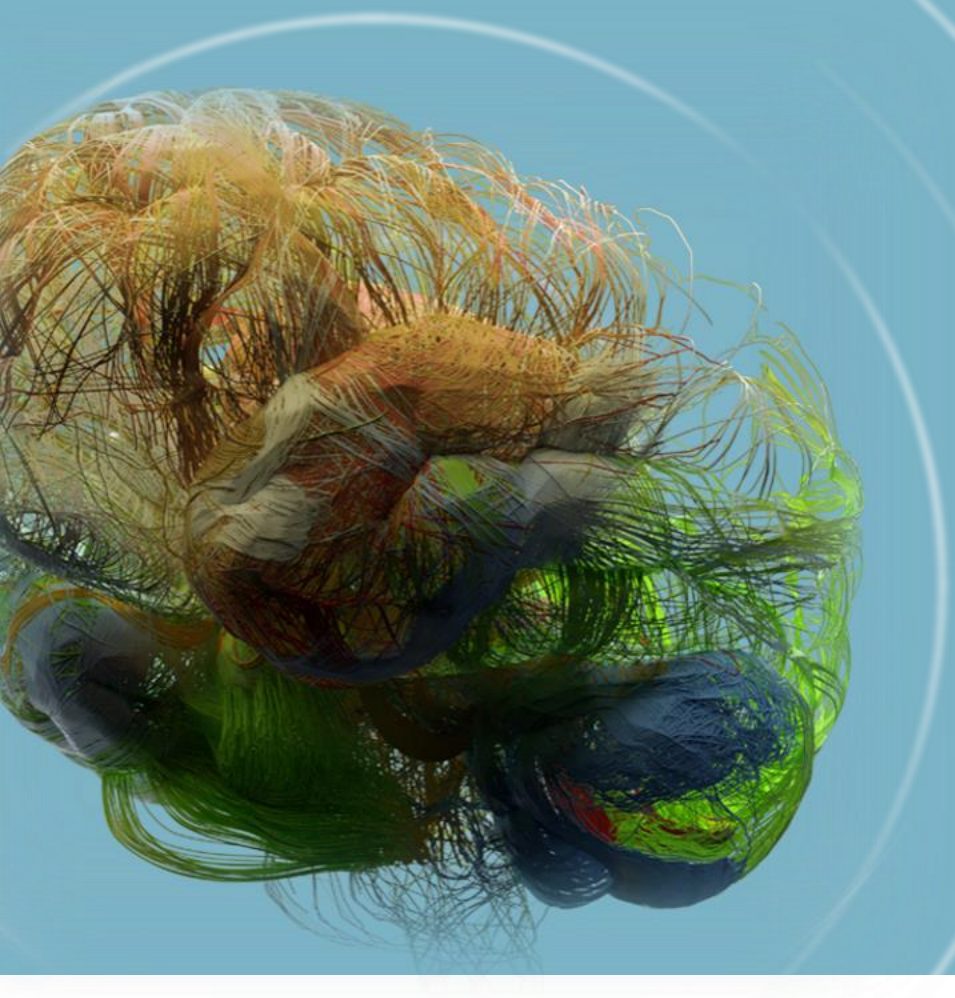

Characterization of M1-Selective and Brain-Penetrant [^{11}C]-PIPE-307 PET Radiotracer in Cynomolgus Monkeys

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May 30th, 2022

Invicro

PIPELINE

Remyelination in Multiple Sclerosis: Unmet Clinical Need

COSTLY AND AFFECTS MANY

1 Million U.S. Patients

$5.6 Million

cost (direct and indirect) of care over lifetime of an MS patient

CURRENT THERAPIES LIMITED

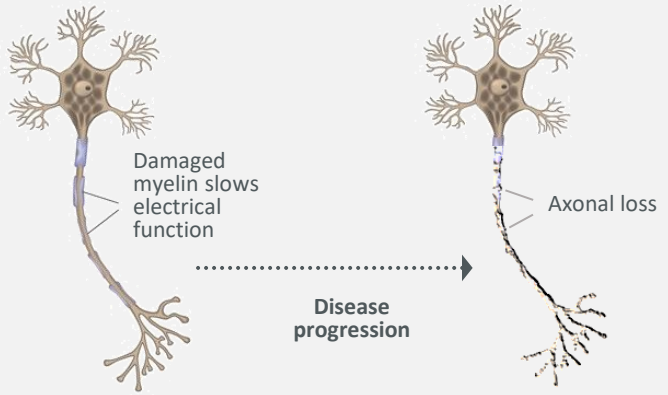
Primary Focus:
Immune Modulation

>20

approved therapies

CURRENTLY UNADDRESSED

Hallmark MS Pathology:
Myelin damage and axonal loss



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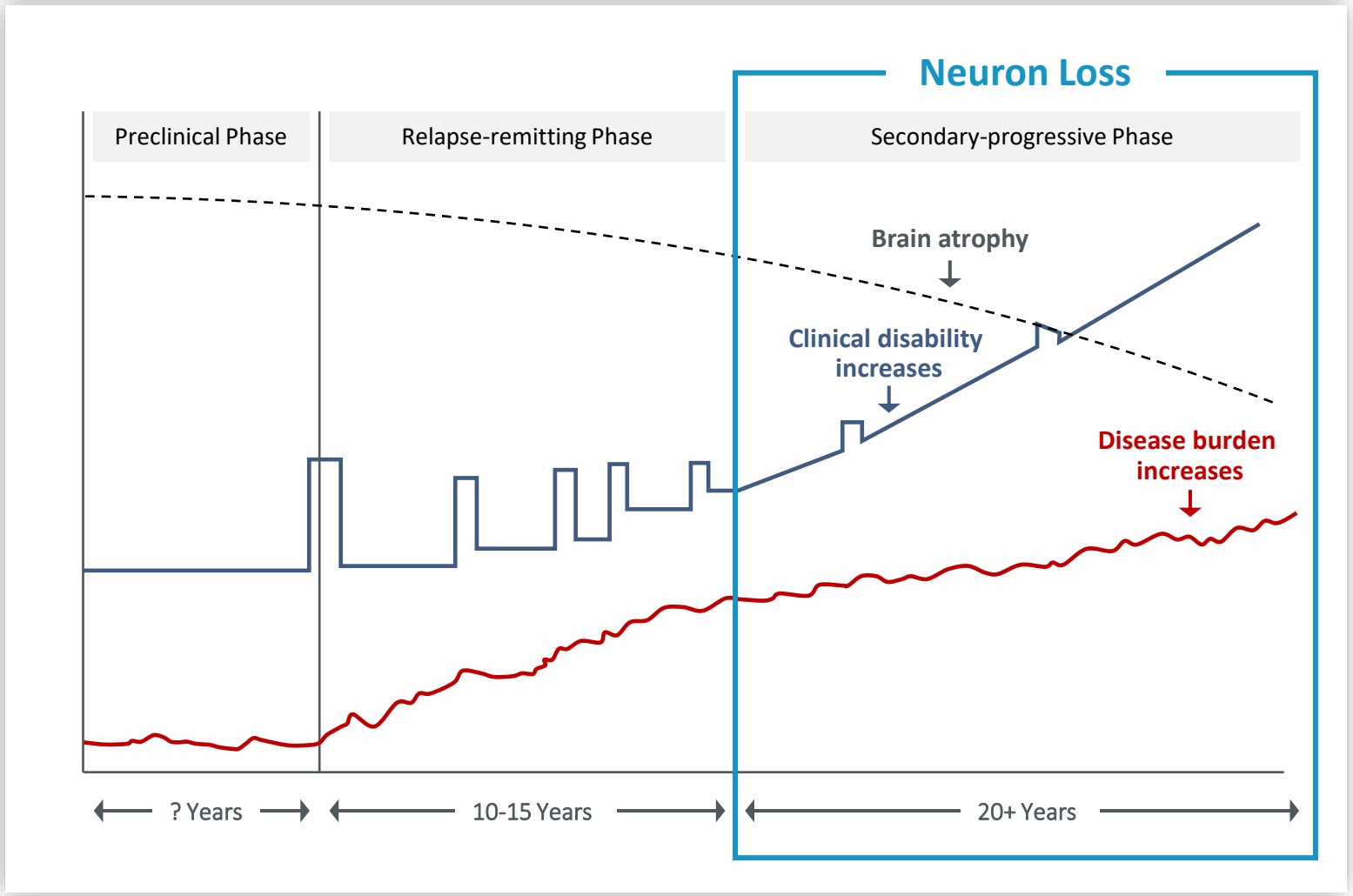
Demyelination and Axonal Degeneration Result in Disease Progression



THE NEED:

Support remyelination throughout the life of MS patients

Reduce disease progression

Restore axonal function

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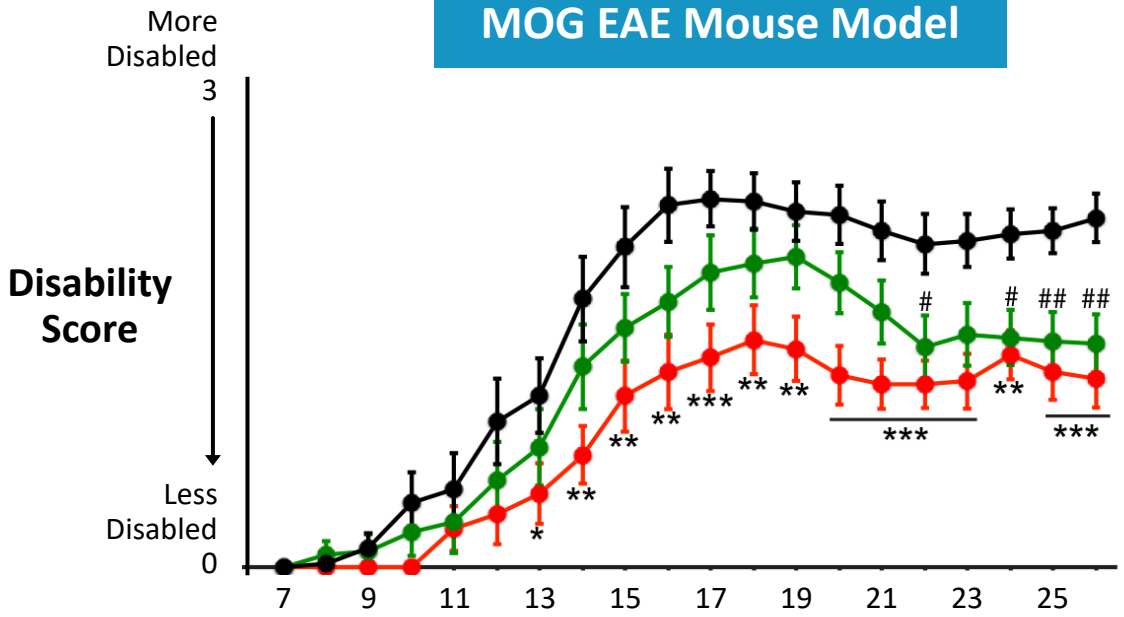
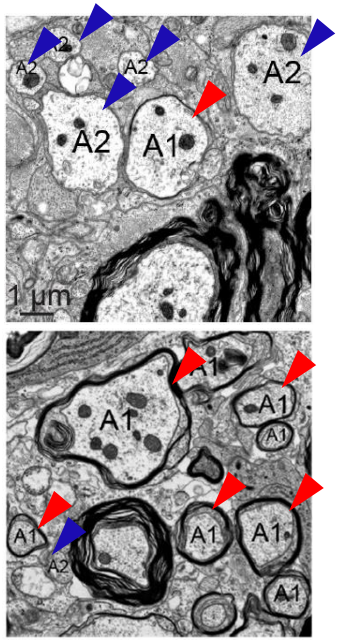
Preclinical Studies Showed M1R to be a Target for Remyelination

MOG EAE Mouse Model



+/- Control

-/- M1R knockout



Red A1 = Remyelinated Axon
Blue A2 = Unmyelinated Axon

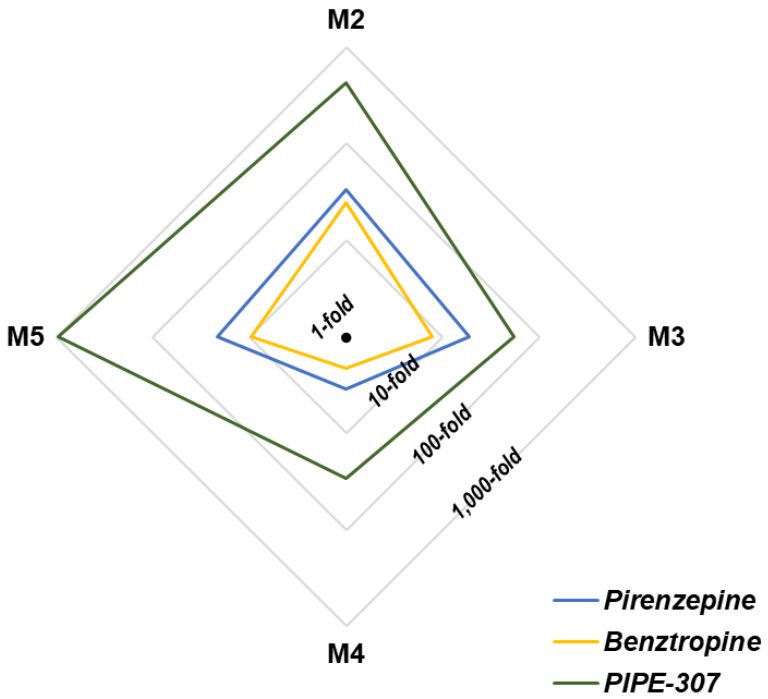
Jonah Chan, UCSF

M1R knockout
reduces clinical disability

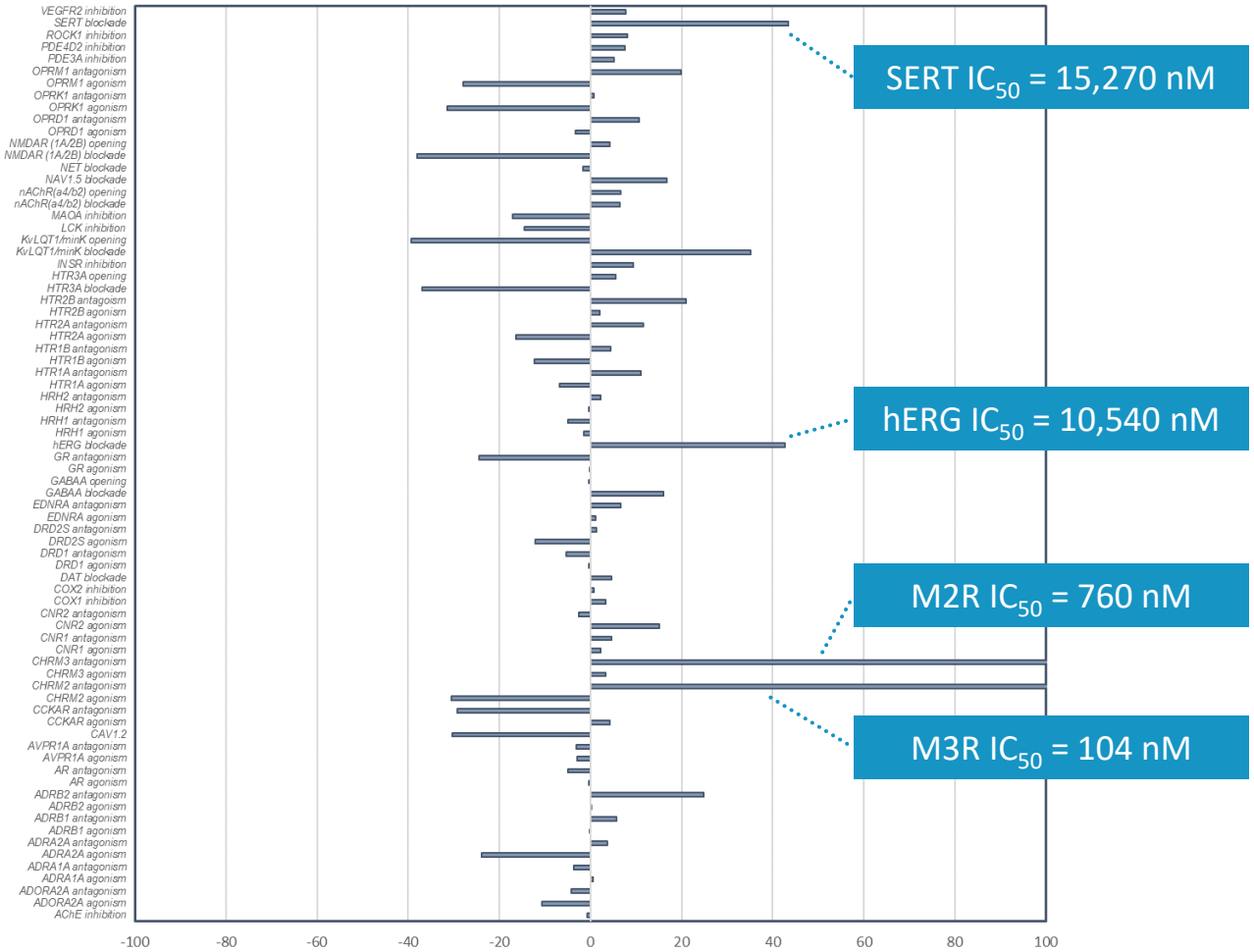
M1R knockout
increases remyelination

PIPE-307: *In Vitro* Profile

Properties	Profile				
Receptor (human)	M1R	M2R	M3R	M4R	M5R
Functional Ca^{2+} flux IC_{50} (nM)	3.8	1,600	210	110	3,600
Fold selectivity vs. M1R	-	420x	55x	29x	950x
Caco-2, P_{app} ($x10^6$ cm sec^{-1})	43 ~ 52				
Caco-2 Efflux Ratio	0.78 ~ 0.81				



Eurofin Functional SAFETYScan



% functional activity vs. control ligand and vehicle @ 10µM of **PIPE-307**

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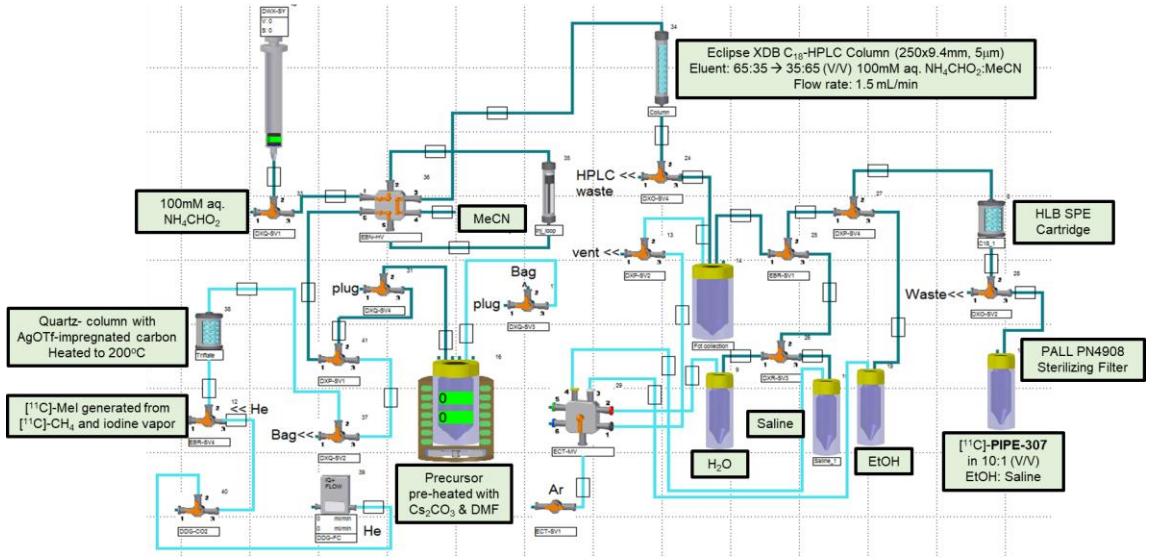
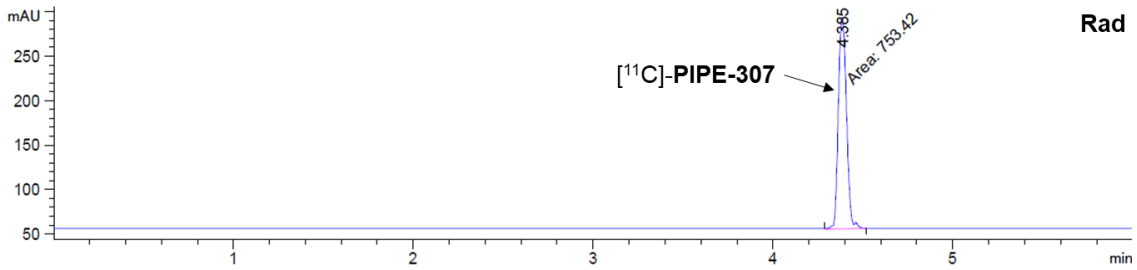
[^{11}C]-PIPE-307: Radiochemistry Implementation

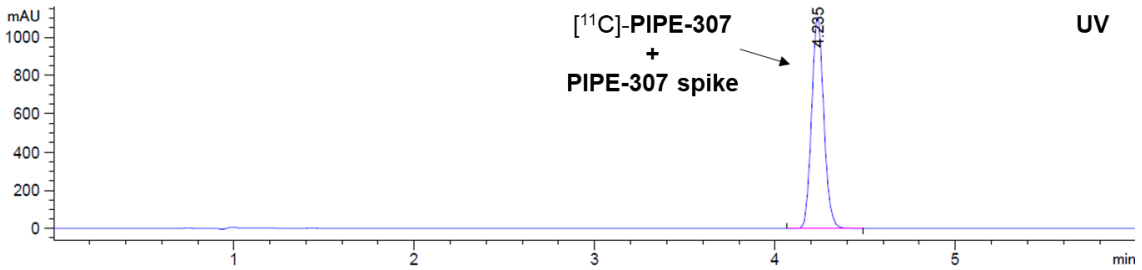
Precursor —(DMF, Cs_2CO_3, 80°C, 70min; then add [^{11}C]-MeOTf, 3min)→ **[^{11}C]-PIPE-307**

Expt	Activity of [^{11}C]-MeOTf	Isolated dose	RCY %	RCP %
1	6.90 GBq	743.3 MBq	11.5	100
2	7.05 GBq	790.8 MBq	11.2	100

Modular Lab™ (*Eckert & Ziegler*)







QC HPLC method: Eclipse XDB-C18 column (150x4.6 mm; 5 µm); Solv. A: AMF pH 8 100 mM; Solv. B= ACN; flow rate: 1.5 mL·min^{-1}; gradient elution: 45 to 90 % Solv. B over 10 min; λ =280 nm.

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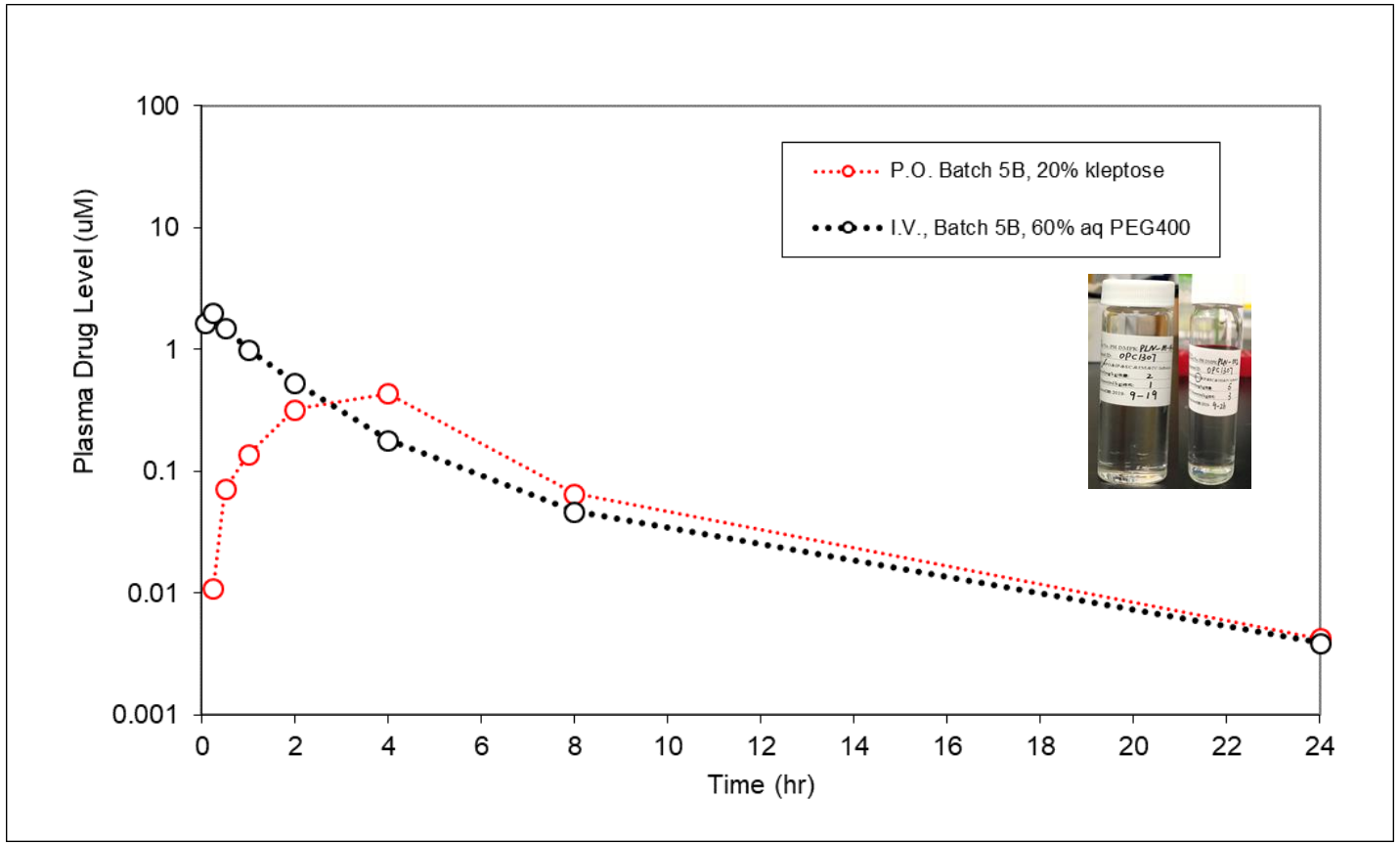
PIPE-307: *In Vivo* ADME Profile

Cynomolgus Macaque, Male (n=3)

P.O. @ 5mpk in 3mL/kg of 20% aq. Kleptose
I.V. @ 2mpk in 1mL/kg of 60% aq. PEG400

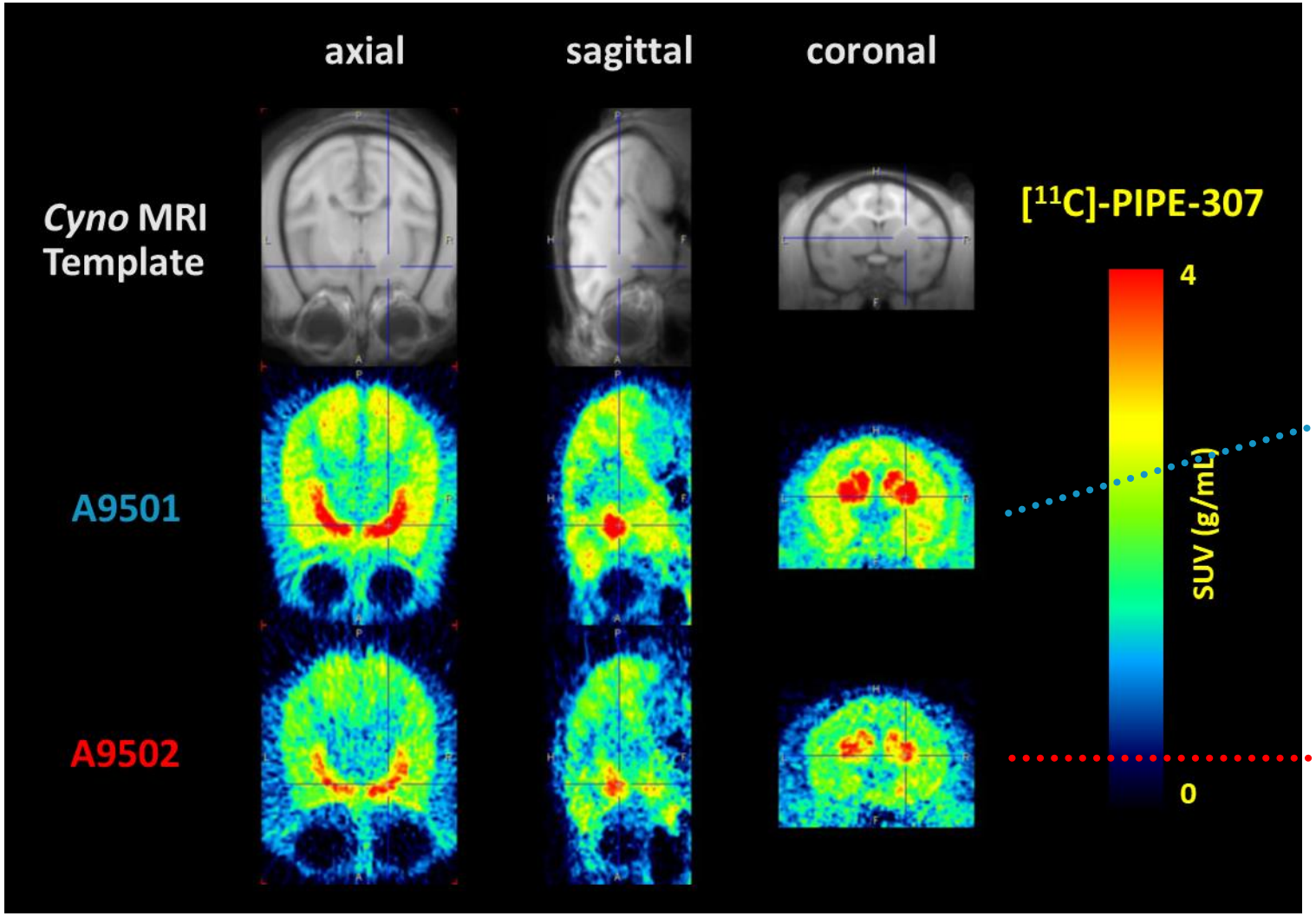


Properties	Profile
$AUC_{PO,\ 0\rightarrow t}$ (µg*h/mL)	1.1
$t_{½,\ PO}$ (h)	3.3
C_{max} (µM)	0.51 @ 3.3 h
F (%)	26
$AUC_{IV,\ 0\rightarrow t}$ (µg*h/mL)	1.6
F (%)	26
CL (mL/min/kg)	20.5
V_{dss} (L/kg)	3.1
$t_{½,\ IV}$ (h)	3.8
PPB (% Free)	13

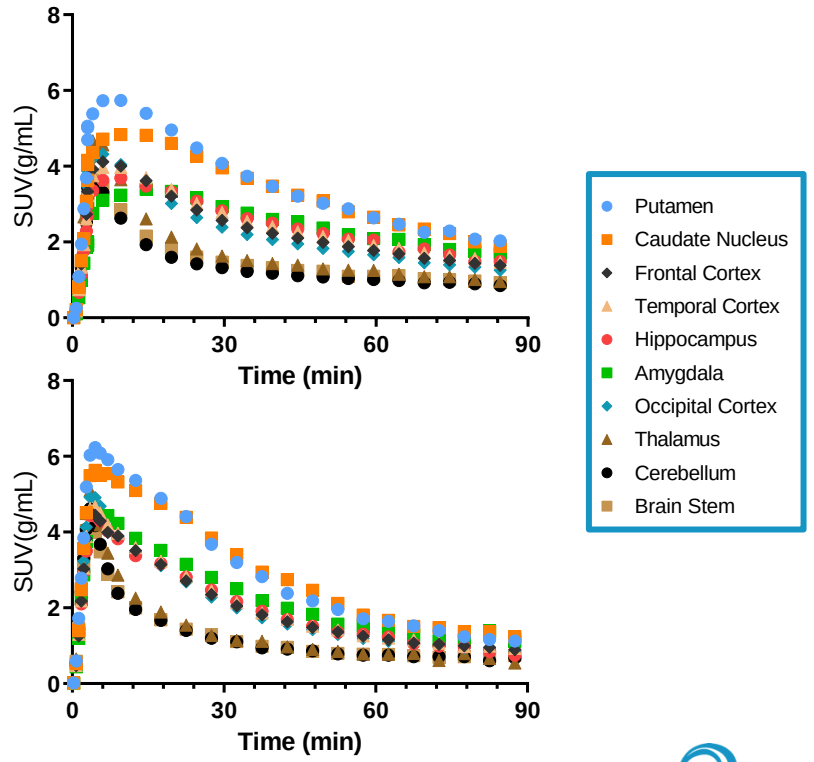
[^{11}C]-PIPE-307: NHP PET Study Design and Analysis Method

- Two subjects (A9501, male; A9502, female) underwent dynamic PET brain imaging.
- [^{11}C]-PIPE-307 radiotracer was administered via the saphenous vein at constant rate over 3 min.
- [^{11}C]-PIPE-307 brain image data were acquired on a Siemens *micro*PET Focus 220 scanner upon the start of tracer administration and continued for 90 min; arterial input function data were also collected.
- Images were normalized to a common *Cynomolgus Macaque* brain template and VOIs (**putamen**, **caudate nucleus**, amygdala, hippocampus, frontal cortex, temporal cortex, occipital cortex, thalamus, cerebellum, and brain stem) were defined on the brain template.
- Time activity curves (kBq/mL) were extracted from VOIs.
- Images and TACs were normalized to both animal weight and injected dose and shown as SUV (g/mL).

[^{11}C]-PIPE-307: NHP PET Baseline Images and Time Activity Curves



Subject	[^{11}C]-PIPE-307 dosed	
	Activity (mCi)	Mass (μg)
A9501	6.5	2.74
A9502	2.6	0.45



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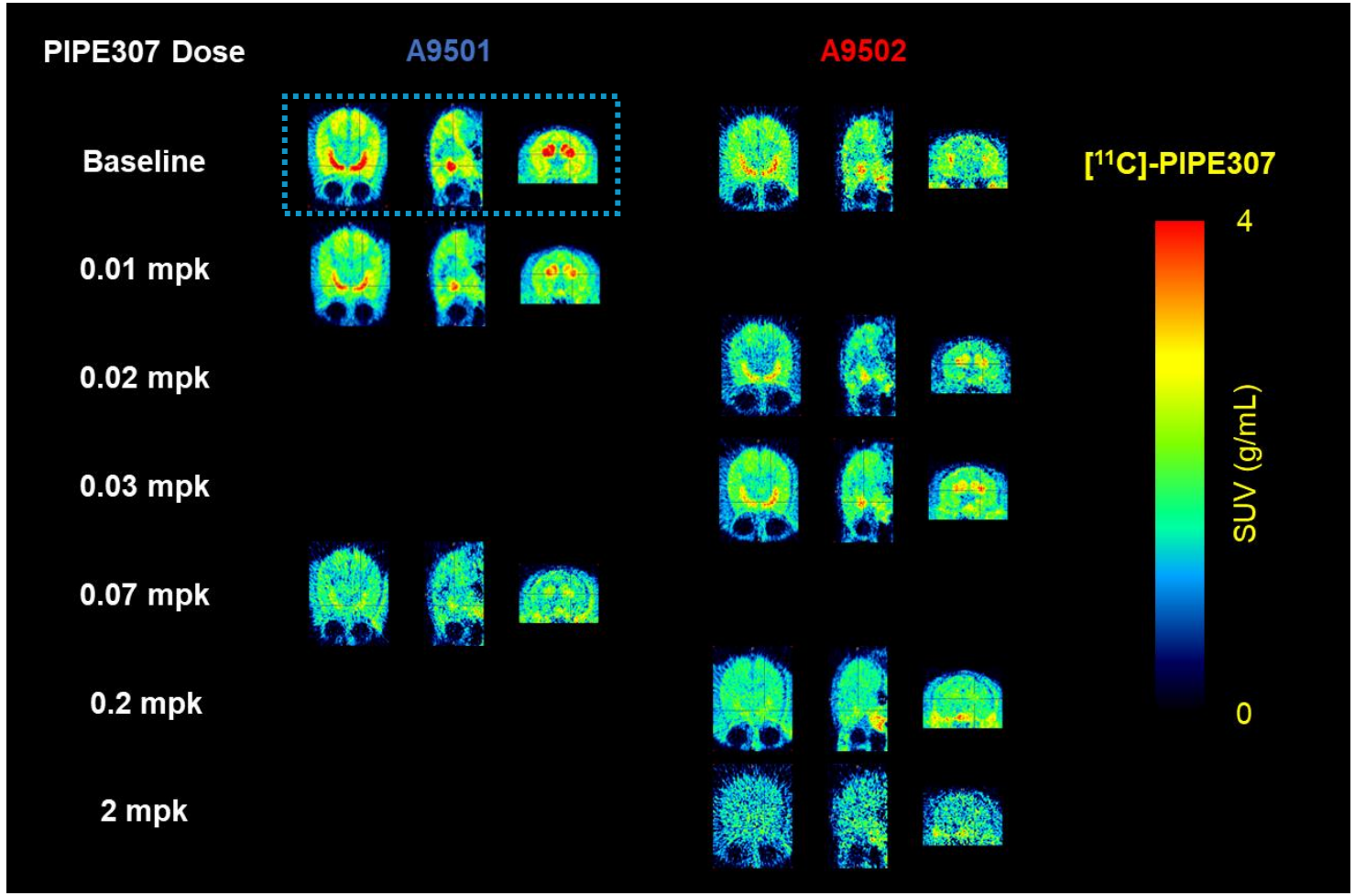
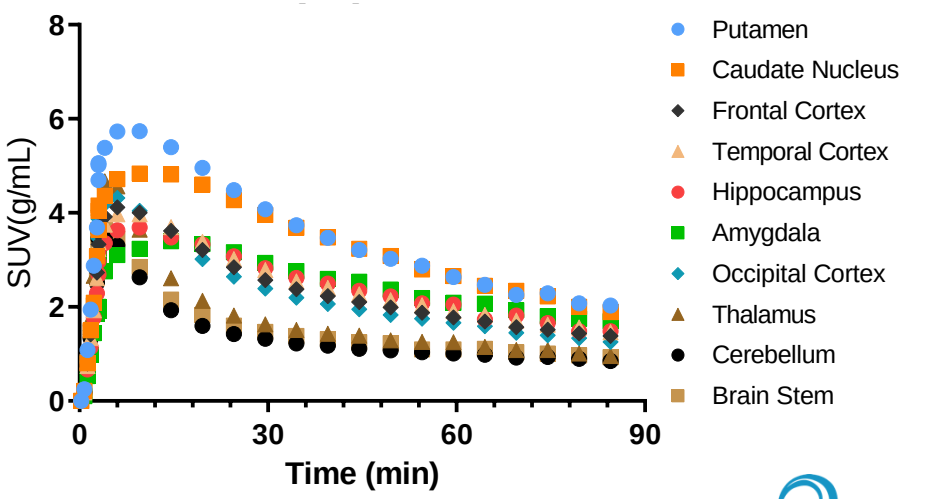
[^{11}C]-PIPE-307: NHP Dose Occupancy Relationship

- Homologous blockade with unlabeled PIPE-307, administered IV as 1.5 min bolus 5 min prior to radiotracer injection.



Blocking Dose with PIPE-307	[^{11}C]-PIPE-307 dosed	
	Activity (mCi)	Mass (μg)
Baseline	6.5	2.74

Time Activity Curve: Baseline



[^{11}C]-PIPE-307: NHP Dose Occupancy Relationship

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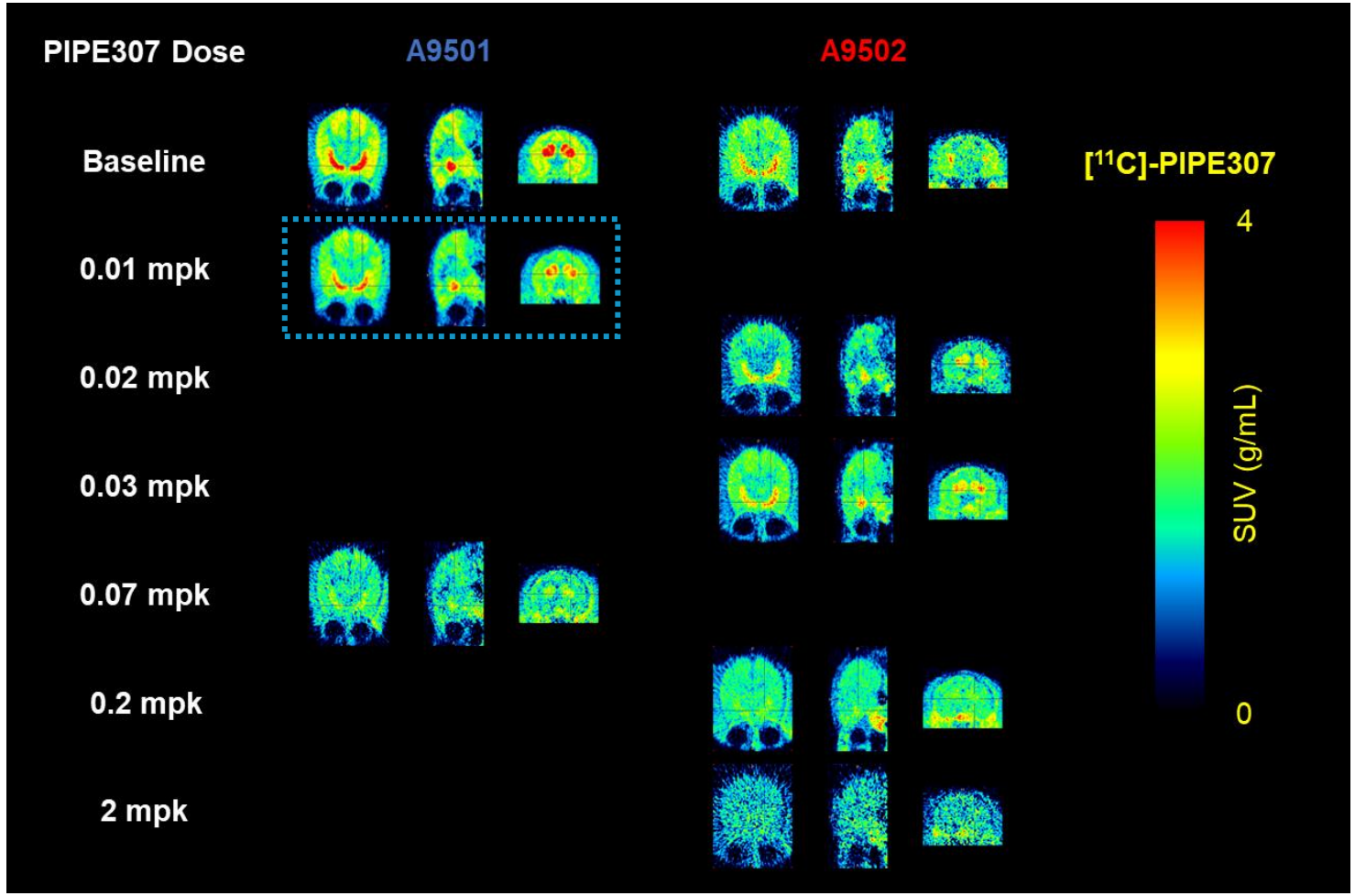
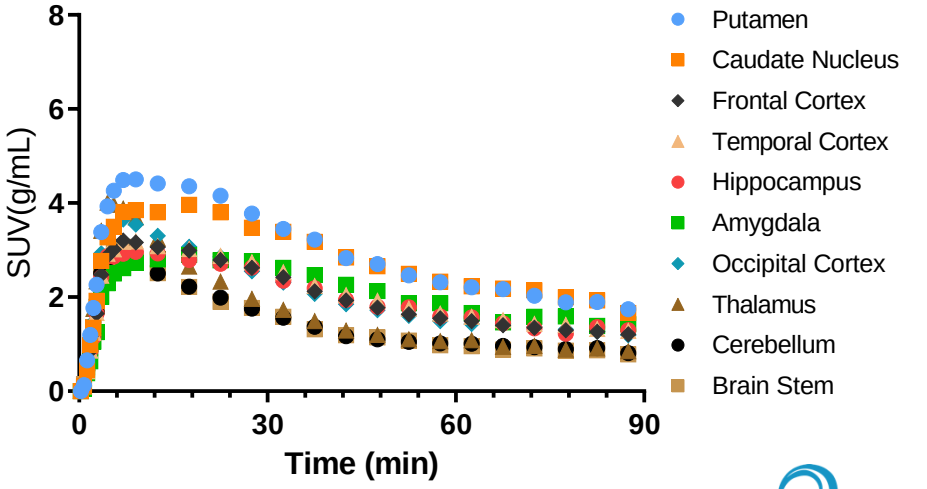


Blocking Dose with PIPE-307	[^{11}C]-PIPE-307 dosed	
	Activity (mCi)	Mass (μg)
Baseline	6.5	2.74
0.01 mpk	5.2	1.02

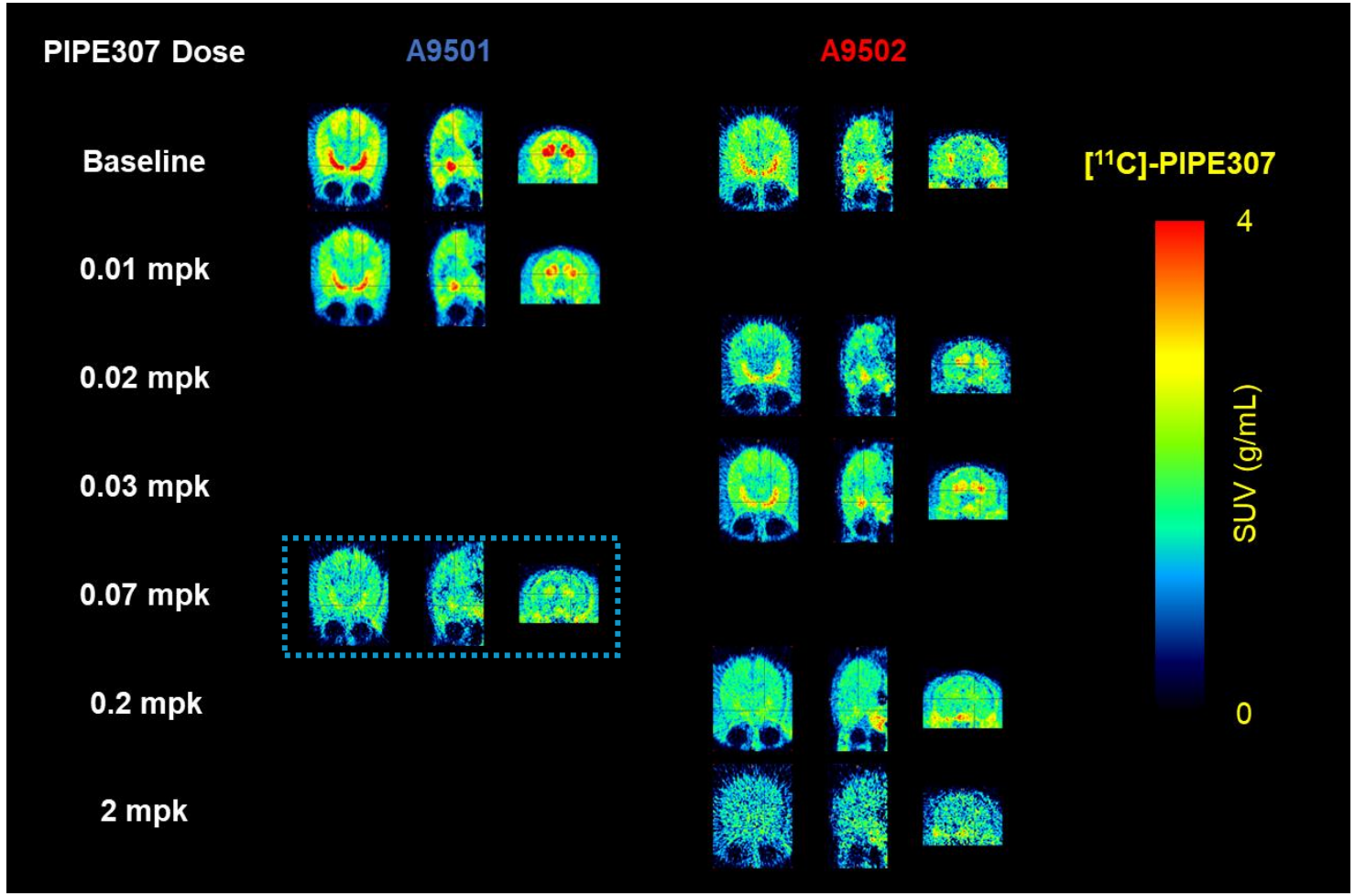
Time Activity Curve: 0.01 mpk PIPE-307



PIPELINE

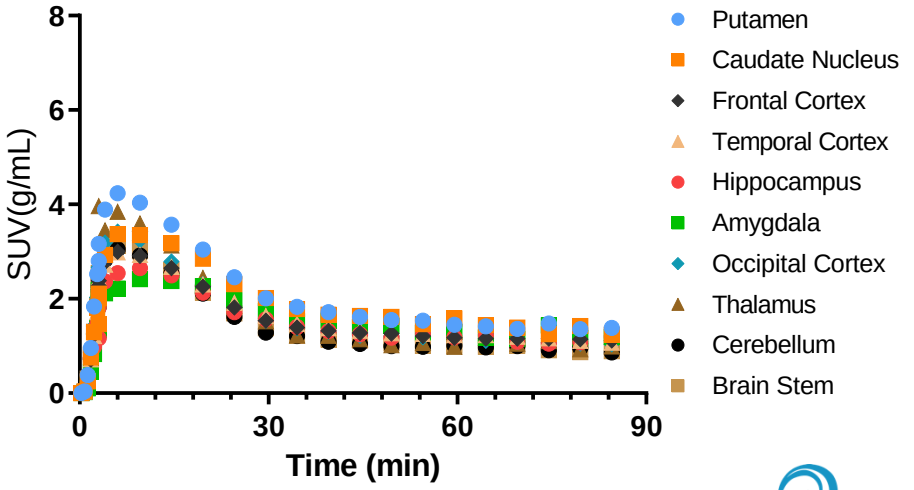
[^{11}C]-PIPE-307: NHP Dose Occupancy Relationship

- Homologous blockade with unlabeled PIPE-307, administered IV as 1.5 min bolus 5 min prior to radiotracer injection.



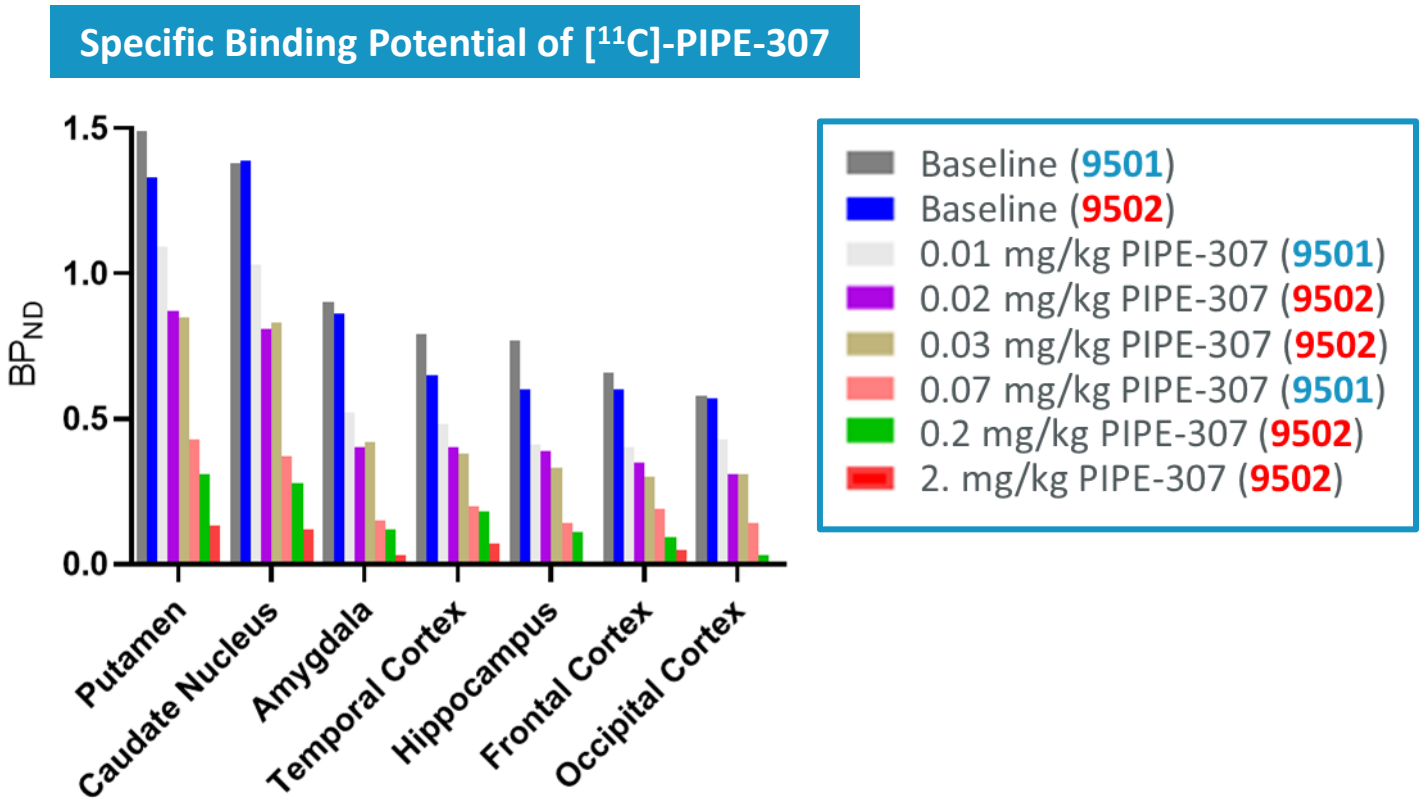
Blocking Dose with PIPE-307	[^{11}C]-PIPE-307 dosed	
	Activity (mCi)	Mass (μg)
Baseline	6.5	2.74
0.01 mpk	5.2	1.02
0.07 mpk	2.2	0.40

Time Activity Curve: 0.07 mpk PIPE-307



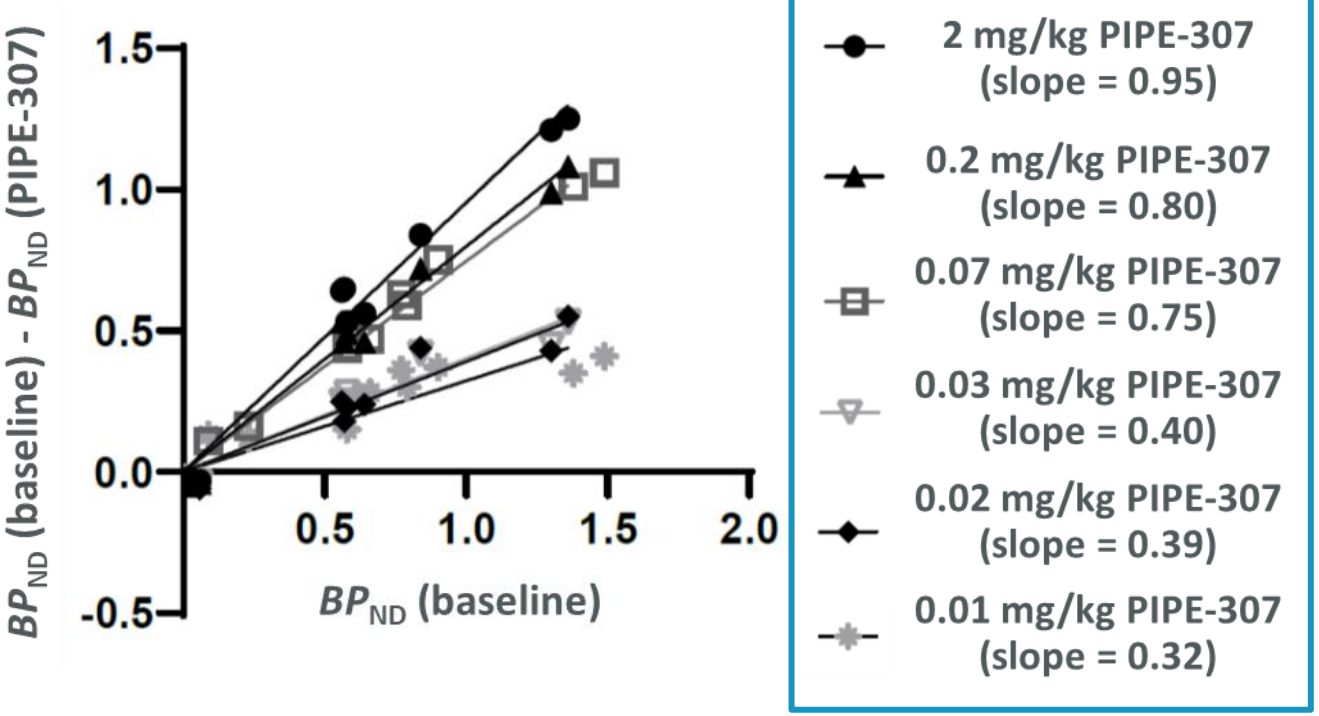
[^{11}C]-PIPE-307: NHP PET Study Outcome Measure

- Total volume of distribution was estimated using Logan graphical analysis with an equilibration cutoff time of 30 min.
- Specific binding potential (BP_{ND}, mL/cm^3) of [^{11}C]-PIPE-307 was determined using cerebellum as the reference region.

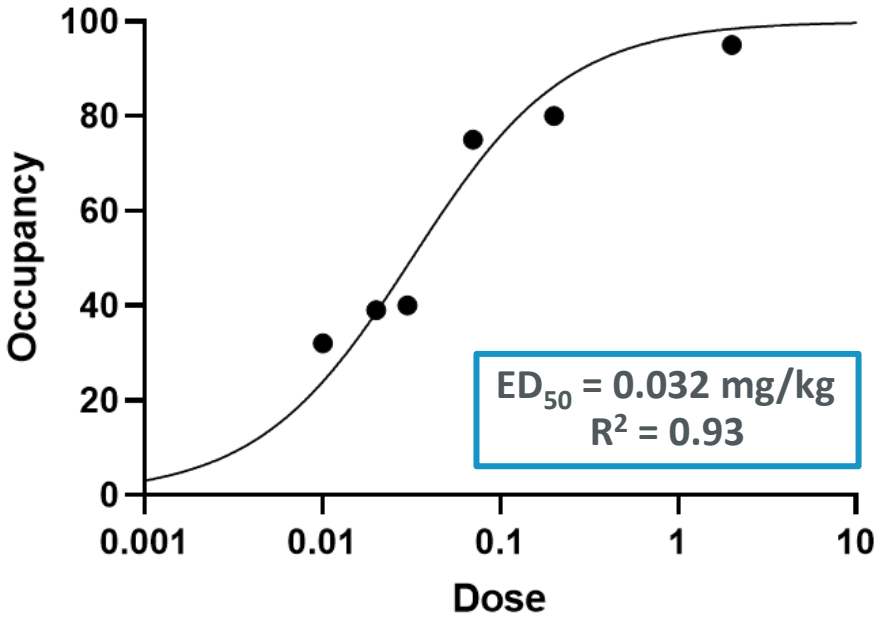


[^{11}C]-PIPE-307: NHP PET Study Outcome Measure

Occupancy Plot

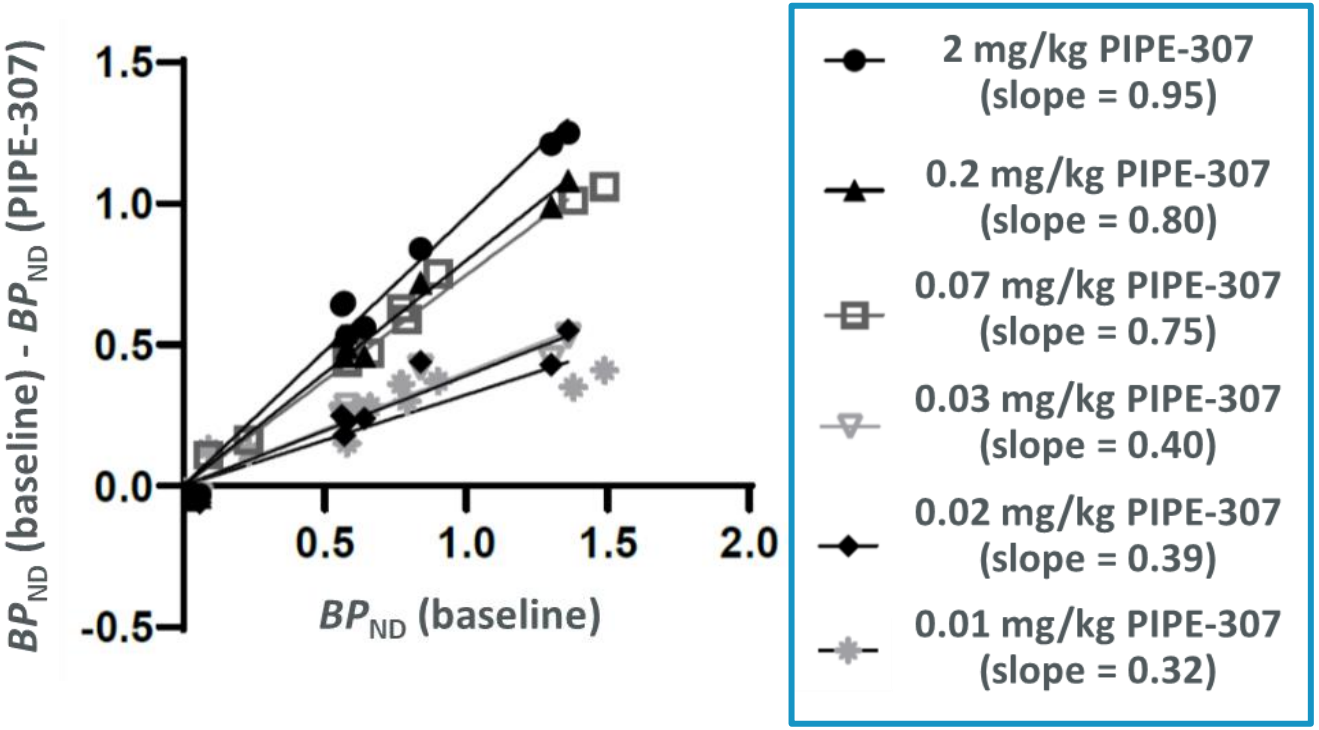


Global M1R Occupancy vs Dose

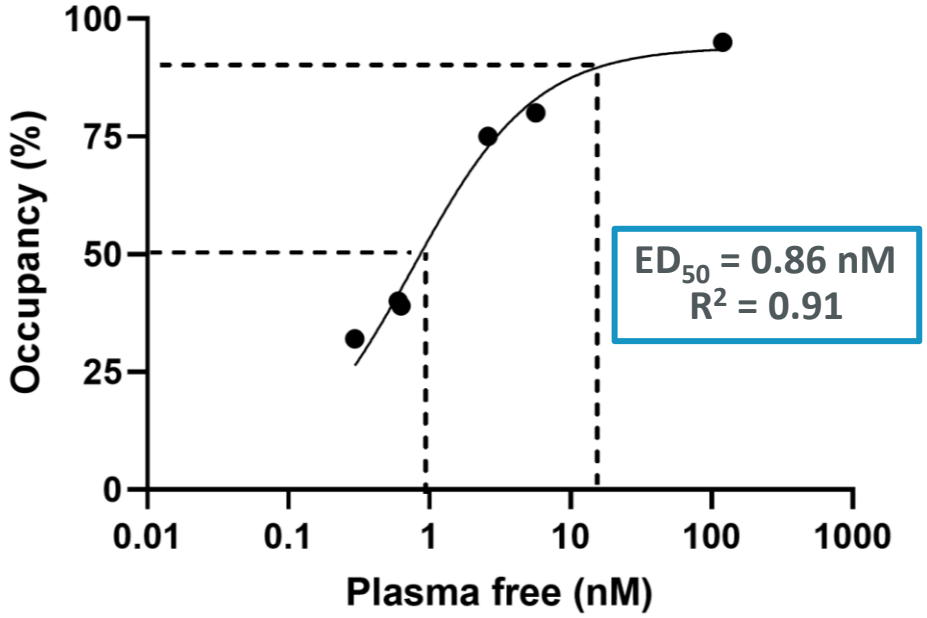


[^{11}C]-PIPE-307: NHP PET Study Outcome Measure

Occupancy Plot



Global M1R Occupancy vs Concentration



[^{11}C]-PIPE-307: Summary and Conclusion

- In cynomolgus monkeys, [^{11}C]-PIPE-307 demonstrated excellent brain uptake, reversible kinetics, and regional distribution consistent with M1 expression.
- BP_{ND} of [^{11}C]-PIPE-307, estimated using Logan graphical analysis with the cerebellum as the reference region, was 1.30 ~ 1.49 in caudate and putamen, and 0.56 ~ 0.90 in amygdala, hippocampus, frontal, occipital, and temporal cortices.
- Test-retest variability of [^{11}C]-PIPE-307 BP_{ND} was 2%, averaged across regions and subjects.
- Unlabeled PIPE-307 decreased BP_{ND} dose-dependently from baseline levels, and the fractional change in regional BP_{ND} was used to estimate global M1R occupancy: the ED_{50} and unbound EC_{50} of PIPE-307 were estimated to be 0.032 mg/kg and 0.86 nM, respectively.
- These results support the use of [^{11}C]-PIPE-307 for the assessment of central M1R occupancy in human subjects.

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