Compartmentalization of plasma TDP-43 in platelets: implications for TDP-43-related biomarker development

Ruth Luthi-Carter | AD/PD™ 2023, March 28 – April 1, 2023

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Conflict of interest disclosure

RLC is an employee of AC Immune and is entitled to stock options

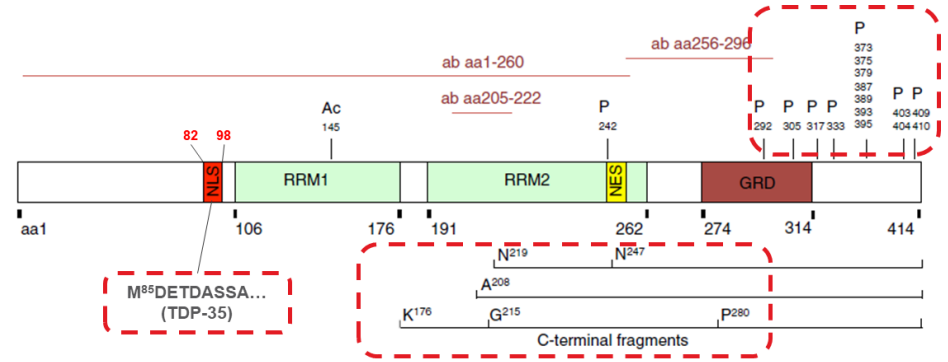
Pathological forms of TDP-43 contribute to the etiology of multiple NDDs[1]

A key target for new therapies and biomarkers

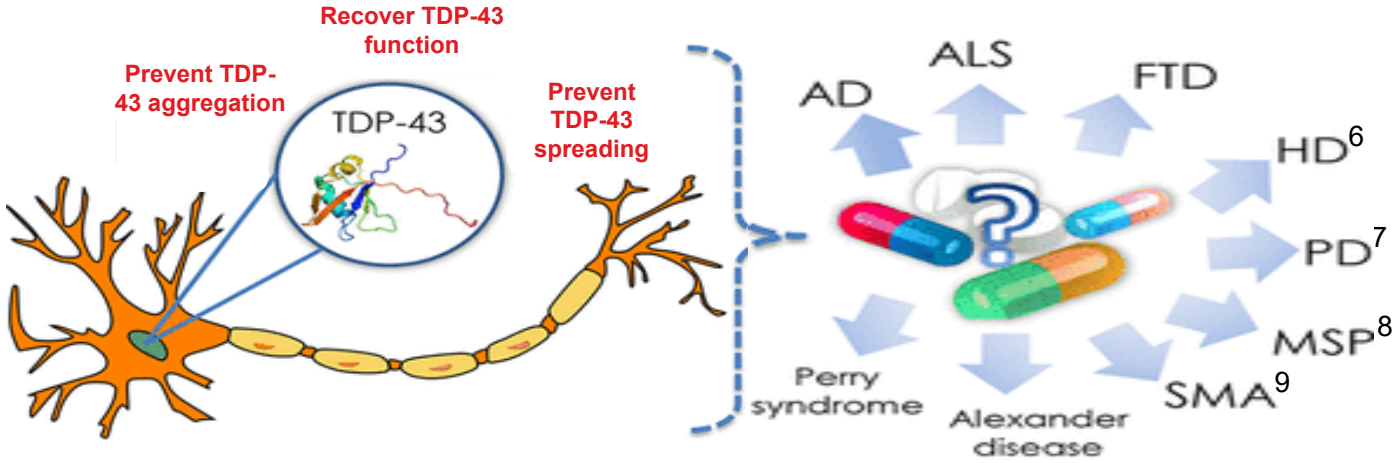
- Heterogeneous, aggregate-prone proteoforms of TDP-43 have been implicated in ALS[2], FTD[3], AD[4], LATE[5] and other NDDs
- TDP-43-based biomarkers are urgently needed for differential diagnosis and to assess target engagement for TDP-43-targeted therapeutics

Transactive response DNA-binding protein of 43 kDa

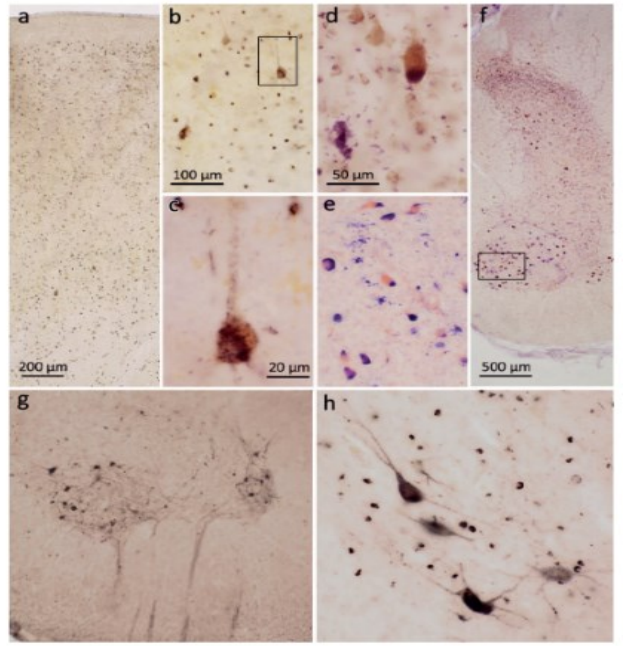
TDP-43



Adapted from Feneberg *et al.*, Mol Neurobiol. 2018



Adapted from Palomo *et al.*, ACS Chem Neurosci. 2019



Brettschneider *et al.*, Ann Neurol. 2013

(1) Neurodegenerative diseases; (2) Amyotrophic lateral sclerosis; (3) Frontotemporal dementia; (4) Alzheimer's disease, (5) Limbic-predominant age-related TDP-43 encephalopathy; (6) Huntington's disease; (7) Parkinson's disease; (8) Multisystem proteinopathy; (9) Spinal muscular atrophy

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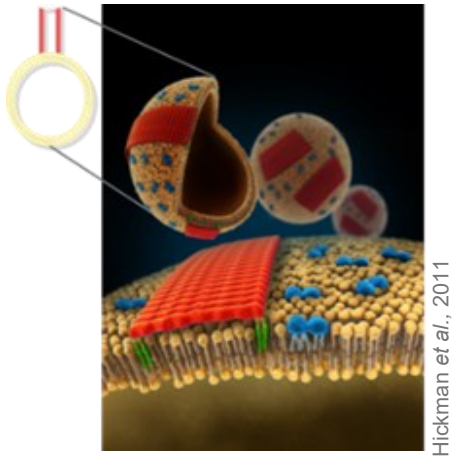
Development of assays for disease-associated forms of TDP-43

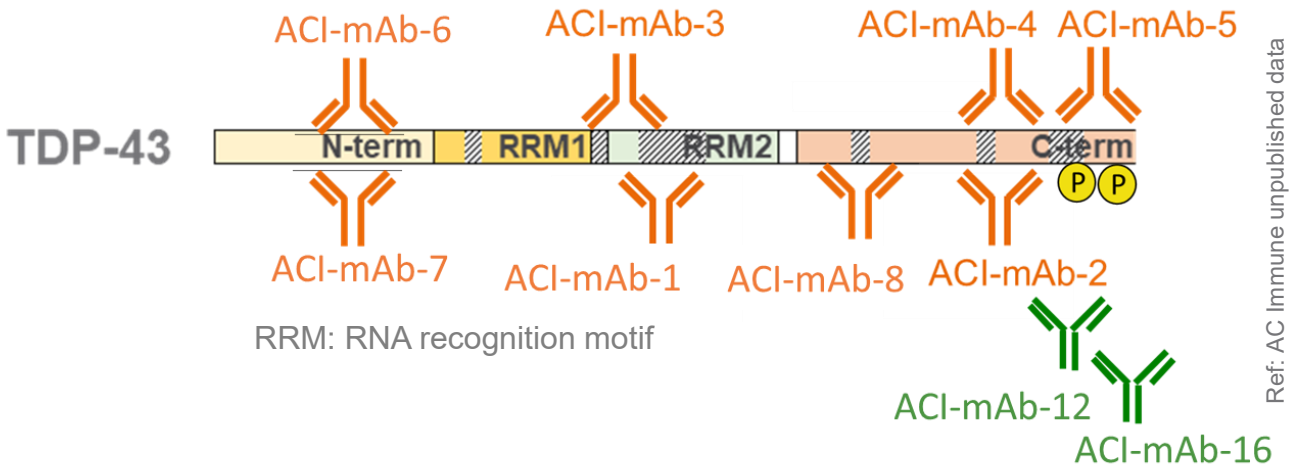
Exploited high-affinity mAbs[1] generated with ACIU's proprietery SupraAntigen® platform

SupraAntigen® platform



Hickman *et al.*, 2011

Binding regions of TDP-43 mAbs



RRM: RNA recognition motif

Ref: AC Immune unpublished data

TARGET ALS

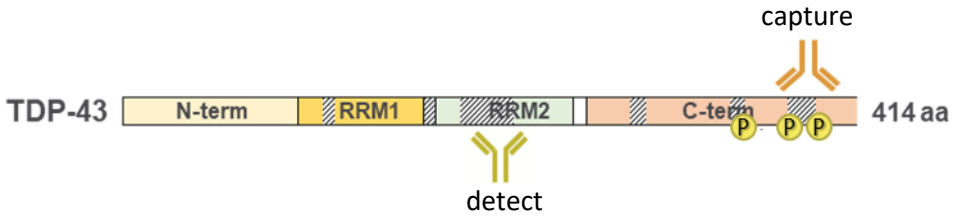
Criteria for assays

1	Detect relevant proteoforms of TDP-43
2	High sensitivity & specificity on SIMOA® bead arrays
3	Good target recovery in biofluids of interest

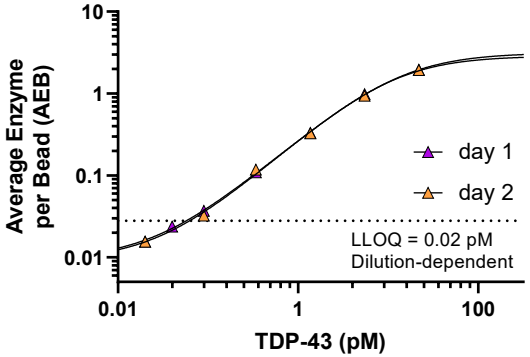
(1) Monoclonal antibodies

Development of C-terminal TDP-43 plasma immunoassays

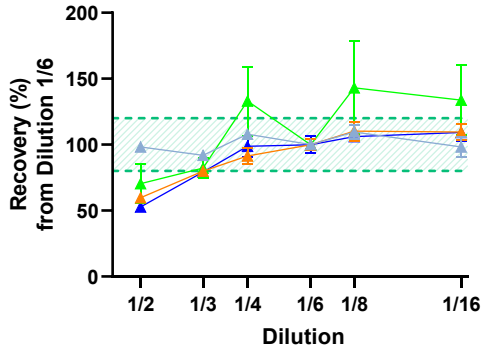
Qualification of a SIMOA® assay in human plasma



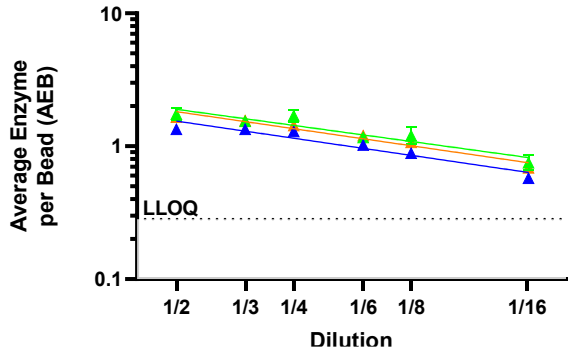
Calibration curve



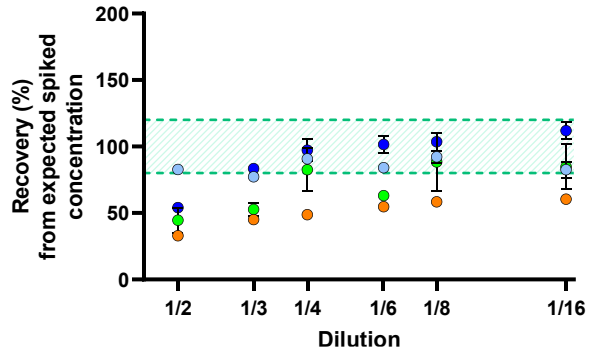
Dilution linearity



Parallelism



Target recovery



Key: (B-D)
- PBS + 11 pM TDP-4
- Plasma A + 11 pM TDP-43
- Plasma A + 5 pM TDP-43
- Plasma B + 11 pM TDP-43

- Assay showed excellent sensitivity (LLOQ[1] ~ 20 fM), specificity, signal-noise ratio, detection range, reproducibility, spike recovery and dilution linearity in human plasma

(1) Lower limit of quantification

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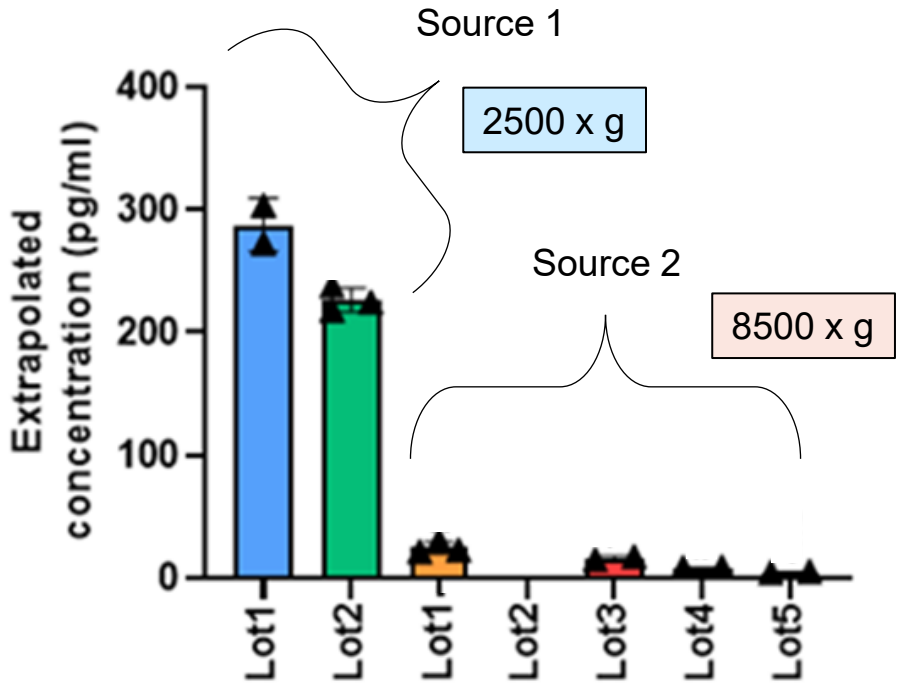
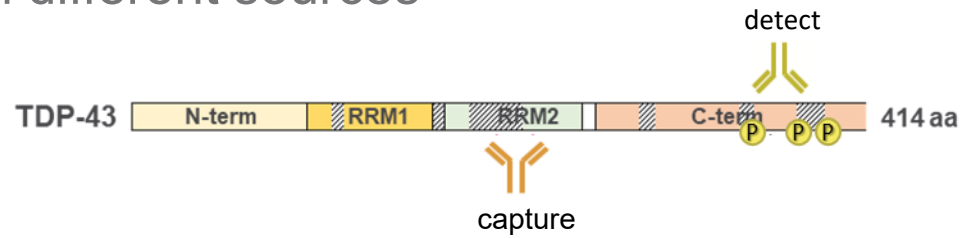
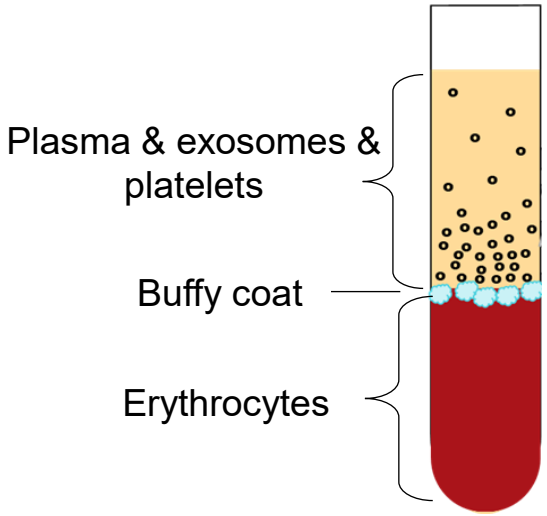
TDP-43 measurements show high variation in human plasma samples

Systematic differences in TDP-43 levels in plasma from different sources



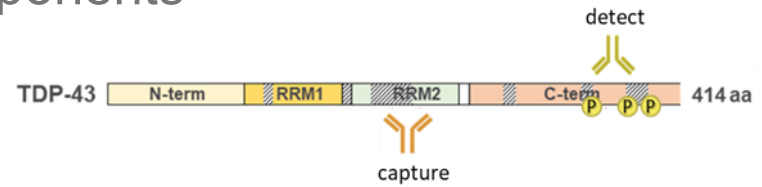


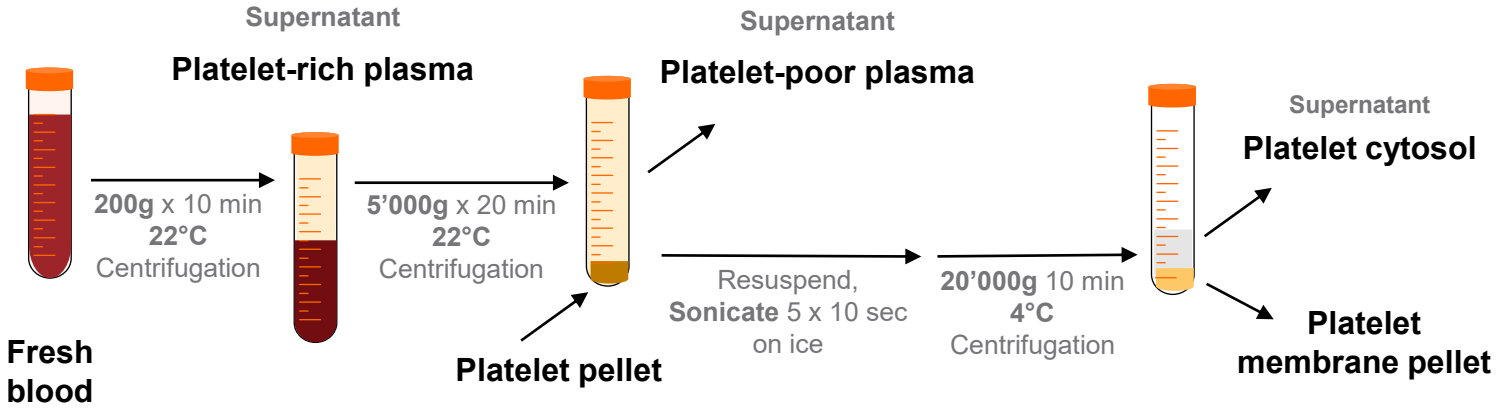


- These findings led us to hypothesize that TDP-43 might be compartmentalized within platelets

Novel assay defines compartmentalization of TDP-43 in plasma

Differential centrifugation separates non-platelet and platelet components





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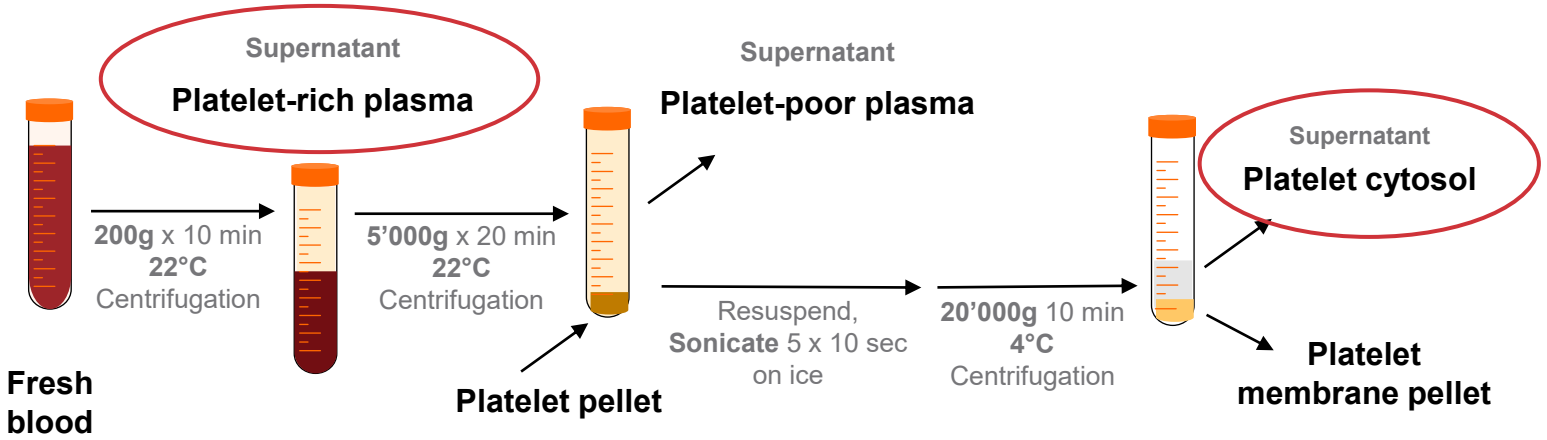
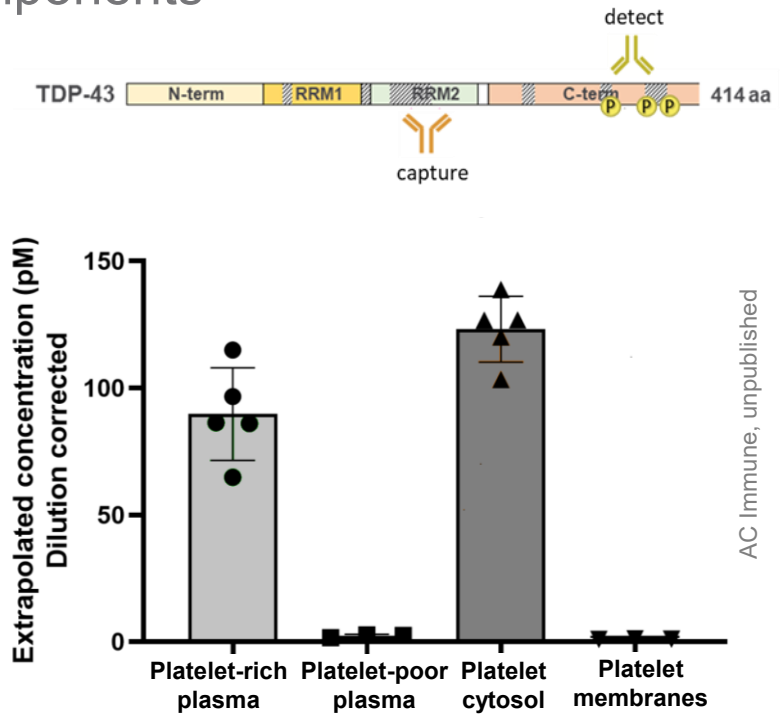
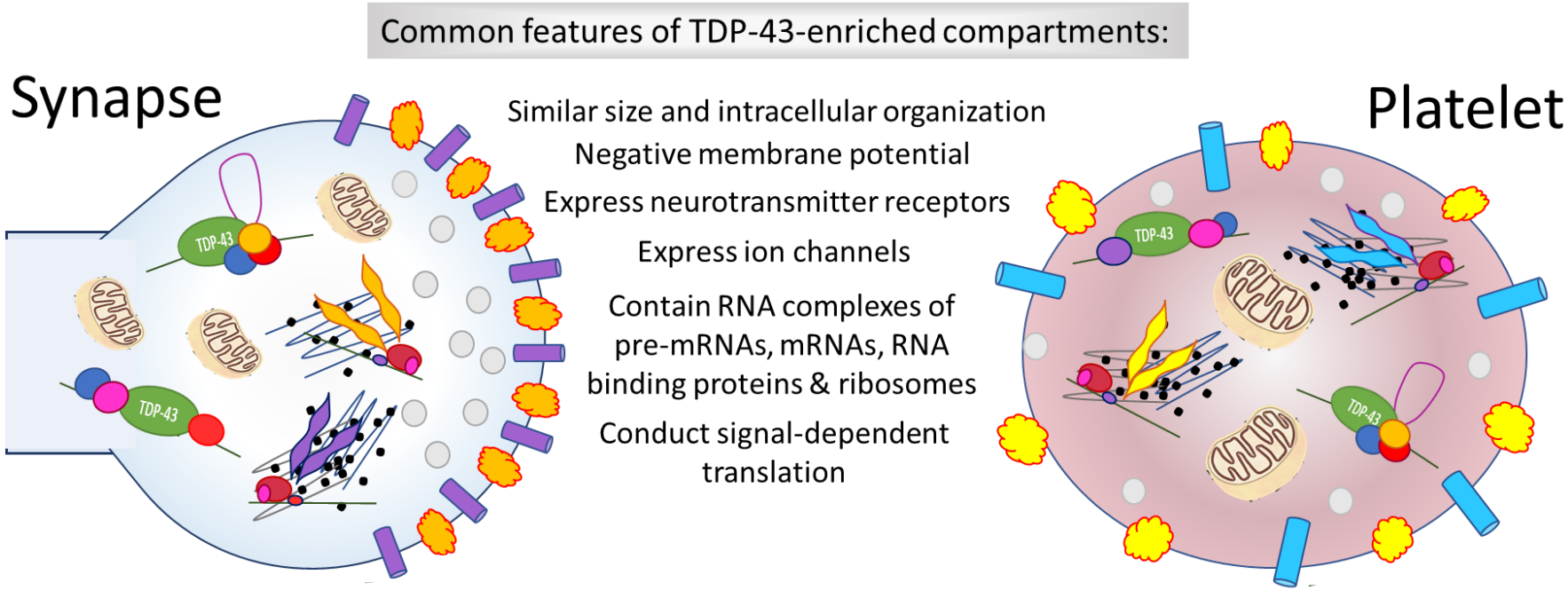




- >95% of TDP-43 is within the cytosol of platelets, comprising a concentrated, accessible source of TDP-43 for prospective biomarker analyses.

Potential disease-relevance of platelet TDP-43 biomarker analyses

TDP-43 may operate similarly in synapses and platelets



AC Immune, unpublished

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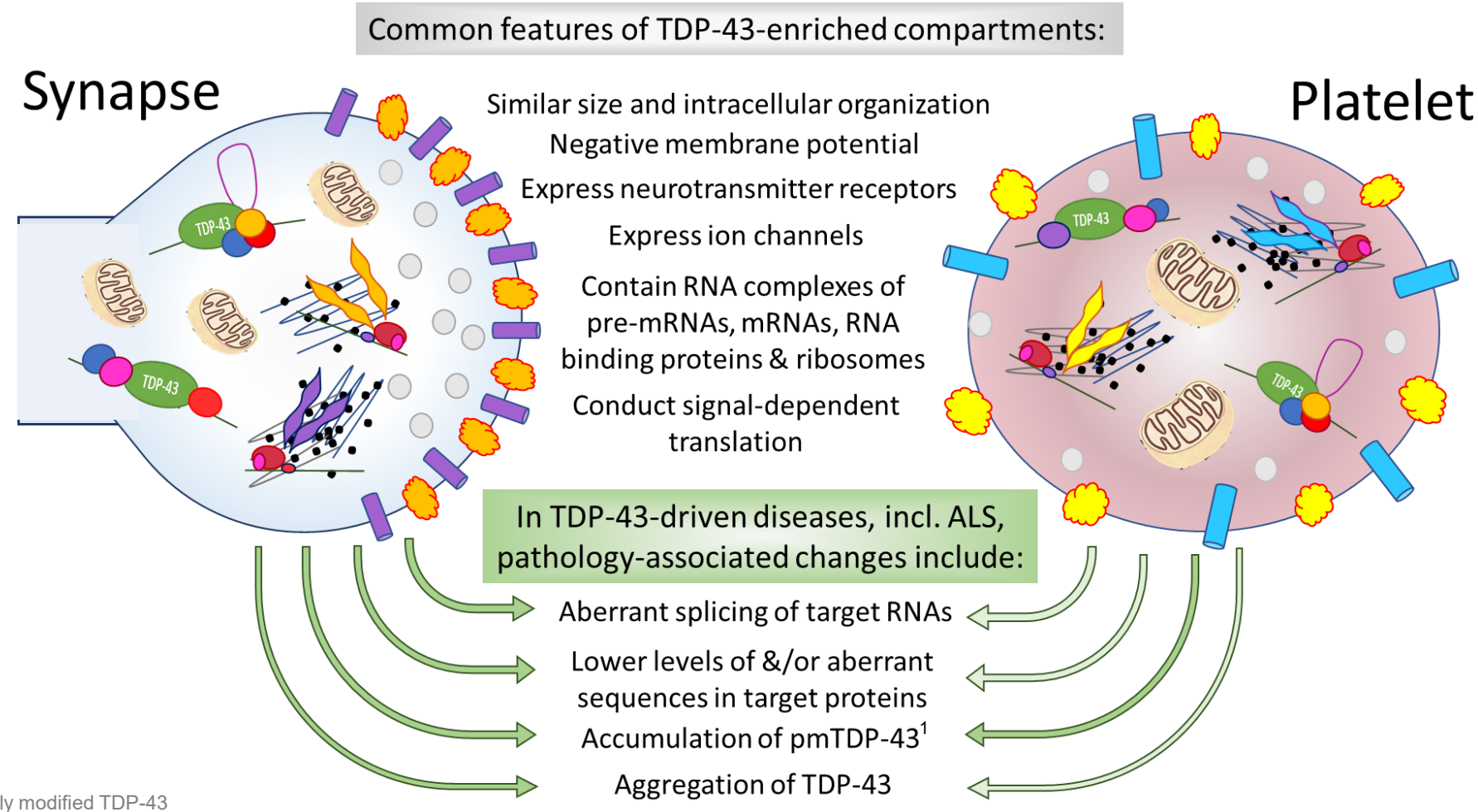


AC Immune, unpublished

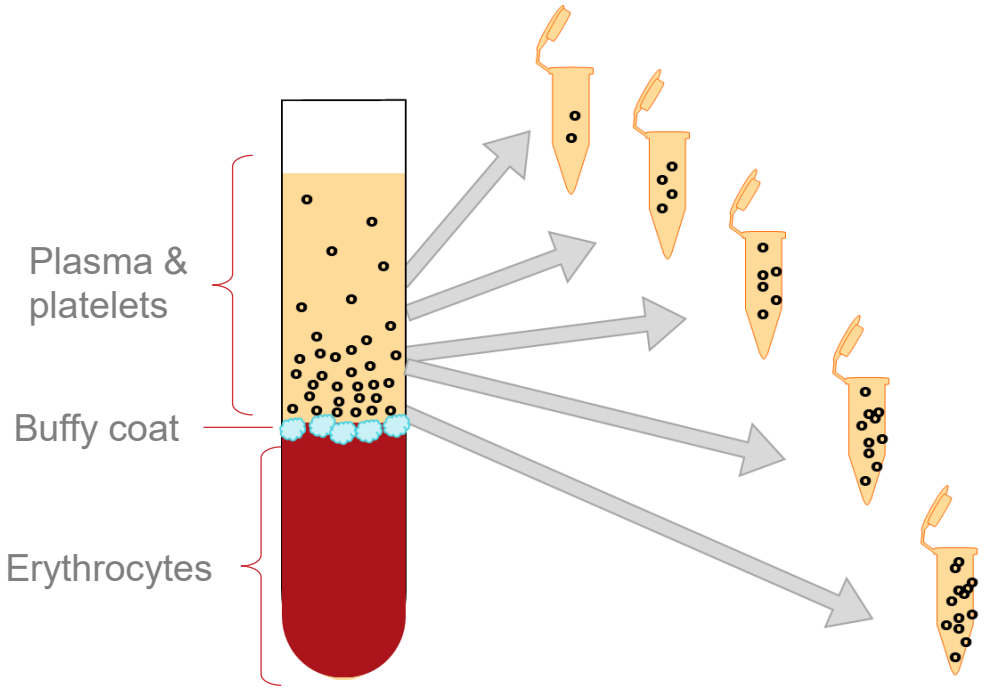
(1) Post-translationally modified TDP-43

Considerations for biobanking protocols and biofluid biomarker assays

Uneven distribution of platelets in 1500 x g plasma confounds biomarker quantification

- Our TDP-43 assay results highlighted a more general issue with plasma sample processing
- Typical biobanking procedures collect samples with varying numbers of platelets
- Residual platelet content may confound measures of plasma protein quantification
- Platelet-rich plasma is inherently unstable due to freeze-thaw effects causing platelet lysis

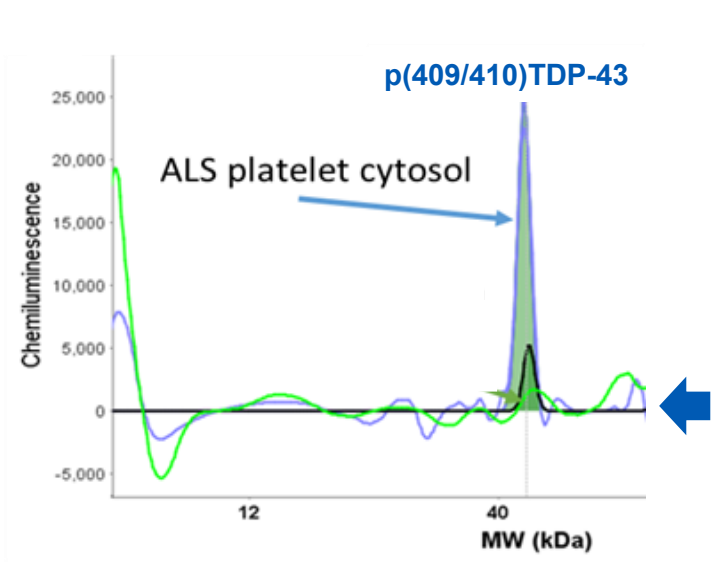
Platelet content varies by tube position

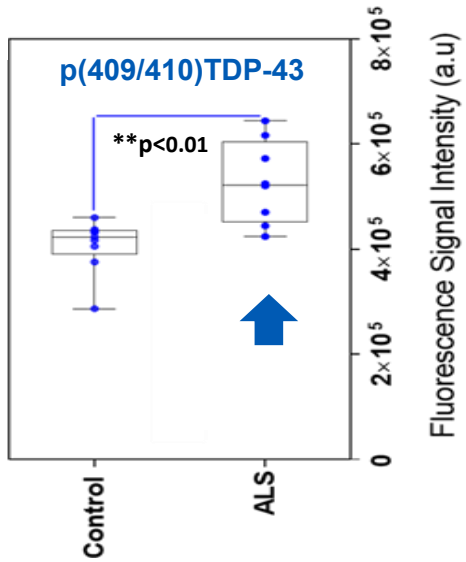


Disease-associated TDP-43 proteoform can be detected in platelets

Ongoing studies as part of a Target ALS consortium project

p(409/410)TDP-43 measured by Capillary Electrophoresis Immunoassay (CEI)





Wilhite *et al.*, Future Sci OA 2017,
Sage *et al.*, *J Vis Exp* 2020,
Agbas *et. al.*, unpublished

- TDP-43 phosphorylated at 409/410, p(409/410)TDP-43, can be detected in platelets and is increased in ALS[1]
- Consortium currently adapting plasma biobanking protocols to facilitate uniform collection of platelets for novel TDP-43 biomarker assays

(1) Amyotrophic lateral sclerosis

Summary

1

- A panel of mAbs[1] from our SupraAntigen® platform were used to develop high-sensitivity assays targeting known proteoforms of TDP-43 with broad epitope coverage
- These include C-terminal TDP-43 assays demonstrating potential clinical utility
- Unexpectedly, the assays revealed that >95% of plasma TDP-43 is contained in the platelet cytosol

2

- Understanding the similarities in the molecular regulation of TDP-43 in synapses and platelets may reveal new aspects of its RNA-, mitochondrial- & plasma membrane signaling-associated functions
- ALS[2] platelet cytosol, like ALS brain, contains elevated levels of p(409/410)TDP-43
- We propose that platelets comprise an interesting substrate for studying disease-associated forms of TDP-43 that might be a useful biomarker for TDP-43 neuropathology

3

- Our results highlighted a more general issue regarding blood processing for biomarker analyses: routine plasma collection procedures produce samples with varying levels of platelets
- Clinical studies need to consider whether measurements of their plasma analytes will be confounded by the presence of platelets and plan collection protocols accordingly
- Samples for platelet TDP-43 analyses are being collected in collaboration with the Target ALS Foundation

(1) Monoclonal antibodies; (2) Amyotrophic lateral sclerosis

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TARGET ALS

NEALS Northeast Amyotrophic Lateral Sclerosis Consortium

Healey Center for ALS

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