RNA Therapeutics: Informational Drugs as a Pandemic Response Tool

Anastasia Khvorova, Ph.D.

April 2020
4 structural proteins: spike, envelope, membrane, and nucleocapsid.

Spike = homotrimeric surface protein (favored target for antibodies/vaccines)
   S1 = binds host cell receptor; S2 = fuses viral and host cell membranes

Complex genome with nine open reading frames and multiple mRNAs

Reducing viral titers is expected to have a profound impact on disease progression

Wikipedia: SARS-CoV-2
The RNAi Therapeutic Mechanism
Informational Drugs: Future of Medicine

Traditional, small molecule drug

**Dianophore**
Ensemble of molecular features that determines PK/PD/ADME

**Pharmacophore**
Ensemble of molecular features that determines target recognition and modulation

**Chemistry of the backbone and ligand**

**Sequence**

Informational drug

**Dianophore**
Ensemble of molecular features that determines PK/PD/ADME

**Pharmacophore**
Ensemble of molecular features that determines target recognition and modulation

*\textit{Dianophore}—from the Greek “διανομή-dianomi” for distribution or delivery

\textit{Khvorova and Watts, 2016, Nature Biotech}
RNAi Has Matured to Become A Defined Therapeutic Class

- **Foundational Biology**
- Focus: minimal modification and LNP delivery
- Focus: extensive modification and conjugate delivery

**Timeline:**
- **Discovery of RNA interference**
- **Nobel Prize to Fire and Mello**
- **Early clinical trials**
- **Big pharma enters**
- **Several advanced clinical trials fail**
- **Big pharma leaves**
- **LNPs continue to show immune effects**
- **Development of stabilized GalNAc siRNAs**
- **First product launched 2018**
Inclisiran® for Cholesterol Lowering Support 6-12 Month Efficacy with Single Injection

Fitzgerald, 2017 NEJM; Khvorova , 2017 NEJM
ORION-11: Efficacy
Durable, potent and consistent effect over 18 months

Percent change in LDL-C over time – observed values ITT patients

Time-averaged Δ 50%
Δ 54%

P-value for placebo – inclisiran comparison at each time point <0.00001

THE MEDICINES COMPANY

Alnylam®
PHARMACEUTICALS
ORION-11: Efficacy
Durable, potent and consistent effect over 18 months

Percent change in LDL-C over time – observed values ITT patients

![Graph showing percent change in LDL-C over time.]

Time-averaged Δ 50%
Δ 54%

Months from start of treatment

P-value for placebo – inclisiran comparison at each time point <0.00001

<table>
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<th>Placebo</th>
<th>Inclisiran</th>
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Reduce SARS CoV 2 or/and host factors in the lung as a treatment and prophylactic of COVID19
Requirements for Development of siRNA-Based COVID19 Therapeutics

• Fully chemically-modified scaffolds supporting multi-month efficacy *in vivo*

• Chemical architectures supporting efficient lung delivery with acceptable safety profile
  • LOCAL- Intratracheal
  • Systemic

• Advanced bioinformatics to identify SARS-CoV2 regions of conservation
  • Cocktail of three compounds is expected to be sufficient to minimized the risk of escape mutants development and full variants coverage

• Lead Compounds efficiently targeting SARS-CoV2

• Experimental systems allowing study of SARS-CoV2 infection in living animals and cells
Increasingly complex requirements for oligonucleotide chemistry

**Scaffold**
- Determines target
- Determines PK/PD/ADME
- Determines mechanism 
  (RNAi, RNase H, Steric blocker, etc)

**Dianophore**
- Determines PK/PD/ADME

**Pharmacophore**
- Determines target

**Steric blocker ASO**
- Nuclease resistance
- Binding affinity
- Bioavailability

**RNase H ASO**
- Nuclease resistance
- Binding affinity
- Bioavailability
- RNase H compatibility
- Specificity of cleavage

**RNAi**
- Nuclease resistance
- Bioavailability
- AGO2 compatibility
- Specificity
siRNAs Modifications in Clinic

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Full Metabolic Stabilization of siRNA is Essential for Conjugate-Mediated Delivery

Hassler, Turanov, Alterman et al, NAR, 2018, Haraszti, Roux, NAR, 2017
Full Metabolic Stabilization is Essential for Systemic Delivery

Hassler, Turanov, Alterman, et al, NAR 2018
Revusiran vs Inclisiran: Composition Defines Potency and Toxicity

- Revusiran: ~50% 2'-fluoro, No 5' PS stabilization, 25 gram a year/patient
- Inclisiran: ~25% 2'-fluoro, 5' PS stabilization, <1 gram a year/patient

Etiology unresolved
Advanced Chemical Modification Scaffolds with Enhanced Stability

Orders of magnitude increase in stability translates in higher tissue accumulation.

**A**

- **revusiran-like**
  - $(mN)$-PO$(mN)$-PO$(mN)$-PO$(mN)$

- **inclisiran-like**
  - $(mN)$-PS$(mN)$-PS$(mN)$-PS$(mN)$

- **Novel scaffolds**
  - $(mN)$-$(xmN)$-$(xmN)$-$(xmN)$

**B**

**In vitro efficacy**

- **mf scaffold:** $IC_{50} \approx 48\, nM$
- **m-rich scaffold:** $IC_{50} \approx 44\, nM$

**C**

**In vivo siRNA Tissue Accumulation**
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Lung Delivery: Intratracheal vs Systemic

Systemic
- Ease of clinical delivery
- Uniform lung distribution through capillary
- No impact of patient clinical condition or lung inflammation
- Significant exposure of other organs

Local
- Non-invasive
- Support delivery to respiratory track (primary point of infection)
- May be affected by patient clinical stage
- Nebulization can increase infection spread
- Highly selective with minimal exposure to other tissues
Path to Success: siRNA structure/stabilization, linker, PK modifier, Conjugate Identity

- Chemical structure
- Hydrophobicity
- Valency

- Covalent / Cleavable
- Length / Hydrophobicity
- Degree of endosomal escape

- Modification Patterns
- Backbone modifications
- Structure
- Sequence selection

- Rate of kidney clearance
- AUC
Lipid Conjugates Support Functional Delivery to Many Tissues

- Improves circulation half-life and bioavailability
- Allows siRNA penetration into a variety of tissues
- Platform for exploring local and systemic delivery

Cholesterol-conjugated siRNA

Turanov et al., Nat Biotechnol., 2018
Yuan et al., Am J Physiol Renal Physiol., 2008
Osborn and Khvorova, Nucleic Acid Ther., 2018
Soutscheck et al. Nature, 2004
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**Important toxicity limiting its therapeutic use**

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- Yuan et al., Am J Physiol Renal Physiol., 2008
- Osborn and Khvorova, Nucleic Acid Ther., 2018
Design of Lipid-siRNA Conjugates with Distinct Physicochemical Properties

**Fatty acids**

**Sterols**

**Vitamins**

Biscans et al., Nucleic Acid Res., 2018
Biscans et al., J. Control. Release, 2019
Osborn et al., Nucleic Acid Res., 2018
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Evaluation of Impact of Conjugate Chemistry on siRNA Delivery

Lipid conjugated siRNA subcutaneous injection

Dose = 20 mg/kg

Day 0

n_{Htt} = 3

Day 2

Distribution studies in 15 tissues

• Fluorescence microscopy
• Antisense strand quantification using PNA hybridization assay

Day 7

n_{Htt} = 8
n_{Ppib} = 8

Efficacy studies

Silencing efficacy of two targets: Htt and Ppib mRNA in several tissues

End
The Structure of the Conjugate Defines Liver/Kidney Distribution Ratio

SC, 20 mg/kg, n = 3, t = 48h. *Leica DMi8 Microscope. 5x Tiled Arrays. DAPI (Blue), siRNA (Red). Scale = 1 mm

Biscans et al., Nucleic Acid Res., 2018
Biscans et al., J. Control. Release, 2019
Increased Hydrophobicity Correlates with Decreased Renal Clearance

Biscans et al., Nucleic Acid Res., 2018
Biscans et al., J. Control. Release, 2019
Hydrophobicity Drives siRNA Partitioning into Distinct Lipoprotein Classes

Osborn et al., Nucleic Acid Res., 2018

Intravenous injection, 20 mg/kg, t = 15 min, n = 2
siRNAs can be engineered to achieve predictable partitioning into lipid transport pathways
The chemical structure of the conjugate defines siRNA tissue distribution

SC, 20 mg/kg, t = 48 h, n=3.

Biscans et al., Nucleic Acid Res., 2018
Biscans et al., J. Control. Release, 2019
Significant siRNA Delivery in Adrenal Glands

SC, 20 mg/kg, t = 48 h, n=3. Leica DMI8 Microscope. 5x Tiled Arrays. DAPI (Blue), siRNA (Red). Scale = 1 mm

Biscans et al., Nucleic Acid Res., 2018
Biscans et al., J. Control. Release, 2019
SC, 20 mg/kg, t = 48 h, n=3.

DCA/PC-DCA improves siRNA extrahepatic tissue accumulation compare to cholesterol.
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End
siRNA Accumulation Leads to Functional Delivery in Multiple Extrahepatic Tissues

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Leica DMi8 Microscope. 40x magnification. DAPI (Blue), siRNA (Red). Scale = 50 μm (SC, 20 mg/kg, n = 3, 48h)

Measurement RNA silencing using QuantiGene Assay. Normalized to Hprt gene and presented as percentage of PBS control (SC, 20 mg/kg, 1 week, n = 6-8)

Biscans et al., Nucleic Acid Res., 2018
Biscans et al., J. Control. Release, 2019
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Local
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The Chemical Scaffolds for Local and Systemic Delivery Are Different

**LOCAL**: n=2, 24h, 5x, Scale=1 mm

- PBS
- siRNA
- Con3-siRNA

**SYSTEMIC, SC**: n=3, 48h, 5x, Scale=1 mm

- PBS
- Con2-siRNA
- Con1-siRNA

Legend:
- DAPI
- Cy3
- Merge
Systemic vs Local Delivery to Lung: Differential Secondary Tissues Exposure Profiles

Intratracheal
Efficient Lung Delivery: Intratracheal and Systemic

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![Graph showing target mRNA expression](image)

\[\text{Target mRNA Expression (\% of Control)}\]
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SARS-CoV-2 (~29.9 kb)

Proportion of SARS-CoV-2 variants targeted (%)

Proportion of siRNAs Selected (%)
Targeting Regions of Conservation for other Human Beta-coronoviruses

SARS-CoV-2
SARS-CoV
MERS-CoV
HCoV-NL63
HCoV-229E
HCoV-HKU1
HCoV-OC43

Homology (%)
Development Algorithms for Modified siRNAs Design

Shmushkovich, Monopoli et al, 2018, NAR
Use a hyperplane (line/multidimensional plane) to classify data

Algorithm:
1. Select features
2. Classify Functional/Nonfunctional sequences (cutoffs)
3. Develop an N-dimensional hyperplane that separates data
4. Obtain an equation with coefficients (weight matrix)

SMV (Support Vector Machine – Kernel Adjustment)
Functional cutoff <30% Target Gene Expression
Recursive Feature Elimination (RFE)
UMMS Nucleic Acid Chemistry Center: Only NFP center in North America with gram-synthesis Capability

**Synthesis:** OligoPilot 100 (GE)  
**Purification:** 1260 HPLC  
**QC:** High Resolution LCMS

4 grams sFLT1_2283/2519/P2 hsiRNAs formulated for baboon injections

Matthew Hassler, PhD

Dimas Echeverria
siRNA Lead Compounds Against Key SARS-CoV2 Regions Identified

A

B

Relative reporter expression (% of control)
Targeting an RNA Virus: siRNA Cocktails

- RNA Viruses mutate
- Rate of SARS-CoV2 mutations is lower
- Use of cocktails (at least three compounds) minimizing escape mutants appearance
- Lead Cocktail:
  - RTI-siCOVID: pp1ab, orf7, N
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Drug Design Concepts for Anti-viral Therapies

- Target conservation regions
- Multiplexing, multiplexing, multiplexing
- Current lead compound: cocktail of three siRNAs
- Highly similar PK/PD and ADME-tox properties makes siRNAs an ideal multiplexing drug
- FDA allow defining a cocktail of several siRNAs as a single drug entity
- Target host factors involved in viral entry
Targeting Host Factors: SARS Infection Cycle- ACE2

Corum and Zimmer, March 13, 2020. NYT.
S1-receptor binding stabilizes prefusion complex for cleavage of S2, which allows for the residues necessary for membrane fusion to be exposed.

SARS-CoV-2 does not produce the protease that cleaves its S protein.
Furin cleaves SARS-CoV-2 spike Protein During Biosynthesis

- Four amino acid residue insertion at the S1/S2 boundary compared to SARS-CoV S and SARSr-CoV S
- This results in the introduction of a furin cleavage site
- SARS-CoV-2 is cleaved at S1/S2 site during biosynthesis in HEK293T, presumably by furin
- SARS-CoV-2 S is not cleaved at S1/S2 site before incorporation into virion

TMPRSS2 can Prime Spike Protein and Modulate SARS-CoV2 Entry

- E-64d is CatL inhibitor
- Camostat mesylate is a clinically proven TMPRSS2 inhibitor
- Vero don’t express TMPRSS2
  - Vero made to express TMPRSS2 rescue SARS-CoV-2 entry from E-64d inhibition

Reduce SARS CoV 2 or/and host factors in the lung as a treatment and prophylactic of COVID19

The Idea

Lung-Conjugate

RTI-siCOVID

Guide Strand

3'

Passenger Strand

5'

RTI-siFURIN
RTI-siACE2
RTI-siTMPRSS2
RTI-IL6/IL6R
Impact of RTI-siCOVID on SARS-CoV2 Infection

• Cells
  • VERO E6 – SARS-CoV2 infected cells (BCL3)
  • SARS-CoV2 infected lung/air epithelia (BCL3)

• Animal models
  • Mouse (ACE2), infected with SARS-CoV2 (BCL3)
  • Ferrets (BCL3)
  • NHPs infected with SARS-CoV2 (BCL4)
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What we have

• Functional lung delivering platform systemic
  • ~ 1% of injected dose accumulates in lungs (systemic)
  • More than 2 month duration of effect following single sc injection
  • Safe in NHPs with a single injection

• Functional lung delivery platform local (intratracheal)
  • Significant lung accumulation (~ 20% )
  • Minimized functional exposure to other tissues (essential for host gene targeting)

• Platform for identification of fully-chemically stabilized “clinical quality siRNAs”
  • Advanced bioinformatics
  • Synthetic capability to make up to 1000 oligonucleotides a week
  • HTS screening platform

• Lead Compounds identified
  • SARS-CoV2 targeting cocktails (IC50<20nM in passive uptake)
  • FURIN, ACE2, TMPRSS2, IL6, IL6R  and other host factors form modulation of entry and inflammatory response

• Concept
  • Target SARS CoV 2 genome in lungs as therapeutics and prophylaxis
  • Target genes involved in SARS CoV 2 cellular transduction (ACE2, FURIN, TMPRSS2 etc) as a combinatorial therapeutics
Major Outstanding Questions and Concerns

- Would incomplete silencing of the virus be sufficient to have a clinically relevant outcome?
- Systemically, lung-delivering siRNAs distribute to many cell types, including monocytes. It is safe in healthy NHPs. Will additional immune cell exposure to siRNAs in the context of activating immune response would exaggerate the inflammatory response?
- What is the optimal time of treatment: prophylactic, early in disease, late in disease. How would you design a clinical trial in a context of active pandemic?
- What is an optimal route of administration?
  - SC, IV, IT
Introduction to EAUs: Emergency Use Authorization

• FDA may issue an EAU if the following four conditions are met:

  1. Serious or life-threatening disease or condition
  2. Evidence of effectiveness ("May be effective" is enough for consideration for EUA)
  3. Risk-Benefit analysis
     • Evidences based on the following (but not limited to): results of domestic and foreign clinical trials, in vivo efficacy data from animal models, in vitro data.
  4. No alternatives or insufficient supply of approved alternatives

• Submission of an IND or IDE is not required for potential EAU products but human data from these studies may support the application for EAU

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- **Month 1**: Leads Design
- **Month 2**: Assays development, Synthesis (~ 300 compounds), Screening
- **Month 3**: Lead identification and validation, Lead compound Optimization
- **Month 4**: Manufacturing (CMC), Lead Scale up, Supply chain, Lead GMP manufacturing (CMO)
- **Month 5**: Animal model validation (efficacy and safety)
- **Month 6**: Regulatory, Understanding regulatory framework, Drafting of EUA, First in patient
Acknowledgments

It takes a village – and a collaborative spirit.

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Chair, Department of Medicine

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