Fixing the Misfolded Proteins That Cause Dementia and Organ Failure

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A Very Brief Personal History

• Growing up in a small Western New York town
• High School Chemistry Teacher—Mr. Tierney
• SUNY College at Fredonia—Professor Phil Kumler
• How did a chemist get interested in neurodegeneration—Rockefeller University?
  • Scripps is a community of fantastic scientists that shaped me
• Where have we been and where are we going with respect to dementia medicines?
Common Neurodegenerative Diseases

• Alzheimer’s disease—Dementia—Economic cost $ 300 billion—Worsened caregiver health, 50 million Patients worldwide, 5th Leading cause of death
  • Impaired ability to remember & think, serious enough to impede daily life

• Parkinson’s disease—Movement disorder & dementia—Economic cost $ 52 billion—Worsened caregiver health, 10 million Patients worldwide, 14th leading cause of death

• Transthyretin Amyloidosis—Polyneuropathy & dementia / cardiomyopathy—Economic Cost $ 10 billion—Worsened caregiver health, ≈ 5 million Patients worldwide
Neurons do not easily regenerate and are thus susceptible to degeneration upon sustained insult, thus a subset of neurons can die in the aging brain.
Thus dying or dysfunctional neurons in the brain impairs connectivity and normal brain function / communication.
Brain Function Requires Neuronal Communication

Several Organ Systems are Compromised!
Mechanistic Take-home message: Neurodegenerative diseases are disorders of protein shape

Proteins having a normal shape are generally spherical (2-3 nm diameter)

Abnormal protein shapes associated with degenerative diseases are rectangular (1-2 nm x ≈ 3000 nm)
On the Origins of Proteins
DNA to RNA to Protein

DNA is transcribed into RNA. RNA is translated into proteins.

Proteins are best thought of as an unlatched pearl necklace, composed of twenty different types of “pearls” or amino acids with distinct chemical properties.

Because of the affinity of a given amino acid for a subset of the twenty other amino acids, proteins adopt shapes by a process known as protein folding that can be spontaneous.
Protein Folding is often Spontaneous—Misassembly Competes

Unfolded Protein
Abnormal Shape

Folded Protein
Normal Shape

Misfolded Protein
Abnormal Shape

Misassembled Protein
Abnormal Shape
Potentially Pathogenic
Intracellular Protein Folding & Protein Degradation Compete

Unfolded Protein
Abnormal Shape

Folded Protein
Normal Shape

Misassembled Protein
Abnormal Shape
Potentially Pathogenic

Misfolded Protein
Abnormal Shape

Cellular Degradation

- Protein folding often inefficient
- Cellular degradation decreases with aging
- Aging is the dominant risk factor for neurodegeneration
Inability to Maintain the Extracellular Folded State Leads to Misfolding and Misassembly and the formation of Many Abnormal Shapes

- Unfolded Protein
- Abnormal Shape
- Folded Protein
- Normal Shape
- Misfolded Protein
- Abnormal Shape
- Misassembled Protein
- Abnormal Shape
- Potentially Pathogenic
- Amyloid Fibrils
Since We Do Not Know Which Abnormal Protein Structures Drive Degeneration, We Posited that Inhibiting All Aggregation of Newly Synthesized Protein Would be Key to Clinical Success.
Stabilizer Binding to the Properly Folded Protein Maintains the Extracellular Folded State Leading to Less Misfolding and less formation of many misassembled or abnormal Shapes
Abnormal shapes confer abnormal functions that damage tissue.

Unstructured low-n oligomers ↔ Unstructured diffuse deposits

Off pathway to fibrils

Pore-forming oligomers, micelles, cylindins, diffusible ligands, etc.

On pathway to fibrils

Structured low-n oligomers ↔ Protofibrils

Amyloid fibrils →

Interfere with transcriptional and other signaling

Compromise organelles like mitochondria

Activate inflammatory and stress-responsive signaling

Interfere with protein trafficking

Consume proteostasis capacity
The Transthyretin (TTR) Amyloidoses Are \textit{in trans} Gain-of-Proteotoxicity Diseases—Sporadic and Autosomal Dominant
Transthyretin (TTR)–Extracellular Circulatory Protein

Transport Thyroxine Retinol Binding Protein

- 127AA β-sheet rich 55 kDa homotetramer
- Present in serum & cerebral spinal fluid
- Ligand-less TTR is the form that aggregates
We Investigated the Detailed Mechanism by Which Transthyretin (TTR) Aggregates—Basic Knowledge Needed for Interventions


Ligand Binding Can Prevent The Tetramer-Amyloidogenic Intermediate Transition Into Misfolded Transthyretin Assemblies

Most Conservative Approach as Clinical Success Dose not Presuppose What the Toxic Species is, Unless its....
Native State Kinetic Stabilization Mediated by Activation Barrier Tuning with Small Molecules

Activation Free Energy

T-I₂ → T-I → Tetramer (T) → Folded Monomer → Unfolded Monomer
Neurologic Impairment Score-Lower Limb Neurological Exam—Sensation, Muscle Strength and Lower Limb Reflexes—First Clinical Trial

Change From Baseline, 2011

- Dr. Richard Labaudiniere
- Dr. Teresa Coelho et al.
- Dr. Donna Grogan

Tafamidis 20 mg once daily

![Tafamidis molecule](image)

![Graph showing change in Neurologic Impairment Score over time with Tafamidis and Placebo groups](graph)

FoldRx

THE FRONT ROW
at Scripps Research
Starting Tafamidis Early in the course of TTR Peripheral and Autonomic Neuropathy offers a substantial advantage to the patient

<table>
<thead>
<tr>
<th></th>
<th>Stable or improved</th>
<th>Progressing at a slow rate (mean progression of NIS-score is 1.1 / year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Started on tafamidis (n=22)</td>
<td>68%</td>
<td>22%</td>
</tr>
<tr>
<td>Started on placebo (n=22) 18 Month Delay in Start of Treatment</td>
<td>46%</td>
<td>27%</td>
</tr>
</tbody>
</table>

Indicates that the earlier patients go on Tafamidis the better; prophylaxis when generic
Long-Term Survival With Tafamidis in Patients With Transthyretin Amyloid Cardiomyopathy

Transthyretin Amyloidosis Lead to Dementia, Focal Neurologic Episodes, Cerebral Vascular Bleeding

The Choroid Plexus Secretes TTR
Into the Cerebral Spinal Fluid

TTR Concentration in Blood ≈ 4 μM
TTR Concentration in CSF ≈ 200 nM

TTR Aggregation in the CSF Destroys the Central Nervous System
Category 1 Drugs Stop Newly Synthesized Proteins from Aggregating, But do Not Clear Amyloid

- **Transthyretin Kinetic Stabilizers**
  - Tafamidis (Regulatory Agency Approval; 2011)
  - Diflunisal (Merck NSAID repurposing as TTR Stabilizer; 2013)

- **Transthyretin mRNA Degraders (Ionis Pharmaceuticals)**
  - Inoteresen, and Eplontersen—Antisense Oligonucleotides (2018)
  - Patisiran and Vutrisiran—RNAi-based drug (2018)

- **Superoxide Dismutatse Amyotrophic Lateral Sclerosis**
  - Tofersen Antisense Oligonucleotide (2023)
Antisense Oligonucleotide Against SOD, Tofersen, to Ameliorate SOD-Amyotrophic Lateral Sclerosis

Richard Smith
Don Cleveland
Frank Bennett
Timothy Miller

The graph shows the adjusted mean change from baseline in VALOR (placebo-controlled) and OLE (open-label tofersen) studies over weeks. The number of participants is indicated for the early-star tofersen and delayed-start tofersen groups. The p-value for the comparison is 0.0272 at 52 weeks.
Category 2 Drugs Clear Amyloid Fibrils, and May Slow the Aggregation of Newly Biosynthesized Proteins

• Alzheimer’s Aβ Aggregate Seeking Monoclonal Antibody
  • Aducanumab (2021; Marketing Withdrawn)
  • Lecanemab (2023)
  • Donanemab (Not an Approved Drug)

• Parkinson’s α-Synuclein Aggregate Seeking Antibody
  – Prasinezumab (Not an Approved Drug, Clinical Results Promising)

These Antibodies recruit Microglial Cells to the cross-β-sheet amyloid fibrils and in some cases other aggregates mediating cellular endolysosomal uptake and an autophagy-mediated degradation.
Lecanemab, an Aβ Oligomer & Amyloid Fibril Removing Antibody, Significantly Slowed Cognitive and Functional Decline

A

![Graph showing CDR-SB Score](chart)

Worsening vs. Adjusted Mean Change from Baseline

B

![Graph showing Amyloid Burden on PET](chart)

Less amyloid vs. Adjusted Mean Change from Baseline (controls)

C

![Graph showing ADAS-Cog14 Score](chart)

Worsening vs. Adjusted Mean Change from Baseline

Lars Lannfelt Discovered Arctic and Swedish Hereditary AD Mutations & Lecanemab in his academic lab
Unifying Hypothesis for how the Dozen Regulatory Agency Approved Neurodegeneration Drugs Function
Step 1: Creating Naked Amyloid Fibrils Inside and Outside of Cells

Goate, Glass, Barres, Cleveland, Holtzman
Step 2: Cell-to-Cell Spreading of Amyloid by Primary Nucleation or Templated Misfolding Increases Naked Amyloid Levels

Jucker, Collinge, Westermark, Diamond, Knowles
Step 3. Secondary Nucleation

Secondary nucleation converts the misfolding-prone proteome into highly dynamic non-amyloid aggregates that dissociate from amyloid and remain soluble.
Protein Aggregation is a Main Driver of Morbidity and Mortality in Amyloid Diseases

- Genetic data in Support of the Aggregation Hypothesis to explain neurodegeneration in the Literature for Decades

- Starting about 10 years ago widespread skepticism owing to clinical trial failures (predominantly in Alzheimer’s disease)

- The hypothesis that protein aggregation causes neurodegeneration lacked substantial support by the scientific and medical communities before the pharmacological clinical trial data was generated
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