Michael Monine

Prediction of PK/PD for Intrathecally-Administered Antisense Oligonucleotides

ROSA Webinar Series

April 17, 2024

Biogen

Disclosure

Michael Monine is an employee of Biogen and holding shares of Biogen

What Are Antisense Oligonucleotides (ASOs)?

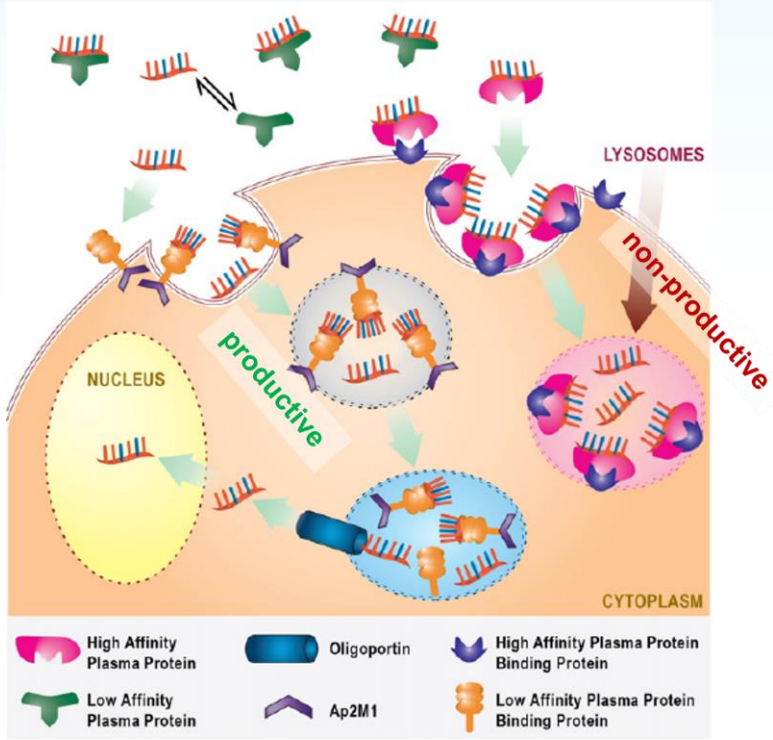
- Short, single-stranded, synthetic nucleic acid chains of 18-20 bps in length; MW range is 6-8 kDa
- 2′-MOE ASOs are hydrophilic, highly water soluble, and poly-anionic
- Designed to bind to RNA based on complementary base pairing to modify protein expression or modify splicing

CNS-targeting ASOs

- Both size and charge for most ASOs prevents distribution across Blood-Brain Barrier (BBB)
- Therefore, ASOs must be administered directly into the central nervous system (CNS) space
- The intrathecal (IT) route is often used to provide a substantial distribution advantage to spinal cord and brain tissues

Mechanisms of action for ASOs

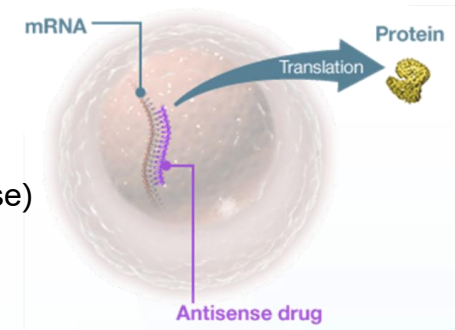
- ASO trafficking within cells may occur through multiple pathways involving **productive** and **non-productive** uptake resulting in varying levels of pharmacodynamic activity
- Once delivered to the target tissue, ASOs need to escape endosomes to engage with the intracellular target (e.g., RNA)
- The non-productive pathway may account for the majority of ASOs accumulating in cells



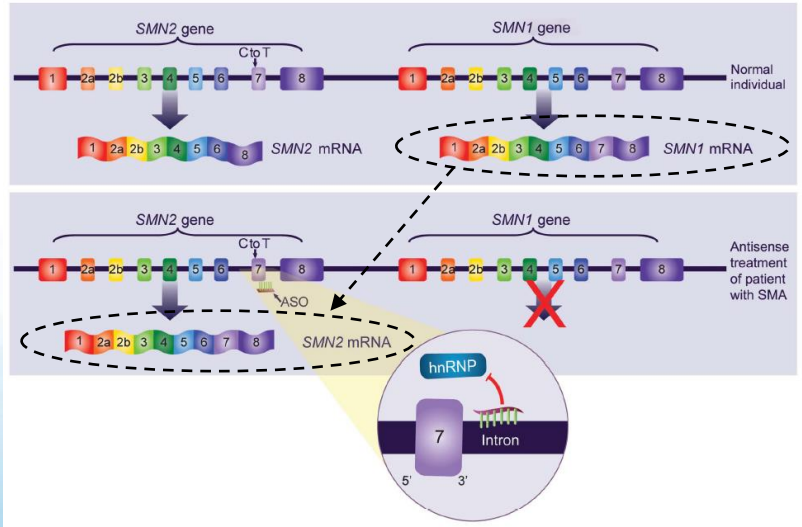
Juliano RL. Nucleic Acid Ther (2018) 28:166–177
Gao et al. Expert Opin Drug Metab Toxicol. (2023) 19:979-990
Koller et al. Nucleic Acids Research (2011) 39: 4795–4807
Bennet, et al. *Annu. Rev. Neurosci*. (2019) 42:385–406
Rigo, et al. *The Journal of Cell Biology* (2012) 199:21-25
Geary, et al. *Adv. Drug Deliv. Rev*. (2015) 87:45-51
Evers, et al. *Advanced Drug Delivery Reviews* (2015) 87:90-103

Examples of IT ASOs and their MoA:

Chimeric 2'-MOE/DNA ASOs ("gapmers")
Enzyme-dependent degradation of targeted mRNA
- *Tofersen* (ALS)
- *Tominersen* (Huntington's disease)
- *Viltolarsen* (Duchene muscular dystrophy)



Full 2'-MOE modified ASOs for splicing modulation
Increase of SMN protein expression by modulating splicing of the *SMN2* precursor mRNA
- *Nusinersen* (SMA)



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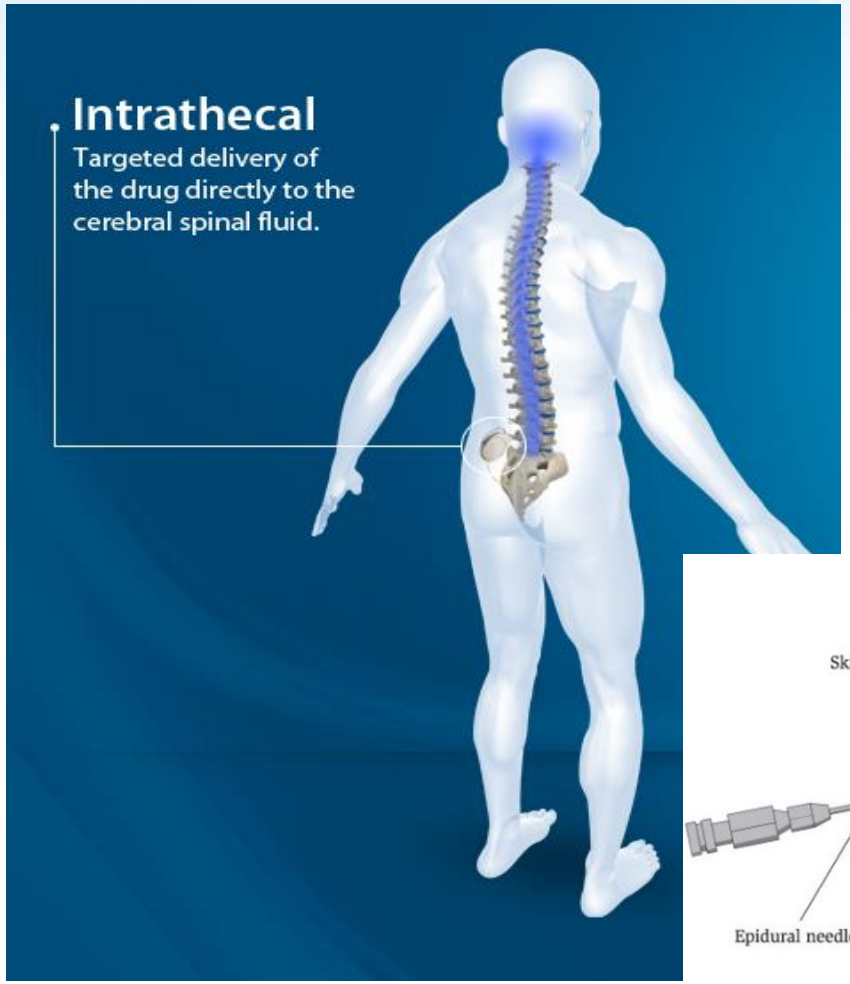
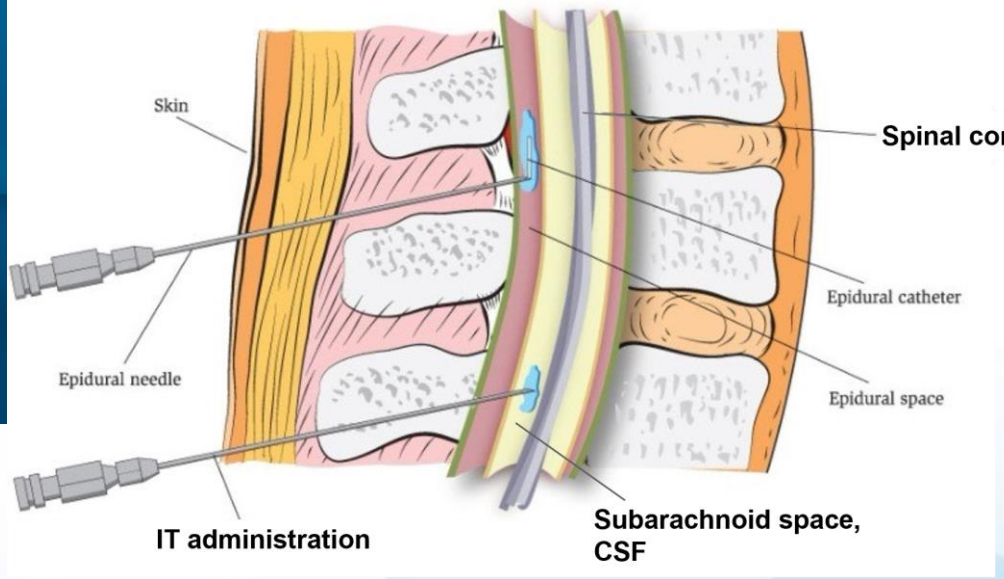
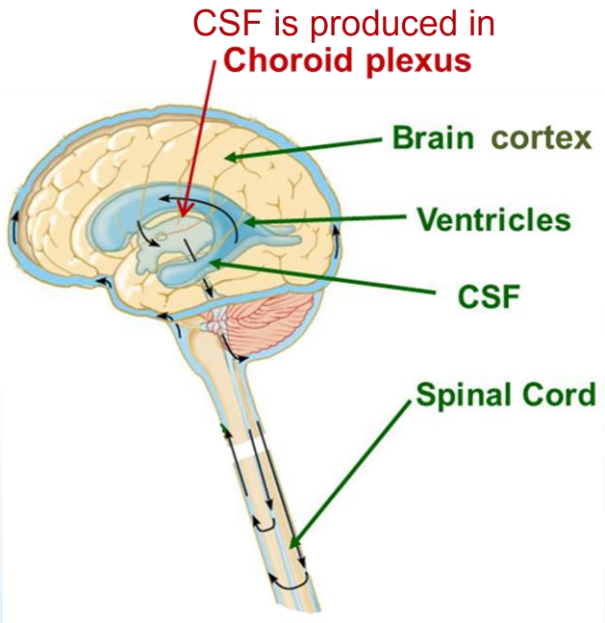
What is intrathecal (IT) administration?



- IT administration allows the drug to bypass BBB
- Cerebrospinal fluid (CSF) is not homogeneous (slowly stirred)
- Heartbeat and breathing rates modulate the frequency and magnitude of pressure oscillations in CSF
- Pressure caused by IT injection and slow CSF bulk movement contribute to the upward distribution of IT drugs
- Drugs can be drained from CNS into blood





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Summary of the CNS-targeted ASO therapeutics launched and under clinical development

Goto et al. Biopharm Drug Dispos 2023;44:26–47

Name	Modality	Indication	Target	Route of administration	Development stage	ClinicalTrials.gov identifier
Nusinersen	ASO	SMA	SMN2	IT	Launched	NCT02462579
Tofersen	ASO	ALS	SOD1	IT	Launched	NCT02623699
ION363	ASO	ALS	FUS	IT	Phase 3	NCT04768972
Zilganersen	ASO	AxD	GFAP	IT	Phase 3	NCT04849741
Tominersen	ASO	HD	HTT	IT	Phase 3	NCT03842969
IONIS-MAPTRx/ BIIB080	ASO	AD, FTD	MAPT	IT	Phase 2	NCT05399888
ION859/BIIB094	ASO	PD	LRRK2	IT	Phase 2	NCT03976349
STK-001	ASO	DS	SCN1A	IT	Phase 2	NCT04740476
GTX-102	ASO	AS	UBE3A-ATS	IT	Phase 1/2	NCT04259281
ION582	ASO	AS	UBE3A-ATS	IT	Phase 1/2	NCT05127226
WVE-003	ASO	HD	HTT	IT	Phase 1/2	NCT05032196
WVE-004	ASO	ALS, FTD	C9orf72	IT	Phase 1/2	NCT04931862
ION541/BIIB105	ASO	ALS	ATXN2	IT	Phase 1	NCT04494256
ION260/BIIB132	ASO	SAT3	ATXN3	IT	Phase 1	NCT05160558
ION464/BIIB101	ASO	MSA, PD	SNCA	IT	Phase 1	NCT04165486
ALN-APP	siRNA	AD, CAA	APP	IT	Phase 1	NCT05231785

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein; AS, Angelman syndrome; ASO, antisense oligonucleotide; ATXN, ataxin; AxD, Alexander disease; C9orf72, chromosome 9 open reading frame 72; CAA, cerebral amyloid angiopathy; CNS, central nervous system; DS, Dravet syndrome; FTD, frontotemporal degeneration; FUS, fused in sarcoma; GFAP, glial fibrillary acidic protein; HD, Huntington's disease; HTT, huntingtin; IT, intrathecal; LRRK, leucine-rich repeat kinase; MAPT, microtubule-associated protein tau; MSA, multiple system atrophy; OT, oligonucleotide therapeutics; PD, Parkinson's disease; SAT3, spinocerebellar ataxia type 3; SCN1A, sodium voltage-gated channel alpha subunit 1; SMA, spinal muscular atrophy; SMN2, survival motor neuron 2; SNCA, synuclein alpha; SOD1, superoxide dismutase 1; UBE3A-ATS, ubiquitin protein ligase E3A-antisense transcript.

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Systemic pharmacology of ASOs

Systemic clearance

- Both IT and SC routes of administration cause rapid absorption of ASOs into the systemic circulation
- Mean plasma concentrations generally decrease ≥90% from Cmax by 24 hours
  - Typically, no accumulation in Cmax or AUC after repeated doses (e.g., monthly)
- ASOs in most chemical classes are metabolized by ubiquitous nucleases
- ASOs are highly bound to plasma proteins (> 95%) and distribute primarily to the liver followed by the kidneys
  - Distributions to other systemic organs/tissues are minimal
- Systemic clearance occurs primarily due to either metabolism in blood or excretion in urine

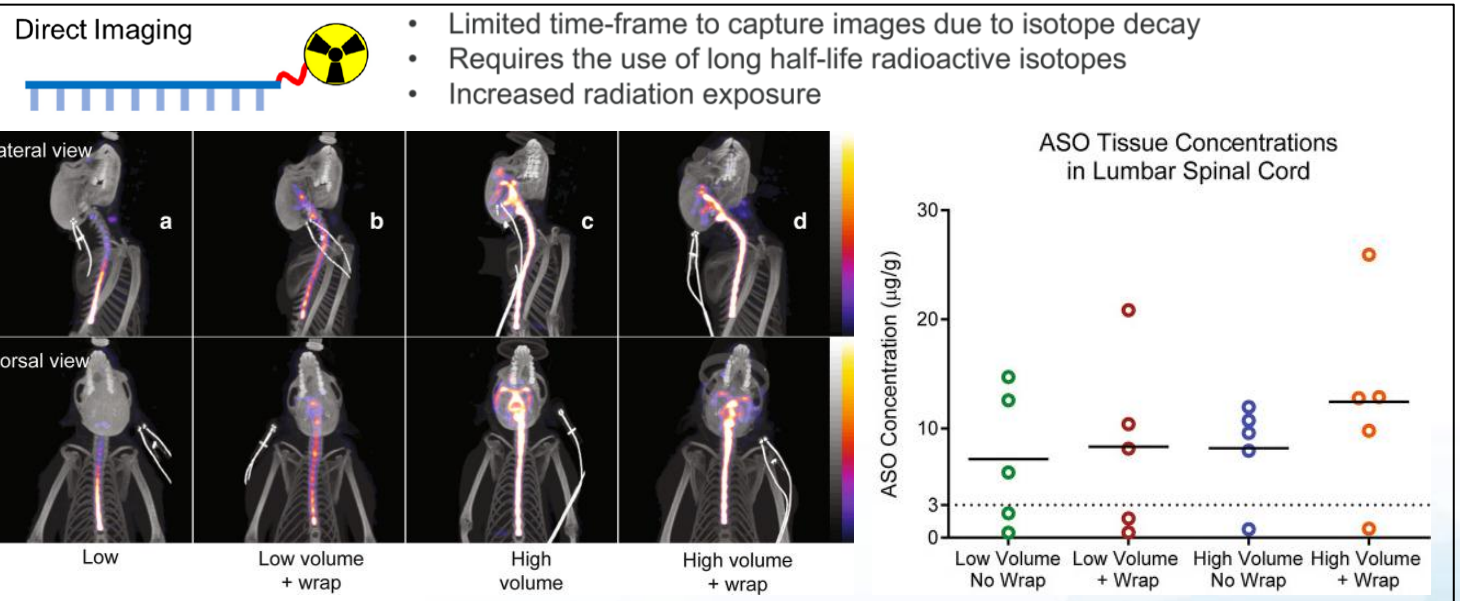
Low risk of DDI and QT prolongation

- Only limited reports of ASOs as substrates, inhibitors or inducers of cytochrome P450 enzymes *in vitro* or *in vivo*
- ASOs are not substrates or inhibitors of uptake or efflux membrane transporters (e.g., OATP, OAT, MDR1, etc.)
- Data from Phase 1 studies of 2'-MOE ASOs at doses up to 400 mg SC or 600 mg IV for 4 weeks suggest a lack of effect on QT intervals

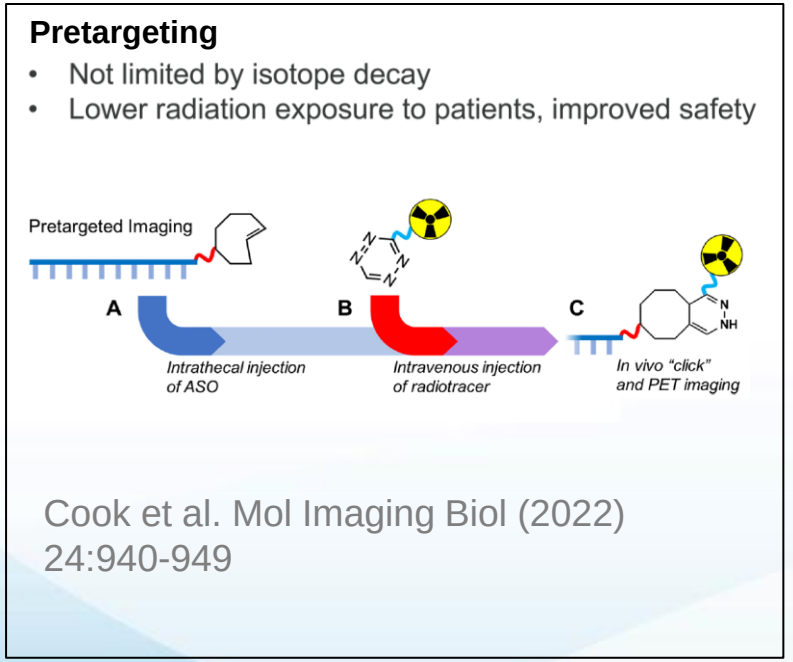
Yu et al. Nucleic Acid Ther (2017) 27:285–294
Gao et al. Expert Opin Drug Metab Toxicol. (2023) 19:979-990

IT administration: approaches to measure ASO exposures in CNS are limited

- Biopsy and microdialysis may be performed under critical conditions
- Sampling from CSF is used as a surrogate
  - Drug concentrations in CSF do not represent target areas
  - May be more closely associated with exposure at the epithelium lining of the ventricular system and spinal cord, but not brain parenchyma or deeper sites of action
- Human applications of PET/CT imaging with radio-labeled molecules and pretargeting technique are in development
  - Still qualitative rather than quantitative



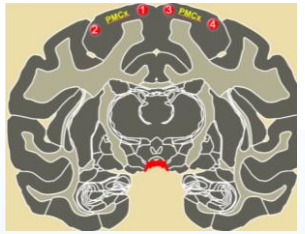
Sullivan et al. J Transl Med (2020)18:309



Cook et al. Mol Imaging Biol (2022) 24:940-949

Preclinical data to characterize distribution of intrathecal ASOs

- Human CNS tissues are practically inaccessible to analyze for drug concentrations *in vivo*
- Animal data and animal-to-human scaling become of critical importance
- Due to close similarity to human (e.g., geometry and upright position of the spinal column), non-human primate (NHP) is a suitable species to evaluate PK of IT-administered ASOs
- PK data is being generated in Cyno monkeys for a range of IT-injected ASOs
- The data typically includes time-dependent PK in the **lumbar CSF**, **spinal cord** regions, **brain** regions, **liver**, **kidneys** and **plasma**
- Plasma and lumbar CSF samples:
  - collected during the study in live animals
- Terminal tissue samples:
  - taken upon animal sacrifice



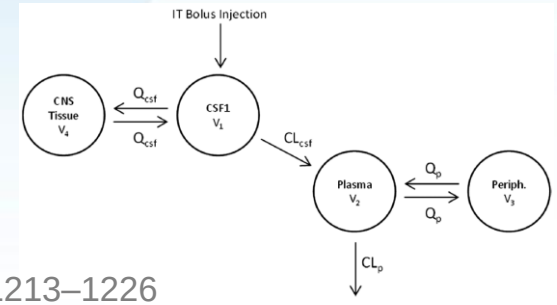
Models of IT ASOs

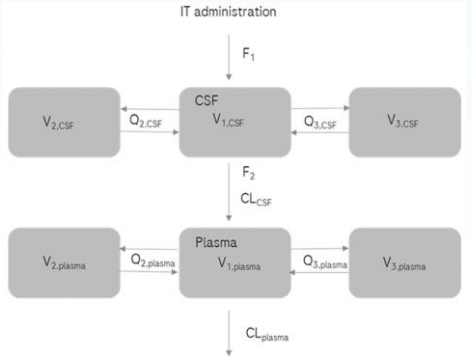
- Compartmental (pop-PK)

Luu et al. J Clin Pharm (2017), 57:1031–1041

MacCannell et al. Neuromuscul Disord (2021) 31: 310–318

Yamamoto et al. CPT Pharmacometrics Syst Pharmacol (2023) 12:1213–1226



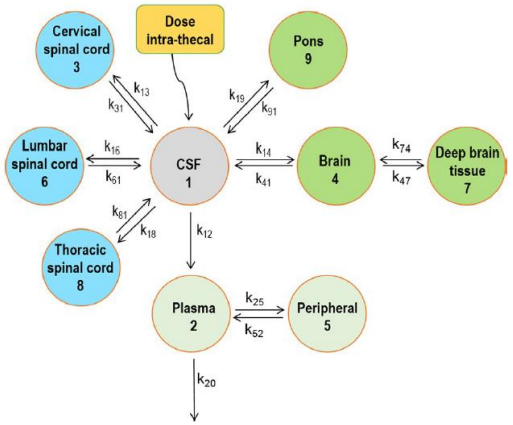


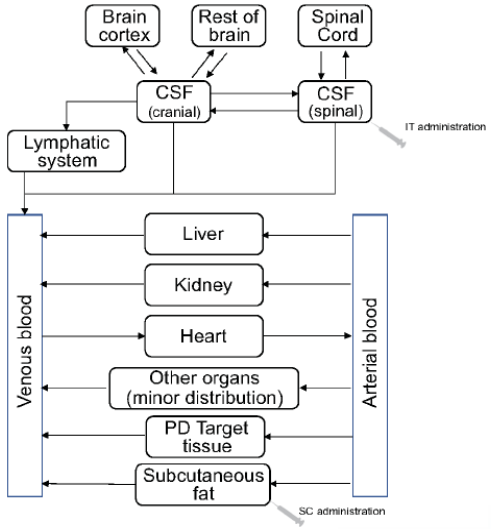
- Physiologically-based PK (PBPK)

Biliouris et al. CPT Pharmacometrics Syst Pharmacol (2018) 7:581–592

Gao et al. Expert Opin Drug Metab Toxicol (2023) 19:979-990

Monine et al. J PKPD (2021) 48:639-654





- Computational Fluid Dynamics (CFD)

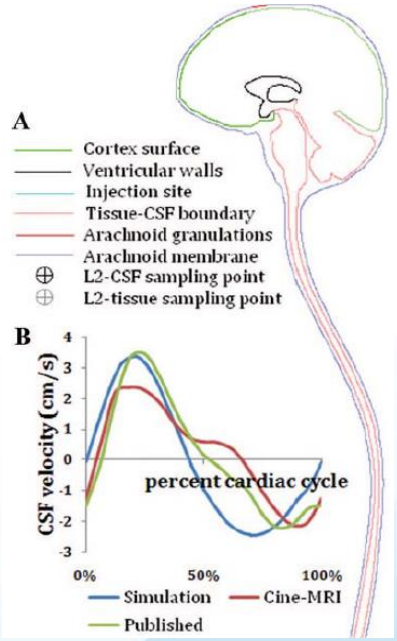
Hsu et al. Anesth Analg (2012) 115:386–394

Linninger et al. Front Physiol (2023) 14:1130925

Khani et al. Fluids Barriers CNS (2022) 19:8

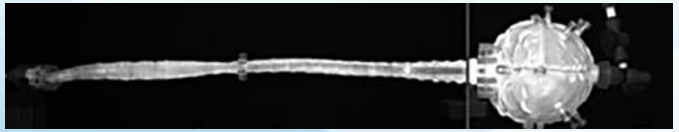
$$\vec{\nabla} \cdot (\rho \vec{u}) = 0$$

$$\frac{\partial \vec{u}}{\partial t} + \vec{u} \cdot \vec{\nabla}\vec{u} = -\frac{1}{\rho}\vec{\nabla}p + \nu\vec{\nabla}^2\vec{u} + \vec{F}$$



- Physical *in vitro* models

Seiner et al. Front Neuroimaging (2022) 1:879098



Utilization of a PBPK model to describe PK of IT ASOs

A physiologically-based pharmacokinetic model to describe antisense oligonucleotide distribution after intrathecal administration

Journal of Pharmacokinetics and Pharmacodynamics (2021) 48:639–654

Michael Monine[1] · Daniel Norris[2] · Yanfeng Wang[2] · Ivan Nestorov[1]

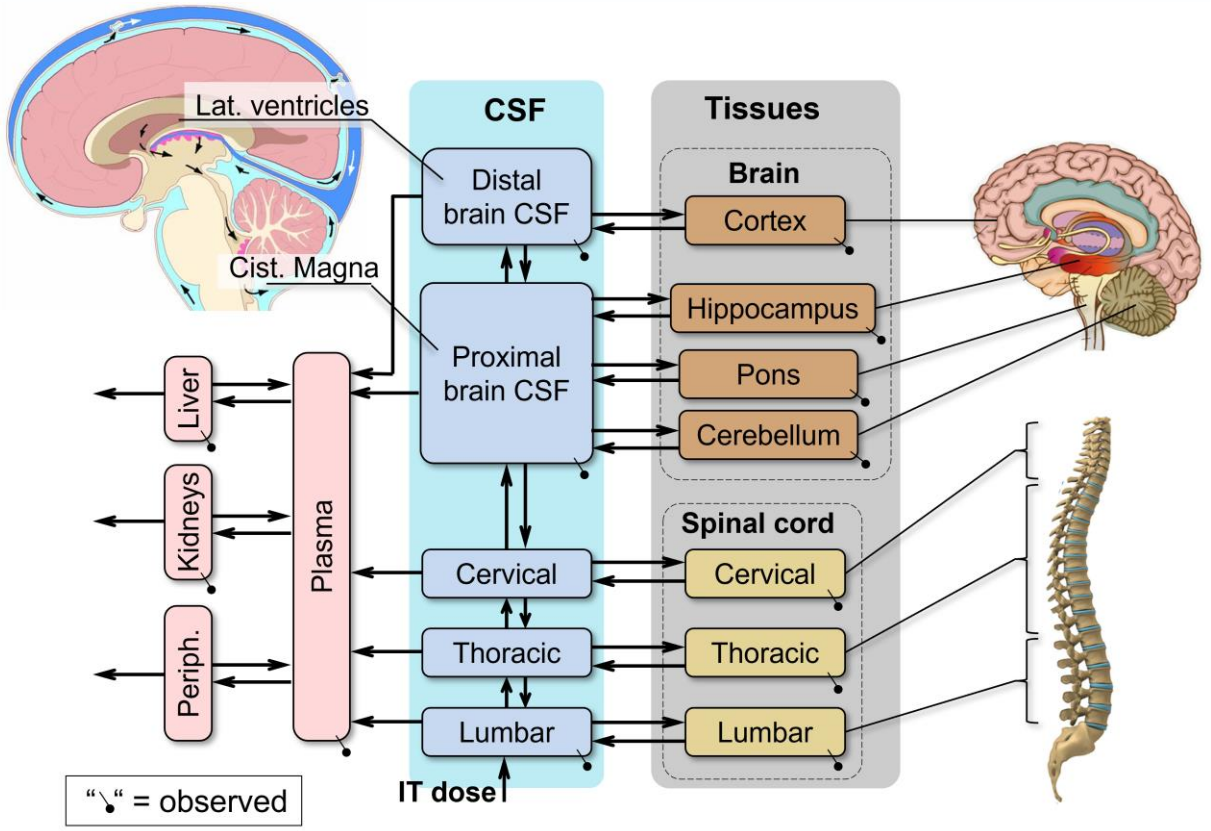
[1]Clinical Pharmacology and Pharmacometrics, Biogen

[2]PK/Clinical Pharmacology, Ionis Pharmaceuticals

NHP data:

- Lumbar CSF and blood (in live)
- Spinal cord and brain regions (terminal)

- Compartmental structure includes observable tissues in non-human primates (NHP)
- All transitions follow the first-order kinetics
- Model parameters are determined based on fitting NHP data



Destructive sampling prohibits estimation of individual (subject-specific) parameters

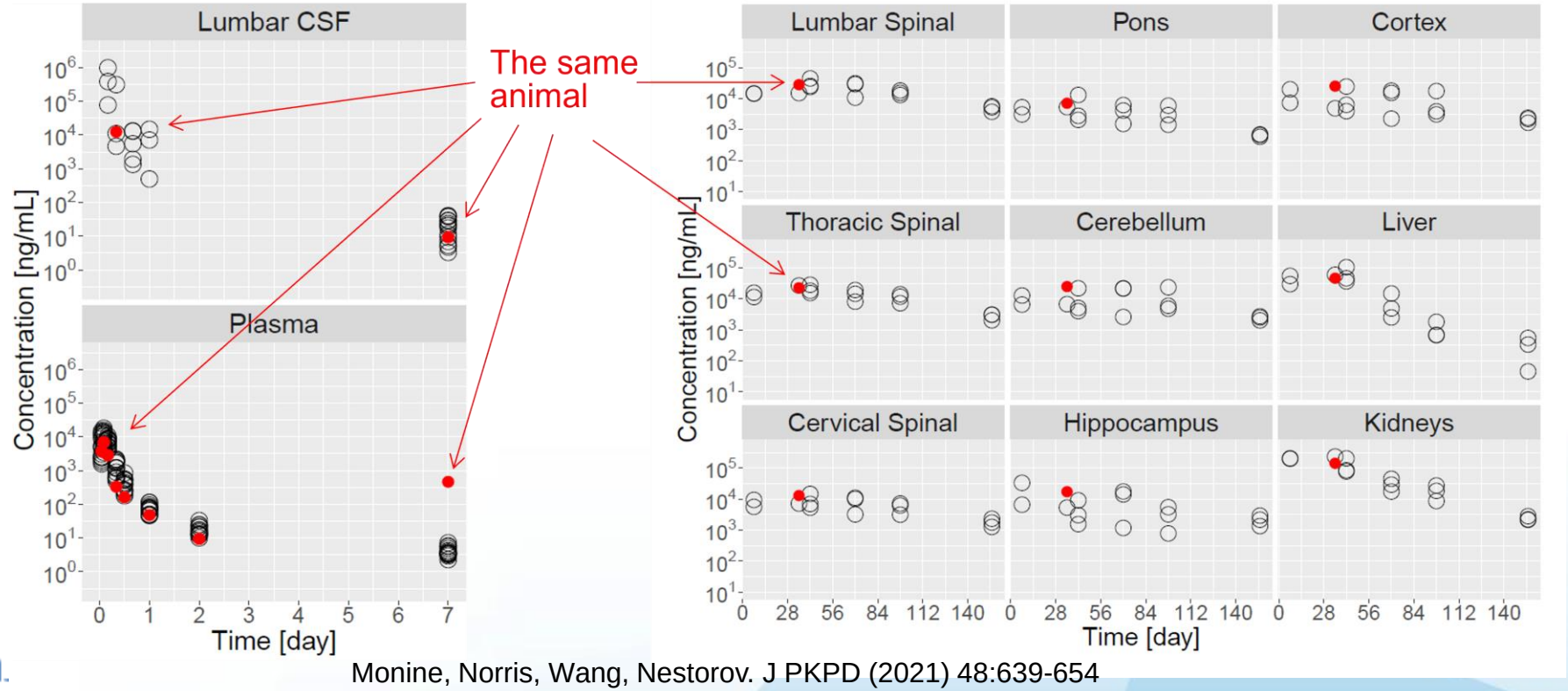
- Inter-subject variability of the animal population could not be adequately estimated
- Therefore, naïve pooled data approach was used to characterize the central tendency
- Observations were averaged across animals at each time point

CSF and Plasma PK:

- Several in-life timepoints for each animal

PK in tissues:

- A single timepoint for each animal after termination



Monine, Norris, Wang, Nestorov. J PKPD (2021) 48:639-654

PBPK model fits average NHP data

- All tested *gapmer* ASOs demonstrated similar PK, which indicates qualitative similarity of biodistribution mechanisms
- Chemical modifications across various ASOs can affect (to some extent) tissue/cellular uptake and elimination rates

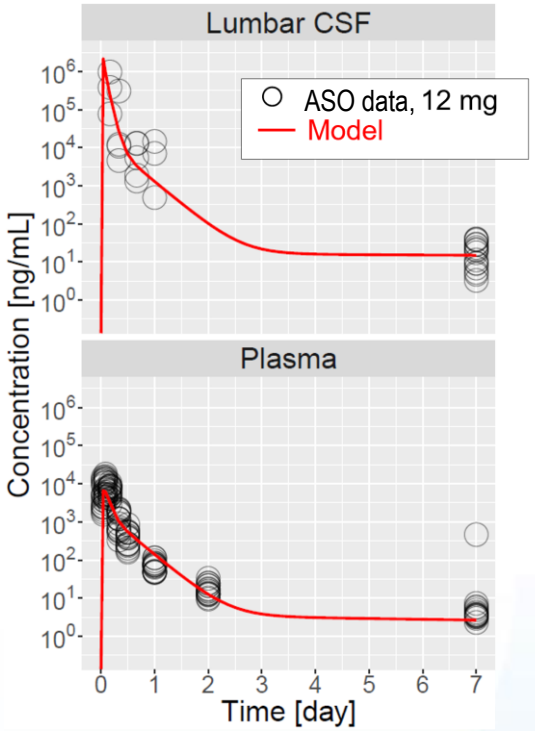
Example: Tofersen ASO

Lumbar CSF

- Distribution phase lasts several days
- Elimination half-life in CSF is controlled by $t_{1/2}$ in CNS tissues

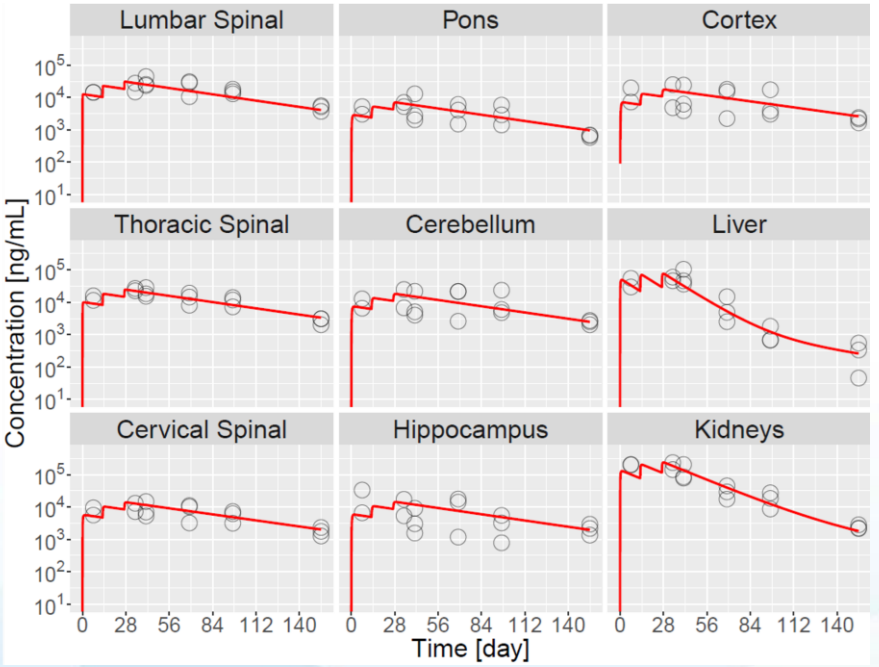
Plasma

- Plasma PK follows the CSF PK with 1-2 hrs delay in T_{max} and lower C_{max}



Tissues

- Elimination $t_{1/2}$ is similar across all CNS tissues (1-2 months)
- Liver and kidneys are major ASO elimination organs



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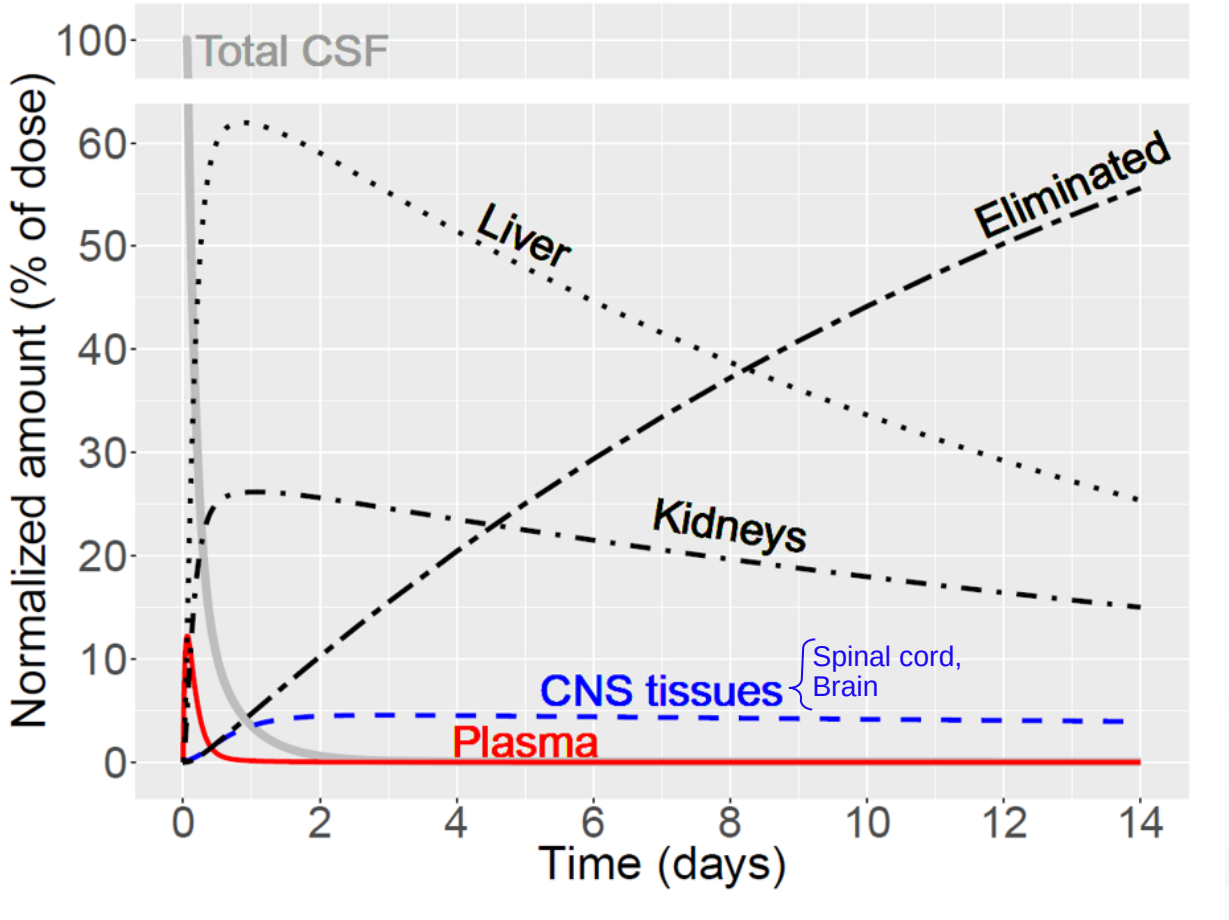
Model predicts CNS exposures reaching ~4% of total IT dose, which is greater than could be achieved via IV route

- Shortly after injection:
  - A major transfer to the systemic circulation takes place
  - Almost instantaneous uptake in liver and kidneys, followed by elimination
- Within two days after IT administration:
  - About 4% of the dose reaches CNS tissues, which still greatly exceeds the amounts delivered by IV dose
  - This result can also be seen as first quantitative justification of IT route over other routes (e.g., IV)



Monine et al. J PKPD (2021) 48:639-654

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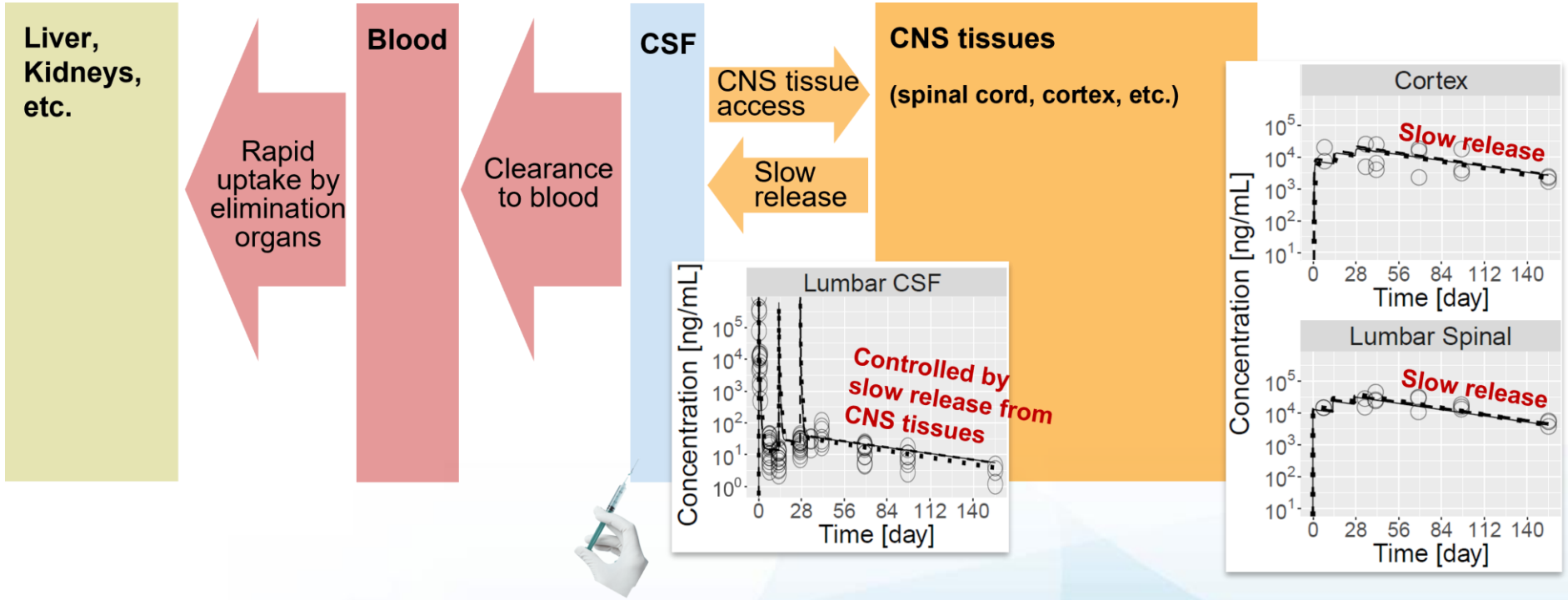
Early time post-dose distribution: elimination half-life is controlled by CNS tissues

- A few hours after injection: major transfer to the systemic circulation takes place
- Rapid uptake in liver and kidneys, followed by elimination
- Reaches maximum in CNS tissues (spinal cord and brain) within 1-2 days after the injection
- Days-weeks: the rate of release from CNS tissues back to CSF controls the elimination phase in all CNS tissues and CSF



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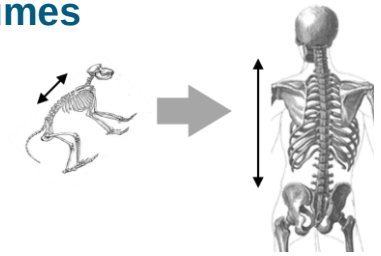
Modeling prospectively predicts human autopsy exposure data in CNS tissues

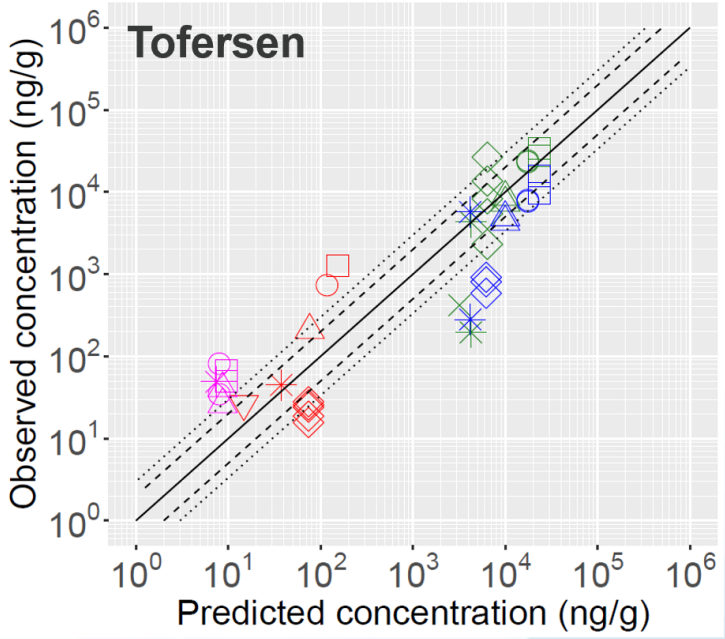
- ASO concentrations in the CNS tissues are scaled by the corresponding physiological tissue volumes (sizes) assuming equivalence of distribution rates between NHP and humans
- Simulations reproduce dosing and post-dose scenarios for participants with ALS who were treated with tofersen or BIIB078 (investigational *C9orf72* ASO), but passed away due to ALS-related conditions
- The model was not fitted to the autopsy data

NHP-to-human scaling by physiological volumes

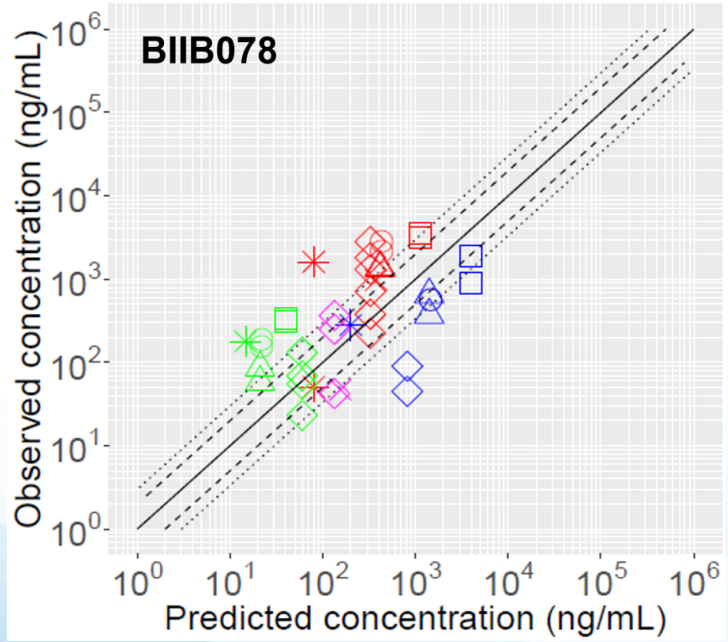


Tissue	NHP	Human	Ratio
CSF, mL	15	140 – 300	≥10
Spinal cord, g	6	35 – 40	~7
Brain cortex, g	54	1000 – 1200	~20
Blood, mL	200 – 250	4500 – 5500	~22

Dose, mg	# doses	From last dose to death
100	18	1.5 months
20	2	8.5 months
100	LTE	1.5 months
20mg x13 + 60mg x5 + 100mg x9		18 months



Dose, mg	# doses	From last dose to death
20	x8	1.5 months
20	x9	5 months
35	x11	3 weeks
10	x5	2 months



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Presented at PAGE 2023

Predicting ASO concentrations and target engagement in support of FIH

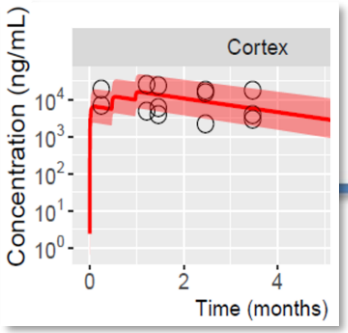
- Key question: what dose levels/regimen would be required to achieve a desired response in a region of interest (e.g., disease–associated mRNA knockdown in cortex)?

1. PK studies in NHP

- Candidate screening
- Toxicology/GLP
- Biodistribution

2. PK modeling

- NHP PBPK model
- NHP-human scaling



3. PD modeling

mRNA knockdown

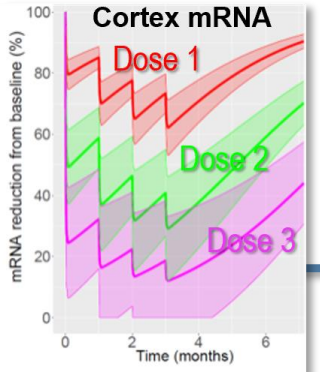
- Assume immediate response
- Potency from transgenic mouse

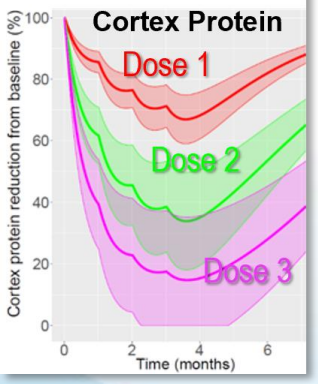
Protein reduction

- Delayed response due to long protein $t_{1/2}$
- Assume same EC_{50} as for mRNA knockdown

mRNA → protein

4. Human dose projection





5. Interpretation of clinical readouts

CSF biomarkers (e.g., protein reduction from baseline)

Clinical function (e.g., ALSFRS-R)

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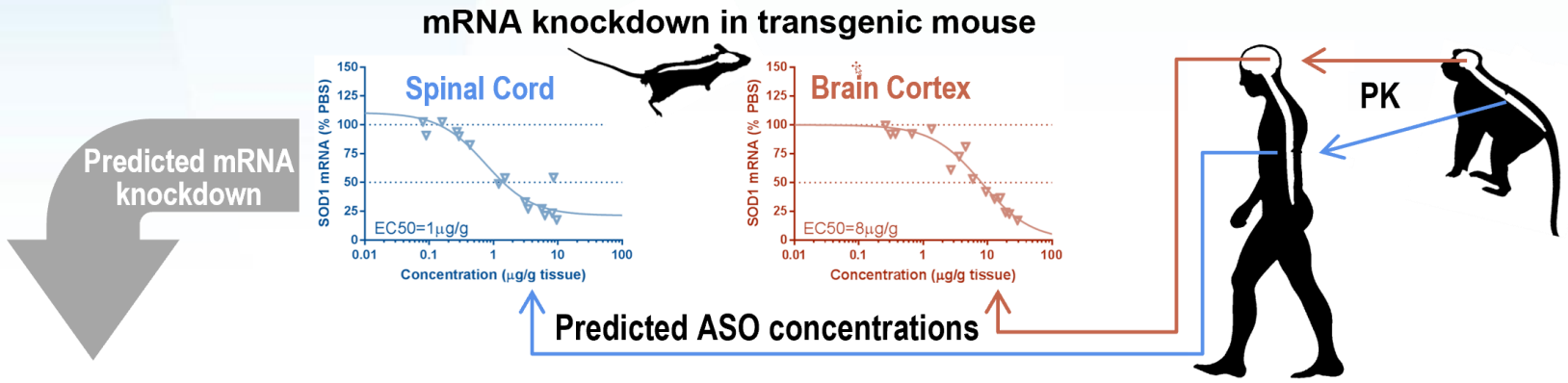
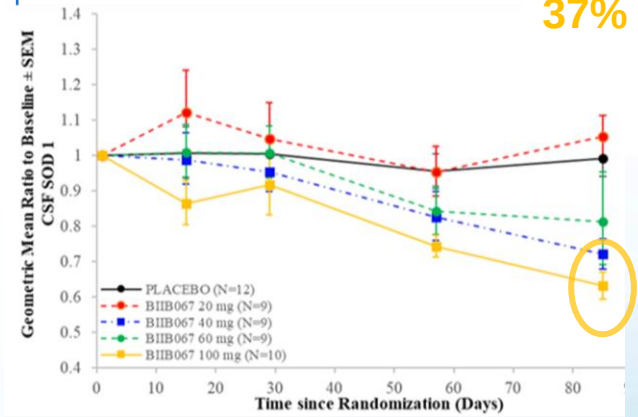
PK/PD: interpretation of CSF protein reduction based on predicted TE in CNS tissues

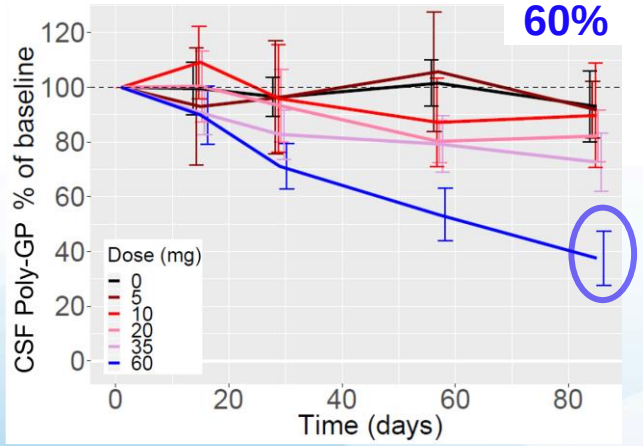
Assumptions
- Potency: human CNS = t.g. mouse CNS
- Protein ↓ = mRNA ↓

- CSF response is proportional to size of target organs:
  - ~ 10% from spinal cord
  - ~ 90% from cortex



	Tofersen (100 mg BIIB067 Q4W)	C9orf72 ASO (60 mg BIIB078 Q4W)
Predicted protein reduction in CSF	~35%	~53%
Observed protein reduction in CSF	37%	60%





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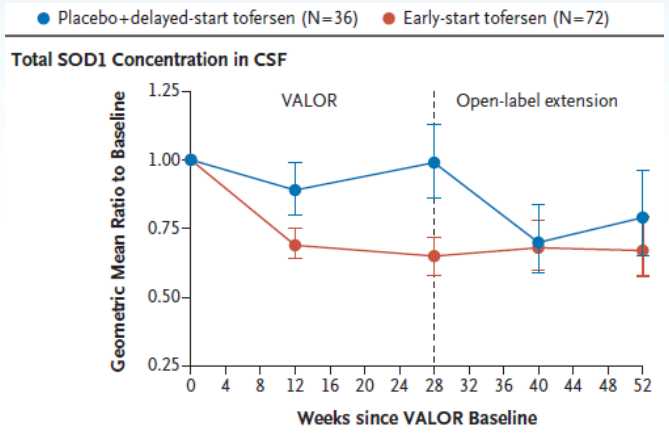
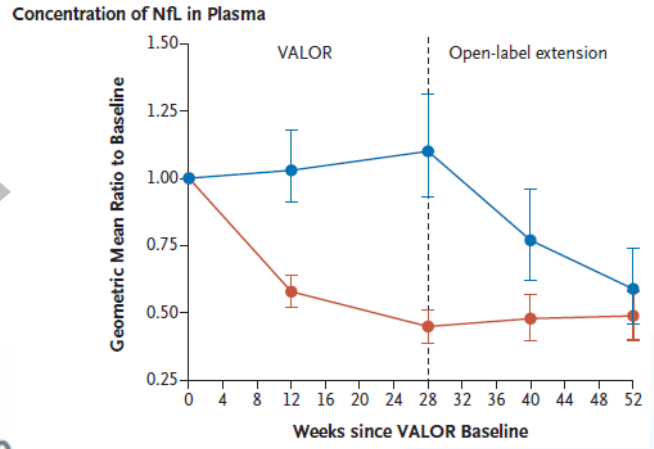
Tofersen/*SOD1* ALS ASO: reduction in SOD1 protein and the associated trends towards improvement in physical functions

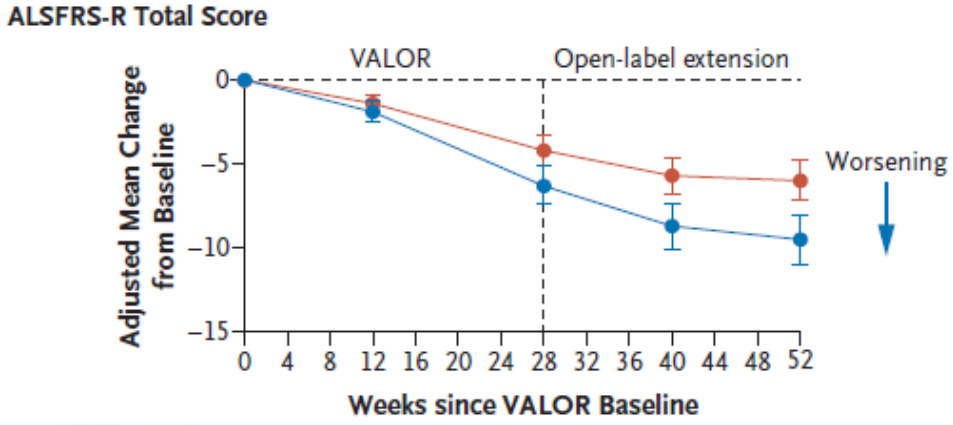
100 mg monthly (IT)



- Neuronal degeneration in *SOD1* ALS disorder is considered to be caused by toxic gain of function of the mutant SOD1 protein
- In persons with *SOD1* ALS, tofersen reduced concentrations of SOD1 in CSF and of neurofilament light chains in plasma over 28 weeks
- Longer term data from the OLE showed improvement in ALSFRS-R specifically in the early-start tofersen group

TE was achieved;
Consistent trend in clinical effect





Miller et al. N Engl J Med 2022;387:1099-110.
DOI: 10.1056/NEJMoa2204705

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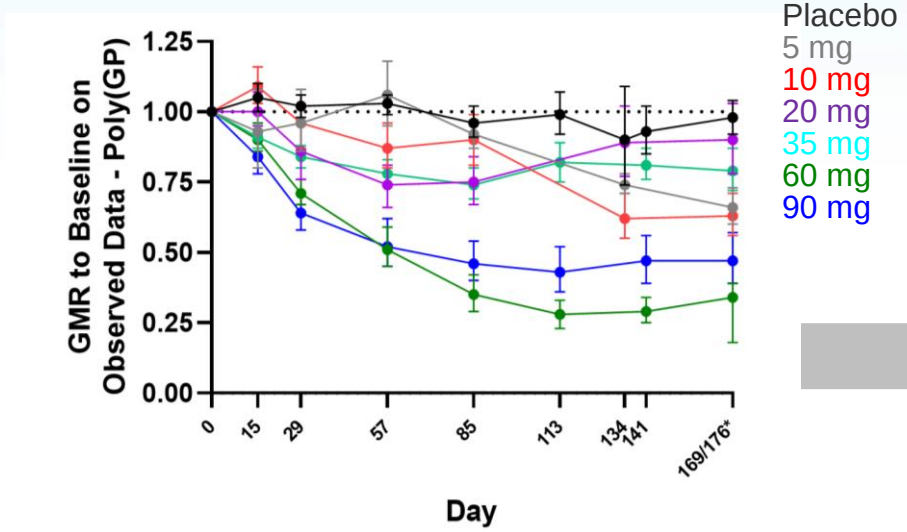
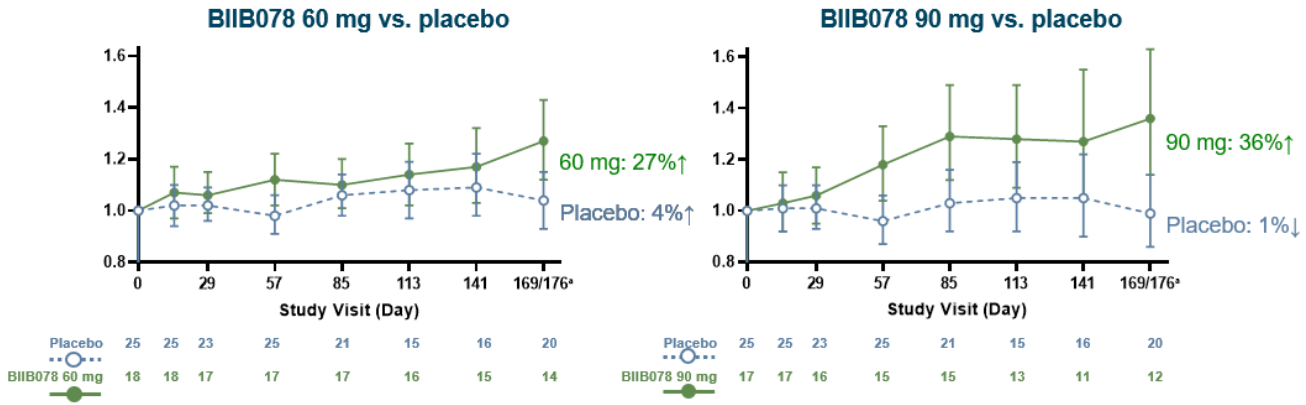
C9orf72 ALS ASO: while treatment led to robust reduction of CSF poly(GP) and poly(GA) proteins, there was no improvement observed in any of the functional scales

5-90 mg monthly (IT)



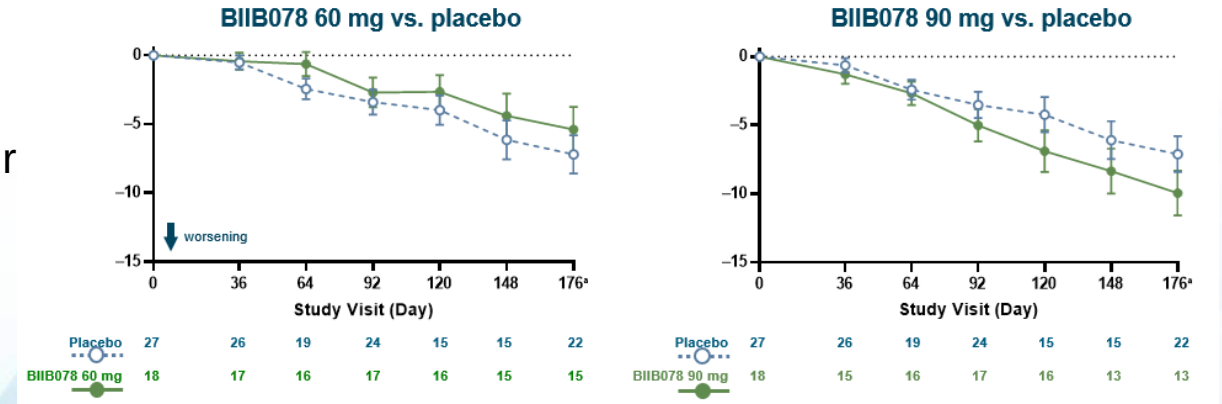
TE was achieved;
However, it did not translate into clinical effect

NfL in CSF



- Based on the results of this Phase 1 study, BIIB078 clinical development has been discontinued, including the open-label extension study
- However, these results will be informative in evolving our understanding of the complex biology of *C9orf72*-ALS

ALSFRS-R total score



Van den Berg et al. Lancet Neurol. 2024. *Accepted*

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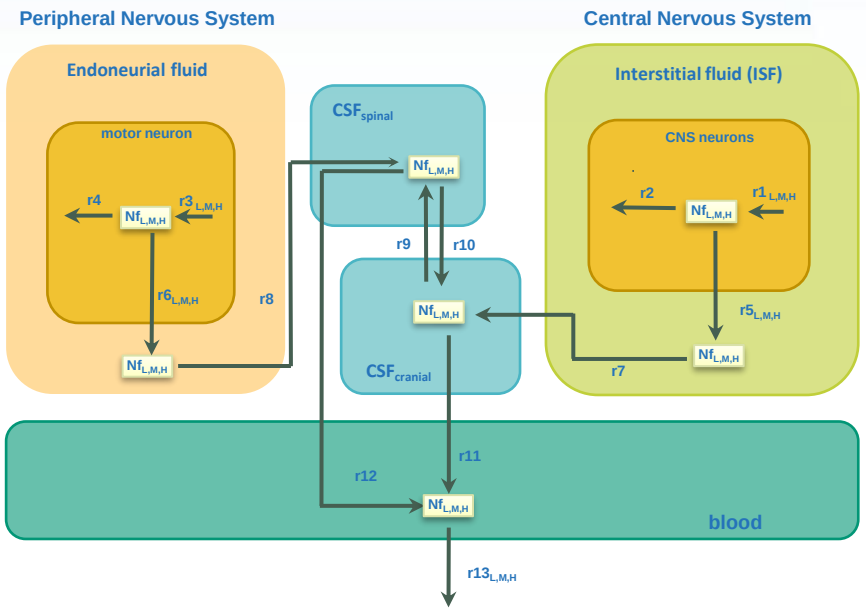
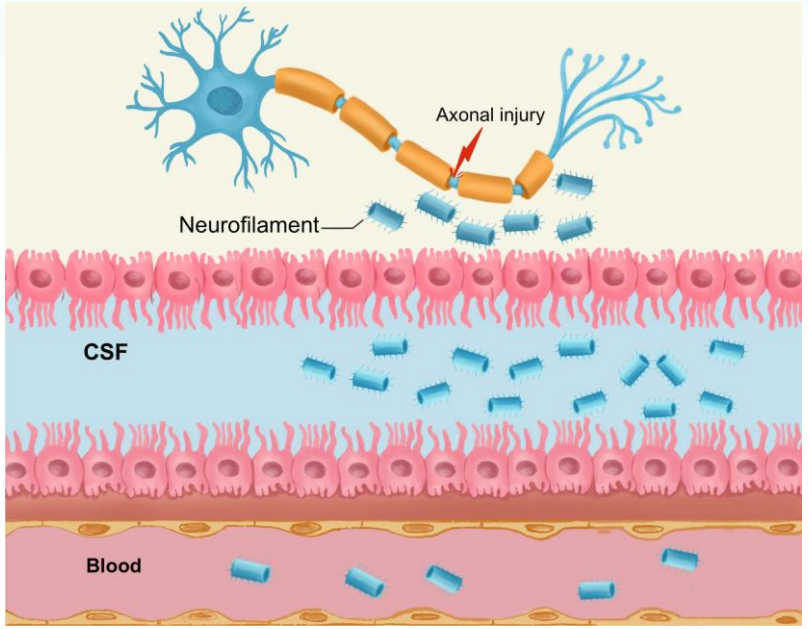
Integrating ASO PK model with QSP approach to predict Nf release

Nf adult healthy model



Paris et al. CPT Pharmacometrics Syst Pharmacol 2022; 11:447-457



- Acknowledged as biomarker of neurodegeneration
- Used in a steadily growing number of clinical trials of different diseases
- Considered for drug approval (tofersen)
- Evaluated as prognostic biomarker

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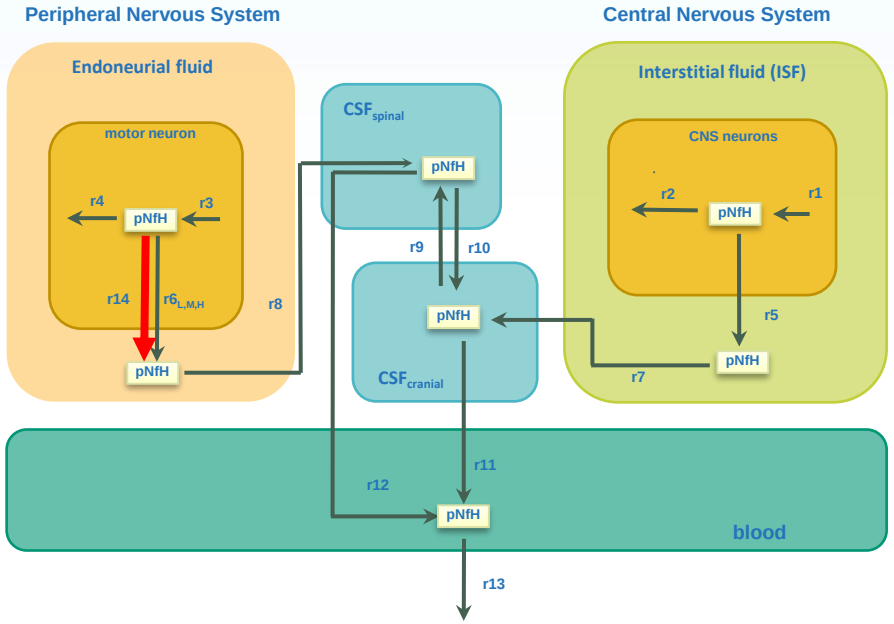
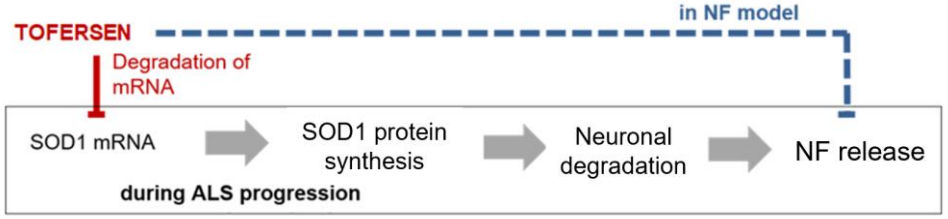
Integrating ASO PK model with QSP approach to predict Nf release

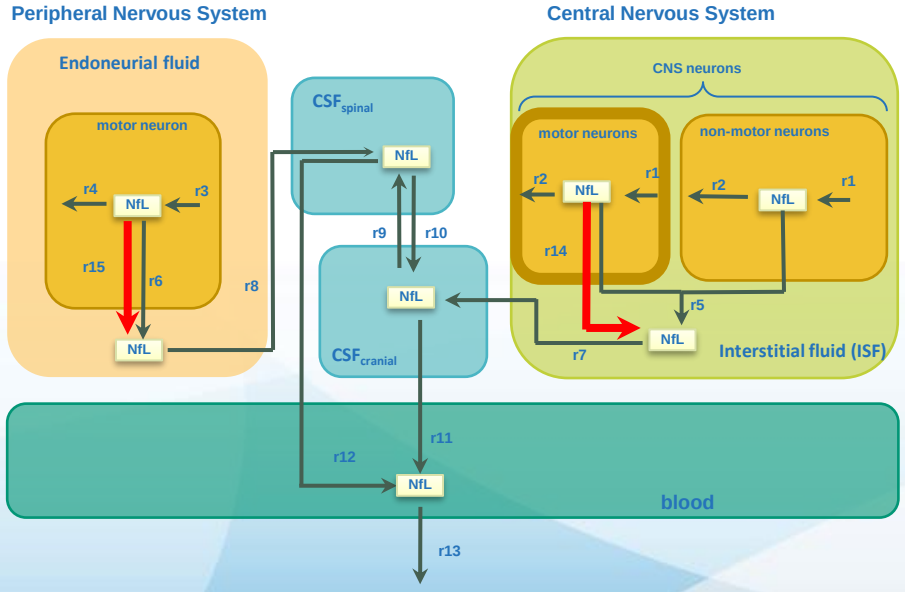
pNfH pediatric SMA model

Paris et al. CPT Pharmacometrics Syst Pharmacol 2023; 12:196-206



NfL adult *SOD1*-ALS model





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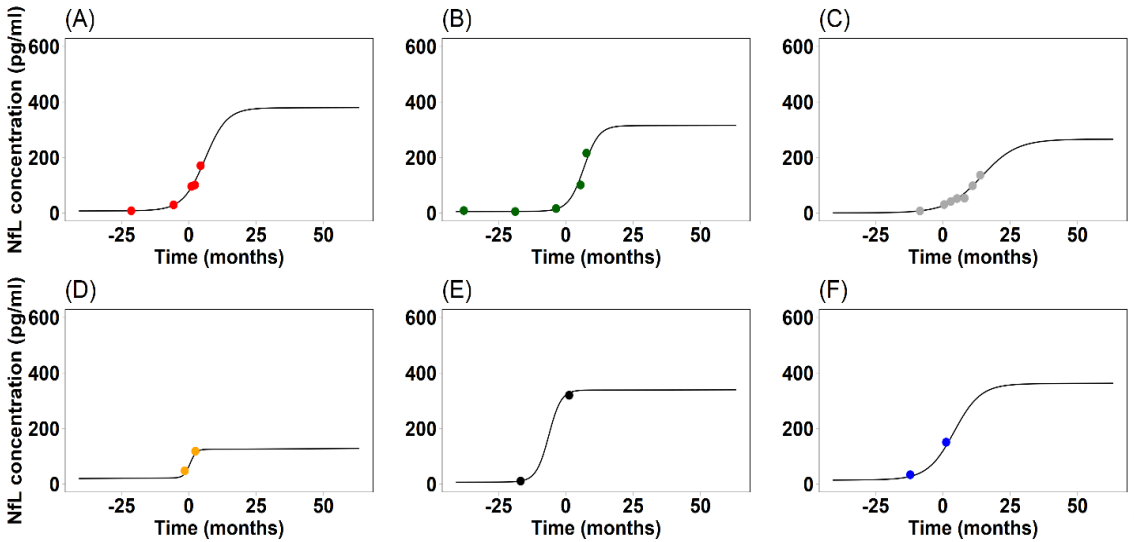
Model application: predicting *SOD1*-ALS disease onset and treatment

Simulation of disease onset

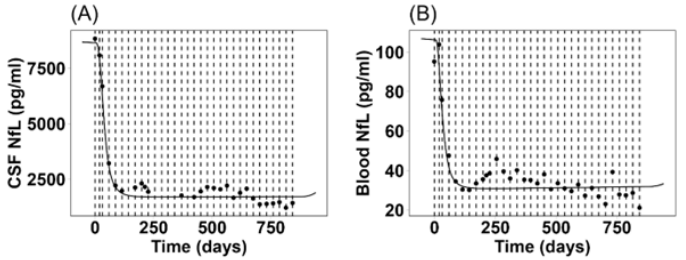
We included in the model a logistic function simulating the increase of the NfL leakage when the people with ALS passes to the symptomatic phase of the disease



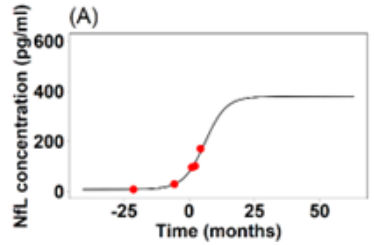
Data from: Benatar et al. *Annals of neurology*, *84*(1), 130-139, 2018

Combination of onset and treatment

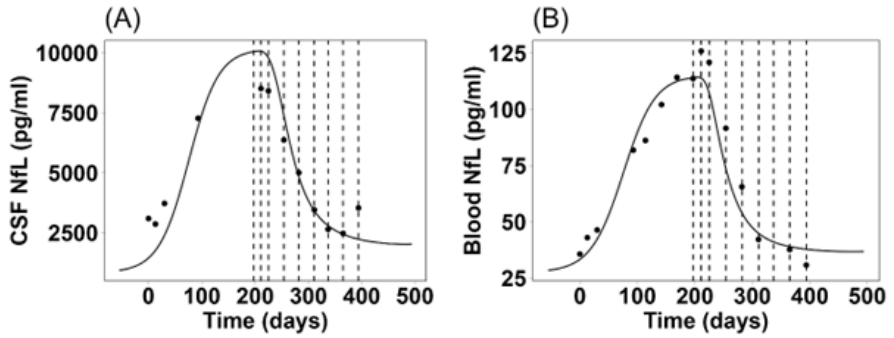
Simulation of treatment



Simulation of disease onset



Simulation of onset and treatment



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Basic conclusions

- Intrathecal (IT) administration of antisense oligonucleotides (ASOs) has become an efficient method for targeting neurodegenerative and neuromuscular disorders
- Dose projection for IT-administered ASOs in humans requires accurate estimation of exposures at target sites within the central nervous system (CNS)
- Since human CNS tissues are practically inaccessible to analyze for ASO concentrations and target engagement in vivo, animal data and animal-to-human scaling become of critical importance in guiding dose selection for first-in-human (FIH) studies
- A preclinical physiologically-based pharmacokinetic (PBPK) model has been developed
  - Describes the whole-body distribution of IT ASOs in non-human primate (NHP) studies
  - Was scaled to human
- Risks remain high due to
  - variability in PK
  - uncertainty in translation of target engagement between species and contribution of pharmacodynamic (PD) response at a tissue level to changes in clinical endpoints
- Integration of the PBPK model with Nf QSP model allowed predicting individual ASO treatment scenarios and the effect on Nf levels

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Acknowledgements

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Ivan Nestorov
Danielle Graham
Toby Ferguson
Steph Fradette
Satish Eraly
Ed Plowey
Jenna Sullivan
Nic Currier
Patrick Trapa
Natasha Penner
Caroline Woodward
Lin Lin
Xiao Tong
Kevin Hallock
Michael Rooney
Kumar Kandadi Muralidharan
Haiming Wu
Eric Masson
Konstantine Skordos

IONIS

Dan Norris
Yanfeng Wang
Holly Kordasiewicz

COSBI

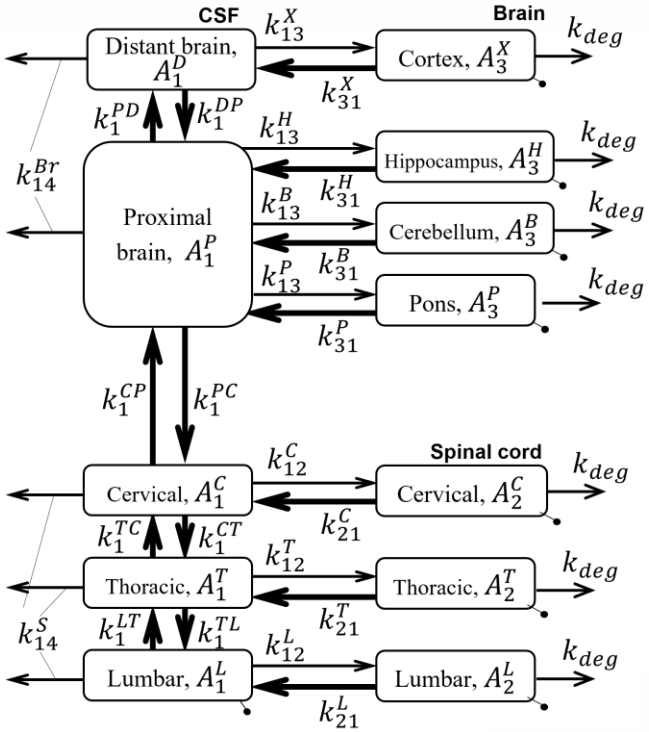
Alessio Paris
Luca Marchetti
Pranami Bora
Silvia Parolo
Enrico Domenici

Backup slides

Dealing with uncertainty and identifiability

Model 1

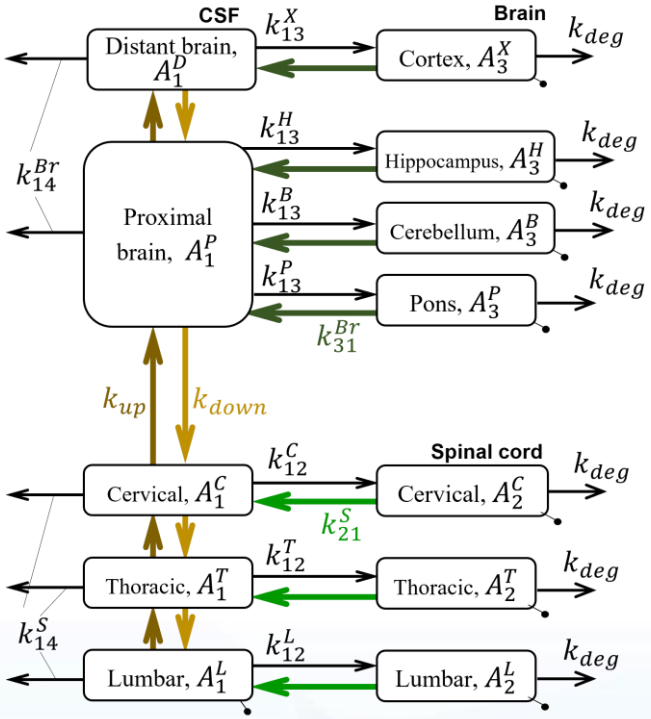
- Each parameter is uniquely defined
- Describes the data nicely
- *Identifiability problem*



Model 1 ($AIC = -18.6$)	
Value, h^{-1}	SE (CV%)
$k_1^{LT} = 0.6942$	–
$k_1^{TC} = 5.464$	–
$k_1^{CP} = 0.09471$	–
$k_1^{PD} = 0.03722$	56.3
$k_1^{TL} = 0.1386$	–
$k_1^{CT} = 2.366$	–
$k_1^{PC} = 0.05027$	88.2
$k_1^{DP} = 2.6 \times 10^{-7}$	–
$k_{12}^L = 1.6 \times 10^{-3}$	29.25
$k_{12}^T = 3.3 \times 10^{-3}$	–
$k_{12}^C = 6.1 \times 10^{-4}$	–
$k_{21}^L = 6.8 \times 10^{-4}$	47.32
$k_{21}^T = 8.3 \times 10^{-4}$	40.41
$k_{21}^C = 7.2 \times 10^{-4}$	45.61
$k_{13}^P = 2.3 \times 10^{-4}$	58.51
$k_{13}^B = 2.5 \times 10^{-3}$	61.84
$k_{13}^H = 4.1 \times 10^{-4}$	67.03
$k_{13}^X = 2.9 \times 10^{-3}$	–
$k_{31}^P = 7.5 \times 10^{-4}$	48.05
$k_{31}^B = 6.3 \times 10^{-4}$	59.49
$k_{31}^H = 7.2 \times 10^{-4}$	56.84
$k_{31}^X = 1.5 \times 10^{-3}$	–
$k_{14}^S = 0.2428$	16.35
$k_{14}^{Br} = 2.3 \times 10^{-3}$	96.86
$k_{deg} = 5.9 \times 10^{-6}$	–

Model 2

- Some parameters are grouped
- Fits the data well
- *More identifiable*



Model 2 ($AIC = -12.5$)	
Value, h^{-1}	SE (CV%)
$k_{up} = 0.2955$	19.3
$k_{down} = 0.08424$	54.86
$k_{12}^L = 9.6 \times 10^{-4}$	16.08
$k_{12}^T = 2.3 \times 10^{-3}$	14.55
$k_{12}^C = 1.8 \times 10^{-3}$	17.5
$k_{21}^S = 7.1 \times 10^{-4}$	19.78
$k_{13}^P = 6 \times 10^{-4}$	23.16
$k_{13}^B = 8.2 \times 10^{-3}$	25.7
$k_{13}^H = 1.2 \times 10^{-3}$	30.09
$k_{13}^X = 0.03179$	24.78
$k_{31}^{Br} = 8.6 \times 10^{-4}$	23.86
$k_{14}^S = 0.2469$	7.469
$k_{14}^{Br} = 0.0403$	32.8
$k_{deg} = 8.4 \times 10^{-8}$	–

Monine, Norris, Wang, Nestorov. *J PKPD* (2021) 48:639-654

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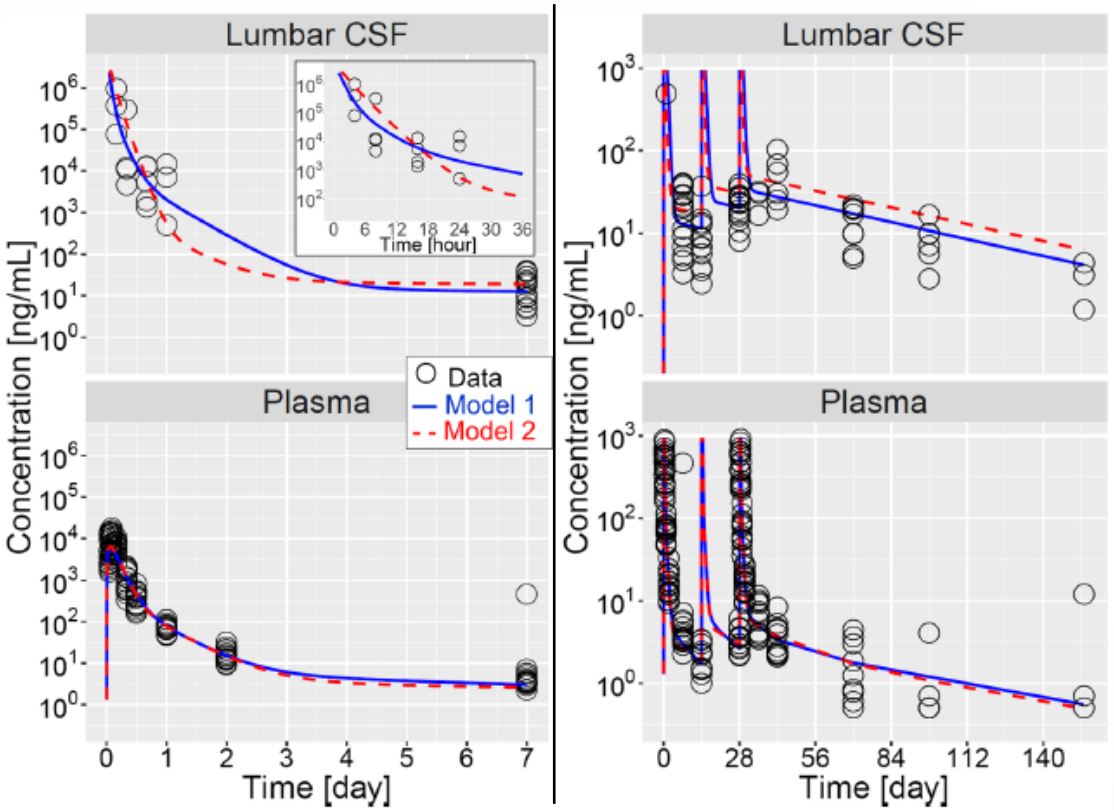
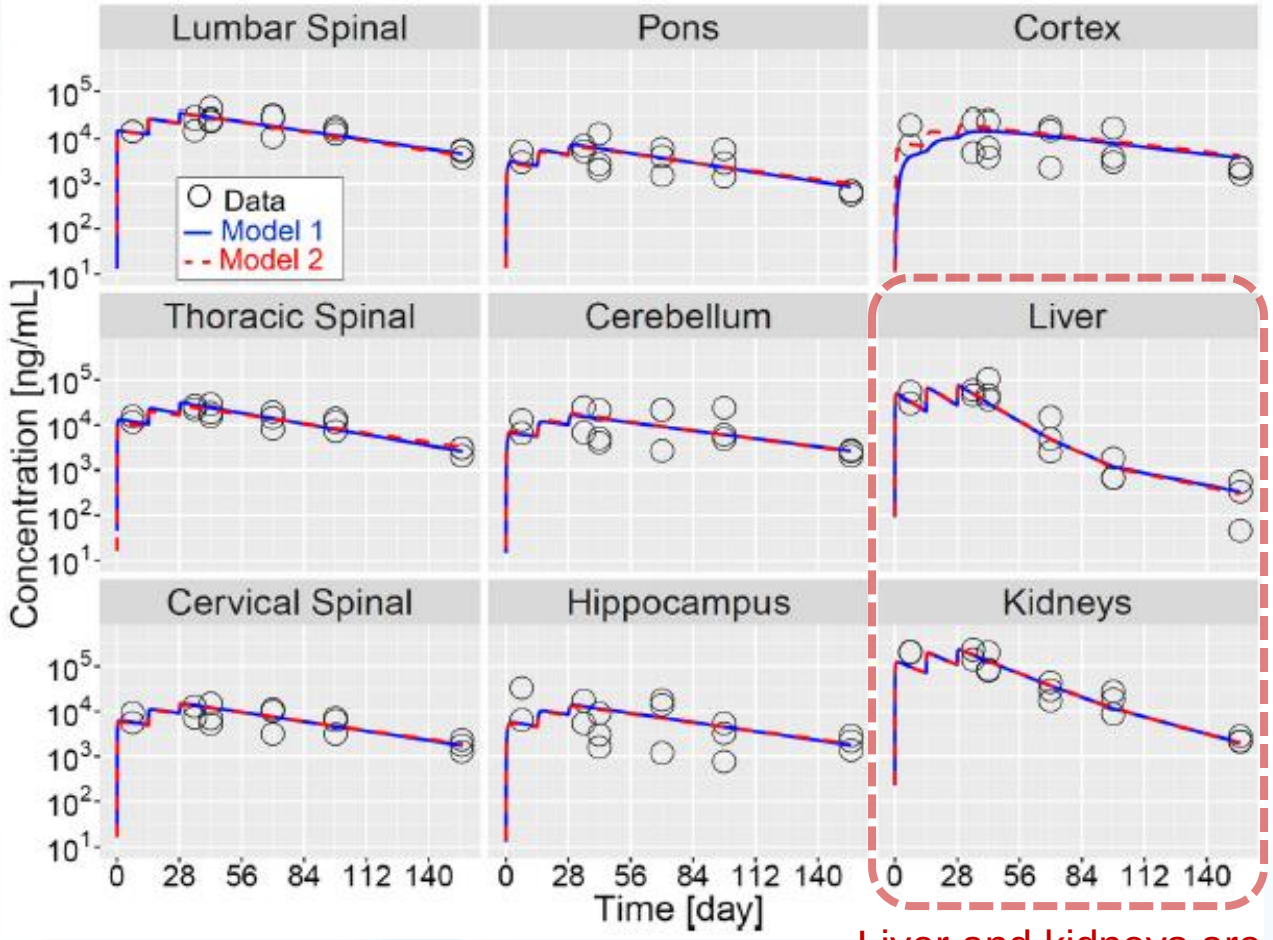
Model performance *vs*. NHP data

- CSF: distribution phase lasts 2-3 days; sharp initial drop
- Plasma PK follows the CSF PK with 1-2 hrs delay in peak concentration
- Long elimination phase detected in CSF (plasma concentrations drop BLQ)



- Terminal half-life is similar across all sampled CNS tissues ($t_{1/2}$~1-1.5 months)



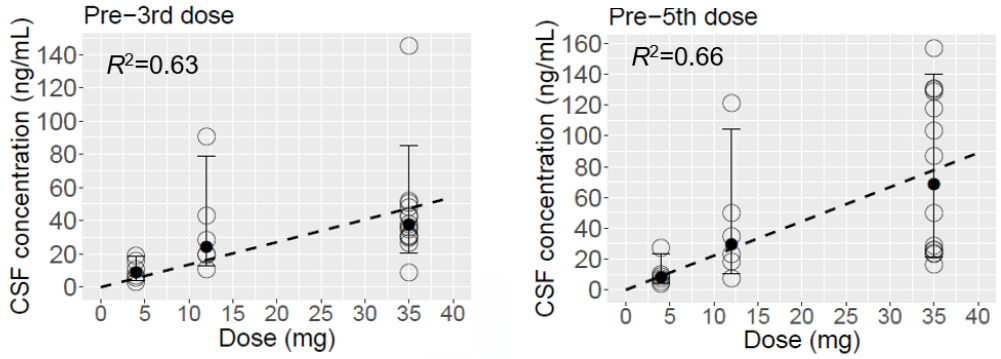
Liver and kidneys are major ASO elimination organs

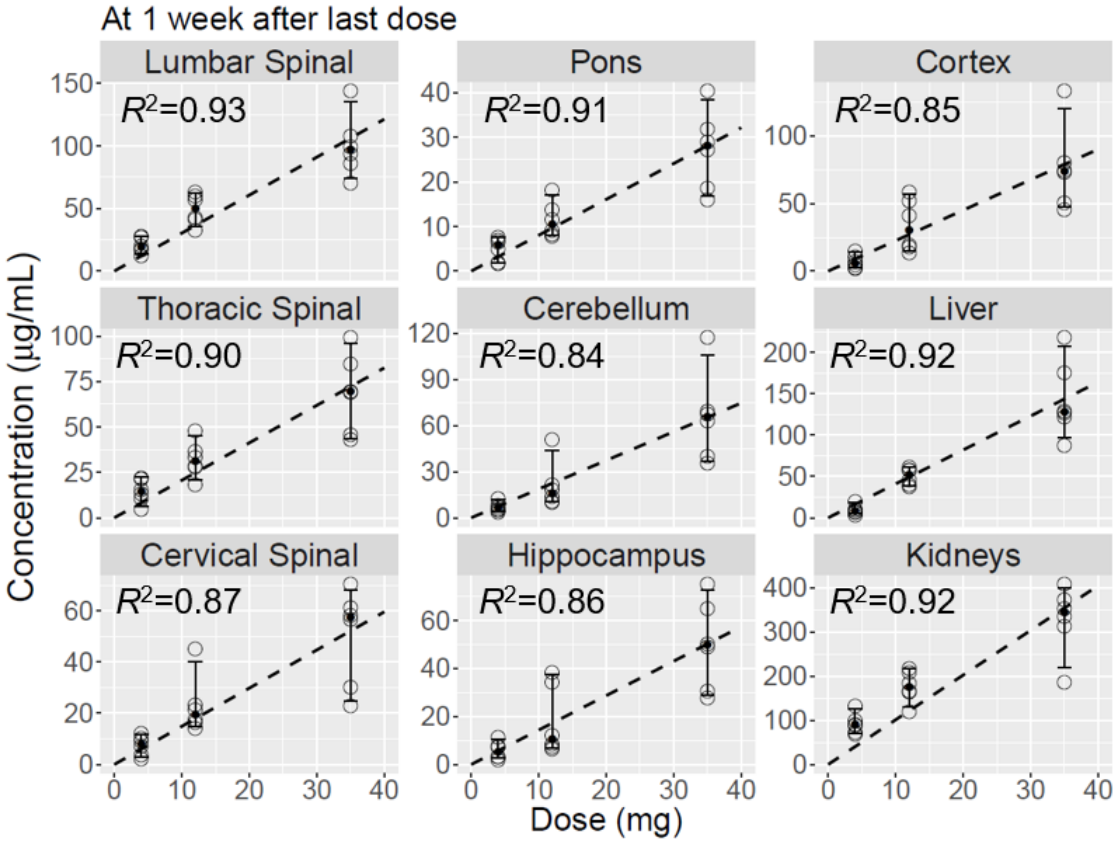
Monine, Norris, Wand, Nestorov. *J PKPD* (2021) 48:639-654

NHP PK data: dose linearity can be assumed

- **Investigational ASO**: 2'-MOE and PS modified gapmer
- 40-50 animals dosed in a typical NHP study
- Infusion via lumbar puncture at level L3-L4 (slow bolus of 1 mL solution over 1 min)

- Dose-linearity check in tissues: Observed ASO concentrations in CNS tissues appear to be linear with dose
- Observed CSF demonstrates slight non-linearity with dose
- **Models assume overall dose linearity within the studied dose range**





Monine, Norris, Wang, Nestorov. *J PKPD* (2021) 48:639-654

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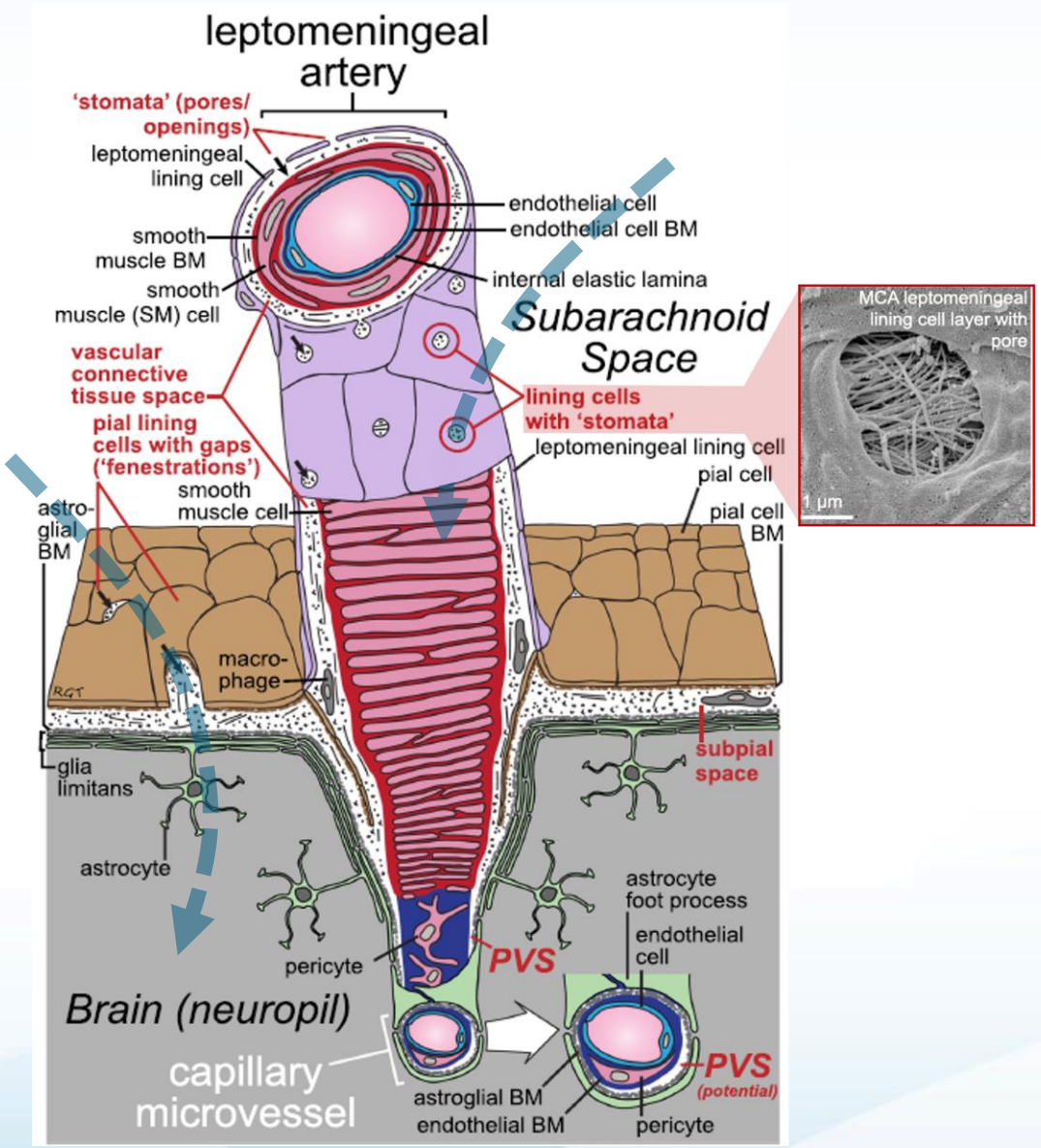
Clearance from CSF to blood and uptake in CNS tissues

- *The perivascular spaces* of cerebral blood vessels have in recent years been the subject of increasing research as pathways for CSF/ISF exchange, but controversy exists over their precise role
- Potential routes of entry from the CSF into the PVS include specialized pores (“stomata”) recently demonstrated on the adventitial lining cells of leptomeningeal vessels
- Similar pores may also exist on the pia, providing an additional route into the PVS via the subpial space



Acta Neuropathologica (2018) 135:387–407

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