Abstracts of papers presented
at the 2010 meeting on

RNA & OLIGONUCLEOTIDE THERAPEUTICS

April 7–April 10, 2010

A

Lower limb

EDB

B

100 bp ladder

Untreated Treated

46 47 51 52

46 47 52

exon 47 exon 52

C

Control Treated Untreated

Dys1

Dysferlin

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

Abstracts of papers presented
at the 2010 meeting on

RNA & OLIGONUCLEOTIDE THERAPEUTICS

April 7–April 10, 2010

Arranged by

Ryszard Kole, *AVI BioPharma*
Adrian Krainer, *Cold Spring Harbor Laboratory*
Bruce Sullenger, *Duke University*

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

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RNA & OLIGONUCLEOTIDE THERAPEUTICS
Wednesday, April 7 – Saturday, April 10, 2010

Wednesday	7:30 pm	Keynote Speaker
Wednesday	8:30 pm	**1** Exon Skipping Therapy
Thursday	9:00 am	**2** siRNA, miRNA and Aptamers as Therapeutics
Thursday	2:00 pm	**3** Poster Session I
Thursday	4:30 pm	Wine and Cheese Party*
Thursday	7:30 pm	**4** Post-transcriptional Control of Gene Expression
Friday	9:00 am	**5** Oligonucleotide Therapy
Friday	2:00 pm	**6** Delivery of RNA Therapeutics
Friday	6:00 pm	Concert
Saturday	9:00 am	**7** Oligonucleotide-induced Immunostimulation and Other Mechanisms

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Poster sessions are located in *Bush Lecture Hall*
**Airslie Lawn,* weather permitting

Mealtimes at Blackford Hall are as follows:
Breakfast 7:30 am-9:00 am
Lunch 11:30 am-1:30 pm
Dinner 5:30 pm-7:00 pm
Bar is open from 5:00 pm until late

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PROGRAM

WEDNESDAY, April 7—7:30 PM

KEYNOTE SPEAKER

Sidney Altman
Yale University

"RNA and therapeutic modes" 1

WEDNESDAY, April 7—8:30 PM

SESSION 1 EXON SKIPPING THERAPY

Chairperson: **R. Kole,** AVI BioPharma, Bothell, Washington

Antisense oligonucleotides to induce exon 51 skipping in boys with Duchenne muscular dystrophy—What we are learning from clinical trials
Francesco Muntoni.
Presenter affiliation: UCL Institute of Child Health, London, United Kingdom. 2

The development of RNA-modulating therapies
Judith C.T. van Deutekom.
Presenter affiliation: Prosensa Therapeutics BV, Leiden, The Netherlands. 3

THURSDAY, April 8—9:00 AM

SESSION 2 siRNA, miRNA AND APTAMERS AS THERAPEUTICS

Chairperson: **B. Sullenger,** Duke University, Durham, North Carolina

Development of universal antidotes to control aptamer activity
Bruce A. Sullenger, Sabah Oney, Jaewoo Lee.
Presenter affiliation: Duke University Medical Center, Durham, North Carolina. 4

RNA oligonucleotides as regulatable antithrombotic agents
Richard C. Becker, Christopher Rusconi, Bruce Sullenger.
Presenter affiliation: Duke Translational Research Institute, Durham, North Carolina. 5

Delivering on the promise of RNA therapeutics
Henrik Ørum.
Presenter affiliation: Santaris Pharma, Copenhagen, Denmark. 6

Small RNA based therapies for the treatment of HIV infection
John J. Rossi.
Presenter affiliation: Beckman Research Institute of the City of Hope, Duarte, California. 7

Prevention of experimental autoimmune encephalomyelitis in mice using an anti-IL-12/23 nucleic acid aptamer
P. Shannon Pendergrast, Robert Silva, Alicia Ferguson, Kristin Thompson, Ryan Boomer, Joe Fraone, Paul Hatala, Jason Killough, Sharon Cload, Karen Olsen.
Presenter affiliation: Archemix Corp, Cambridge, Massachusetts. 8

Creating RNA aptamers to modulate functions of human estrogen receptor alpha
Daiying Xu, Antonis Kourtidis, Douglas Conklin, Hua Shi.
Presenter affiliation: SUNY New York University at Albany, Albany, New York. 9

Preclinical development of osteopontin aptamer as adjuvant therapy for metastatic breast cancer
Zhiyong Mi, Virginia Burns, William Zamboni, Paul Kuo.
Presenter affiliation: Duke Tranlsational Medicine Institute, Durham, North Carolina. 10

THURSDAY, April 8—2:00 PM

SESSION 3 POSTER SESSION I

Modelling and gene silencing in Spinocerebellar ataxia type 7
Sandro Alves, Morwena Latouche, Sandra Duqué, Thibaut Marais, Alice Chort, Alexis Brice, Giovanni Stevanin, Martine Barkats, Annie Sittler.
Presenter affiliation: UPMC/Univ. Paris 6, Inserm UMRS 975, CNRS 7225, Centre de Recherche - Institut du Cerveau et de la Moelle, Paris, France. 11

RNA aptamers targeting the receptor-binding domain of the cancer- associated protease urokinase plasminogen activator
Lisbeth M. Andersen, Daniel M. Dupont, Jeppe B. Madsen, Jørgen Kjems, Peter A. Andreasen.
Presenter affiliation: Aarhus University, Aarhus, Denmark. 12

Regulation of STAT3 alternative splicing and its anti-tumorigenic potential
Francesca Zammarchi, Luca Cartegni.
Presenter affiliation: Memorial Sloan-Kettering Cancer Center, New York, New York. 13

Fluoresecent nanodiamond-assisted delivery of dsRNAs
Han-Yi E. Chou, Jia-Ying Lee.
Presenter affiliation: National Taiwan University, Taipei, Taiwan. 14

Binding, stabilization, and delivery of therapeutic RNA
Lisa Cillessen, Matthew Warner, Stephanie Barber, Brooke Parker, Jenna McNew, Adam Wanekaya, Kartik Ghosh, Lifeng Dong, Michael Craig, Richard Garrad, Garry Glaspell, Robert DeLong.
Presenter affiliation: Missouri State University, Springfield, Missouri. 15

Efficient siRNA delivery into primary cells by a peptide transduction domain-dsRNA binding domain fusion proteins
Akiko Eguchi, Hiroyuki Michiue, Yung-Chi Chang, Bryan R. Meade, Craig T. Fredrickson, Karl Willert, Nitin Puri, Steven F. Dowdy.
Presenter affiliation: Howard Hughes Medical Institute, Chevy Chase, Maryland; UCSD, La Jolla, California. 16

Synthesis of the RNA analog 2’-O-methyl-3’-amino-3’-deoxyribonucleic acid
Benjamin D. Heuberger, Jack W. Szostak.
Presenter affiliation: Howard Hughes Medical Institute, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts. 17

Overcoming immunostimulatory issues associated with therapeutic siRNA for topical delivery to the lung
Stephen A. Hughes, Kenneth L. Clark, Jill Coates, Doug I. Ball, Kitty E. Moores, Joel D. Parry, Steffanos I. Ioannidis, Paul A. Wilson, Mark R. Edbrooke.
Presenter affiliation: GlaxoSmithKline, Stevenage, United Kingdom. 18

Effective inhibition of core gene of HCV 3a genotype using synthetic and vector derived siRNAs
Saba Khaliq, Shah Jahan, Bushra Ijaz, Sajida Hassan.
Presenter affiliation: University of the Punjab, Lahore, Pakistan. 19

The generation and characterization of nucleic acid aptamers that selectively inhibit human IL-23 versus human IL-12 in vitro
Jason Killough, Sharon T. Cload, Kristin M. Thompson, John L. Diener, Alicia Preiss, Daniel Lagasse, Shuhao Zhu, P. Shannon Pendergrast.
Presenter affiliation: Archemix Corp, Cambridge, Massachusetts. 20

High-resolution Northern blot for reliable analysis of microRNAs and their precursors
Edyta Koscianska, Julia Starega, Marta Olejniczak, Wlodzimierz J. Krzyzosiak.
Presenter affiliation: Institute of Bioorganic Chemistry, Poznan, Poland. 21

Intracellular delivery of an antisense oligonucleotide via targeting gastrin-releasing peptide receptor
Xin Ming, Md. Rowshon Alam, Michael Fisher, Xiaoyuan Chen, Rudolph L. Juliano.
Presenter affiliation: The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina. 22

Small molecule ligands target a conjugated oligonucleotide to the sigma receptor in cancer cells
Osamu Nakagawa, Xin Ming, Michael Fisher, Leaf Huang, Rudolph L. Juliano.
Presenter affiliation: The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina. 23

Nucleoside modifications regulate activation of the protein kinase PKR in an RNA structure-specific manner
Subba Rao Nallagatla, Christie N. Jones, Linda L. Spremulli, Philip C. Bevilacqua.
Presenter affiliation: Pennsylvania State University, University Park, Pennsylvania. 24

Efficient gene silencing by gymnotic delivery of locked nucleic acid antisense oligonucleotides
Jacob Ravn, Johnathan Lai, Cy A. Stein, Luba Benimetskaya, Paul Miller, Daniela Castanotto.
Presenter affiliation: Santaris Pharma A/S, Hørsholm, Denmark. 25

Development of novel RNAi based therapeutics against cancer
Shaguna Seth, Yoshiyuki Matsui, Kathy Fosnaugh, Yan Liu, Narendra Vaish, Roger Adami, Pierrot Harvie, Rachel Johns, Gregory Severson, Yan Chen, Tod Brown, Susan Bell, Brian Granger, Akihide Takagi, Michael Houston, Alan So, Michael Templin, Barry Polisky.
Presenter affiliation: MDRNA Inc., Bothell, Washington. 26

Improved shRNA activity encoded within oncolytic Ad vectors
David Sharon, Michael Schuemann, Mary M. Hitt.
Presenter affiliation: University of Alberta, Edmonton, Canada. 27

Development of aptamer-based cancer therapeutics targeting transmembrane glycoprotein NMB (GPNMB)
Johannes H. Urban, Chien-Tsun Kuan, Bruce A. Sullenger.
Presenter affiliation: Duke University Medical Center, Durham, North Carolina. 28

The profiling of microRNAs and its target genes on MDSCs in tumor-bearing mouse
Sungsook Yu, Young H. Kim, Won Hyun Song, Byoung S. Kwon.
Presenter affiliation: National Cancer Center, Goyang, Gyeongido, South Korea. 29

Elucidating the kinetics of siRNA-based therapeutics
Jonathan E. Zuckerman, Chung Hang J. Choi, Mark E. Davis.
Presenter affiliation: Caltech, Pasadena, California. 30

THURSDAY, April 8—7:30 PM

Wine and Cheese Party

THURSDAY, April 8—7:30 PM

SESSION 4 POST TRANSCRIPTIONAL CONTROL OF GENE EXPRESSION

Chairperson: **C. Thornton,** University of Rochester, New York

Oligonucleotide therapeutics in myotonic dystrophy
Charles A. Thornton, Thurman M. Wheeler, Sobczak Krzysztof.
Presenter affiliation: University of Rochester, Rochester, New York. 31

Allele selective inhibition of human huntingtin protein expression
David Corey.
Presenter affiliation: University of Texas Southwestern Medical Center, Dallas, Texas. 32

Epigenetic reprogramming of tumor suppressor genes by Artificial Transcription Factors
Pilar Blancafort.
Presenter affiliation: University of North Carolina, Chapel Hill, North Carolina. 33

Therapeutic gene modification via triplex-forming peptide nucleic acids
Peter M. Glazer.
Presenter affiliation: Yale University, New Haven, Connecticut. 34

Targeted degradation of toxic RNA in myotonic dystrophy
Johanna E. Lee, Thomas A. Cooper.
Presenter affiliation: Baylor College of Medicine, Houston, Texas. 35

Silencing of prolonged CAG repeat containing alleles in Huntington's disease using antisense oligonucleotides
Melvin Evers, Judith van Deutekom, Annemieke Aartsma-Rus, Paolo Paganetti, Johan den Dunnen, Gert-Jan van Ommen, Willeke van Roon-Mom.
Presenter affiliation: Leiden University Medical Center, Leiden, Netherlands. 36

FRIDAY, April 9—9:00 AM

SESSION 5 OLIGONUCLEOTIDE THERAPY

Chairperson: **A. Krainer,** Cold Spring Harbor Laboratory, New York

Antisense correction of SMN2 splicing in the central nervous system of mouse models of spinal muscular atrophy
Adrian Krainer.
Presenter affiliation: Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 37

Current progress with the systemic administration trial of AVI-4658, a novel Phosphorodiamidate Morpholino Oligomer (PMO) skipping dystrophin exon 51 in Duchenne muscular dystrophy (DMD)
Stephen B. Shrewsbury, Francesco Muntoni, Sebahattin Cirak, Michela Guglieri, Katie Bushby.
Presenter affiliation: AVI BioPharma Inc, Bothell, Washington. 38

RNA-targeting with second generation antisense drugs—Bench to clinic
Richard Geary.
Presenter affiliation: Isis Pharmaceuticals Inc., Carlsbad, California.

RNA-based therapeutics for emerging infectious disease
Patrick Iversen, Sina Bavari, Travis Warren, Thomas Voss, Dan Mourich
Presenter affiliation: AVI BioPharma Inc., Bothell, Washington. 39

Long term systemic antisense-mediated exon skipping in dystrophic mouse models
Annemieke Aartsma-Rus, Christa L. de Winter, Hans A. Heemskerk, Maaike van Putten, Judith C. van Deutekom, Gert-Jan B. van Ommen.
Presenter affiliation: Leiden University Medical Center, Leiden, Netherlands. 40

Repeat dose toxicology evaluation of AVI-4658 PMO in monkeys and mice
Peter L. Sazani, Doreen L. Weller, Stanley S. Stadnicki, Steven B. Shrewsbury.
Presenter affiliation: AVI BioPharma, Bothell, Washington. 41

Antisense oligonucleotides—Potential therapeutic strategy against breast cancer
Rajesh K. Gaur, Shikha Gaur, John Shively, Yun Yen.
Presenter affiliation: Beckman Research Institute, Duarte, California. 42

FRIDAY, April 9—2:00 PM

SESSION 6 DELIVERY OF RNA THERAPEUTICS

Chairperson: **S. Dowdy,** Howard Hughes Medical Institute, University of California, San Diego

siRNA delivery—The 800 pound gorilla
Steven F. Dowdy.
Presenter affiliation: Howard Hughes Medical Institute, La Jolla, California. 43

Delivering the punch—Targeted delivery of siRNA in vivo
Paloma H. Giangrande, William H. Thiel, Kristina W. Thiel, Justin P. Dassie, Xiuying Liu, Katie R. Stockdale, William M. Rockey, James O. McNamara II.
Presenter affiliation: University of Iowa, Iowa City, Iowa. 44

Advances in the therapeutic use of RNA interference (RNAi)
P. Pavco, K. Bulock, L. Libertine, J. Cardia, K. Flannery-Rossi, J. Metterville, T. Drew, M. Byrne, J. Kamens, G. Ford, A. Rodgers, A. Khvorova.
Presenter affiliation: RXi Pharmaceuticals, Worcester, Massachusetts. 45

RNAi therapeutics—Discovery, delivery and development
Muthiah Manoharan.
Presenter affiliation: Alnylam Pharmaceuticals, Cambridge, Massachusetts.

Evaluation of lipid nanoparticle-mediated systemic delivery of siRNA in live mice by non-invasive bioluminescence imaging
Joe Davide, Mingmei Cai, Guo-Jun Zhang, Vicki South, Laura Sepp-Lorenzino, Weikang Tao.
Presenter affiliation: Merck Research laboratories, West Point, Pennsylvania. 46

Anti-tumor activity of splice-switching oligonucleotides in vivo
John Bauman, Shyh-Dar Li, Angela Yang, Leaf Huang, Ryszard Kole.
Presenter affiliation: University of North Carolina School of Medicine, Chapel Hill, North Carolina. 47

Directing miRNA-regulatory PNAs to breast cancer cells with EGF analogs
Yuan-Yuan Jin, Chang-Po Chen, Rui-Yan Jing, Eric Wickstrom.
Presenter affiliation: Thomas Jefferson University, Philadelphia, Pennsylvania. 48

FRIDAY, April 9

BANQUET

Cocktails 6:00 PM Dinner 6:45 PM

SATURDAY, April 10—9:00 AM

SESSION 7 OLIGONUCLEOTIDE INDUCED IMMUNOSTIMULATION AND OTHER MECHANISMS

Chairperson: **S. Agrawal,** IDERA Pharmaceuticals, Cambridge, Massachusetts

Modulation of TLR-mediated immune responses with synthetic oligonucleotides
Sudhir Agrawal.
Presenter affiliation: Idera Pharmaceuticals, Inc., Cambridge, Massachusetts. 49

Understanding the immune effects of therapeutic nucleic acids
Arthur M. Krieg.
Presenter affiliation: Pfizer, Cambridge, Massachusetts. 50

Improving the therapeutic pipeline
Andrew D. Ellington.
Presenter affiliation: University of Texas at Austin, Austin, Texas. 51

Pro- to anti-angiogenic switch—Changing VEGFR activity by splicing/polyadenylation interference
Sandra Vorlova, Luca Cartegni.
Presenter affiliation: Memorial Sloan-Kettering Cancer Center, New York, New York. 52

Gene silencing by synthetic U1 adaptors
Rafal Goraczniak, Mark A. Behlke, Chris Miller, Michael Neubauer, Wen-Pin Yang, Samuel I. Gunderson.
Presenter affiliation: Rutgers University, Piscataway, New Jersey. 53

The p53 target Wig-1 regulates mRNA stability and is necessary for normal embryonic development
Anna Vilborg, Björn Rozell, Cinzia Bersani, Klas G. Wiman, Margareta T. Wilelm.
Presenter affiliation: Karolinska Institutet, Stockholm, Sweden. 54

The regulation of transcription-driven R-loop formation and its therapeutic implications
Tsai-Kun Li, Shu-Yu Huang, Chen-Yu Wen, Yu-Chen Yang.
Presenter affiliation: College of Medicine, National Taiwan University, Taipei, Taiwan. 55

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RNA AND THERAPEUTIC MODES

Sidney Altman
Yale University, Department of Molecular, Cellular and Developmental Biology, New Haven, CT, 06520

RNA plays a central role in biology. Its primacy, and variety of sizes in vivo, indicates that it may be useful as a target and a means of developing a therapeutic tool. Some of these problems involve extensive and detailed analysis of metabolic pathways in vivo, which is a classic tool for drug development, and others are centered on a simple analysis of a particular situation and how to deal with the RNAs involved. The size of RNA and its negative charge present problems in targeting diseased cells in this regard. However, different ways of approaching all these issues are available. These aspects of developing a clinical tool will be discussed.

ANTISENSE OLIGONUCLEOTIDES TO INDUCE EXON 51 SKIPPING IN BOYS WITH DUCHENNE MUSCULAR DYSTROPHY: WHAT WE ARE LEARNING FROM CLINICAL TRIALS

Francesco Muntoni

UCL Institute of Child Health, The Dubowitz Neuromuscular Centre, 30 Guilford Street, London, WC1N 1EH, United Kingdom

The UK MDEX Consortium (http://www.mdex.org.uk/) has been involved since 2005 in preclinical studies and in clinical trials using antisense oligonucleotides to induce exon skipping in boys with Duchenne muscular dystrophy (DMD). In close collaboration with AVI Biopharma, in 2008 we completed a two doses-escalation study of a morpholino oligonucleotide (AVI-4658) which induces skipping exon 51 in dystrophin mRNA in seven patients with DMD. In 2009 we have started and now almost completed, also in close collaboration with AVI Biopharma, an open label, dose escalation study in ambulant DMD boys aged 5-15 years with deletions that benefit from skipping exon 51. This study consists of 12 weekly administrations of AVI-4658 followed by a muscle biopsy to assess dystrophin expression at baseline and 14 weeks.

During the last few years we have optimised methodologies for the identification of lead antisense compounds; we have elected to skip exon 51 based on our experience of the mild resulting in-frame mutations; we have developed techniques to detect and quantitate small changes in dystrophin protein expression, and of its associated protein partners in the dystrophin glycoprotein complex.

The encouraging results of the previous IM study, and the interim analysis of the ongoing systemic IV study, together with the excellent tolerability profile of the morpholino antisense oligonucleotides, clearly suggest that this approach has the potential to lead to the development of a drug that could play a role in the treatment of DMD.

A number of challenges remain, ranging from the optimal administration regimens of the antisense oligonucleotides, to the initiation of studies targeting other exons, to the validation and adoption by the international scientific community of clinical and biochemical outcome measures which could allow to compare and learn from the different studies which are being planned or executed, to the regulatory complexity of antisense oligonucleotides as indeed the level of personalised approach that these compounds bring is currently without precedent for a genetic disease.

In my presentation will discuss what we have learned since 2005 in relations to these various issues and how this is informing our future approach to exon skipping in DMD.

THE DEVELOPMENT OF RNA-MODULATING THERAPIES

Judith C.T. van Deutekom
Prosensa Therapeutics BV, VP Discovery Leiden, 2300, Netherlands

RNA-modulating therapeutics like antisense oligonucleotides (AONs) provide an innovative tool for targeted modulation of gene expression and/or to correct mutated mRNA causing life threatening disorders. An increasing number of studies show that AONs can interfere with splicing in order to induce exon skipping, enhance exon inclusion, or correct splicing mutations, can remove mutant RNA or protein domains, or block RNA expression. Prosensa Therapeutics applies the AON technology platform to develop RNA-modulating therapies for a variety of genetic diseases, including neuromuscular and neurodegenerative disorders such as Duchenne Muscular Dystrophy (DMD), Myotonic Dystrophy (DM1), Huntington's Disease (HD) and Spinal Muscular Atrophy (SMA). First proof-of-concept was recently obtained with PRO051 (GSK2402968) for DMD. Based upon promising results from two clinical phase 1/2 studies the compound is now moving into phase 3. We will present highlights and challenges from our development program.

DEVELOPMENT OF UNIVERSAL ANTIDOTES TO CONTROL APTAMER ACTIVITY

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With an ever-increasing number of people taking numerous medications, the need to safely administer drugs and limit unintended side effects has never been greater. Antidote control remains the most direct means to counteract acute side effects of drugs but unfortunately it has been challenging and cost prohibitive to generate antidotes for most therapeutic agents. We will describe the development of a set of antidote molecules that are capable of counteracting the effects of an entire class of therapeutic agents, aptamers. These universal antidotes exploit the fact that when systemically administered, aptamers are the only free extracellular oligonucleotides naturally found in circulation. We demonstrate that protein and polymer-based molecules that capture oligonucleotides can reverse the activity of several aptamers in vitro and counteract aptamer activity in vivo. The availability of universal antidotes to control the activity of any aptamer suggests that aptamers may be a particularly safe class of therapeutics.

RNA OLIGONUCLEOTIDES AS REGULATABLE ANTITHROMBOTIC AGENTS

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αThrombosis serves as focal point of interface for tissue injury, inflammation and immunity, and represents a unifying phenotype for both inherited and acquired disorders of impaired vascular homeostasis. Actively controlled and fully reversible antithrombotics potentially expand the current therapeutic paradigm for clinicians, providing flexibility and both patient- and condition-specific treatment options. Aptamers are single-stranded nucleic acids that bind with high affinity and specificity to a target protein or small molecule. A complementary oligonucleotide, directed to a portion of the aptamer can elicit a structural change, preventing an interaction and reversing the drugs' pharmacodynamic activity. A family of coagulation factor IXa aptamers was identified using iterative in vitro techniques (SELEX) against a library of 1014 nucleic acid sequences. The lead compound, RB006 and its complementary active reversal agent, RB007(figure) have undergone extensive pre-clinical and clinical testing, to include first-in-human phase 1a, b and c studies, a phase 2A pilot study and an ongoing, international phase 2B investigation of patients with an acute coronary syndrome undergoing percutaneous coronary intervention.

DELIVERING ON THE PROMISE OF RNA THERAPEUTICS

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Short, high affinity oligonucleotides based on the LNA chemistry are able to potently and specifically inhibit mRNA and miRNA *in-vitro* and in *in-vivo*. Target inhibition can be achieved in many different tissues upon systemic delivery and can be sustained by very infrequent dosing. Notably, short LNA oligonucleotides do not require any form of delivery vehicle to exert their potent pharmacology in cell cultures or experimental animals. To date, four LNA oligonucleotides have advanced to clinical trials in cancer and infectious diseases. The most advanced compound, SPC3649, targets miRNA-122 and is being developed for the treatment of HCV. The presentation will provide an overview of the LNA technology platform and the clinical candidates, with particular emphasis on SPC3649.

SMALL RNA BASED THERAPIES FOR THE TREATMENT OF HIV INFECTION

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Despite the widespread use of chemotherapy for the treatment of HIV infection there still exists a need for new approaches for treatment of HIV infection due to viral drug resistance and toxicity problems. To address these problems we have developed and tested a novel combination of small RNA based inhibitors which inhibit the virus as well as cellular transcripts. One approach involves the use of small Pol III expression cassettes in a gene therapy stem cell setting. The inhibitory RNAs consist of an RNAi triggering anti-HIV shRNA, a hammerhead ribozyme targeting a cellular co-receptor and a nucleolar localizing TAR decoy. This triple combination is currently in a first in man clinical trial for AIDS/lymphoma patients using gene modified autologous hematopoietic stem cells in a stem cell transplant setting. The background and current status of this trial will be discussed. The second approach involves synthetic anti-HIV envelope aptamer-siRNA combinations which have a dual inhibitory function. The aptamer neutralizes free virus, but also serves as a vehicle for delivery of anti-HIV and cellular targeted Dicer substrate siRNAs into HIV infected cells. These novel inhibitors have been tested in a humanized mouse model for their ability to inhibit HIV replication and spread. The animals were first challenged with HIV followed several weeks later by intravenous injection of the aptamer-siRNA chimeras. Remarkably this dual inhibitory approach resulted in up to 1 million fold inhibition of viral replication after the first administration. More importantly, the dual inhibitors resulted in complete protection from T-cell depletion well beyond the last injection. This is in contrast to the untreated animals and controls which suffered substantial CD4 Tcell depletion. These results will be discussed in the context of possible use in humans.

PREVENTION OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS IN MICE USING AN ANTI-IL-12/23 NUCLEIC ACID APTAMER

P. Shannon Pendergrast, Robert Silva, Alicia Ferguson, Kristin Thompson, Ryan Boomer, Joe Fraone, Paul Hatala, Jason Killough, Sharon Cload, Karen Olsen

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Nucleic acid aptamers, short oligonucleotides which fold into well-defined three-dimensional structures capable of specifically binding proteins, have emerged as a new class of therapeutic compounds. While numerous aptamers have been shown to inhibit a variety of targets, no systemically administered aptamer has shown efficacy in an inflammatory disease model. IL-23 is a critical cytokine for the development of mouse experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis characterized by immune inflammation of the CNS. It is a heterodimeric protein, and antibodies specific for either the p40 or the p19 sub-units protect mice from EAE. A non-vascular site of action for IL-23 in EAE is supported by expression data and the observation that EAE induction is rescued in IL-23 knock-out mice by ectopic expression of IL-23 in the CNS but not in the vascular compartment. We have generated an aptamer that inhibits mouse IL-23 and the related cytokine mouse IL-12. The aptamer inhibits mIL-23-dependent STAT3 activation of human PHA blasts by either mIL-23 or mIL-12 (IC50s for both at ~6nM). More importantly, we have found that systemic chronic dosing of our aptamer prevents PLP(139-151)-induced EAE in mice. The aptamer reduces symptoms of the disease in a dose-dependent manner; with the highest dose (50 mg/kg) completely preventing the onset of disease. This is the first demonstration of efficacy by a systemically administered aptamer in an inflammatory disease model and significantly expands the range of indications for this powerful class of therapeutic compounds.

CREATING RNA APTAMERS TO MODULATE FUNCTIONS OF HUMAN ESTROGEN RECEPTOR ALPHA

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A significant number of breast cancers are estrogen receptor (ER)-positive and treatable by ER antagonists. The human estrogen receptor (hER) alpha protein is composed of multiple domains and bears multiple sites interacting with other factors or elements. However, all ER antagonists currently in use target the ligand-binding pocket of ER. We hypothesized that some other sites on hER alpha may be validated as new drug targets for treating breast cancer and other estrogenopathies. To test this hypothesis, we used RNA aptamers as a means for modulation of hER alpha functions through specific protein surface occlusion.

Two classes of high affinity RNA aptamers have been developed for unliganded full length hER alpha through the method of in vitro selection. Class I aptamers bind to both isoforms of hER, alpha and beta, with similar affinities, while class II aptamer only binds to hER alpha with high affinity. These results suggested that the two classes bind to different sites on hER alpha. To study the efficacy of the aptamers in breast cancer cell lines, genetic systems have been constructed to produce aptamers through transcription from synthetic genes delivered into the cells. In ER-positive breast cancer cell line MCF 7, the expressed aptamers reduced hER alpha-driven luciferase gene activity by 30-50%.

PRECLINICAL DEVELOPMENT OF OSTEOPONTIN APTAMER AS ADJUVANT THERAPY FOR METASTATIC BREAST CANCER

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Breast cancer remains one of the most common and devastating malignancies worldwide. Clinically, over 95% of women with breast cancer have no overt metastatic disease at initial diagnosis, yet half of these women will eventually die from breast cancer metastasis in the absence of systemic therapy. Osteopontin (OPN) is a secreted tumor protein which is recognized as a mediator of breast cancer growth and metastasis, thus it represents an attractive therapeutic target for RNA aptamer-based therapy. An RNA aptamer (OPN-R3) directed against OPN has been identified using SELEX. This aptamer has shown to significantly decrease local progression and metastases in a xenograft model of human breast cancer through ablation of OPN surface. Preliminary evidence indicated that OPN-R3 had a high potential for translational impact and further Preclinical studies have been initiated to translate this innovative therapy into clinical trials. Thus far, the team has successfully completed: (1) Optimization studies of the OPN-R3 aptamer for increased biostability (2) In vitro and In vivo studies to evaluate K_d and $t_{1/2}$ for the optimized OPN-R3 aptamer, (3) Evaluation of various dosing strategies, (4) Confirmation of aptamer function through further efficacy studies using Murine xenograft models (MDA-MB231 human breast cancer cells). Herein we discuss the current results from these studies and how the data will fuel the next phase of preclinical development for this innovative breast cancer therapeutic.

MODELLING AND GENE SILENCING IN SPINOCEREBELLAR ATAXIA TYPE 7

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Spinocerebellar ataxia type 7 is a dominantly inherited neurodegenerative disorder. The mutation has been identified as a CAG trinucleotide repeat expansion in the coding region of the ATXN7 gene which encodes the ataxin-7 protein (ATXN7). In the present work we engineered lentiviral vectors encoding either truncated wild-type and truncated mutant human ataxin-7 to develop an SCA7 in vivo model. In this study, we demonstrate that overexpression in the cerebellum of adult mice of mutant but not wild-type human ataxin-7 is associated with the formation of ubiquitinated ataxin-7 aggregates, loss of the calbindin and parvalbumin markers, disruption of the neurofilaments and microtubule-associated protein type 2, and strong activation of astrocytes, suggesting neuronal dysfunction.
Gene silencing by RNA interference (RNAi) is a process that suppresses the expression of a gene at the RNA level. RNAi holds promise as a potential therapy to treat dominantly inherited human diseases, many of which are currently untreatable, such as SCA7. Thus, miRNA-based approaches may provide more appropriate biological tools for expressing inhibitory RNAs in the brain, the implications of which are crucial to the development of RNAi for therapeutic applications. In the present work, we engineered lentiviral vectors encoding a microRNAs (miRNA) cassette targeting human ATXN7. The efficacy of these miRNAs was assessed by co-transfecting human 293T cells with plasmids expressing truncated wild-type or truncated mutant human ATXN7 (trATXN7-10Q or trATXN7-100Q) with the miRNAs vectors and analyzed by immonufluorescence, western-blot and RT-PCR. We are currently testing these miRNAs in the lentiviral-based mouse model of SCA7 in order to check if we obtain a reduction in the SCA7 neuropathological readouts of neurotoxicity.

RNA APTAMERS TARGETING THE RECEPTOR-BINDING DOMAIN OF THE CANCER- ASSOCIATED PROTEASE UROKINASE PLASMINOGEN ACTIVATOR

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Systematic evolution of ligands by exponential enrichment (SELEX) is a relatively new approach for generating potential therapeutic and diagnostic agents. The technique combines the ability of RNA or DNA oligonucleotides to fold into a variety of three-dimensional structures, with the possibility of selecting, from very large pools of random sequences (~1015), the ones capable of binding to a target of interest. We have generated a library of serum-stable 2´-F-pyrimidine modified RNA oligonucletides and used it in a SELEX experiment to select sequences, or so-called aptamers, binding to human urokinase plasminogen activator (uPA). The aptamers have been analysed by surface plasmon resonance (SPR) and bind to the amino terminal fragment of human but not murine uPA, with KD-values in the low nanomolar range. SPR analyses and cell assays have shown that aptamer binding block the association of uPA with its receptor uPAR and in consistency with the probable binding area, they do not inhibit the uPA proteolytic activity directly. However, the aptamers inhibit cell-dependent plasminogen activation. uPA-binding aptamers represent a promising principle for interfering with the pathophysiological functions of the plasminogen activation system and targeting the receptor-ligand interaction through uPA may be beneficial as uPAR-directed agents may not be antagonists of all functions of uPAR.

REGULATION OF STAT3 ALTERNATIVE SPLICING AND ITS ANTI-TUMORIGENIC POTENTIAL

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Signal transducer and activator of transcription (STAT) proteins are a family of signalling molecules implicated in growth factors and cytokines signalling. STATs are latent transcription factors thatare sequestered in the cytoplasm in an inactive form. Upon JAK/SRC dependent phosphorylation, they dimerize, translocate to the nucleus, and activate transcription of target genes. In normal physiological conditions, STAT proteins have a limited activation period, whereas they (especially STAT3) show a persistent activation in many human cancers. This promotes growth and survival of tumor cells, induces tumor angiogenesis and suppresses anti-tumor immune responses.

Because of its pivotal position at the convergence of many oncogenic tyrosin-kinase signaling pathways, STAT3 seems to be particularly suitable as a molecular target for cancer therapy, especially considering that tumor cells tend to become addicted to persistent STAT3 signaling.

A naturally occurring alternative splicing variant, STAT3-beta, uses an alternative acceptor site within exon 23 and leads to the production of a truncated isoform, which lacks the C-terminal trans-activation domain (TAD). STAT3-beta can act as a dominant negative regulator of transcription and promote apoptosis.

We are characterizing the cis-elements and trans-acting factors that regulate STAT3 exon 23 alternative splicing and have used modified antisense oligonucleotides to specifically induce a shift from the abundant, active STAT3-alpha to the anti-tumorigenic STAT3-beta isoform.

Induction of the endogenous STAT3-beta leads to cell death and apoptosis in cell lines addicted to STAT3 activation and causes tumor regression *in vivo*.

FLUORESECENT NANODIAMOND-ASSISTED DELIVERY OF DSRNAS

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The incorporation of targeting, imaging, and ablation/therapeutic capabilities on a biocompatible RNA delivery platform is a powerful approach for cancer therapy. Matching these multiple requirements, type Ib fluorescent nanodiamonds (FNDs) can emit no photobleaching and no photoblinking fluorescence from their nitrogen-vacancy point defects, at a spectral range well suited for long term observation in living cells. Here we report that FNDs can assist delivery of siRNAs, resulting in enhanced efficacy of gene silencing at very low dose of siRNA. This is achieved via caveolin-mediated endocytosis of FND-siRNA particles, which are rapidly released from endosome compartments as shown by electron microspe studies. Time-dependent studies also indicate that the siRNA delivered attain target gene silencing in a more rapid, as well as prolong kinetics than conventional delivery systems. Moreover, long-term presence of FNDs do not show detectable impact on cell viability and proliferation, while providing excellent indication for positive delivery and cell tracing. The possible mechanisms involved are discussed.

BINDING, STABILIZATION, AND DELIVERY OF THERAPEUTIC RNA

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Our current group focus is on the binding, stabilization, and delivery of therapeutic RNA via nanomaterials. We have synthesized gold, manganese and zinc oxide nanoparticles by chemical and physical methods and characterized their interaction with RNA. Using UV/Vis and dynamic laser light spectroscopic measurements we have been able to determine association of the RNA to the Au, MnO and ZnO nanoparticles. Absorbance differences and shifts in the nucleic acid or nanomaterial spectra reveal these interactions. A measurable change in surface or zeta potential is observed upon their association as well. The therapeutic utility and pharmacologic potential of macromolecular RNA in particular is greatly limited by its instability as it is subject to rapid chemical and enzymatic degradation. Therefore, one must focus on the stabilization of RNA before imagining delivery. Thus recently we have begun experiments aimed at determining the nanomaterials ability to stabilize RNA when subjected to such accelerated degradation treatment. The kinetics and extent of degradation are monitored by electrophoretic and HPLC determination and thus protection of the RNA by binding to biomaterials and nanoparticles can be measured. Protection against chemical, RNase, and nucleases present in serum and various tissue extracts is sought. Once a stabilizing condition for the RNA bionanoparticles is devised, one can begin to envision the delivery of splice-site switching oligonucleotide or siRNA against model or therapeutic RNA targets.

EFFICIENT SIRNA DELIVERY INTO PRIMARY CELLS BY A PEPTIDE TRANSDUCTION DOMAIN-DSRNA BINDING DOMAIN FUSION PROTEINS

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Targeted mRNA degradation by siRNA-induced RNA interference (RNAi) allows for selective manipulation of cellular phenotypes and has great potential to treat human disease. However, due to their size (~14,000 Dalton) and charge, siRNAs have no bioavailability to enter unperturbed cells. Moreover, current delivery approaches fail to deliver siRNAs into the cast majority of cell types, especially primary cells. Here we report a siRNA delivery approach that targets 100% of cells by Peptide Transduction Domain-dsRNA Binding Domain (PTD-DRBD) fusion proteins. DRBDs bind siRNAs in a sequence-independent manner that masks the negative charge, allowing for cationic PTD-mediated siRNA delivery. PTD-DRBD delivered siRNAs induced RNAi responses in the entire population of all 23 cell types tested, including primary human umbilical vein endothelial cells (HUVEC), primary fibroblasts, keratinocytes, primary T cell, macrophage and human embryonic stem cells (hESCs). Indeed, PTD-DRBD-siRNA mediated knockdown of the Oct4 pluripotent transcription factor induced hESC differentiation. Moreover, we observed no cytotoxicity, minimal off-target transcriptional changes and no induction of innate immune responses. Using transgenic ROSA26 mice expressing luciferase in the nasal and tracheal passages, we showed that PTD-DRBD–delivered Luc siRNA led to an extensive reduction of luciferase expression throughout the nasal and tracheal passages. In addition, PTD-DRBD delivered EGFRvIII siRNAs resulted in EGF Receptor knockdown in intracerebral glioblastoma tumors in vivo. PTD-DRBD combinatorial delivery of EGFR and Akt2 siRNAs synergized to dramatically increase survival and decrease tumor volume in pre-clinical models of glioblastoma. Taken together, these observations demonstrate a near ubiquitous siRNA delivery approach that has the potential to perform RNAi manipulation on currently difficult cell types for screening and in vivo therapeutic applications.

SYNTHESIS OF THE RNA ANALOG 2'-O-METHYL-3'-AMINO-3'-DEOXYRIBONUCLEIC ACID

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The synthesis of 2'-O-methyl-3'-amino-3'-deoxyuridine is described. This RNA analog formally derives from the combination of previously separate RNA modifications known individually to enhance the thermal stability of a standard Watson-Crick double helix. The novel polymer may enjoy an enhanced level of known advantages imparted by the individual modifications – those primarily being nuclease resistance and thermal stability. Initial nonenzymatic primer extension experiments indicate ready compatibility with RNA as evidenced by efficient templated oligomerization. Future experiments assessing its potential, as an antisense therapeutic candidate will be pursued.

OVERCOMING IMMUNOSTIMULATORY ISSUES ASSOCIATED WITH THERAPEUTIC SIRNA FOR TOPICAL DELIVERY TO THE LUNG

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The Immunostimulatory side effects of siRNAs must be taken into account when considering the application of siRNAs for therapeutic applications. Toll-like receptors (TLR3, TLR7, TLR8 and TLR9) can recognise siRNA leading to immune activation. This immune activation by siRNAs can be abrogated by the careful selection and screening of siRNA sequences. In order to circumvent any downstream developability issues we have established a methodology for screening out any potential immunostimulatory activity using a combination of bioinformatic, in vitro and in vivo studies in rodents. This methodology will be presented together with data for both nasal and pulmonary targets showing that siRNAs can be well tolerated in vitro and when delivered topically to the airways in vivo. The implications of this data for the development of siRNAs for other nasal and pulmonary targets will be discussed

EFFECTIVE INHIBITION OF CORE GENE OF HCV 3A GENOTYPE USING SYNTHETIC AND VECTOR DERIVED SIRNAS

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HCV has been considered a significant risk factor in HCV induced liver diseases and development of hepatocellular carcinoma, with genotype 3a responsible for most of the cases in Pakistan. Current combination treatment of interferon-α and ribavirin has shown limited efficiency, poor tolerability and significant expense so alternative options are desperately needed. RNA interference (RNAi) is a novel regulatory and powerful silencing approach for molecular therapeutics through a sequence-specific RNA degradation process to inhibit virus infection or replication. Core gene of HCV comes in immediate contact with cells during infection and play complex roles in regulation of cells growth and host genes expression essential for infectivity. Therefore, Core gene of HCV might be a relevant target for new drug development. In the present study, full-length HCV Core gene of Pakistani HCV-3a isolates were amplified and sequenced from serum samples as there was previously no available sequence of the local HCV-3a genotype. For in vitro analysis, mammalian expression plasmids expressing HCV-3a Core gene was constructed by insertion of amplified gene into pCR3.1/FlagTAG. The focus was on inhibition of HCV by using consensus small interfering RNAs (siRNAs) against HCV-3a genotype which were designed and constructed. The activity of HCV-specific siRNAs were determined through co-transfection with mammalian expression vectors of HCV-3a into Huh-7 cells. The expression of Core gene was dramatically reduced both at mRNA and protein levels as compared with Mock transfected and control siRNAs treated cells in a dose dependent manner. The potential of siRNAs specificity to inhibit HCV 3a replication in serum-infected Huh-7 cells followed by a combined treatment of effective siRNAs for counteracting viral escape by mutation variants and a significant decrease in HCV viral copy number and protein expression was observed. For long lasting effect of siRNAs, vector based siRNAs (shRNAs) were designed and tested against HCV-3a Core (most effective siRNAs) which resulted in a similar pattern of inhibition on RNA and protein expression of HCV Core as synthetic siRNAs. Moreover, to evaluate the efficacy of shRNA against the whole virus, the cell culture tested shRNA were tested for inhibition of HCV replication and found to be equally effective like siRNAs using HCV-3a serum infected Huh-7 cells. This proof-of-principle study clearly demonstrates that the RNAi-mediated silencing of HCV Core resulting from mRNA degradation be among the first of their type for the development of effective siRNA based therapeutic opportunities against HCV-3a genotype.

THE GENERATION AND CHARACTERIZATION OF NUCLEIC ACID APTAMERS THAT SELECTIVELY INHIBIT HUMAN IL-23 VERSUS HUMAN IL-12 IN VITRO

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IL-23 is a heterodimeric cytokine consisting of the p40 subunit of IL-12 and a unique p19 subunit. IL-23 binds to activated T and NK cells via a distinct receptor from IL-12 and has distinct affects, including the stimulation of IL-17. Studies using knock-out mice indicate that IL-23 rather than IL-12 is the more relevant cytokine for mouse models of multiple sclerosis, rheumatoid arthritis, and IBD. We have used SELEX™ (Systematic Evolution of Ligands by Exponential enrichment) to generate nucleic acid aptamers to IL-23. Several selections yielded a family of aptamers that bind with low nanomolar affinity and demonstrate selectivity for IL-23 over IL-12. Isolation and optimization of the core family sequence produced a 34 nucleotide derivative, ARCX, with a G-quartet structure and subnanomolar affinity for IL-23. PEGylation of ARCX generated ARCY, which is capable of completely inhibiting IL-23-dependent activation of the STAT3 signal transduction pathway of T-cells in vitro (IC50 = 1.5 nM). Furthermore, ARCY completely inhibits IL-23-dependent IFN-γ and IL-17a release from immune cells with low nanomolar IC50s. However, ARCY inhibits IL-12-dependent IFN-γ release to a much less extent; with IC50 > 50X higher than for IL-23 and only 60% inhibition at 1 uM. This aptamer is an intriguing lead for the development of novel IL-23 specific anti-inflammatory agents for in vitro studies and possible therapeutic purposes.

HIGH-RESOLUTION NORTHERN BLOT FOR RELIABLE ANALYSIS OF MICRORNAS AND THEIR PRECURSORS

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MicroRNAs (miRNAs) are posttranscriptional regulators of gene expression in all multicellular organisms. These tiny ~20 nt RNAs regulate the majority of physiological processes and the deregulation of miRNA expression is a hallmark of many diseases. Therefore, many efforts are being made to develop new, more sensitive methods of miRNA analysis and improve the existing methods to make miRNA characteristics more accurate. The most standardized and widely used method is Northern blot hybridisation which is considered the “gold-standard” in miRNA detection. However, all Northern blot protocols are aimed at enhancing the sensitivity of the method rather than at increasing the signal resolution. These protocols are focused on miRNAs leaving many questions regarding effective pre-miRNA detection unanswered. Our protocol fulfils this gap and provides a detailed methodology for the robust detection of both miRNAs and pre-miRNAs. We show how to perform the Northern blot analysis of miRNAs and their precursors with a single-nucleotide resolution allowing us to detect individual length variants and to evaluate their relative quantities in cells. Our protocol is applicable for all analyses of any kind of endogenous and exogenous RNAs, falling within 20-30 nt and 50-80 nt length ranges, corresponding to the miRNA and pre-miRNA lengths respectively. We discuss all steps of the procedure from the perspective of the enhanced resolution of short RNAs and suggest its possible applications. We demonstrate how to use high-resolution Northern blot in the investigation of miRNAs biogenesis and function, as well as in the diverse applications of RNAi and miRNA technologies, when the molecular size of reagents released from vectors needs to be precisely defined. Specifically, using the protocol we show how to evaluate the precision of Drosha and Dicer cleaveages and how to determine the processing of short hairpin RNA constructs (shRNAs) composed of CNG repeats.

INTRACELLULAR DELIVERY OF AN ANTISENSE OLIGONUCLEOTIDE VIA TARGETING GASTRIN-RELEASING PEPTIDE RECEPTOR

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Gastrin-Releasing Peptide Receptor (GRPR) had a long and successful history being utilized for receptor-mediated delivery of cytotoxins, immunotoxins, and radioactive compounds, and this study was intended to extend it into delivery of oligonucleotides. A 2'-*O*-Me phosphorothioate antisense oligonucleotide (termed 623), which corrects abnormal splicing when delivered into nucleus, was conjugated to a bombesin (BBN) peptide at its 5'-end. Intracellular delivery of oligonucleotides was tested in GRPR expressing PC3 cells which had been stably transfected with a reporter comprised of the firefly luciferase gene interrupted by an abnormally spliced intron. The BBN-623 conjugate produced significant higher luciferase expression compared to the ‘free’ 623, and this increase was reversed in the present of excess amount of free BBN peptide. The BBN-623 conjugate produced a gradual increase followed by a gradual decline, while the cationic lipid-623 complex caused a rapid increase followed by a monotonic decline. Dose-dependent uptake study revealed a combination of a saturable, receptor-mediated endocytosis and non-saturable pinocytosis for uptake of BBN-623 in PC3 cells. In addition, the K_m value for satuable uptake of the conjugate was compared favorably to the EC_{50} value for the pharmacological response, which indicated that the saturable, receptor-mediated endocytosis mainly contributes to the effectiveness of the conjugate. Effects of selective endocytosis inhibitors, and of transfection with a dominant-negative mutant dynamin, on cellular uptake showed that BBN-623 utilized a clathrin-dependent, actin-dependent, and dynamin-dependent pathway to enter PC3 cells. The subcellular distribution of the BBN-623 conjugate was partially co-localized with a marker for clathrin. Membrane vesicles containing BBN-623 were colocalized with those marked by transiently expressed GFP chimeras of Rab7 and Rab9, demonstrating that BBN-623 was transported to late endosomes and then the *trans*-Golgi network. These observations suggest that the BBN peptide-oligonucleotide conjugate enters cells via a process of receptor-mediated endocytosis mediated by GRPR.

SMALL MOLECULE LIGANDS TARGET A CONJUGATED OLIGONUCLEOTIDE TO THE SIGMA RECEPTOR IN CANCER CELLS

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Sigma receptor is a membrane protein that is over-expressed in some cancer cells such as prostate, lung and breast tumor cells.[1] Several small molecules including anisamide, haloperidol, SA4503 and opipramol have been reported as high affinity sigma-receptor ligands. Recently, it was reported that anisamide targeted liposomes effectively delivered cargos to sigma receptor-expressing tumor cells.[2,3] Herein, we report the synthesis and evaluation of novel mono- and multi-valent anisamide-oligonucleotide (ON) conjugates. Anisamide was effectively converted to a phosphoramidite precursor that can be directly introduced into solid phase ON synthesis. Using the phosphoramidite, mono- and tri-valent anisamide conjugates were synthesized in satisfying yields. The mono-anisamide was directly conjugated to the 5'-terminal of the ON, while the tri-anisamide was introduced using a three-branched linker. To evaluate biological function, we utilized a reporter system wherein effective delivery of a splice-switching antisense oligonucleotide (SSO) to the nucleus results in up-regulation of luciferase expression. Cellular uptake of unconjugated SSO or anisamide conjugates were evaluated in sigma-receptor expressing human lung carcinoma H460 cells. The mono-anisamide conjugate showed slightly higher uptake compared to the unconjugated SSO, while trivalent-anisamide conjugate showed significantly greater uptake than the unconjugated SSO. The trivalent-anisamide conjugate also displayed high luciferase expression as compared to the unconjugated or mono-conjugated SSO. Thus, we have demonstrated that the multi-valent anisamide conjugates enhanced cell uptake and improved biological effects by direct mediation of the sigma receptor.

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NUCLEOSIDE MODIFICATIONS REGULATE ACTIVATION OF THE PROTEIN KINASE PKR IN AN RNA STRUCTURE-SPECIFIC MANNER

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Protein kinase PKR is a key component of innate immunity. It is activated by long stretches of dsRNA and functions to inhibit translation initiation. Many cellular and viral transcripts contain nucleoside modifications which could affect PKR activation. For example, a 5'-triphosphate confers the ability of relatively unstructured transcripts to activate PKR. Herein, PKR activation by ssRNA and dsRNA containing internal nucleobase, sugar, and phosphodiester modifications is analyzed. We find that for 5'-triphosphate-containing ssRNA, most base and sugar modifications abrogate activation, although 2'-fluoro-modified ssRNA does not, indicative of a critical role for hydrogen bonding at the ribose sugar. In the case of dsRNA, a more limited set of nucleoside modifications affect PKR activation (1-3). Surprisingly, GU wobble pairs also largely abrogate dsRNA-mediated activation when present at modest levels. In order to understand the biological implications of nucleoside modifications on PKR activation, we have extended the study to biologically relevant modified cellular tRNAs and less-modified mitochondrial tRNAs (mt-tRNA). We find that a T7 transcript of unmodified yeast $tRNA^{phe}$ and natively extracted bovine liver mitochondrial tRNAs activate PKR, whereas heavily modified native yeast $tRNA^{phe}$ does not. Overall, the findings indicate that nucleoside modifications and wobble pairing may serve to discriminate self-RNA and pathogenic RNA in innate immunity, and also help in designing non-immune stimulated small RNA therapeutics.

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EFFICIENT GENE SILENCING BY GYMNOTIC DELIVERYOF LOCKED NUCLEIC ACID ANTISENSEO LIGONUCLEOTIDES

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For the past 15-20 years, the intracellular delivery and silencing activity of oligonucleotideshas been essentially completely dependent on the use of a delivery technology (e.g., lipofection). We have developed a method (called “gymnosis”) that does not require the use of any transfectionreagent or any additives to serum whatsoever, but rather takes advantage of the normal growth properties of cells in tissue culture in order to promote productive oligonucleotideuptake. This robust method permits the sequence-specific silencing of multiple targets in a large number of cell types in tissue culture, both at the protein and mRNA level, at concentrations in the low micromolarrange. Optimum results were obtained with locked nucleic acid (LNA) phosphorothioategap-mers, but other chemical modifications are also active. By appropriate manipulation of oligonucleotidedosing, this silencing can be continuously maintained with no toxicity for >240 days. High levels of oligonucleotidein the cell nucleus is not a requirement for gene silencing, contrary to long accepted dogma. In addition, gymnoticdelivery can efficiently deliver oligonucleotidesto suspension cells that are known to be very difficult to transfect. Finally, the pattern of gene silencing of in-vitrogymnotically-delivered oligonucleotidescorrelates particularly well with in-vivosilencing. The establishment of this link is of particular significance to those in the academic research and drug discovery and development communities.

DEVELOPMENT OF NOVEL RNAI BASED THERAPEUTICS AGAINST CANCER

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Silencing aberrant gene expression through RNA interference is an emerging class of medicine for targeting incurable diseases such as cancer. Survivin, is a unique member of the inhibitor of apoptosis protein family, involved in the control of mitotic progression and inhibition of apoptosis. PLK1 is a serine/threonine kinase that plays a central role in cell division and cell cycle progression and its up-regulation is positively correlated with a cancer phenotype. The selective overexpression of survivin and PLK1 in cancerous tissues compared to normal tissues has been associated with disease progression and increased resistance to chemotherapy. Successful delivery of such siRNAs to their intracellular targets depends on the development of efficient systemic delivery systems. MDRNA has developed siRNAs targeting survivin and PLK1 that are highly specific and efficacious when delivered systemically with our novel Di-Lipo amino-acid (DiLA2) based liposomes to both subcutaneous and orthotopically placed liver tumors established in immuno-compromised mice. Repeat administration of siRNAs in DiLA2 liposomes resulted in significant decrease in mRNA expression in the human-derived liver tumors and a dramatic reduction in tumor volume. Furthermore, Survivin and PLK1 siRNAs encapsulated in DiLA2 liposomes delivered locally via intravesical instillation in an orthotopic bladder cancer mouse model elicited significant downregulation of mRNA expression that correlated with a substantial decrease in tumor volume in a dose dependent manner sustained over a period of three weeks. We are further screening potential targets that exploit multiple pathways leading to cancer phenotypes for enhanced efficacy and prolonged therapeutic effect against a variety of cancers.

IMPROVED SHRNA ACTIVITY ENCODED WITHIN ONCOLYTIC AD VECTORS

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Adenoviral (Ad) vectors have been extensively utilized to target shRNAs to cancer cells. These vectors can infect many cell types, both proliferating and quiescent, they do not integrate into the host chromosome and are easy to construct and grow to high titers. Furthermore, oncolytic Ad vectors armed with an shRNA have also recently been constructed. Oncolytic Ad vectors are designed to specifically replicate and lyse cancer cells through deletions, mutations or additions of genes to the Ad genome. Due to the replication of these vectors in cancer cells, shRNAs levels encoded within these vectors would increase specifically in these cells. Previous studies have shown that the addition of oncogene-targeted shRNAs to oncolytic Ad vectors increased the anti-tumour effect of the oncolytic vectors. However, current oncolytic Ad vector design include adenoviral genes that code for RNA molecules called virus associated RNAs (VA-RNAs). These highly complex RNA structures are expressed at high levels during Ad viral replication and are thought to be important in inhibiting the intracellular antiviral immunity. Recent studies have also found that VA-RNAs are processed into viral miRNAs by the RNAi pathway. Due to the high levels of VA-RNA expression, the RNAi pathway was found to be saturated, reducing the ability of the key RNAi enzymes to process other RNA molecules to si- and miRNAs. These results suggest that the activity of shRNAs encoded within oncolytic Ad vectors may be reduced when the VA-RNAs are expressed. Therefore, we are currently determining whether the levels and activity of shRNAs encoded within oncolytic Ad vectors could be increased when the VA-RNAs genes are deleted from the oncolytic Ad genome. We have designed breast cancer specific oncolytic Ad vectors encoding a luciferase-targeted shRNA with or without the VA-RNA genes. These oncolytic Ad vectors were constructed to encode the E1a Ad gene and the luciferase-targeted shRNA within the same transcript. This large transcript is controlled by the breast cancer specific promoter, mammaglobin. The increase in shRNA levels will be determined through RNA analysis using northern blots of a panel of breast cancer and normal cell lines infected with the oncolytic vectors with or without VA-RNA expression. Additionally, increase in shRNA activity will also be measured using the luciferase reporter assay system on the infected panel of cell lines transiently expressing the luciferase enzyme. Future *in vitro* and *in vivo* studies with these oncolytic Ad vectors will allow for the construction of a potent oncogene-targeted shRNA delivered to breast cancer using oncolytic Ad vectors.

DEVELOPMENT OF APTAMER-BASED CANCER THERAPEUTICS TARGETING TRANSMEMBRANE GLYCOPROTEIN NMB (GPNMB)

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Transcript profiling and immunohistochemical analysis recently identified transmembrane glycoprotein NMB (GPNMB) as a tumor-associated antigen overexpressed in glioblastoma multiforme and melanoma cell lines and patient tumor samples. Even though the physiological function of GPNMB is currently unknown, elevated levels in tumor specimens seem to be a valuable prognostic marker and have been correlated with poor prognosis. Moreover, its restricted expression in normal tissue makes GPNMB a promising candidate for targeted tumor therapy. Therefore our objective is to develop high affinity ligands that specifically recognize this receptor and to subsequently transform these ligands into anti-tumor therapeutics. Starting from a synthetic RNA library, modified with 2´fluoro modified pyrimidines for increased nuclease resistance, we selected aptamers that bind to recombinant GPNMB protein produced in SF-9 insect cells with high affinity. To ensure aptamer binding not only to purified protein *in vitro*, but also to GPNMB in its native cell surface expressed context, additional selection rounds were conducted on living cells overexpressing GPNMB. After eight rounds of selection, aptamers have been identified with binding affinities in the nanomolar range (Kd < 50 nM). Flow cytmometry analysis using fluorophore labeled apatmers revealed that selected oligonucleotides specifically recognize cells expressing GPNMB and are internalized into subcellular compartments, an important prerequisite for their further engineering into a bioactive therapeutic. In our onging work we will focus on the development of bifunctional GPNMB-aptamer/siRNA chimeras that combine the tumor-selective targeting properties of the aptamer domain with a cytotoxic activity associated with an siRNA moiety. Herein, an siRNA targeting cellular mRNAs crucial for cell survival will be fused to the GPNMB-aptamers and the anti-tumor effects of the chimeras assessed *in vitro* and *in vivo*.

THE PROFILING OF MICRORNAS AND ITS TARGET GENES ON MDSCS IN TUMOR-BEARING MOUSE

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Immune tolerance to tumors is often associated with accumulation of myeloid-derived suppressor cells (MDSC) and an increase in the number of T-regulatory cells (Treg). The inhibitory activity of MDSC is achieved by the production of arginase, reactive oxygen species, inducible nitric oxide synthase, and interleukin-10. In tumor-bearing mice, MDSCs can facilitate the generation of tumor-specific Tregs. We set out to determine the expression of microRNA and its target genes in MDSCs that are required for induction of Treg accumulation in tumor-bearing mouse. To begin to define how miRNAs could be involved in the regulation of gene expression on MDSC, microarray method was used to determine the miRNAs expressed highly on MDSC in tumor-bearing mouse. We demonstrated five miRNAs from MDSCs and Treg in melanoma-bearing mouse. The expression pattern of identified miRNAs was confirmed by real-time PCR. Potential target genes of microRNA were selected by Computational predictions using databases. The expression of potential target genes was checked by real-time PCR. The correlationships with microRNA and its target gene were determined. We postulate that identified miRNAs might play a role in certain basic functions on MDSC in melanoma-bearing mouse.

ELUCIDATING THE KINETICS OF SIRNA-BASED THERAPEUTICS

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The pharmacokinetics and pharmacodynamics of systemically administered siRNA-based therapeutics can almost be completely decoupled. Once delivered to the desired site of therapeutic action, siRNA can affect gene knockdown for long time spans (c.a., one month for non-dividing cells), orders of magnitude longer than the plasma half-life of the siRNA or siRNA-delivery vehicle complex. Thus, determining dosing regimens for siRNA-based therapeutics is not straightforward due to the aforementioned phenomena. Our current work is focused on elucidating the kinetics of the siRNA-based therapeutics in animal cancer models. Using numerous types of in vivo studies, we are in the process of elucidating the kinetics of circulation, whole animal biodistribution, organ distribution, cellular distribution and gene inhibition in order to understand their relationships to anti-tumor outcomes.

OLIGONUCLEOTIDE THERAPEUTICS IN MYOTONIC DYSTROPHY

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Dominantly-inherited neurodegenerative diseases usually involve a deleterious gain-of-function by the mutant protein. By contrast, in myotonic dystrophy type 1 (DM1) there is a toxic gain-of-function by the mutant RNA (RNA dominance). The genetic basis for this effect is an expansion of CTG repeats in the 3′ untranslated region of *DMPK*, a gene encoding a protein kinase. Transcripts that contain an expanded CUG repeat (CUG^{exp}) accumulate in nuclear foci and initiate a complex cascade of downstream events. For example, CUG^{exp} binding proteins, such as, splicing factors in the Muscleblind-like (MBNL) family, become sequestered on the mutant RNA, causing misregulated alternative splicing of pre-mRNA. CUG^{exp} transcripts also activate signaling pathways, compounding the problem with misregulated gene expression. This presentation will focus on the therapeutic use of antisense oligonucleotides (ASOs) in mouse models DM1. For example, splice blocker ASOs can be used to enforce the correct splicing of exons that are misregulated in the disease. However, this is a piecemeal approach that must be applied to each misregulated exon in turn. Blocker ASOs can also be used to bind to the CUG^{exp} tract, thereby releasing proteins that are sequestered on the mutant RNA. This has several beneficial consequences, including a general correction of the alternative splicing defect. Finally, siRNAs can be used to target the toxic RNA for cleavage. Taken together, these observations suggest that oligonucleotide drugs offer a promising approach to develop an effective treatment for myotonic dystrophy.

ALLELE SELECTIVE INHIBITION OF HUMAN HUNTINGTIN PROTEIN EXPRESSION

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Many neurological disorders are caused by expanded trinucleotide repeats, including Machado-Joseph Disease (MJD) and Huntington Disease (HD). MJD and HD are caused by expanded CAG repeats within the ataxin-3 (ATXN3) and huntingtin (HTT) genes. Inhibiting expression of ATXN3 or HTT are promising therapeutic strategies, but indiscriminant inhibition of wild-type and mutant alleles may lead to toxicity. We hypothesized that expanded triplet repeat mRNA might be preferentially recognized by complementary oligomers. We observe selective inhibition of mutant ataxin-3 and HTT protein expression by peptide nucleic acid (PNA) and locked nucleic acid (LNA) oligomers targeting CAG repeats. Duplex RNAs were less selective, suggesting an advantage for single-stranded oligomers. Inhibiting mutant HTT expression protected cultured striatal neurons from an HD mouse model against glutamate-induced toxicity. Antisense oligomers that discriminate between wild-type and mutant genes on the basis of repeat length offer new options for treating MJD, HD, and other hereditary diseases.

EPIGENETIC REPROGRAMMING OF TUMOR SUPPRESSOR GENES BY ARTIFICIAL TRANSCRIPTION FACTORS

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Mammary serine proteinase inhibitor (maspin) is a multifaceted tumor suppressor gene controlling tumor cell proliferation, angiogenesis and metastatic spread for many types of cancer, including breast and lung cancer. Unlike other tumor suppressor genes, maspin is not mutated or deleted in tumor cells but its promoter is found epigenetically silenced in metastatic cells by aberrant promoter hypermethylation. In contrast, normal breast cells express high levels of maspin and the promoter is not found methylated. The epigenetic silencing of maspin in tumor cells offers a unique opportunity for therapeutic intervention by re-activation of the endogenous gene. Our lab has developed a novel approach to specifically re-activate maspin using engineered artificial transcription factors (ATFs). We have constructed several ATFs made of six-zinc finger (ZF) sequence-specific DNA binding domains and linked to a transactivator domain. These ATFs target unique 18 base pair sites in the human proximal maspin promoter. We have shown that the ATFs are able to reactivate maspin expression in cell lines carrying a hypermethylated promoter. Furthermore, we have shown that ATFs synergize with chromatin remodeling agents presently in clinical trials to reactivate maspin expression, indicating that epigenetic silencing marks act as a partial blockade for the ATFs, probably by affecting the accessibility of the ATF-binding site. Furthermore, we have demonstrated that the ATFs are able to induce endogenous maspin expression in xenografts and metastasis models of breast cancer in nude mice. Concomitantly to maspin re-activation, we have shown that the ATFs induced tumor growth reduction ad metastasis suppression in vivo. In summary our work document the power of a novel therapeutic approach that uses ATFs to overcome the endogenous epigenetic silencing of tumor suppressors.

THERAPEUTIC GENE MODIFICATION VIA TRIPLEX-FORMING PEPTIDE NUCLEIC ACIDS.

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Triple helix-forming peptide nucleic acids (PNAs) can bind to polypurine regions in DNA in a sequence-specific manner. The resulting triplexes constitute an altered DNA structure that can stimulate DNA repair and recombination in a site-specific manner in human cells. Transfection of human cells with triplex-forming PNAs plus short single-stranded donor DNAs can produce targeted gene modification at frequencies of 2% or more in a single treatment. We have developed triplex-forming PNAs that bind with high affinity and specificity to a selected site in the CCR5 gene, which encodes a co-receptor for R5-tropic human immunodeficiency virus-1 (HIV-1) that must be present at the cell surface for R5-viral entry. Individuals who possess a homozygous delta32 mutation in CCR5 express a truncated protein, reducing its expression at the cell surface and inhibiting HIV-1 from entering the cell. These individuals are almost completely resistant to R5-tropic HIV-1 infection and show no significant adverse phenotypes. One therapeutic strategy to mimic this naturally occurring inactivating mutation is PNA-mediated modification of CCR5. In a cell line model, we have shown the ability of a triplex-forming PNAs to induce recombination in the CCR5 gene at a frequency of 2.46% based on direct evaluation of 1870 single-cell derived clones. Gene targeting was verified by isolation of modified clones and analysis at the DNA, RNA, and protein levels. Importantly, PNA-mediated CCR5 modification was also shown to confer resistance to infection with HIV-1. This approach to target CCR5 was also tested in primary human CD34+ hematopoietic stem cells (HSCs), and successful modification was demonstrated at both the DNA and RNA levels. CCR5 targeted human CD34+ HSCs were transplanted into NOD-scid IL2Rγ^{null} HLA A2.1 mice, and persistence of the gene modification was detected in donor-derived splenic CD4 lymphocytes more than four months post-transplantation. This work suggests a potential therapeutic strategy for permanently inactivating the CCR5 co-receptor in human HSCs from HIV-1 infected individuals, thereby creating a reservoir of virus-resistant cells to preserve immune system function.

TARGETED DEGRADATION OF TOXIC RNA IN MYOTONIC DYSTROPHY

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Myotonic dystrophy (DM) is the primary cause of adult onset muscular dystrophy that affects 1 in 8000 individuals worldwide. The main symptoms in DM include myotonia, insulin resistance, cardiac conduction defects, cataracts and altered central nervous system function. Myotonic dystrophy type 1 (DM1), which is the more common form in the United States, is caused by a CTG repeat expansion (to >4000 repeats) in the 3' UTR of the dystrophia myotonica protein kinase (DMPK) gene. The expanded DMPK allele is transcribed to produce mRNAs containing long CUG repeats that accumulate in nuclear foci. The CUG repeat-containing RNA has a toxic gain-of-function that is the main cause of DM1 pathogenesis; therefore, eliminating the toxic RNA is anticipated to have a therapeutic effect.

We chose to use antisense oligonucleotides (ASOs) as our approach to target degradation of the repeat RNA. ASOs containing CAG repeats are expected to hybridize to the expanded CUG repeats, forming a DNA-RNA hybrid that is a substrate for RNase H-mediated degradation of the mutant RNA. ASOs can be modified to form chimeric oligonucleotides termed "gapmers", which contain phosphorothioate-modified DNA in the center flanked with three or four locked nucleic acids (LNA) on both ends. This provides both nuclease resistance and highly efficient mRNA cleavage. We tested three different ASO gapmers in cells transiently expressing 960 CUG repeats, and all of them resulted in a >90% knock-down of the repeat RNA. We are currently using electroporation to deliver gapmer ASOs into skeletal muscle tissue of a DM1 mouse model developed in our lab [PNAS 105, 2646 (2008)] to determine their efficacy in vivo. Recently, RNase-H-inactive CUG-repeat-binding morpholinos have been shown to promote the release of proteins bound to the long repeats and dissociate RNA foci [Science 325, 336 (2009)]. We are testing the possibility that combined administration of morpholinos with the gapmers will enhance the knock-down efficiency.
There is currently no known cure for DM1, hence the goal of our studies is to test a potential therapeutic approach for this disease which may provide insight for other RNA-dominant disorders.

This project is supported by the Muscular Dystrophy Association.

SILENCING OF PROLONGED CAG REPEAT CONTAINING ALLELES IN HUNTINGTON'S DISEASE USING ANTISENSE OLIGONUCLEOTIDES

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Huntington's disease (HD) is a devastating disease for which currently no therapy is available. It is a progressive autosomal dominant neurodegenerative disorder that is caused by a CAG repeat expansion in the *HD* gene, which results in an expansion of polyglutamines at the N-terminal end of the huntingtin (htt) protein, and the accumulation of cytoplasmic and nuclear aggregates in neurons. The polyglutamine expansion results in a toxic gain of function for the huntingtin protein and plays a central role in the disease. The size of this expansion has a direct link to the aggregation-proneness as well as the severity of pathological and clinical features. Here, we make use of fully modified 2'OMePS antisense oligonucleotides (AONs) as a therapeutic strategy to effectively reduce both htt transcript (agarose gel and quantitative PCR) and protein levels (Western Blot and time resolved FRET) in patient derived HD fibroblasts. The (CUG)7 AON is a promising therapeutic tool to reduce mutant htt in HD. Evidence in a Spinocerebellar Ataxia 3 (SCA3) fibroblast cell line suggests that (CUG)7 AON could also be effective in other polyglutamine neurodegenerative diseases with a prolonged pure CAG repeat.

ANTISENSE CORRECTION OF SMN2 SPLICING IN THE CENTRAL NERVOUS SYSTEM OF MOUSE MODELS OF SPINAL MUSCULAR ATROPHY

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Spinal Muscular Atrophy (SMA) is a genetic disease characterized by progressive degeneration of motor neurons in the anterior horn of the spinal cord, which in turn leads to severe muscle weakness and atrophy. SMA is caused by deletion or loss-of-function mutations in the Survival-of-motor-neuron *(SMN1)* gene. The paralogous *SMN2* gene, present in one or more copies in all SMA patients, attenuates the severity of SMA, but expresses only a low level of full-length SMN protein, due to alternative splicing that results in inefficient inclusion of exon 7. Increasing the extent of *SMN2* exon 7 inclusion to express more full-length, functional SMN protein in motor neurons is a promising approach to treat SMA. Previously, we identified an optimal 2'-*O*-(2-methoxyethyl) (MOE) 18mer antisense oligonucleotide (ASO) that targets an hnRNP A1 bipartite motif in an intron-7 splicing silencer (ISS-N1) and efficiently promotes *SMN2* exon 7 inclusion in liver and kidneys of transgenic mice after systemic administration. Because ASOs do not cross the blood-brain barrier, we explored direct delivery to the mouse central nervous system. Using a surgically implanted micro-osmotic pump, the ASO (dubbed ISIS-SMN_{Rx}) was delivered into cerebrospinal fluid through the right lateral ventricle in adult $Smn^{-/-}$ type-III SMA mice carrying a human *SMN2* transgene. Dose-response studies revealed that intracerebroventricular (ICV) infusion of the 18mer ASO increased *SMN2* exon 7 inclusion in spinal cord to ~90%, compared to ~10% in saline-treated mice. Western blotting and immunohistochemical analysis demonstrated a robust increase of the human transgenic SMN protein levels in spinal-cord motor neurons. We are using this and other ICV delivery methods, in combination with available SMA mouse models, to optimize the effectiveness of the ASO, characterize phenotypic improvement, and establish a time window for effective treatment. Our data show that ISIS-SMN_{Rx} is a promising drug candidate for SMA therapy.

CURRENT PROGRESS WITH THE SYSTEMIC ADMINISTRATION TRIAL OF AVI-4658, A NOVEL PHOSPHORODIAMIDATE MORPHOLINO OLIGOMER (PMO) SKIPPING DYSTROPHIN EXON 51 IN DUCHENNE MUSCULAR DYSTROPHY (DMD)

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Objective: AVI BioPharma in collaboration with the MDEX consortium have identified a PMO to skip dystrophin exon 51 in DMD patients, restore the reading frame and enable dystrophin expression. Here, we test 6 PMO doses to select an effective, well tolerated dose for subsequent registration.
Method: Open label, dose escalation study in ambulant DMD boys aged 5-15 years with relevant deletions, of 12 weekly administrations of AVI-4658; 14 week follow up with muscle biopsy to assess dystrophin expression. Clinical efficacy (6 minute walk), skeletal muscle, pulmonary and cardiac function is being assessed. Safety assessment includes adverse events, physical examinations and laboratory tests – including hematology, coagulation studies, chemistry and anti-dystrophin antibodies. A DSMB guided dose escalation decisions (across 6 doses: 0.5, 1.0, 2.0, 4.0, 10.0 and 20.0 mg/kg).
Results: Study fully enrolled 19 patients by Dec 2009. All doses well tolerated (ongoing at 20mg/kg). No Drug Related SAEs or severe AEs reported so far. To date, maximum single dose is 900mg and cumulative PMO dose 10800mg. Biopsies from first 4 cohorts showed exon skipping at 2 and 4 mg/kg and 1 patient with 20% increase in number of dystrophin positive fibres.
Conclusion: Study drug well tolerated to date. Dosing and follow up continue on schedule. These preliminary data bode well for safe long term administration in Duchenne patients, and suggests clinically meaningful dystrophin expression can be expected following systemic administration. Preliminary, laboratory data from the remaining cohorts is due in 2Q and full clinical results in 3Q 2010.

RNA-BASED THERAPEUTICS FOR EMERGING INFECTIOUS DISEASE

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Background: Single-stranded RNA viruses represent the leading edge of emerging infectious disease. The challenges to drug discovery and development are numerous and include: a) efficient in replication to produce high viral titers, b) present unstable genome sequences as quaisispecies, and c) the infections interfere with effective immune responses. Methods: Studies were initiated to survey targeting genomes of single-stranded RNA viruses from a variety of viral families to determine effective strategies. The most extensive investigations have led to therapeutics in development for Zaire Ebolavirus (AVI-6002) and Lake Victoria Marburgvirus (AVI-6003). The strategies have utilized a novel phosphorodiamidate morpholiono oligomer with two to six positively charged linkages referred to as PMOplus. Results: Screening the viral genome of Zaire Ebolavirus led to effective targeting of VP35 and VP24 which are combined in AVI-6002. Similar screening in Lake Victoria Marburgvirus led to VP24 and NP targets which are combined in AVI-6003. The PMOplus agents have been effective in mouse, guinea pig and nonhuman primate lethal challenge studies. Both AVI-6002 and AVI-6003 demonstrate dose dependent antiviral activity, preservation of the immune response and provide significant survival benefit. Further, the position of the positive linkages in PMOplus agents offers an effective counter to viral resistance. More recent studies have explored the RNA-therapeutic approach to manipulation of the immune response to provide a more comprehensive approach to development of therapeutics for emerging infectious disease. Conclusion: Together, the strategies for targeting viral the genome as an antiviral and the host immune response to infection can be employed for effective and rapid therapeutic development that is necessary to combat emerging infective diseases.

LONG TERM SYSTEMIC ANTISENSE-MEDIATED EXON SKIPPING IN DYSTROPHIC MOUSE MODELS

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Antisense-mediated reading frame restoration is presently the most promising therapeutic approach for Duchenne muscular dystrophy (DMD). In this approach, antisense oligoribonucleotides (AONs) induce specific exon skipping during pre-mRNA splicing to restore the disrupted open reading frame and allow synthesis of internally deleted, partly functional Becker-like dystrophin proteins. The approach is theoretically applicable to over 70% of all patients. Proof of concept has been achieved in cultured muscle cells from patients carrying different mutation types, in the mdx mouse and dog models and recently in patients as well. In a first trial in 2006, we showed exon 51 skipping and dystrophin restoration in each patient after local intramuscular AON injections. A subsequent trial where patients are treated systemically has recently been completed successfully and a 6-months followup trial using the most effective dosage is underway by Prosensa (van Deutekom *et al*. This meeting).
Due to AON turnover, repeated treatment is necessary. Therefore, long term safety and efficacy of 2'O-methyl phosphorothioate AON treatment was tested in mouse models with varying levels of severity: *mdx* mice (mild phenotype) and *mdx* mice with one utrophin allele (*mdx +/-*; intermediate phenotype). Comparison of different routes of administration revealed that the AON load in muscle was similar for intravenous (IV), intraperitoneal (IP) and subcutaneous (SC) delivery, while the load in liver and kidney was much reduced for IP and SC injections. Thus, mice were treated with weekly SC injections of 200 mg/kg for up to 6 months. This was well tolerated during treatment and liver and kidney weights and serum parameters were similar for 4, 8, 12 and 24 week treated mice compared to saline treated controls at the end of treatment. In the *mdx* and *mdx +/-* mice treatment resulted in significantly improved serum creatine kinase (marker for muscle quality) and rotarod running time (marker for muscle function) compared to the controls. In the more severely affected *mdx +/-* mice the therapeutic effect was larger. Time course experiments revealed that exon skipping levels increased for the first 12 weeks, but remained constant after that. Protein analysis revealed a similar pattern.
These results indicated that long term treatment with 2'-O-methyl phosphorothioate AONs is safe and efficient in dystrophic mouse models, which is encouraging for future long term trials in patients.

REPEAT DOSE TOXICOLOGY EVALUATION OF AVI-4658 PMO IN MONKEYS AND MICE

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Duchenne muscular dystrophy (DMD) affects 1 in every 3500 males worldwide and results from a mutation of the dystrophin gene. AVI BioPharma is currently in clinical development with AVI 4658, a Phosphorodiamidate Morpholino Oligomer (PMO) drug, designed to induce exon 51 skipping and restore dystrophin expression in a subset of DMD patients. The objective of these preclinical studies was to evaluate the potential toxicity of AVI-4658 upon repeated administration. Cynomolgus monkeys were dosed once weekly for 12 weeks with AVI-4658 at levels up to and including the maximum feasible dose of 320 mg/kg/injections, either intravenously or subcutaneously. AVI-4658 was well tolerated at dose including 320 mg/kg/injection, with no adverse effects. Findings were generally limited to the kidney, and included basophilic granules, basophilic tubules and instances of vacuolation at the highest dose levels. No evidence of kidney function change was detected, as evidenced by clinical chemistry and urinalysis evaluations. Kidney findings were generally reversible, as evidenced in the 28 day recovery groups. This study demonstrates the remarkable safety of AVI-4658 in non-human primates. In the murine study, wild type and dystrophic mdx mice were dosed once weekly for 12 weeks with AVI-4658 up to 960 mg/kg/injections (maximum feasible in mice), either intravenously or subcutaneously. AVI-4658 was well tolerated at all doses including 960 mg/kg/injection, with no adverse effects. Findings were generally limited to the kidney, and included basophilic granules, basophilic tubules and instances of vacuolation at the highest dose levels. No evidence of kidney function change was detected, as evidenced by clinical chemistry and urinalysis evaluations. Kidney findings were generally reversible, as evidenced in the 28 day recovery groups. This study demonstrates the remarkable safety of AVI-4658 in both healthy and dystrophic rodents. Similar findings were seen in a separate study in which AVI-4225, designed to restore dystrophin expression in mdx mice, was administered in the identical manner and doses. In addition, mechanism of action driven improvement in muscle pathology was detected in a dose dependent manner in the AVI-4225 study. The above data demonstrate the extraordinary preclinical safety profile of PMOs compounds, and in particular AVI-4658, for use as exon skipping drugs for the treatment of DMD.

ANTISENSE OLIGONUCLEOTIDES: POTENTIAL THERAPEUTIC STRATEGY AGAINST BREAST CANCER

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Carcinoembryonic antigen related cell adhesion molecule-1 (CEACAM1) is a cell surface glycoprotein mainly expressed on the luminal surface of epithelial cells. In humans, CEACAM1 pre-mRNA undergoes alternative splicing to produce 11 splice variants. The two major splice variants of CEACAM1 are generated by the inclusion (CEACAM1-L) or exclusion (CEACAM1-S) of exon 7. The link between CEACAM1 downregulation and breast cancer has been known for more than a decade. However, CEACAM1 was overlooked as a target for therapeutic intervention apparently because its activity is downregulated in only 30% of breast cancers. We looked at the existing paradigm from a different perspective. We hypothesized that a physiological S:L ratio is required for normal breast morphology and altered ratio may disrupt tissue architecture thereby initiating tumorigenesis. To test our hypothesis, we asked the following questions: (1) Will an alteration in the ratio of CEACAM1 splice variants induce phenotypic changes in acinar morphogenesis of the non-malignant human mammary epithelial cell line (MCF10A)? (2) Will changing the relative balance of CEACAM1 splice isoforms in ZR75, a tumor mammary epithelial cell line that does not form lumena when grown in Matrigel, revert to normal morphology or lose transformed phenotype? We found that antisense oligonucleotide mediated shift in S:L ratio disrupted acinar morphology in MCF10A cells, a salient feature of early glandular epithelial cancer. Importantly, altered S:L ratio significantly reduced invasive potential of malignant breast cancer cell line ZR75. Insights from this study will not only be important to understand the role of isoform ratios in 70% of breast cancers that continue to express CEACAM1 but may also identify new target for breast cancer intervention.

SIRNA DELIVERY: THE 800 POUND GORILLA

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siRNA induced RNAi responses have great potential to treat human disease, especially cancer and viral infections. However, siRNAs are negatively charged, 14,000 Dalton macromolecules with no bioavailability to enter cells and siRNA cellular delivery remains a significant rate-limiting step for development of RNAi therapeutics. To address the siRNA delivery problem, we developed a Peptide Transduction Domain-dsRNA Binding Domain (PTD-DRBD) fusion protein. DRBDs bind siRNAs in a sequence-independent manner that masks the negative charge, allowing for PTD-mediated siRNA delivery. PTD-DRBD mediated delivered of EGFR and Akt2 siRNAs induced a synthetic lethal RNAi response that significantly increased longevity in intracerebral glioblastoma mouse models in vivo. PTD-DRBD fusion proteins begin to address the siRNA delivery problem with a high efficiency and low toxicity, biodegradable platform.

DELIVERING THE PUNCH: TARGETED DELIVERY OF SIRNA IN VIVO

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Systemic delivery of small interfering RNA (siRNA) to target tissues remains a major hurdle for the widespread therapeutic application of RNAi. We have recently demonstrated delivery of cytotoxic siRNAs to prostate cancer cells following systemic administration (Dassie, 2009). The siRNAs were conjugated to a synthetic RNA ligand (aptamer) with affinity for prostate specific membrane antigen (PSMA), an antigen expressed on prostate cancer cells.

While encouraging, the extended use of RNA aptamers as a delivery tool for siRNAs awaits the identification of RNA aptamer sequences capable of targeting and entering the cytoplasm of many different cell types. To enable the isolation of cell-type specific cell-internalizing RNA aptamers, we have developed a modified cell-based SELEX (systematic evolution of ligands by exponential enrichment) methodology which we refer to as cell-internalization SELEX. This approach allows for the rapid isolation of RNA aptamer sequences that are efficiently internalized into the target cells. We demonstrate the usefulness of this approach for isolating cell-internalizing RNA aptamers that target specific cell types (e.g. cancer cells vs. normal cells) or specific surface antigens expressed on cells. Conjugation of these cell-internalizing aptamers to siRNAs results in efficient delivery of the siRNA cargo into the cytoplasm of the target cells enabling robust gene-specific silencing. The development and future refinement of this technology may facilitate the widespread use of RNAi for clinical applications.

Dassie JP, Liu XY, Thomas GS, Whitaker RM, Thiel KW, Stockdale KR, Meyerholz DK, McCaffrey AP, McNamara JO 2nd, Giangrande PH. Systemic administration of optimized aptamer-siRNA chimeras promotes regression of PSMA-expressing tumors. Nat Biotechnol. 27(9):839-49 (2009). PMCID: PMC2791695.

ADVANCES IN THE THERAPEUTIC USE OF RNA INTERFERENCE (RNAi)

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Just over a decade has passed since the discovery of the RNAi mechanism (Fire et al., 1998, Nature 391:806-11). In this relatively short time, numerous siRNA-based candidates have moved from discovery research to clinical testing in humans. This presentation will include an overview of this progression and discuss steps being taken to advance improved RNAi compounds into therapeutic development.

Early human trials focused on clinical indications for which direct administration of siRNA to the site of disease was possible. These included the eye (2004, direct intraocular injection) and lung (2006, inhalation). Trials using 'direct delivery' to the kidney via intravenous administration (2007) and transdermal injection for skin (2008) followed. Early siRNAs often included chemically modified nucleotides (2'-O-methyl, 2'-fluoro) and phosphorothioate linkages to improve stability while maintaining potency. Continued research on chemistry configuration as well as selection and design parameters has led to RNAi compounds with enhanced therapeutic oligonucleotide properties and resolution of some potential issues of non-specificity and immune stimulation.

A major hurdle in the development of RNAi as a broad therapeutic class is delivery of the RNAi compound to the appropriate tissue and efficient intracellular uptake. Many delivery modalities are being explored, most of which involve formulating the RNAi compound with additional components, often lipids, to form complex delivery vehicles intended to improve the compound's PK parameters and allow increased distribution to the appropriate tissues. Formulations used in human trials to date include a targeted cyclodextrin-based 'polymer' to deliver the siRNA payload to specific tumor cells (2008) and a lipid nanoparticle for improved delivery to the liver (2009).

Our alternative delivery approach is to alter the structural and chemical composition of siRNA to create novel RNAi compounds with efficient cellular uptake. These 'self-delivering' RNAi compounds (sd-rxRNA™) retain potent activity, stability, and reduced immune stimulation and are rapidly and effectively taken up by cells without a transfection reagent or delivery vehicle. Specific proprietary chemical modifications and patterns and reduced oligonucleotide content are required. Analyses of fluorescently-labeled sd-rxRNAs demonstrate efficient cellular internalization in a variety of cultured and primary cells in vitro and following direct administration in vivo. Significant reduction of targeted mRNA in vivo following local administration supports the use of sd-rxRNAs for clinical applications where direct administration is possible.

EVALUATION OF LIPID NANOPARTICLE-MEDIATED SYSTEMIC DELIVERY OF SIRNA IN LIVE MICE BY NON-INVASIVE BIOLUMINESCENCE IMAGING

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Mouse models with liver-specific expression of firefly luciferase have been developed that enable a real-time, longitudinal and non-invasive assessment of siRNA-mediated gene silencing in hepatocytes of live animals via bioluminescence imaging. Using these models, a set of lipid nanoparticles (LNP), composed of cationic lipids, a PEG lipid and cholesterol, were tested for their abilities in delivering a luciferase siRNA to the liver via systemic administration. A dose-dependent luciferase knockdown by LNP/siRNA assemblies was measured by in vivo bioluminescence imaging, which well correlated with the results from parallel ex vivo analyses of luciferase mRNA and protein levels in the liver. RNAi-mediated target silencing was further confirmed by detection of the target mRNA cleavage site. A single dose of LNP02L at 3 mg/kg (siRNA) caused >90% reduction of luciferase expression and the target repression lasted for at least 10 days. With identical components, LNPs containing 2% PEG are more potent than those with 5.4% PEG. Our results demonstrate that these mouse models with tissue-specific expression of luciferase provide a powerful tool for a high throughput evaluation of siRNA delivery vehicles and that the molar ratio of PEG lipid can affect the efficacy of LNPs in silencing liver targets via systemic administration.

ANTI-TUMOR ACTIVITY OF SPLICE-SWITCHING OLIGONUCLEOTIDES IN VIVO

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Alternative splicing has emerged as an important target for molecular therapies. Splice-switching oligonucleotides (SSOs) modulate alternative splicing by hybridizing to pre-mRNA sequences involved in splicing and blocking access by splicing factors. Recently, the efficacy of SSOs has been established in various animal disease models; however, the application of SSOs against cancer targets has been hindered by poor *in vivo* delivery of antisense therapeutics to tumor cells. We previously showed that redirection of Bcl-x pre-mRNA splicing from anti-apoptotic Bcl-xL to pro-apoptotic Bcl-xS induced apoptosis and enhanced chemosensitivity in breast and prostate cancer cells. In this study, the effect of SSO-induced Bcl-x splice-switching on metastatic melanoma was assessed in cell culture and B16F10 tumor xenografts. SSOs were delivered *in vivo* using a liposome-polycation-DNA nanoparticle. Administration of nanoparticle with Bcl-x SSO resulted in modification of Bcl-x pre-mRNA splicing in lung metastases and reduced tumor burden, while nanoparticle alone or formulated with a control SSO had no effect. Our findings demonstrate *in vivo* anti-tumor activity of SSOs that modulate Bcl-x pre-mRNA splicing.

DIRECTING miRNA-REGULATORY PNAS TO BREAST CANCER CELLS WITH EGF ANALOGS

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Breast cancer, one of the leading causes of cancer death worldwide, must be treated by drugs with multiple activities due to its complex nature. Disregulated in many types of cancer, miRNAs are being studied intensely as targets for breast cancer therapeutics.
For example, elevated miR-21 reduced the protein expression levels of tumor suppressor proteins including PDCD4, TPM1 and PTEN in breast carcinoma, contributing to increased breast cancer cell proliferation, microfilament destabilization, survival, invasiveness, and metastatic transformation. In contrast, attenuation of let-7 enabled increased KRAS and NRAS proto-oncogene expression, both in vitro and in vivo. The differential expression of some let-7 isoforms correlates with progesterone receptor status, lymph node metastasis, or high proliferation index in breast cancer. Similarly, attenuation of miR-17-5p elevated expression of AIB1 protein, which acts as a rate-limiting factor for estrogen-mediated and E2F1-mediated growth in breast cancer cells.
We hypothesize that complementary oligomers that can inhibit or mimic the above miRNAs will re-establish the balance of homeostasis. Breast cancer cell-restricted miRNA regulation will be tested by introducing sequence-specific peptide nucleic acid (PNA) 12-mers conjugated to a C-terminal epidermal growth factor (EGF) analog to enable EGFR-mediated endocytosis into those cells that overexpress EGF receptor (EGFR, Her-1, erb-B1). We have validated breast cancer cell uptake and cytoplasmic localization of a fluorescently labeled peptide previously reported to bind to EGFR.
Uptake and intracellular trafficking of fluorophore-PNA-EGF analogs vs. PNA and peptide mismatch controls are underway. The miRNA target protein expression levels in breast cancer cells following treatments will be compared to those in cells treated with controls. Changes in breast cancer cell migration, microfilament organization, proliferation and survival will be analyzed in cells treated with PNA-EGF analogs, vs. mismatch controls.
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MODULATION OF TLR-MEDIATED IMMUNE RESPONSES WITH SYNTHETIC OLIGONUCLEOTIDES

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Synthetic oligonucleotides comprising various nucleotide sequences, lengths, and chemical modifications are currently being studied as therapeutic agents based on various mechanisms of action. Oligonucleotides are transported into intracellular compartments by endocytosis. Toll-like receptors (TLR) 3, 7, 8, and 9 are expressed in endosomes, and are receptors that recognize foreign RNA and DNA. TLR3 recognizes double-stranded RNA, TLR7 and TLR8 recognize single-stranded RNA and TLR9 recognizes DNA containing unmethylated CG dinucleotides . Under certain pathological conditions TLR7 and TLR9 are also known to recognize self RNA and DNA present within immune complexes. Through extensive structure-activity relationship studies, we have observed that TLR7-, 8-, and 9-mediated immune responses can be modulated depending on the synthetic DNA and RNA sequences, nucleotide motifs, secondary structures, accessibility of the 5'- and 3'-ends, and chemical modifications. Activation of immune responses through these receptors has broad therapeutic potential for the treatment of a range of diseases, including cancer, infectious diseases, asthma and allergies, and use as adjuvants with vaccines. In addition, blocking immune responses mediated through TLR7 and TLR9 has applications for potential treatment of autoimmune diseases, including lupus, arthritis, and psoriasis. Several compounds designed through these programs are at various stages of clinical trials for a broad range of diseases.

UNDERSTANDING THE IMMUNE EFFECTS OF THERAPEUTIC NUCLEIC ACIDS

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Immune stimulatory effects have complicated the therapeutic development of antisense oligodeoxynucleotides (ODNs) and small interfering RNAs (siRNAs). Both antisense and siRNA molecules can induce innate immunity through Toll-like receptors (TLRs) and/or the cytoplasmic retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs). A subset of TLRs, including TLR3, TLR7, TLR8, and TLR9, are expressed intracellularly within one or more endolysosomal compartments, and detect single-stranded CpG DNA (TLR9) and certain single-stranded RNA motifs (TLR7 and 8), or double-stranded RNA (TLR3). Signaling via these receptors can activate several types of immune and non-immune cells, with resultant innate immune stimulation and the production of type I interferon and pro-inflammatory cytokines and chemokines. Unless appropriate controls are performed, the TLR- and RLR-driven immune stimulatory effects of molecules designed as antisense ODN or RNAi can be mistakenly attributed to the intended mechanism of action.

IMPROVING THE THERAPEUTIC PIPELINE

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While it is uncertain whether and what academics have to contribute to drug development pipelines, the fact remains that nucleic acid therapeutics show great promise. However, nucleic acids as a therapeutic class must now be subjected to a long and arduous accumulation of knowledge that may lead to their adoption as drugs. In this, they are at a severe disadvantage relative to protein drugs, such as antibodies, which started their commercial lives much closer to therapeutic relevancy and are already in use for a variety of indications. I briefly consider the relatively minor role that discovery plays in the pipeline, and then move on to academic endeavors that address (a) possibilities for the large-scale production of complex nucleic acid therapeutics, (b) the still largely unexplored realms of nucleic acid pharmacokinetics and bioavailability, and (c) the unique side-effects that will likely be associated with nucleic acid pharmaceuticals and that will have to be dealt with into the future.

PRO- TO ANTI-ANGIOGENIC SWITCH: CHANGING VEGFR ACTIVITY BY SPLICING/POLYADENYLATION INTERFERENCE

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The targeting of angiogenic signal transduction pathways represents a promising emerging strategy in solid tumor therapy. Vascular endothelial growth factor (VEGF) is a potent vascular endothelial cell mitogen and a crucial mediator of tumor angiogenesis.

VEGF expression is elevated in tumors and it acts on endothelial cells, which express two high-affinity receptors, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR). A soluble truncated isoform of VEGFR-1 (sFlt-1) contains only the first six, extra-cellular Ig-like domains but lacks the transmembrane and TK domains due to alternative splicing/polyadenylation. This endogenously encoded soluble receptor functions as a specific, high-affinity antagonist of VEGF signaling.

We developed a new strategy for an antisense-based therapy by re-directing VEGFR-1 and VEGFR-2 alternative splicing/polyadenylation to the soluble VEGFR-1 isoform (sFlt-1) and a newly identified soluble VEGFR-2 (sKDR). Treatment exerts negative regulatory effects by three main mechanisms: elimination of the active VEGFR isoform, sequestering VEGF (similar to the action of VEGF Trap) and inducing dominant-negative receptor heterodimers with remaining full length VEGFRs.

We identified target mRNA sequences in VEGFR-1 and VEGFR-2 to modulate sFlt-1 and sKDR expression and developed specific antisense compounds (morpholinos), covalently coupled to cell-delivering moieties, to modulate endogenous VEGFR splicing/polyadenylation and.

This novel anti-angiogenic strategy based on alternative splicing/polyadenylation offers the advantage of simultaneously knocking down the pathological target while introducing a natural dominant-negative isoform.

GENE SILENCING BY SYNTHETIC U1 ADAPTORS

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Historically, there have been two major types of gene silencing platforms, RNAi and traditional antisense. Here we present new data on a recently-published alternative gene silencing method called U1 Adaptor that is based on a novel mechanism. The U1 Adaptor is a single stranded oligonucleotide having an annealing domain that targets gene-specific pre-mRNA in the terminal exon by conventional basepairing and a U1 domain that basepairs to the U1 snRNA component of the U1 snRNP splicing factor. This tethering of U1 snRNP to pre-mRNA inhibits 3' end processing (ie. polyA tail addition), hence leading to reduced mRNA levels. U1 Adaptors specifically inhibit both reporter and endogenous genes with IC50 values of <1nM routinely being achieved. Targeting a single gene with multiple U1 Adaptors or an Adaptor and siRNA leads to enhanced "deep" silencing, the latter consistent with their having distinct mechanisms that act in distinct cellular compartments. Such deep silencing will be especially useful for those genes where modest levels of silencing do not achieve the desired outcome. New microarray studies, comparing U1 Adaptors with siRNAs, show U1 Adaptors are to a large degree as specific as siRNAs and have identified U1 Adaptor-response specific genes, ie. those genes whose changes in expression levels depends on the basic U1 Adaptor design. U1 Adaptors have distinct advantages over other methods including: 1) the ability to maintain activity even when all bases of the molecule are modified and when covalently bound to cell-specific targeting groups (such as peptides), 2) lack of immune stimulation, and 3) lack of off-target effects on particular cellular processes. Through use of delivery agents, we will present data on silencing of target genes in mice.

THE P53 TARGET WIG-1 REGULATES MRNA STABILITY AND IS NECESSARY FOR NORMAL EMBRYONIC DEVELOPMENT

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The p53 tumor suppressor triggers cell cycle arrest, apoptosis, or a variety of other responses after exposure to cellular stress. p53 exerts its biological function at least in part through transcriptional transactivation of target genes. One such gene is wig-1 (for wild-type p53 induced gene 1). Our biochemical studies have revealed that the Wig-1 protein binds double stranded (ds) RNA with high affinity in vitro and in living cells. We have also shown that Wig-1 binds to a U-rich region in p53 mRNA and stabilizes it by preventing deadenylation, the first and rate limiting step of mRNA degradation. We show here that Wig-1 is an essential protein, since knockout mice die during embryonic development and the ratio of heterozygous to wildtype mice is less than expected. Many factors involved in processes such as embryonic development and cancer are regulated at the level of mRNA stability, through AU-rich elements as well as through other mechanisms. Our preliminary data indicate that Wig-1 regulates other mRNA containing U-rich or AU-rich sequences apart from p53, some of which, alongside with p53, are potentially interesting targets for cancer therapy. Once verified, we aim to use RNA therapeutics to modify their regulation by Wig-1.

THE REGULATION OF TRANSCRIPTION-DRIVEN R-LOOP FORMATION AND ITS THERAPEUTIC IMPLICATIONS

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The RNA editing and DNA deamination model have been proposed as the action mechanisms of activation-induced deaminase (AID)-mediated immunoglobulin (Ig) recombination for antibody diversification. Here, we present experimental results supporting that the R-loop, a RNA-DNA hybrid with a single-stranded DNA region, as the targeting substrate for AID. A bacterial genetic model was further employed to explore the regulations and recombination pathways involved in this AID-mediated post-transcriptional control of gene expression. To our surprise, we have observed that AID expression also led to bacterial killing and the DNA damage-associated cellular filamentation. Thus, unregulated deamination and subsequent DNA breaks by AID are mutagenic and lethal to cells. This is in agreement with the potential adverse effect of AID that its activity on non-Ig genes is responsible for the generation of chromosome translocations and to lymphomagenesis. In addition, transcripts of Ig switch regions have been shown to preferentially form R-loop both In Vitro and in cells. Currently, the potential of nuclear RNAs, particularly for those containing sequences from Ig switching regions, as anti-cancer agents in the absence and presence of AID is under investigation. Preliminary results have showed that transfection of RNA oligos caused chromosomal DNA breakage and cell killing. With advanced knowledge on the newly identified roles of small RNAs, it is therefore important to understand the underlying mechanism(s) for the anti-tumor activity of RNA oligos and the potential contribution of R-loop/AID in this RNA-mediated cell killing.

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VISITOR INFORMATION

EMERGENCY	CSHL	BANBURY
Fire	**(9) 742-3300**	**(9) 692-4747**
Ambulance	**(9) 742-3300**	**(9) 692-4747**
Poison	**(9) 542-2323**	**(9) 542-2323**
Police	**(9) 911**	**(9) 549-8800**
Safety-Security	**Extension 8870**	

Emergency Room **Huntington Hospital** 270 Park Avenue, Huntington	**631-351-2300** **(1037)**
Dentists Dr. William Berg Dr. Robert Zeman	 **631-271-2310** **631-271-8090**
Doctor MediCenter 234 W. Jericho Tpke., Huntington Station	**631-423-5400** (**1034**)
Drugs - 24 hours, 7 days Rite-Aid 391 W. Main Street, Huntington	**631-549-9400** (**1039**)

Free Speed Dial
Dial the four numbers (****) from any **tan house phone** to place a free call.

GENERAL INFORMATION

Books, Gifts, Snacks, Clothing, Newspapers

BOOKSTORE 367-8837 (hours posted on door)
Located in Grace Auditorium, lower level.

Photocopiers, Journals, Periodicals, Books, Newspapers

Photocopying – Main Library
Hours: 8:00 a.m. – 9:00 p.m. Mon-Fri
10:00 a.m. – 6:00 p.m. Saturday
Helpful tips - Obtain PIN from Meetings & Courses Office to enter Library after hours. See Library staff for photocopier code.

Computers, E-mail, Internet access

Grace Auditorium
Upper level: E-mail only
Lower level: Word processing and printing.
STMP server address: mail.optonline.net
To access your E-mail, you must know the name of your home server.

Dining, Bar

Blackford Hall
Breakfast 7:30–9:00, Lunch 11:30–1:30, Dinner 5:30–7:00
Bar 5:00 p.m. until late
Helpful tip - If there is a line at the upper dining area, try the lower dining room

Messages, Mail, Faxes

Message Board, Grace, lower level

Swimming, Tennis, Jogging, Hiking

June–Sept. Lifeguard on duty at the beach. 12:00 noon–6:00 p.m.
Two tennis courts open daily.

Russell Fitness Center

Dolan Hall, west wing, lower level
PIN#: **Press 64315 (then enter #)**

Concierge

On duty daily at Meetings & Courses Office.
After hours – From tan house phones, dial x8870 for assistance

Pay Phones, House Phones

Grace, lower level; Cabin Complex; Blackford Hall; Dolan Hall, foyer

CSHL's Green Campus

Cold Spring Harbor Laboratory is pledged to operate in an environmentally responsible fashion wherever possible. In the past, we have removed underground oil tanks, remediated asbestos in historic buildings, and taken substantial measures to ensure the pristine quality of the waters of the harbor. Water used for irrigation comes from natural springs and wells on the property itself. Lawns, trees, and planting beds are managed organically whenever possible. And trees are planted to replace those felled for construction projects.

Two areas in which the Laboratory has focused recent efforts have been those of waste management and energy conservation. The Laboratory currently recycles most waste. Scrap metal, electronics, construction debris, batteries, fluorescent light bulbs, toner cartridges, and waste oil are all recycled. For general waste, the Laboratory uses a "single stream waste management" system, removing recyclable materials and sending the remaining combustible trash to a cogeneration plant where it is burned to provide electricity, an approach considered among the most energy efficient, while providing a high yield of recyclable materials.

Equal attention has been paid to energy conservation. Most lighting fixtures have been replaced with high efficiency fluorescent fixtures, and thousands of incandescent bulbs throughout campus have been replaced with compact fluorescents. The Laboratory has also embarked on a project that will replace all building management systems on campus, reducing heating and cooling costs by as much as twenty-five per cent.

Cold Spring Harbor Laboratory continues to explore new ways in which we can reduce our environmental footprint, including encouraging our visitors and employees to use reusable containers, conserve energy, and suggest areas in which the Laboratory's efforts can be improved. This book, for example, is printed on recycled paper.

1-800 Access Numbers

AT&T	**9-1-800-321-0288**
MCI	**9-1-800-674-7000**

Local Interest

Fish Hatchery	631-692-6768
Sagamore Hill	516-922-4447
Whaling Museum	631-367-3418
Heckscher Museum	631-351-3250
CSHL DNA Learning Center	x 5170

New York City

Helpful tip -

Take Syosset Taxi to Syosset Train Station ($8.00 per person, 15 minute ride), then catch Long Island Railroad to Penn Station (33rd Street & 7th Avenue). Train ride about one hour.

TRANSPORTATION

Limo, Taxi

Syosset Limousine	516-364-9681 (**1031**)
Super Shuttle	800-957-4533 (**1033**)
To head west of CSHL - Syosset train station	
Syosset Taxi	516-921-2141 (**1030**)
To head east of CSHL - Huntington Village	
Orange & White Taxi	631-271-3600 (**1032**)
Executive Limo	631-696-8000 (**1047**)

Trains

Long Island Rail Road	822-LIRR
Schedules available from the Meetings & Courses Office.	
Amtrak	800-872-7245
MetroNorth	800-638-7646
New Jersey Transit	201-762-5100

Ferries

Bridgeport / Port Jefferson	631-473-0286 **(1036)**
Orient Point/ New London	631-323-2525 **(1038)**

Car Rentals

Avis	631-271-9300
Enterprise	631-424-8300
Hertz	631-427-6106

Airlines

American	800-433-7300
America West	800-237-9292
British Airways	800-247-9297
Continental	800-525-0280
Delta	800-221-1212
Japan Airlines	800-525-3663
Jet Blue	800-538-2583
KLM	800-374-7747
Lufthansa	800-645-3880
Northwest	800-225-2525
United	800-241-6522
US Airways	800-428-4322