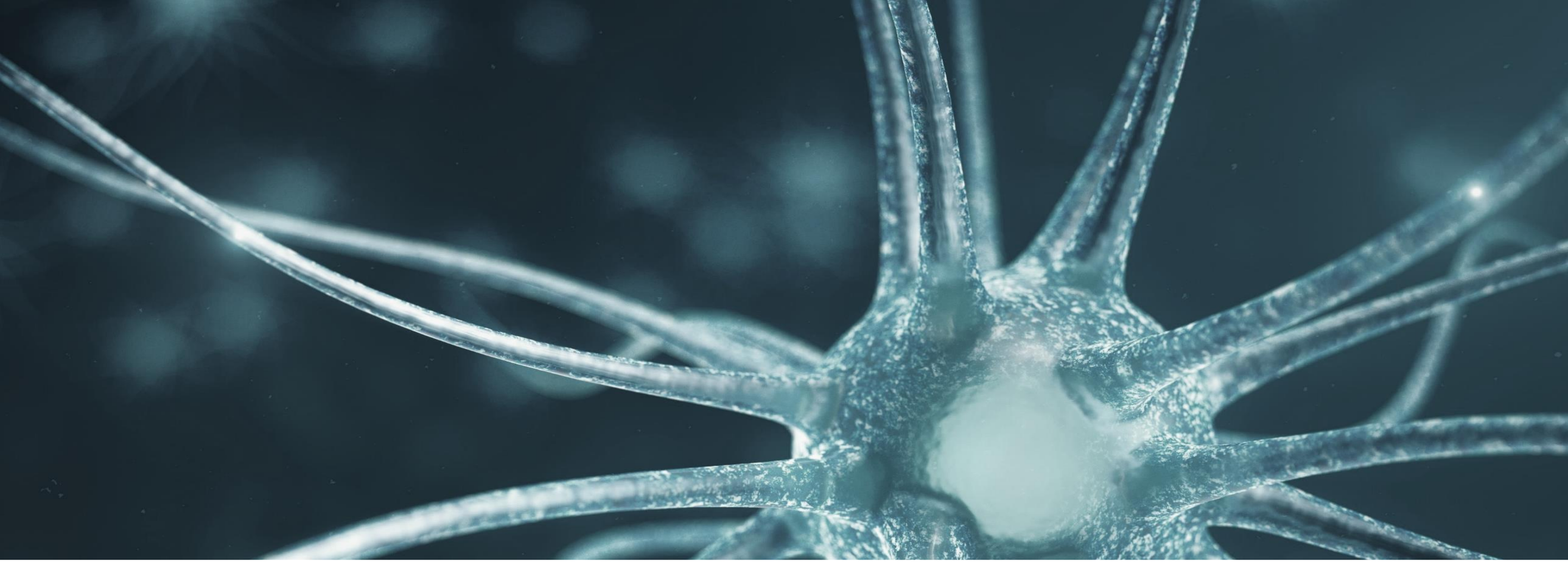


TDP-43 IMMUNOTHERAPY DECREASES NEUROPATHOLOGY AND CONFERS NEUROPROTECTION THROUGH MICROGLIAL ENGAGEMENT IN MOUSE MODELS OF ALS/FTD

Tariq Afroz, PhD | AD/PD™ 2022 | 18 March

AC Immune

Disclaimer

This presentation may contain statements that constitute "forward-looking statements" within the meaning of Section 27A of the Securities Act of 1933 and Section 21E of the Securities Exchange Act of 1934. Forward-looking statements are statements other than historical fact and may include statements that address future operating, financial or business performance or AC Immune's strategies or expectations. In some cases, you can identify these statements by forward-looking words such as "may," "might," "will," "should," "expects," "plans," "anticipates," "believes," "estimates," "predicts," "projects," "potential," "outlook" or "continue," and other comparable terminology. Forward-looking statements are based on management's current expectations and beliefs and involve significant risks and uncertainties that could cause actual results, developments and business decisions to differ materially from those contemplated by these statements. These risks and uncertainties include those described under the captions "Item 3. Key Information—Risk Factors" and "Item 5. Operating and Financial Review and Prospects" in AC Immune's Annual Report on Form 20-F and other filings with the Securities and Exchange Commission. Forward-looking statements speak only as of the date they are made, and AC Immune does not undertake any obligation to update them in light of new information, future developments or otherwise, except as may be required under applicable law. All forward-looking statements are qualified in their entirety by this cautionary statement.

SupraAntigen® is a registered trademark of AC Immune SA in the following territories: AU, EU, CH, GB, JP and RU.

Conflict of interest disclosure

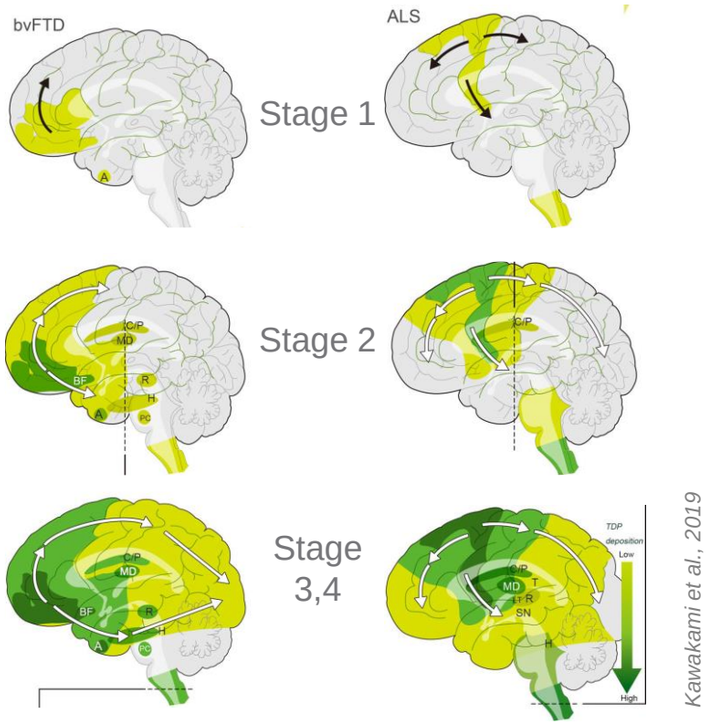
Tariq Afroz is an employee of AC Immune entitled to stock options

TDP-43 immunotherapy

To mitigate TDP-43 pathology and ameliorate associated cellular dysfunctions

TDP-43 pathology appears to spread in a defined pattern in ALS/FTD

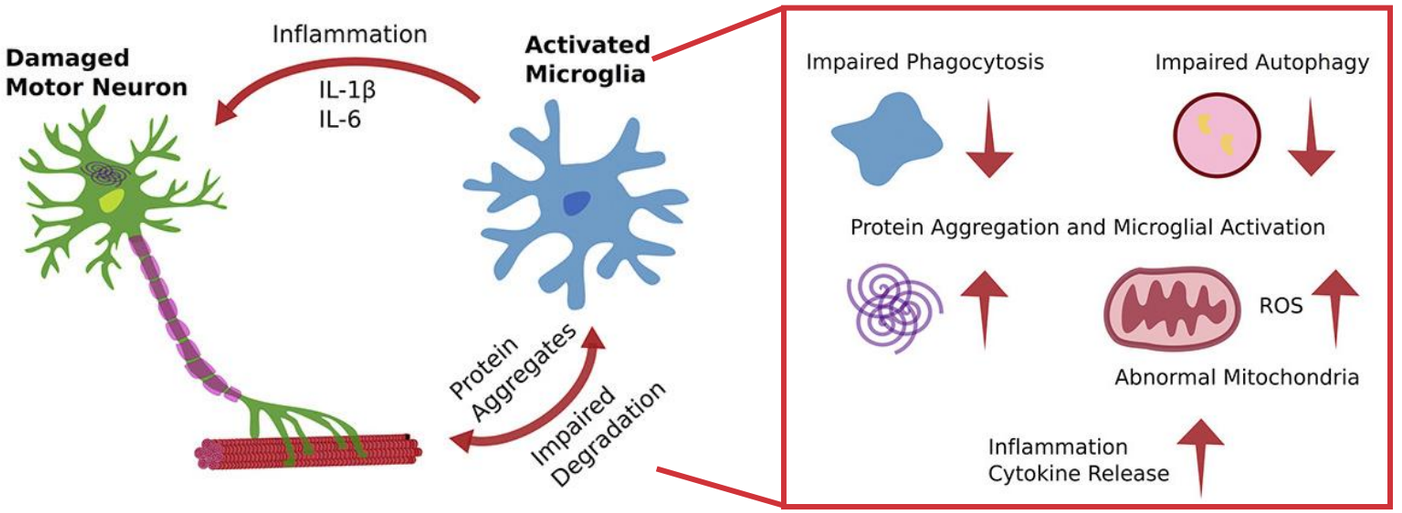
(*Brettschneider et al., 2013, Kawakami et al., 2019)*



Kawakami et al., 2019

Microglial dysfunction in ALS/FTD

(*Haukedal et al., 2019, Quek et al., 2022)*



Haukedal et al., 2019

- Reactive microglia facilitate TDP-43 pathology clearance and exert neuroprotective functions in rNLS8 mouse model of TDP-43 proteinopathies

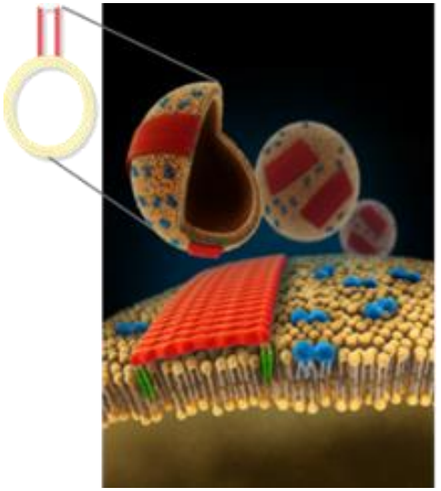
(*Spiller et al., 2018)*

- Extracellular TDP-43 species involved in spreading are promising targets for an antibody-based therapeutic approach

Generation and selection of TDP-43 mAbs

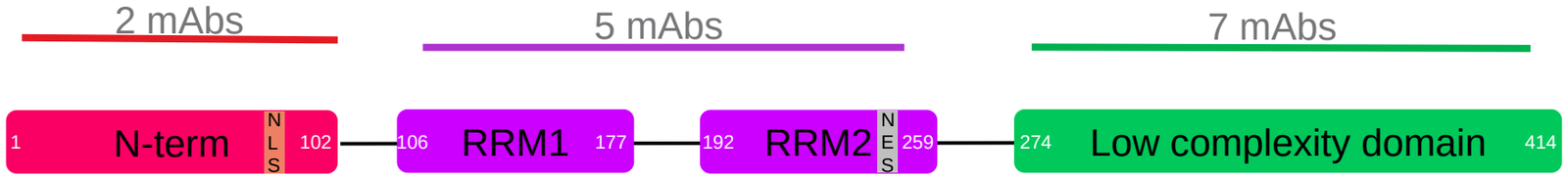
Using SupraAntigen® platform

SupraAntigen® platform



Hickman et al., 2011

Binding regions of TDP-43 mAbs



RRM: RNA recognition motif
NLS: Nuclear localization signal
NES: Nuclear export signal

Criteria for mAb selection

1	Biological relevance and target engagement
2	Target affinity
3	Functional efficacy

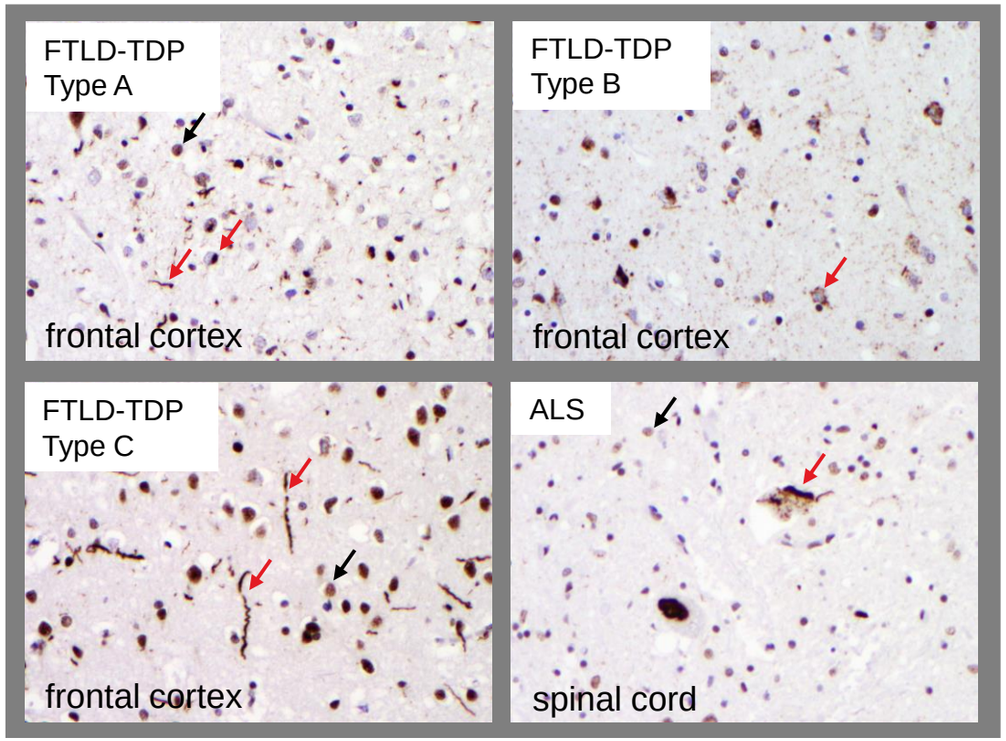
- Liposome and adjuvant-based presentation of either full-length or specific peptides
- Importantly, coverage of different domains involved in aggregation
- Evaluation of functional efficacy of mAbs by both systemic administration and vectorized approach

Ref: AC Immune unpublished data

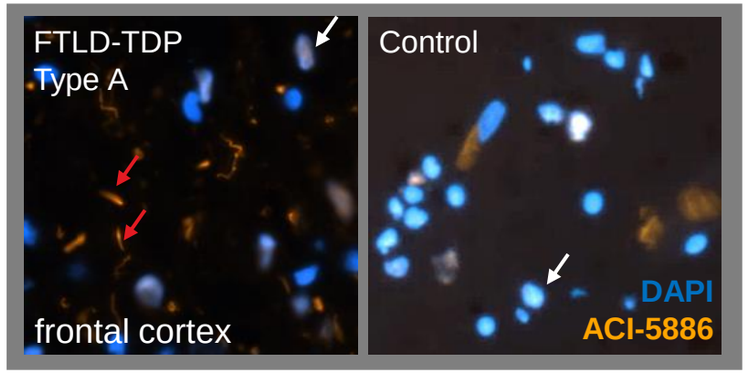
Target engagement and affinity of TDP-43 mAbs

By immunohistochemistry and surface plasmon resonance (SPR)

ACI-5891



ACI-5886



Antibody	Binding affinity to TDP-43, K_D (pM)
ACI-5891	182 ± 94
ACI-5886	691 ± 340

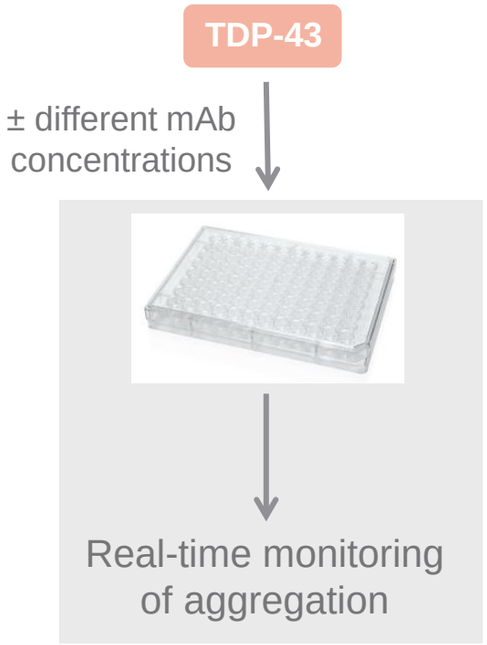
Ref: T. Afroz *et al.*, manuscript submitted

- ACI-5891 (binding in C-terminal domain) and ACI-5886 (binding in RRM domains) are pan TDP-43 mAbs that recognize both physiological and aggregated forms of TDP-43 in patient tissues
- Both mAbs bind TDP-43 with high affinity with K_D for TDP-43 in sub-nanomolar range

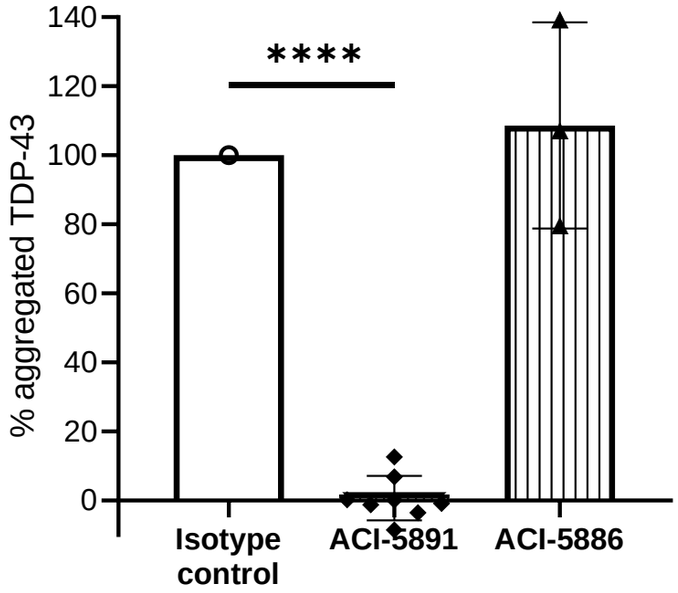
ACI-5891 inhibits TDP-43 aggregation *in vitro*

Assessed using recombinant TDP-43

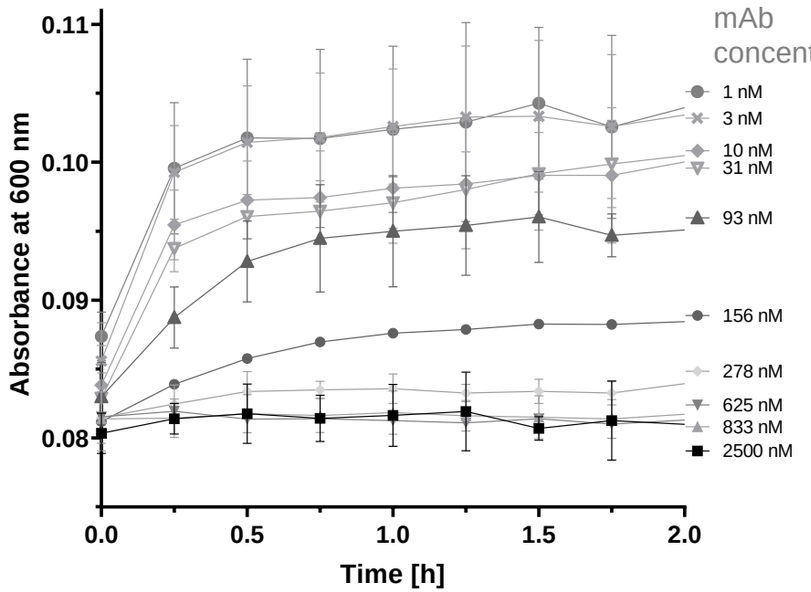
TDP-43 aggregation assay



% aggregated TDP-43 at 24 h



ACI-5891 dose-dependent inhibition of TDP-43 aggregation



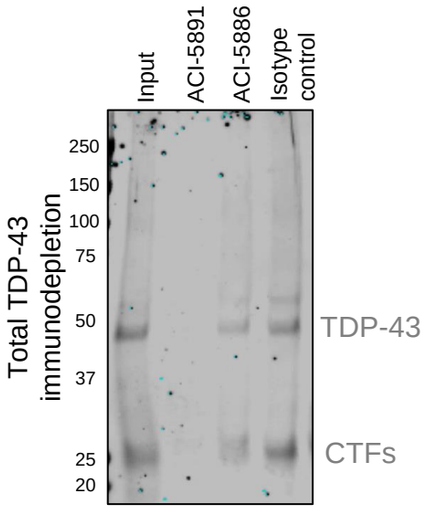
Ref: T. Afroz *et al.*, manuscript submitted

- ACI-5891 inhibits TDP-43 aggregation whereas ACI-5886 shows no significant effect
- Dose-dependent inhibition of TDP-43 aggregation is observed with ~ 98% inhibition achieved at highest dose

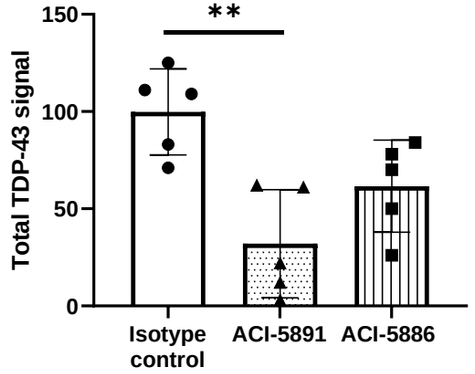
ACI-5891 inhibits TDP-43 templated-aggregation *in vitro*

Using patient brain-derived TDP-43 seeds

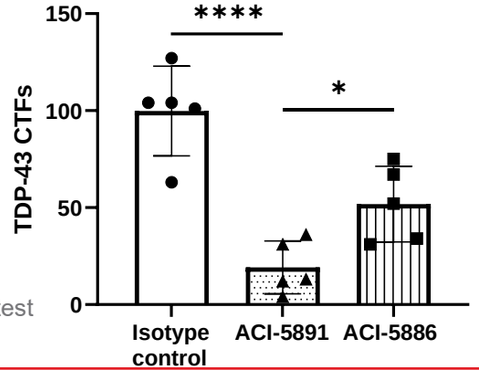
TDP-43 immunodepletion



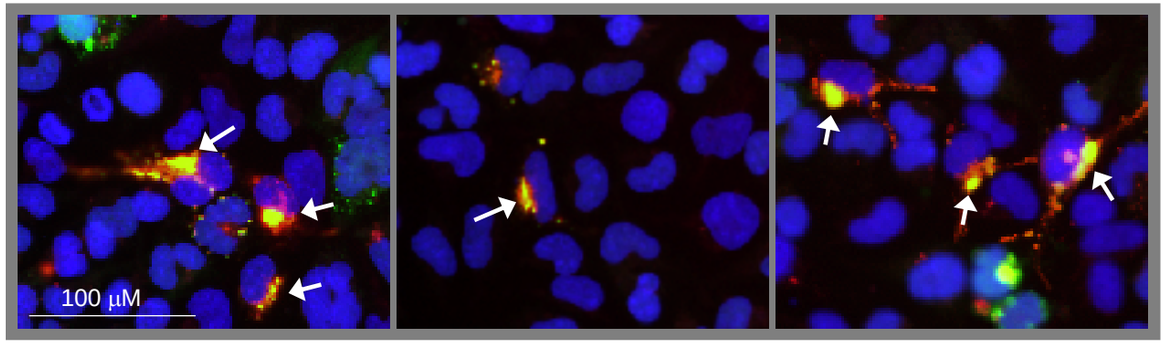
Total TDP-43



C-terminal fragment (CTFs)

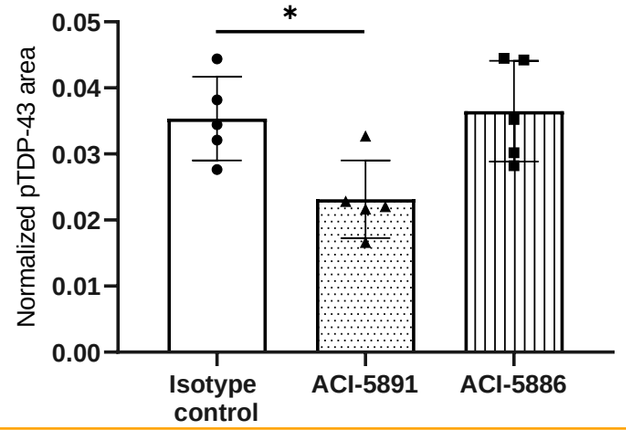


- Data shown as Mean±SD
- Statistics: One-way ANOVA with Tukey's test
- *p<0.05 **p<0.01 ****p<0.0001



QBI-293A GFP-TDP-43 NLSm

GFP-TDP-43
pTDP-43



- Immunodepletion demonstrates ACI-5891 target engagement to disease-related forms of TDP-43
- ACI-5891 demonstrates significant inhibition of FTLD-TDP brain-derived TDP-43 templated-aggregation as compared with ACI-5886 and isotype control

Ref: T. Afroz *et al.*, manuscript submitted

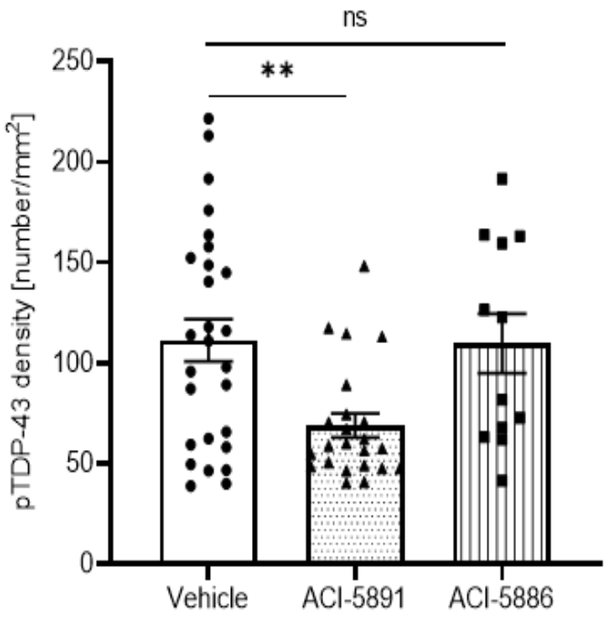
AC Immune

ACI-5891 reduces TDP-43 pathology in rNLS8 mice

By immunohistochemistry and biochemistry

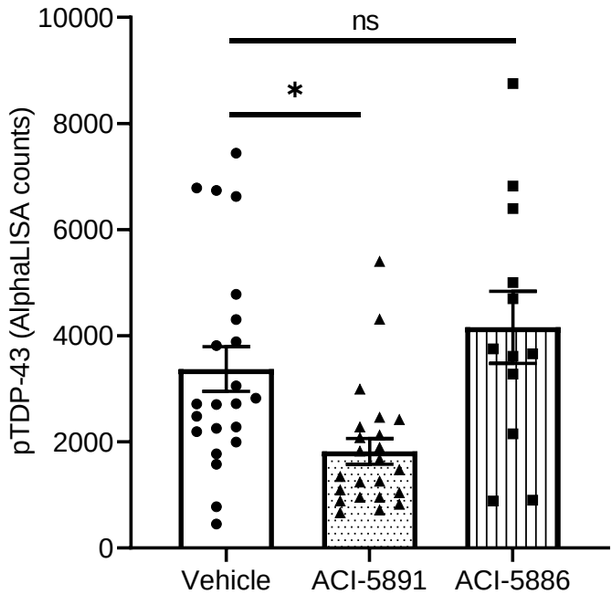
Phosphorylated TDP-43 (pTDP-43)

(in cortex by IHC)



- Data shown as Mean±SEM
- Statistics: One-way ANOVA with Fisher's LSD *post hoc* test
- ** P < 0.01, *P < 0.05

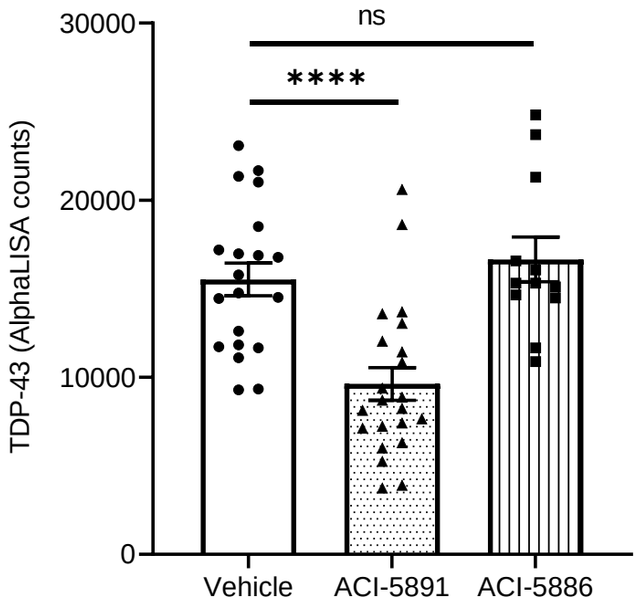
(in cortex, RIPA-insoluble*)



*Insoluble fractions were prepared from mouse brain cortex using RIPA buffer: 25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS

Aggregated TDP-43

(in cortex, RIPA-insoluble)



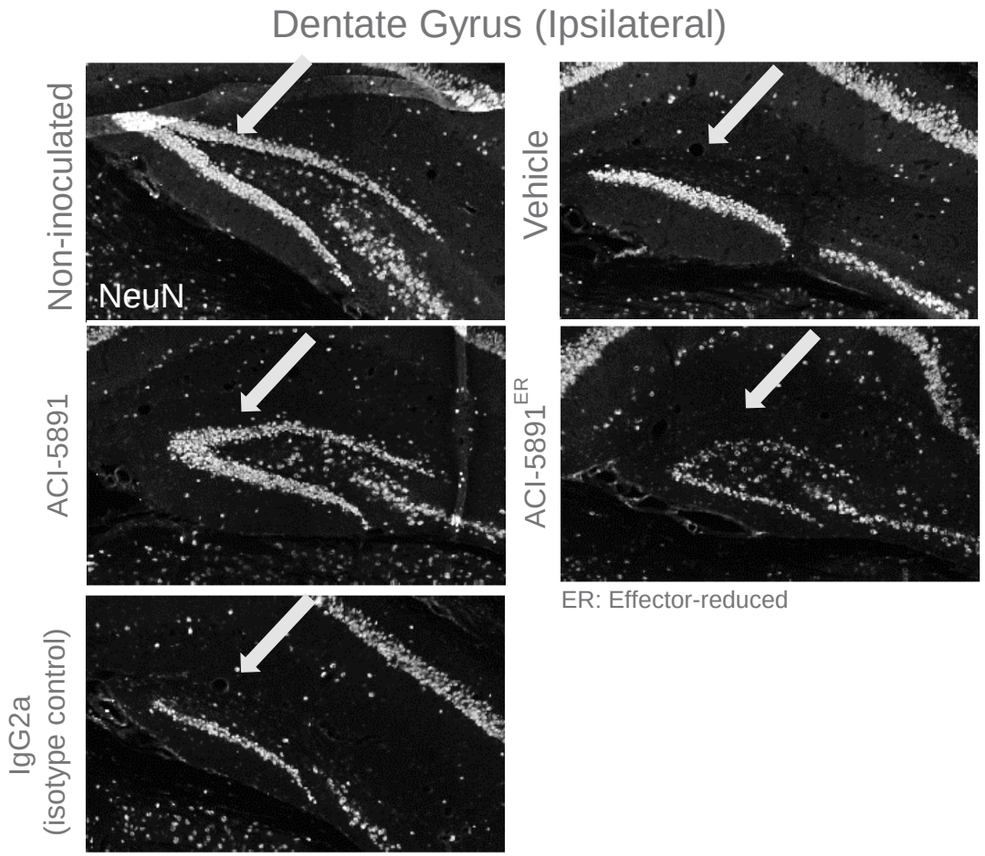
- Data shown as Mean±SEM
- Statistics: One-way ANOVA with Dunnett's *post hoc* test
- * P < 0.05, **** P < 0.0001

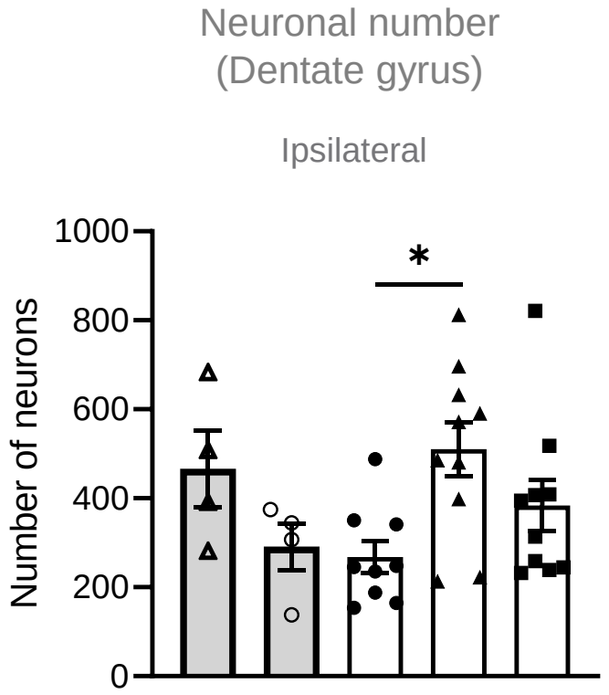
Ref: T. Afroz *et al.*, manuscript submitted

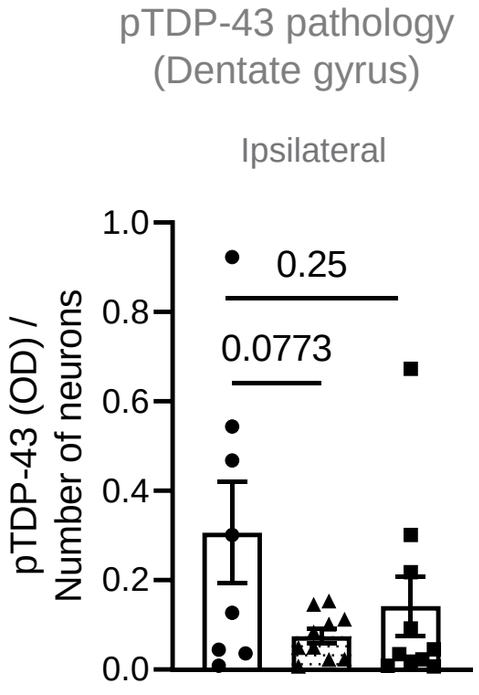
- ACI-5891 decreases TDP-43 pathology in cerebral cortex while ACI-5886 has no significant effect
- These data demonstrate *in vivo* relevance of targeting C-terminal epitope

ACI-5891 reduces neurotoxicity in CamKIIa mice

With FTLD-TDP brain extract injection







- Non-inoculated
- Vehicle
- Isotype control
- ACI-5891
- ACI-5891ER

*One-way ANOVA + Tukey post-hoc
ER: Effector-reduced

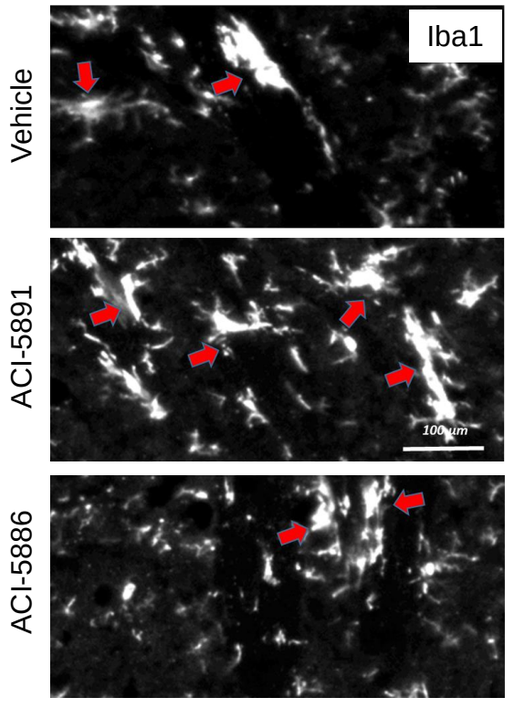
Ref: T. Afroz *et al.*, manuscript submitted

- ACI-5891 treatment significantly reduces neuronal loss in the dentate gyrus as compared with effector-reduced variant

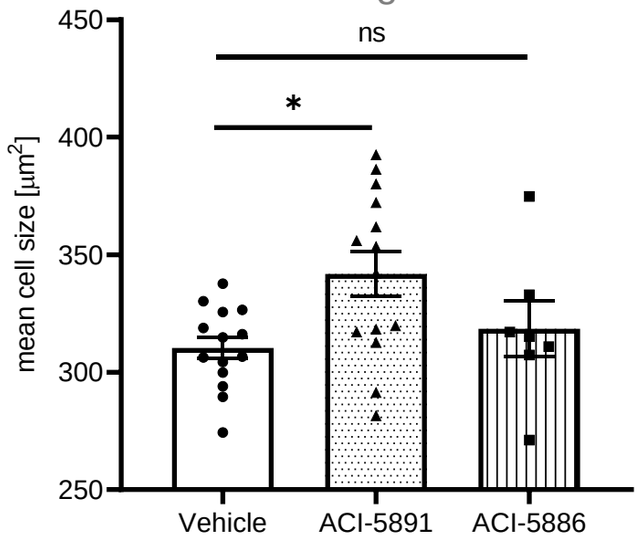
ACI-5891 promotes microglial activity *in vivo*

Assessed by immunohistochemistry on brain sections

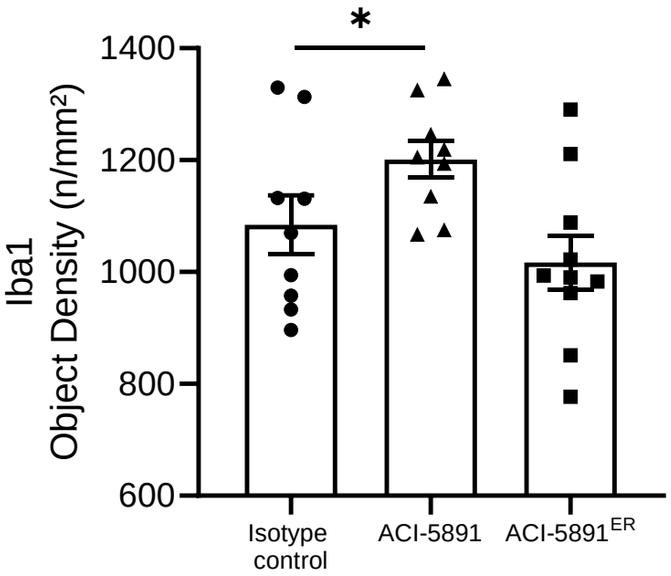
Microglia in brain cortex (rNLS8 model)



Large hypertrophic microglia



Microglia in dentate gyrus (CamKIIa model)



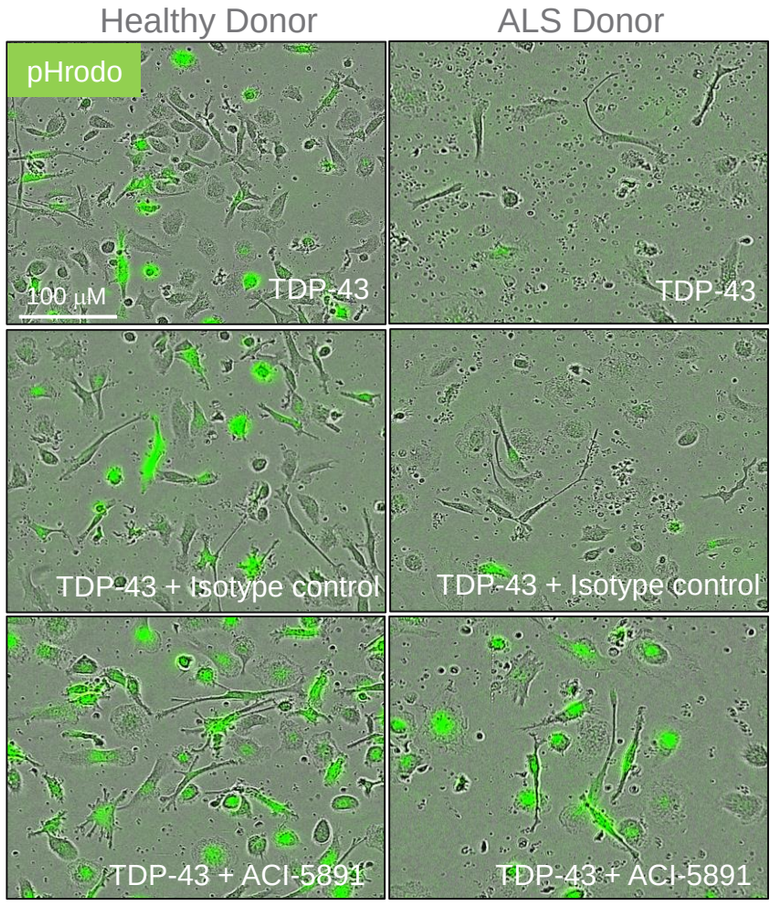
- Data shown as Mean±SEM
- Statistics: One-way ANOVA with Fisher's LSD *post hoc* test, *P < 0.05
- ER: Effector-reduced

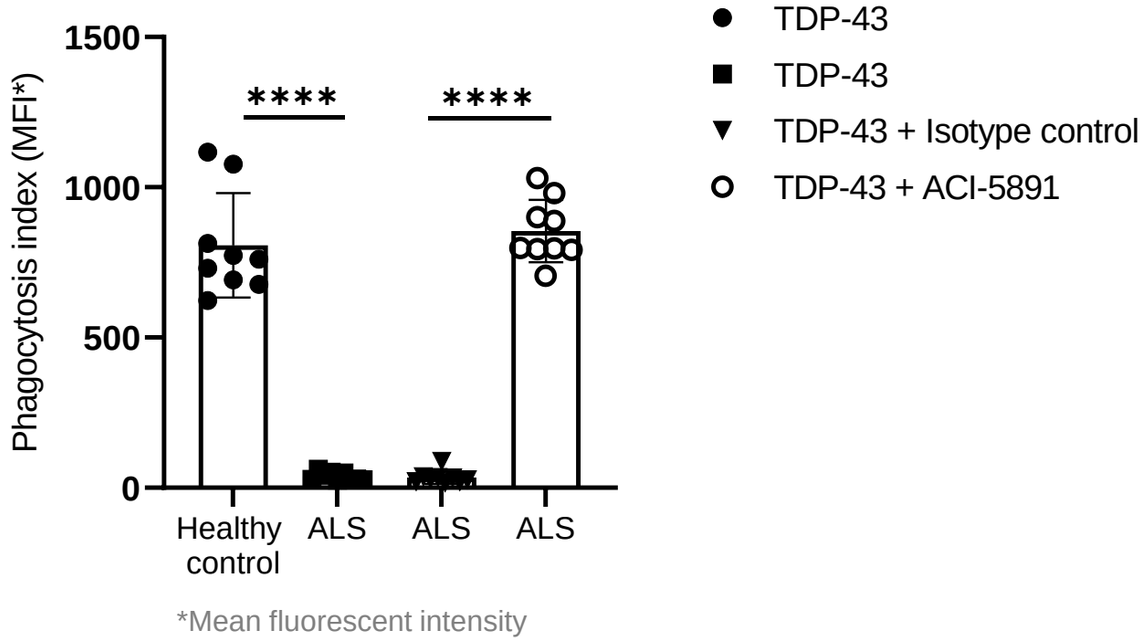
Ref: T. Afroz *et al.*, manuscript submitted

- ACI-5891 increases the mean cell size of large hypertrophic microglia in rNLS8 mice suggesting a mechanism for the reduction of TDP-43 pathology by increased phagocytic activity
- ACI-5891 increases microglial density as compared with effector-reduced mAb in CamKIIa mice

ACI-5891 promotes TDP-43 uptake by ALS patient cells

TDP-43 aggregate internalization by ALS monocyte-derived microglia



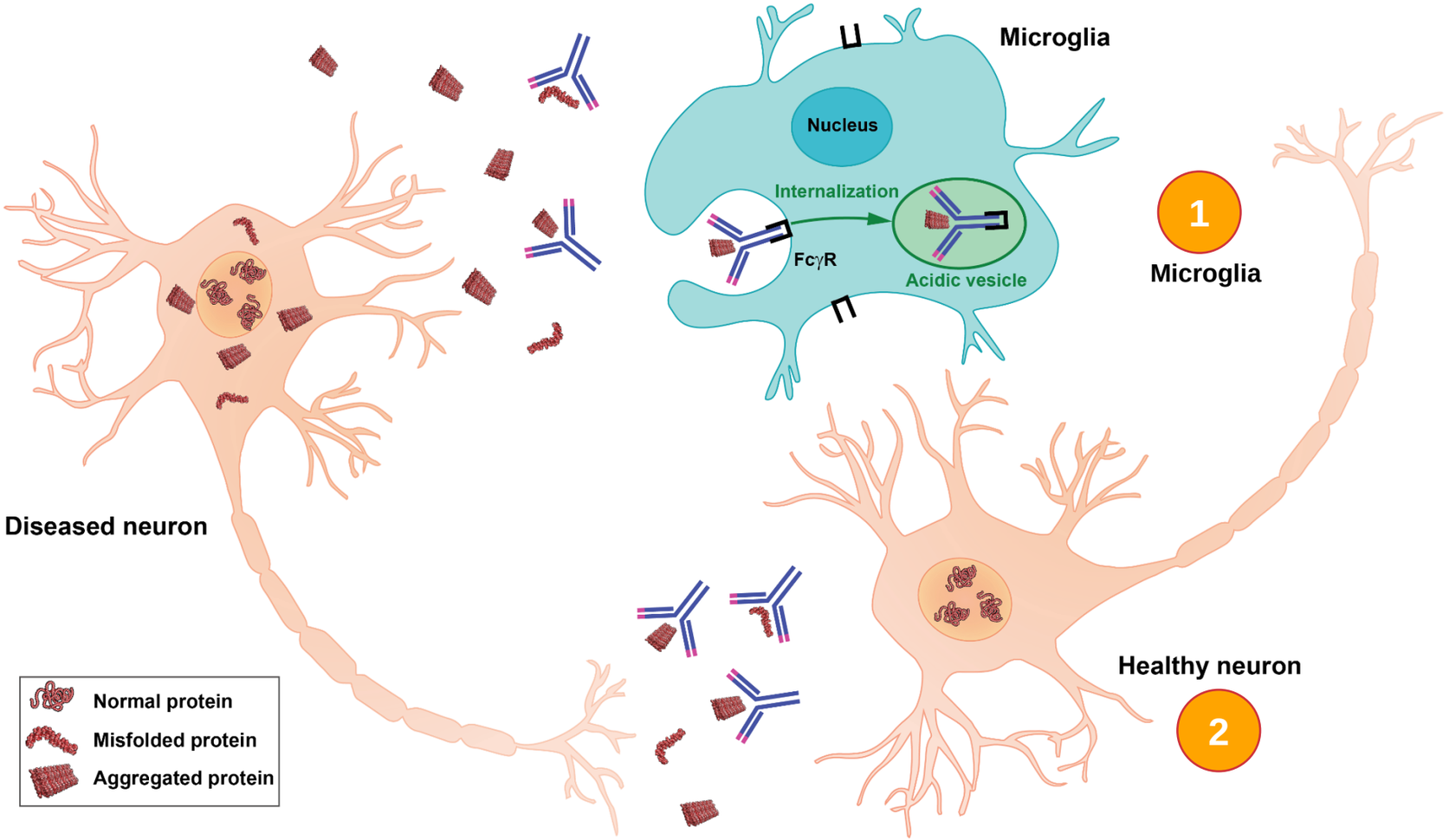


*Mean fluorescent intensity

Ref: AC Immune unpublished data

- ACI-5891 promotes phagocytosis of TDP-43 aggregates in microglia derived from ALS patients

ACI-5891 mode of action



Ref: T. Afroz *et al.*, manuscript submitted

- *In vivo* and *in vitro* data demonstrate microglia-directed phagocytic clearance and inhibition of TDP-43 templated-aggregation and thereby spreading as a potential mode of action of ACI-5891

Conclusions

TDP-43 mAb therapeutic program

1	▪ A panel of mAbs with broad epitope coverage obtained using the SupraAntigen® platform

2	▪ Antibody with picomolar affinity targeting C-terminal domain of TDP-43 showed efficacy *in vitro:*
	  ▪ inhibiting TDP-43 aggregation by 98%
	  ▪ efficiently depleting TDP-43 seeds from patient brain extracts to decrease templated-aggregation within cells
	▪ Two distinct *in vivo* models of TDP-43 pathology demonstrated:
	  ▪ C-terminal mAb altered pathology in contrast with the RRM targeting mAb
	  ▪ significantly reduced TDP-43 mediated pathology and neuronal loss
	  ▪ mechanism involves microglia and Fc-mediated phagocytosis and therefore the need of full effector in the therapeutic candidate
	▪ Importantly using cells from ALS patient demonstrates that ACI-5891 also rescues phagocytic impairments

3	▪ ACI-5891 humanization and manufacturability assessment completed

Acknowledgements

AC Immune

Andrea Pfeifer
Marie Kosco-Vilbois
Tamara Seredenina
Bojana Portmann
Tamar Ziehm
Christopher Dumayne
Alberto Silva
Romain Ollier
Francesca Capotosti
Marija Vukicevic
Kasia Piorkowska
Mickael Audrain

Valérie Eligert
Paolo Montanari
Damien Nevoltris
Jan Stoehr
Pierre Heuzé
Elodie Chevalier
Anne-Laure Egesipe
Lorène Mottier
Inmaculada Rentero
Ritwik Burai
Marie-Gabrielle Beuzelin
Anthony Gesbert

Valerie Alonso
Jacqueline Kocher
Mayank Chauhan
Celine Petit
Pilar Lopez
Nicolas Piot
Maxime Ayer
Aline Fuchs
Oskar Adolfsson
Samjhana Thapa

Penn
University of Pennsylvania

Prof. Virginia Lee
Late Prof. John Trojanowski
Dr. Silvia Porta

DZNE
German Center for Neurodegenerative Diseases within the Helmholtz Association

Dr. Manuela Neumann

- **Neurodegenerative Disease Brain Bank,** UCSF
- **Netherlands Brain Bank**, Netherlands Institute for Neuroscience, Amsterdam
- **Queen Square Brain Bank** for Neurological Disorders, UCL
- **Banner Sun Health Research Institute's** Brain and Body Donation Program
- Brain Bank affiliated with the **German Center for Neurodegenerative Diseases** (DZNE) and the **University Hospital of Tübingen**

AC Immune

We continue to shape the future of neurodegeneration by discovering and developing breakthrough therapies through pioneering science and precision medicine

Web: https://www.acimmune.com/

Social media: www.linkedin.com/company/ac-immune

Presenter: tariq.afroz@acimmune.com

Business development: bd@acimmune.com

Investors and Media: communications@acimmune.com