Search for Therapeutics for Prion Diseases

Rapidly Progressive Dementias
San Francisco, CA

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Neurodegenerative Diseases

• The neurodegenerative diseases include Alzheimer’s (AD), Parkinson’s (PD), Huntington’s (HD), and Creutzfeldt-Jakob (CJD) diseases as well as the frontotemporal dementias (FTDs) and chronic traumatic encephalopathy (CTE).
• Over 5 million Americans suffer from Alzheimer’s and 1 million from Parkinson’s. The greatest risk factor for AD and PD is age.
• Most AD and PD cases are sporadic, but ~15% are familial due to mutations in disease-specific genes.
• Each neurodegenerative disease is caused by a different misprocessed protein; there are no medicines that either slow or halt any of these fatal disorders.
• Though ~500,000 Americans with AD die annually—similar to the number of Americans dying of either cancer or heart disease—only ~1.5% of the National Institutes of Health (NIH) budget supports AD investigations.

Spectrum of Neurodegenerative Diseases

Accumulation of disease specific proteins

• Prion diseases – PrPSc (amyloid plaques)
• Alzheimer’s disease – Aβ (amyloid plaques)
• Frontotemporal dementias (tauopathies) – tau (Pick bodies and neurofibrillary tangles)
• Parkinson’s disease – α-synuclein (Lewy bodies)
• Huntington’s disease – huntingtin (nuclear inclusions)
• ALS – SOD, TDP43, FUS (ubiquinated, cytoplasmic inclusions)
Defining the features of prions

- A prion is a protein that can adopt at least two conformational states, one of which is self-perpetuating.
- The self-perpetuating conformation of prions seems to prefer a multimeric, aggregated state.
- For mammalian PrPsc prions, the self-perpetuating, infectious state may be as small as a trimer.
- All prions appear to assemble into fibrils with the tinctorial and ultrastructural features of amyloids; PrPsc and fungal prions polymerize into amyloid fibrils as does the Aplysia prion comprising the cytoplasmic polyadenylation element binding (CPEB) protein.

Prions are self-propagating proteins that feature in metabolic processes and disease states

- Prion proteins adopt at least two conformational states, one of which is self-perpetuating and assembles into fibrils with the tinctorial and ultrastructural properties of amyloids.
- In neurodegenerative diseases, PrP, α-synuclein, APP and tau proteins accumulate in the brain intracellularly as Pick or Lewy bodies or NFTs, or extracellularly as Aβ or PrP amyloid plaques.
- Increasing evidence argues each aberrantly processed protein spreads and accumulates in brain through a prion-like mechanism.

Discovering prions form amyloid suggested plaques and tangles in other neurodegenerative diseases might also be etiologic...

Western Blot

PrPc (lane 1) is hydrolyzed by limited proteolysis (lane 2). PrPsc (lane 3) is N-terminally truncated to form PrP 27-30 (lane 4).

α-PrP antibodies were used to stain proteins extracted from normal (lanes 1 and 2) and prion-infected (lanes 3 and 4) Syrian hamster brains.

OCCASIONAL NOTES

Some Speculations about Prions, Amyloid, and Alzheimer’s Disease

The finding that prions aggregate into amyloid-like rods suggests the possibility that amyloid deposition in the brains of patients with Alzheimer’s disease represents something more than a pathologic byproduct of the disease process. For nearly half a century, amyloid plaques have generally been thought to represent waste collections of pathologic macromolecules. We must now ask whether or not the amyloid deposits in Alzheimer’s disease may represent accumulations of the etiologic agents, as they appear to in Creutzfeldt–Jakob disease, kuru, and the Gerstmann–Sträussler syndrome. However, if prions exist in Alzheimer’s disease, then they must be different from the agents causing Creutzfeldt–Jakob disease. Differences in trans...
1930: familial CJD pedigree drawn.

1973: CJD from familial cases transmitted to apes and monkeys. Several explanations were offered:
1. CJD virus transmitted among family members living in close proximity.
2. Genetic predisposition to ubiquitous CJD virus.
3. Vertical transmission of CJD virus from parent to offspring.

1989: familial CJD and GSS are caused by PrP gene mutations. Mutant PrP<sup>Sc</sup> more readily refolds into PrP<sup>Sc</sup> than does wild-type PrP<sup>c</sup>.

How can diseases be both genetic and infectious?

Spectrum of Human Prion Diseases

<table>
<thead>
<tr>
<th>Manifestation (Frequency)</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sporadic (85 - 90%)</td>
<td>Creutzfeldt-Jakob disease (CJD); incidence of 1 - 2 cases per million population worldwide.</td>
</tr>
<tr>
<td>2. Inherited (10 - 15%)</td>
<td>Gerstmann-Sträussler-Scheinker disease, familial CJD and fatal familial insomnia have 50% of family members afflicted.</td>
</tr>
<tr>
<td>3. Infectious (&lt;1%)</td>
<td>Kuru among New Guinea natives transmitted by cannibalism. Iatrogenic CJD caused by growth hormone derived from human pituitaries. New variant CJD caused by BSE prions from contaminated beef in Great Britain.</td>
</tr>
</tbody>
</table>

Creutzfeldt-Jakob Disease is Alzheimer’s on fast forward (>>)

- Creutzfeldt-Jakob (CJD) and Alzheimer’s Diseases (AD) are uniformly fatal, dementing illnesses that increase with age.
- CJD and AD patients lose their short-term memory initially followed by progressive deterioration of intellect.
- PrP<sup>Sc</sup> causes mad cow disease and CJD. The Aβ 42 peptide causes AD, and modified tau protein accumulates in AD, tauopathies and repeated traumatic brain injuries (TBI).
- People with CJD die within 3–6 months from diagnosis while most with AD live ~10 years.
**Could They All Be Prion Diseases?**

Recent studies renew interest in the idea that many neurodegenerative diseases may involve prionlike mechanisms.

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**Pharmacotherapeutic strategies for clearing self-propagating proteins causing CNS disease**

- Prion proteins adopt at least two conformational states, one of which is self-perpetuating. Some prions are toxic to cells.
- Strategies for decreasing prions: (1) decrease the precursor, (2) inhibit conversion into the prion state and (3) enhance prion clearance.
- Once the level of the prion form is sufficiently low, cells will clear the remaining pathological conformers.
- Administration of multiple anti-prion drugs for limited periods of time will likely prove the best therapeutic strategy.

1. Precursor
2. Prion form
3. Fibrils

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**Spectrum of Neurodegenerative Diseases**

**Self-propagating protein conformations**

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>Prion Diseases</th>
<th>Alzheimer’s Disease</th>
<th>FTDP</th>
<th>Parkinson’s Disease</th>
<th>Huntington’s Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporadic</td>
<td>sCJD sFl</td>
<td>AD</td>
<td>FTD, CBD, PSP, Picks, CTE</td>
<td>PD, LBD</td>
<td></td>
</tr>
<tr>
<td>Inherited</td>
<td>FJD</td>
<td>IAD</td>
<td>FTDP17, CBD, PSP, Picks</td>
<td>IPD</td>
<td>All HD</td>
</tr>
<tr>
<td>Infectious</td>
<td>CJD</td>
<td>Experimental AD</td>
<td>Experimental tauopathy</td>
<td>Lewy bodies in fetal grafts in striatum of PD patients</td>
<td>Cytoplasmic polyglutamine aggregation</td>
</tr>
<tr>
<td>Self-propagating proteins</td>
<td>PrP</td>
<td>Aβ</td>
<td>Tau</td>
<td>α-Synuclein</td>
<td>Huntingtin</td>
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</tbody>
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**From Targets to Clinical Candidates**

- Hit Identification
  - 2006 from ChemDiv: 10,000 compounds
  - 2010 from SPECS and ChemBridge: ~58,000 compounds
- Lead Generation
  - 2006 among them
  - 2010 ~16 new leads
- Lead Optimization
  - Potency
  - Toxicity
  - Solubility
  - Permeability
  - Brain Penetration
  - Metabolic Stability
- Clinical candidates
  - Aminothiazoles (AMT) selected from initial Medchem campaign
**Brain C\(_{\text{max}}\):EC\(_{50}\) ratio of aminothiazoles**

- Over 175 AMTs made; 3 with good “drug properties” now in mouse bioassay.

**CJD Program: Discovering “Hits” and “Confirmed Hits” in Diverse Chemical Compounds from Libraries and Pharma**

- 58,000 diverse chemical compounds (54,000 from ChemBridge and SPECS and 4,100 from Pharma companies), and 1,703 FDA drugs screened; hits/confirmed hits are compounds resulting in a ≥30% reduction in PrP\(\text{Sc}\).

**New Scaffolds Identified:**
- 14 from Dividing Cell Assay
- 9 from Non-Dividing Cell HTS Assay

**Example:** Benzoxazole Scaffold

**Scaffold Analysis for PrP\(\text{Sc}\) in dividing cells**
Kinetics of GFAP deposition in astrocytes of Tg(Gfap-luc) mice after inoculation with RML prions

<table>
<thead>
<tr>
<th>NIBL</th>
<th>RML</th>
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<tbody>
<tr>
<td>63</td>
<td>21</td>
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<tr>
<td>35</td>
<td>42</td>
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<td>49</td>
<td>56</td>
</tr>
<tr>
<td>63</td>
<td>117</td>
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</tbody>
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GFAP mRNA (blue)
Bioluminescence (red) and control (black)

Compound B delays GFAP upregulation and extends survival by ~150 days in FVB Tg(Gfap-luc) female mice

<table>
<thead>
<tr>
<th>Sex</th>
<th>Status</th>
<th>Drug (M)</th>
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Compounds B and AMT 163

Rx of RML-infected mice with phenylhydrazone and aminothiazole

Compd B
AMT 163
PrP<sub>Sc</sub> GFAP Actin

AMT 082

AMT 082 and 163 extend survival and reduce PrP<sub>Sc</sub> levels in FVB mice

AMT 082 AMT 163

Building a robust pipeline for CJD

AMT 082 Rx FVB Females AMT 082 Rx FVB Males

Brain [82] (µM) 1.3 1.5 4.8 4.6 2.4 5.4 4.5 5.0 TBD TBD

CJD project: Scaffolds in/scheduled for efficacy, PK and EC<sub>50</sub> studies

Efficacy Pharmacokinetic studies EC<sub>50</sub> studies

IND-0054304 6 Pyrazole, 6 Thiazole 13 Diverse amide 13 Diverse biaryl 8 Benzoxazole

3 AMTs 2 Thiazole 1 Guanidine 4 Piperazine 25-30 several scaffolds

250382 1 Quinoline 2 Stilbene 25-30 several scaffolds

255163 1 Miscellaneous 1 Miscellaneous
**AD/Tau Program: Discovering “Hits” that lower the levels of APP* and Tau+**

*48,960 diverse chemical compounds and 1,703 FDA-approved drugs were screened in APP assay; confirmed hits are those resulting in a ≥30% reduction.

*48,720 diverse chemical compounds and 1,703 FDA-approved drugs were screened in Tau assay; confirmed hits are those resulting in a ≥30% reduction.

**Widespread Aβ deposition throughout the brain in AD-inoculated Tg(APP23) mice**

**Bioluminescence imaging in bigenic AD mice**

11-12 months post-inoculation Stain: Aβ-40
Morbidity and mortality associated with Alzheimer’s disease over a 20-year time frame (MCI is mild cognitive impairment)

U.S.A. Alzheimer’s Disease: Incidence, Cost to Medicare/Medicaid & Federal Research Funding

Federal Gov’t Expenditures

People in US with Alzheimer’s Disease

Alzheimer’s Disease Facts and Figures (March 2020)

MCI & early AD

Severe AD

In care, Care/Research (current trends)

Number of Americans with AD (millions)

Complete

Under Construction

Projected

Proposed