



Phosphoryl guanidine (PN)-containing oligonucleotides support exon skipping in skeletal muscle in mice and boys with DMD

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Disclosures

Pachamuthu Kandasamy¹, Graham McClorey, Laurent Servais, Craig Campbell, Xiao Shelley Hu¹, Andrew Hart¹, Joseph Haegele¹, Kuldeep Singh¹, Jeanette Rheinhardt¹, Anamitra Ghosh¹, Mamoru Shimizu¹, Nayantara Kothari¹, Naoki Iwamoto¹, Michael Byrne¹, Fangjun Liu¹, Chikdu Shivalila¹, Carlo Rinaldi, Hailin Yang¹, Danlin Xu¹, Stephen Lake¹, Michael Panzara¹, Anne-Marie Li-Kwai-Cheung¹, Matthew Wood, Chandra Vargeese¹

¹Employees of Wave Life Sciences

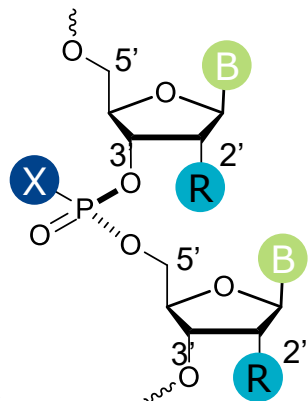
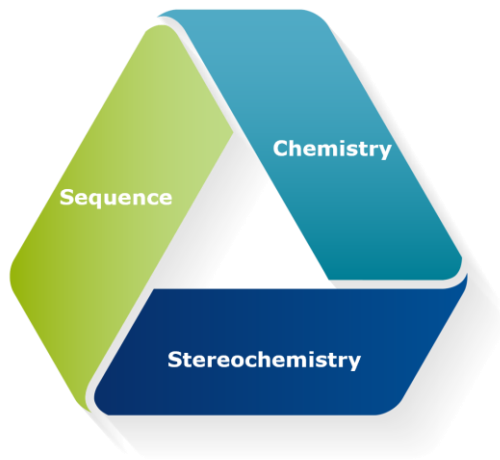
Laurent Servais has provided consultancy and lectures, and attended advisory boards for Sarepta, Dyne, Pfizer, Santhera, RegenxBio, Affinia, and Fibrogen

Craig Campbell has served as site investigator for Acceleron, AMO, Biogen, Dyne, Fibrogen, Pfizer, Roche, PTC, Sarepta, Cytokinetics, Ultragenix, and Wave and has acted as a Data Safety Monitoring Board member for Catabasis, Edgewise, and Solid. Additionally, he has received investigator-initiated grants from Genzyme, PTC Therapeutics, and Biogen

Matthew Wood is a cofounder of PepGen

The data in this presentation are from preliminary analyses of an ongoing clinical trial

PRISM™ platform enables rational drug design

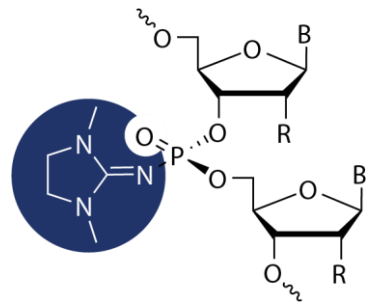


- X:**
- O:** Phosphodiester
- S:** Phosphorothioate
- N:** Phosphoryl guanidine

B Base

R 2'-ribose modification

X Stereochemistry & backbone modification



Phosphoryl guanidine

Duchenne muscular dystrophy and WVE-N531

Duchenne Muscular Dystrophy

A rapidly progressive, rare disease characterized by irreversible muscle degeneration and weakness, loss of ambulation and upper body function, and cardiorespiratory complications

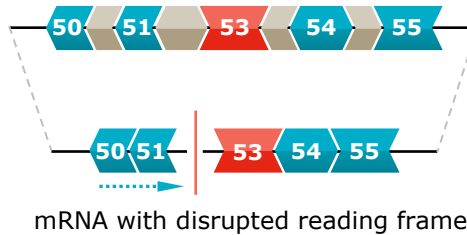
WVE-N531

- Investigational exon-skipping oligonucleotide
- Contains PN chemistry
- Designed to skip exon 53
- 8-10% of patients with DMD are amenable to exon 53 skipping¹

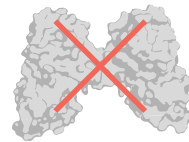
Disease State

Dysfunctional Splicing

Mutant pre-mRNA



Translation halted

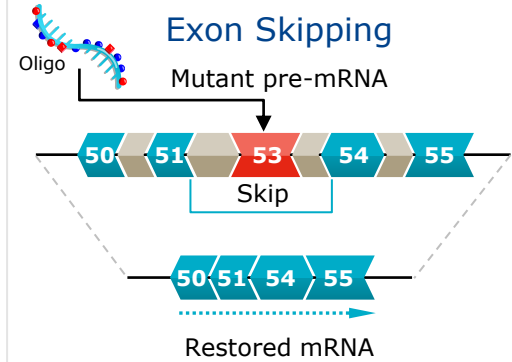


No dystrophin protein produced

Restored State

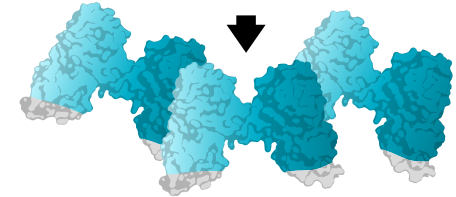
Exon Skipping

Mutant pre-mRNA



Restored mRNA

Translation continues



Functional dystrophin produced

PN chemistry improves pharmacological properties in multiple preclinical model systems



***mdx23* mouse**

Protein	Pathology
✓ Utrophin	Mild No decrease in survival
✗ Dystrophin	



Double knockout mouse (dKO)

Protein	Pathology
✗ Utrophin	Severe muscular dystrophy
✗ Dystrophin	Premature death



Nonhuman primate (NHP)

Protein	Pathology
✓ Utrophin	Healthy
✓ Dystrophin	

WVE-N531 surrogate yields excellent muscle exposure, exon skipping and dystrophin protein expression in *dKO* mouse model

- PBS
- WVE-N531 surrogate for mouse Dmd exon 23



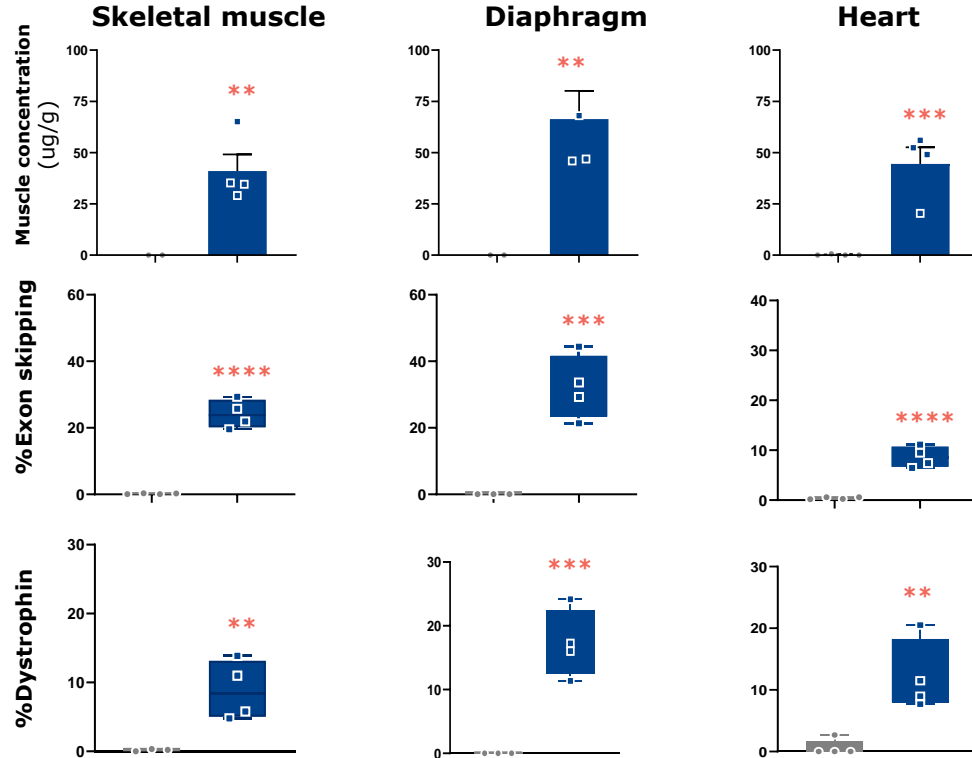
***dKO**
mouse**

D0 D7 D14 D21 D28 D35 D37


↑ dose ↑ ↑ ↑ ↑ ↓



*Similar results observed in *mdx23* mouse

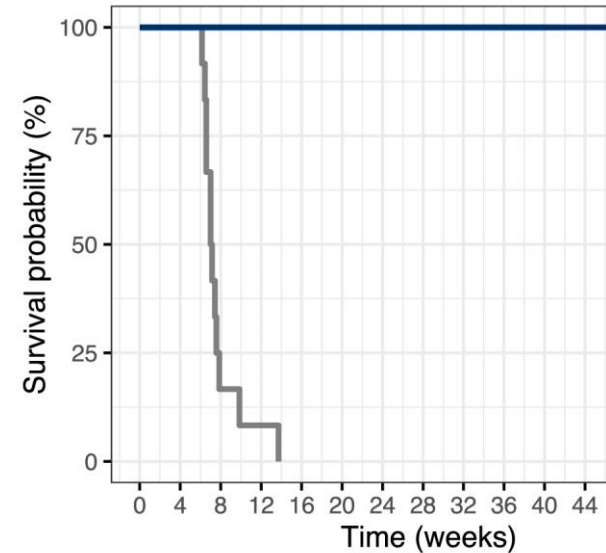


Biweekly administration of WVE-N531 surrogate improves survival and muscle function in dKO mouse model

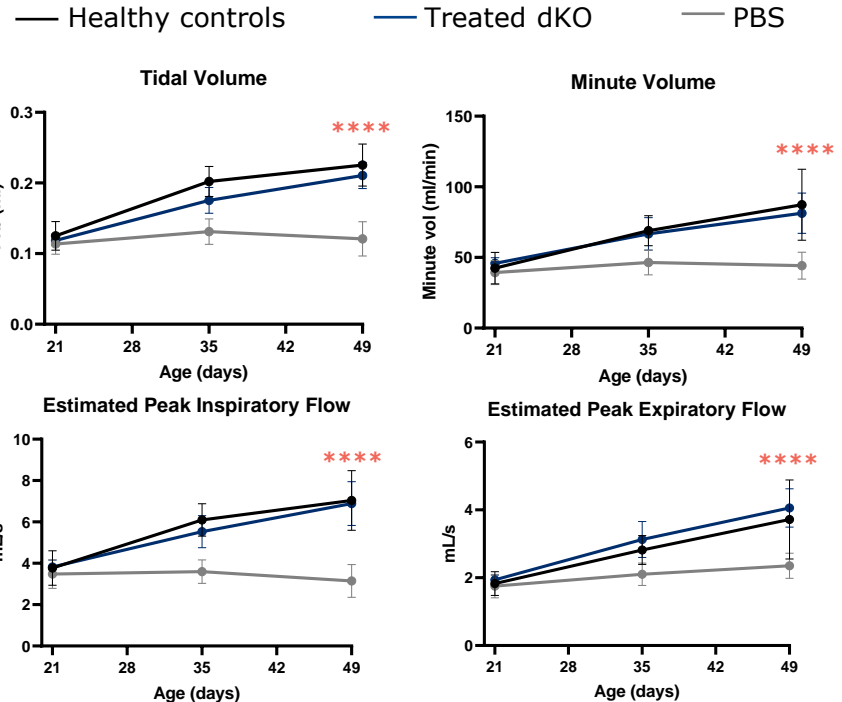


Median survival	
PBS	49 days
WVE-N531 surrogate	280 days

$P=2 \times 10^{-11}$



Respiratory function comparable to age-matched healthy controls
(6 x weekly doses, dKO mouse)

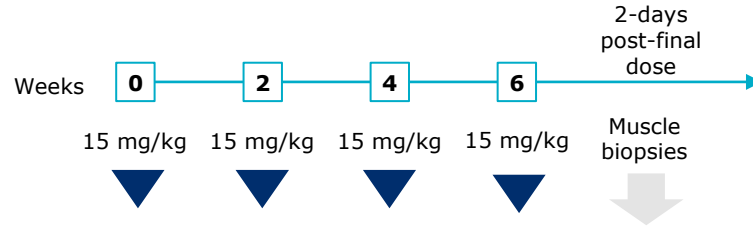


WVE-N531 reached high concentrations in heart and diaphragm in NHP



NHP

Dosing at 15 mg/kg biweekly



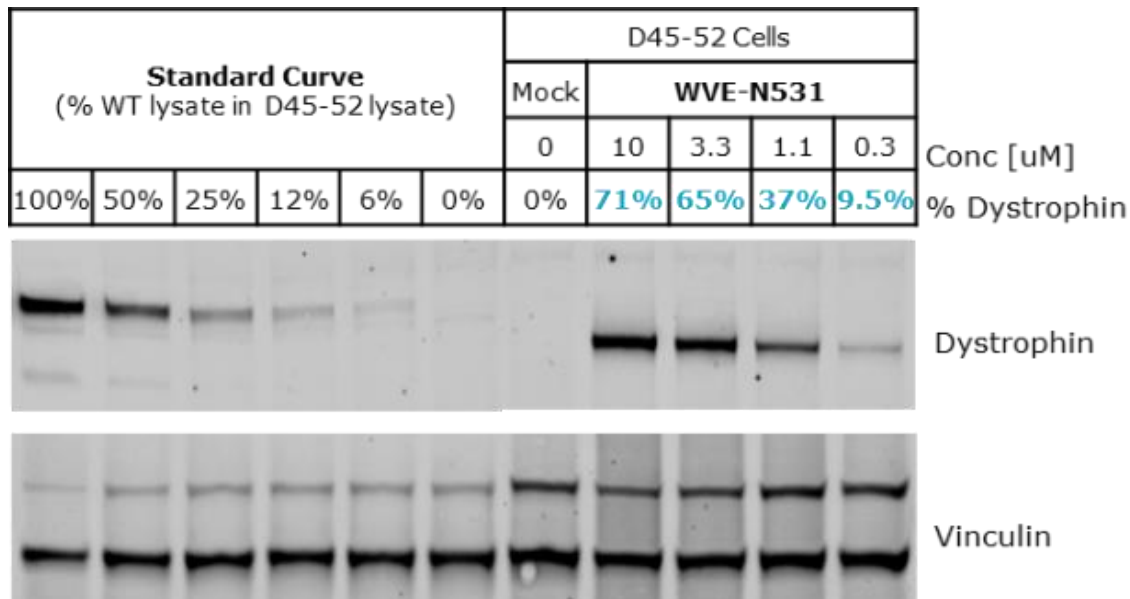
	Mean Tissue Concentration		
	Skeletal muscle	Diaphragm	Heart
15 mg/kg* IV dose	2.17 ug/g	10.8 ug/g	57.2 ug/g

*approximately equivalent to 10 mg/kg in patients based on plasma AUC values

WVE-N531 restores dystrophin expression in patient-derived myoblasts

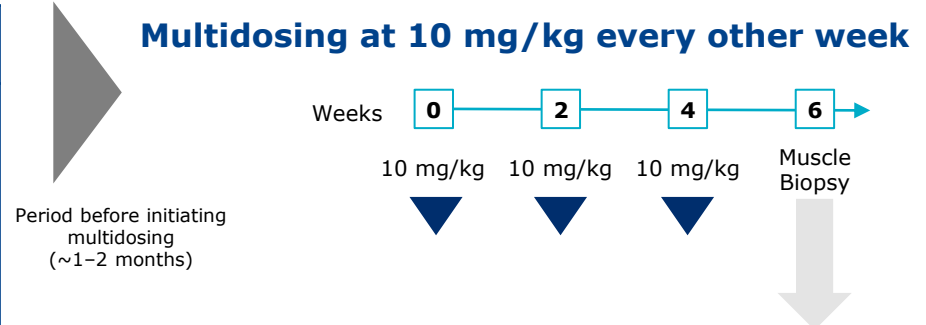
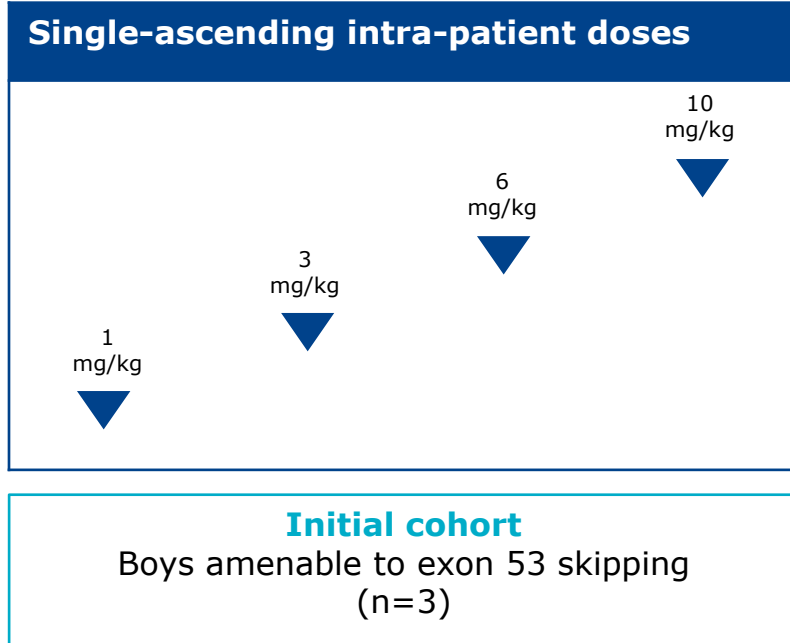
Dystrophin protein restoration up to 71% *in vitro*

western blot normalized to
primary healthy human myoblast lysate



WVE-N531: First-in-human study design

WVE-N531-001: First-in-human study



Endpoints:

Primary	<ul style="list-style-type: none">• Safety and tolerability of WVE-N531
Secondary	<ul style="list-style-type: none">• WVE-N531 muscle concentrations• WVE-N531 localization• Exon skipping• Dystrophin protein

Muscle concentrations and exon skipping indicate WVE-N531 is engaging target

Plasma $t_{1/2}$: 25 days (10 mg/kg single dose)

Patient	Tissue Source	Tissue concentration ($\mu\text{g/g}$)	% Exon skipping by RT-PCR	Dystrophin by western blot (% of normal)
1	Deltoid	85.5	61.5	0.24
2	Deltoid	33.5	49.8	0.23
3	Bicep	8.3	47.9	0.34

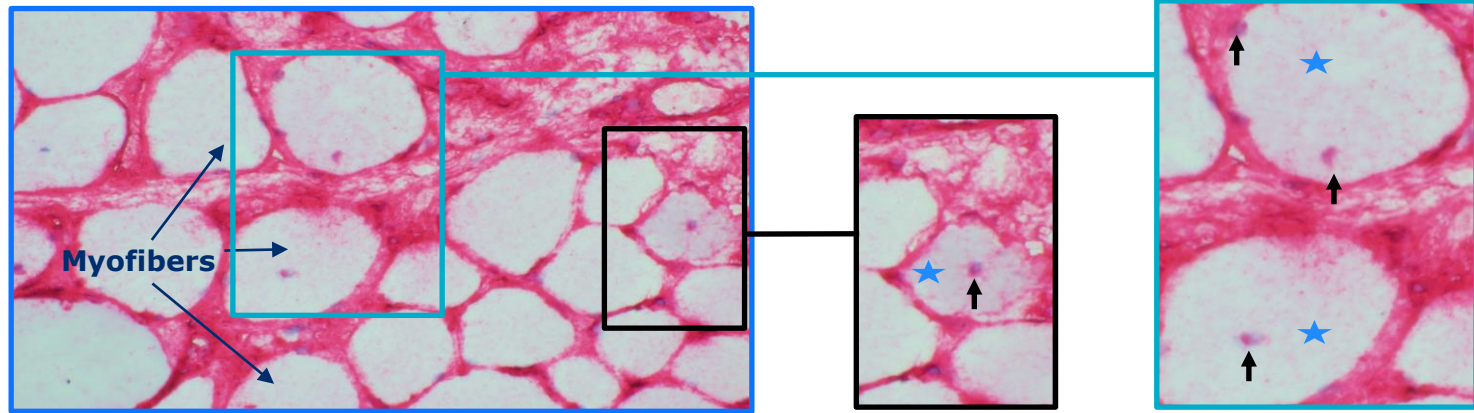
Mean muscle concentration:
42 $\mu\text{g/g}$

Mean exon skipping:
53%

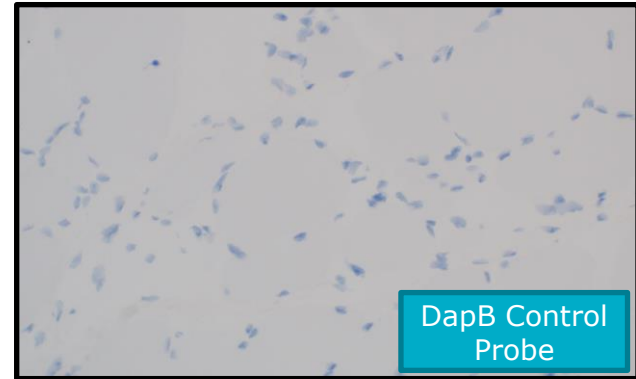
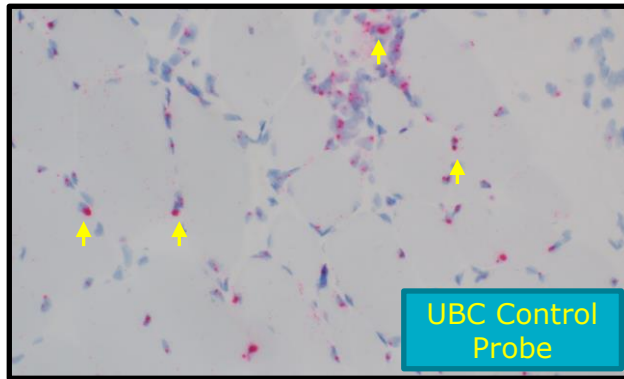
Mean dystrophin:
0.27% of normal
(BLQ)

WVE-N531 was generally safe and well-tolerated

Intracellular WVE-N531 enabling exon 53 skipping



WVE-N531 (in red) in myofiber cytoplasm (stars) and nuclei (black arrows)



Summary

- WVE-N531 surrogate increased muscle concentration, percentage of exon skipping and dystrophin expression in *mdx23* and dKO mouse models compared with control
- In dKO model, WVE-N531 surrogate substantially extended survival and rescued respiratory function
- In NHPs, WVE-N531 reached high concentrations in heart and diaphragm
- In boys with DMD, WVE-N531 reached high concentrations in skeletal muscle (mean 42 µg/g), and induced 53% mean exon skipping following three biweekly 10 mg/kg doses
- Half-life supports biweekly or less frequent dosing
- WVE-N531's safety profile supports further development
- Wave is initiating Part B: Phase 2 open-label clinical trial for boys amenable to exon 53 skipping, with data expected in 2024

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Investigators

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- Craig Campbell

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Silencing - antisense

NAR Breakthrough Article

Impact of guanidine-containing backbone linkages on stereoregular antisense oligonucleotides in the CNS

Pachamuthu Kandasamy^{1,†}, Yuanjing Liu^{1,†}, Vincent Aduda, Sandheep Akare, Rowshon Alam, Amy Andreucci, David Boulay, Keith Bowman, Michael Byrne, Megan Cannon, Onanong Chivataram, Julli Dilip Shelke, Naoki Iwamoto, Tomomi Kawamoto, Jayakanthan Kumarasamy, Sarah Lamore, Muriel Lemaitre, Xuena Lin, Kenneth Longo, Richard Looby, Subramanian Marappan, Jake Metterville, Susovan Mohapatra, Bridget Newman, Ik-Hyeon Park, Saurabh Pathi, Erin Purcell-Estabrook, Mamoru Shimizu, Pochi Shum, Stephany Standley, Kris Taborn, Snehlata Tripathi, Hallin Yang, Yuan Yin, Xiansi Zhao, Elena Dena and Chandra Vargeese^{1,2*}

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Silencing - siRNA

Impact of stereoregular chimeric backbone chemistries on the potency and durability of gene silencing by RNA interference

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ABSTRACT

Herein, we report the systematic investigation of stereoregular phosphorothioate (PS) and phosphoroyl guanidine (PG) linkages on siRNA-mediated silencing. The incorporation of appropriately positioned and configured stereoregular PS and PG linkages

INTRODUCTION

In 1998, the RNA interference (RNAi) pathway, or the mechanism by which short double-stranded RNAs lead to the degradation of specific mRNA was first discovered (1), and since thereafter, confirmation that this gene silencing pathway is conserved in mammalian cells was reported (2). To increase this endogenous mechanism for

Splicing

Control of backbone chemistry and chirality boost oligonucleotide splice switching activity

Pachamuthu Kandasamy^{1,†}, Graham McCloye^{2,†}, Mamoru Shimizu¹, Nayantara Kothari¹, Rowshon Alam¹, Naoki Iwamoto¹, Jayakanthan Kumarasamy¹, Gopal R. Bommineni¹, Adam Bezigian¹, Onanong Chivataram¹, David C. D. Butler¹, Michael Byrne¹, Katarzyna Chwatienia², Kay E. Davies⁴, Jigar Desai¹, Julli Dilip Shelke¹, Ann F. Durbin¹, Ruth Ellerington³, Ben Edwards⁴, Jack Godfrey¹, Andrew Hoss⁵, Fangjun Liu¹, Kenneth Longo¹, Genliang Lu¹, Subramanian Marappan¹, Jacopo Olien¹, Ik-Hyeon Park¹, Erin Purcell-Estabrook¹, Chikou Shivavilli¹, Merve Tischebin¹, Tomomi Kawamoto¹, Carlo Rinaldi^{1,2,4}, Joana Rajko-Saravaj¹, Snehlata Tripathi¹, Hallin Yang¹, Yuan Yin¹, Xiansi Zhao¹, Cong Zhou¹, Jason Zhang¹, Luciano Apponi¹, Matthew J. A. Wood^{2,3,4} and Chandra Vargeese^{1,1*}

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RNA Editing

Endogenous ADAR-mediated RNA editing in non-human primates using stereoregular chemically modified oligonucleotides

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Technology that recruits and directs the activity of endogenous RNA-editing enzymes to specific cellular RNAs have therapeutic potential, but translating them from cell culture to animal models has been challenging. Here we describe short, chemically modified oligonucleotides called **AIMers** that direct efficient and specific A-to-I editing of endogenous transcripts by endogenous adenine deaminases acting on RNA (ADAR) enzymes, including the ubiquitous and constitutively expressed ADAR1 p150 isoform. We show that fully chemically modified AIMers with chimeric backbones containing stereoregular phosphorothioate