Inhibiting extracellular vesicle release for the treatment of Alzheimer's disease

CAROLYN TALLON, PHD

POSTDOCTORAL FELLOW

JOHNS HOPKINS DRUG DISCOVERY

Outline

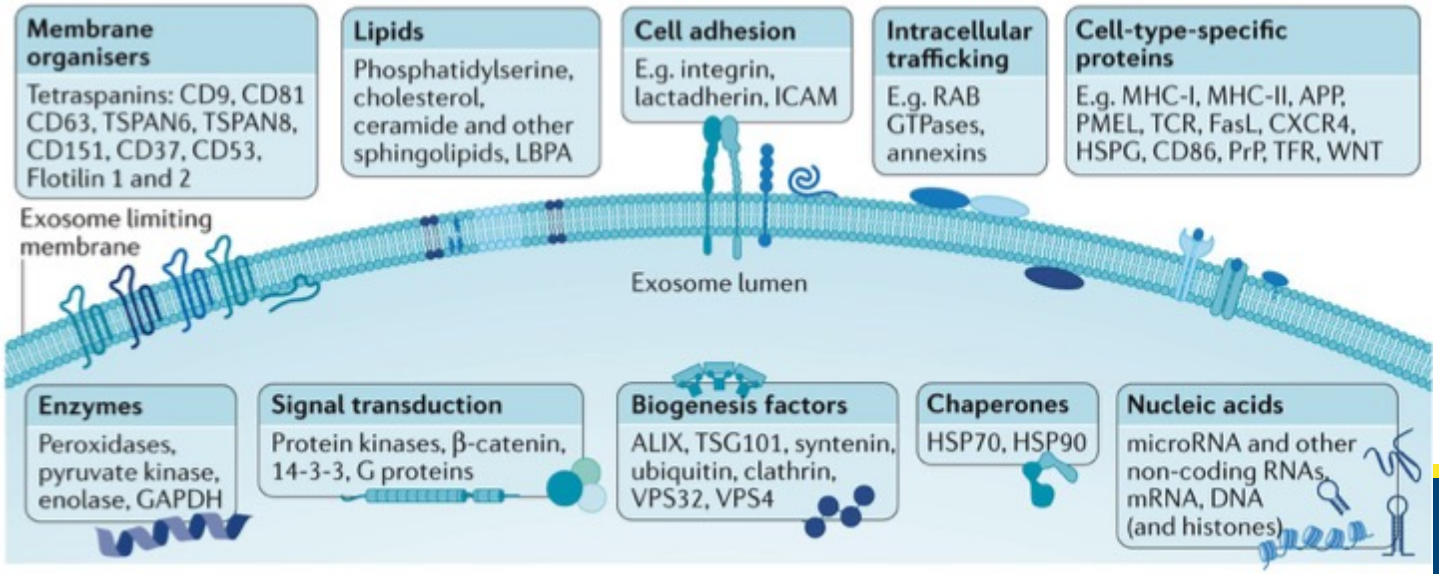
1. Introduction on extracellular vesicles and Alzheimer’s disease
2. Development and characterization of PDDC for the treatment of Alzheimer’s disease
3. Future plans
4. Summary

Outline

1. **Introduction on extracellular vesicles and Alzheimer's disease**
2. Development and characterization of PDDC for the treatment of Alzheimer's disease
3. Future plans
4. Summary

Extracellular vesicles: More than just garbage disposal

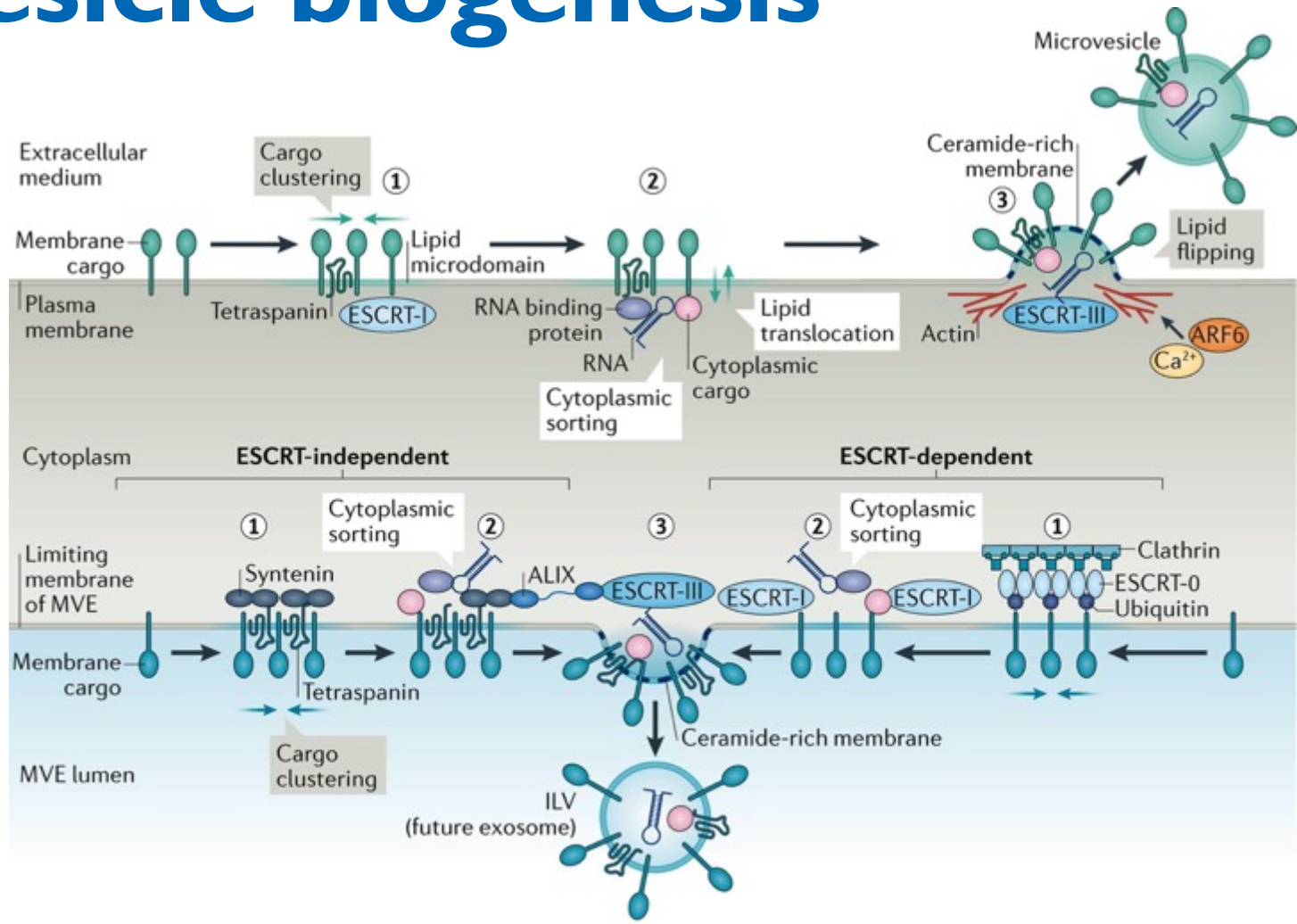
- Membrane bound vesicles originating from endosome and plasma membrane
- Range from 50-500nm in diameter
  - Exosomes are a subgroup of extracellular vesicles ranging from 50-150nm
- Important for numerous biological functions
  - Removal of waste, cell-cell communication, extracellular signaling
- Carry wide variety of cargo



Nature Reviews Molecular Cell Biology volume 19, pages213–228(2018)

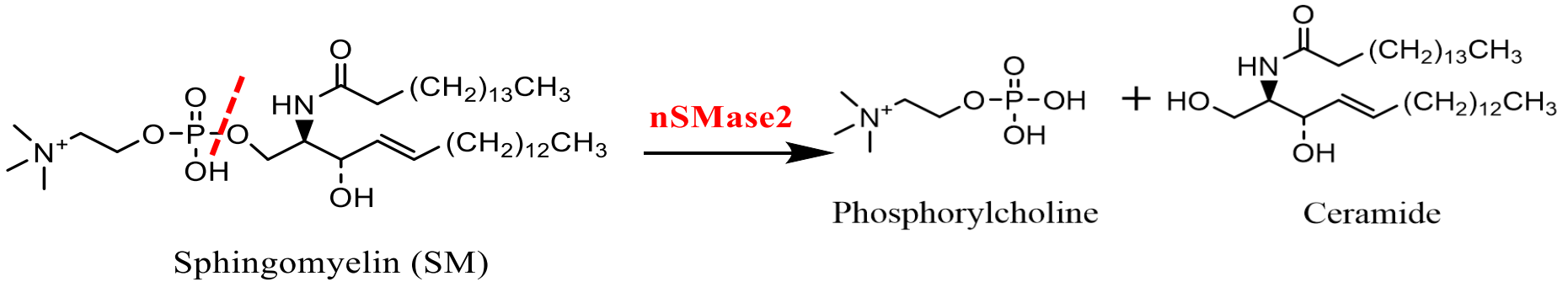
Extracellular vesicle biogenesis

- Canonical pathway utilizes the endosomal sorting complex required for transport (ESCRT) machinery
  - Proteins recruited to pinch vesicle off of the membrane
- Non-canonical pathways utilize physical properties of altering membrane lipid composition
  - Enriching for ceramides causes bending of membrane to form vesicles



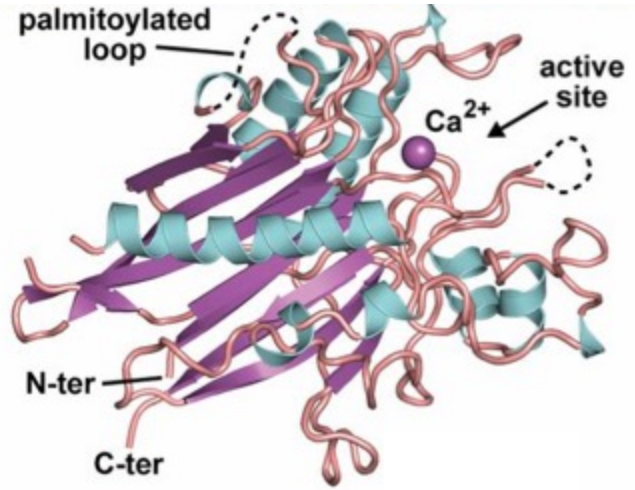
Nature Reviews | Molecular Cell Biology

Neutral Sphinghomyelinase 2 (nSMase2) catalyzes the formation of ceramide



Sphingomyelin (SM)

- Transmembrane protein situated on the inner leaflet
- Localized to the plasma membrane and golgi apparatus
- Highly expressed in the brain
- Has a hydrophobic N-terminal domain and catalytic C-terminal domain



Proceedings of the National Academy of Sciences Jul 2017, 114 (28) E5549-E5558; DOI: 10.1073/pnas.1705134114

Exosomes have been implicated in numerous diseases

Neutral Sphingomyelinase 2 (nSMase2)-dependent Exosomal Transfer of Angiogenic MicroRNAs Regulate Cancer Cell Metastasis*

Received for publication, December 18, 2012, and in revised form, February 18, 2013 Published, JBC Papers in Press, February 25, 2013, DOI 10.1074/jbc.M112.446831

The Neutral Sphingomyelinase Pathway Regulates Packaging of the Prion Protein into Exosomes*

Received for publication, August 15, 2014, and in revised form, December 1, 2014 Published, JBC Papers in Pre

Belinda B. Guo[‡§], Shayne A. Bellingham[‡§1], and Andrew F. Hill[‡§2]

From the ‡Department of Biochemistry and Molecular Biology, The University of Melb

Capture and transfer of HIV-1 particles by mature dendritic cells converges with the exosome-dissemination pathway

*Nuria Izquierdo-Useros,[1] *Mar Naranjo-Gómez,[2] Jacob Archer,[3] Steven C. Hatch,[3] Itziar Erkizia,[1] Julià Blanco,[1] Francesc E. Borràs,[2] Maria Carmen Puertas,[1] John H. Connor,[3] Maria Teresa Fernández-Figueras,[4] Landon Moore,[5] Bonaventura Clotet,[1] †Suryaram Gummuluru,[3] and †Javier Martinez-Picado[1,6]

Exosomes mediate Zika virus transmission through SMPD3 neutral Sphingomyelinase in cortical neurons

Evidence for secretion of Cu,Zn superoxide dismutase via exosomes from a cell model of amyotrophic lateral sclerosis

Catarina Gomes[a], Sascha Keller[b], Peter Altevogt[b], Júlia Costa[a,*]

Cell-Produced α-Synuclein Is Secreted in a Calcium-Dependent Manner by Exosomes and Impacts Neuronal Survival

[illegible][b] Michael R. Sherman[b,c] Girish Neelakanta[a] and Hameeda Sultana[a,d]

[illegible]kas H. Margaritis,[3] Leonidas Stefanis,[1,4] and Kostas Vekrellis[1]

[illegible]isions of [1]Basic Neurosciences and [2]Biotechnology, Biomedical Research Foundation of the Academy of Athens, Athens 11527, Greece, [3]Department of

Alzheimer's disease β-amyloid peptides are released in association with exosomes

Lawrence Rajendran*, Masanori Honsho*, Tobias R. Zahn*, Patrick Keller†, Kathrin D. Geiger‡, P and Kai Simons*§

*Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany; †Meso Scale MD 20977; and ‡Department of Neuropathology, Institute of Pathology, University Clinic, University of Technology, 01307 D

Exosome-associated Tau Is Secreted in Tauopathy Models and Is Selectively Phosphorylated in Cerebrospinal Fluid in Early Alzheimer Disease*

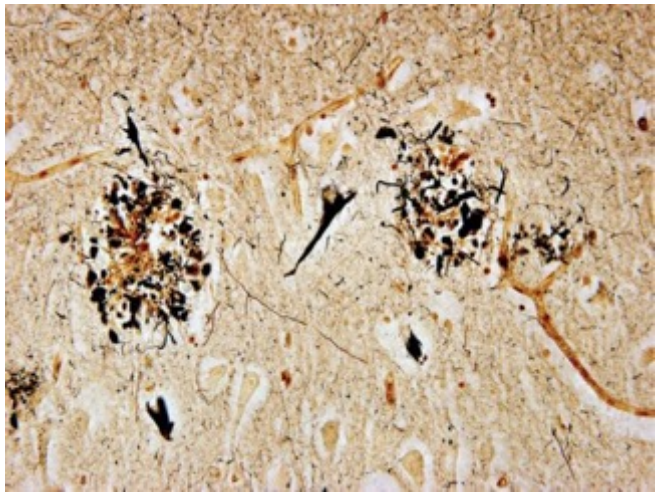
Received for publication, June 28, 2011, and in revised form, November 2, 2011 Published, JBC Papers in Press, November 4, 2011, DOI 10.1074/jbc.M111.277061

Sudad Saman[‡], WonHee Kim[‡], Mario Raya[§], Yvonne Visnick[§] Suhad Miro[§], Sarmad Saman[§], Bruce Jackson[§], Ann C. McKee[¶‖], Victor E. Alvarez[¶‖], Norman C. Y. Lee[**], and Garth F. Hall[‡1]

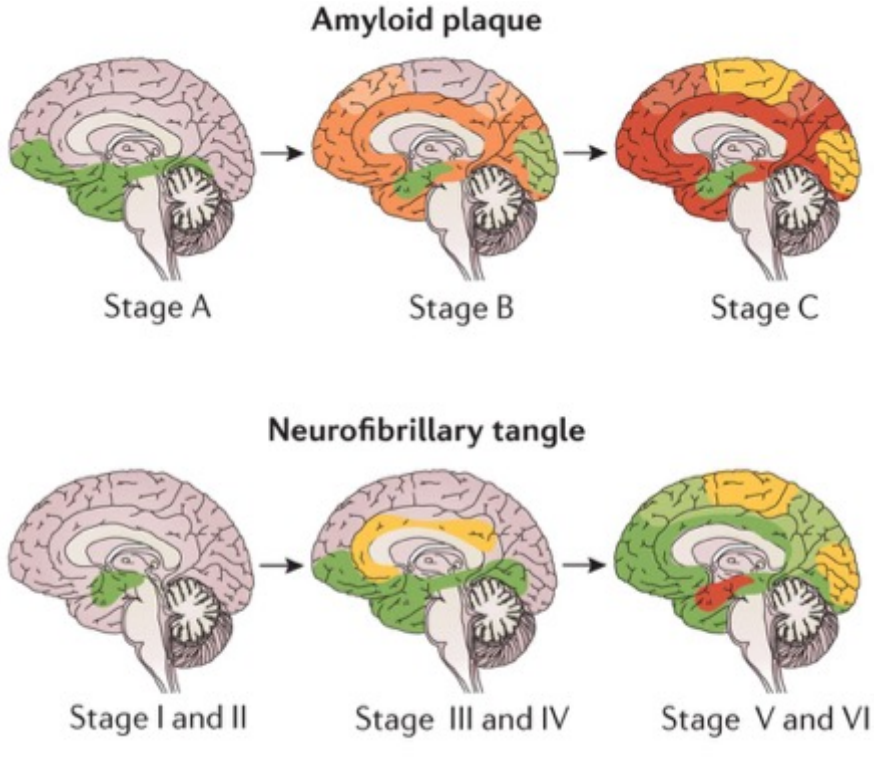
*From the ‡Department of Biological Sciences, University of Massachusetts, Lowell, Massachusetts 01854, the §MassBay Community College Science Department STEM Division, Wellesley Hills, Massachusetts 02481, the ¶GRECC Unit, Veterans Affairs Medical Center, Bedford, Massachusetts 01730, the ‖Departments of Neurology and Pathology, Boston University School of Medicine, Boston, Massachusetts 02215, and the **Chemical Instrumentation Center, Department of Chemistry, Boston University, Boston, Massachusetts 02215*

Alzheimer's disease

- Progressive neurodegenerative disease
  - Causes memory impairment and ultimately leads to death
- Major pathologies are amyloid plaques and neurofibrillary tangles
  - Familial AD cases have mutations leading to faulty amyloid β processing and tau mis-folding
- 5.8 million living with AD in US
  - Expected to increase to ~14 million by 2050
- No cure or treatment to slow disease progression
  - Treatments only target symptoms



Mt Sinai J Med. 2010;77(1):32–42.



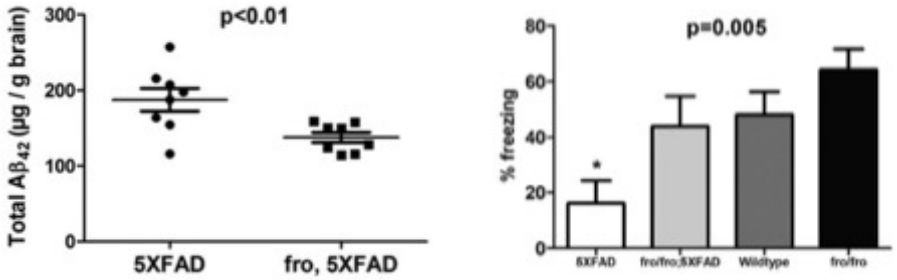
Nat Rev Dis Primers **1,** 15056 (2015)

Exosomes and neutral sphingomyelinase 2 in Alzheimer's disease

- Elevated ceramide levels have been detected in AD patient plasma and CSF (*Neuroscience* **130**, 657-666 (2005); *Neurology* **79**, 633-641 (2012))
- Neuronal exosomes isolated from Alzheimer's disease patient plasma have increased AD associated proteins such as phosphorylated tau (*Alzheimers Dement. 2015;11(6):600–7.e1; JAMA Neurol. 2019;76(11):1340–1351.)*
  - Analysis of neuronally derived exosome content had some predictive abilities
- In the case of Alzheimer's disease, isolated exosomes carrying tau are able to seed tau aggregation *(J Biol Chem 291,24 (2016): 12445-66)*

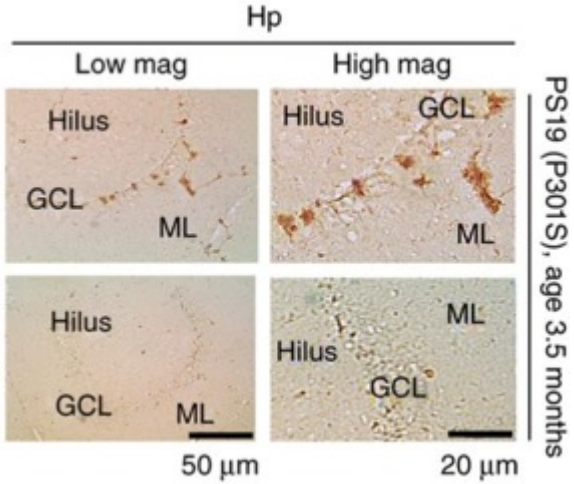
Genetic and pharmacological reduction of nSMase2 improves AD mouse models

Genetic deletion of nSMase2 in the 5XFAD mouse reduces Aβ; improves memory in contextual fear conditioning



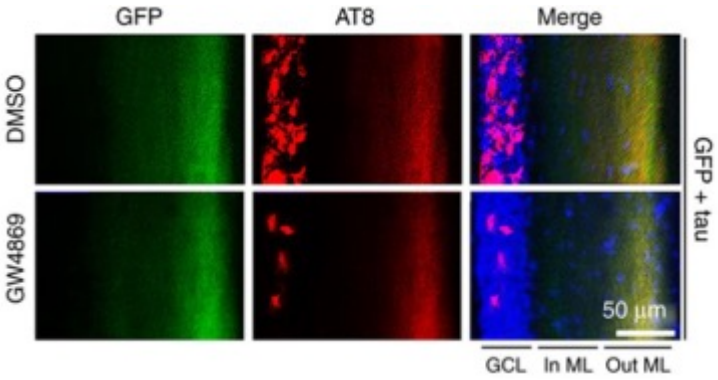
Dinkins et al., *J Neurosci (2016)*

nSMase2 inhibition reduces tau accumulation in the PS19 mouse model



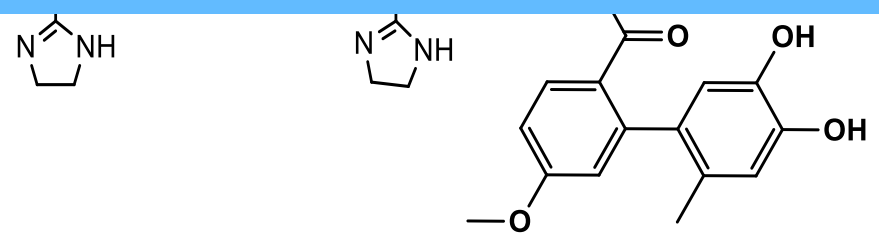
Asai et al. *Nat Neurosci* (2015)

nSMase2 inhibition slows propagation in an AAV-tau injection model



Asai et al. *Nat Neurosci* (2015)

No drug-like nSMase2 inhibitors

SMase inhibitor	Chemical Structure	IC_{50} (μM)	MW	Ref
3-O-				l
GW				l 9
Altenusin		28	290	1999 J Antibiot (Tokyo) Vol 52 p572-4

OBJECTIVE

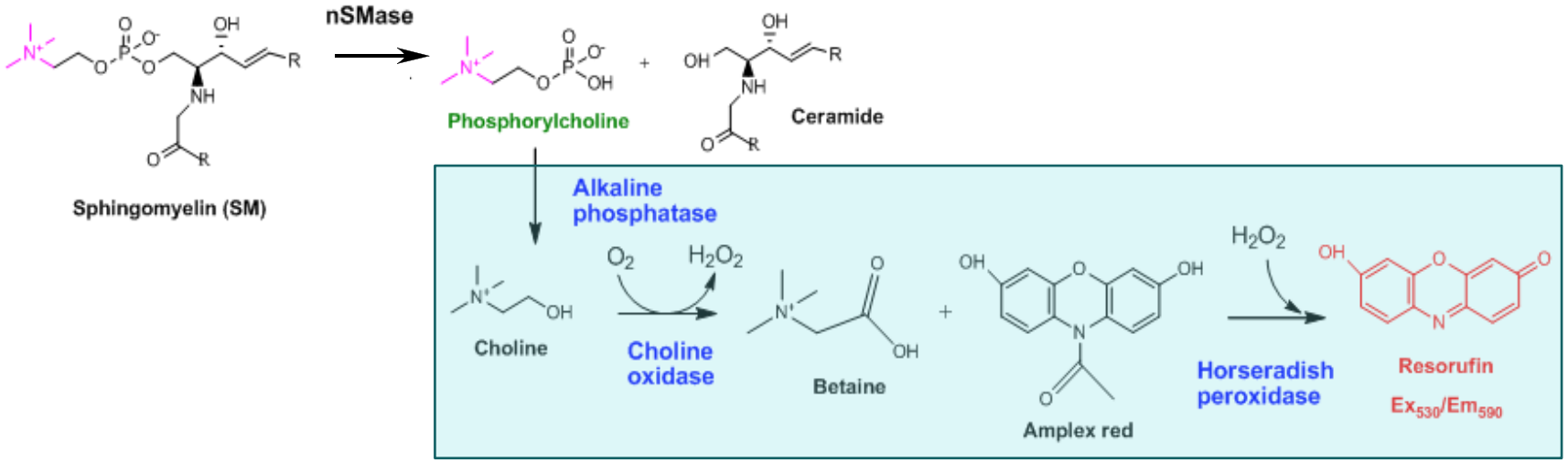
IDENTIFY INHIBITORS OF nSMase2

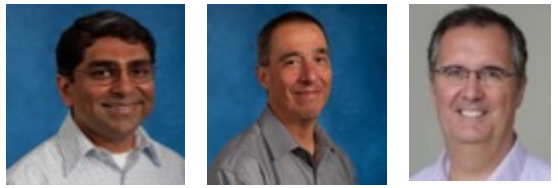
Insoluble, high molecular weight, low potency

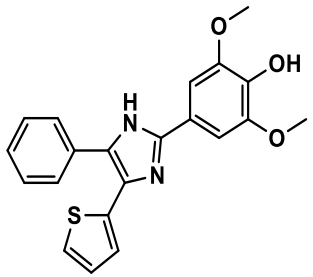
Outline

1. Introduction on extracellular vesicles and Alzheimer's disease
2. **Development and characterization of PDDC for the treatment of Alzheimer's disease**
3. Future plans
4. Summary

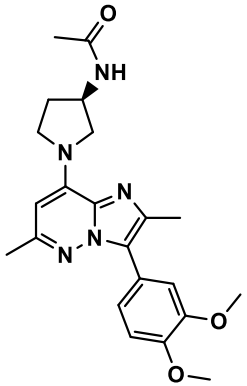
Screened > 365,000 Compounds for human nSMase2 Inhibitors





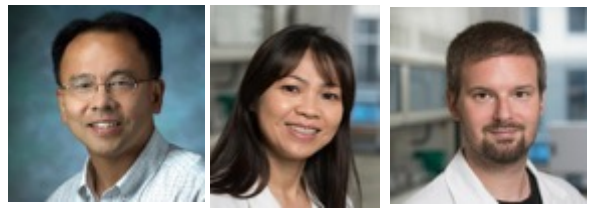


DPTIP (NCATS)

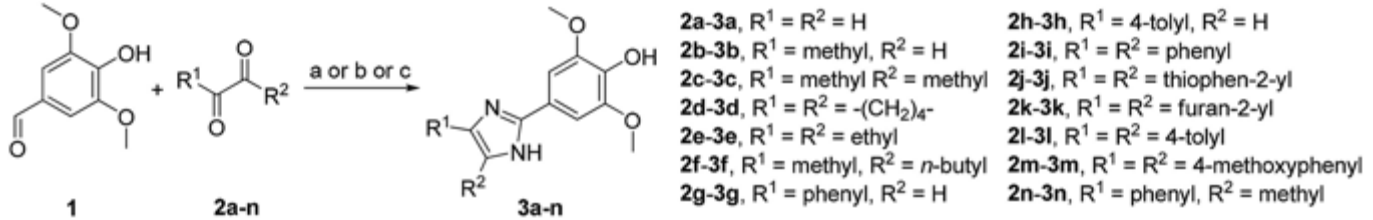


MCK380 (Internal Library)

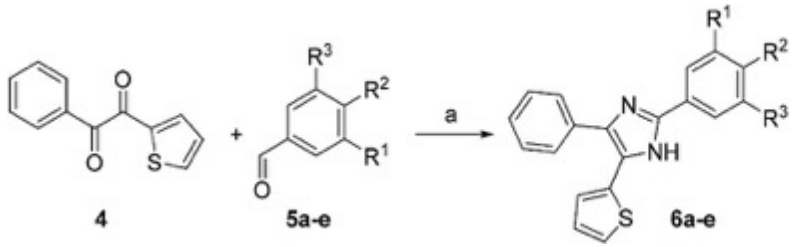
Extensive chemistry efforts around hit "DPTIP"



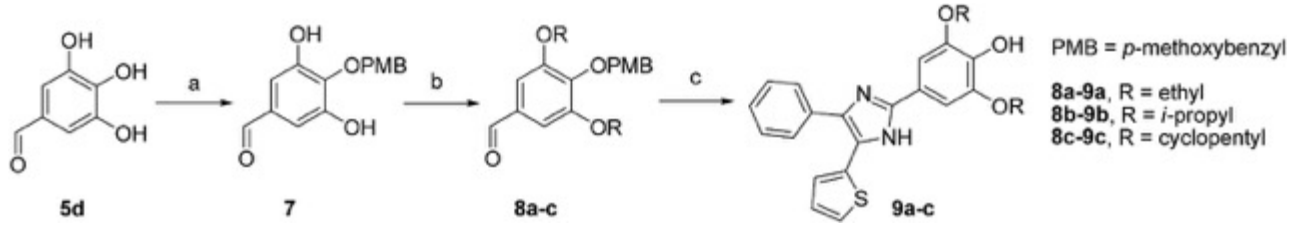
T Tsukamoto, N Hin, O Stepanek



2a-3a, $R^1 = R^2 = H$
2b-3b, R^1 = methyl, $R^2 = H$
2c-3c, R^1 = methyl R^2 = methyl
2d-3d, $R^1 = R^2 = -(CH_2)_4-$
2e-3e, $R^1 = R^2$ = ethyl
2f-3f, R^1 = methyl, R^2 = *n*-butyl
2g-3g, R^1 = phenyl, $R^2 = H$
2h-3h, R^1 = 4-tolyl, $R^2 = H$
2i-3i, $R^1 = R^2$ = phenyl
2j-3j, $R^1 = R^2$ = thiophen-2-yl
2k-3k, $R^1 = R^2$ = furan-2-yl
2l-3l, $R^1 = R^2$ = 4-tolyl
2m-3m, $R^1 = R^2$ = 4-methoxyphenyl
2n-3n, R^1 = phenyl, R^2 = methyl

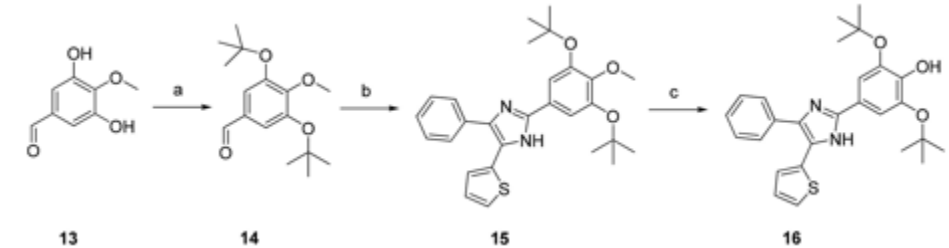


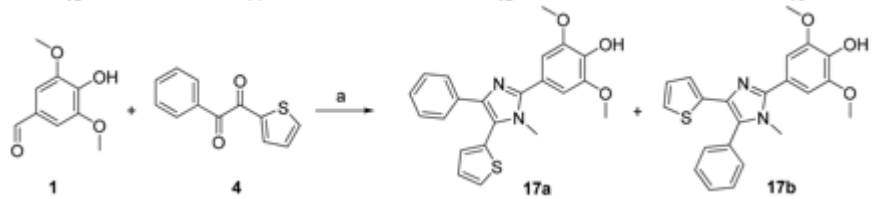
5a-6a, R^1 = OMe, R^2 = OH, $R^3 = H$
5b-6b, $R^1 = H$, R^2 = OH, $R^3 = H$
5c-6c, R^1 = OMe, R^2 = OH, R^3 = OH
5d-6d, R^1 = OH, R^2 = OH, R^3 = OH
5e-6e, R^1 = Cl, R^2 = OH, R^3 = Cl

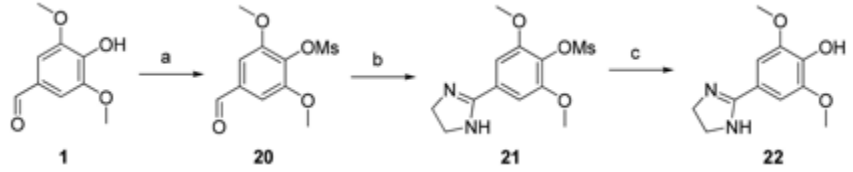


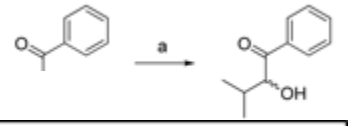
PMB = *p*-methoxybenzyl

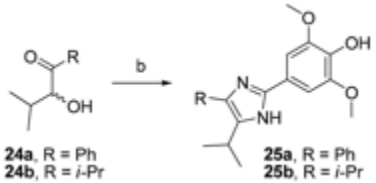
8a-9a, R = ethyl
8b-9b, R = *i*-propyl
8c-9c, R = cyclopentyl

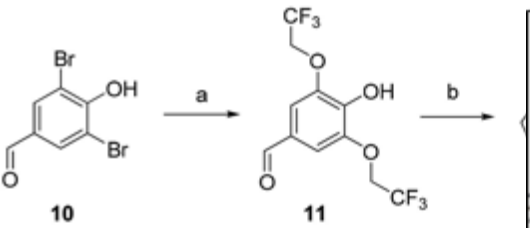


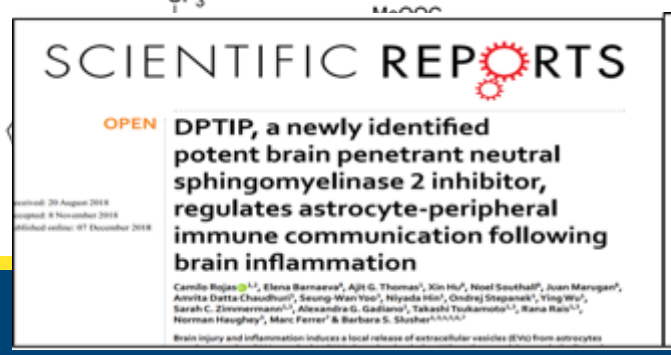


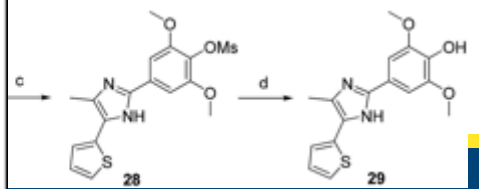




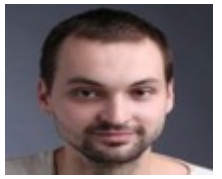






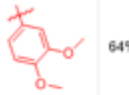
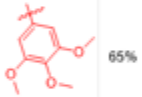
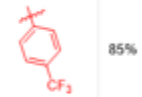
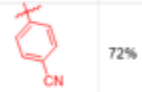
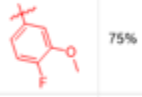
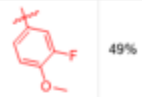
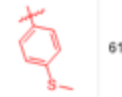


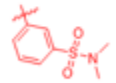
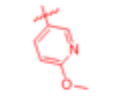
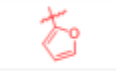
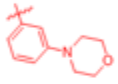
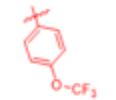
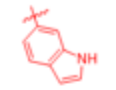
Extensive chemistry around hit “MCK380”

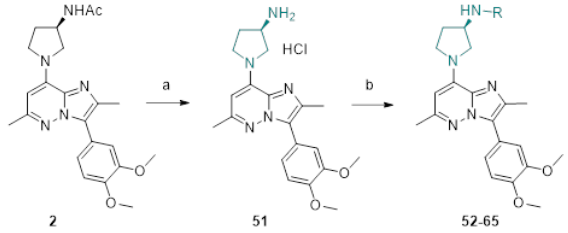


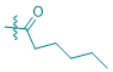
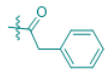
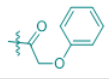
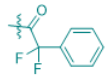
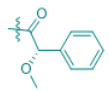
Radim Nencka

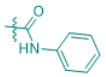
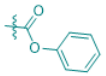
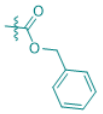
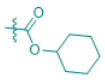
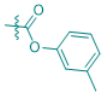
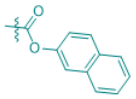
Michal Sala

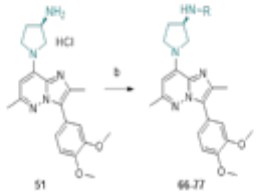
Compnd	Method	Ar	Yield
2	A		64%
35	A		93%
36	A		65%
37	A		85%
38	A		72%
39	A		75%
40	A		49%
41	A		61%

Compnd	Method	Ar	Yield
42	A		50%
43	A		45%
44	B		68%
45	B		52%
46	B		62%
47	A		82%
48	A		84%
49	A		40%



Compnd	Method	R	Yield
52	A		86%
53	A		65%
54	A		59%
55	A		74%
56	A		69%
57	B		48%
58	B		75%

Compnd	Method	R	Yield
59	C		47%
60	D		49%
61	E		72%
62	E		41%
63	F		36%
64	F		49%
65	F		45%



Compnd	Method	R	Yield
66	A		67%
67	A		35%
68	A		69%
69	A		77%
70	A		86%
71	A		76%

Compnd	Method	R	Yield
72	A		80%
73	A		75%
74	A		76%
75	A		
76	A		
77	B		

Compnd	R	Yield compnd a	Yield compnd b	Yield compnd c
78		79%	65%	73%
79		81%	85%	93%
80		73%	65%	86%
81		94%	65%	40%
82		97%	92%	68%

Received: 16 August 2019 | Revised: 12 June 2019 | Accepted: 13 June 2019

DOI: 10.1111/bph.14789

RESEARCH PAPER

BJP BRITISH JOURNAL OF PHARMACOLOGY

A novel and potent brain penetrant inhibitor of extracellular vesicle release

Camilo Rojas[1,2] | Michal Sala[8] | Ajit G. Thomas[1] | Amrita Datta Chaudhuri[3] | Seung-Wan Yoo[3] | Zhigang Li[3] | Ranjeet P. Dash[1] | Rana Rais[1,3] | Norman J. Haughey[3] | Radim Nencka[8] | Barbara Slusher[1,3,4,5,6,7]

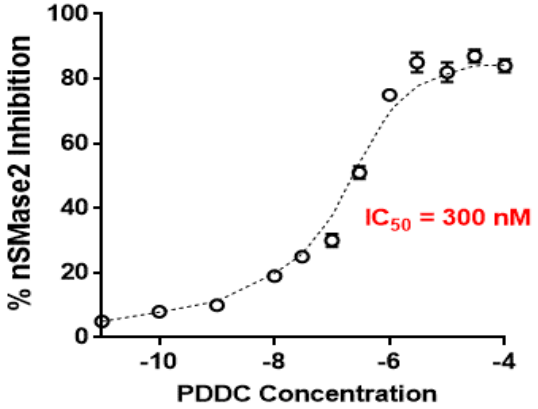
[1]Johns Hopkins Drug Discovery, Johns Hopkins School of Medicine, Baltimore, Maryland
[2]Department of Molecular and Comparative Pathobiology, Johns Hopkins School of Medicine, Baltimore, Maryland
[3]Department of Neurology, Johns Hopkins School of Medicine, Baltimore, Maryland
[4]Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine,

Background and Purpose: Extracellular vesicles (EVs) are constitutively shed from cells and released by various stimuli. Their protein and RNA cargo are modified by the stimulus, and in disease conditions can carry pathological cargo involved in disease progression. Neutral sphingomyelinase 2 (nSMase2) is a major regulator in at least one of several independent routes of EV biogenesis, and its inhibition is a promising new therapeutic approach for neurological disorders. Unfortunately, known inhibitors exhibit μM potency, poor physicochemical properties, and/or limited brain

Hit optimization led to our lead compound - PDDC

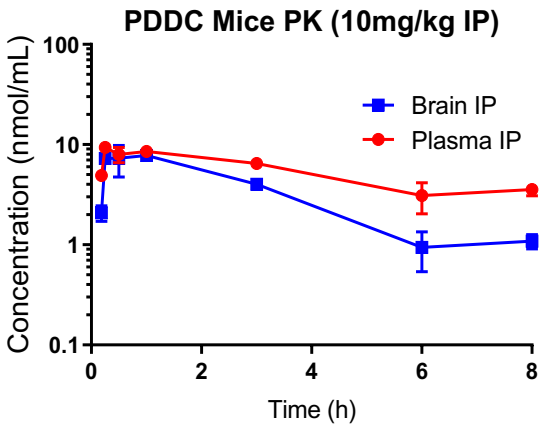
potent, brain penetrable, orally bioavailable, selective nSMase2 inhibitor

nM POTENCY



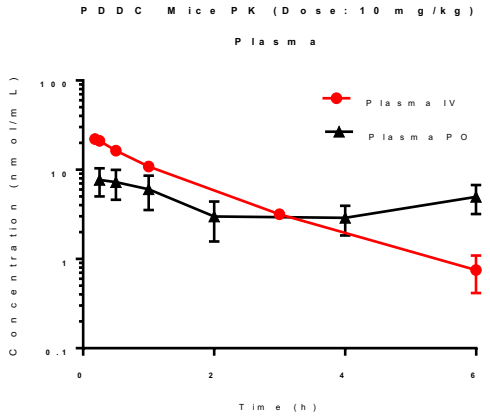
BRAIN PENETRABLE

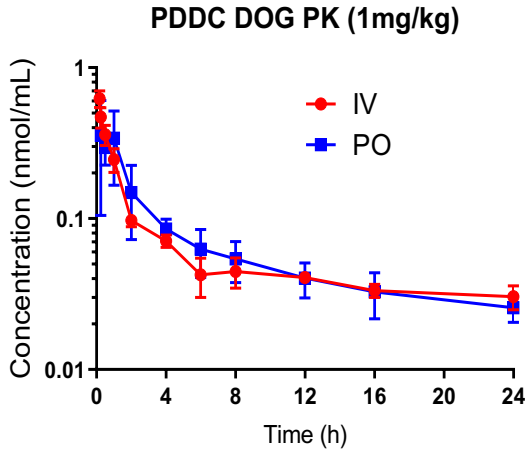
AUC_{brain}/AUC_{plasma}=0.6



ORALLY BIOAVAILABLE IN MICE AND DOGS

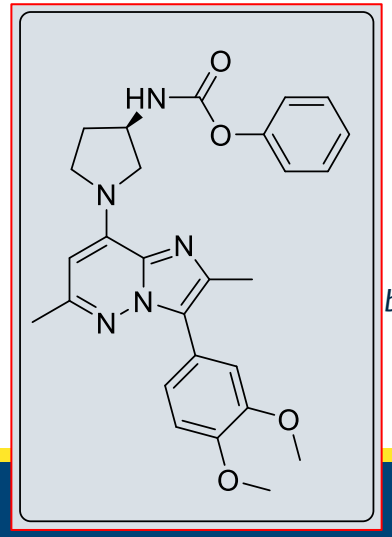
F=~90%



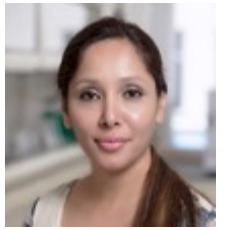


SELECTIVE

Clean in Eurofin's SafetyScreen44 evaluating targets identified by 4 major pharmaceutical companies based on their experience with off target activities that could hinder/stop development (Nature Reviews 2012, 909).



phenyl (R)-(1-(3-(3,4-dimethoxyphenyl)-2,6-dimethylimidazo[1,2-b]pyridazin-8-yl)pyrrolidin-3-yl)carbamate



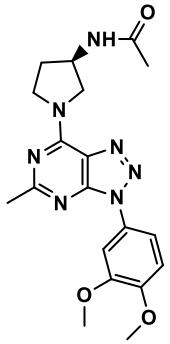
Rana Rais

Ranjeet Dash

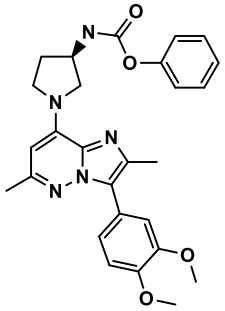
PDDC inhibited extracellular vesicle release *in vitro*

Amrita Datta Chaudhuri
JHU - Neurology

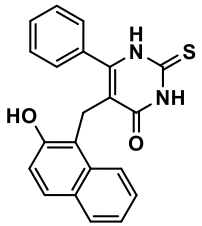
- Primary glial culture was stimulated (- FBS) ± PDDC, cambinol, or inactive compound 5 for 2h
- Collected media / isolated EVs / measure conc. with nanoparticle tracker



Compound 5
IC_{50} > 100

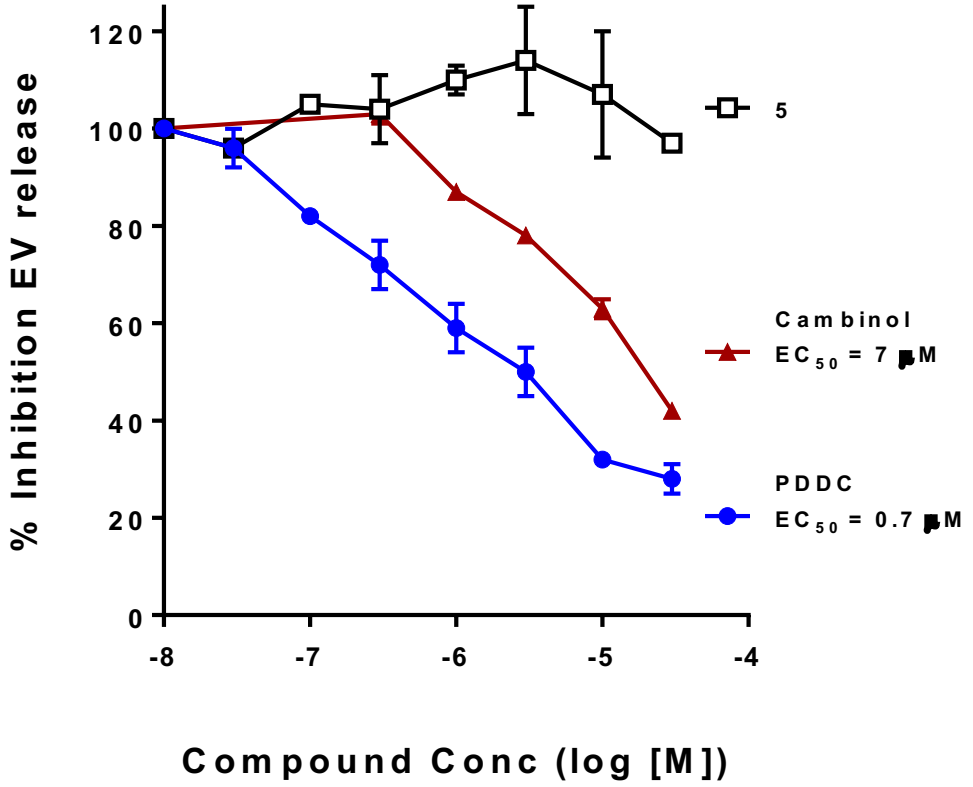


PDDC
IC_{50} = 300nM



Cambinol
Tool compound

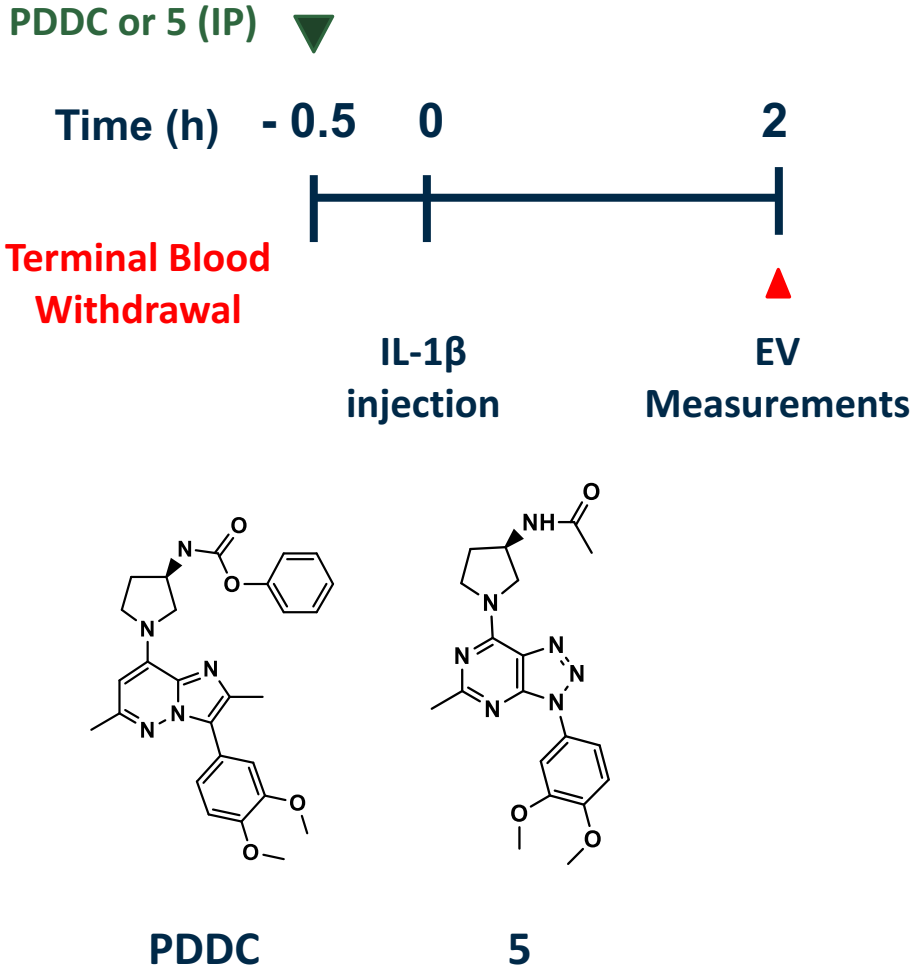
PDDC reduces EV release;
Inactive analog had no effect

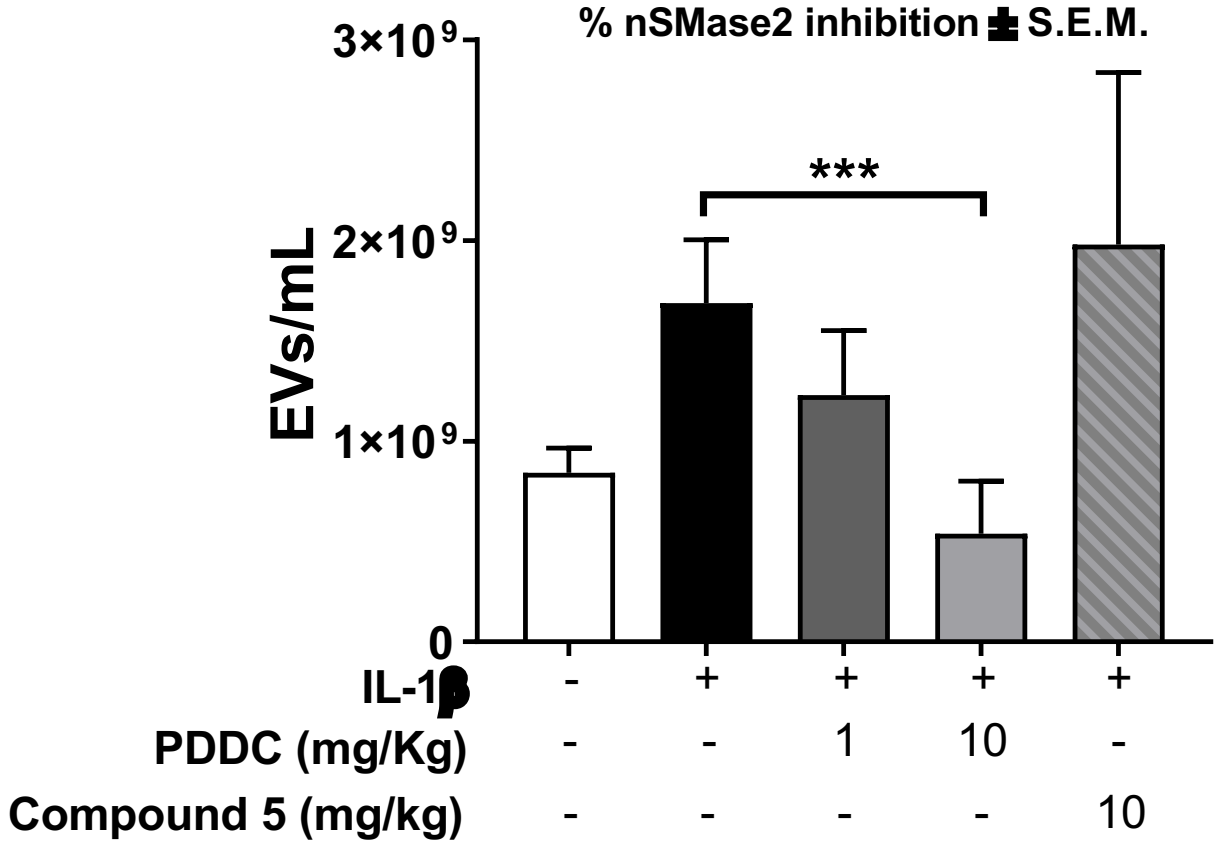


PDDC inhibits exosome release *in vivo*

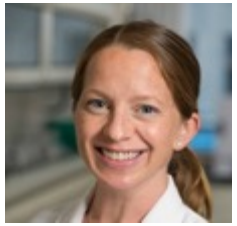
Inactive compound had no effect

Seung-Wan Yoo
JHU- Neurology





Initial analysis in 5XFAD AD model shows body weight and behavioral improvements



Kristen Hollinger
JHDD

Begin treating
10mg/kg IP daily

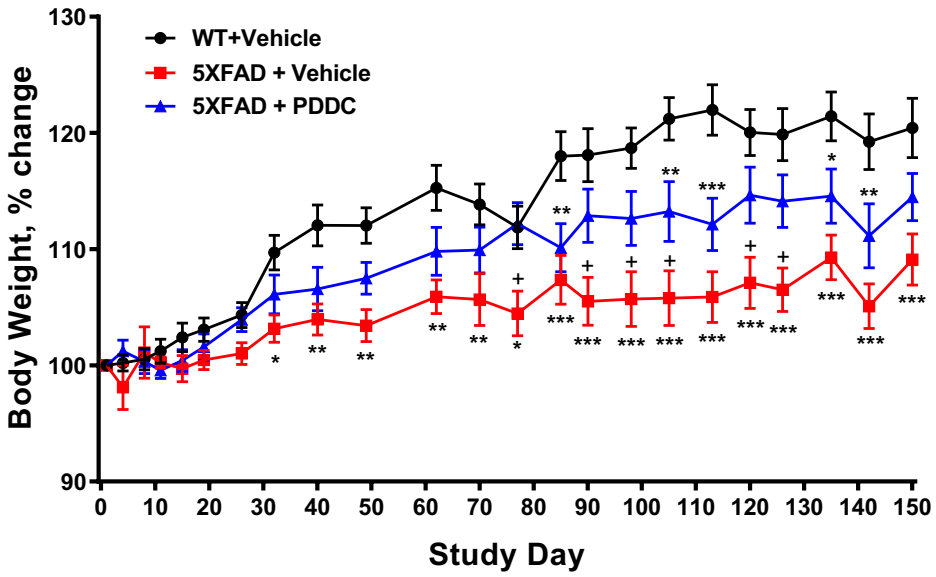
Fear conditioning
behavioral analysis

Weekly body weight

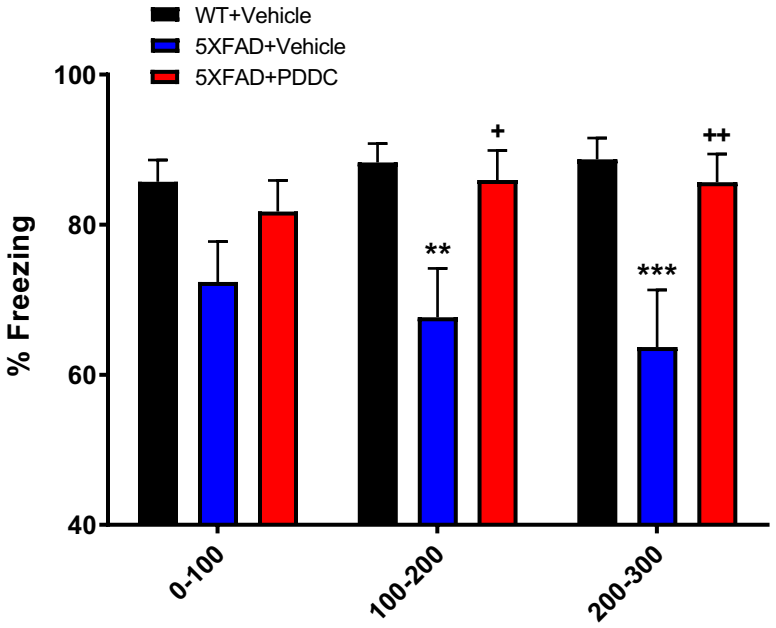
3 months old

8 months old

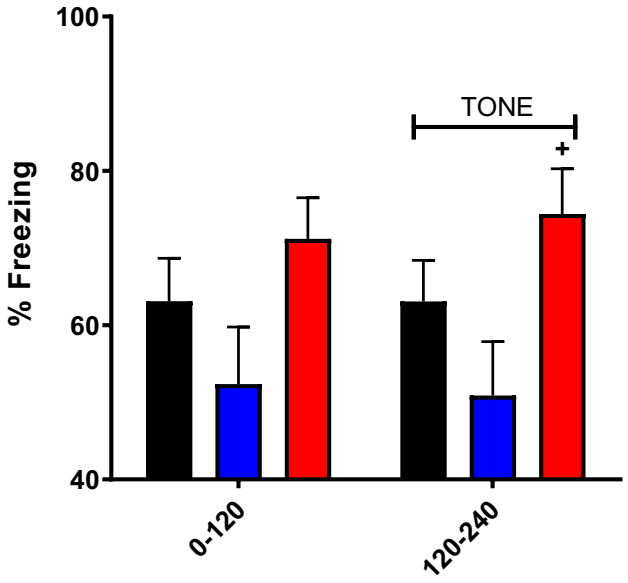
PDDC treatment improves body weight



Contextual fear conditioning is enhanced



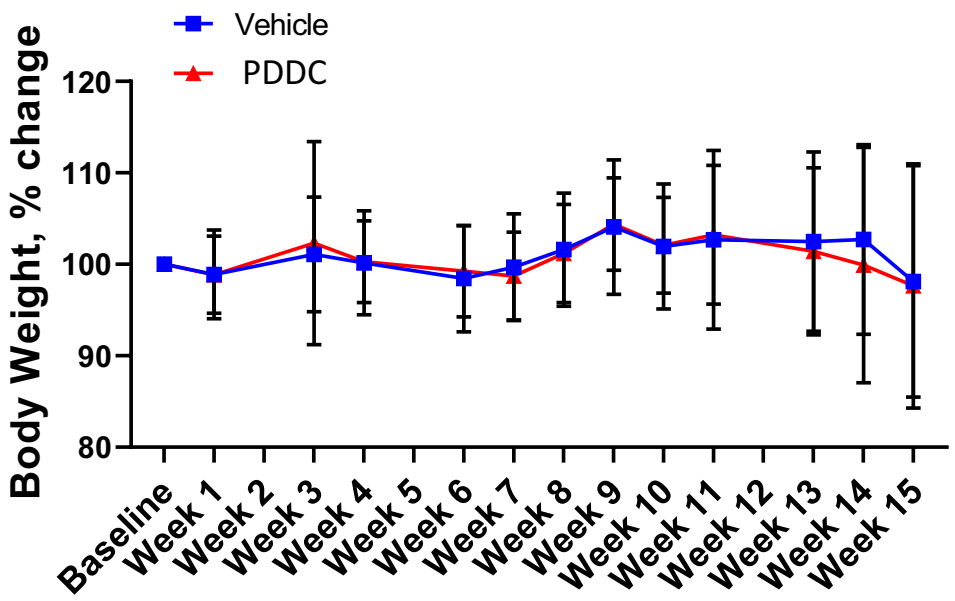
Cued fear conditioning is enhanced



PDDC is well tolerated in mice

- Mice were treated with 10mg/kg IP PDDC for 15 weeks
- Body weight monitored weekly and clinical chemistries were evaluated after 15 weeks

Chronic PDDC does not cause weight loss



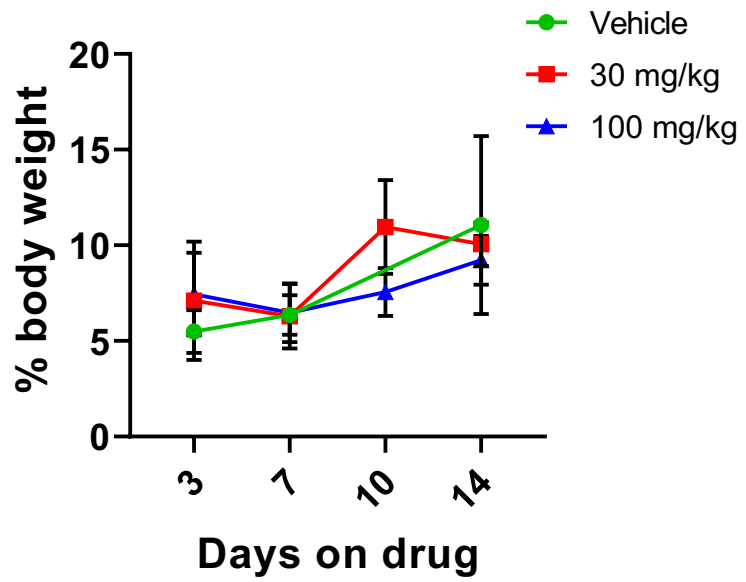
Chronic PDDC does not alter clinical chemistries

Parameter	Vehicle	PDDC	SEM	Normal Range	P value
AST	80.67	55.67	14.17	54 - 269	0.1525
ALT	21.33	18.67	4.46	26 - 77	0.5821
BUN	18.67	18.67	3.249	8 - 33	0.9999
ALP	72.67	62.33	13.37	35 - 96	0.4829
CA	10.5	10.33	0.1944	7.1 - 10.1	0.4395
GLU	216.7	220	23.36	62 - 175	0.8934
LDH	225	173.7	50.45		0.3665
GGT	3.333	4	0.6667		0.3739
TP	5.633	5.6	0.1333	3.5 - 7.2	0.8149
ALB	3.167	3.133	0.04714	2.5 - 3.0	0.5185
TBILI	0.2667	0.2333	0.04714	0.1 - 0.9	0.5185
CREAT	0.1667	0.2333	0.04714	0.2 - 0.9	0.2302
CK	245	134.7	88.41	63 - 445	0.2801
PHOS	6.867	7.367	0.3448		0.2206

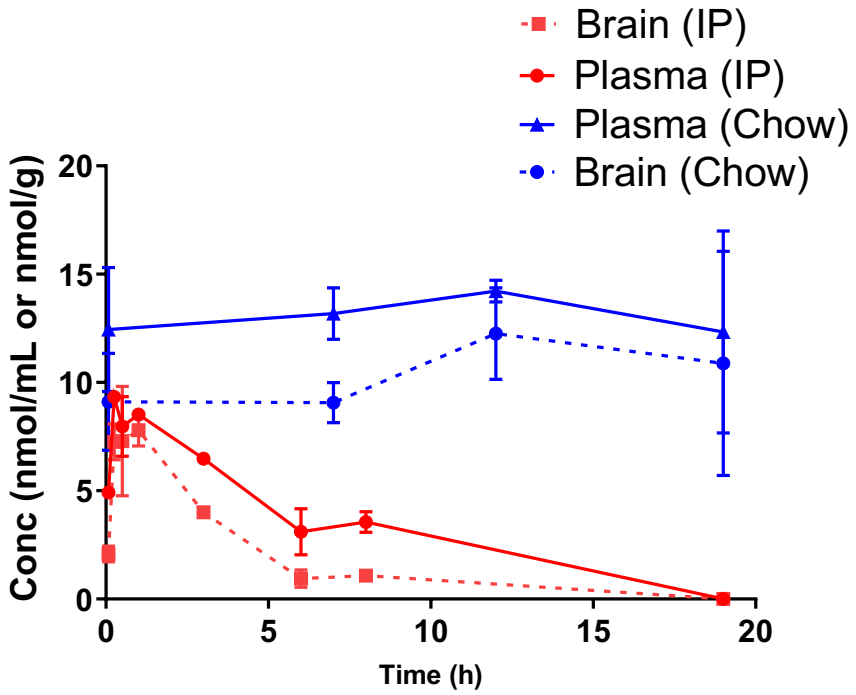
PDDC can be administered orally via mouse chow

- 6 month old WT mice were fed with 30mg/kg and 100 mg/kg PDDC chow for 2 weeks
- PDDC was measured in plasma and brain by LC-MS

No changes in body weight

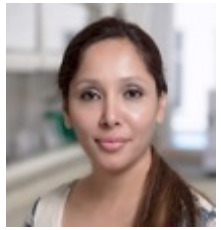


4-fold plasma increase; 5-fold brain increase



	Plasma	Brain	Plasma (Chow)	Brain (Chow)
Total Area	63.07	33.61	250.1	197.1
Std. Error	5.694	2.793	19.89	22.06

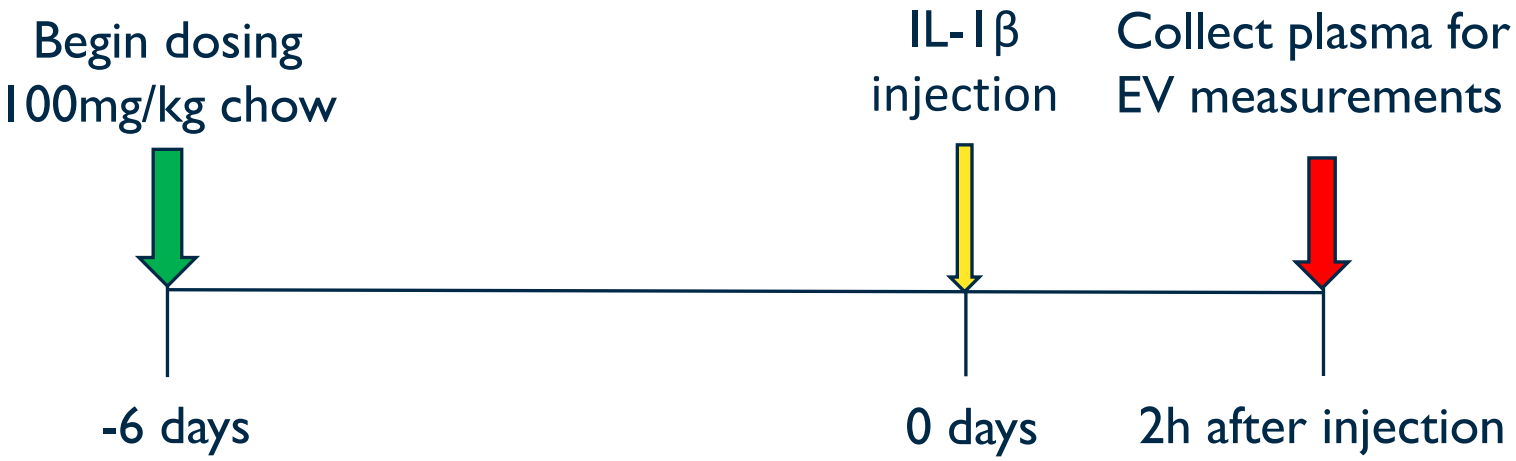
Jesse Alt



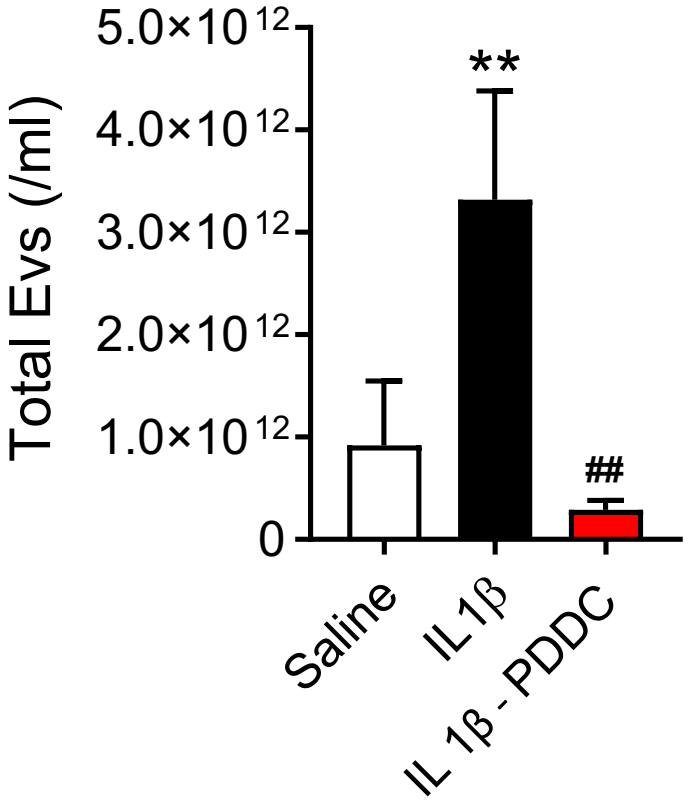
Rana Rais

100mg/kg PDDC chow reduces EV release in acute *in vivo* assay

Seung-Wan Yoo
JHU- Neurology



91% reduction of EV release

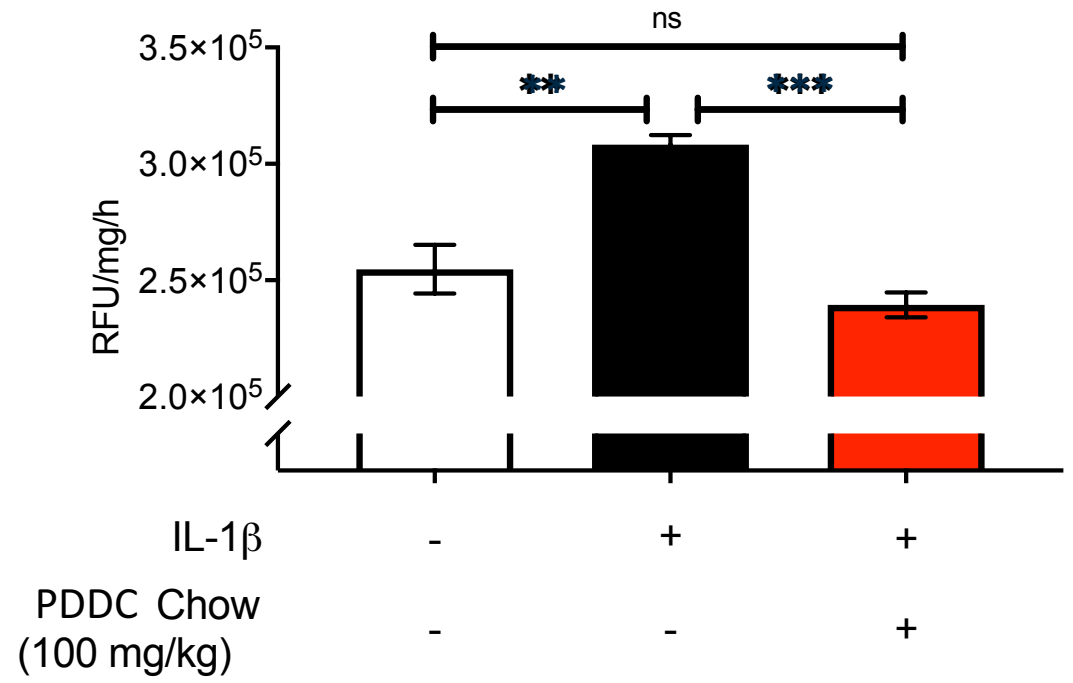


100mg/kg PDDC chow significantly inhibits *ex vivo* brain nSMase2 activity


Ajit G Thomas

- *Ex vivo* nSMase2 brain activity was measured in the IL-1β injected mice following 6 days of 100mg/kg PDDC chow treatment

PDDC significantly reduces nSMase2 activity



Outline

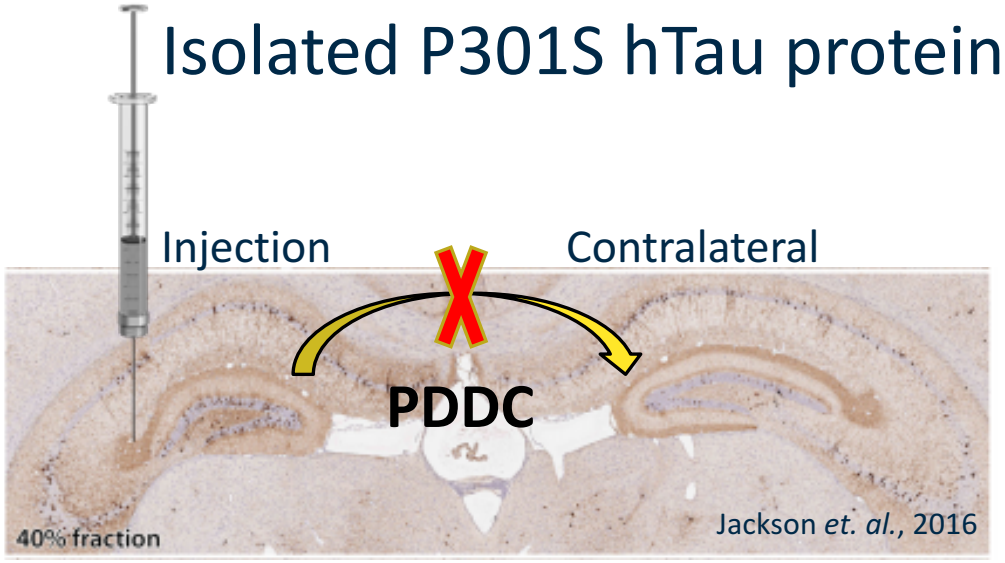
1. Introduction on extracellular vesicles and Alzheimer’s disease
2. Development and characterization of PDDC for the treatment of Alzheimer’s disease
3. **Future plans**
4. Summary

PDDC chow treatment in a tauopathy mouse model of Alzheimer's

- 5XFAD mouse model does not have significant tau pathology
- Phosphorylated tau levels in isolated exosomes may predict AD more effectively
- Will test PDDC in the P301S (PS19) transgenic tauopathy mouse model

Evaluate 2 new models of tau propagation

Isolated P301S hTau protein

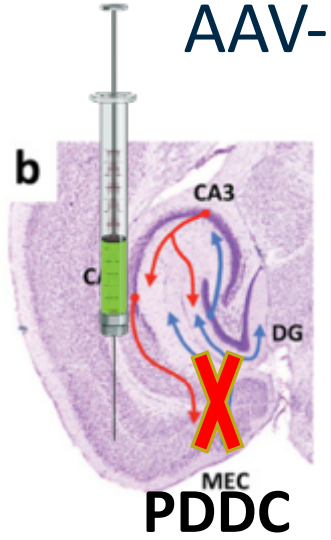


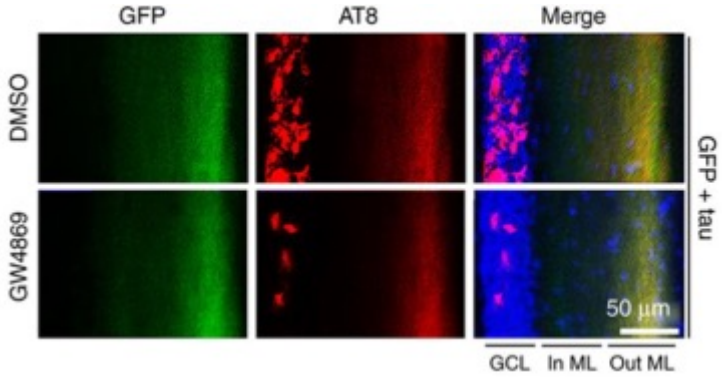
PS19/3xTg mice

Isolated P301S hTau seeded into PS19 mice via unilateral injection into CA3 hippocampus, propagation evaluated in contralateral hippocampus after 3 months.

- Ahmed *et.al.*, *Acta Neuropathol*, 2014.
- Jackson *et. al. J. Neurosci*, 2016.
- Iba . Al., *J. Neurosci*, 2013.
- Goedert, Eisenberg, Crowther, *Annu Rev Neurosci*, 2017

AAV-P301LhTau-GFP





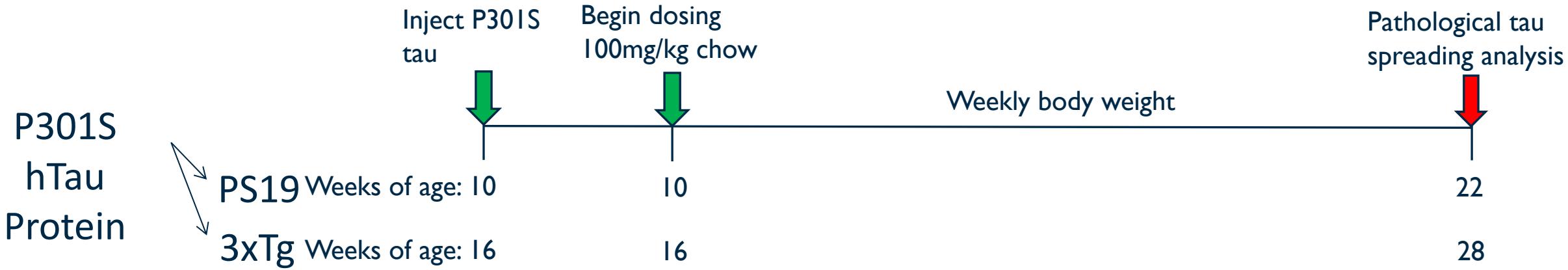
Asai *et. al.*, 2016

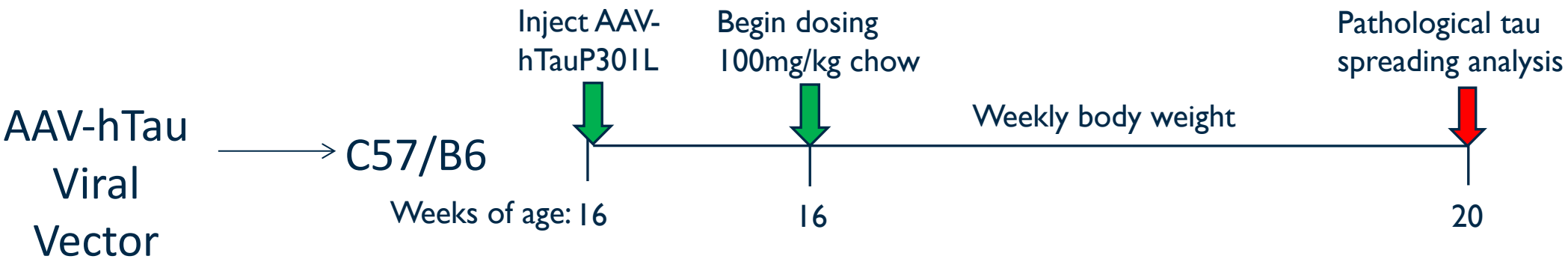
C57/B6

P301L hTau DNA introduced into MEC of WT mice via AAV-vector, tau propagation observed across the dentate gyrus of hippocampus after 1 month.

- Asai *et. al.*, *Nat Neurosci*, 2015.
- Cook *et. al.*, *Hum Mol Genet*, 2015
- Jaworski *et. al.*, *Plos One*, 2009

Next steps


Inject P301S tau
Begin dosing 100mg/kg chow
Pathological tau spreading analysis
Weekly body weight
P301S hTau Protein
PS19 Weeks of age: 10
10
22
3xTg Weeks of age: 16
16
28


Inject AAV-hTauP301L
Begin dosing 100mg/kg chow
Pathological tau spreading analysis
Weekly body weight
AAV-hTau Viral Vector
C57/B6
Weeks of age: 16
16
20

Summary

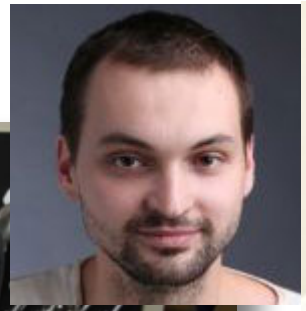
- Identified PDDC, a potent, selective, orally available and brain penetrant nSMase2 inhibitor
- PDDC dose-dependently inhibited extracellular vesicle release both *in vitro* and *in vivo*
- In preliminary studies, PDDC led to improved cognition in an amyloid mouse model of AD
- Ongoing studies examining tauopathy mouse models and more targeted tau propagation mouse models

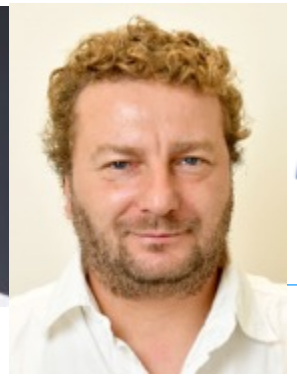
Acknowledgements

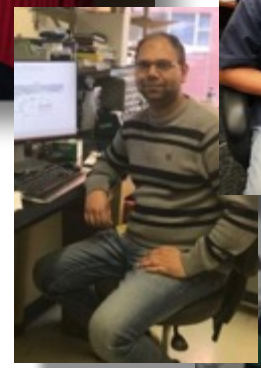
Johns Hopkins Drug Discovery
Barb Slusher
Camilo Rojas
Rana Rais
Ajit Thomas
Ondrej Nesuta
Takashi Tsukamoto
Ondrej Stepanek
Kristen Hollinger
Jesse Alt
Ben Bell
Amanda Donoghue
Hiba Farah
Lyndah Lovell

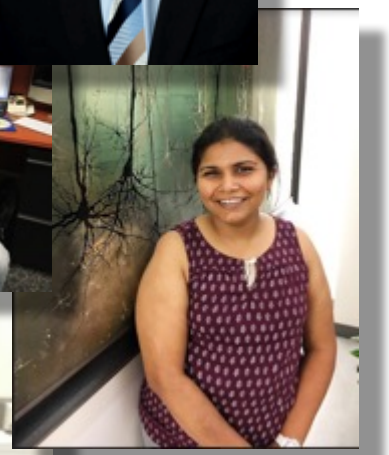
Haughey Lab:
Seung-Wan Yoo
Asit Kumar
Mohammed Moniruzzaman
Amrita Datta Chaudhuri

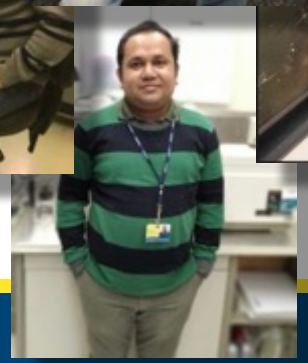
IOCB Prague
Radim Nencka
Michal Sala
Evzen Boura











These studies were funded by:
NIMH Center for Novel Therapeutics for HIV: **MH075673**
R01MH110246 **R01MH077542**
R01DA040390 **R01AG057420**
P01MH105280 **R01MH096636**
Bluefield Innovations