(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 September 2013 (12.09.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/131182 A1

(51) International Patent Classification:
A61K 31/711 (2006.01) *A61P 31/14* (2006.01)

(21) International Application Number:
PCT/CA2013/050123

(22) International Filing Date:
15 February 2013 (15.02.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/599,653 16 February 2012 (16.02.2012) US

(71) Applicants: **UNIVERSITY OF TORONTO** [CA/CA]; MaRS Centre, Heritage Building, 101 College Street, Suite 320, Toronto, Ontario M5G 1L7 (CA). **HOSPITAL FOR SICK CHILDREN** [CA/CA]; 555 University Avenue, Room 1237, Black Wing, Toronto, Ontario M5G 1X8 (CA).

(72) Inventors: **HEGELE, Richard George**; 45 Howland Avenue, Toronto, Ontario M5R 3B2 (CA). **MASTRANGELO, Peter**; 48 Lloyd George Avenue, Etobicoke, Ontario M8W 3W6 (CA). **MORAES, Theo**; 43 Woodlawn Avenue East, Toronto, Ontario M4T 1B9 (CA).

(74) Agent: **MACINS, Andris**; Smart & Biggar, 2300 - 1055 West Georgia Street, Vancouver, British Columbia V6E 3P3 (CA).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2013/131182 A1

(54) Title: GUANOSINE-RICH OLIGONUCLEOTIDE (GRO) COMPOSTIONS, METHODS AND USES FOR TREATING RESPIRATORY SYNCYTIAL VIRUS INFECTION

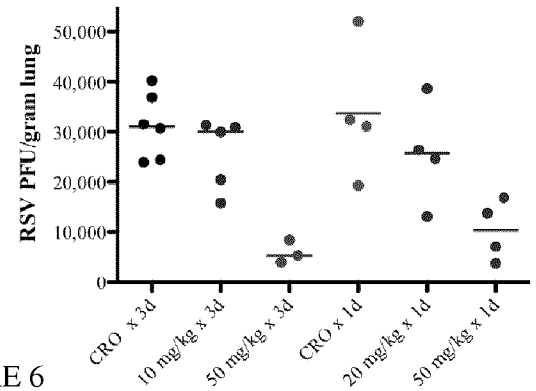


FIGURE 6

(57) Abstract: Provided herein is a method of treating Respiratory Syncytial Virus (RSV) infection in a cell. The method involves administering the guanosine-rich oligonucleotide (GRO) AS 1411 (5'- GGTGGTGGTGG TTGTGGTGGTGGTGG-3' - also known as GR026B and AGRO100) to the cell.

GUANOSINE-RICH OLIGONUCLEOTIDE (GRO) COMPOSTIONS, METHODS AND USES FOR TREATING RESPIRATORY SYNCYTIAL VIRUS INFECTION

TECHNICAL FIELD

[0001] The invention is in the field of treatment of Respiratory Syncytial Virus (RSV) infection, and particularly methods, uses, and guanosine-rich oligonucleotide (GRO) compositions for treating RSV infection.

CROSS REFERENCE TO RELATED APPLICATIONS

[0002] This application claims the benefit of U.S. Provisional Patent Application Serial No. 61/599,653 entitled "GUANOSINE-RICH OLIGONUCLEOTIDE (GRO) COMPOSTIONS, METHODS AND USES FOR TREATING RESPIRATORY SYNCYTIAL VIRUS INFECTION" filed on 16 February 2012.

BACKGROUND

[0003] Human respiratory syncytial virus (RSV) is an enveloped, single-stranded, negative-polarity RNA *Pneumovirus* of the family *Paramyxoviridae*. It is a common cause of respiratory tract infections worldwide, including bronchiolitis and other serious illnesses (see, for *e.g.,* Collins and Graham, 2008; Wu *et al.,* 2008). Control and prevention of RSV infection is a global health priority; almost all children are infected with RSV during the first two years of life (see, for *e.g.,* Domachowske and Rosenberg, 1999). In the United States alone, over 2 million RSV-infected infants require medical attention annually (Hall *et al.,* 2009). Infants hospitalized for RSV are also at risk for developing recurrent wheezing and asthma (Escobar *et al.,* 2010).

[0004] Polarized, ciliated respiratory epithelial cells are a major target for RSV infection *in vivo* (see, for *e.g.,* Johnson *et al.,* 2007). RSV infection of ciliated respiratory epithelial cells *in vitro* occurs on the apical (luminal) aspect (see, for *e.g.,* Zhang *et al.*, 2002). Viral replication in a host cell initiates with attachment of the virus to the plasma membrane via receptor-mediated binding (see, for *e.g.,* Marsh and Helenius, 2006). Candidate RSV receptors have been proposed (see, for *e.g.,* Krusat and Streckert, 1997; Behera *et al.,* 2001; and, Malhotra *et al.*,

2003). Laboratory-adapted strains of RSV show increased efficiency of infection by binding to cell surface glycosaminoglycans (GAGs); however, cells deficient in GAGs are permissive to infection (see, for *e.g.,* Feldman *et al.,* 2000) and the role of GAGs in cellular infection by wild-type, community isolates of RSV is unclear (see, for *e.g.,* Hallak et al., 2007).

[0005] Options for treatment are limited, vaccination poses numerous obstacles, and passive prophylactic treatment using anti-RSV antibodies (*e.g.,* palivizumab) is expensive, is not 100% effective, and is limited to those at high risk for severe infection (Wu *et al.,* 2008).

[0006] The RSV envelope contains three proteins: small hydrophobic (SH), glycoprotein (G) and fusion (F) (Collins and Graham, 2008). SH protein is not required for virus binding (see, for *e.g.,* Techaarpornkul *et al.,* 2001). RSV G, the heterogeneity of which characterizes RSV subtypes A and B, binds to cell surface glycosaminoglycans (GAGs) at high affinity (see, for *e.g.,* Hallak *et al.,* 2007), but is not an absolute requirement for infection, since mutant RSV deficient in G glycoprotein (RSV ÄG) remains infectious (see, for *e.g.,* Techaarpornkul *et al.,* 2002). Moreover, cells deficient in cell surface GAGs or with chemically modified GAGs are permissive to RSV, albeit at lower levels than cells expressing abundant GAGs (see, for *e.g.,* Techaarpornkul *et al.,* 2002; and Hallak *et al.,* 2000).

[0007] Nucleolin is a ubiquitous nucleolar phosphoprotein involved in fundamental aspects of transcription regulation, cell proliferation and growth (see, for *e.g.,* Tuteja *et al.*, 1998; and Chen *et al.*, 2008). Nucleolin has also been described as a shuttling molecule between nucleus, cytosol and the cell surface. Studies have demonstrated that surface nucleolin may serve as a receptor for various extracellular ligands, for instance those implicated in cell proliferation, differentiation, adhesion, mitogenesis and angiogenesis. Nisole *et al.* (1999), US20040002457A1, and US20020076693A1 disclose that nucleolin is involved in binding of HIV virus to host cells. Nucleolin peptide, nucleotide antibodies, and nucleolin RNAi have all been shown used for inhibiting RSV infection (WO/2011/123945).

SUMMARY

[0008] The present invention is based in part on the discovery that nucleolin plays a role in RSV infection, and that the administration of guanosine-rich oligonucleotide (GRO) AS1411 - 5'-GGTGGTGGTGG TTGTGGTGGTGGTGG-3' (SEQ ID No: 1) (also known as GR026B and AGRO100) to a cell may be useful in the treatment of RSV infection. Such a treatment may cause interference with the interaction between RSV protein F and a cells endogenous nucleolin.

[0009] In a first aspect, a method is provided for of treating Respiratory Syncytial Virus (RSV) infection in a cell, the method including administering AS1411 to the cell.

[0010] In a further aspect, there is provided a use of AS1411 having a sequence of SEQ ID NO:1 for the treatment of RSV infection.

[0011] In a further aspect, there is provided a use of AS1411 having a sequence of SEQ ID NO:1 in the preparation of a medicament for the treatment of RSV infection.

[0012] In a further aspect, AS1411 is provided for of treating Respiratory Syncytial Virus (RSV) infection in a cell.

[0013] In a further aspect, there is provided a commercial package, comprising: (a) AS1411; and (b) instruction for use in the treatment of RSV.

[0014] The cell may be a human cell. The human cell may be an epithelial cell. The human cell may be a mucosal cell. The human cell may be a cell of the respiratory tract. The human cell may be a ciliated respiratory epithelial cell. The cell may be in a subject having or at risk of developing an RSV infection. The AS1411 molecule may be administered intravenously. The AS1411 molecule may be topically administered to a mucosal membrane of the subject. The AS1411 molecules may be mixed with lipid particles prior to administration.

The AS1411 molecules may be encapsulated in liposomes prior to administration. The AS1411 molecule may also be administered via nasal spray or intramuscularly or intravenously.

[0015] The treatment may be of a human subject. The treatment may be of a bovine subject. The treatment may be of an ovine subject. The treatment may be of an equine subject. The treatment may be of a porcine subject. The treatment may be of a murine subject.

[0016] The RSV may be a human strain of RSV. The RSV may be a bovine strain of RSV. The RSV may be an ovine strain of RSV. The RSV may be an equine strain of RSV. The RSV may be a porcine strain of RSV.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] **FIGURE 1a** shows a graph of fluorescence signal over a wide dosing range of AS1411 and CRO (control) in RSV-infected HEp-2 cells, where the cells were pre-treated with oligo (prophylactic experiment) 24 hrs before exposure to RSV (MOI = 1.0).

[0018] **FIGURE 1b** shows a graph of fluorescence signal over a wide dosing range of AS1411 and CRO (control) in RSV-infected HEp-2 cells, where the cells were exposed to RSV 24 hrs (MOI = 0.1) before adding the oligo (therapeutic experiment).

[0019] **FIGURE 2** shows representative fluorescence micrographs of HEp-2 cells from a therapeutic experiment treated with 17.5 μM AS1411 (panel A) and 17.5 μM CRO control (panel B), wherein the reduction in fluorescence signal is due to reduction in the overall number of infected cells (rather than a decreased amount of infection per cell).

[0020] **FIGURE 3** shows a graph of the effect of increasing concentrations of AS1411 (left column) or CRO (right column) after RSV infection of MDCK cells (n=6; two-tailed paired t test), where the cells were exposed to RSV (MOI = 0.1) 24 hrs prior to treating with AS1411/CRO and 17.5 μM and 70 μM of AS1411 was sufficient to get a 87% and 97%

decrease in RSV fluorescence respectively, 24 hrs after addition to virus-infected MDCK cell cultures ($P < 0.0001$).

[0021] **FIGURE 4** shows the effect of increasing concentrations of AS1411 or CRO after RSV infection of MDCK cells in a therapeutic experiment, where MDCK cells were exposed to RSV (MOI = 0.1) 24 hrs prior to treating with AS1411/CRO, and where an MTS assay was performed 24 hrs after treating with the oligos to assess cellular toxicity.

[0022] **FIGURE 5** shows fluorescence micrographs of representative cell culture wells from a therapeutic experiment with MDCK treated with 17.5 μM AS1411 (panel A) and 17.5 μM CRO control (panel B), wherein with the AS1411 treatment, the reduction in fluorescence signal is due to reduction in the overall number of infected cells.

[0023] **FIGURE 6** shows a plot of RSV lung titers following AS1411 and a control oligo (CRO) delivery intranasally (at either 30 or 50 mg/kg and either 1 or 3 times per day) to mice who had received 5×10^6 PFU RSV on day 0 and either AS1411 or CRO starting day 1, when the mice were euthanized on day 4 RSV lung titres were determined.

[0024] **FIGURE 7** shows a plot of lung weight for the same groups shown in FIGURE 6, where the AS1411 groups had heavier and visibly redder lungs and no overt histopathology.

[0025] **FIGURE 8** shows a graph of % initial body weight for the same groups shown in FIGURE 6, where the where the AS1411 is associated with less recovery of weight loss.

[0026] **FIGURE 9** shows a graph of RSV PFUs/g in lung tissue following treatment with clinical grade AS1411 delivered intranasally (single doses at 20mg/kg and 50 mg/kg as compared to RSV alone).

[0027] **FIGURE 10** shows a graph of % initial body weight for the same groups shown in FIGURE 9, where the where the AS1411 is associated with less recovery of weight loss especially at 50mg/kg.

FIGURE 11 shows a plot of RSV PFU/g in lung tissue following treatment with clinical grade AS1411 delivered by intraperitoneal injection (IP).

DETAILED DESCRIPTION

[0028] Any terms not directly defined herein shall be understood to have the meanings commonly associated with them as understood within the present field of art. Certain terms are discussed below, or elsewhere in the specification, to provide additional guidance to the practitioner in describing the compositions, devices, methods and the like of embodiments, and how to make or use them. It will be appreciated that the same thing may be said in more than one way. Consequently, alternative language and synonyms may be used for any one or more of the terms discussed herein. No significance is to be placed upon whether or not a term is elaborated or discussed herein. Some synonyms or substitutable methods, materials and the like are provided. Recital of one or a few synonyms or equivalents does not exclude use of other synonyms or equivalents, unless it is explicitly stated. Use of examples in the specification, including examples of terms, is for illustrative purposes only and does not limit the scope and meaning of the embodiments described herein.

[0029] A method is provided for "treating" Respiratory Syncytial Virus (RSV) infection in a cell, wherein treating is meant to encompass preventing RSV infection, ameliorating RSV infection, and eradicating RSV infection. The term "treating" as used herein is also meant to include the prophylactic administration of a compound prior to infection of RSV or in anticipation of RSV infection, the administration of a compound when there is an ongoing infection with RSV, or if the RSV is in a latent state. Those skilled in the art would appreciate that the term "preventing" would include the avoidance of infection with RSV or minimizing RSV infection once an exposure to RSV occurs. A person of skill in the art would appreciate that the term "ameliorating" is meant to include the prospect of making an infection more

tolerable for a subject afflicted with an RSV infection (for example, by reducing viral load in a subject). A person of skill in the art would also appreciate that the term "eradication" with regards to RSV infection would include elimination of the RSV from a subject or removal of the infection (for example by the treatment itself or in conjunction with another treatment(s) and/or the subjects immune response). Accordingly, as used herein "treatment" may refer to the prevention of an RSV infection, the amelioration of an RSV infection, the eradication of RSV from a subject, or combinations thereof. Furthermore, a "treatment" may be prophylactic, whereby the treatment is administered prior to infection or the appearance of symptoms in a subject or in anticipation of an exposure to RSV. Similarly, "treatment" may be administered following RSV infection or the onset of RSV infection symptoms.

[0030] As used herein, the term "RSV" refers to Respiratory Syncytial Virus, which is an enveloped, single-stranded, negative-polarity RNA *Pneumovirus* of the family *Paramyxoviridae*. Human RSV strains are significant causes of bronchiolitis and other serious lower respiratory tract infections worldwide, especially in children, the elderly, and in immunocompromised adults. The term RSV may include, without limitation, respiratory syncytial viruses of the *Pneumovirus* genus which have specificity for other host species, such as bovine respiratory syncytial virus, ovine respiratory syncytial virus and murine pneumonia virus, which is also known in the art as PVM (see, for *e.g.,* Bem *et al.,* 2009). As used herein, the term "infection" refers to a state in which an infectious agent, such as a virus, and including without limitation RSV, is established within a cell or within a subject, as the case may be. In certain embodiments, the compounds may be used for treating RSV infection. Such methods and compounds are described herein.

[0031] Human RSV strains may be classified as A or B group viruses (*i.e.,* RSV-A and RSV-B) based on MAb binding to the RSV glycoprotein (G protein) (see, for *e.g.,* (Anderson *et al.*, 1985; Coates *et al.*, 1966; Hendry *et al.*, 1986; Mufson *et al.*, 1985) and by genetic analysis (see, for *e.g.,* Garcia *et al.*, 1994; Sullender *et al.*, 1991; Sullender *et al.*, 1993). The RSV G protein is thought to be the most variable of the RSV proteins (see, for *e.g.,* Garcia-Barreno *et al.*, 1989; Johnson *et al.*, 1987; Mufson *et al.*, 1985), and its C-terminal region (the second

hypervariable region) accounts for strain-specific epitopes (see, for *e.g.,* Cane, 1997; Cane, 2001; Cane and and Pringle, 1995; Garcia *et al.*, 1994; Johnson *et al.*, 1987; Peret *et al.*, 1998; Rueda *et al.*,1991). By contrast the F protein of RSV is much less variable between strains and is thus a more attractive target for a broad based RSV treatment.

[0032] Nucleolin is a ubiquitous nucleolar phosphoprotein involved in fundamental aspects of transcription regulation, cell proliferation and growth (see, for *e.g.,* Tuteja *et al.*, 1998; and Chen *et al.*, 2008). Nucleolin has also been described as a shuttling molecule between nucleus, cytosol and the cell surface. Studies have demonstrated that surface nucleolin may serve as a receptor for various extracellular ligands, for instance those implicated in cell proliferation, differentiation, adhesion, mitogenesis and angiogenesis.

[0033] The term "nucleolin" refers, in part, to the polypeptide expression product of the nucleolin gene, the human orthologue corresponding to EntrezGene # 4691, GenPept Protein Accession #P19338.3. Homologous nucleolin proteins are found in other species which bear sequence similarity to human nucleolin and may thus find utility herein, including but not limited to mouse (EntrezGene # 17975; GenPept # P09405.2), Guinea Pig (EntrezGene # 100735210; GenPept # XP_003474646) and rat (EntrezGene # 25135; GenePept # P13383.3). The term "nucleolin" also refers to the nucleolin gene itself, and includes reference to the nucleolin gene as it is present in a variety of genomes including, without limitation, human, bovine, ovine, equine, porcine, and murine. The term "nucleolin" also includes gene products (*e.g.,* mRNA, protein) produced from the underlying nucleolin gene. It will be appreciated by a person skilled in the art that the term "nucleolin peptide" qualifies the understanding of the term "nucleotide" by making specific reference to a "peptide" as it is defined herein. For example, SEQ ID NO:2 provides an amino acid sequence of human nucleolin. The term "nucleolin" also includes post-translationally modified forms of the protein.

[0034] Active GROs have been shown to bind to cell surface Nucleolin and are often referred to as "Nucleolin aptamers." Encompassed herein is the use of a guanosine-rich oligonucleotide (GRO) called **AS1411** for administration to a cell. AS1411 (as described in

WO2009098464) has the sequence 5'-GGTGGTGGTGGTTGTGGTGGTGGTGG-3' (SEQ ID No: 1) and is also known as GR026B and AGRO100. AS1411 is a 26-mer DNA aptamer, which is currently undergoing clinical evaluation in acute myeloid leukemia (AML). AS1411 is a guanine-rich aptamer has unmodified phosphodiester linkages and forms a G-quadruplex structure (Dapic, V. *et al.* 2003) that is resistant to degradation by serum enzymes (Dapic, V. *et al.* 2002). AS1411 shows high affinity and specificity for nucleolin.

[0035] Since the identification of Nucleolin as a receptor for RSV infection (Tayyari, F. *et al.*, 2011) AS1411 was tested for an effect on RSV infection using a fluorescence focus assay (FFA). In FFA, the amount of infected cells per well after 24 hrs exposure to the virus is converted to a fluorescent signal.

[0036] The compounds, as described herein, may be in isolation, or may be linked to or in combination with tracer compounds, liposomes, carbohydrate carriers, polymeric carriers or other agents or excipients as will be apparent to one of skill in the art. In alternate embodiments, such compounds may further comprise an additional medicament, wherein such compounds may be present in a pharmacologically effective amount.

[0037] The term "medicament" as used herein refers to a composition that may be administered to a patient or test subject and is capable of producing an effect in the patient or test subject. The effect may be chemical, biological or physical, and the patient or test subject may be human, or a non-human animal, such as a rodent or transgenic mouse, or a, mouse, rat, dog, cat, cow, sheep, horse, hamster, guinea pig, rabbit, cotton rat, or pig. The medicament may be comprised of the effective chemical entity alone or in combination with a pharmaceutically acceptable excipient.

[0038] The term "pharmaceutically acceptable excipient" may include any and all solvents, dispersion media, coatings, antibacterial, antimicrobial or antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. An excipient may be suitable for intravenous, intraperitoneal, intramuscular, subcutaneous, intrathecal, topical or

oral administration. An excipient may include sterile aqueous solutions or dispersions for extemporaneous preparation of sterile injectable solutions or dispersion. Use of such media for preparation of medicaments is known in the art.

[0039] Compositions or compounds according to some embodiments described herein may be administered in any of a variety of known routes. Examples of methods that may be suitable for the administration of a compound include orally, intravenous, inhalation, intramuscular, subcutaneous, topical, intraperitoneal, intra-rectal or intra-vaginal suppository, sublingual, and the like. The compounds described herein may be administered as a sterile aqueous solution, or may be administered in a fat-soluble excipient, or in another solution, suspension, patch, tablet or paste format as is appropriate. A composition comprising the compounds described herein may be formulated for administration by inhalation. For instance, a compound may be combined with an excipient to allow dispersion in an aerosol. Examples of inhalation formulations will be known to those skilled in the art. Other agents may be included in combination with the compounds described herein to aid uptake or metabolism, or delay dispersion within the host, such as in a controlled-release formulation. Examples of controlled release formulations will be known to those of skill in the art, and may include microencapsulation, embolism within a carbohydrate or polymer matrix, and the like. Other methods known in the art for making formulations are found in, for example, "Remington's Pharmaceutical Sciences", (19th edition), ed. A. Gennaro, 1995, Mack Publishing Company, Easton, Pa.

[0040] The dosage of the compositions or compounds of some embodiments described herein may vary depending on the route of administration (oral, intravenous, inhalation, or the like) and the form in which the composition or compound is administered (solution, controlled release or the like). Determination of appropriate dosages is within the ability of one of skill in the art. As used herein, an "effective amount", a "therapeutically effective amount", or a "pharmacologically effective amount" of a compound refers to an amount of the AS1411 present in such a concentration to result in a therapeutic level of the compound delivered over the term that the compound is used. This may be dependent on the mode of delivery, time

period of the dosage, age, weight, general health, sex and diet of the subject receiving the compound. Methods of determining effective amounts are known in the art. It is understood that it could be potentially beneficial to restrict delivery of the compounds described herein to the target tissue or cell in which inhibition of RSV infection is desired. It is also understood that it may be desirable to target the compounds described herein to a desired tissue or cell type. The compounds described herein may thus be coupled to a targeting moiety. The compounds may be coupled to a cell uptake moiety. The targeting moiety may also function as the cell uptake moiety.

METHODS AND MATERIALS

[0041] The following methods and materials were employed with respect to the Examples described herein.

[0042] **Cells.** HEp-2 (product # is ATCC CCL-23) and MDCK cells (product number is ATCC CCL-34) were obtained from (American Type Culture Collection (ATCC), Rockville, MD) were maintained in Dulbecco's modified essential medium (Invitrogen™, Carlsbad, CA) supplemented with 1% L-glutamine (Invitrogen™) and 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Invitrogen™), at 37°C. All cell cultures were kept in a humidified incubator containing 5% CO_2.

[0043] **Viruses.** Working stocks of RSV A2 and RSV B (ATCC), RSV lacking G protein (RSV ΔG) (gift of Dr. P.L. Collins, National Institutes of Health, Bethesda, MD), RSV expressing GFP (Hallak *et al.,* 2000) were prepared as previously described (Kaan and Hegele, 2003).

[0044] **RSV propagation (*in vivo* experiments).** Cells involved with RSV propagation were routinely screened for mycoplasma and LPS. 2 x 10^7 HEp-2 cells (ATCC) were seeded in 10% FBS EMEM in a T-150 tissue culture flask. Cells were left to grow in 37°C, 5% CO_2 incubator overnight. The next day (Day 0), cells were rinsed twice with clean PBS. RSV-A2 (ATCC) was added in 12 mL of serum free EMEM at an MOI ~ 0.1. Cells/RSV underwent

incubation for 2 to 4 h while rotating flask every 15 to 30 min. Twenty-eight mL 6% FBS EMEM were added to flasks and cells incubated for 3 to 4 days. By day 4, 50% cells usually detached and virus was harvested and purified.

[0045] **RSV harvesting and purification.** Infected HEp-2 cells were scraped and collected with media into 50mL Falcon tubes, and then underwent centrifugation at 820 x g for 10 min. All solutions and cells were kept on ice or at 4°C unless otherwise stated. Supernatant was decanted and placed in a cold 50 mL falcon tube. The cell pellet was resuspended with 5 mL medium, then quick frozen in liquid nitrogen, and melted at 37°C with constant agitation. This freeze-thaw cycle was repeated once. The resuspended pellet underwent sonication on ice for 20 s twice and centrifugation at 820 x g for 10 min. The cell pellet was discarded and the supernatant was combined with the original decanted media for ultracentrifugation (SW28 rotor) at 110,000 x g for 45 min, over a sucrose cushion (30% sucrose in 0.1 M sodium chloride, 0.01 M Tris-HCl, 0.001 M EDTA, 1 M urea, pH = 7.5). After this spin a virus pellet was clearly visible and supernatant was decanted and disposed. The pellet was resuspended in 100-150 µL LPS-free PBS per T-150 flask (yield: 2x108 PFU RSV/mL) and stored at -80°C or in liquid nitrogen.

[0046] **Aptamers.** A guanosine-rich oligonucleotide (GRO) called **AS1411** having the sequence 5'-GGTGGTGGTGGTTGTGGTGGTGGTGG-3' (SEQ ID No: 1) was used herein. As a negative control the same oligo, but with all the guanosines substituted with cytosine residues (CRO) 5'-CCTCCTCCTCCTTCTCCTCCTCCTCC-3' (SEQ ID No: 3) was used. Others have already shown that CRO serves as a negative control for AS1411's anti-cancer cell activity. AS1411 and CRO were obtained from Life Technologies™.

[0047] **Fluorescence focus assay** (Malhotra *et al.*, 2003): Briefly, HEp-2 cells cultured in EMEM supplemented with 10% foetal bovine serum (FBS) and antibiotics are plated onto black flat clear-bottomed 96 well plates (1 x 10^4 cells/well). The next day virus is added and incubated for 24 hrs at 37°C and 5% CO_2 to allow at least one full round of virus replication. The cells are then fixed with 75% cold acetone in PBS for 5 minutes. Following 3 washes with

PBS/ 0.1% Tween-20™ (PBS-Tween) cells were incubated with a 1:10 dilution of NCL-RSV3 (Novacastra™; mouse anti-RSV monoclonal pool) in PBS-Tween/10% FBS for 30 minutes at room temperature. Cells were washed again as before and incubated with 1:400 dilution (5 µg/mL) of Alexa Fluor 488™ anti-mouse IgG (Life Technologies™) in PBS-Tween/10% FBS. After washing again as before with PBS-Tween, cells were counterstained with 0.1% naphthol blue black for 10 minutes at room temperature. Then, after a final washing, fluorescence was detected with a Tecan Infinite M200Pro™ plate reader (ex. 488 nm, em. 519 nm).

[0048] In this assay the amount of infected cells per well after 24 hrs exposure to RSV is converted to a fluorescent signal. AS1411 was applied to the wells in triplicate starting at 70 µM with serial dilutions down to 0.55 µM. As a negative control we used a derivative of the same compound that does not interact with Nucleolin (CRO). In "prophylactic" experiments we pre-treated cells with oligo 24 hrs before exposure to RSV (MOI = 1.0). In "therapeutic" experiments we exposed cells to RSV (MOI = 0.1) 24 hrs before adding the compound.

[0049] In experiments with MDCK cells, cells were plated at 4 x 10^4 cells/well and maintained for 4-5 days with daily medium changes to allow cells to polarize before using them in our assay.

[0050] To assay cellular toxicity, MTS assay (Promega™) was performed according to manufacturer instructions.

[0051] ***In vivo*** **experiments**: Animal studies were approved by the Hospital for Sick Children Animal Care Committee with the regulation of the Canadian Council on Animal Care. Female Balb/C mice, age 6 to 8 weeks old were obtained from Charles River Laboratories (St. Constant, QC, Canada). Animals were kept in a specific pathogen free environment and fed food and water ad libitum.

[0052] For all intra-nasal instillation, lightly sedated mice (isoflurane) readily inhaled 50µL of instillate applied to their nares with a P-200 pipetter while their mouth was held closed. On

day 0 mice received 5 x 10^6 PFU of RSV via intranasal installation (n = 4 to 6 per group). Mice were monitored and weighed daily.

[0053] For the AS1411 intraperitoneal (IP) injection, the methods were the same as for the intranasal instillations, with the exception of the AS1411 administration (standard mouse IP injection protocols were followed without sedation).

[0054] The RSV used (RSV-A2, ATCC) was amplified in HEp-2 cells and purified over sucrose in an ultracentrifuge.

[0055] On day 1, mice received AS-1411 via intranasal installation (at indicated dose; ranged from 10 mg/kg to 50 mg/kg) diluted in PBS. Control group received 50 mg/kg of CRO. In one experiment, AS-1411 administration was repeated on day 2 and 3. On day 4, mice were euthanized and the lungs removed, weighed and homogenized for viral titres. Titres were determined by standard plaque assay on HEp-2 cells.

[0056] The following examples are provide for illustrative purposes and are not intended to be limiting, as such:

EXAMPLES

[0057] EXAMPLE 1: Effect of Increased Concentration of AS1411 on Viral Infection of HEp-2 Cells.

[0058] In prophylactic experiments conducted over a wide dosing range of AS1411, the RSV-associated fluorescence signal was reduced by ~70%, without overt cytotoxicity (FIGURE 1a; P = 0.004). In therapeutic experiments we found that treating RSV-infected HEp-2 cell cultures with as little as 17.5 μM AS1411 resulted in a 50% decrease in fluorescence signal (FIGURE 1b; P = 0.0014), attributable to a reduction in the overall number of infected cells relative to the control (FIGURE 2 panels A and B). Furthermore, CRO-treated control wells had more clusters of positive cells (FIGURE 2, right panel B), indicative of cell-

to-cell spread of RSV infection. These findings suggest that AS1411 acts *in vitro* not only to stop new infection events, but also to slow the spreading of infection outward from an initial focus of infection.

[0059] In both experiments (1 and 2 – FIGURES 1a and 1b) 35 μM of AS1411 was sufficient to get a 60-70% decrease in fluorescent foci. In these experiments, the HEp-2 cells were pre-treated with the oligos for 24 hrs prior to adding the virus. Fluorescent foci where then detected 24 hrs after adding the virus.

[0060] EXAMPLE 2: Effect of Increased Concentration of AS1411 on Viral Infection of MDCK Cells

[0061] Similar to EXAMPLE 1, the effect of increasing concentrations of AS1411 or CRO after RSV infection was tested on MDCK cells (therapeutic experiment; n=6; two-tailed paired t test). MDCK cells were exposed to RSV (MOI = 0.1) 24 hrs prior to treating with AS1411/CRO. FIGURE 3 shows that 17.5 μM and 70 μM of AS1411 is sufficient to get a 87% and 97% decrease in RSV fluorescence respectively, 24 hrs after addition to virus-infected MDCK cell cultures ($P < 0.0001$).

[0062] FIGURE 4 shows the effect of AS1411 or CRO on MDCK cellular toxicity as measured by MTS assay (Promega™) performed 24 hrs after treating with the oligos. MDCK cells were exposed to RSV (MOI = 0.1) 24 hrs prior to treating with AS1411/CRO. No difference is seen between AS1411 and CRO in terms of cellular proliferation and cellular health.

[0063] FIGURE 5 shows fluorescence images of cell culture wells from MDCK therapeutic experiment treated with 17.5 μM of AS1411 and CRO, respectively. As with FIGURE 2, AS1411 treatment resulted in the reduction in fluorescence signal is due to reduction in the overall number of infected cells. Wells containing CRO show more clusters of virus-positive cells.

[0064] EXAMPLE 3: Effect of AS1411 (Intranasal) on Viral Infection of Mouse Lung Tissue

[0065] AS1411 was also tested in live mice tissues to determine the effectiveness against RSV infection. FIGURE 6 shows mice that were administered AS1411 or CRO delivered intranasally, following receipt of 5×10^6 PFU RSV on day 0 and treatment with AS1411 or CRO on day 1. Mice given AS1411 showed reduced RSV titres and repeated dosing had only a modest effect on lung titers. The mice given 50mg/kg showed the most pronounced effect.

[0066] Furthermore, the AS1411 groups had heavier and visibly redder lungs, without any overt histopathology (FIGURE 7). However, the AS1411 groups also were associated with less recovery of weight loss (see FIGURE 8). In most cases, such body weight recovers when the animal recovers from the illness.

[0067] The mouse experiment was repeated using clinical grade AS1411 (see FIGURE 9 - also, delivered intranasally) and showed results similar to those shown in FIGURE 6.

[0068] TABLE 1: Tukey's Multiple Comparison Test:

	Mean Diff.	**Q**	**P value**	**95% CI of diff**
RSV vs RSV + 20mg/kg AS1411 x 1	166.7	0.1662	P > 0.05	-4009 to 4343
RSV vs RSV + 50mg/kg AS1411 x 1	8500	8.478	P < 0.01	4324 to 12680
RSV + 20mg/kg AS1411 x 1 vs RSV + 50mg/kg AS1411 x 1	8333	10.18	P < 0.001	4924 to 11740

[0069] Similarly, FIGURE 10 shows that the % of initial weight is similar for clinical grade AS1411 as it is for non-clinical grade AS1411.

[0070] EXAMPLE 4: Effect of AS1411 (Intraperitoneal Injection (IP)) on Viral Infection of Mouse Lung Tissue

[0071] For comparison purposes, FIGURE 11 shows that IP injections of AS1411 (at 50mg/kg) have no effect on RSV lung titers when compared to CRO control.

[0072] Although embodiments described herein have been described in some detail by way of illustration and example for the purposes of clarity of understanding, it will be readily apparent to those of skill in the art in light of the teachings described herein that changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

REFERENCES

Anderson *et al.* (1985). Antigenic characterization of respiratory syncytial virus strains with monoclonal antibodies. *J. Infect. Dis.* **151**, 626-633.

Behera *et al.* (2001). Blocking intercellular adhesion molecule-1 on human epithelial cells decreases respiratory syncytial virus infection. *Biochem. Biophys. Res. Commun.* **280**, 188-195.

Bem *et al.* (2009). Mechanical ventilation enhances lung inflammation and caspase activity in a model of mouse pneumovirus infection. *AJP – Lung Physiol.* **296**, L46-L56.

Cane, P.A. and Pringle, C.R. (1995). Evolution of subgroup A respiratory syncytial virus: evidence for progressive accumulation of amino acid changes in the attachment protein. *J. Virol.* **69**, 2918-2925.

Cane P.A. (1997). Analysis of linear epitopes recognised by the primary human antibody response to a variable region of the attachment (G) protein of respiratory syncytial virus. *J. Med. Virol.* **51**, 297-304.

Cane, P.A. (2001). Molecular epidemiology of respiratory syncytial virus. *Rev. Med. Virol.* 11:103-116.

Chen *et al.* (2008). Cell surface nucleolin serves as receptor for DNA nanoparticles composed of pegylated polylysine and DNA. *Mol. Ther.* **16**, 333-342.

Coates *et al.* (1966). An antigenic analysis of respiratory syncytial virus isolates by a plaque reduction neutralization test. *Am. J. Epidemiol.* **83**, 299-313.

Collins, P.L. & Graham, B.S. (2008). Viral and host factors in human respiratory syncytial virus pathogenesis. *J Virol.* **82**, 2040-2055.

Dapic, V. *et al.* (2002) Antiproliferative activity of G-quartet-forming oligonucleotides with backbone and sugar modifications. Biochemistry 41: 3676–85.

Dapic, V. *et al.* (2003) Biophysical and biological properties of quadruplex oligodeoxyribonucleotides. Nucleic Acids Res 31: 2097–107.

Domachowske, J.B. and Rosenberg, H.F. (1999). Respiratory syncytial virus infection: immune response, immunopathogenesis, and treatment. *Clinical Microbiol. Rev.* **12**, 298-309.

Escobar, G.J., *et al.* (2010). Recurrent wheezing in the third year of life among children born at 32 weeks' gestation or later: relationship to laboratory-confirmed, medically attended infection with respiratory syncytial virus during the first year of life. *Arch. Pediatr. Adolesc. Med.* **164**, 915-922.

Feldman *et al.* (2000). The fusion glycoprotein of human respiratory syncytial virus facilitates virus attachment and infectivity via an interaction with cellular heparan sulfate. *J. Virol.* **74**, 6442-6447.

Garcia *et al.* (1994). Evolutionary patterns of human respiratory syncytial virus (subgroup A): cocirculating lineages and correlation of genetic and antigenic changes in the G glycoprotein. *J. Virol.* **68**, 5448-5459.

Garcia-Barreno *et al.* (1989). Marked differences in the antigenic structure of human respiratory syncytial virus F and G glycoproteins. *J. Virol.* **63**, 925-932.

Hall, C.B., *et al.* (2009). The burden of respiratory syncytial virus infection in young children. *N. Engl. J. Med.* **360**, 588-598.

Hallak *et al.* (2000). Glycosaminoglycan sulfation requirements for respiratory syncytial virus infection. *J. Virol.* **74**, 10508-10513.

Hallak, L.K., Kwilas, S.A. & Peeples, M.E. (2007). Interaction between respiratory syncytial virus and glycosaminoglycans, including heparan sulfate. *Methods Mol Biol* **379**, 15-34.

Hendry *et al.* (1986). Concurrent circulation of antigenically distinct strains of respiratory syncytial virus during community outbreaks. *J. Infect. Dis.* **153**, 291-297.

Johnson *et al.* (1987). The G glycoprotein of human respiratory syncytial viruses of subgroups A and B: extensive sequence divergence between antigenically related proteins. *Proc. Natl. Acad. Sci. USA* **84**, 5625-5629.

Johnson *et al.* (2007). The histopathology of fatal untreated human respiratory syncytial virus infection. *Mod. Pathol.* **20**, 108-119.

Kaan, P.M. and Hegele R.G. (2003). Interaction between respiratory syncytial virus and particulate matter in guinea pig alveolar macrophages. *Am. J. Respir. Cell. Mol. Biol.* **28**, 697-704.

Krusat, T. and Streckert, H.J. (1997). Heparin-dependent attachment of respiratory syncytial virus (RSV) to host cells. *Arch. Virol.* **142**, 1247-1254.

Malhotra *et al.* (2003). Isolation and characterisation of potential respiratory syncytial virus receptor(s) on epithelial cells. *Microbes Infect* **5**, 123-133.

Marsh, M. and Helenius, A. (2006). Virus Entry: Open Sesame. *Cell* **124**, 729–740.

Mufson *et al.* (1985). Two distinct subtypes of human respiratory syncytial virus. *J. Gen. Virol.* **66**, 2111-2124.

Nisole *et al.* (1999). The anti-HIV pseudopeptide HB-19 forms a complex with the cell-surface-expressed nucleolin independent of heparan sulfate proteoglycans. *J. Biol. Chem.* **274**, 27875-84.

Peret *et al.* (1998). Circulation patterns of genetically distinct group A and B strains of human respiratory syncytial virus in a community. *J. Gen. Virol.* 79, 2221-2229.

Rueda *et al.* (1991). Premature stop codons in the G glycoprotein of human respiratory syncytial viruses resistant to neutralization by monoclonal antibodies. *J. Virol.* **65**, 3374-3378.

Sullender *et al.* (1991). Genetic diversity of the attachment protein of subgroup B respiratory syncytial viruses. *J. Virol.* **65**, 5425-5434.

Sullender *et al.* (1993). Analysis of respiratory syncytial virus genetic variability with amplified cDNAs. *J. Clin. Microbiol.* **31**, 1224-1231.

Tayyari, F. *et al.* (2011). Identification of nucleolin as a cellular receptor for human respiratory syncytial virus. Nat Med. 17(9):1132-5.

Techaarpornkul, S., Barretto, N. & Peeples, M.E. (2001). Functional analysis of recombinant respiratory syncytial virus deletion mutants lacking the small hydrophobic and/or attachment glycoprotein gene. *J. Virol.* **75**, 6825-6834.

Techaarpornkul, S., Collins, P.L. & Peeples, M.E. (2002). Respiratory syncytial virus with the fusion protein as its only viral glycoprotein is less dependent on cellular glycosaminoglycans for attachment than complete virus. *Virology* **294**, 296-304.

Tuteja *et al.* (1998). Nucleolin: a multifunctional major nucleolar phosphoprotein. *Crit. Rev. Biochem. Mol. Biol.* **22**, 407-436.

Wu, H., Pfarr, D.S., Losonsky, G.A. & Kiener, P.A. (2008). Immunoprophylaxis of RSV infection: advancing from RSV-IGIV to palivizumab and motavizumab. *Curr Top Microbiol Immunol* **317**, 103-123.

Zhang, L., Peeples, M.E., Boucher, R.C., Collins, P.L. & Pickles, R.J. (2002). Respiratory syncytial virus infection of human airway epithelial cells is polarized, specific to ciliated cells, and without obvious cytopathology. *J Virol* **76**, 5654-5666.

INFORMAL SEQUENCE LISTINGS

SEQ ID NO: 1: Description: AS1411

```
1   GGTGGTGGTG GTTGTGGTGG TGGTGG  26
```

SEQ ID NO: 2: Description: human nucleolin

```
1   MVKLAKAGKN QGDPKKMAPP PKEVEEDSED EEMSEDEEDD SSGEEVVIPQ KKGKKAAATS
61  AKKVVVSPTK KVAVATPAKK AAVTPGKKAA ATPAKKTVTP AKAVTTPGKK GATPGKALVA
121 TPGKKGAAIP AKGAKNGKNA KKEDSDEEED DDSEEDEEDD EDEDEDEDEI EPAAMKAAAA
181 APASEDEDDE DDEDDEDDDD DEEDDSEEEA METTPAKGKK AAKVVPVKAK NVAEDEDEEE
241 DDEDEDDDDD EDDEDDDDED DEEEEEEEEE EPVKEAPGKR KKEMAKQKAA PEAKKQKVEG
301 TEPTTAFNLF VGNLNFNKSA PELKTGISDV FAKNDLAVVD VRIGMTRKFG YVDFESAEDL
361 EKALELTGLK VFGNEIKLEK PKGKDSKKER DARTLLAKNL PYKVTQDELK EVFEDAAEIR
421 LVSKDGKSKG IAYIEFKTEA DAEKTFEEKQ GTEIDGRSIS LYYTGEKGQN QDYRGGKNST
481 WSGESKTLVL SNLSYSATEE TLQEVFEKAT FIKVPQNQNG KSKGYAFIEF ASFEDAKEAL
541 NSCNKREIEG RAIRLELQGP RGSPNARSQP SKTLFVKGLS EDTTEETLKE SFDGSVRARI
601 VTDRETGSSK GFGFVDFNSE EDAKAAKEAM EDGEIDGNKV TLDWAKPKGE GGFGGRGGGR
661 GGFGGRGGGR GGRGGFGGRG RGGFGGRGGF RGGRGGGGDH KPQGKKTKFE
```

SEQ ID NO: 3: Description: CRO

```
1   CCTCCTCCTC CTTCTCCTCC TCCTCC  26
```

WHAT IS CLAIMED IS:

1. A method of treating respiratory syncytial virus (RSV) infection in a cell, the method comprising administering AS1411 (SEQ ID NO:1) to the cell.

2. The method of claim 1, wherein the cell is a human cell.

3. The method of claim 2, wherein the human cell is an epithelial cell.

4. The method of claim 2 or 3, wherein the human cell is a ciliated respiratory epithelial cell.

5. The method of any one of claims 1-4, wherein the cell is in a human subject having or at risk of developing an RSV infection.

6. The method of any one of claims 1-5, wherein AS1411 molecule is administered intranasally.

7. The method of any one of claims 1-5, wherein AS1411 molecule is administered intravenously.

8. The method of any one of claims 1-6, wherein AS1411 is topically administered to a mucosal membrane of the subject.

9. The method of any one of claims 1-8, wherein AS1411 is mixed with lipid particles prior to administration.

10. The method of any one of claims 1-8, wherein AS1411 is encapsulated in liposomes prior to administration.

11. AS1411 (SEQ ID NO:1) for use in the treatment of RSV infection.

12. The AS1411 of claim 11, wherein the AS1411 is formulated for intravenous or intranasal administration.

13. Use of AS1411 (SEQ ID NO:1) in the preparation of a medicament for the treatment of RSV infection.

14. Use of AS1411 (SEQ ID NO:1) for the treatment of RSV infection.

15. The use of claim 12 or 13, wherein the treatment is of an animal subject.

16. The use of any one of claims 12-14, wherein the treatment is of a human subject.

17. A commercial package, comprising:
    a. AS1411; and
    b. Instructions for the treatment of RSV infection.

18. The commercial package of claim 15, wherein RSV is a human strain of RSV.

19. The commercial package of claim 15, wherein RSV is a bovine strain of RSV.

20. The commercial package of claim 15, wherein the RSV is an ovine strain of RSV.

21. The commercial package of claim 15, wherein the RSV is an equine strain of RSV.

22. The commercial package of claim 15, wherein the RSV is a porcine strain of RSV.

FIGURE 1

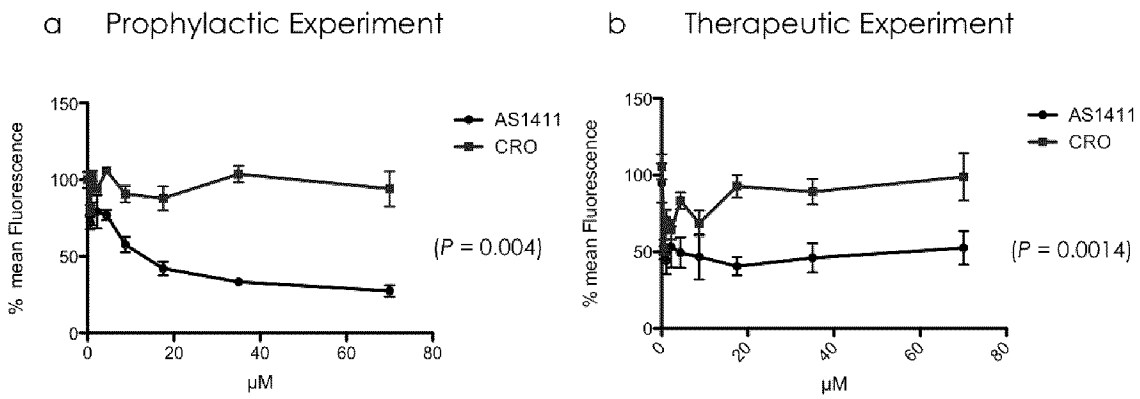
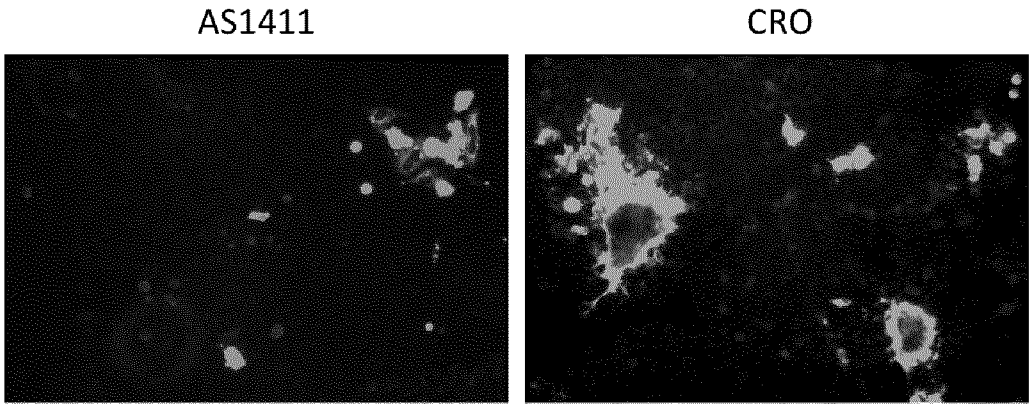

a Prophylactic Experiment
b Therapeutic Experiment
% mean Fluorescence
150
100
50
0
0
20
40
60
80
µM
AS1411
CRO
(P = 0.004)
(P = 0.0014)

FIGURE 2



A B

FIGURE 3

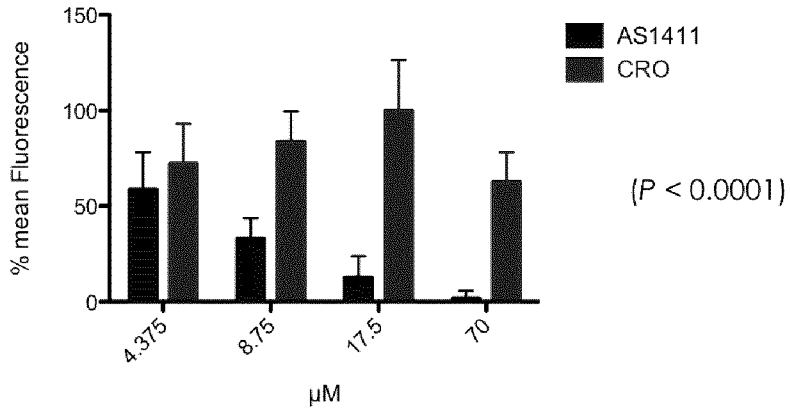

150
100
50
0
% mean Fluorescence
4.375
8.75
17.5
70
µM
AS1411
CRO
(P < 0.0001)

FIGURE 4

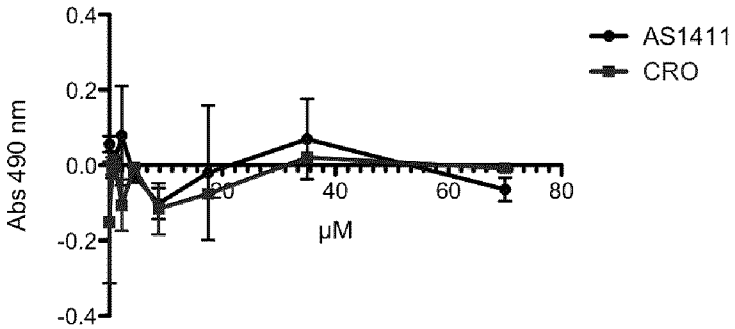
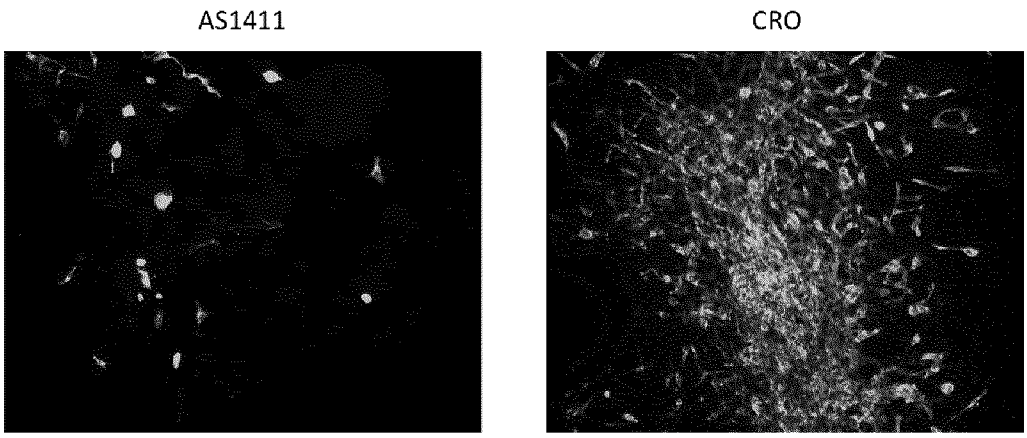


FIGURE 5



A B

FIGURE 6

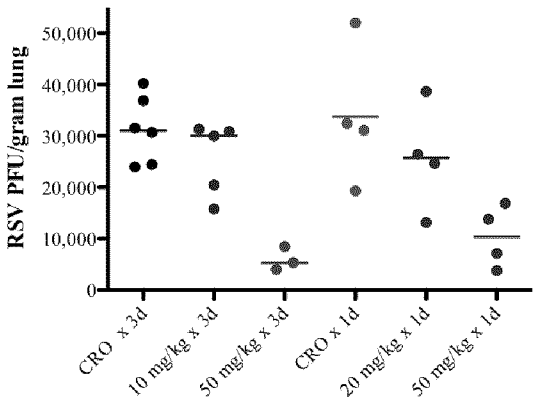

RSV PFU/gram lung
50,000
40,000
30,000
20,000
10,000
0
CRO x 3d
10 mg/kg x 3d
50 mg/kg x 3d
CRO x 1d
20 mg/kg x 1d
50 mg/kg x 1d

FIGURE 7

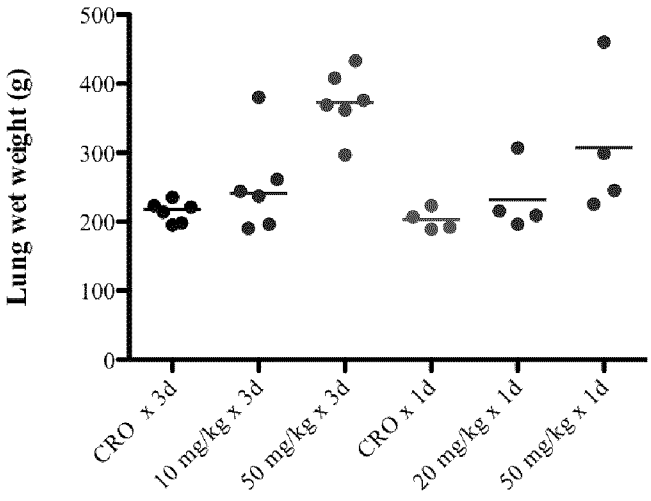

Lung wet weight (g)
500
400
300
200
100
0
CRO x 3d
10 mg/kg x 3d
50 mg/kg x 3d
CRO x 1d
20 mg/kg x 1d
50 mg/kg x 1d

FIGURE 8

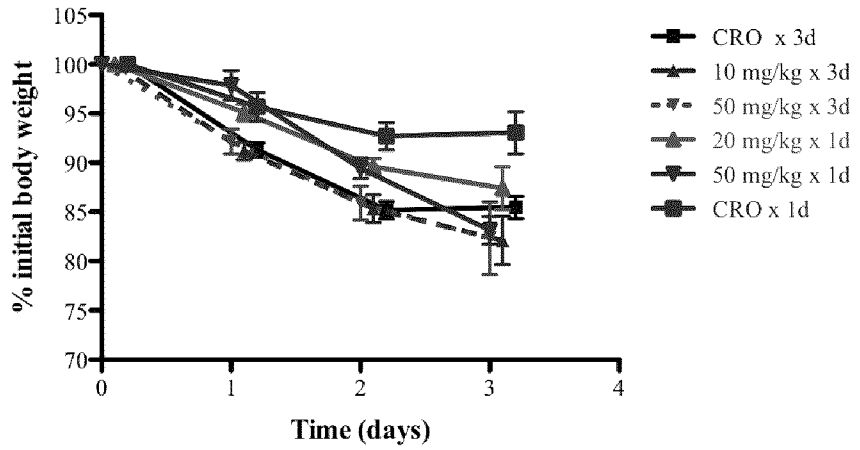

105
100
95
90
85
80
75
70
% initial body weight
0
1
2
3
4
Time (days)
CRO x 3d
10 mg/kg x 3d
50 mg/kg x 3d
20 mg/kg x 1d
50 mg/kg x 1d
CRO x 1d

FIGURE 9

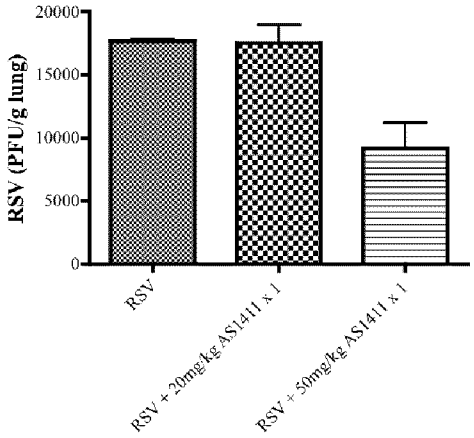

RSV (PFU/g lung)
20000
15000
10000
5000
0
RSV
RSV + 20mg/kg AS1411 x 1
RSV + 50mg/kg AS1411 x 1

FIGURE 10

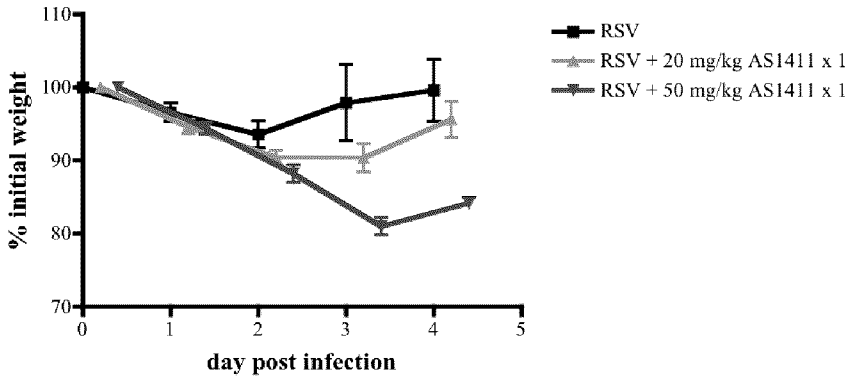
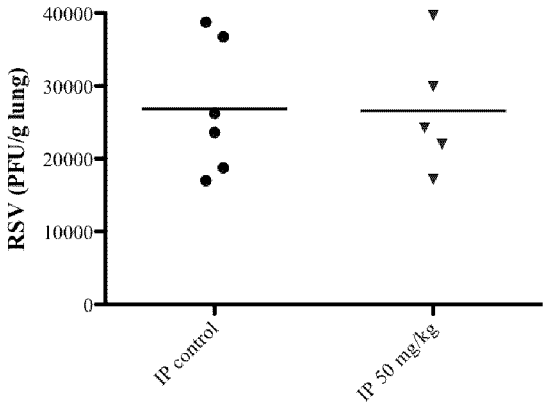

110
100
90
80
70
% initial weight
0
1
2
3
4
5
day post infection
RSV
RSV + 20 mg/kg AS1411 x 1
RSV + 50 mg/kg AS1411 x 1

FIGURE 11



INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2013/050123

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 31/711*** (2006.01) , ***A61P 31/14*** (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: ***A61K 31/711*** (2006.01) , ***A61P 31/14*** (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Biosis, CAplus, Medline, EPOQUE (epodoc, cl txte), Canadian Patent Database, GenomeQuest (SEQ ID NO:1 using GQPat, GeneSeq);
Keywords: respiratory syncytial virus, oligonucleotide(s), aptamer(s), nucleolin, AS1411, GRO26B, AGRO100

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO2009098464A2 (ACTON, G. et al) 13 August 2009 (13-08-2009) - SEQ ID NO:1; Figure 1; cited in the description	11, 12, 17-22
A		1-10, 13-16
X	US2009131351A1 (GREEN, C. and JONES, D.) 21 May 2009 (21-05-2009) - SEQ ID NO:12; Figures 1, 4	11, 12, 17-22
A		1-10, 13-16
X	US2011178161A1 (TRENT, J.O. et al) 21 July 2011 (21-07-2011) - SEQ ID NO:12;	11, 12, 17-22
A		1-10, 13-16
X	WO2011133142A1 (BATES, P.J. and ISLAM, M.A.) 27 October 2011 (27-10-2011) - SEQ ID NO:1	11, 12, 17-22
A		1-10, 13-16

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 June 2013 (28-06-2013)	05 July 2013 (05-07-2013)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Michael W. De Vouge (819) 997-2952

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO2011129494A1 (LEE, J.-H. et al) 20 October 2011 (20-10-2011) - SEQ ID NO: 2	11, 12, 17-22
A		1-10, 13-16
X	WO2007044851A2 (KMIEC, E.B. and PAREKH-OLMEDO, H.) 19 April 2007 (19-04-2007) -SEQ ID NO:11	11, 12, 17-22
A		1-10, 13-16
P, X	MASTRANGELO, P. and HEGELE, R.G. The RSV fusion receptor: not what everyone expected it to be. Microbes and Infection, issue of November 2012 (11-2012), Volume 14, No. 13, pages 1205-1210, ISSN 1286-4579. - Section 5.2	1-22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2013/050123

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 1-10
because they relate to subject matter not required to be searched by this Authority, namely :

- indicated on **Extra Sheet**

2. [ ] Claim Nos. :
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

...continued from **Box No. II**:

Claims 1-10 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search. However, this Authority has carried out a search based on the alleged effect or purpose/use of the product defined in claim 11. It should be noted that methods of administering AS1411 to a cell are considered as methods of medical treatment unless the cell is explicitly stated to be *in vitro*.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2013/050123

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2009098464A2	13 August 2009 (13-08-2009)	EP2268284A2	05 January 2011 (05-01-2011)
		GB0802075D0	12 March 2008 (12-03-2008)
		GB0808956D0	25 June 2008 (25-06-2008)
		GB2460086A	18 November 2009 (18-11-2009)
		JP2011511051A	07 April 2011 (07-04-2011)
		US2011105422A1	05 May 2011 (05-05-2011)
		WO2009098464A3	14 January 2010 (14-01-2010)
US2009131351A1	21 May 2009 (21-05-2009)	None	
US2011178161A1	21 July 2011 (21-07-2011)	AT375358T	15 October 2007 (15-10-2007)
		AU775412B2	29 July 2004 (29-07-2004)
		AU4212900A	14 November 2000 (14-11-2000)
		CA2370000A1	19 October 2000 (19-10-2000)
		DE60036700D1	22 November 2007 (22-11-2007)
		DE60036700T2	24 July 2008 (24-07-2008)
		DK1181304T3	11 February 2008 (11-02-2008)
		EP1181304A1	27 February 2002 (27-02-2002)
		EP1181304A4	02 October 2002 (02-10-2002)
		EP1181304B1	10 October 2007 (10-10-2007)
		EP1930340A1	11 June 2008 (11-06-2008)
		ES2296615T3	01 May 2008 (01-05-2008)
		JP2002541264A	03 December 2002 (03-12-2002)
		PT1181304E	23 January 2008 (23-01-2008)
		US7314926B1	01 January 2008 (01-01-2008)
		US2008318888A1	25 December 2008 (25-12-2008)
		US7960540B2	14 June 2011 (14-06-2011)
		US2008318887A1	25 December 2008 (25-12-2008)
		US8114850B2	14 February 2012 (14-02-2012)
		US2008318889A1	25 December 2008 (25-12-2008)
		US2008318890A1	25 December 2008 (25-12-2008)
		US2009326047A1	31 December 2009 (31-12-2009)
		WO0061597A1	19 October 2000 (19-10-2000)
		WO0061597A9	29 November 2001 (29-11-2001)
WO2011133142A1	27 October 2011 (27-10-2011)	None	
WO2011129494A1	20 October 2011 (20-10-2011)	US2010317723A1	16 December 2010 (16-12-2010)
WO2007044851A2	19 April 2007 (19-04-2007)	AU2006302022A1	19 April 2007 (19-04-2007)
		CA2625035A1	19 April 2007 (19-04-2007)
		EP1940861A2	09 July 2008 (09-07-2008)
		EP1940861A4	09 September 2009 (09-09-2009)
		JP2009511023A	19 March 2009 (19-03-2009)
		US2007105805A1	10 May 2007 (10-05-2007)
		US7807648B2	05 October 2010 (05-10-2010)
		US2008051363A1	28 February 2008 (28-02-2008)
		WO2007044851A3	22 November 2007 (22-11-2007)