the back skin of the SCID mouse, it is convenient to monitor the activity and toxicity of an anti-HPV compound throughout the course of the experiment.In this model (17), human foreskin fragments were infected with HPV6 and implanted in the left and right flanks on the back of SCID mouse. The HPV-infected xenograft was left to grow for 6 weeks at the end of which a warty growth appeared (Fig. 11). During the next 6 weeks of wart growth, ORI-1001 was administered under each flank intradermally twice weekly under the graft. At the end of the 6-week treatment period, mice were sacrificed and grafts removed, measured and processed. The graft size growth (GSG) changes during the treatment period, expressed as the percentage of change of the composite geometric mean diameter of the two grafts borne by the animal, was the primary endpoint. The vehicle and a mismatch oligonucleotide were used as negative controls.

In two separate studies in this model, ORI-1001 caused a dose-dependent reduction of wart growth with shrinkage of the warts in the higher dose groups (Fig. 12). The highest mean GSG was measured in the vehicle control group (36%) with a decrease of the GSG as the dose of ORI-1001 was increased (10, –2.5, –15%), consistent with a dose-related efficacy of ORI-1001 (18,19). The corresponding mismatch oligonucleotide did not show dose-dependent reduction in GSG. However, in this model, the

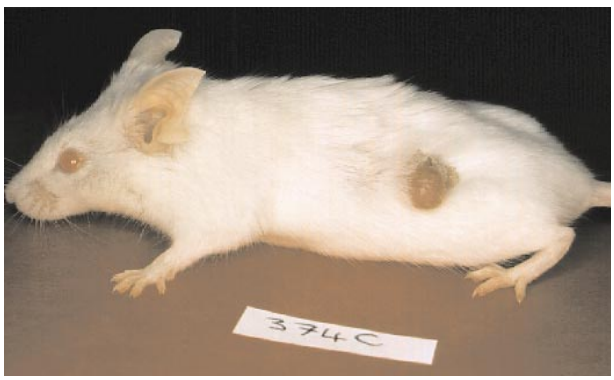

Fig. 11. Appearance of HPV-induced wart in the SCID mouse.

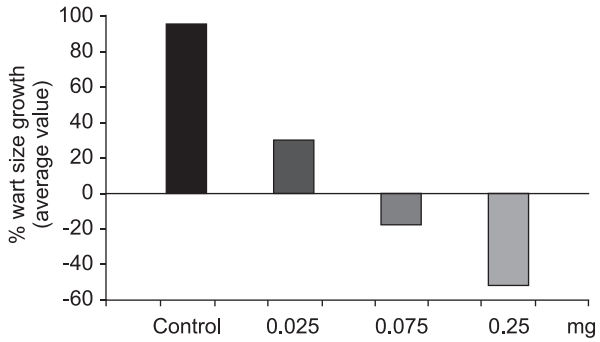

Fig. 12. Efficacy of ORI-1001 in orthotopic human xenograft SCID mouse model. Human foreskin infected with HPV6 is implanted on the left and right flanks of SCID mice; 6 replicate groups each for vehicle and each treatment dose. Warts were allowed to develop for 6 weeks. After 6 weeks, mice were treated with ORI-1001 or mismatch control at doses of 0.025, 0.075 and 0.25 mg/graft/dose, intradermally, twice weekly for 6 weeks. Graft sizes (composite geometric mean diameter) were measured before, during and after treatment. The efficacy endpoint was the dose-dependent reduction in graft size growth (GSG) treatment with ORI-1001.

dose-dependent reduction in GSG by ORI-1001 did not correlate with the disappearance of HPV genome expression as determined by histology, presence of capsid protein or viral transcripts. Hence, according to studies in this model, ORI-1001 may have an antiviral activity that only affects the cellular proliferative properties of the virus. Preliminary studies (20) using topically applied ORI-1001 gel have also been carried out using this model. Indeed, topical treatment with ORI-1001 gel showed a clear trend towards dose-related reduction in GSG.

Product Development

ORI-1001 is a 20-mer-hybrid oligonucleotide comprised of 2'-OMe-ribonucleoside and deoxyribonucleoside phosphorothioate segments (Fig. 13). ORI-1001 has 19 phosphorothioate linkages, and the resulting chirality at the phosphorous center gives rise to 2^{19} isomers. ORI-1001 is manufactured in an automated synthesizer using solid-phase phosphoramidite chemistry.

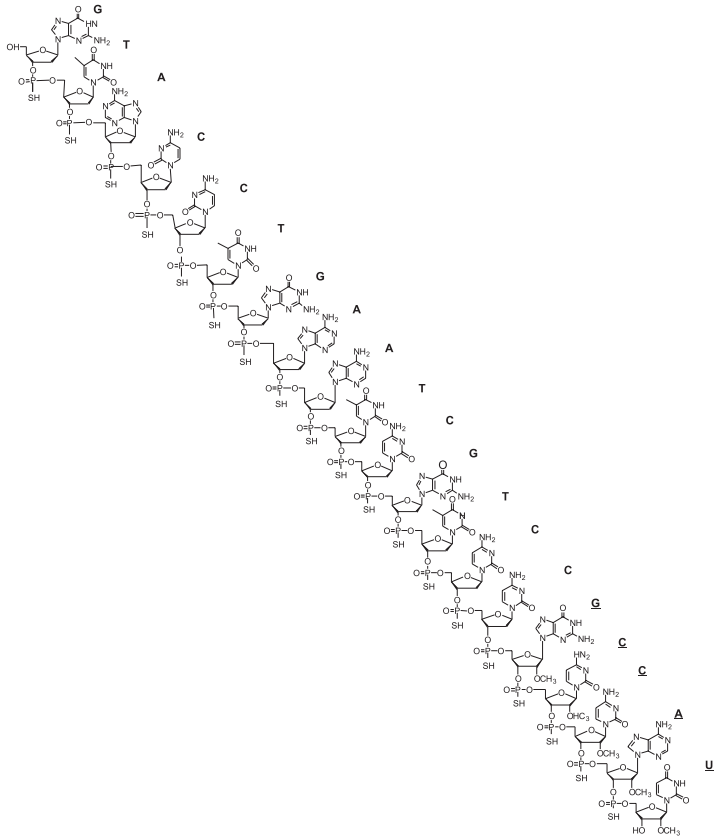

Fig. 13. Structure and properties of ORI-1001: $C_{197}H_{254}N_{71}O_{105}P_{19}S_{19}$; molecular weight: 6494.2956; elemental analysis: C, 36.43; H, 3.94; N, 15.31; O, 25.87; P, 9.06; S, 9.38. All internucleotide linkages are phosphorothioates. The underlined sequence is 2'-OMe ribonucleoside linkage. Manufactured by automated solid-phase synthesis.

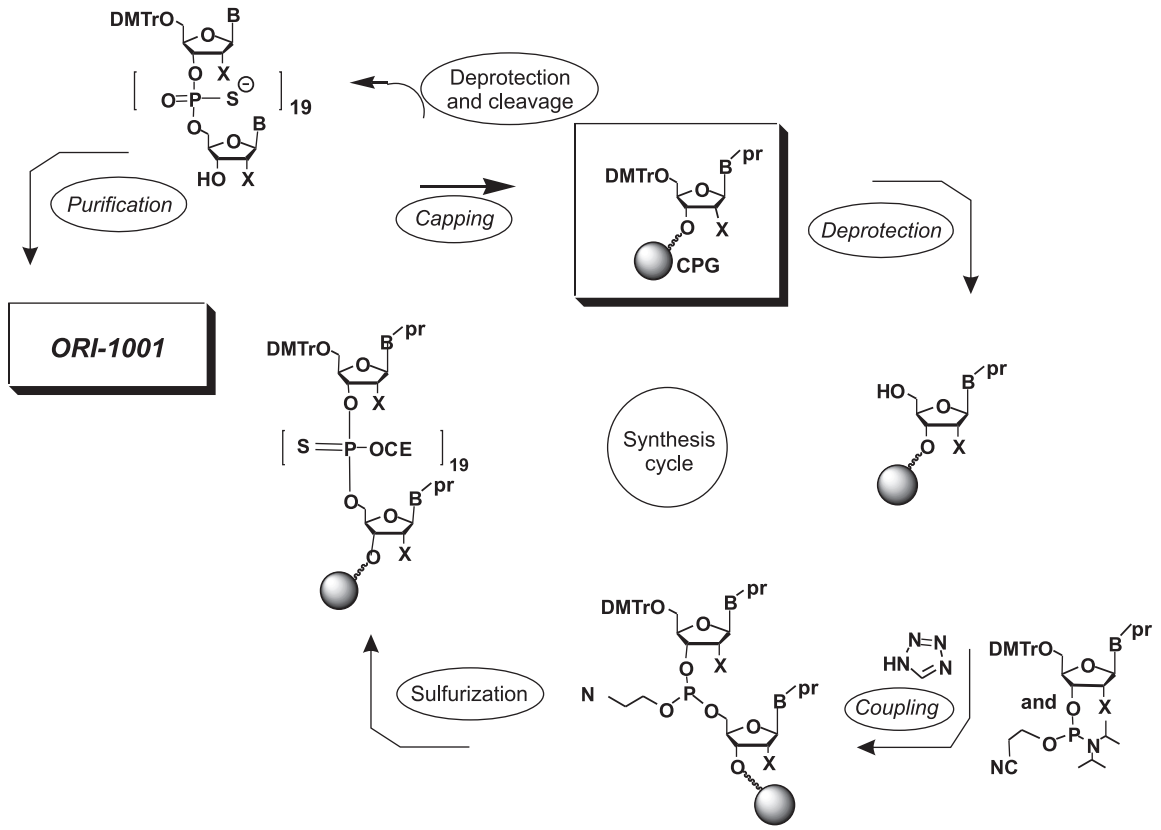

Fig. 14. Manufacture of ORI-1001 by automated solid-phase synthesis using phosphoramidite chemistry.

The assembly of ORI-1001 is carried out from the 3' to 5' direction (Fig. 14). Thus, the 3'-hydroxy group of the terminal nucleoside is attached via a succinyl linker to an amino-functionalized solid support. Each cycle of oligonucleotide synthesis is comprised of sequential steps that include deblock (deprotection), coupling, oxidative sulfurization and capping. The deblock step removes the acid-labile 5'-DMTr group. The resultant unmasked 5'-hydroxyl group from the deblock step is reacted with the nucleoside phosphoramidite building block during the coupling step to form the internucleotidic phosphite triester bond. The building block for each cycle is a nucleoside 3'-*O*-(betacyanoethyl) phosphoramidite that carries a 4,4'-dimethoxytrityl group (DMTr) at the 5'-hydroxyl group, as well as a protecting group for the exocyclic amino group of the nucleobase. The internucleotidic phosphite triester bond is converted to the phosphorothioate group via sulfurization. Finally, the capping step in each cycle masks any unreacted 5'-hydroxyl groups of the growing oligonucleotide chain. The synthetic cycle is repeated until the full length oligonucleotide has been assembled.

Following the completion of the synthesis, the DMTr group remains attached to the 5'-terminus of the oligonucleotide. Any capped failure sequence will lack the hydrophobic 5'-DMT group. Thus, the hydrophobic handle facilitates the separation of the full-length oligonucleotide from the "failure sequences" that lack this handle when reversed-phase HPLC is employed in the purification of the oligonucleotide.

Following the assembly of the oligonucleotide, the solid support is treated with 28% aqueous ammonium hydroxide. During this step, the base and phosphate protecting groups are removed and the oligonucleotide is also simultaneously cleaved off from the support. The resultant crude DMTr-on oligonucleotide is extensively purified by HPLC, DMTr group removed by 80% acetic acid treatment, desalted and lyophilized to obtain the active pharmaceutical ingredient.

ORI-1001 is intended for therapeutic use as a topical agent. Accordingly, a number of formulations of ORI-1001 were prepared and evaluated for physical and chemical stability, excipient compatibility and delivery of ORI-1001 into the epidermis – the target tissue. Based on these studies, a gel formulation was developed for ORI-1001. The formulation showed high metabolic stability of ORI-1001 in viable pigskin and ORI-1001 was found in significant concentration in epidermis – the site of action – and in stratum corneum that could act as a reservoir for the drug.

Pharmacology and Toxicology Studies

ORI-1001 did not show any genotoxicity in in vitro assays. The Ames test showed the compound to be non-mutagenic in both metabolism-competent and -noncompetent systems. The compound also did not show any evidence of chromosome aberrations in standard mammalian cell assays.

The ORI-1001 drug substance and gel formulation were also evaluated in several in vitro and in vivo toxicology studies. Where applicable, pharmacokinetics and tissue distribution were also ascertained. A single-dose dermal irritation study in rabbits indicated that ORI-1001 gel had negligible irritation rating. The ORI-1001 gel, applied topically, was also found to be nonsensitizing in the guinea pig model of delayed type hypersensitivity. The pharmacokinetics and tissue distribution using ^{35}S-labeled ORI-1001 were examined in Sinclair minipigs after single-dose topical and s.c. administrations. Following topical application, there was minimal systemic absorption. Following s.c. administration, the drug was absorbed and distributed mainly in the plasma, with approximately 10% found in liver and kidneys.

Repeat-dose toxicology studies were also performed. A 14-day i.v. administration in rats up to 30 mg/kg/day and 14-day s.c. administration in minipigs up to 7.5 mg/kg/day showed the compound to be well tolerated. There were no findings of toxicological importance. A 14-day toxicity study after topical administration of 0, 5, 15 and 30 mg/kg/day in rats indicated no findings of toxicological importance. The ORI-1001 gel was also locally well tolerated in rats after 14-day penile and genital applications. Repeat-dose toxicology studies were carried out in minipigs following cutaneous application of ORI-1001 gel and s.c. administration of ORI-1001 drug substance, both at doses ranging from 1.25-7.5 mg/kg/day. No findings of toxicological importance were noted in these studies.

Clinical Studies

A phase I clinical trial of ORI-1001 gel in normal healthy volunteers was completed in the last quarter of 2001. The product was evaluated for skin irritation and dermal sensitization. The ORI-1001 gel was found to be safe and well tolerated as a topical agent. Phase II efficacy trials of ORI-1001 are being planned for the first quarter of 2003.

Source

Origenix Technologies, Inc. (US).

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