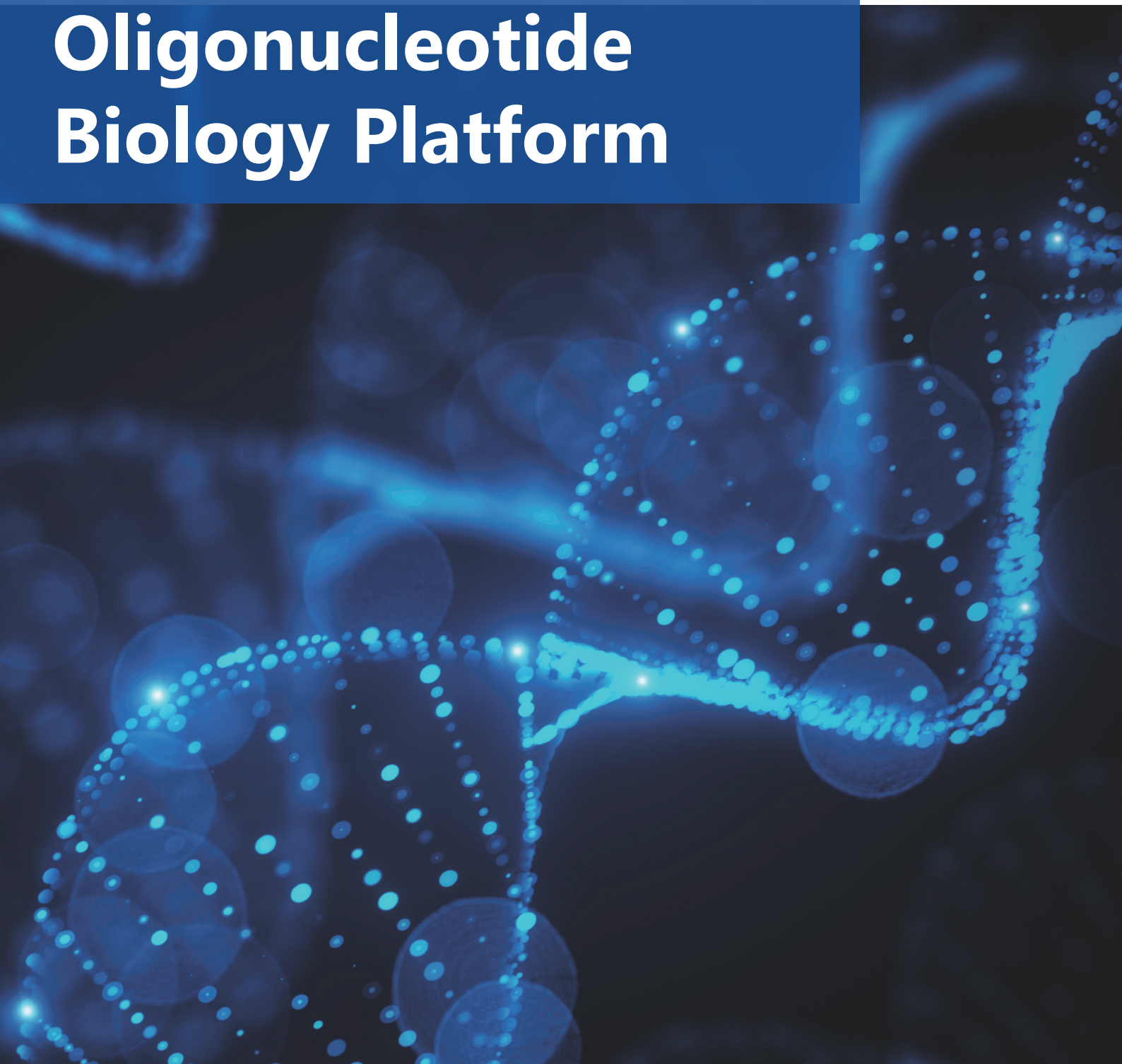
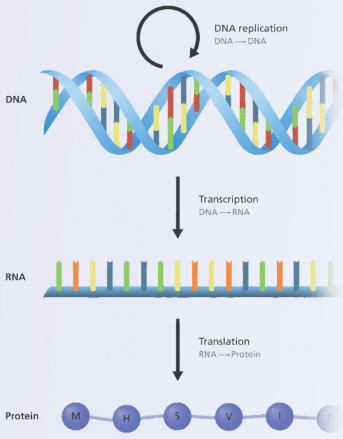


WuXi AppTec Oligonucleotide Biology Platform



Oligonucleotide drugs



Central Dogma

Item	Molecule type	MOA
ASO	12-30 nt, ss	Bind to mRNA, miRNA or pre-mRNA, induce mRNA degradation, regulate splicing, or block translation
siRNA	19-25 nt dsRNA	Bind to mRNA with fully complementary to the target mRNA sequence, induce mRNA degradation with RNAi machinery
microRNA (miRNA)	~22 nt ssRNA	Bind to mRNA with partially complementary to the target mRNA sequence, which usually occurs at the seed region (nt 2-7 of the 5' end) of miRNA and the 3' UTR of the target mRNA, result in mRNA degradation and inhibition.
Aptamer	25-60 nt, ss	Bind to the target protein for inhibition or activation

An example workflow for the discovery of oligonucleotide drugs

Example workflow: liver target

Confirm target and feasibility

Choose candidate sequence, show efficacy

Confirm PK-PD and safety

Idea Generation Hit Finding

- Target validation
- in silico sequence selection
- Assay development
- Check mRNA/Protein expression and turnover

Oligonucleotide Optimization and Validation

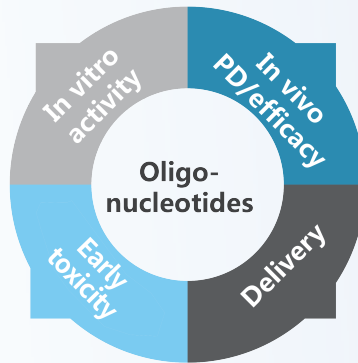
- Oligonucleotide screening array
- in vitro screening for mRNA and protein KD
- Stability testing on siRNA and conjugate (e.g. GalNAc)
- Sequence, substitution and back bone optimization
- In vivo efficacy in rodent, functional end points, check off-target tissue beds, check cellular location of KD

NHP PK-PD, safety, scale-up

- Optimize conjugation for delivery and release
- In vivo PK-PD in NHP, determine duration of KD
- Develop toxicology and safety dataset
- Scale up synthesis and CMC evaluation

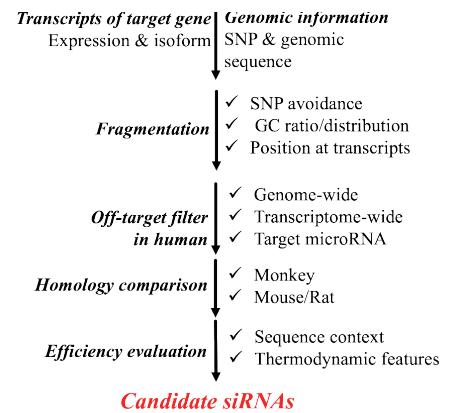
WuXi Biology oligonucleotide capabilities

- Sequence design
- Knockdown effect in cell lines and primary cells (transfection and free uptake)
- Splicing analysis
- Functional assays
- Other assays: Tm determination
- Cytotoxicity
- Immunotoxicity
 - Reporter assays
 - Cytokines
- Off-target effect
 - In silico analysis
 - RNAseq analysis
 - psiCheck reporter



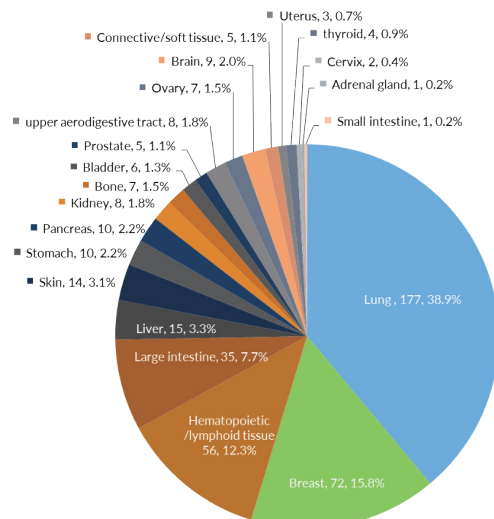
- "Humanized" mouse models
 - Transgenic, HDI and AAV mouse models
- NHP models
- Short and longterm KD efficacy/ pharmacology readouts
- GalNAc-ASGPR delivery system
- SPR screening
- Customized assays for other delivery systems
- In vitro stability and protein-protein binding
- Delivery/RISC PK in mouse, rat, NHP

Sequence designs



Cell-based KD assessments

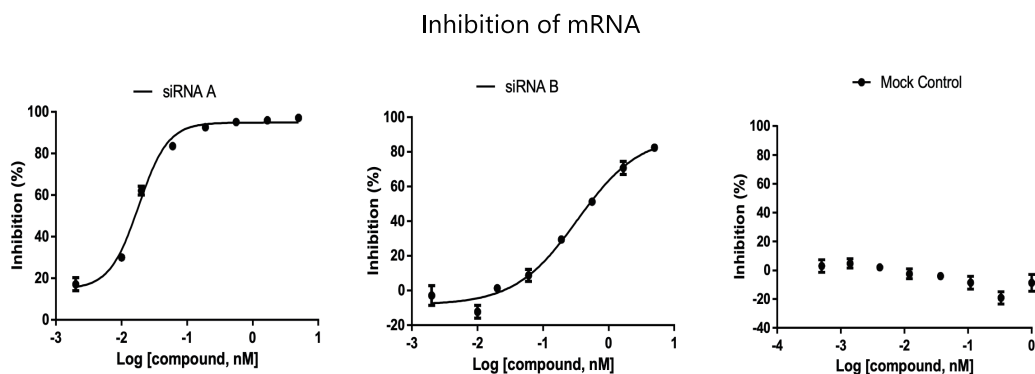
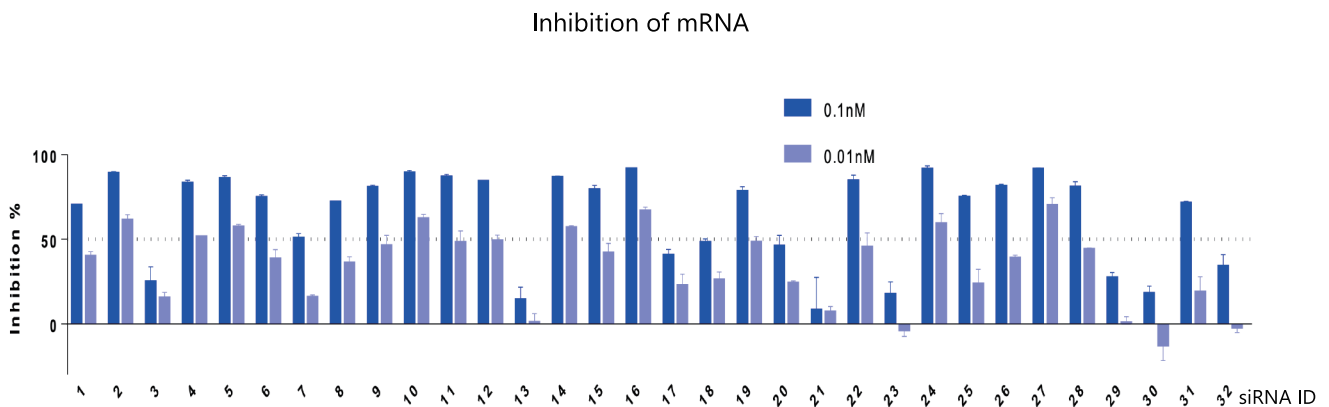
- **Assessment of mRNA and protein**
 - Cell lines
 - >400 cell lines in WuXi Biology cell bank
 - Patient derived fibroblast cell lines
 - Customized stable cell lines with lentivirus systems
 - Primary cells
 - Primary human hepatocytes (fresh and cryopreserved)
 - Primary mouse, rat, dog and monkey hepatocytes
 - Customized other primary cell assays upon request
- **psiCHECK reporter assay**
- **Transfection: commercial transfection reagents and electroporation**



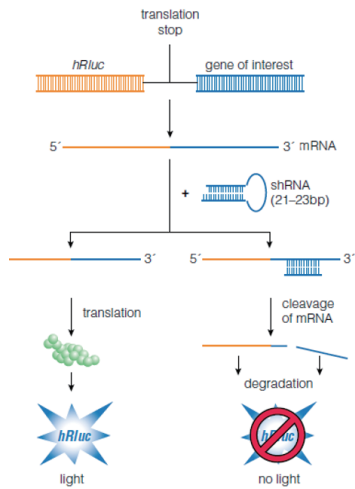
In vitro assay platform to support oligonucleotide screening

- Evaluation of knockdown effect
 - mRNA: RT-qPCR, bDNA, ddPCR, capillary electrophoresis, sequencing
 - Protein: ELISA, Luminex, MSD, CBA, Western/capillary Western, FACS, HCS, IF, HiBit luciferase reporter assay, AlphaLISA, ICW, etc.
- Cell-based functional assays
 - GPCR & other receptor assays
 - Ion channels & transporters
 - Apoptosis/Cell cycle
 - Angiogenesis
 - Downstream signaling pathway analysis
 - Cell structure and morphology analysis
 - Virus infection assays
 - Others

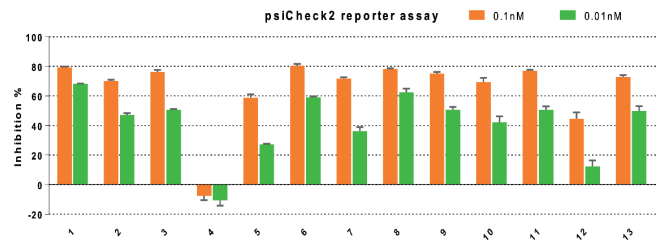
KD effect of siRNA in cell lines assessed by RT-qPCR



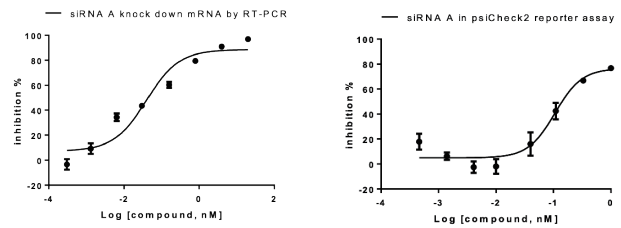
Activity of siRNA in psiCheck reporter assay



Cited from Promega manual

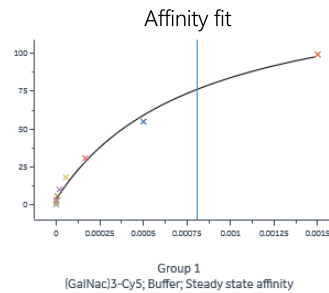
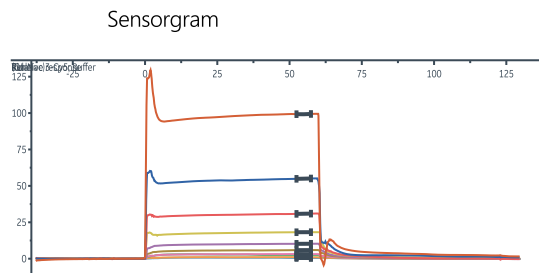


Comparison of siRNA activity in RT-PCR and reporter assays



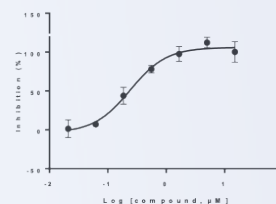
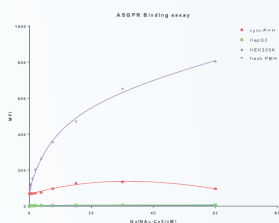
Evaluation of in vitro delivery activity

SPR



		Affinity fit		
Ligands	Analyte	KD(M)	Rmax (RU)	Chi ² (RU ²)
ASGPR-1	(GalNAc) ₃ -Cy5	8.11e-04	145.1	1.20e+01

Cell-based assay

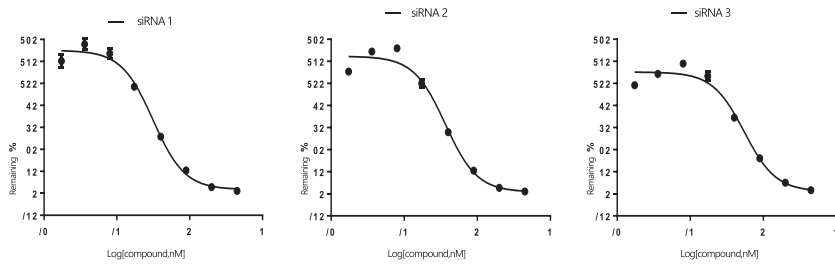


- Both fresh mouse primary hepatocytes (MPH) and cryopreserved primary human hepatocytes (PHH) showed potent ASGPR binding activity

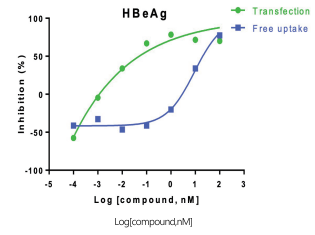
- Cy5 labeled GalNac3 was mixed with non-labeled GalNac conjugated compound, and incubated with PHH
- The uptake of Cy5 labeled GalNac3 was evaluated by measuring the median fluorescence intensity (MFI) of Cy5

Evaluation of free uptake of combined efficiency of the sequence and delivery tool

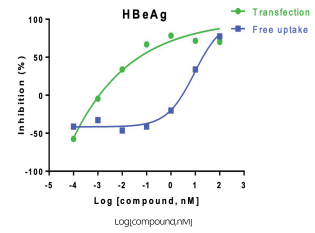
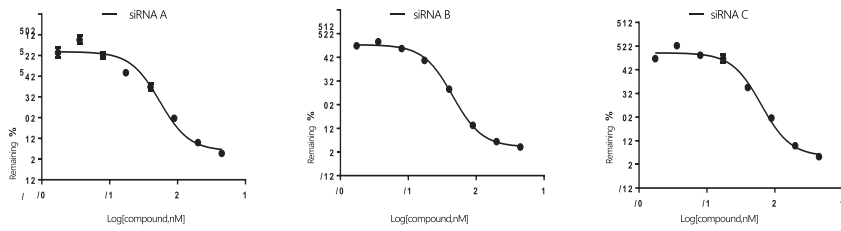
Primary human hepatocytes



Primary mouse hepatocytes

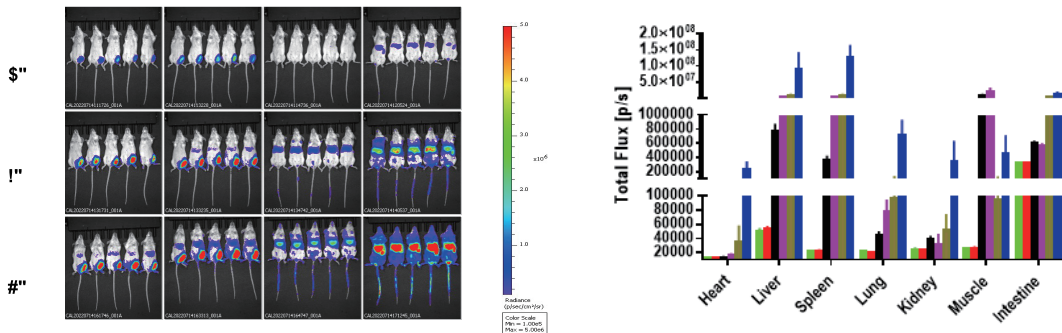


Primary cyno monkey hepatocytes

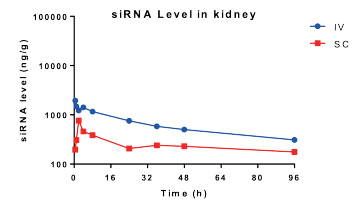
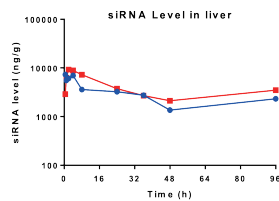
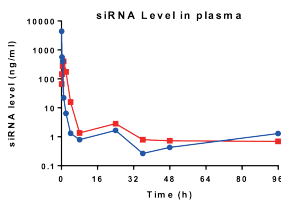


In vivo biodistribution/PK study

Evaluation of in vivo distribution with LNP delivery system in mouse

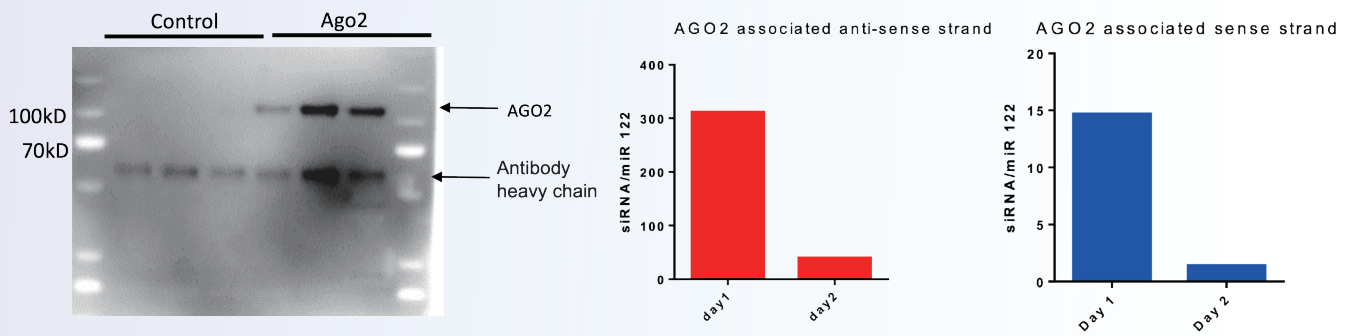


siRNA concentration in mouse plasma, kidney and liver

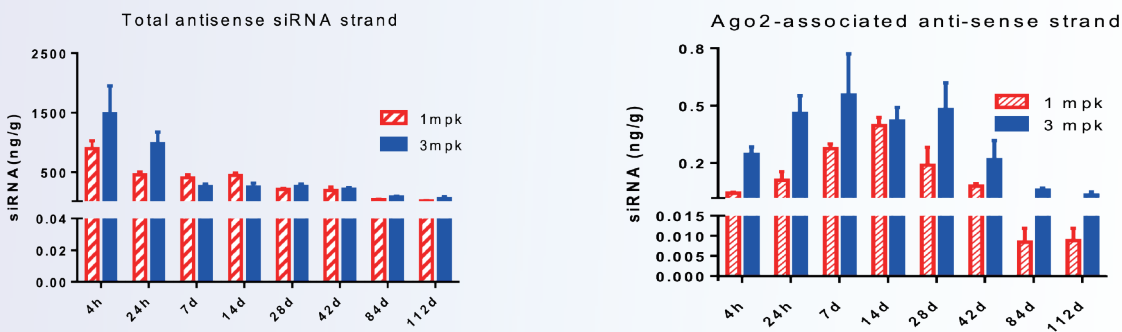


siRNA in RISC complex

Quantification of siRNA in RISC complex in hepa1-6 cells by SL-qPCR

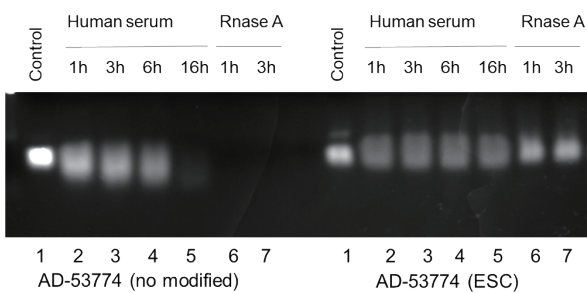


Quantification of total and Ago associated siRNA in liver by SL-qPCR



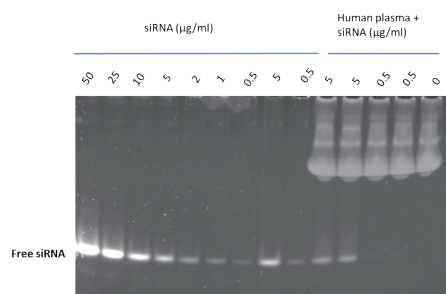
siRNA stability and PPB by electrophoresis assays

Stability



Plasma protein binding

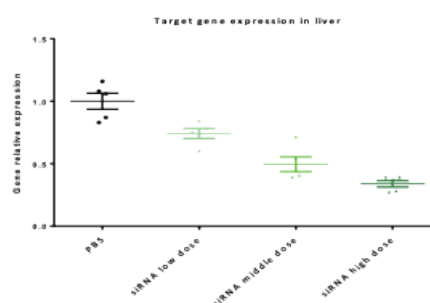
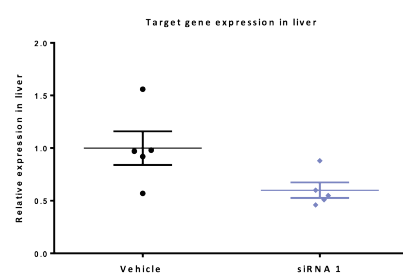
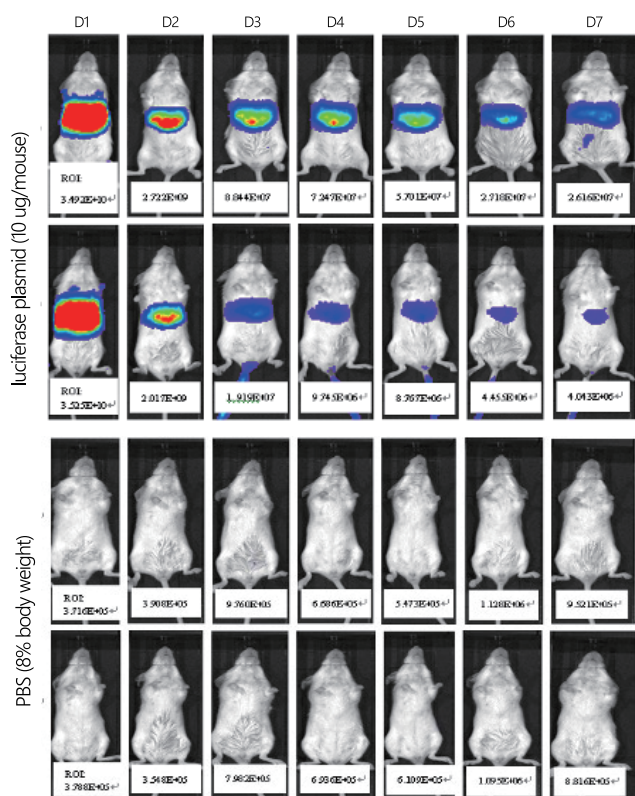
Electrophoretic mobility shift assay



“Humanized” mouse models for evaluation of oligonucleotides

Model	Target tissue/gene	Pros	Cons
Hydrodynamic injection mouse (HDI) model	Liver	Short timeline for model establishment	Transient expression of target human genes
AAV mouse model	Liver and non-liver targets	Stable expression of exogenous genes; various AAV serotypes which lead to different tissue targeting	Limited package size of AAV
Transgenic mouse model	One transgenic mouse strain is specific for a target gene	Stable expression of exogenous human genes; no lead time to establish the model compared to AAV model	Long lead time to generate/purchase the animals
Liver humanized mouse	Liver	Not only on-target effect but also suitable for off-target effect	High cost, long lead time to purchase animals, immune deficient

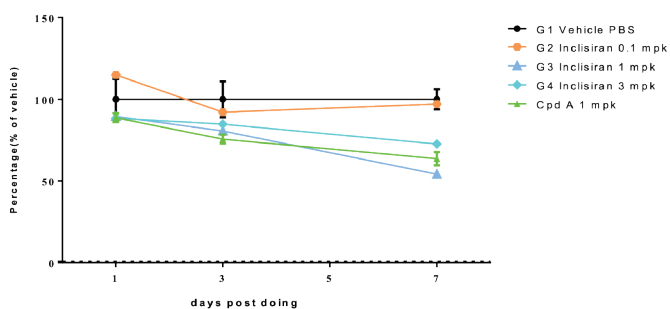
PD study of oligonucleotides in an HDI mouse model



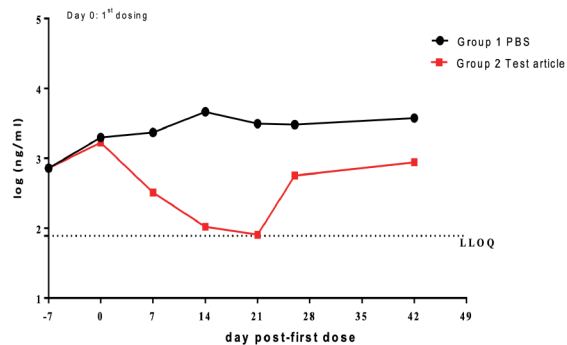
- Balb/c mice were dosed with siRNA
- Target plasmid DNA was injected by HDI
- Target gene mRNA in liver was determined by RT-qPCR

PD study of oligonucleotides in transgenic and AAV mouse models

hPCSK9 in plasma



Target protein level

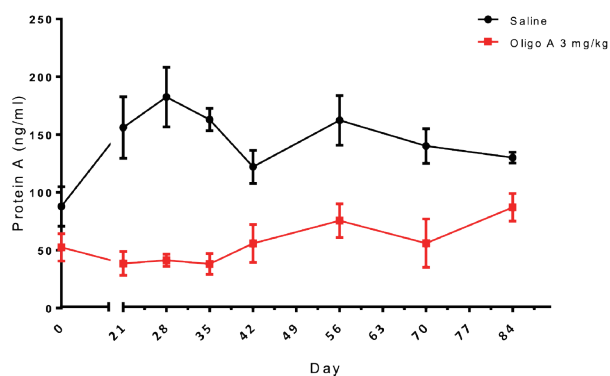


- hPCSK9 transgenic mice were dosed with siRNA once
- Serum hPCSK9 was determined by ELISA

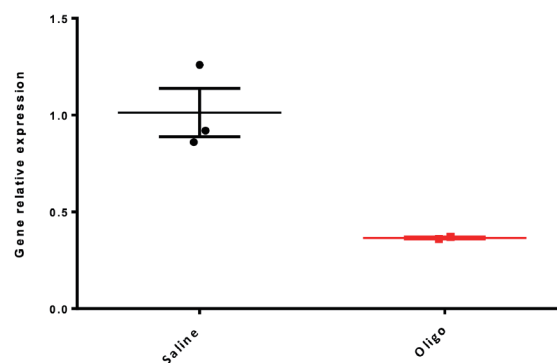
- C57BL/6j mice were infected with rAAV8
- Target protein in serum was determined by ELISA

PD study of an oligonucleotide in NHP

Target protein level in plasma



mRNA of target gene in liver by RT-qPCR



- Monkeys were dosed with vehicle or test article at day 0
- Plasma was collected at the indicated times. Target protein was determined by ELISA
- Livers were collected at the terminal time. Target mRNA was determined by RT-qPCR

Animal study capabilities

Dosing route	Abbreviation
Intraperitoneal injection	IP
Intravenous injection	IV
Retro-orbital injection	/
Intramuscular injection (with or without EP)	IM
Subcutaneous injection	
Intradermal injection	
Intracranial injection	IC
Intra-cerebroventricular injection	ICV
Intrathecal injection	IT
Thalamus injection	/
Intravitreal injection	/



- IT (intrathecal) injection
 - Mouse is anesthetized with isoflurane
 - An injection site is shaved and sterilized
 - A needle is inserted between two vertebrae (the lumbar spinal cord, L5-L6) into the IT space.
 - Solution is injected slowly over a 5 min period

Types of samples for collection and analysis

- Blood, plasma, serum, urine, BAFL, bone marrow
- Liver, lungs, kidneys, heart, brain, spleen, eyes, skin, skeletal muscle, intestinal tract
- Cerebral cortex, cerebellum, striatum, hippocampus, brain stem, cervical spinal cord, thoracic spinal cord, lumbar spinal cord, retina
- Gonads, adrenal glands, white fat, thyroids, artery and vein, etc.
- Other samples based on a specific study design

- Readouts
 - PK in blood, CSF and various tissues
 - PD/biomarkers in blood and tissues
 - Pathology, immunohistochemical staining
 - Hematology, blood chemistry, coagulation
 - Pharmacology readouts

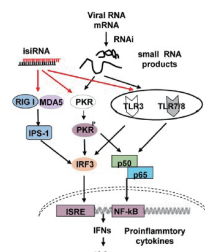
Assessments of off-target effect and safety of oligonucleotides

Cytotoxicity assays

- Cytotoxicity
 - Cancer cell lines (HUH-1, HEPG-2)
 - Non-cancer cell lines (HEK-293, HK-2, HFL-1, HUVEC)
 - Cryopreserved PHH
- Cell viability and apoptosis
 - CellTiter Glo
 - LDH assay
 - caspaseGlo 3/7

In vitro immunogenicity assays

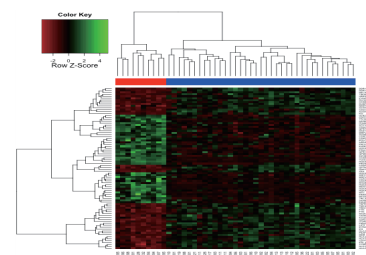
- HEK-Blue-hTLR3, 5, 7, 8, 9, RIG-I, MAD5 reporter cell lines
- Cytokine induction and immune cell activation in PBMCs
 - PBMCs from human and various animal species
 - Analysis of cytokines by ELISA, Luminex, MSD, CBA, FACS



Frontiers in Immunol., 2017, 8:1

Off target profiling

- Sequence in silico analysis for off-target prediction and by RT-qPCR assay
- RNAseq/smRNAseq for siRNA/ASO off-target
- Off-target evaluation using psiCheck reporter assay



Analysis of biomarkers to support oligo PD/efficacy studies

Genomics platforms

- Qiasymphony, Agilent 2100/Qubit
- ABI 7900HT, QuantStudio™ 6/7 Real-Time PCR System
- bDNA
- Southern and Northern blot
- ddPCR QX200 Droplet Digital PCR System
- Sanger sequencing and 2nd generation sequencing: 3730xl Genetic Analyzer and NextSeq 550

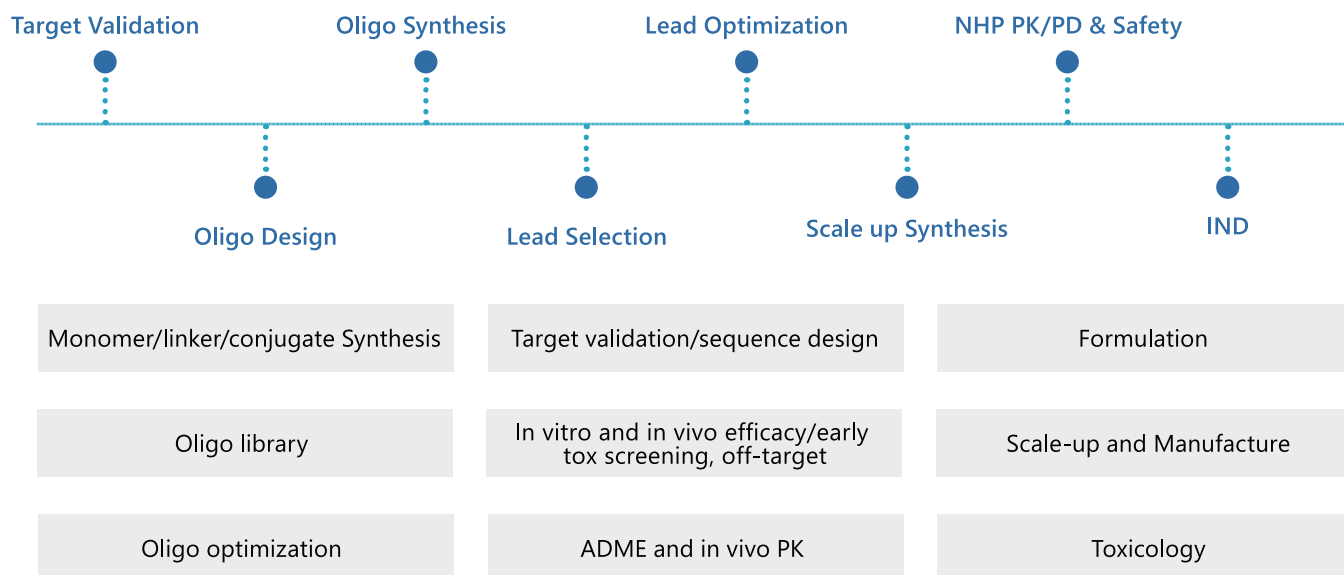
Protein analysis platforms

- Beckman COULTER Chemistry Analyzer, Cobas e601
- Flow cytometry: BD FACS Canton II, Aria III, Fortessa
- Bio-Plex 200 system, MESO SECTOR S600, AID ELISpot Reader, SpectraMax M2e, Envision, Biotek Synergy2, etc.
- Odyssey, LAS4000, WES system
- Histology platform: HistoCore PEARL, HistoCore Arcadia H - Heated Paraffin Embedding Station, etc

LC/MS platforms

- Small and large molecules
- Internal and external analytes

WuXi AppTec oligonucleotide capabilities



About WuXi AppTec


As a global company with operations across Asia, Europe, and North America, WuXi AppTec provides a broad portfolio of R&D and manufacturing services that enable the global pharmaceutical and healthcare industry to advance discoveries and deliver ground breaking treatments to patients. Through its unique business models, WuXi AppTec's integrated, end-to-end services include chemistry drug CRDMO (Contract Research, Development and Manufacturing Organization), biology discovery, preclinical testing and clinical research services, and cell and gene therapies CTDMO (Contract Testing, Development and Manufacturing Organization), helping customers improve the productivity of advancing healthcare products through cost-effective and efficient solutions.

About WuXi Biology

A full spectrum of biology services and solutions, supporting stand-alone and integrated projects, from target discovery to candidate selection and into the clinic.

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