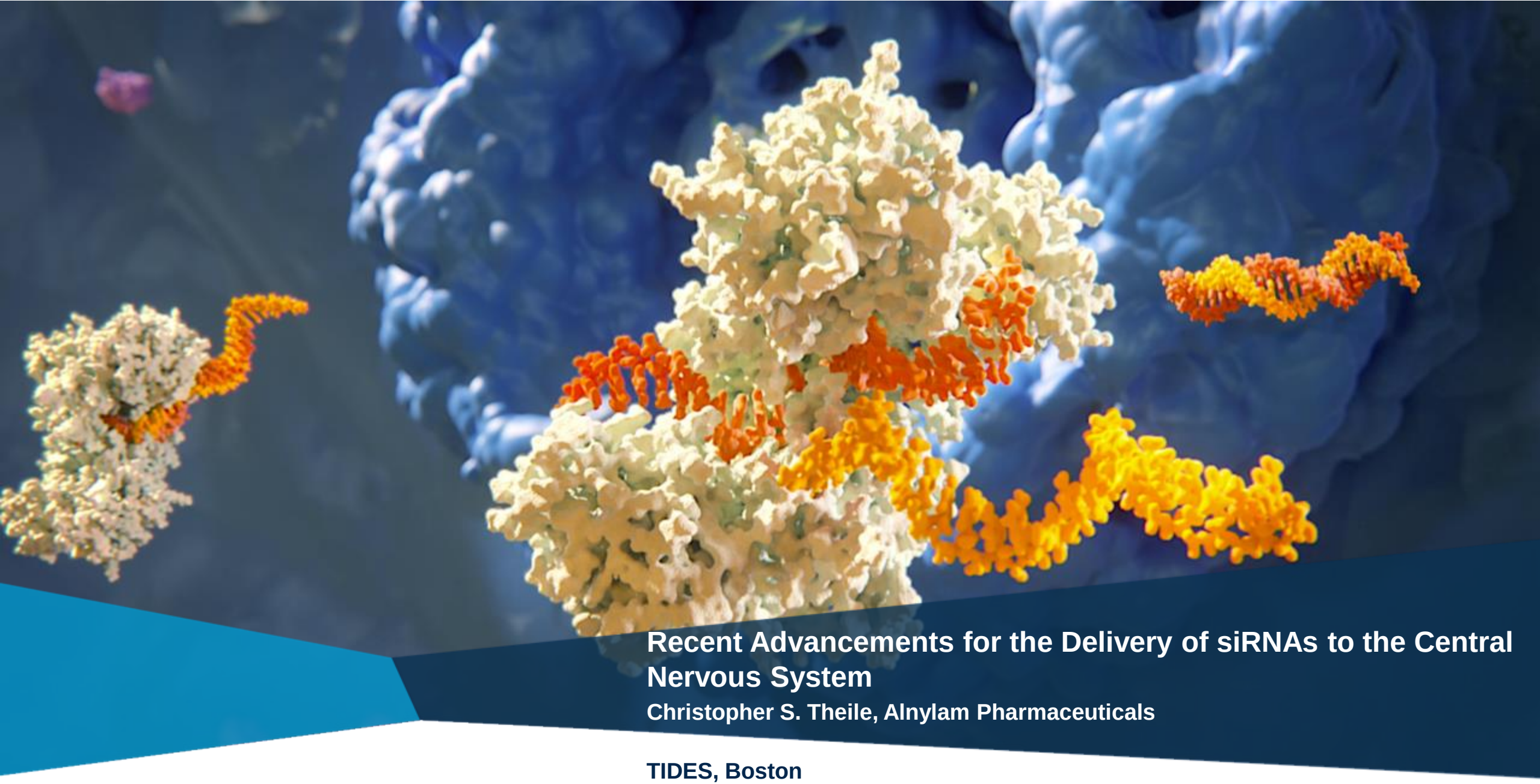


Recent Advancements for the Delivery of siRNAs to the Central Nervous System

Christopher S. Theile, Alnylam Pharmaceuticals

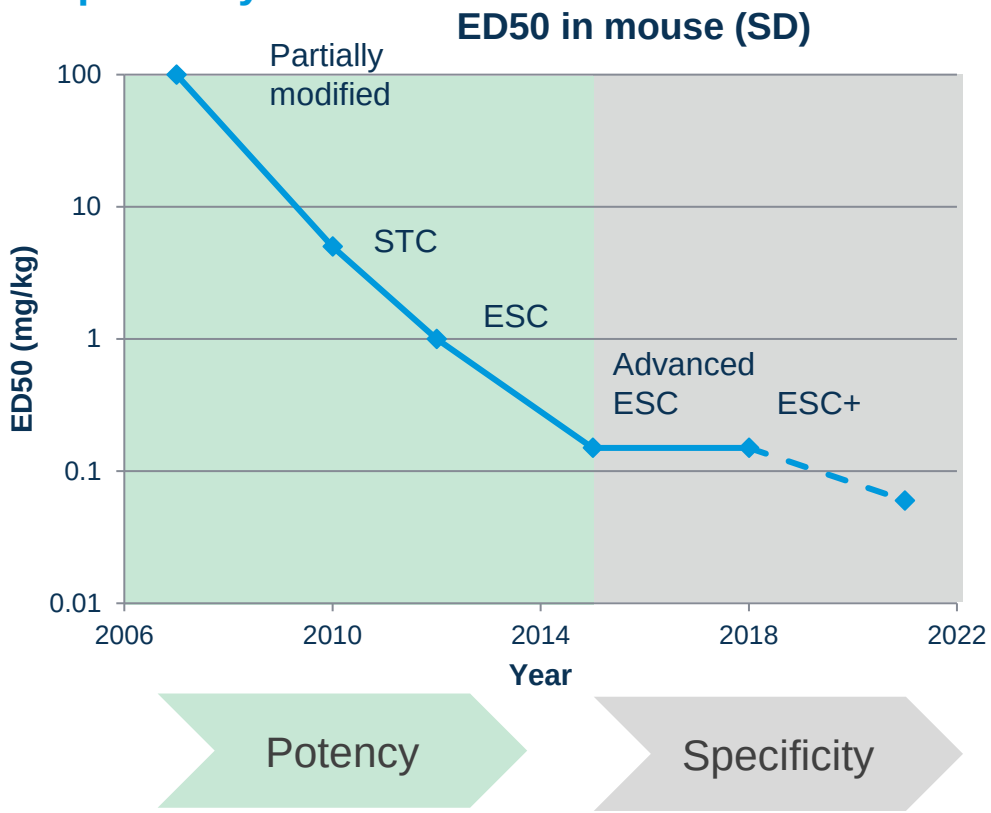
TIDES, Boston

May 11th, 2022

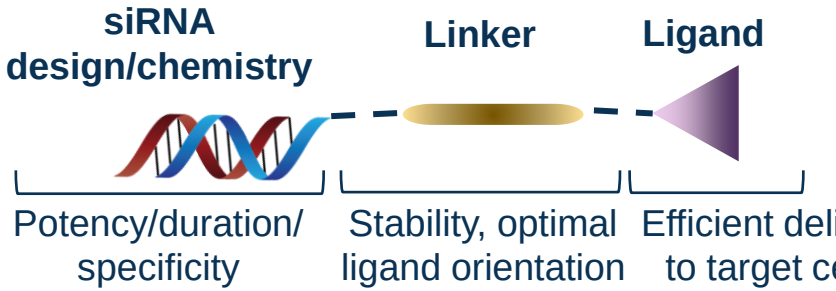
Alnylam@20

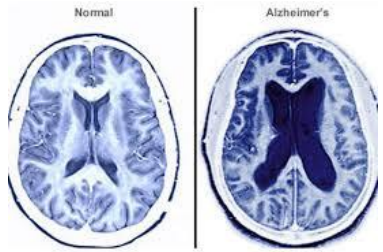
Advancements in Conjugate-Based Delivery Serve as a Blueprint for Extrahepatic Applications

Evolution of conjugate design with improved potency and specificity



- **> 10 GalNAc-siRNAs with human PoC; 3 approved so far**





Investigational RNAi therapeutics for CNS

Investigational RNAi Therapeutics for CNS Diseases

Devastating diseases with enormous burden and unmet need

Many dominantly inherited neurodegenerative diseases:

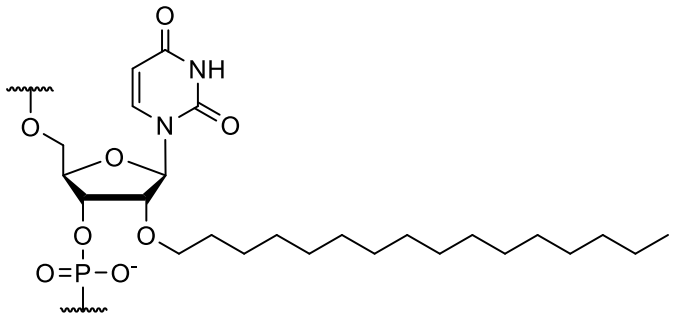
- Alzheimer's disease
- Amyotrophic lateral sclerosis (ALS)
- Cerebral amyloid angiopathy
- Frontotemporal dementia
- Huntington's disease
- Multi-system atrophy
- Parkinson's disease
- Spinocerebellar ataxia

A large number of genetically validated targets are known but few disease modifying therapies for these devastating, life threatening disorders

RNAi therapeutics directed to disease-causing, CNS-expressed genes represent an opportunity to address diseases with some of the greatest unmet need

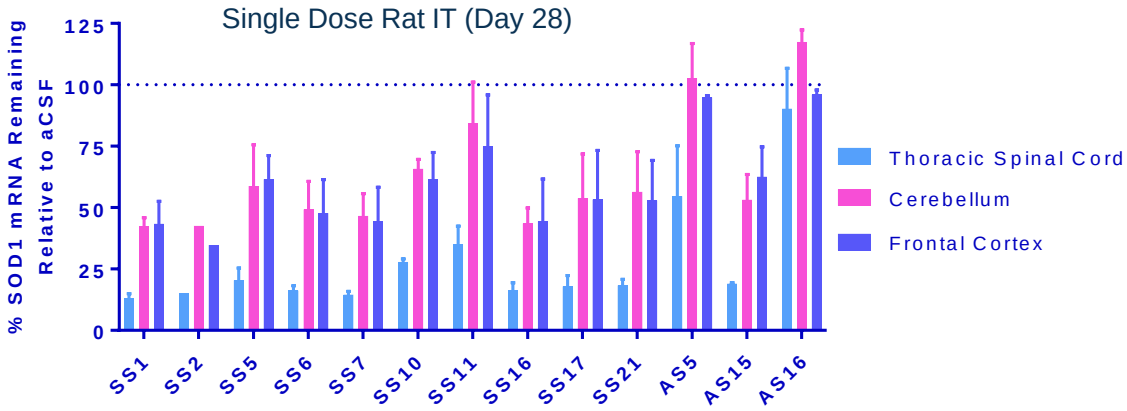
Conjugation of 2'-*O*-palmityl (C16) to siRNAs Enables Robust and Durable Target Knockdown in the Rat CNS

Optimization of siRNA lipophile, position and design chemistry for CNS delivery

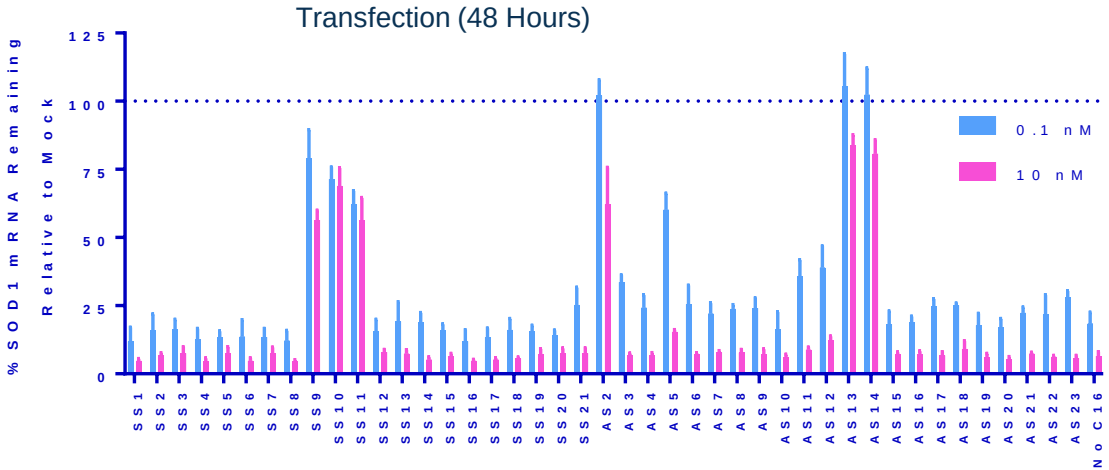


2'-*O*-palmityl Uridine: All nucleobases amenable to C16 modification

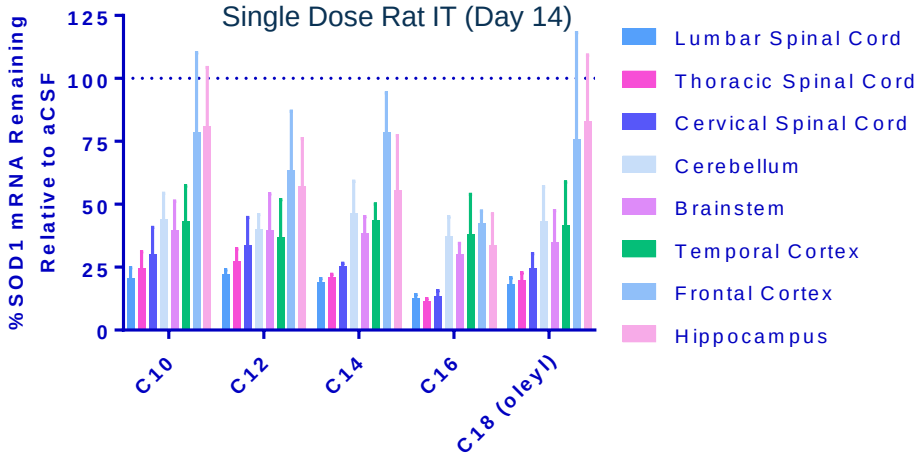
Optimal position for C16 benefit *in vivo*



Single nt walk with C16: Impact on inherent RNAi activity

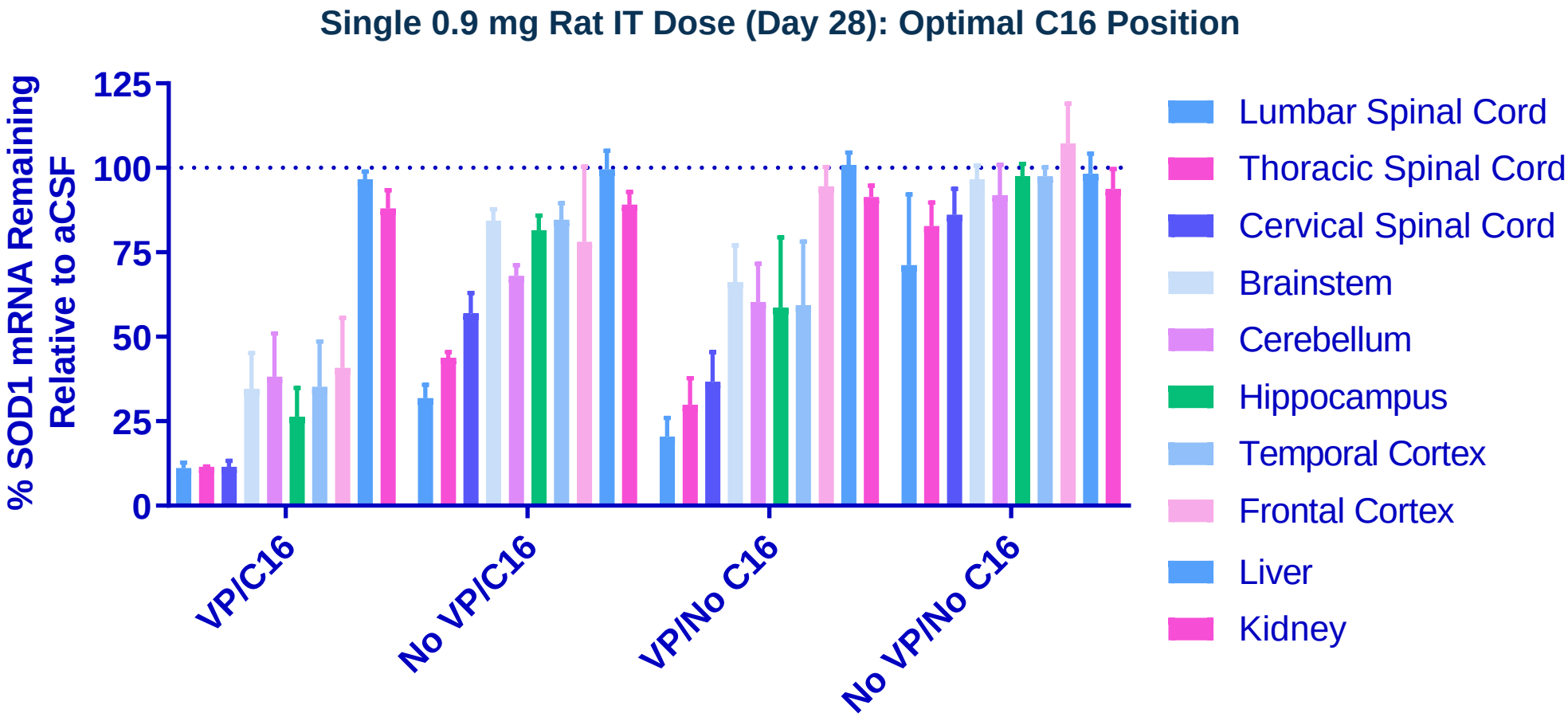


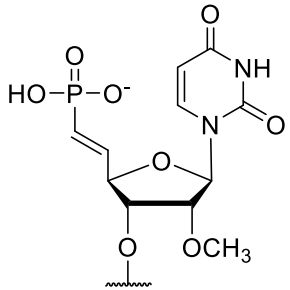
Comparison of lipophiles



Nature Biotechnology 2022, In press

Greatest Potency Across Rat CNS with siRNA Combining Both 2'-C16 and 5'-VP: a Stable Phosphate Mimic





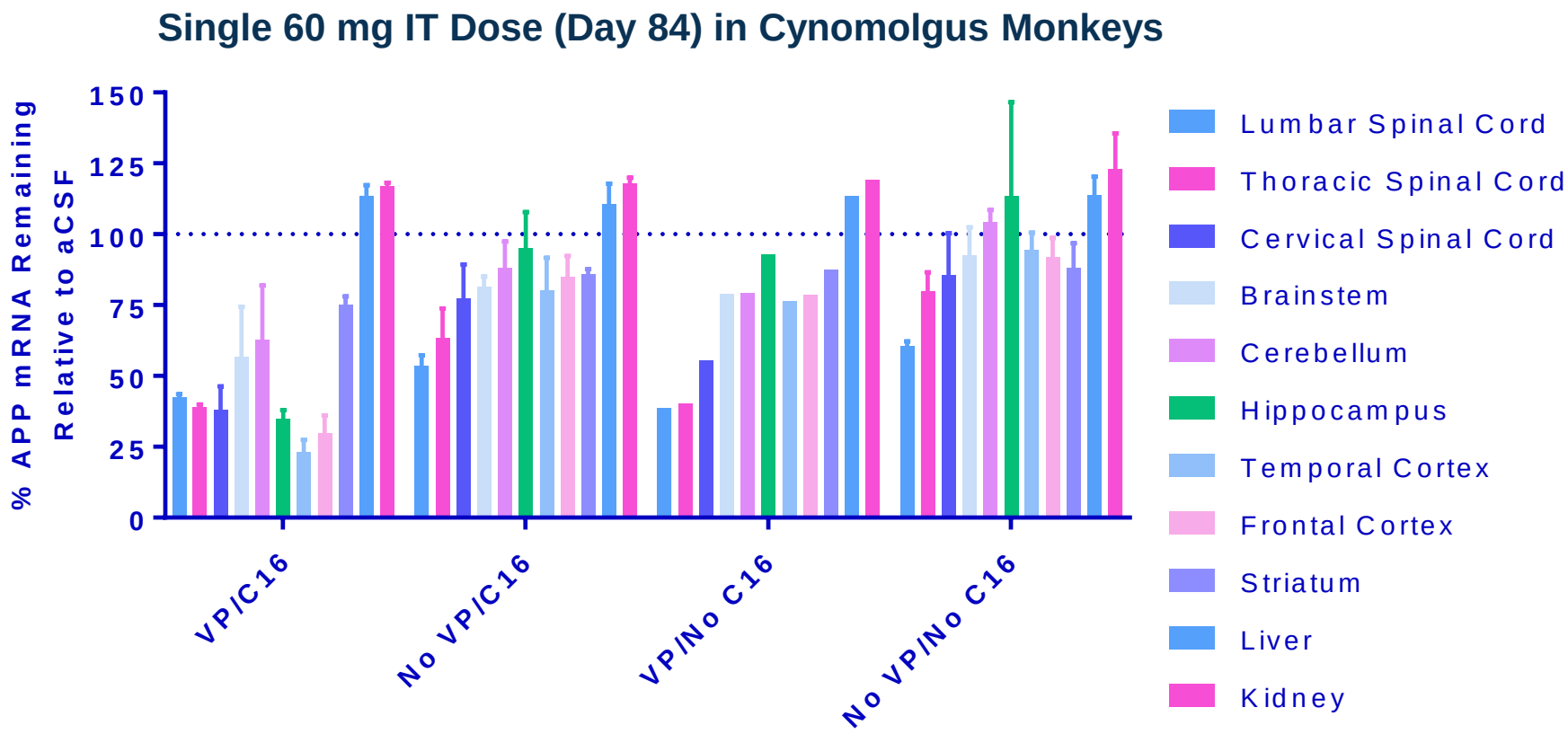
5'-(*E*)-Vinylphosphonate (VP)

ChemBioChem. 2016;17:985
Bioorg Med Chem Lett.2016; 26:2817

Nucleic Acids Res.2017; 45:3528.
Tetrahedron. 2018; 74: 6182.

Nature Biotechnology 2022, In press

Potency benefit of 2'-C16 and 5’-VP Translates to Higher Species



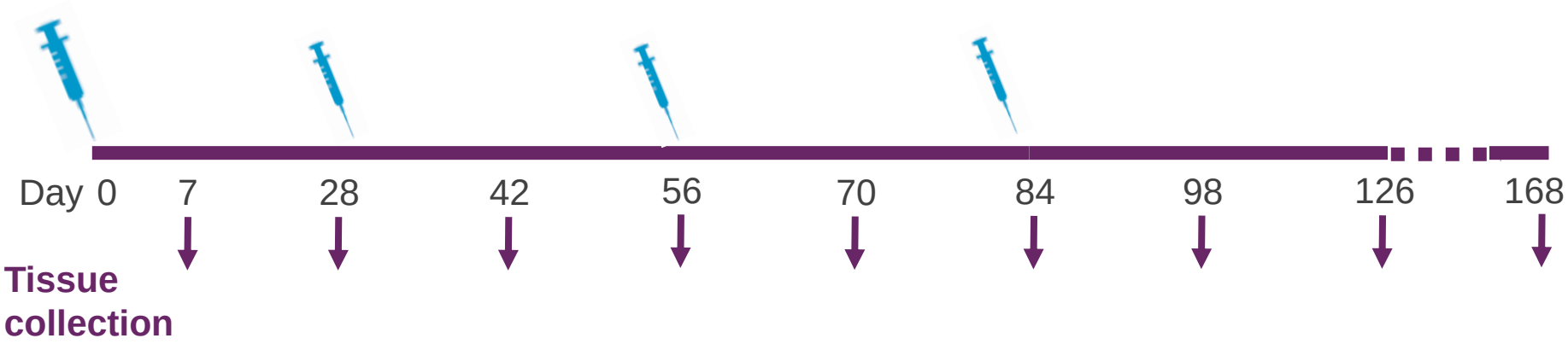
Nature Biotechnology 2022, In press

Potency and Durability of Intrathecal Dosed Optimized siRNA Conjugates

Single and multi-dose response in rat

siRNAs targeting SOD1 in single dose or dose response

- Single siRNA conjugate doses of 0.9 mg, 0.3 mg, 0.07 mg
- Multidose arm - 0.3 mg monthly × 5
- Time points through 6 months for SOD1



Tissues: Spinal cord: **Lumbar, thoracic and cervical**

Brain: **prefrontal cortex, cerebellum and remaining brain**

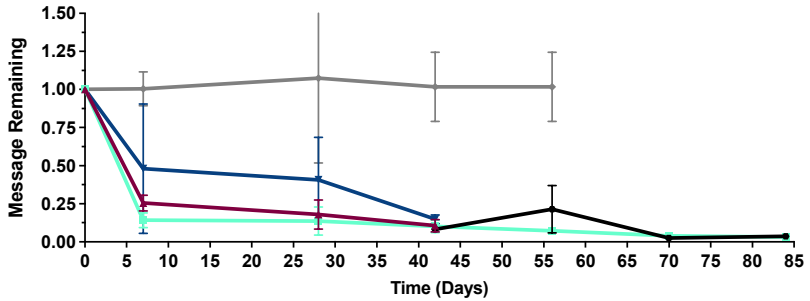
Fluids: **CSF and plasma**

Assays: **mRNA, tissue siRNA levels, Histology**

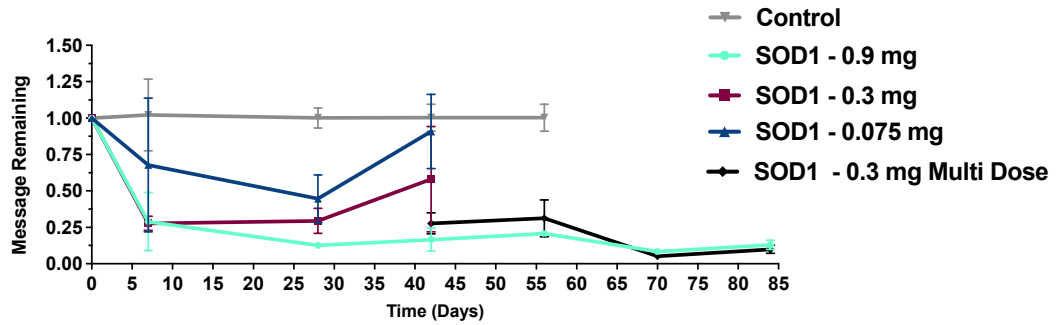
Robust and Durable Silencing Demonstrated Following a Single IT Dose

Silencing of SOD1 following a single or multiple IT doses

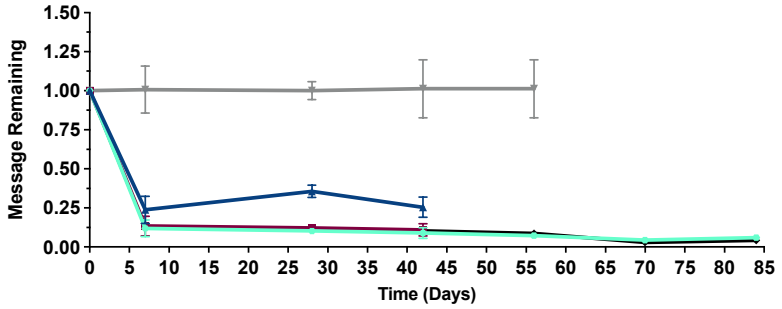
Lumbar



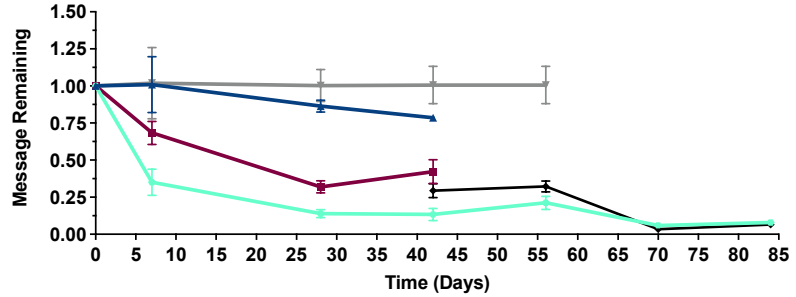
Cerebellum



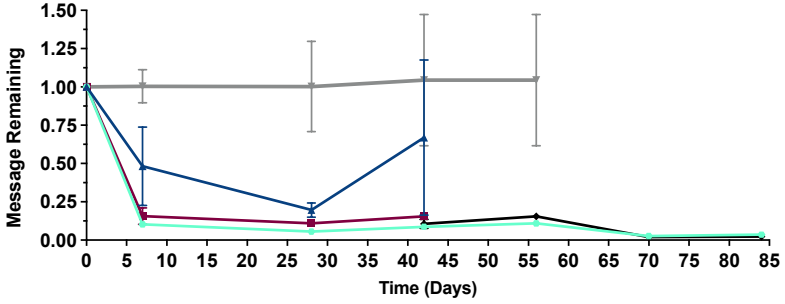
Thoracic



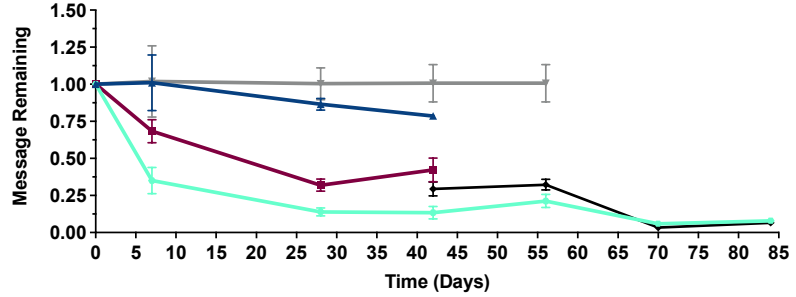
Frontal Cortex



Cervical

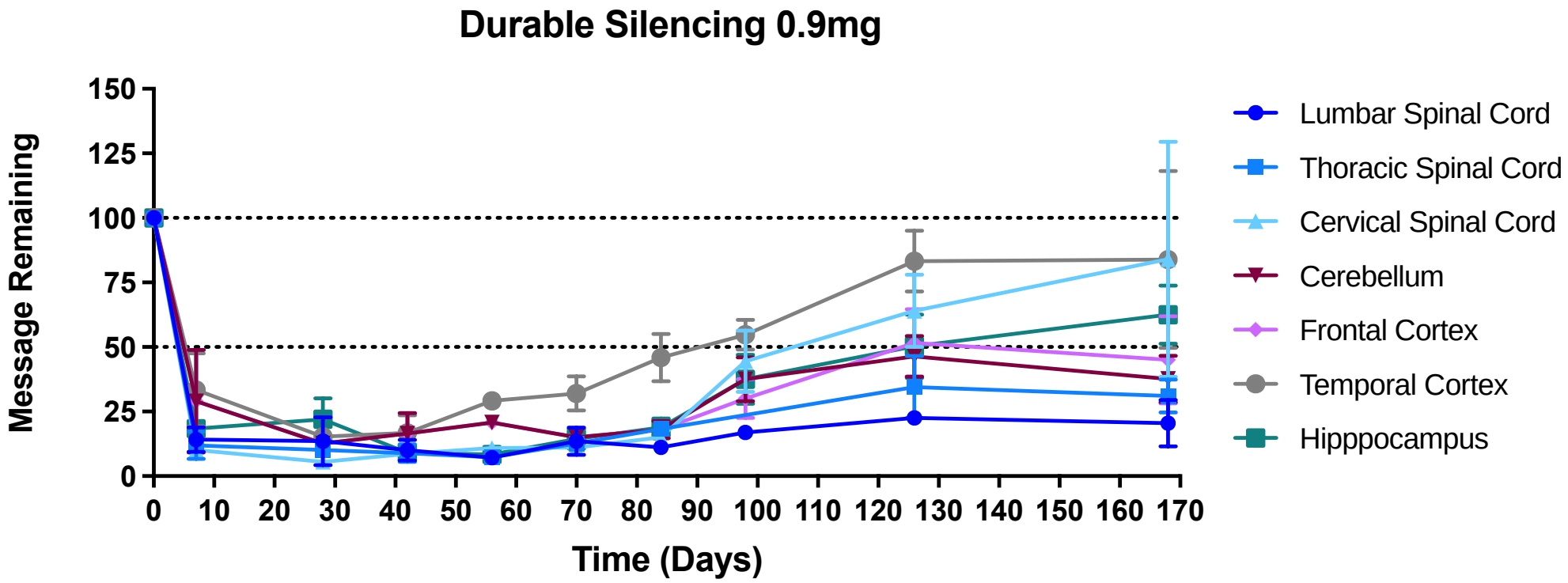


Temporal Cortex



Robust Silencing of *SOD1* Throughout the Brain Post Single IT-Dose

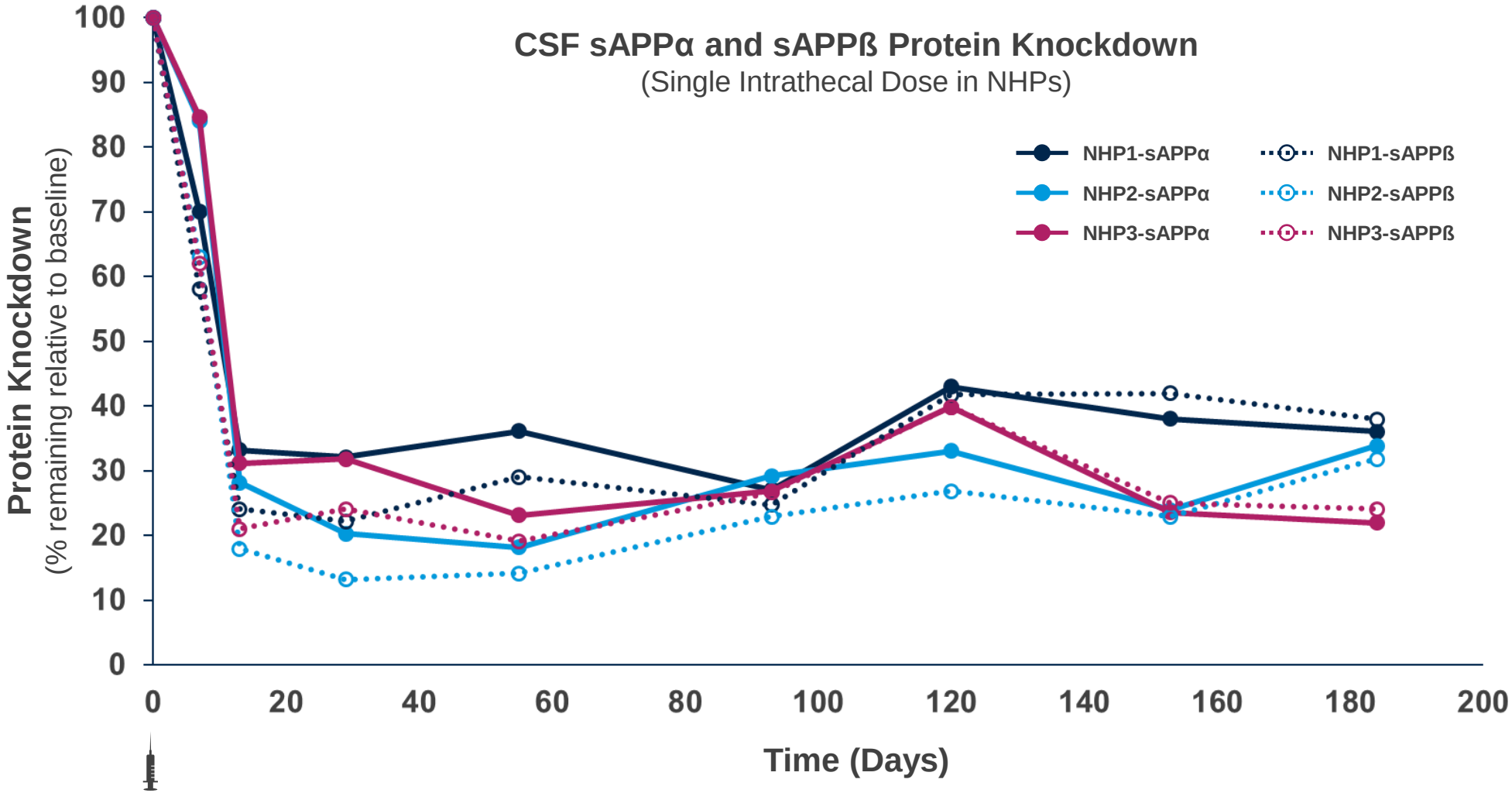
Intrathecal delivery of siRNA provides durable knockdown throughout CNS



Consistent lowering across animals in most regions of the brain

Highly Durable Amyloid Precursor Protein (APP) Knockdown in NHP

Single Intrathecal Dose of ALN-APP Supports Bi-Annual or Less Frequent Regimen



REGENERON®

Using Radio Imaging to Assess Distribution of siRNA in the CNS and Periphery

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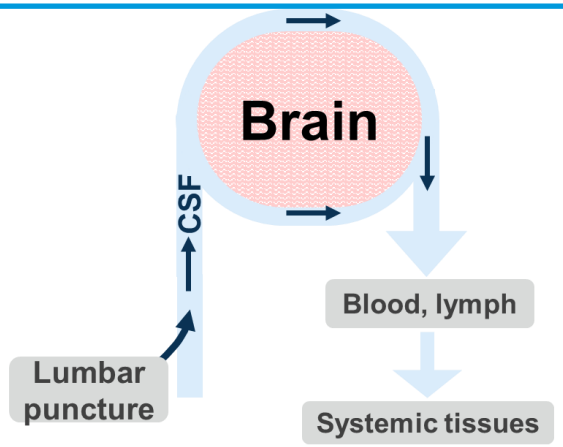
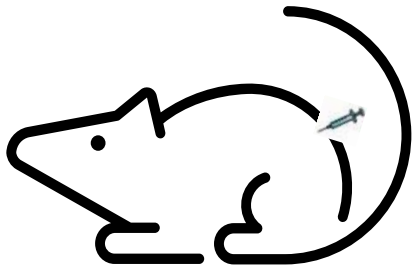
Rat CNS Distribution of Radiolabeled Conjugate-siRNA

IT drug delivery



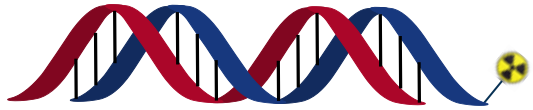
Study Groups

Three groups:
Lumbar IT catheter
N = 2 animals/siRNA
3 toolkit siRNAs

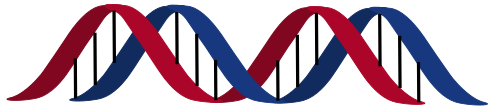


Dose Formulation

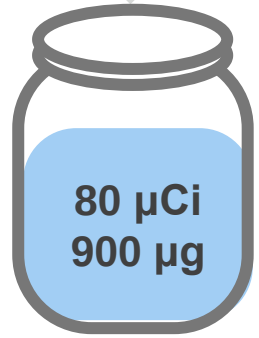
[^{111}Indium]-DTPA-conjugated siRNA



Unlabeled siRNA



Dosing (per animal)

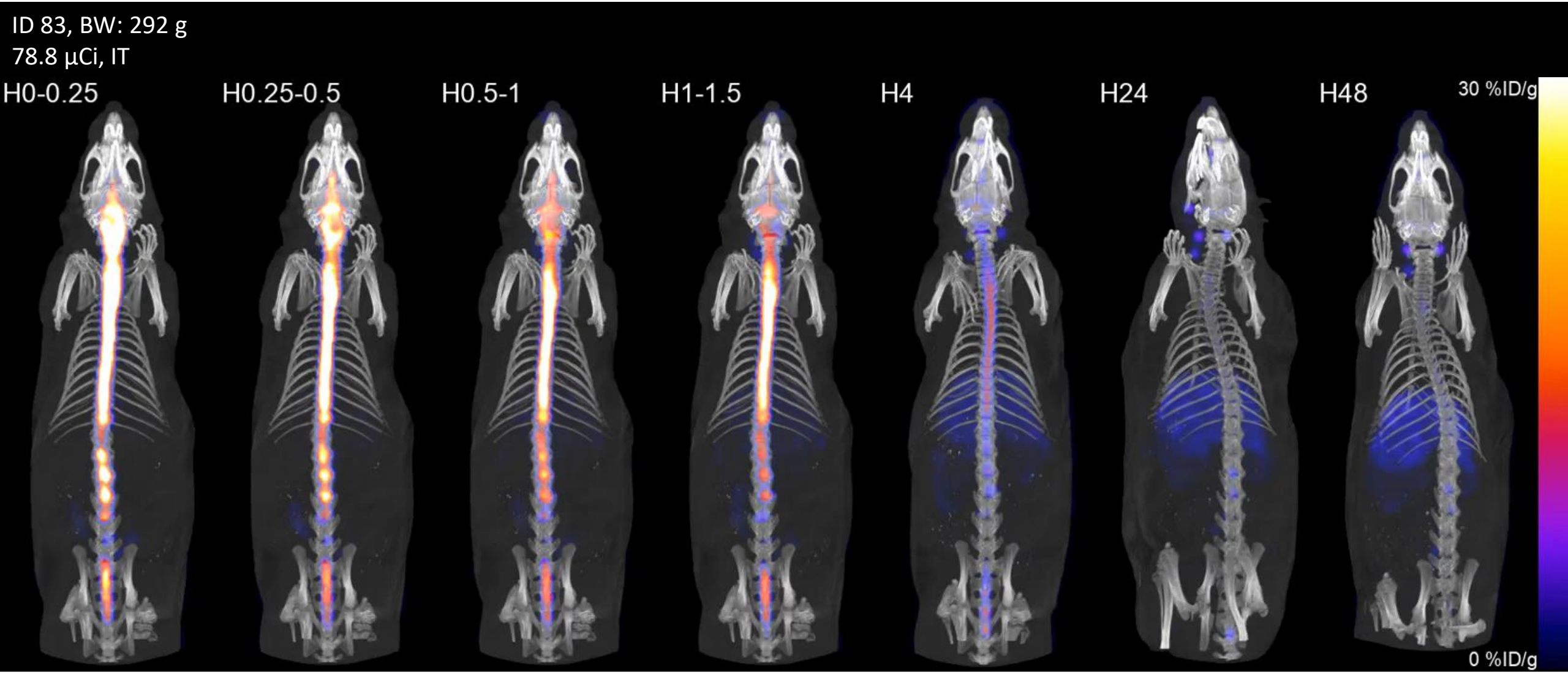


^{111}In $t_{1/2}$ = 2.8 days

SPECT/CT Imaging	Sampling	Radiological quantification
0-1.5 (dynamic), 4, 24 and 48 h	Whole-organ and blood gamma counting, and immunohistochemistry for siRNA	^{111}In whole-body uptake per timepoint, and uptake by 13 brain regions

Biodistribution of IT-Dosed ^{111}In-siRNA in Rodents

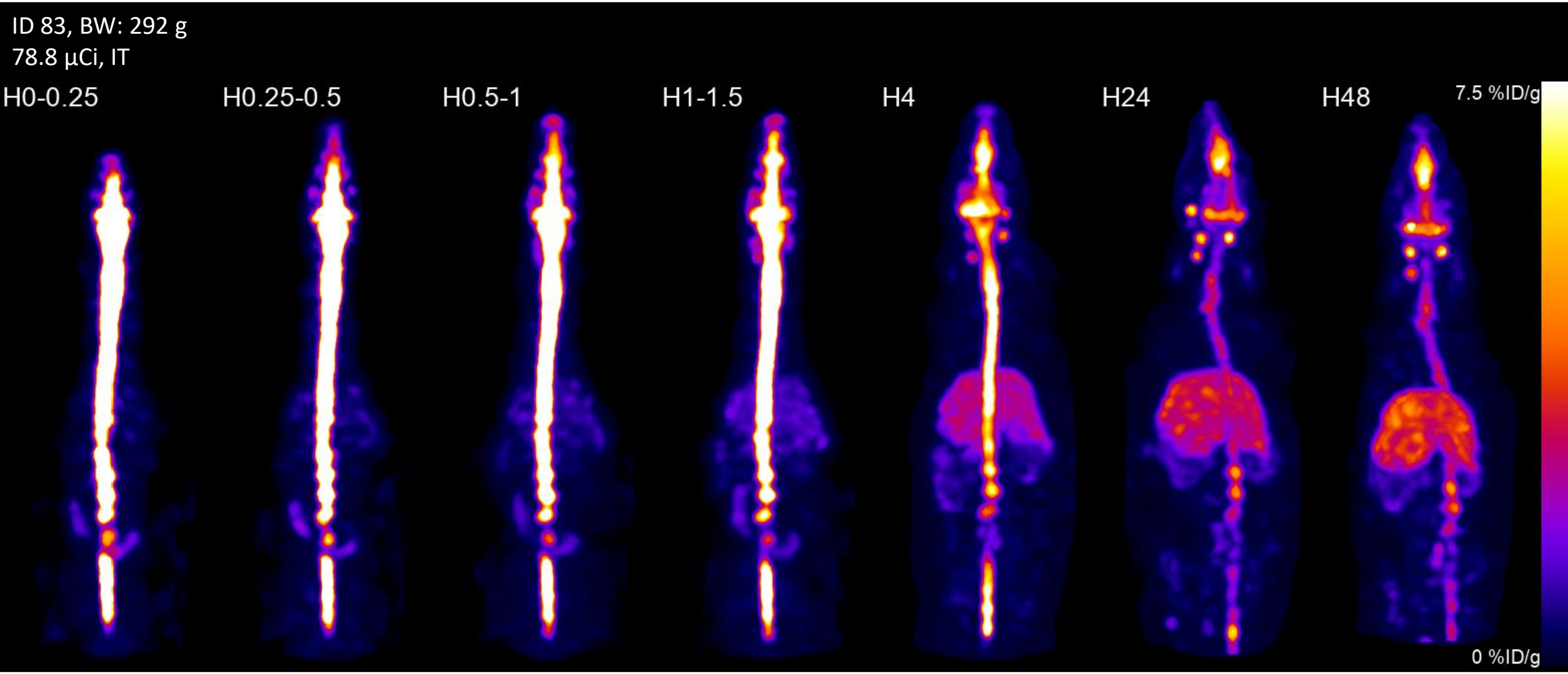
Co-registered SPECT/CT images facilitate anatomical orientation



MIP: Maximum intensity projections derived from projecting the voxel with the highest value on every view throughout the volume onto a 2D image.

Biodistribution of IT-Dosed ^{111}In-siRNA in Rodents

SPECT reveals rapid movement through CSF to brain (<1 h) followed by drainage to systemic circulation

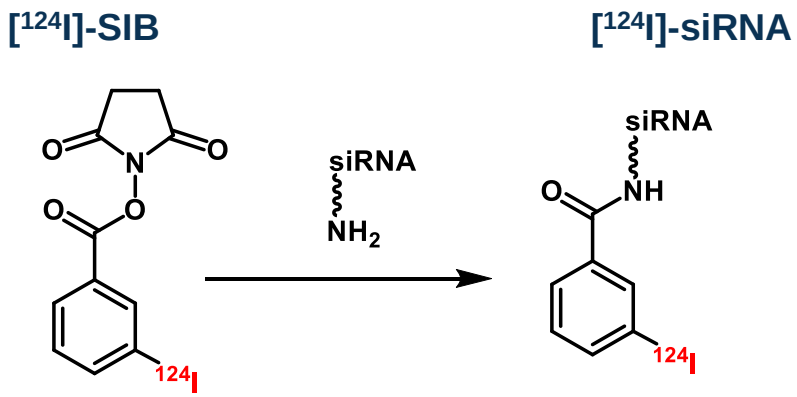


MIP: Maximum intensity projections derived from projecting the voxel with the highest value on every view throughout the volume onto a 2D image.

NHP CNS Distribution of Radiolabeled siRNAs: PET Imaging

Objective: Use higher resolution PET imaging to study the distribution of siRNAs in cynomolgus monkeys

[^{124}I]-SIB conjugation to amine



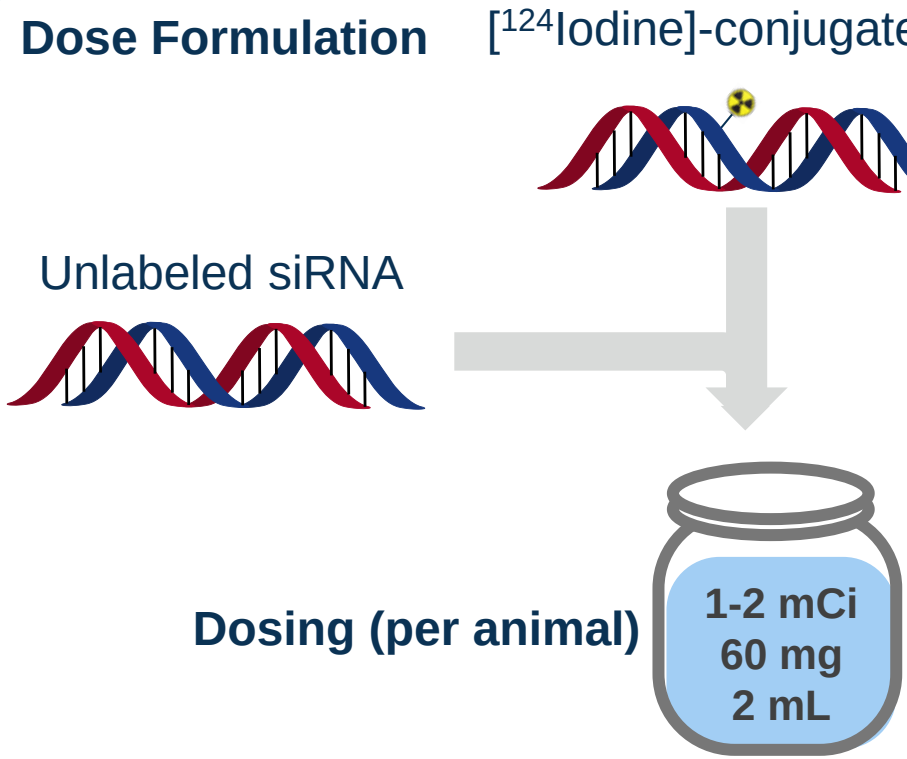
SIB = succinimidyl [^{124}I]-iodobenzoate

Iodine-124 (^{124}I)

- High energy, high specific-activity positron emitter
- $t_{1/2}$ = 4.18 days; ^{125}I-siRNA stability$_{aCSF}$ = 14 days

**Zalutsky 1988 Cancer Res 15, 1446-50; Chen 2014 Pharm Res 31, 2810-21.

Dose Formulation

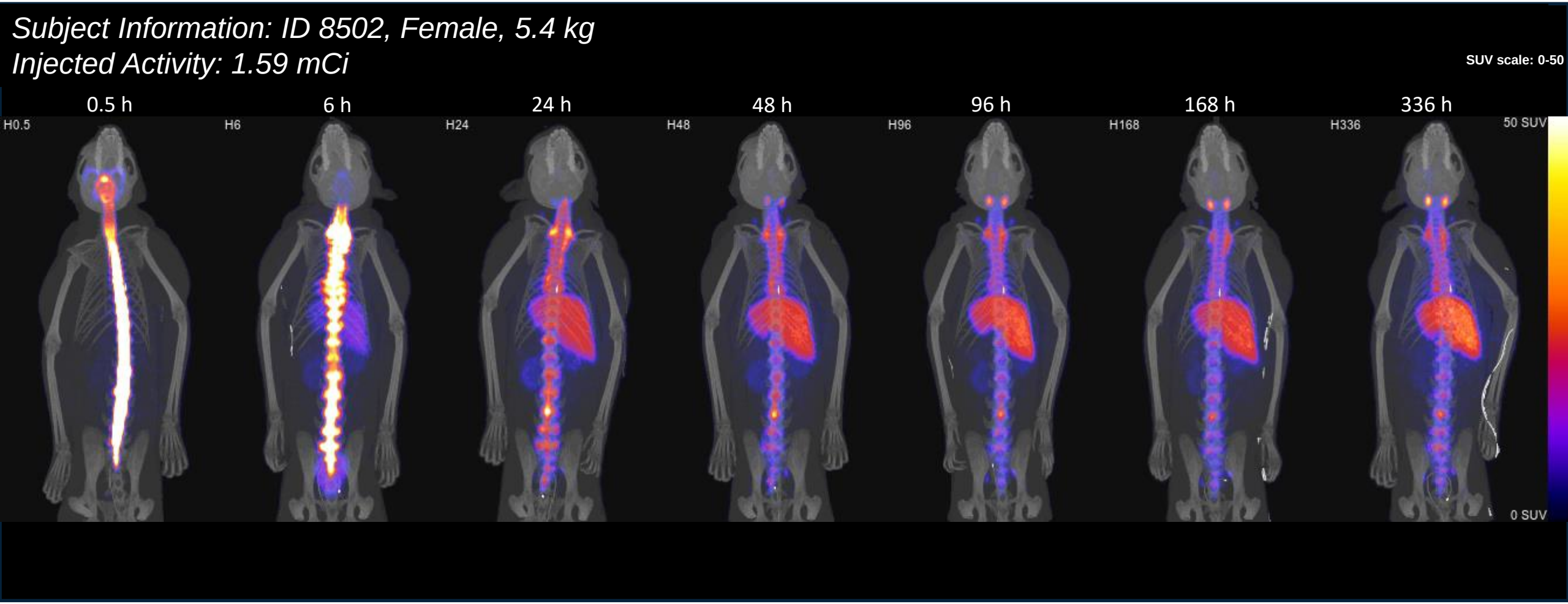


PET Imaging

0.5-1.5, 6, 24, 48, 96 h, 7 & 14 days

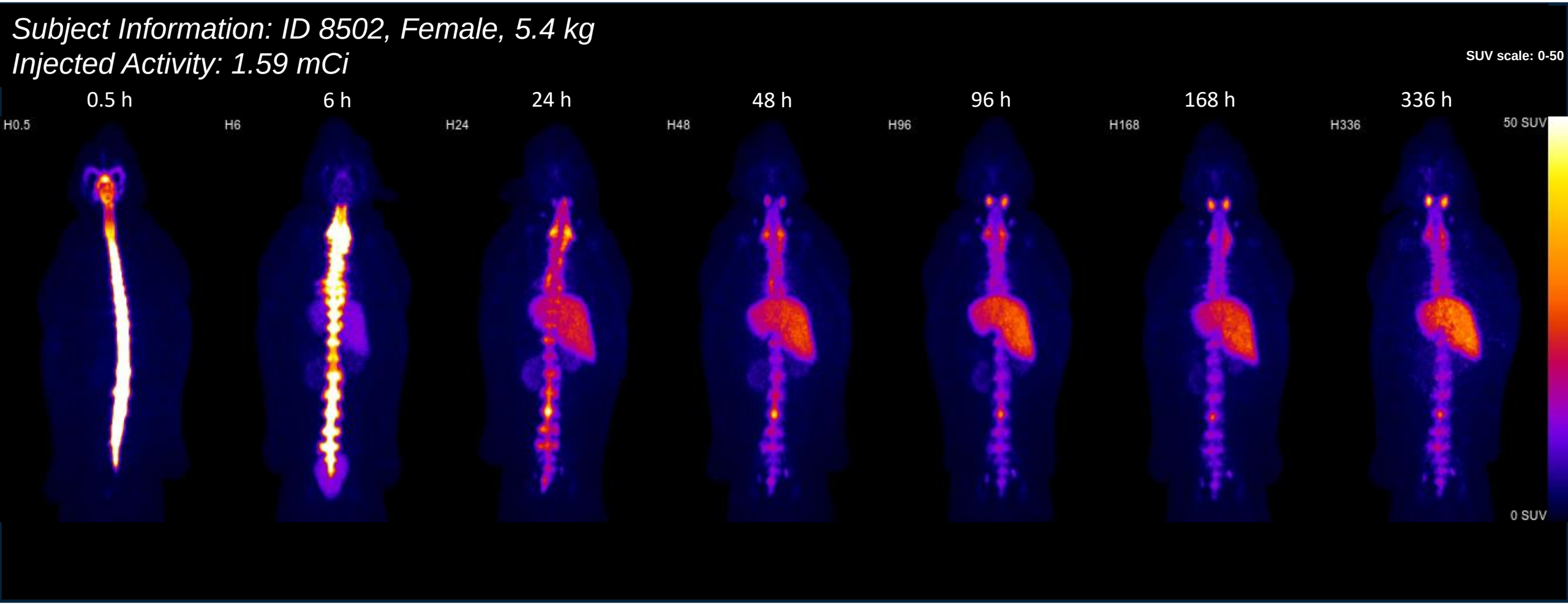
Representative Images Following IT Dosing

Co-registered PET/CT images facilitate anatomical orientation



Representative Images Following IT Dosing

PET reveals rapid movement through CSF to brain (<1h) followed by drainage to systemic circulation

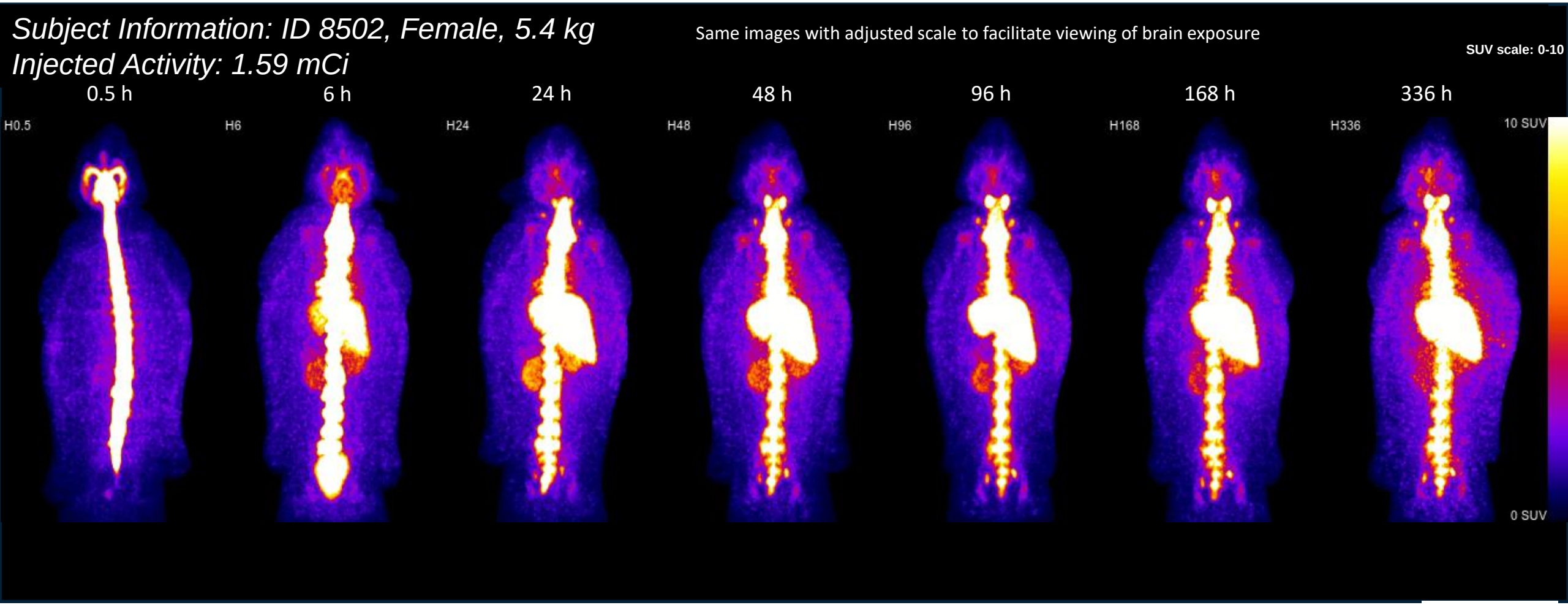


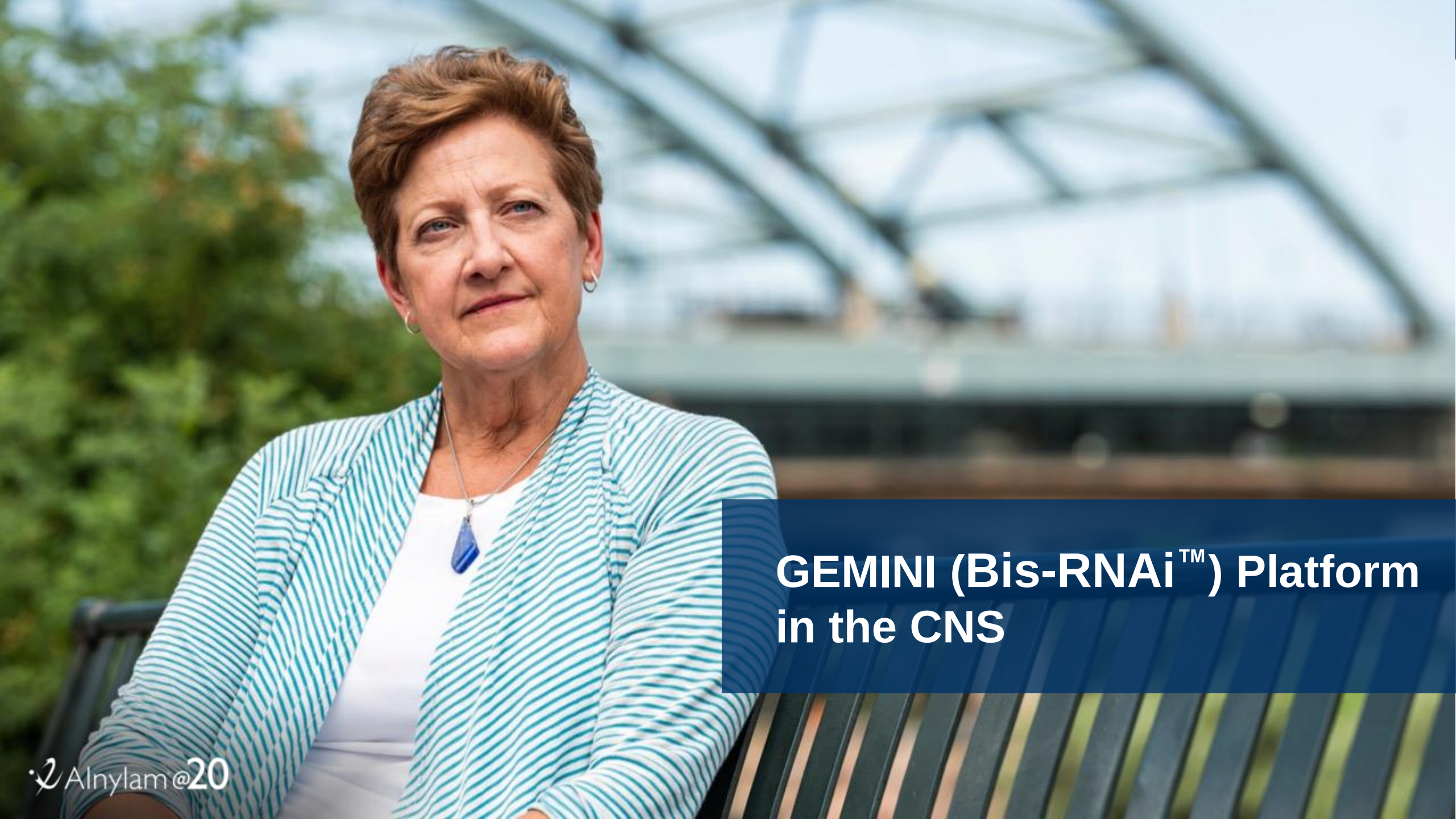
Representative Images Following IT Dosing

PET at higher-sensitivity scaling shows wide distribution across the body, yet long retention within the CNS

Subject Information: ID 8502, Female, 5.4 kg
Injected Activity: 1.59 mCi

Same images with adjusted scale to facilitate viewing of brain exposure





GEMINI (Bis-RNAi™) Platform in the CNS

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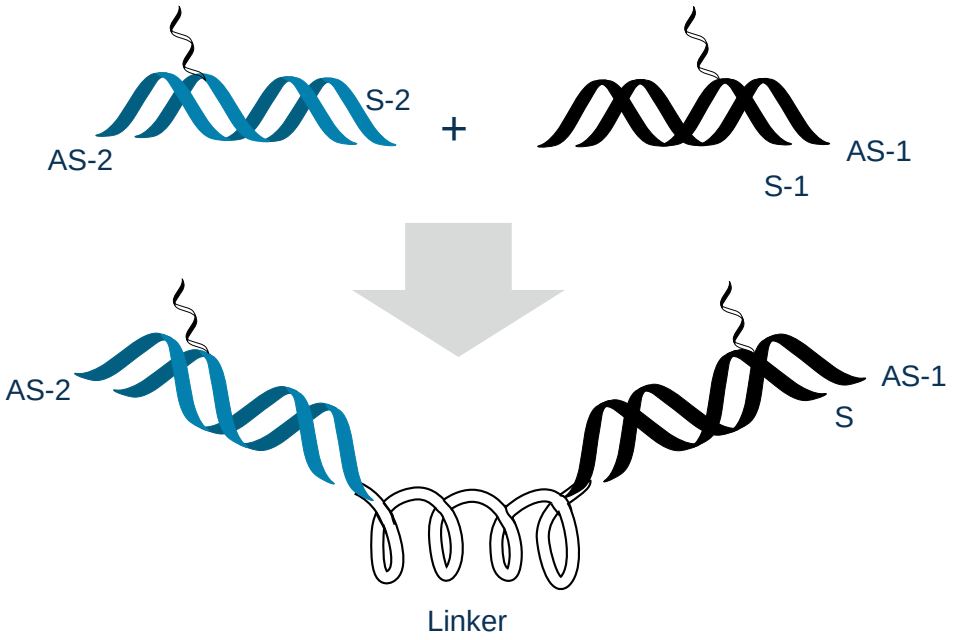
GEMINI Platform

Objective

- Effectively combine conjugate siRNAs for the simultaneous silencing of two transcripts or same (e.g. for viruses) using single chemical entity



Three-strand 2xC16 CNS design

CNS Gemini Study 1: Mouse ICV

Objective: to evaluate the efficacy of multiple bis siRNA with varying nucleotide linkers after a single intracerebroventricular dose administration in C57BL/6 mice

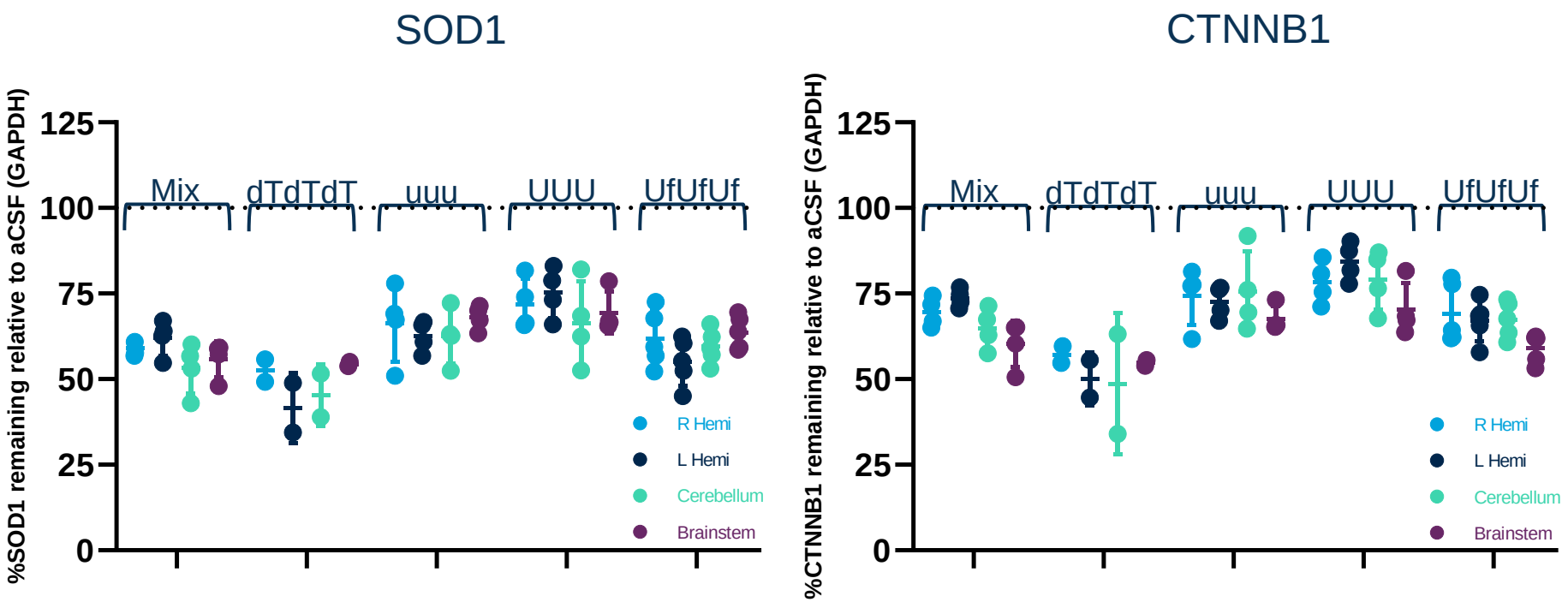
Group #	Treatment	Linker	n	Time Point	ICV Dose (ug) in 5ul	Readouts
1	aCSF	-	4	D21	--	qPCR: right hemisphere, left hemisphere, cerebellum, brainstem
2	Duplex Mixture	-			50ug + 50ug	
3	Multiplex 1	dTdTdT (DNA)			100ug	
4	Multiplex 2	uuu (2’ OMe)				
5	Multiplex 3	UUU (RNA)				
6	Multiplex 4	UfUfUf (2’ F)				

- Predicted metabolic very stable (stable in plasma, liver cytosol and tritosome)
- Predicted metabolic medium stable (2' F linker cytosol cleaved in liver, dTdTdT cleaved in cytosol and tritosome)
- Predicted metabolic unstable (rapidly cleaved in plasma)

Comparison of Linker Chemistry Following ICV Administration

Best activity seen with the DNA (dTdTdT) linker

- DNA (dTdTdT) linker performed best
  - 50%+ KD of mSOD1 and mCTNNB1
- DNA>Mix>2'F>2'OMe>RNA



Summary

- Advancements in siRNA chemistry together with improvements in mechanistic understanding have been the predominant drivers behind the evolution of the conjugate platform technology
- Conjugation of 2'-*O*-palmityl (C16) to siRNAs along with 5'-VP enables safe, robust and durable target knockdown in the CNS of preclinical species
- Alnylam has developed an understanding of siRNA delivery, distribution and activity throughout the CNS across preclinical species.
  - siRNA conjugates are active across CNS regions
  - Radiolabeled studies show distribution of the siRNA throughout the CNS following IT administration through the primary CSF flow routes within 30 minutes
  - Dose clears quickly, likely due to systemic drainage, with less than 5% remaining in the CNS at 48 hrs
  - Rapid and substantial tissue peripheral distribution (highest concentration in liver)
- GEMINI platform can be used to target two separate transcripts in the CNS with a single drug entity