Comment

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The potential of TDP-43 PET ligands for a biological diagnosis of TDP-43 proteinopathies

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Candidate PET ligands targeting pathological TDP-43 aggregates are characterized by Vokali and colleagues in a series of human tissue, cell/animal model, and non-human primate experiments. Their preclinical data suggests favorable specificity and pharmacokinetic profiles of their two candidate tracers, which could translate into a disease-specific biomarker in TDP-43 proteinopathies.

Clinical and pathologic heterogeneity of TDP-43 proteinopathies

Vokali et al. address a central obstacle in the development of disease-modifying therapies for TDP-43 proteinopathies by multimodal evaluation of novel compounds with binding affinities for aggregated TDP-43 inclusions that could potentially be adapted for in vivo PET imaging detection and tracking of TDP-43 pathology in the human brain. If successful, a TDP-43-specific PET tracer would be a major advance for the field by providing a means for a biological diagnosis and enhanced clinical trial design across the spectrum of TDP-43 proteinopathies.

TDP-43 proteinopathies encompass a heterogenous spectrum of age-associated neurodegenerative disorders, including a range of predominantly cognitive disorders, such as frontotemporal lobar degeneration (FTLD-TDP)[1] and limbic predominant age-related TDP-43 encephalopathy neuropathologic change (LATE-NC)[2], and motor predominant syndrome of amyotrophic lateral sclerosis (ALS)[3]. There are currently no FDA-approved therapies for FTLD-TDP or LATE-NC, and ALS therapies target either *SOD-1* or non-specific pathways. Rapid and accurate diagnosis is challenging because 1) subtle heterogenous symptoms limit detection of early stages, particularly in sporadic disease, 2) FTLD-TDP can often present with clinically-similar or indistinguishable syndromes from primary tauopathies and atypical variants of Alzheimer's disease (AD)[1], and 3) clinical diagnosis of LATE-NC is less certain in the presence of mixed AD neuropathologic change (i.e., LATE-NC + ADNC)[4]. Thus, diagnosis is not definitive without autopsy-confirmation and there is an urgent need for biomarkers that are specific to TDP-43.

Pathologically, these disorders can be further classified based on morphological characteristics of TDP-43 inclusions (Fig. 1), as well as by genetic associations (e.g., pathogenic variants in *TARDBP*, *PGRN*, *C9orff72*, *VCP* and others)[1,5]. These disorders share the common features at autopsy of loss of nuclear TDP-43 along with cytoplasmic phosphorylated TDP-43 inclusions and associated neuronal loss within the neuroaxis, but there is significant biological heterogeneity, which could potentially affect compound binding, as observed by the authors here. For example, FTLD-TDP type C has unique features of TDP-43 inclusions that colocalize with the phospholipid-binding protein, annexin-A11 (ANXA11)[6] in hybrid heterofilaments composed of both proteins[7]. FTLD-TDP associated with *GRN* haploinsufficiency has shared features with the lysosomal storage disease, neuronal ceroid lipofuscinosis caused by homozygous *GRN* null variants[8]. *C9orf72*-associated FTLD-TDP and ALS have additional pathology from aberrant transcription of the pathogenic hexanucleotide expansion into intranuclear RNA foci and translation into di-peptide repeat inclusions[9]. Finally, LATE-NC + AD can include TDP-43 inclusions within the same neurons bearing neurofibrillary tangles[2,10]. Therefore, it is critical to rigorously validate candidate TDP-43 specific biomarkers in an array of diverse clinical, genetic and pathological subtypes in autopsy-confirmed samples for generalizability and interpretation in living cohorts.

Characterization of specificity to pathogenic TDP-43 aggregations

Vokali et al. identified lead compounds (ACI-19278 and ACI-19626) by screening a library of small molecular-weight, brain-penetrant compounds with affinity for protein amyloids (i.e., beta-sheet conformation). For these two lead compounds, they perform a series of autoradiography experiments in frontal and/or temporal cortex tissue from FTLD-TDP subtypes A, B and C, LATE-NC + AD, and healthy control tissue. The primary motor cortex of ALS was also evaluated. They find evidence of displaceable binding for both compounds in FTLD-TDP A, B and LATE-NC + AD tissues, but not controls, which qualitatively corresponded to TDP-43 inclusion density on adjacent sections. Interestingly, both ALS motor cortex with low TDP-43 inclusion burden, and FTLD-TDP C tissues with abundant TDP-43 inclusions, did not bind to either compound. High-resolution autoradiography experiments helped confirm these results at the cellular level, where, additionally, there was binding observed in the low-density inclusions in ALS.

To test the binding affinity of these compounds, Vokali et al. next performed a saturation binding assay in a subset of tissues and cross-validated this with a radiobinding assay from detergent-insoluble brain extracts of pathological TDP-43, finding favorable binding properties of both compounds. Similar to autoradiography above, they also find a lack of binding for FTLD-TDP C and controls, and low-level non-saturable binding in ALS extracts. As mentioned by the authors in the discussion, these data raise interesting questions into the unique nature TDP-43/ANXA11 co-filaments in FTLD-TDP C and potential limits

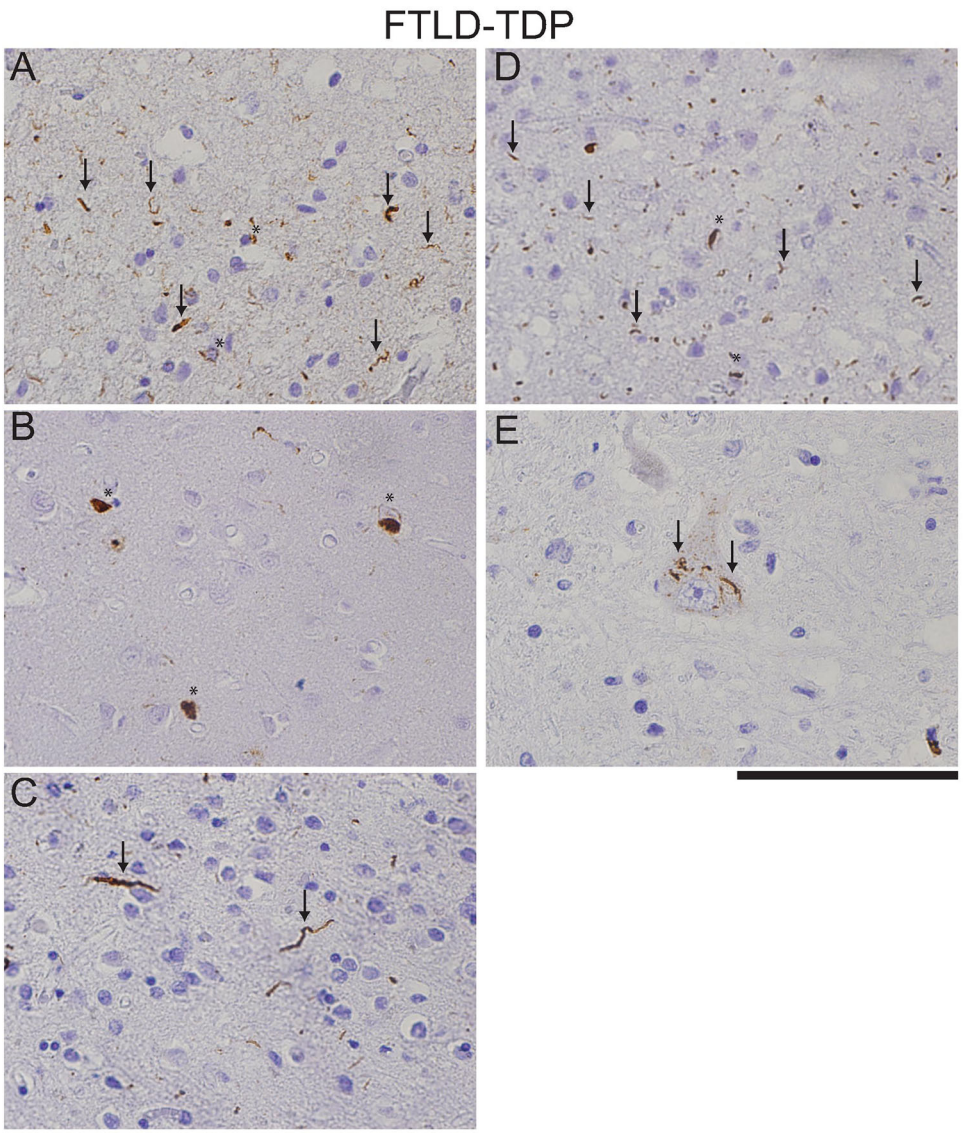

Fig. 1 | Heterogeneity of TDP-43 inclusions in FTLD-TDP and ALS. Photomicrographs of representative morphologies for FTLD-TDP subtypes (**A**–**D**) and ALS (**E**) tissue immunostained for phosphorylated TDP-43 (pS409/410). **A** FTLD-TDP morphological subtype A with superficial layer short dystrophic neurites (arrows) and neuronal cytoplasmic inclusions (asterisks) containing pathological TDP-43, **B** FTLD-TDP subtype B with mainly neuronal cytoplasmic inclusions (asterisks), **C** FTLD-TDP subtype C with long ropy dystrophic neurites (arrows), and **D** FTLD-TDP subtype D associated with pathogenic variants in *valosin-containing protein* (*VCP*) with superficial layer lentiform neuronal intranuclear inclusions (asterisks) and short dystrophic neurites (arrows) while **E** ALS shows skin-like inclusions (arrows) in anterior horn motor neuron. Scale bar = 100 µm. Reproduced and modified from Irwin et al.[16].

of detection for low levels of inclusion burden in specific brain regions, subtypes and stages of disease.

Finally, complimentary experiments using surface plasmon resonance spectroscopy in human brain extracts and a cell-based model of TDP-43 exon splicing function helped determine lack of compound binding to insoluble (non-pathologic) TDP-43 and lack of interreference in TDP-43 physiologic function in vivo, respectively. Moreover, the auto-fluorescent ACI1278 compound showed specificity for TDP-43 fibrils and not endogenous TDP-43 in a cell model system.

Testing compound specificity compared to other proteinopathies

After establishing binding affinity for pathologic TDP-43 in a subset of TDP-43 proteinopathies, the authors next test compound specificity using autoradiography in ADNC without TDP-43 and primary tauopathies, comparatively with established tracers for Aβ and tau. Moreover, in mixed pathology LATE-NC + AD tissue, candidate compounds showed relative selectivity for TDP-43 inclusions compared to Aβ and tau tracer binding to plaques and tangles, respectively. Finally, binding affinity experiments using insoluble fractions of brain extracts from

ADNC and Parkinson's disease found neither compound reacts with pathological tau, Aβ or alpha-synuclein-enriched extracts. To help exclude other sources of off-target binding, the authors also screened a panel of cellular proteins, including monoamine oxidase A and B, without evidence of binding for either compound. These data help establish potential utility in the common clinical setting of mixed pathologies and suggest low-likelihood of off-target binding in healthy brain.

Pharmacokinetic profile of candidate ligands

The authors present complimentary data from experiments in both rodents and a non-human primate to characterize the pharmacokinetic profile of each compound. After radio-labeling with fluorine-18, infusion into a rhesus macaque and scanning for up to 180 min, favorable parameters for brain uptake, washout and metabolism were observed for both tracers.

There were subtle differences between compounds, with advantages of ACI-19626 over ACI19278, including greater specificity for TDP-43 inclusions compared to other pathologies, an absence of non-specific white matter binding, and less lipophilic properties with greater homogeneity of brain uptake in the macaque that the authors posit will result in greater signal-to-noise and faster washout. As a result, ACI19626 is currently being testing in first-in-human clinical trial (NCT06891716).

Concluding remarks

The authors should be applauded for their multidisciplinary approach to carefully account for the heterogenous biology of TDP-43 proteinopathies, as well as the potential confounds from the complexities of mixed-pathologies in aging. Another strength of this study is the use of complementary assays to cross-validate results. With guarded optimism for the future, there are still unanswered questions for the long-term translation of these pre-clinical data into a clinical biomarker.

While the authors performed detailed postmortem work on a range of patient samples, considering the vast heterogeneity of TDP-43 disorders (Fig. 1), there were a relatively small number of patient samples and regions evaluated, which is a somewhat inherent limitation to postmortem studies. Indeed, some genetic and pathological subtypes were not studied, including FTLD-TDP subtype E, which has unique features of diffuse grain-like and granulofilamentous neuronal cytoplasmic inclusions[5]. Moreover, additional regional analyses in ALS cases with varying levels of cortical pathology may help further clarify the limits of detection for sparse pathology; however, as the authors thoughtfully note, further work in a larger array of diverse samples across brain regions will help further establish greater generalizability of their findings. Finally, the lack of binding in FTLD-TDP C tissue further emphasizes the unique biology of this subtype, which warrants further study and consideration in future biological classification schemes for TDP-43 disorders.

TDP-43 proteinopathies have unique histopathological features compared to ADNC, which may influence eventual in vivo human PET data interpretation. TDP-43 inclusions are relatively sparse and less reactive to amyloid-binding dyes[11] compared to ADNC. Moreover, TDP-43 lacks "ghost pathology" left behind from degenerated neurons, and regions with very advanced neurodegeneration have low levels of neuronal TDP-43 inclusions[12,13], suggesting the in vivo temporal dynamics of inclusion formation and tracer uptake could diverge from the patterns of additive regional progression observed for ADNC tau[14]. Thus, future analyses of diagnostic accuracy and regional uptake for potential PET TDP-43 ligands will need to carefully account for the clinical stage of disease, phenotype and cortical volumes. Finally, TDP-43 proteinopathies have variable inclusions in white matter oligodendroglia[5], especially in tracts associated with gray matter neuron loss[13], thus selection of ACI-19626 with limited nonspecific binding may be helpful to detect this biological signal in humans.

Despite these potential caveats, a successful PET TDP-43 tracer has the potential to achieve a biologic diagnosis in vivo, which thereby would reduce diagnostic delays and facilitate a precision medicine approach as inclusion criteria and/or outcome measure for clinical trials targeting TDP-43 mechanisms. Moreover, PET TDP-43 imaging would facilitate enhanced models of biological disease progression and accelerate validation of future biofluid and other biomarkers of TDP-43 biology.

In summary, there is a recent paradigm shift to biological definitions of neurodegenerative disease[15], but TDP-43-specific biomarkers are currently an important missing piece to the diagnostic puzzle. Here, Vokali et al. present early preclinical data in candidate PET ligands demonstrating specific binding affinity in a subset of TDP-43 proteinopathies with the potential to address this critical gap.

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