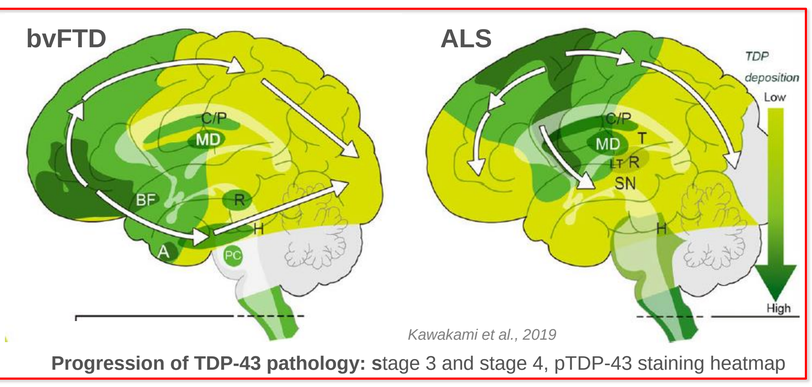
Optimization of affinity, selectivity and pharmacokinetic profile of TDP-43 PET ligands

Tariq Afroz[1], Efthymia Vokali[1], Nicolas Dreyfus[1], Elodie Chevalier[1], Mathieu Clavel[1], Monisha Ratnam[1], Tania Melly[1], Thomas Jaquier[1], Harro Seelaar[2], Jia Newcombe[3], Heiko Kroth[1], Andrea Pfeifer[1], Marie Kosco-Vilbois[1], Tamara Seredenina[1]

[1] AC Immune SA, EPFL Innovation Park, 1015 Lausanne, Switzerland; [2] Department of Neurology and Alzheimer Center Erasmus MC University Medical Center, Rotterdam, The Netherlands; [3] NeuroResource, UCL Queen Square Institute of Neurology, London WC1N 1PJ

Background

- TDP-43 pathology is common in patients with frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) and is present as co-pathology in other neurodegenerative diseases
- Selective and sensitive biomarkers of TDP-43 pathology currently not available
- Direct detection of TDP-43 aggregates by positron emission tomography (PET) holds promise for better diagnosis, patient stratification and assessment of therapeutic efficacy in clinical trials
- Building on our expertise in TDP-43 biology and extensive experience with developing CNS PET tracers, we use our Morphomer® platform to identify PET ligands binding to aggregated TDP-43



Kawakami et al., 2019

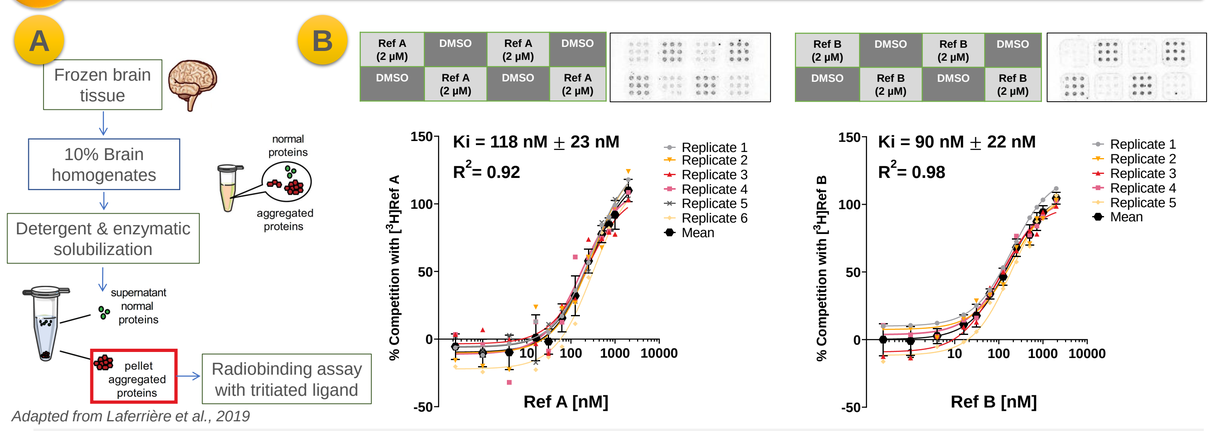
Progression of TDP-43 pathology: stage 3 and stage 4, pTDP-43 staining heatmap

1 Screening cascade for clinical candidate selection

Multiple chemical series for risk-reduced development	Morphomer library, scaffold hopping, rational design, focused screening
Affinity on TDP-43 (Ki) Selectivity over Aβ, Tau and α-synuclein	Radiobinding assay on insoluble fractions from FTLD-TDP, AD and PD brains
In vitro ADME and mouse PK	Solubility, permeability, microsomal stability, chemical stability, neuroPK
[3H] radiolabeling Affinity (Kd) Target engagement	Radiobinding assay and autoradiography on brains with FTLD-TDP
[18F] radiolabeling [18F] PK in mice and NHP	Assessment of properties for CNS PET-tracer
CC	Clinical candidate IND-enabling studies

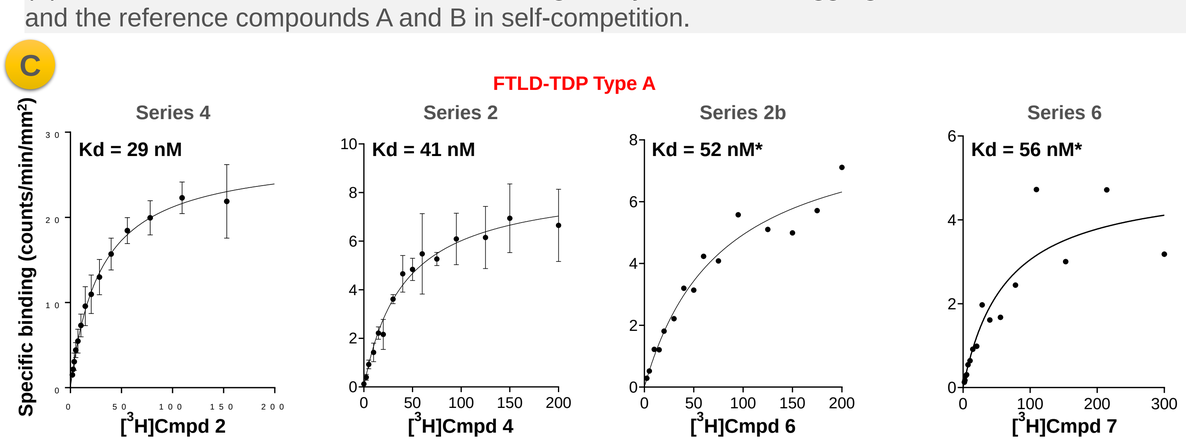
AD – Alzheimer's disease; PD – Parkinson's disease; ADME – absorption, distribution, metabolism and excretion; PK – pharmacokinetics; NHP – non-human primates; CC – clinical candidate; CNS – central nervous system; IND – investigational new drug

2 Binding to TDP-43 aggregates from patient brain

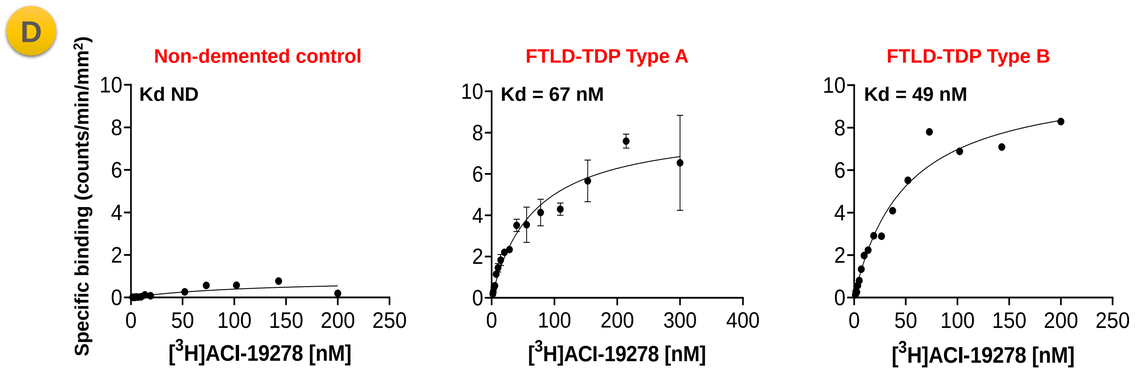


Adapted from Laferrière et al., 2019

(A) Enrichment of TDP-43 aggregates from human brain allowing elimination of physiological TDP-43
(B) Validation of the miniaturized radiobinding assay on TDP-43 aggregates from FTLD-TDP brain and the reference compounds A and B in self-competition.

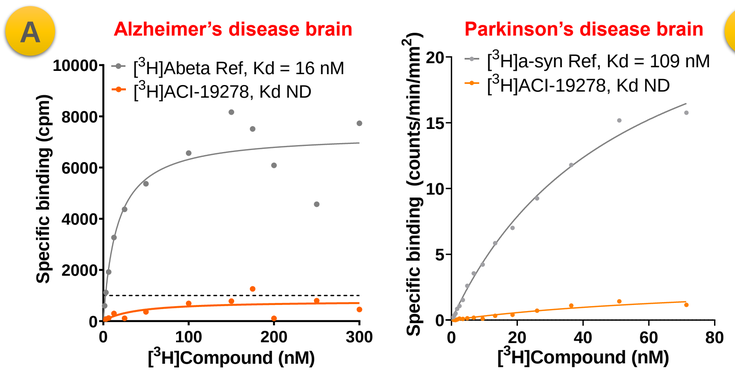


(C) Binding profiles showing nanomolar affinity (Kd) for hits from different chemical series on insoulble fractions from FTLD-TDP Type A brain samples. *Representative curve, mean Kd of n=2 experiments.



(D) Binding profiles for [3H]ACI-19278 to insoluble fractions from FTLD-TDP type A, B and control brains. ND – not determined.

3 Selectivity profiling



B

Target	Result
MAO A, Ki	>2000 nM
MAO B, Ki	767 nM
Bioprint panel of 132 proteins	No hits with > 50% inhibition

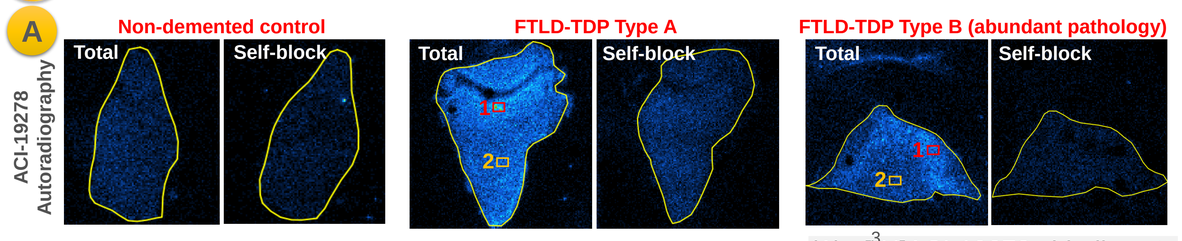
(A) ACI-19278 displays good to excellent selectivity over Abeta and a-synuclein in patient brain homogenates; cpm – counts per minute. ND – not defined. (B) ACI-19278 shows no binding to MAO A and MAO B in filter binding assay in competition with tritium-labeled Harmine and L-deprenyl.

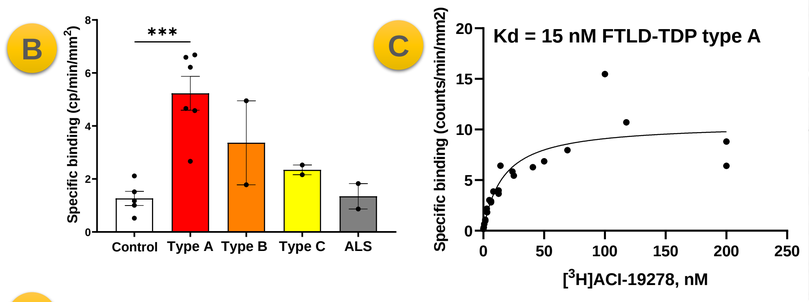
Acknowledgements: Neurodegenerative Disease Brain Bank UCSF, Prof. William Seeley (funding support from NIH grants P01AG019724 and P50AG023501, the Consortium for Frontotemporal Dementia Research, and the Tau Consortium).
Netherlands Brain Bank, Netherlands Institute for Neuroscience, Amsterdam. All Material has been collected from donors from whom a written informed consent for brain autopsy and the use of the material and clinical information for research purposes had been obtained by the NBB.
Queen Square Brain Bank for Neurological Disorders, UCL, Prof. Tammaryn Lashley.

ImageTDP43

Grant ID: MJFF-022232

4 Target engagement on FTLD-TDP brain



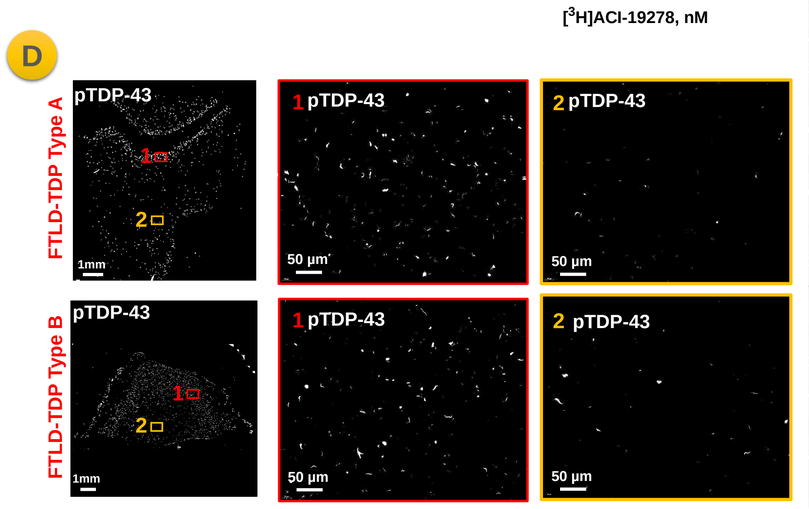


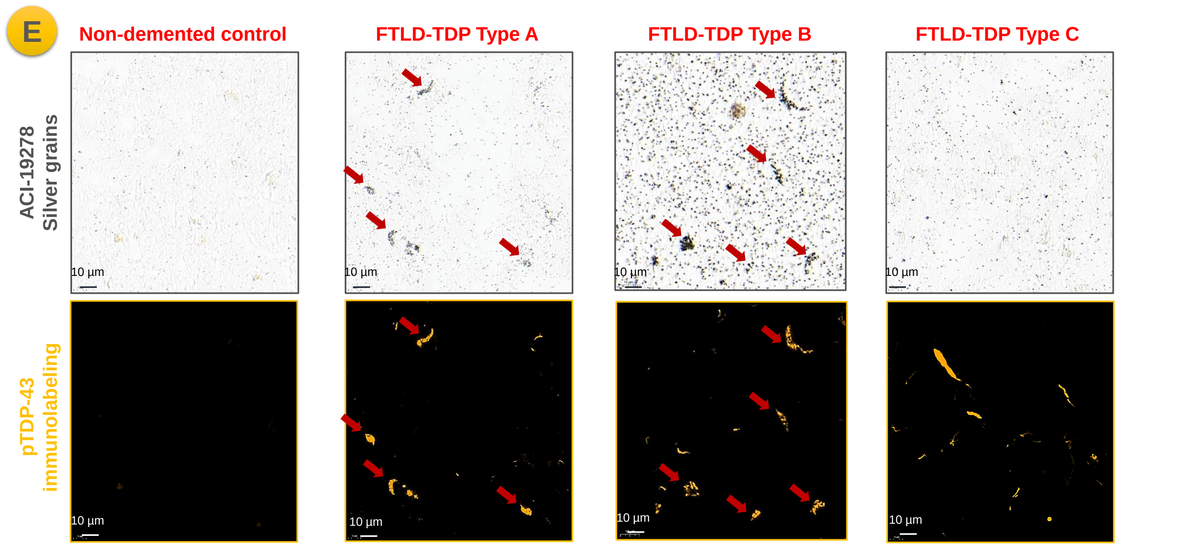
(A) [3H]ACI-19278 binding to FTLD-TDP Type A and B by classical autoradiography (ARG) on frozen sections from frontal cortex. Total signal: 20 nM [3H]ACI-19278; non-specific binding: pre-blocking with 2 µM unlabeled ACI-19278.

(B) Specific binding: total activity minus non-displaceable signal. One-Way ANOVA with Dunett's test for multiple comparison. ***p<0.001. Each point represents a different donor. Mean ± SEM.

(C) ACI-19278 affinity from saturation binding on sections from FTLD-TDP Type A case.

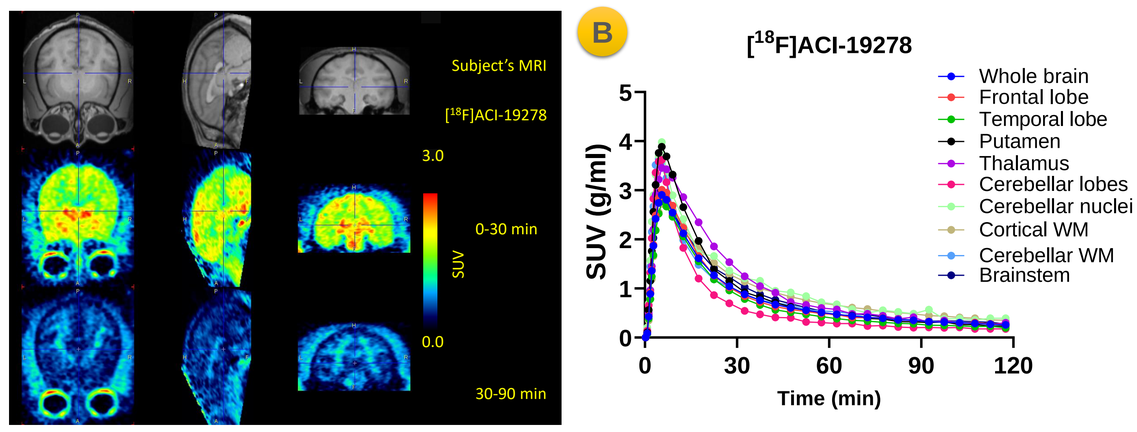
(D) The intensity of radioactive signal correlates with the abundance of pTDP-43 pathology (immunolabeling on adjacent sections, 1D3 pS409/410 TDP-43 antibody).





(E) Target engagement by high resolution ARG on frozen brain sections of frontal cortex with FTLD-TDP Type A, B and C pathology. Co-localization of [3H]ACI-19278 (sliver grain accumulation) with pTDP-43 antibody labeling (orange, 1D3 pS409/410), red arrows.

5 PET pharmacokinetic profile in WT non-human primates



- [18F]ACI-19278 showed robust and rapid brain uptake in NHP upon intravenous administration
- Peak whole-brain standardized uptake value (SUV) > 2.5 % injected dose
- Fast washout suitable for human PET

Summary

- Several hit series with distinct chemical scaffolds identified that bind to pathological TDP-43
- First-in-class TDP-43 ligand ACI-19278 shows:
  - differentiation of FTLD-TDP from non-demented cases by classical ARG
  - 15 nM affinity on FTLD-TDP brains sections by classical ARG
  - selectivity over Aβ and α-synuclein
  - proven fast and robust brain uptake and fast washout in non-human primates
- Medicinal chemistry optimization ongoing to increase affinity and explore SAR