# Investigational Therapeutics in CJD

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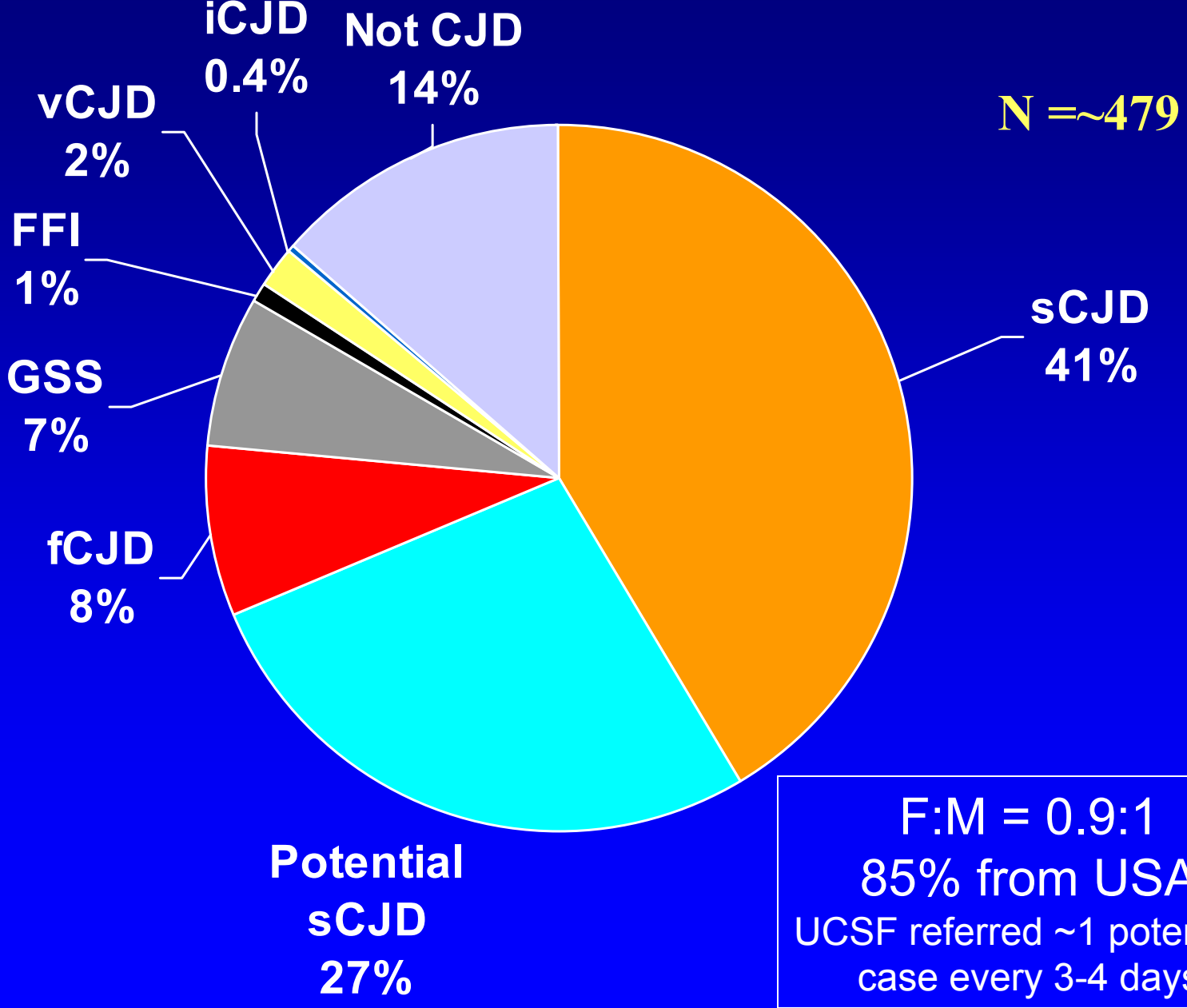
# Outline

- Therapeutic Treatment of CJD
  - Quinacrine experience
    - Observational human data
    - UCSF mouse data
  - Other potential therapeutic compounds
  - Identifying lead compounds - Screening assays
  - Rational Drug Design
- UCSF Diagnostic Studies
  - Diagnostic utility of MRI
  - First Symptom
  - If time, Conformation Dependent Immunoassay (CDI)

# Diagnostic Breakdown of UCSF Potential CJD Contacts Over past 4+ Years



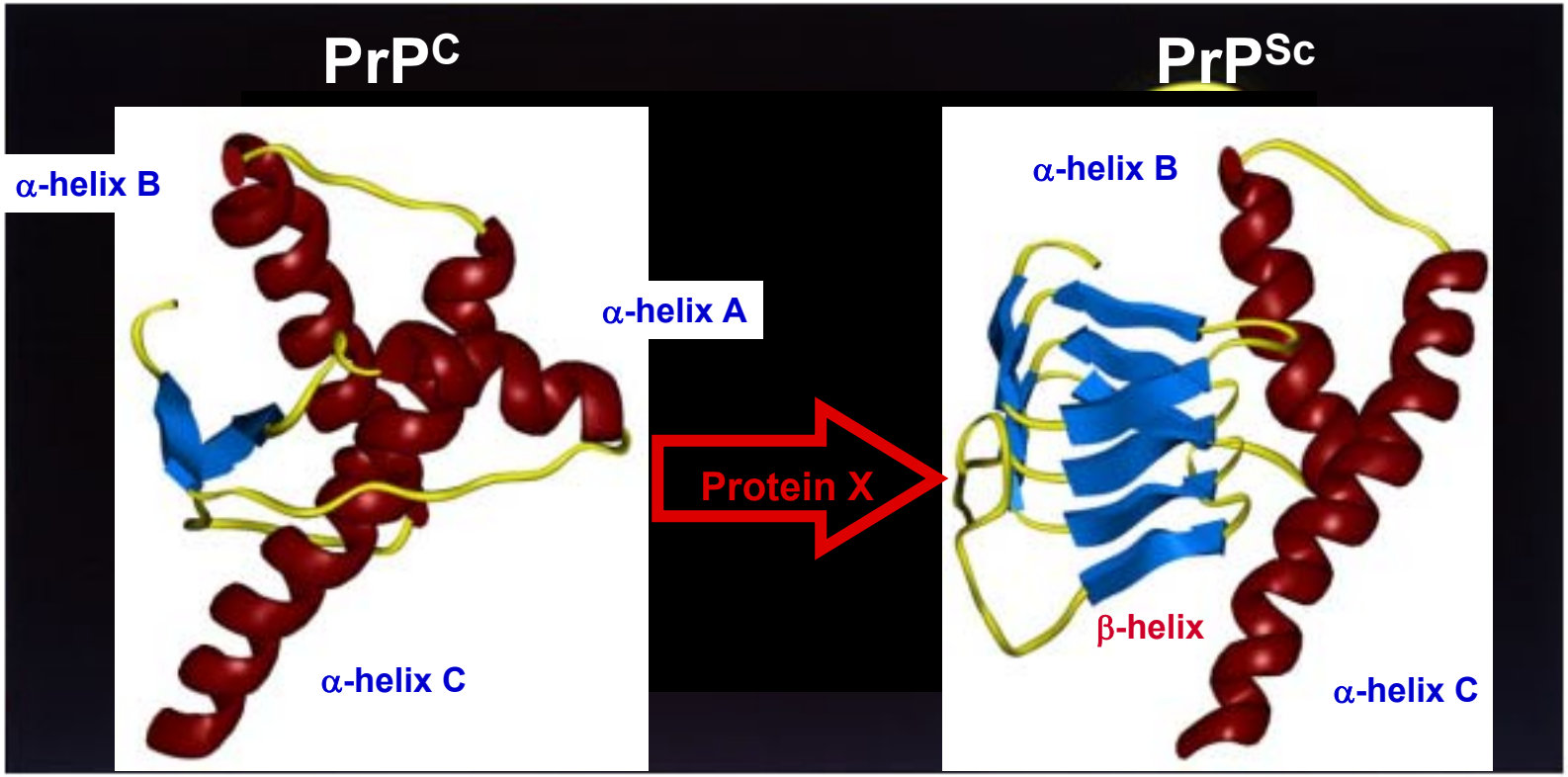

N =~479

F:M = 0.9:1

85% from USA

UCSF referred ~1 potential case every 3-4 days

# Conformational Changes Feature in Prion Replication

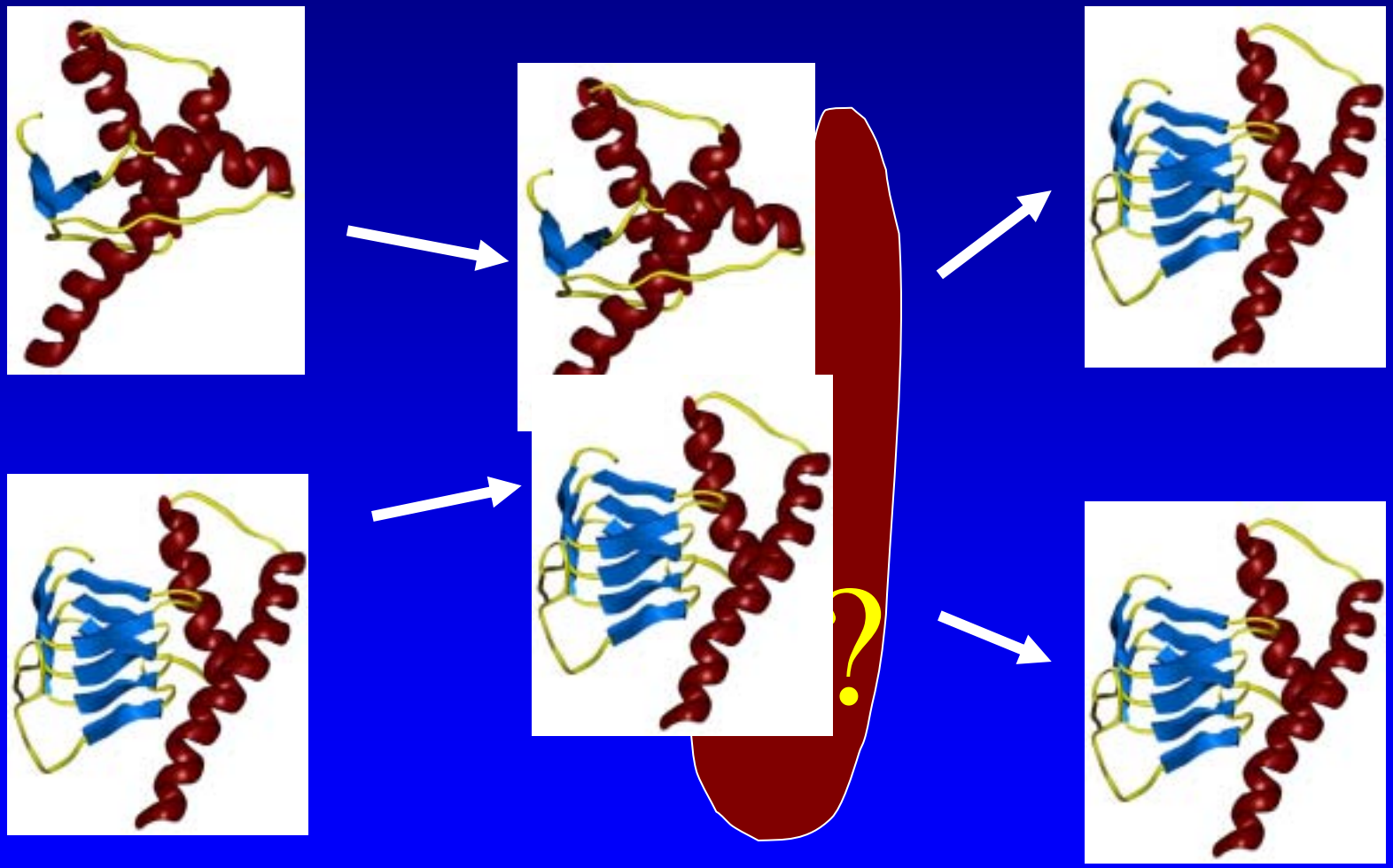



## Secondary Structure (%)

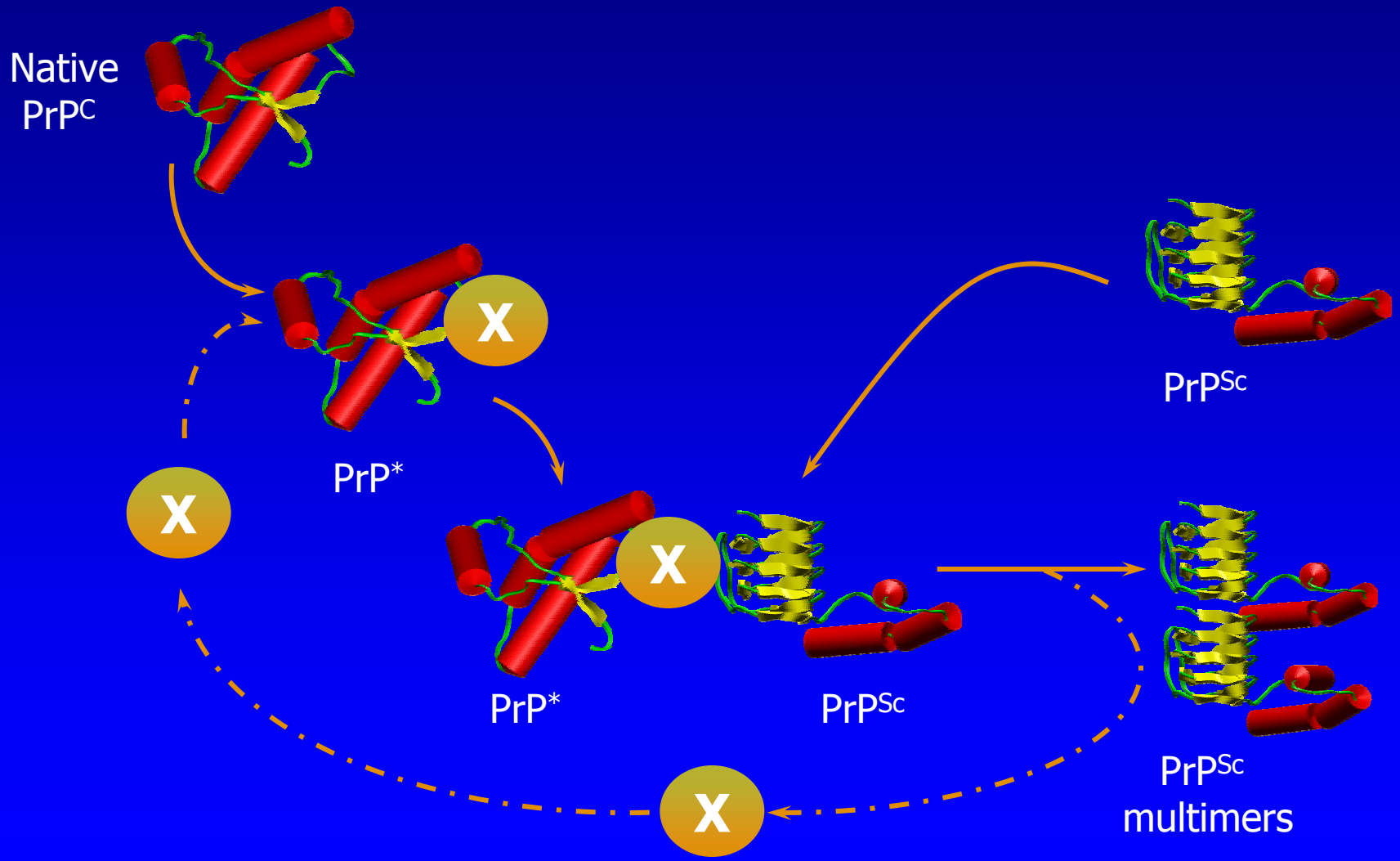
| | | |
|---|---|---|
| 42 | α-helix | 30 |
| 3 | β-sheet | 43 |
| Negative | Scrapie infectivity | Positive |

Prion models courtesy of Fred Cohen

# A Model of Prion Replication



# More Detailed Model of Prion Propagation



# Mechanisms of Treatment of Prion Diseases

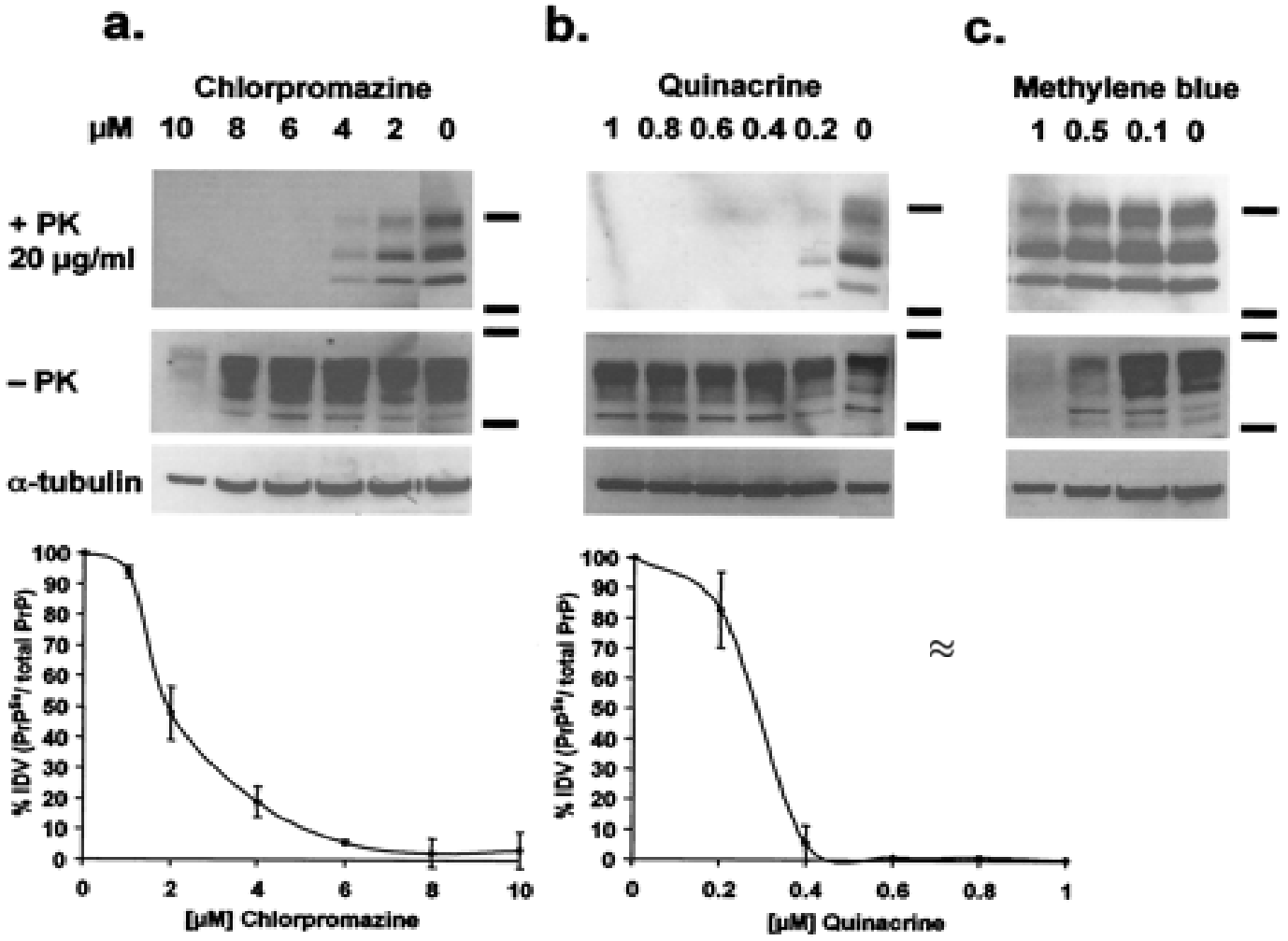
- General Mechanisms
  - Prevention of accumulation of $PrP^{Sc}$
  - Promote clearance of $PrP^{Sc}$
  - Combination
- Specific Treatment Mechanisms
  - Mimicking $PrP^{C}$ Protective Sites
  - Blocking binding of Protein X ($PrP^{C}$ & $PrP^{Sc}$)
  - Promoting lysosomal clearance?

# Some Anti-prion Agents

| | |
|---|---|
| Heteropolyanion 23 (ammonium 5-tungsto-2-antimoniate) | Kimberlin & Walker (1983, 1986) |
| Dextran sulphate 500 | Ehlers & Diringer (1984), Farquhar & Dickinson (1986) |
| Dextran sulphate 5 | Ehlers & Diringer (1984) |
| Pentosan polysulphate | Ehlers & Diringer (1984), Dringer & Ehlers (1991); Dohura et al. (2004) |
| Amphotericin B | Pocchiari et al. (1989); Demaimay et al (1997) |
| Congo red | Caughey & Race (1992), Caspi et al. (1998) |
| Anthracycline | Tagliavini et al. (1997) |
| Branched polyamines (poly amidoamide dendrimers, Polupropyleneimine, polyethyleneimine) | Supattapone et al. (1999) |
| Porphyrin and Phthalocyanine | Priola et al.(2000) |
| Cp-60 | Perrier et al. (2000) |
| E-64d | Doh-Ura et al. (2000) |
| Beta-breaker | Soto et al (2000) |
| Quinacrine | Korth et al. (2001), Doh-Ura et al. (2000), Haik et al (2004) |
| Anti-prion antibodies | Enari et al (2001); Peretz et al (2001) |
| Bis-quinacrine | May et al. (2003) |
| Flupirtine | Otto et al (2004) |

See Brown 2002 Neurology

# Quinacrine & Chlorpromazine effective *in vitro*




Korth C, May BC, Cohen FE, Prusiner SB. Acridine and phenothiazine derivatives as pharmacotherapeutics for prion disease. PNAS. 98(17):9836-41

# French Quinacrine Observational Data

- 8/01-12/02
- 1000 mg po over 30 hours then 100 tid po
- Followed monthly with Rankin (& toxicicty)
- 9 patients > 30 days of treatment
- Trend toward improved survival, but not significant (8.8 vs 7 months)*
- No difference in pathology
- Side effects
  - 6 pts - Transaminase elevation 2 pts - skin eruption
  - 1 pt - digestive intolerance
  - 1 pt - leukopenia in one.

| | Treated |
|---|---|
| Total sCJD | 30 |
| N alive (6/03) | 9 |
| Time from Onset to Quinacrine | 6.4 mo (1-17) |
| Mean Treatment Duration (range) | 36 d (1-265) |
| Mean Survival From Stop Quinacrine to Death | 40d (range 0-176) |

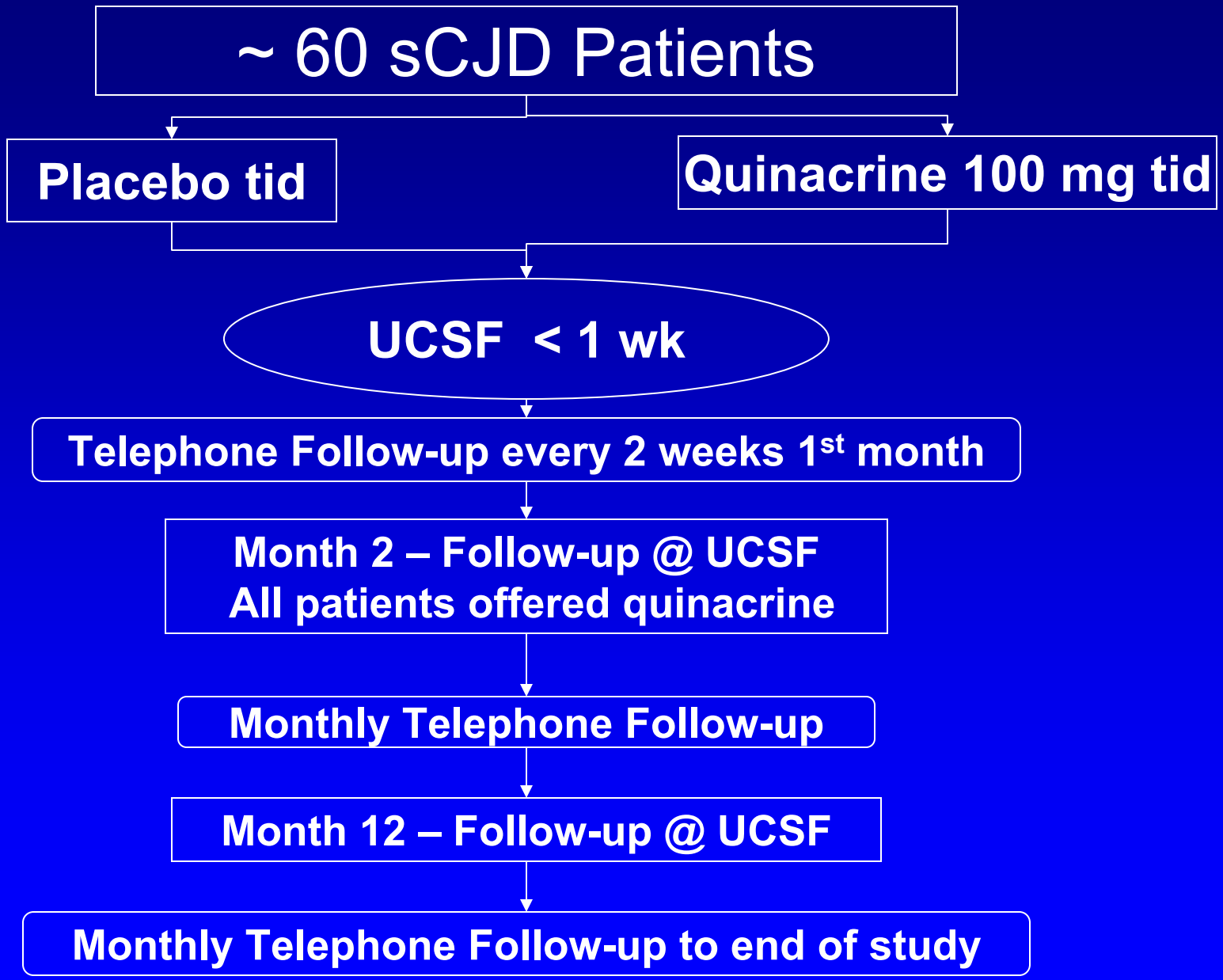
* N.B. different codon 129 ratios between groups

Haik 2004 Neurology

# UCSF Observations of sCJD Patients on Quinacrine

- It appears sCJD patients who contacted UCSF who started quinacrine (on a compassionate basis by their local physician), may have survived slightly longer than those who patients who did not take quinacrine
- This was not scientific study. No controlled, randomized study was done; therefore we do not know if quinacrine improves, worsens, or has no effect on survival or quality of life in patients with sCJD.
- Only a randomized, double blinded, placebo controlled study can answer this question. This study will start in 5/05.

# CJD Quinacrine Treatment Study




NIH-funded

# Outcomes of UCSF CJD Quinacrine Trial

- Primary
  - Median Survival (or time to feeding tube)
- Secondary
  - Change on neurologic exam, cognitive testing, and ADL scales
  - Change in
    - EEG scale
    - MRI (atrophy & DWI change)
- **Potential Variables Affecting Efficacy of Treatment**
  - Stage & rate of decline of disease
  - Dose tolerated/Toxicity

# Comparison of UCSF vs UK Quinacrine Prion Trials

| | UCSF CJD Quinacrine | UK MRC PRION1 |
|---|---|---|
| **Cohort** | sCJD* | All prion |
| **N** | 60 | 90 |
| **1° Outcome** | Survival | Survival |
| **Duration** | ~3 yrs | 3 yrs |
| **Method** | **Stratified, Randomized, blinded, placebo controlled** | 1. **Similar** (but no placebo)<br>2. **Open Treatment**<br>3. **Open No Treatment** |

*If *PRNP* mutation later found, will continue in trial

# CJD Quinacrine Study

- Toxicity
  - Liver – reversible elevated transaminases
  - Blood dyscrasias
  - Rashes
  - Behavior
- Benefits of Study
  - Scientifically evaluate quinacrine – survival, neurologic & other function
  - Prospectively follow course of CJD

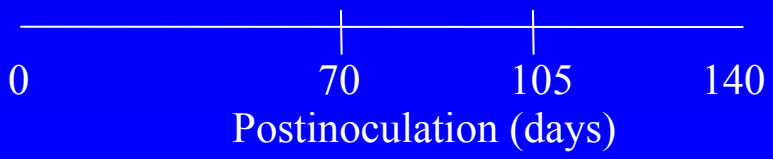
# In vivo experiments for evaluating quinacrine efficacy against prion disease

| | |
|---|---|
| Mouse strains: | Wild type (non-transgenic) strain ; FVB, CD-1<br>Transgenic strain ; Tg(MoPrP-A)FVB-B4053 |
| Inoculation: | Intracerebral inoculation with RML prion |
| Drug | Quinacrine (racemate and each enantiomer) |
| Delivery: | Oral administration using drug mixed liquid diet |

Legname, Ryou, Prusiner, et al.

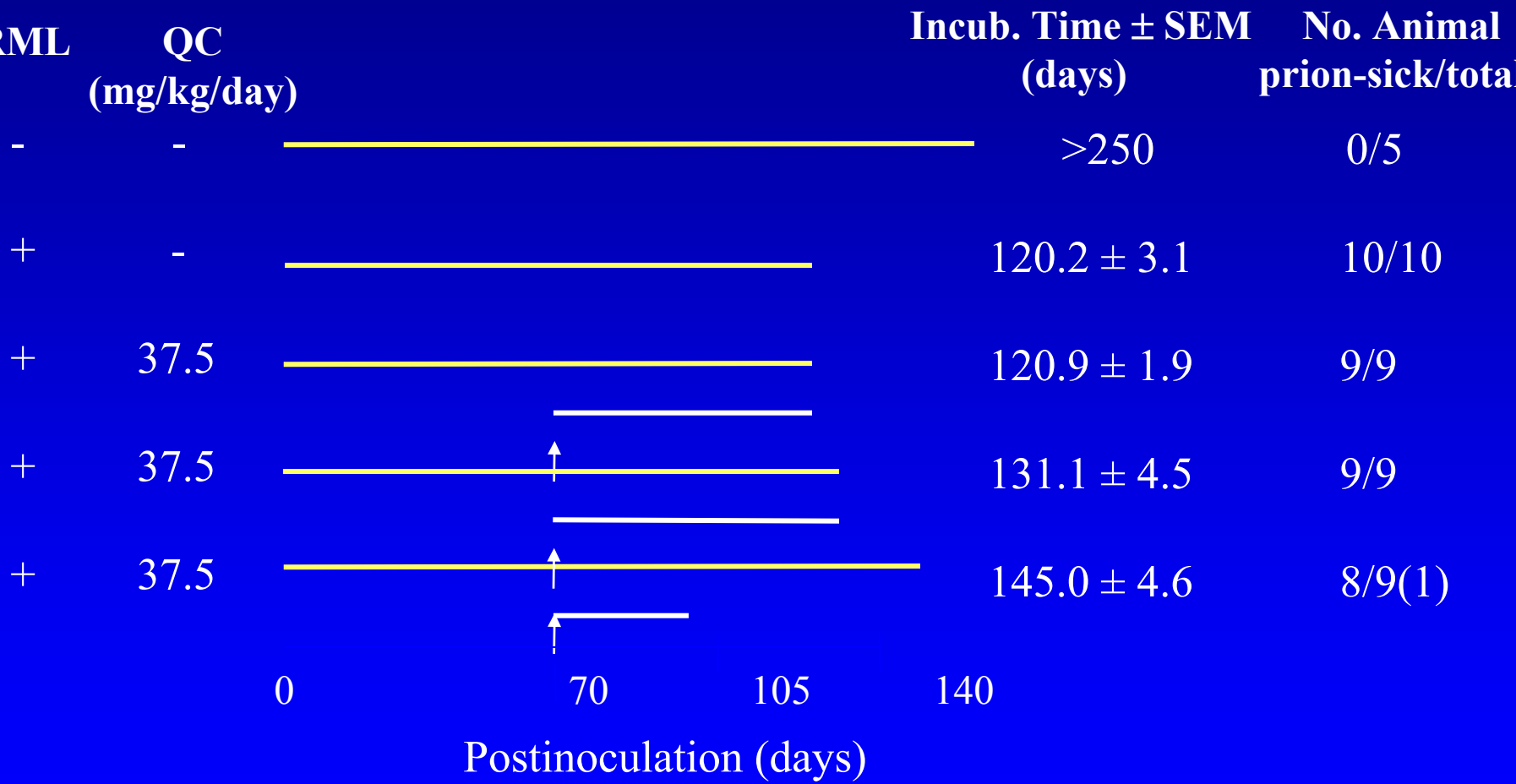
# Treatment of RML-inoculated CD-1 mice (life long Rx with various doses)

| RML | QC (mg/kg/day) | Incub. Time ± SEM (days) | No. Animal prion-sick/total |
|---|---|---|---|
| - | - | >250 | 0/5 |
| + | 75 | 126.8 ± 3.0 | 9/9 |
| + | 75 | 121.3 ± 2.6 | 9/9 |
| + | 37.5 | 120.9 ± 1.9 | 9/9 |
| + | 37.5 | 121.4 ± 1.9 | 9/9 |
| + | 18.75 | 121.0 ± 4.3 | 9/9 |
| + | 18.75 | 124.8 ± 3.2 | 9/9 |
| + | PBS | 120.2 ± 3.2 | 10/10 |



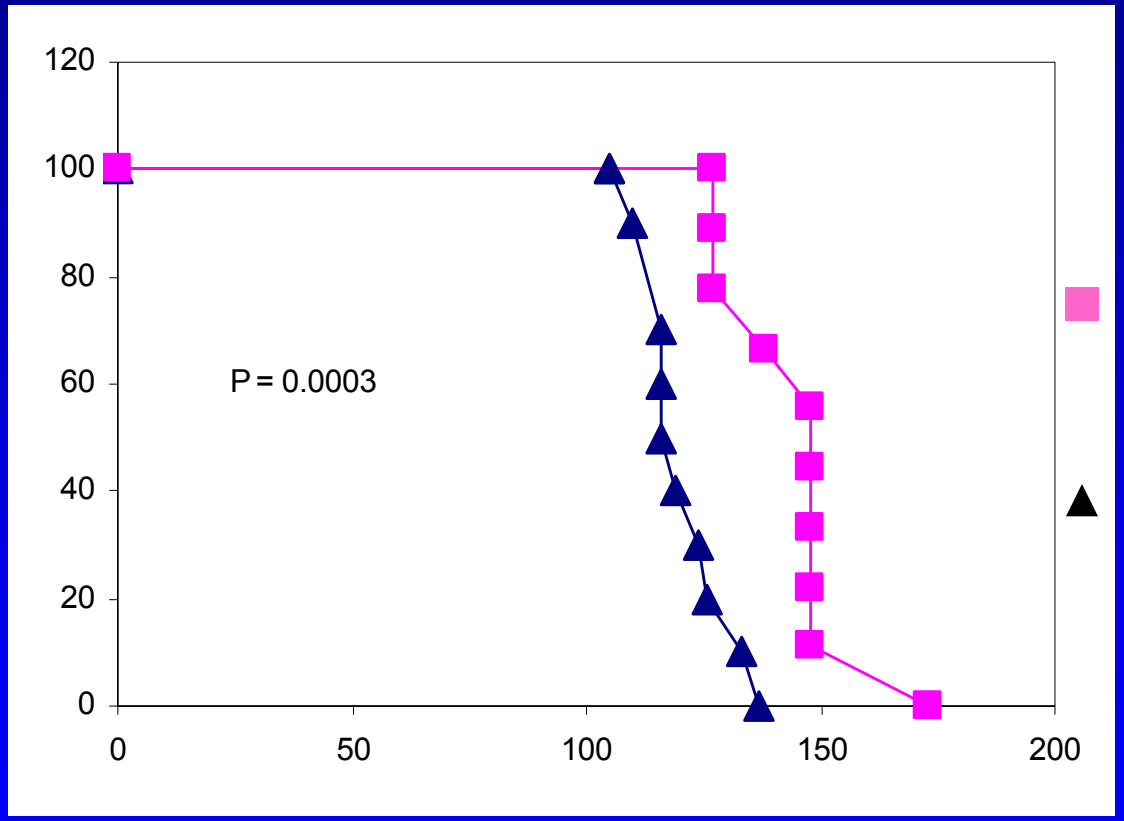

Legname, Ryou, Prusiner, et al.

# Treatment of RML-inoculated CD-1 mice (Duration of Rx)




Legname, Ryou, Prusiner, et al.

# Survival curve for CD-1 mice treated for 30 days with quinacrine starting at 70 days post-inoculation

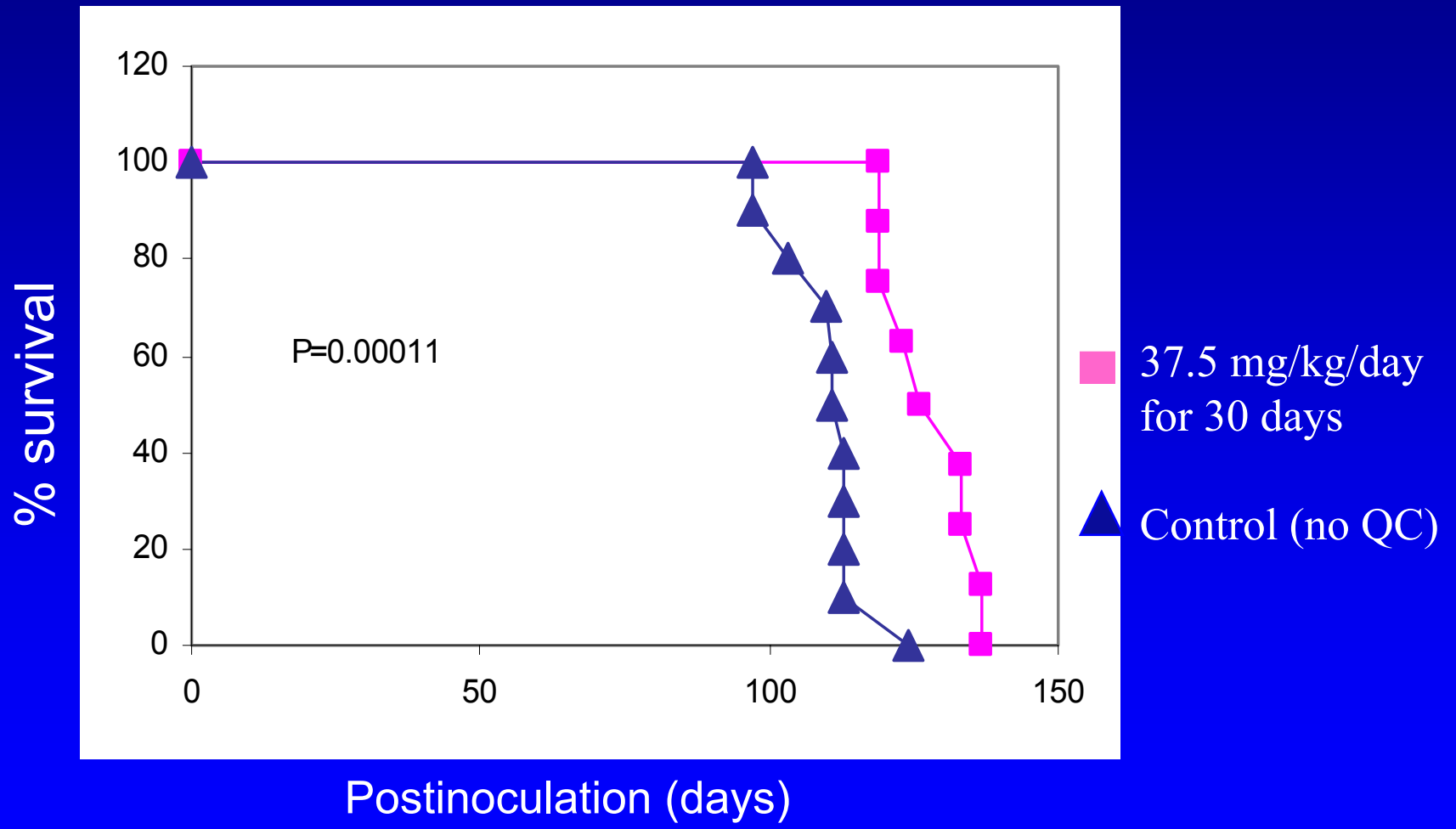



37.5 mg/kg/day for 30 days

Control (no QC)

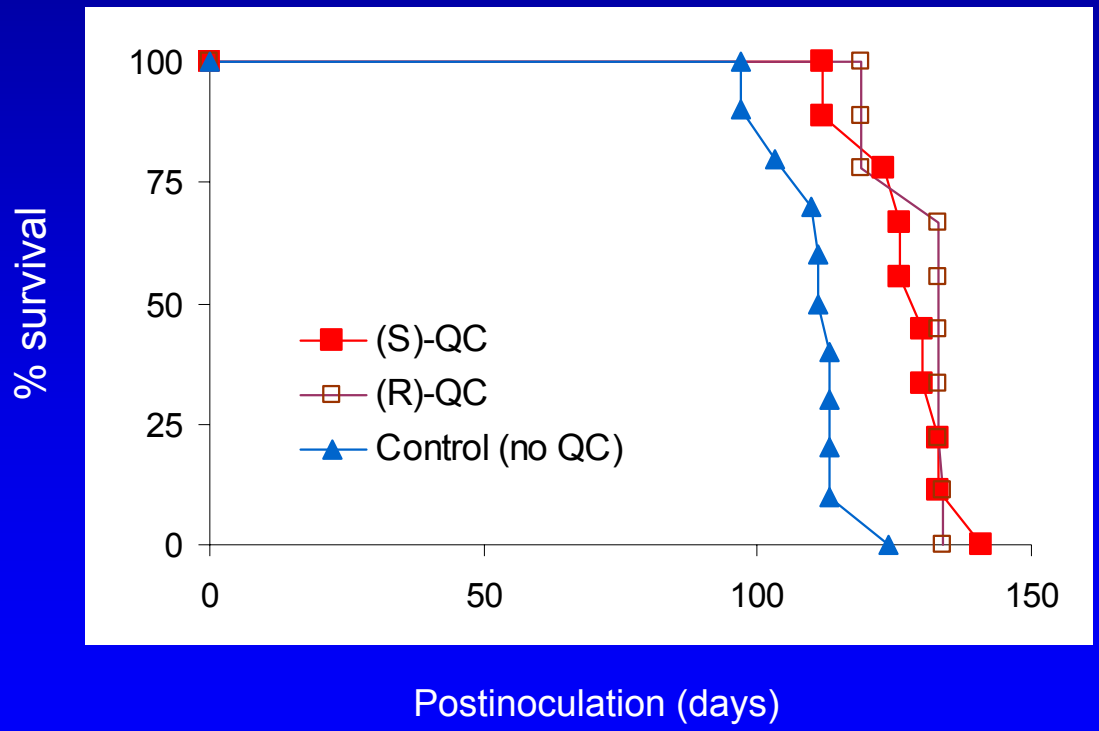
Legname, Ryou, Prusiner, et al.

# Survival curve for FVB mice treated for 30 days with quinacrine starting at 60 days postinoculation




Legname, Ryou, ...., Prusiner

# Survival curve of RML-inoculated FVB mice treated for 30 days with quinacrine enantiomers starting at 60 days postinoculation (18.75mg/kg/day)




Legname, Ryou, ...., Prusiner

# Summary of in vivo quinacrine studies

- Treatment of prion-inoculated mice with quinacrine prolongs the incubation time, suggesting that quinacrine is effective *in vivo* for delaying the progress of the disease.
- Prolongation of the incubation time was statistically significant. (2.5-3.5 weeks of prolongation [16-20 % of the incubation time])
- Anti-prion drug effective even when the treatment begins long after the infection has occurred
- Treatment for a short period (30 days) is effective in delaying disease onset
- Questions: how to apply mouse data on time and dose window to people?!

Legname, Ryou, ...., Prusiner

# Pentosan Polysulfate (PPS) as Treatment for Prion Disease

- Mice expressing hamster PrP innoculated w/ 263K prion hamster brain homogenate
- I.T. admin of PPS given 7, 10, 21 and 35 dpi x 4 weeks
- High dose PPS improved incubation 141% at 10 dpi and 71 % at 35 dpi
- Decreased pathology also noted at side of infusion, but not contralaterally
- Subcutaneous infusion – no benefit
- Safety - 2/6 dogs on 345 g/kg/day and 3/4 dogs receiving 460 g/kg/day suffered seizures shortly after infusion (1/group survived)
- Also looked at I.T. quinacrine (no benefit) and amphotericin B (26% improvement only at early administration; no benefit at late administration)
- 5 UK vCJD patients; 2 US patients on compassionate use PPS

Dohura 2004 J Virol

# Use of Flupirtine to treat CJD

- Flupirtine maleate –
  - centrally acting, non-opioid analgesic
  - In PrP infected cells (and AD models) reduces apoptotic cell death
  - Generally safe, well tolerated drug
- Study done in Germany
- Primary outcome – change in baseline cognitive scale (ADAS-cog); Secondary outcomes – survival
- Placebo controlled. 28 patients total
- MMSE 19-20
- Significantly less decline in ADAS-cog with flupirtine (p=0.02)
- No difference in survival (median 106 vs 107 d; mean 141 vs 97 d), although study not powered for survival
- One conclusion of authors: “It is clear the main challenge in CJD is early diagnosis”

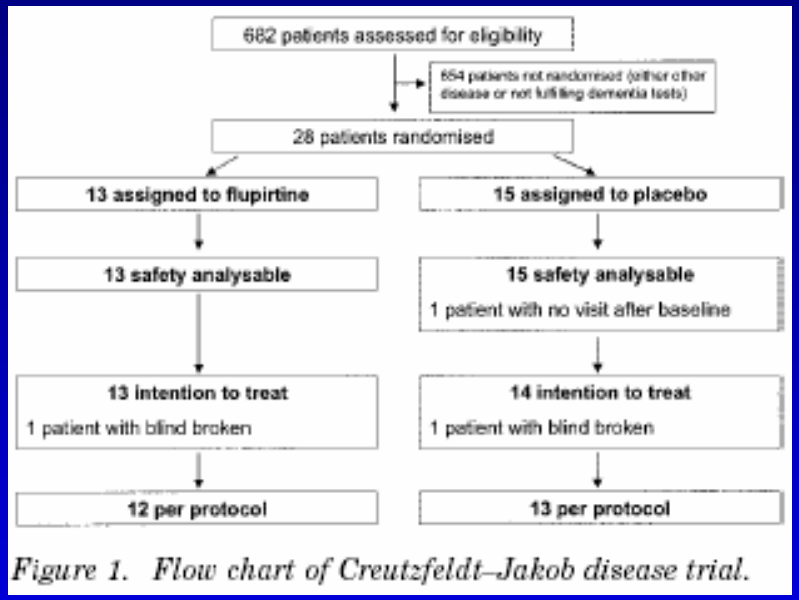



*Figure 1. Flow chart of Creutzfeldt–Jakob disease trial.*

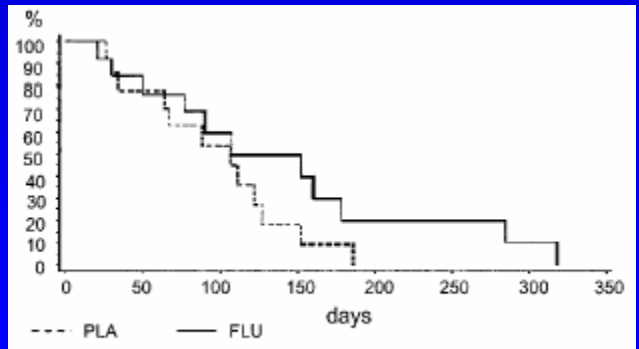



*Figure 2. Survival analysis (time to death of intention-to-treat cohort). PLA = placebo; FLU = flupirtine.*

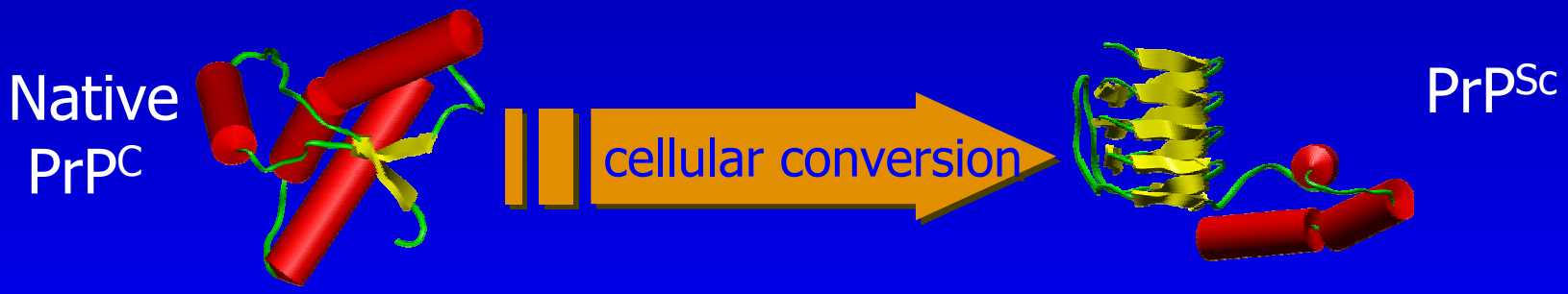
Otto 2004 Neurology

# Mechanisms of Treatment of Prion Diseases

- General Mechanisms
  - Prevention of accumulation of $PrP^{Sc}$
  - Promote clearance of $PrP^{Sc}$
  - Combination
- Specific Treatment Mechanisms
  - Mimicking $PrP^{C}$ Protective Sites
  - Blocking binding of Protein X ($PrP^{C}$ & $PrP^{Sc}$)
  - Promoting lysosomal clearance?
  - Removal of $PrP^{C}$?

# Drug screening using a cell model of prion infection

Chronically prion infected cells simulate a biologically relevant conversion of $PrP^{C}$ to $PrP^{Sc}$

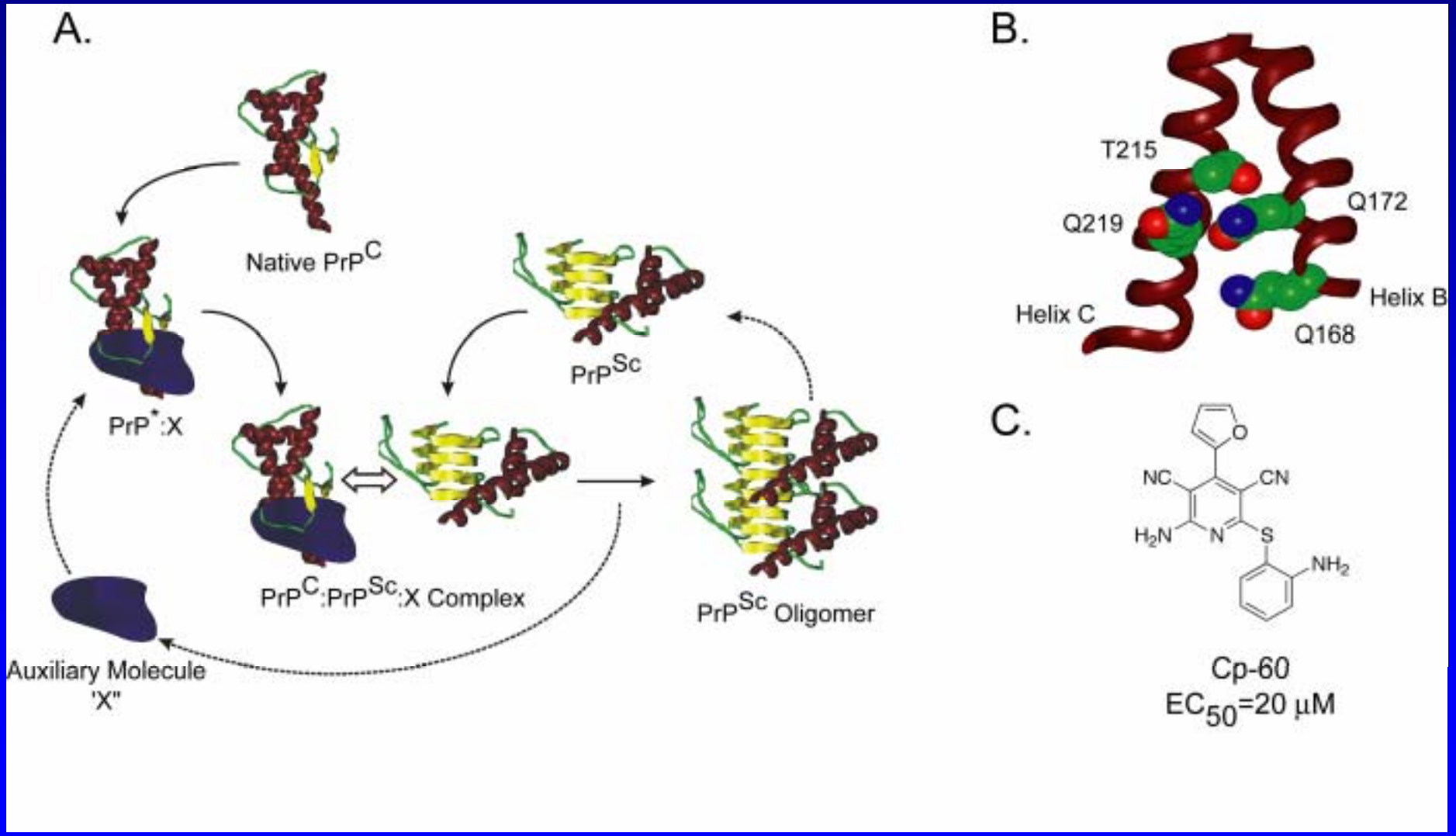



Use cell-based assays as initial screen for efficacy against prion activity

In vitro screens may also be effective

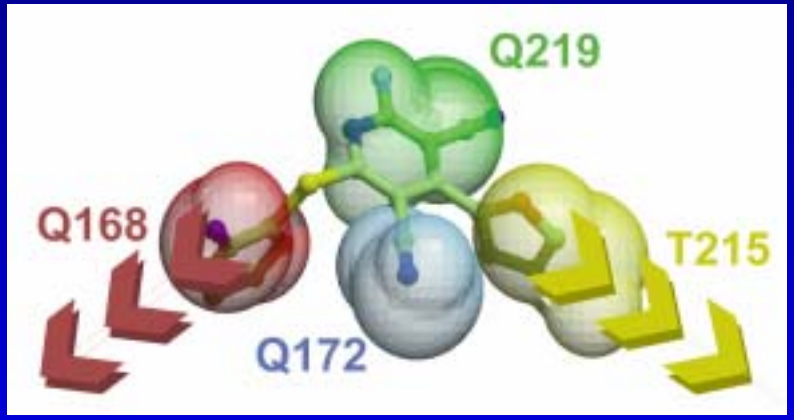
Courtesy of Barney May

# Rational Drug Design: blocking prion conversion through small molecule mimicry of "protective" sites




Courtesy of Barney May

# Small molecule mimicry reduces $PrP^{Sc}$ in cells!

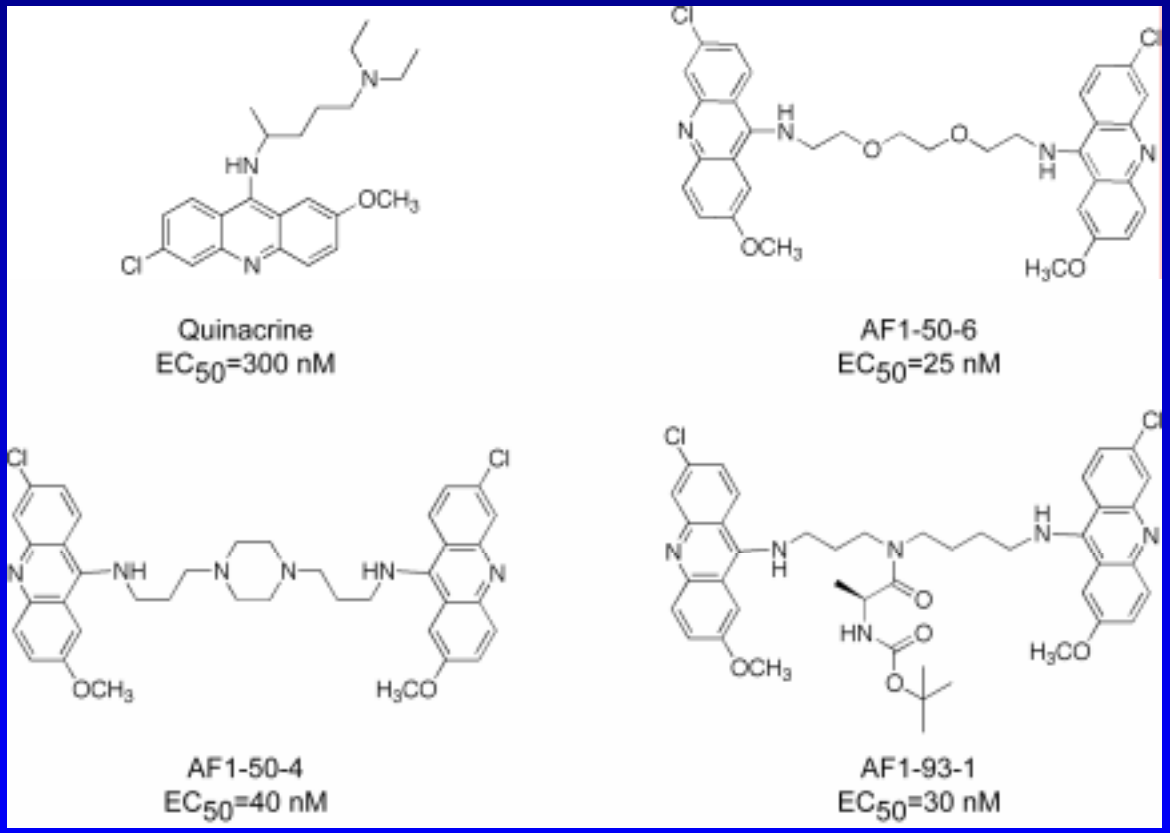




Courtesy of Barney May

# Improving upon a lead compound

Lead compound, quinacrine, was the focus of study to improve potency in culture




Dimeric analogs of quinacrine, "bis-acridines," are 10-times more potent than quinacrine!

Courtesy of Barney May

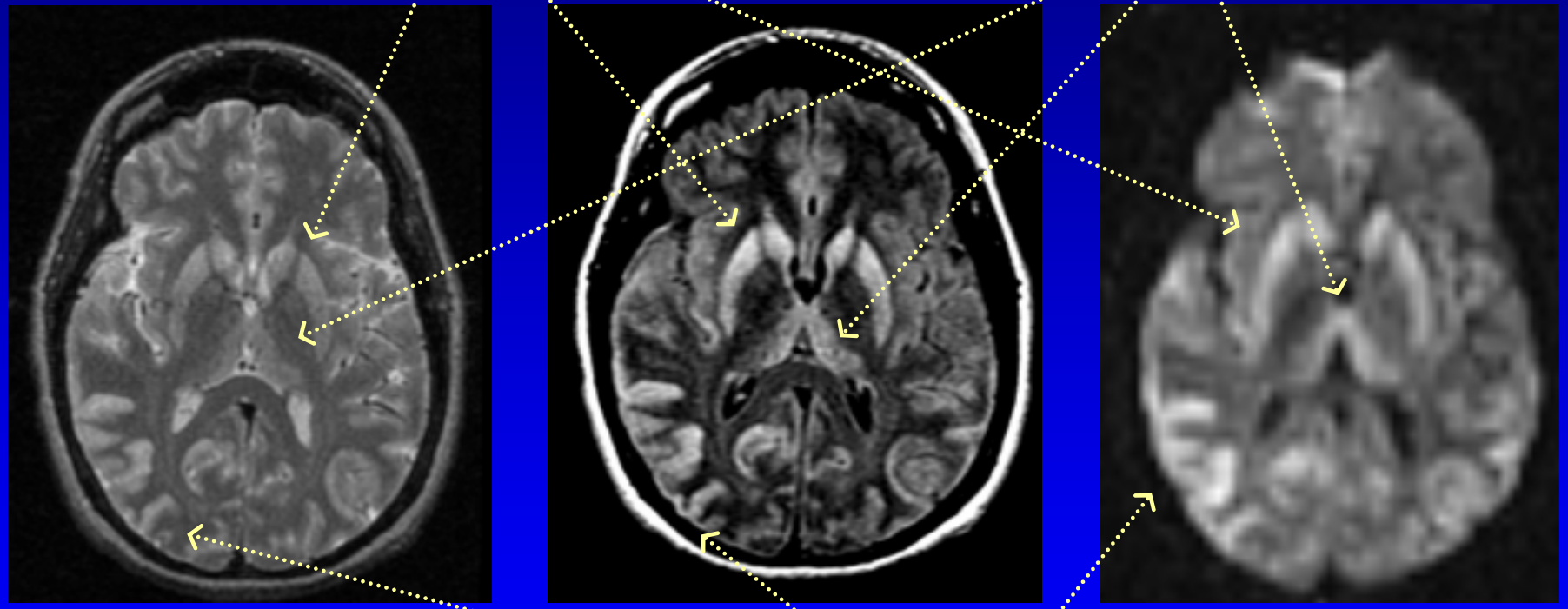
# Must diagnose CJD earlier!

- Earlier diagnosis, better chance of treatment
- Identify earliest symptoms
  - Prior studies, retrospective, autopsy
  - chart review
- Identify the very first symptoms
  - Pseudo-prospective, direct contact with patients and families
- On whom to use new diagnostic tests?

# Classic MRI Findings in Sporadic CJD

Basal Ganglia Hyperintensities

Thalamic Hyperintensities
(less common)



T2 FLAIR DWI

Cortical Ribboning

# Goals of UCSF CJD MRI Study

- What is the sensitivity and specificity of DWI and FLAIR MRI for CJD?
- Difference between electronic and film reading of MRIs?
- Spectrum of MRI involvement in CJD?



Young, Geschwind et al AJNR 2005

# Methods - UCSF CJD MRI Study

- CJD cases - 40 serial pts 7/01-11/02 23 definite and 11 probable sCJD
  - 6 genetic CJD (4 E200K, 1 octapeptide repeat, 1 GSS)
- Controls - 53 patients, UCSF dementia clinic
- T1, DWI and FLAIR MRI
  - 12 CJD and 28 controls reviewed on a digital system
  - 28 CJD and 25 control cases viewed on film
- Two neuroradiologists read 93 MRIs blinded, random
  - Interpreted whether CJD or not CJD
  - Noted abnormal areas in each sequence



Young, Geschwind, et al AJNR 2005

# Combined Diagnostic Utility of FLAIR and DWI MRI in CJD* is High

| Image Display Technique | Sensitivity | Specificity | Accuracy |
|---|---|---|---|
| Film | 0.88 | 0.98 | 0.93 |
| Digital | 1.00 | 0.93 | 0.95 |
| Combined | 0.91 | 0.95 | 0.94 |

*Based on the average of both readers.



Young, Geschwind, et al AJNR 2005

# MRI readings were reproducible

| Reproducibility of MRI Readings | | | | |
|---|---|---|---|---|
| | Intra-Reader* | Inter-Reader | | |
| Image Display Technique | Film | Film | Digital | Combined |
| Observed Agreement | 0.93 | 0.92 | 1.00 | 0.96 |
| Predicted Chance Agreement | 0.53 | 0.50 | 0.56 | 0.51 |
| Kappa | 0.86 | 0.85 | 1.00 | 0.91 |



Young, Geschwind, et al AJNR 2005

*Digital not included due to 100% inter-reader agreement

# Areas of Gray Matter Abnormality

| | Percent of Cases with Abnormality | |
|---|---|---|
| Region | CJD | Controls |
| Neocortex (N) | 88 | 17 |
| Frontal | 84 | 9 |
| Rolandic | 0 | 1 |
| Parietal | 72 | 3 |
| Temporal | 65 | 11 |
| Primary Visual | 9 | 1 |
| Occipital | 39 | 2 |
| Limbic (L) | 79 | 25 |
| Striatum (S) | 69 | 4 |
| Thalamus (T) | 34 | 0 |

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# Patterns of Abnormality In CJD Cohort

- 68% had both cortical & subcortical (striatal +/- thalamic) abnormalities
- 24% had isolated cortical– "cortical ribboning" – without subcortical abnormalities
- 5% had only subcortical (striatal +/- thalamic) abnormalities



Young, Geschwind, et al AJNR 2005

# Summary of Some Criteria for Probable sCJD

## Masters

Dementia w/
1 of 5 following:

1. Myoclonus
2. Pyramidal
3. Extrapyramidal (EP)
4. Cerebellar
5. Typical EEG

(Masters Ann Neurol 1979)

## WHO Revised*

Dementia w/
2 of 4 following:

1. Myoclonus
2. Pyramidal/EP
3. Visual/Cerebellar
4. Akinetic Mutism

AND

Typical EEG or CSF 14-3-3 (if < 2 year duration)*

* or MRI

## UCSF

Rapid cognitive decline w/
2 of 6 following:

1. Myoclonus
2. Pyramidal/EP
3. Visual
4. Cerebellar
5. Akinetic Mutism
6. Other focal cortical sign (neglect, aphasia, apraxia)

AND

Typical EEG or MRI

# First Symptom Study: Methods

- Reviewed relational database of UCSF “CJD” referrals for probable or definite sCJD cases
- Identified more than 100 potential symptoms/signs
- Records review; recorded in CJD database
- Scored quality of first symptom data (0-5)
  - Required clear identification of first symptom(s) by caregiver/patient and/or medical records
- 116 probable or definite sCJD cases with credible first symptom data
- Eliminated 2 patients with >4 first symptoms
- 114 cases for analysis

# Demographics of First Symptom Study

| | |
|---|---|
| Total patients | 114 |
| Definite sCJD | 78 (67%) |
| Probable sCJD | 36 (33%) |
| Modified Revised WHO (MRI) | 33 (92%) |
| Master's | 3 (8%) |
| Mean Age | 62 +/- 10 (26-80) |
| Men | 49% |
| Women | 51% |



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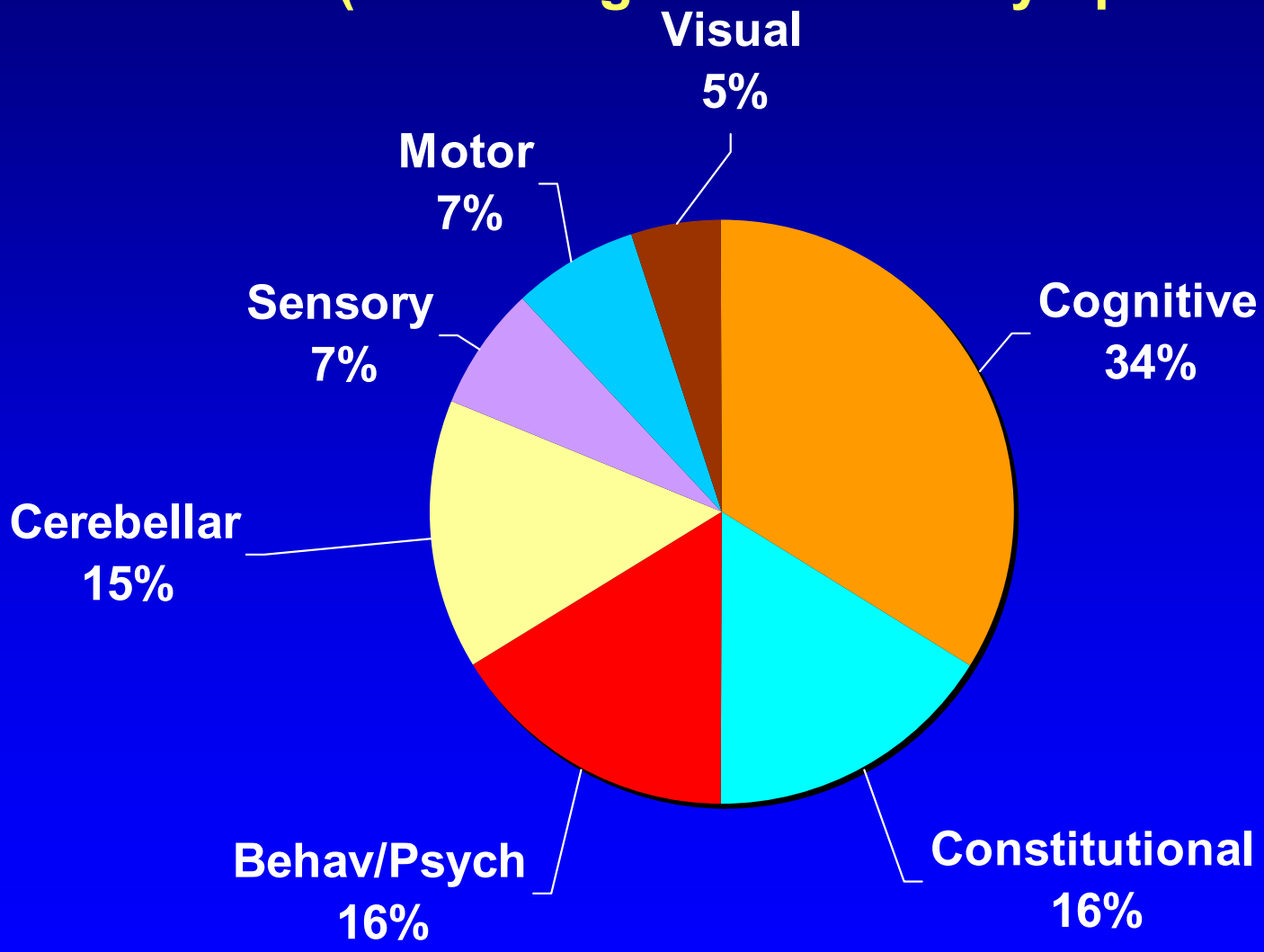
# Distribution of First Symptoms

## (Percentage of all First Symptoms)




114 patients with 165 1st Symptoms

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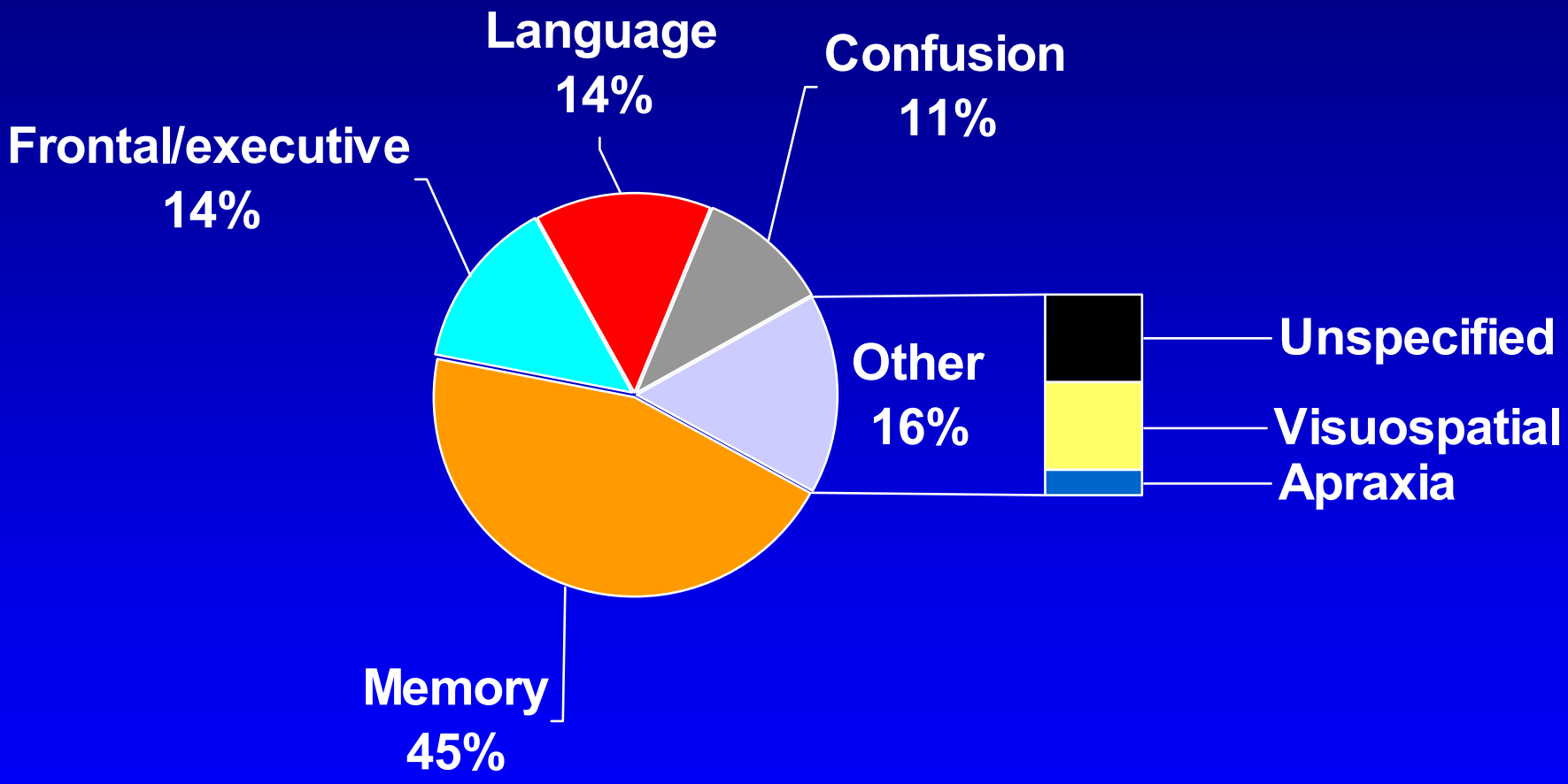
# Comparison of Studies
## (Percentage of Patients)

| | 1st Symptom | Early Symptoms | | | |
|---|---|---|---|---|---|
| Symptom /Study | UCSF '00-04 N=114 | France '68-82 N=230 | UK '90-94 N=144 | Belgium '61-98 N=100 | Japan '75-78 N=63 |
| Cognitive | 49% | 46% | 19% | 14% | 29% |
| Constitutional | 24% | 17% | N/A | 17% | 5% |
| Behavioral | 24% | 29% | 15% | 42% | 36% |
| Cerebellar | 21% | 34% | 39% | 45% | 41% |
| Sensory | 11% | 5% | N/A | 4% | 6% |
| Motor | 10% | 5% | 6% | 23% | 16% |
| Visual | 7% | 17% | 10% | 6% | 13% |

# Distribution of Cognitive Symptoms




N = 56

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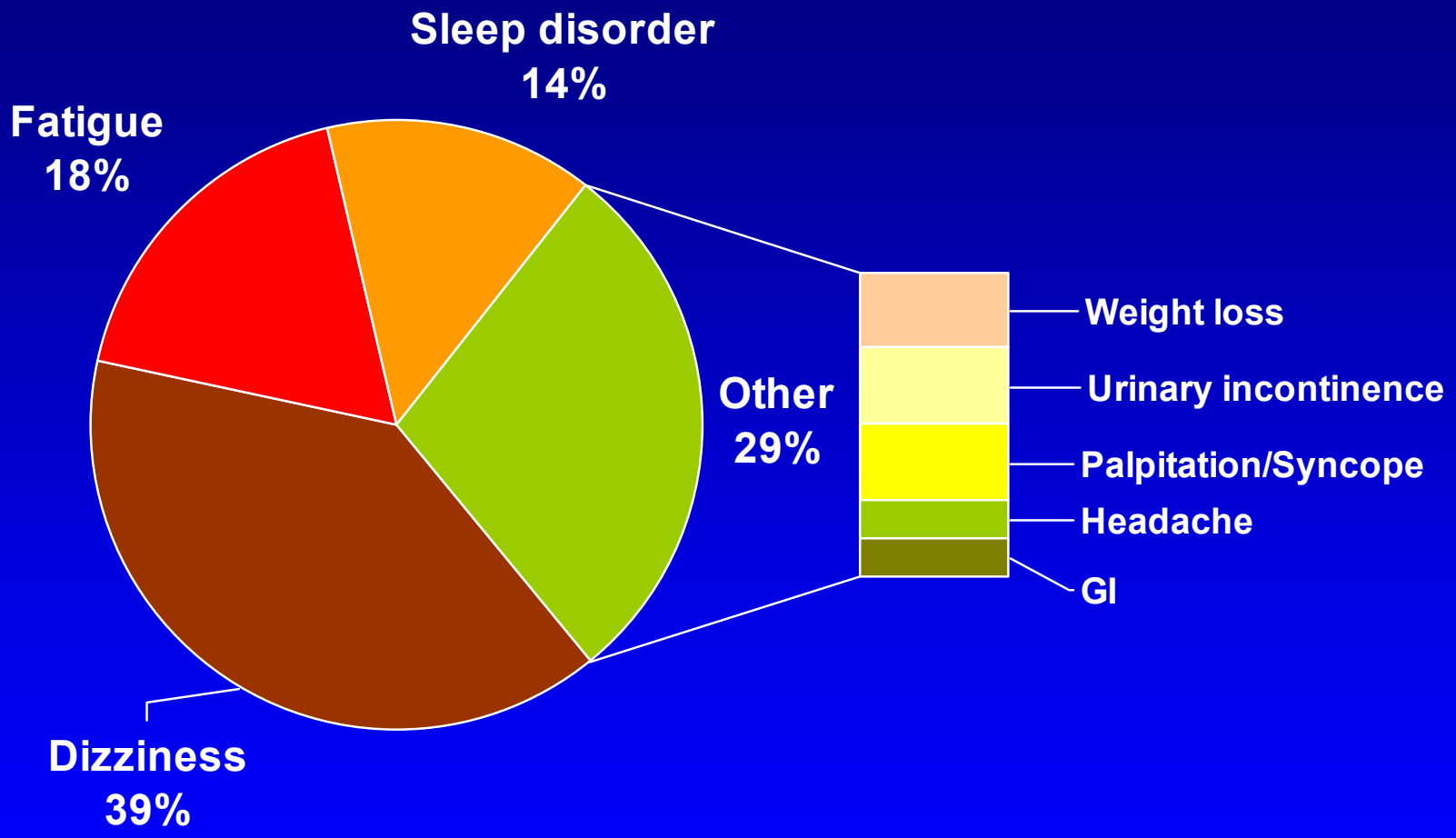
# Distribution of Constitutional Symptoms




N = 27

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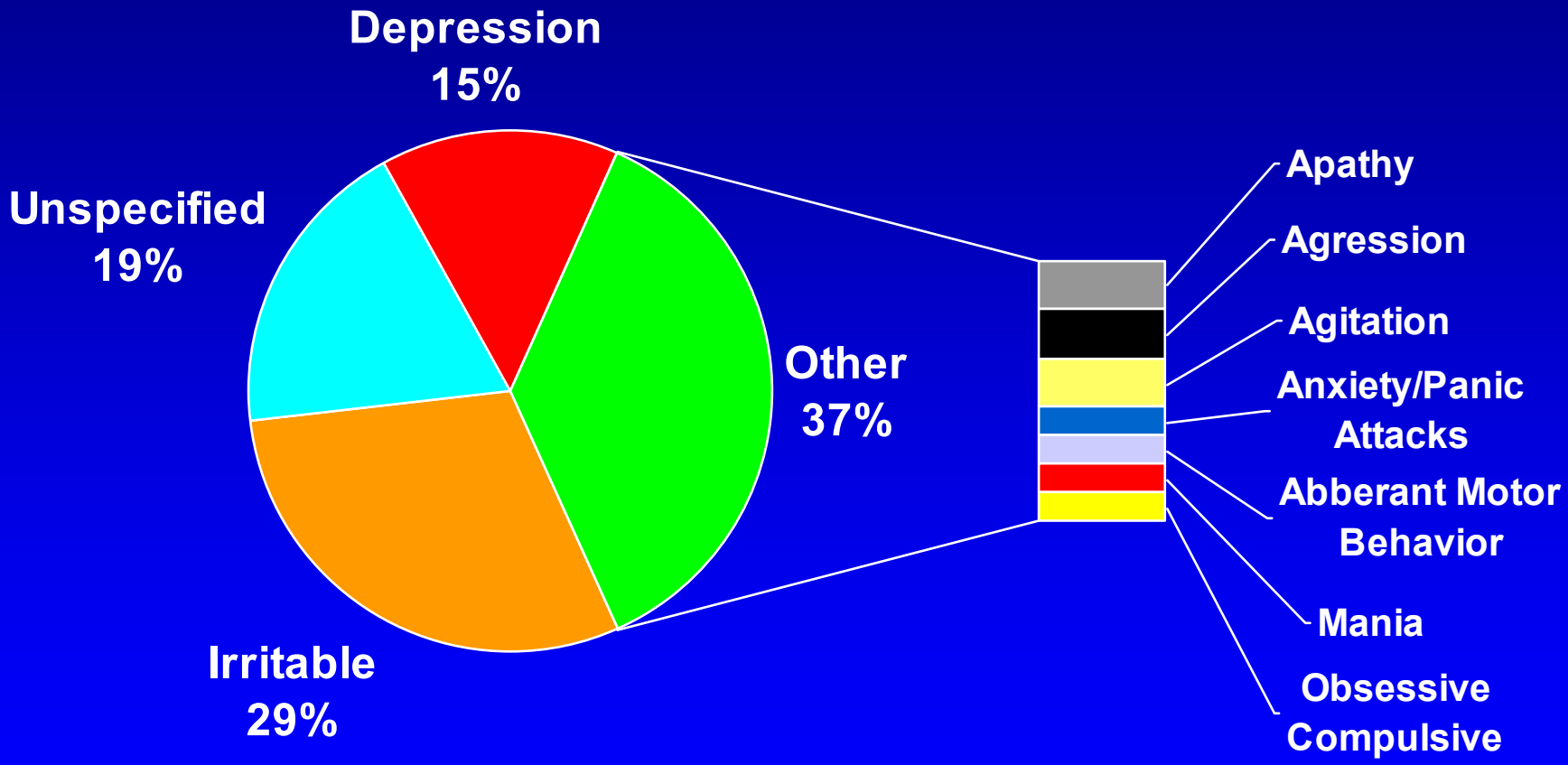
# Distribution of Behavioral/Psychiatric Symptoms




N = 27

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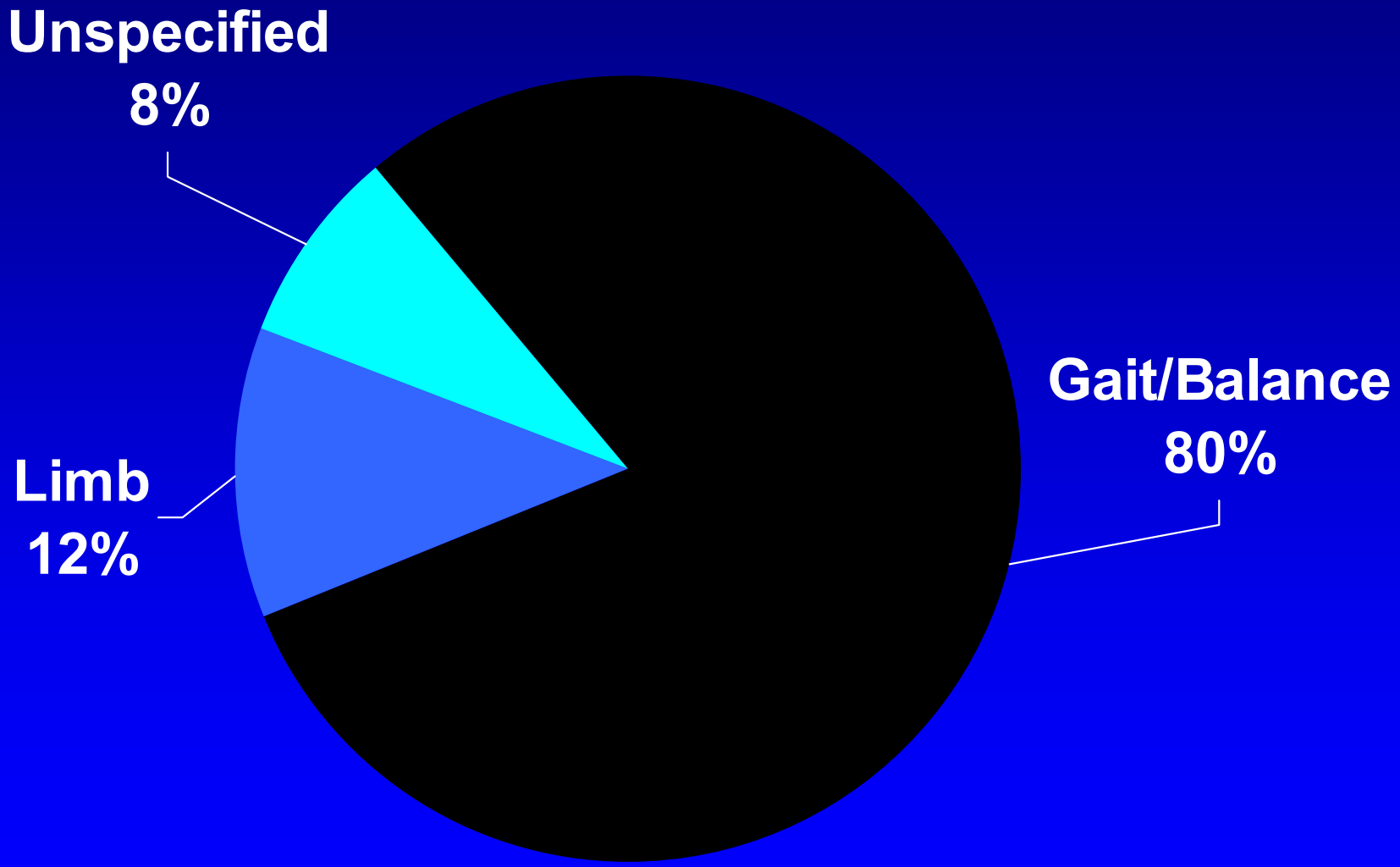
# Distribution of Cerebellar Symptoms




N = 24

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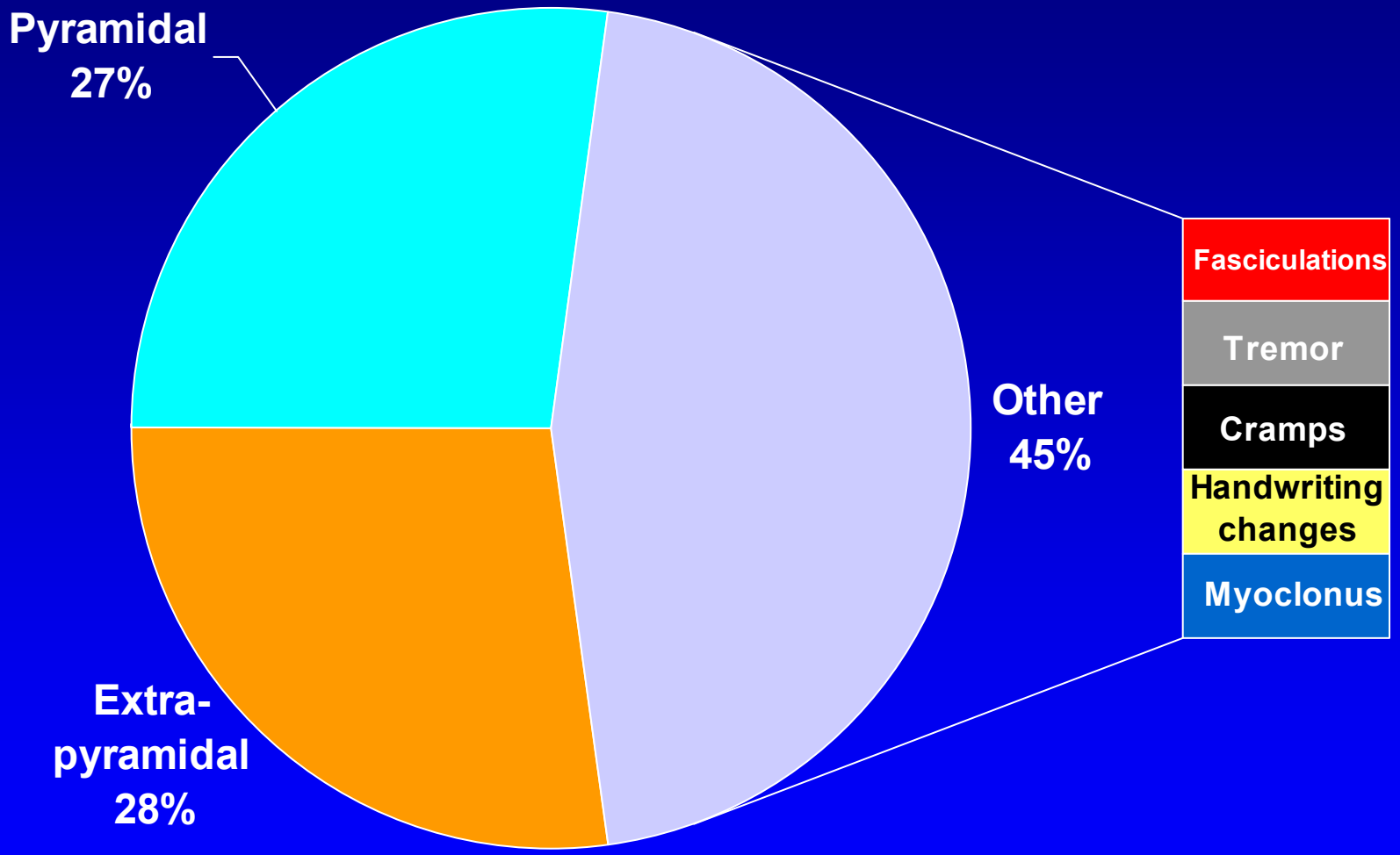
# Distribution of Motor Symptoms




N = 11

Rabinovici et al AAN 2005

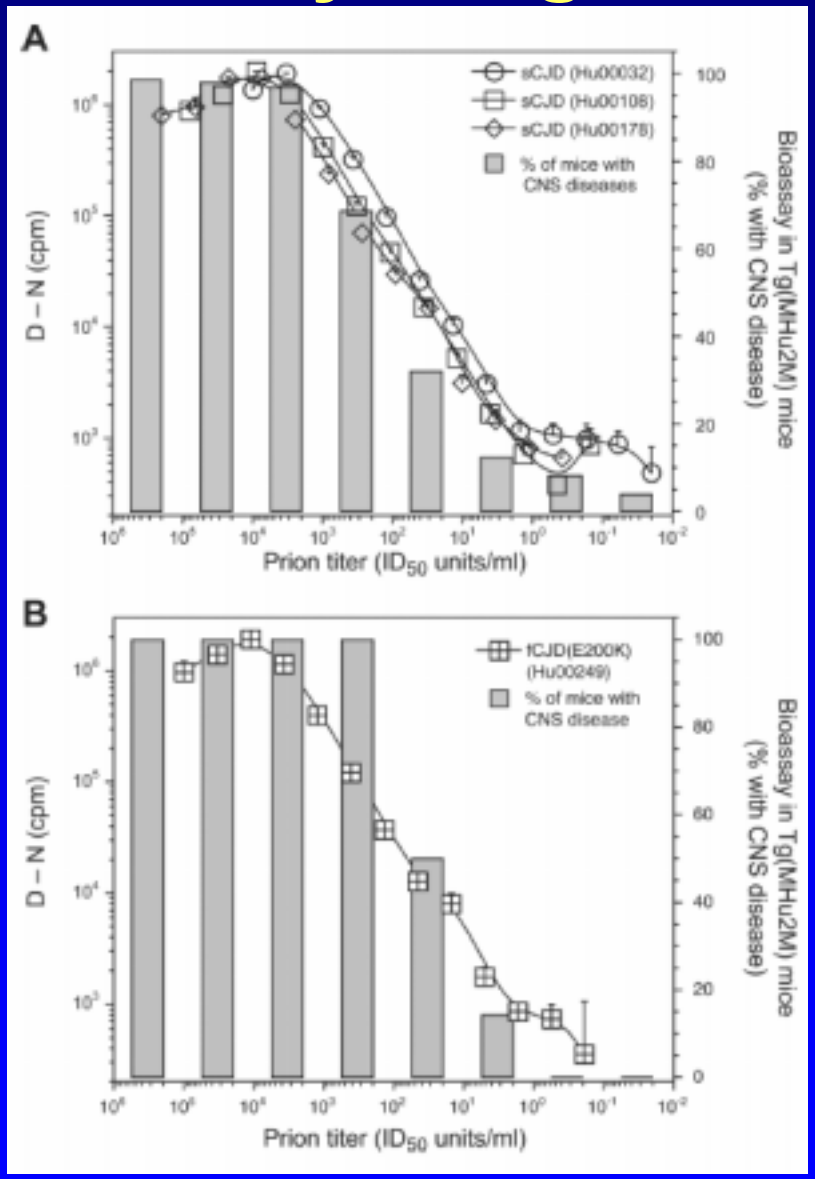
# Conformation Dependent Immunoassay (CDI) as diagnostic test

- CDI is an Elisa-based assay for detection of the prion
- It has been shown to be more sensitive and specific than any existing technique for detecting prions in brain tissue
- Work is being done to see if it can detect prions in bodily fluids

# CDI is better than gold standard of bioassay in Tg mice

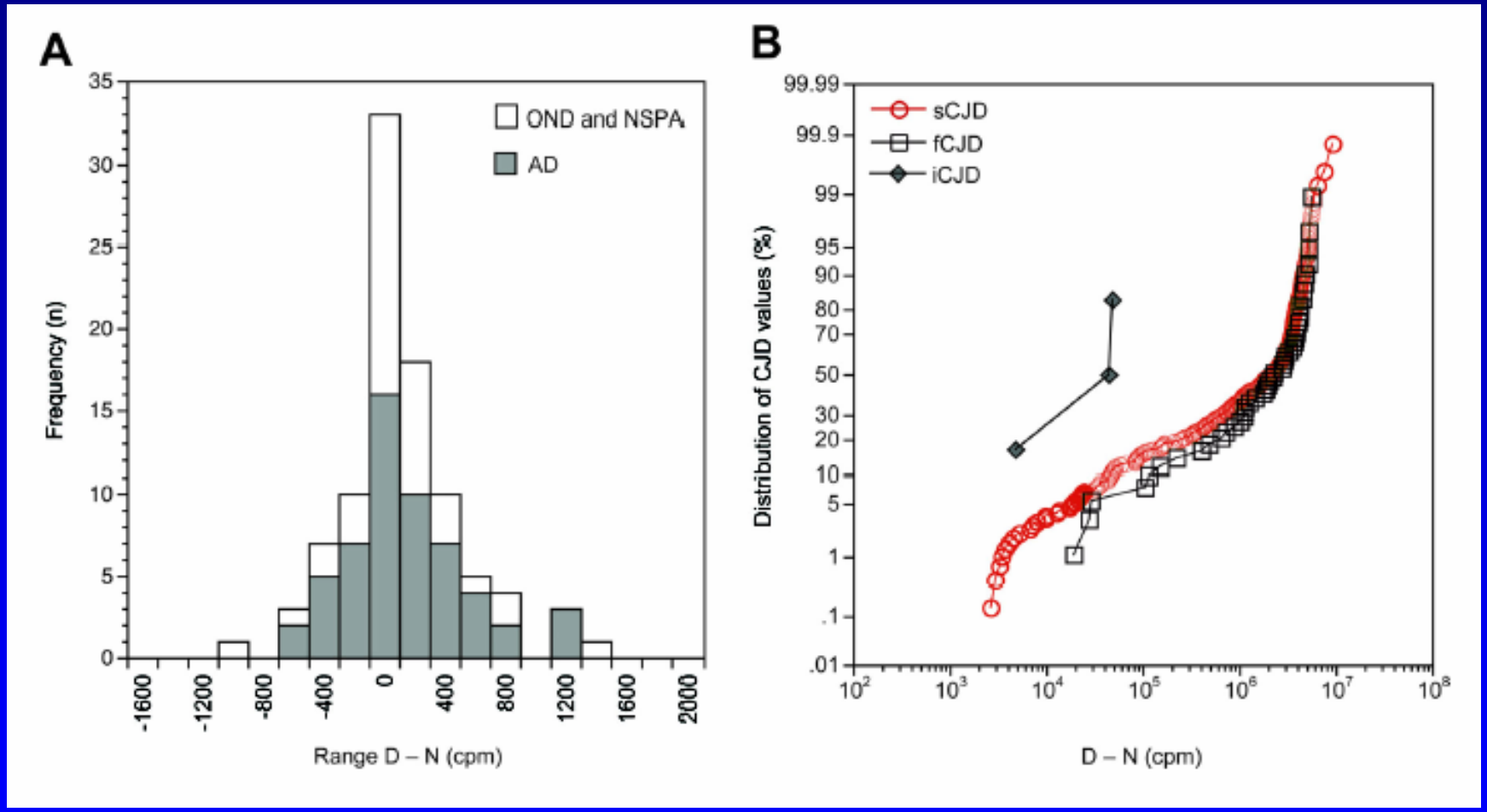
3 sCJD cases

fCJD case



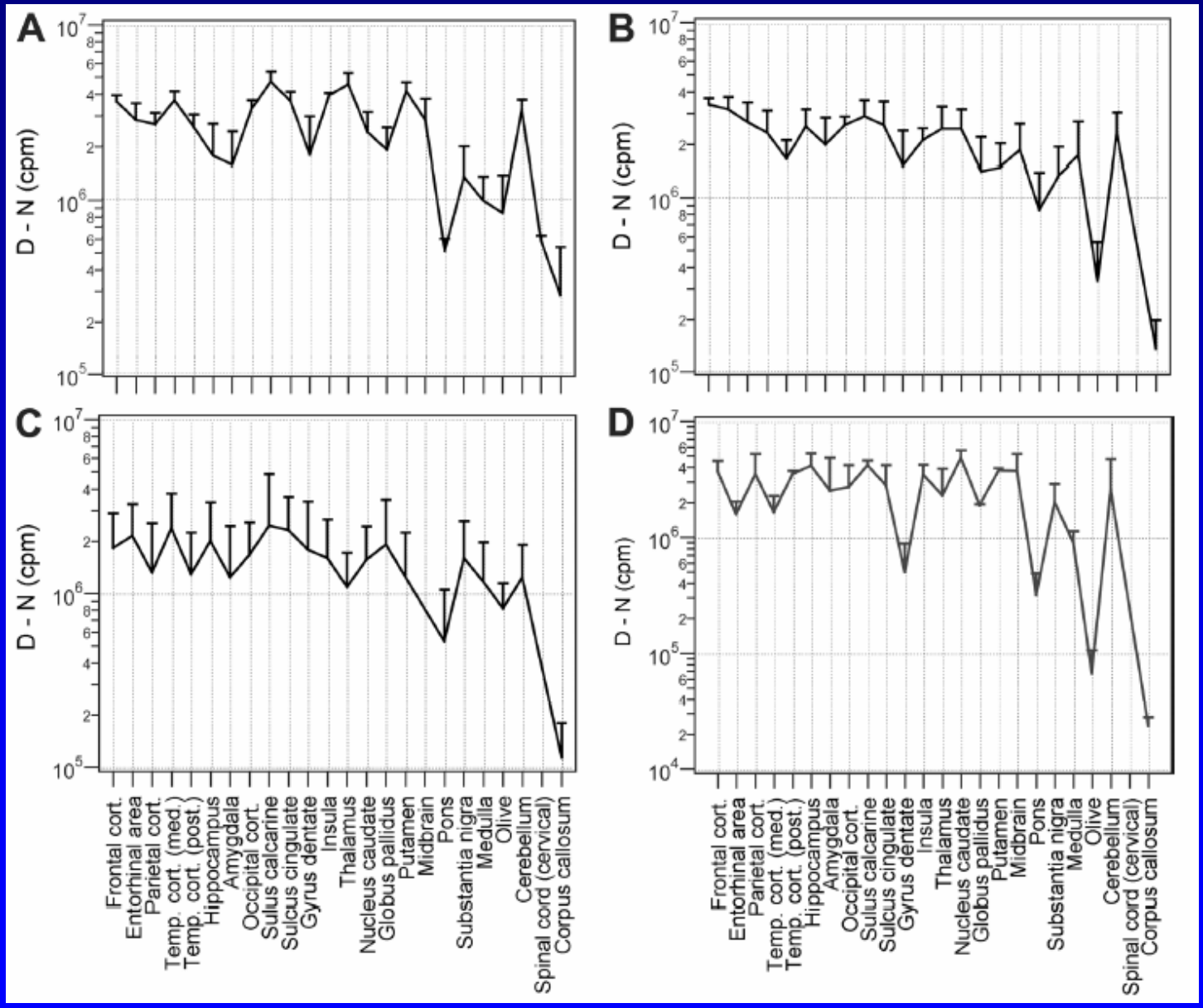

Safar et al. PNAS 2005

# Can CDI differentiate prion from non-prion disease brain tissue?



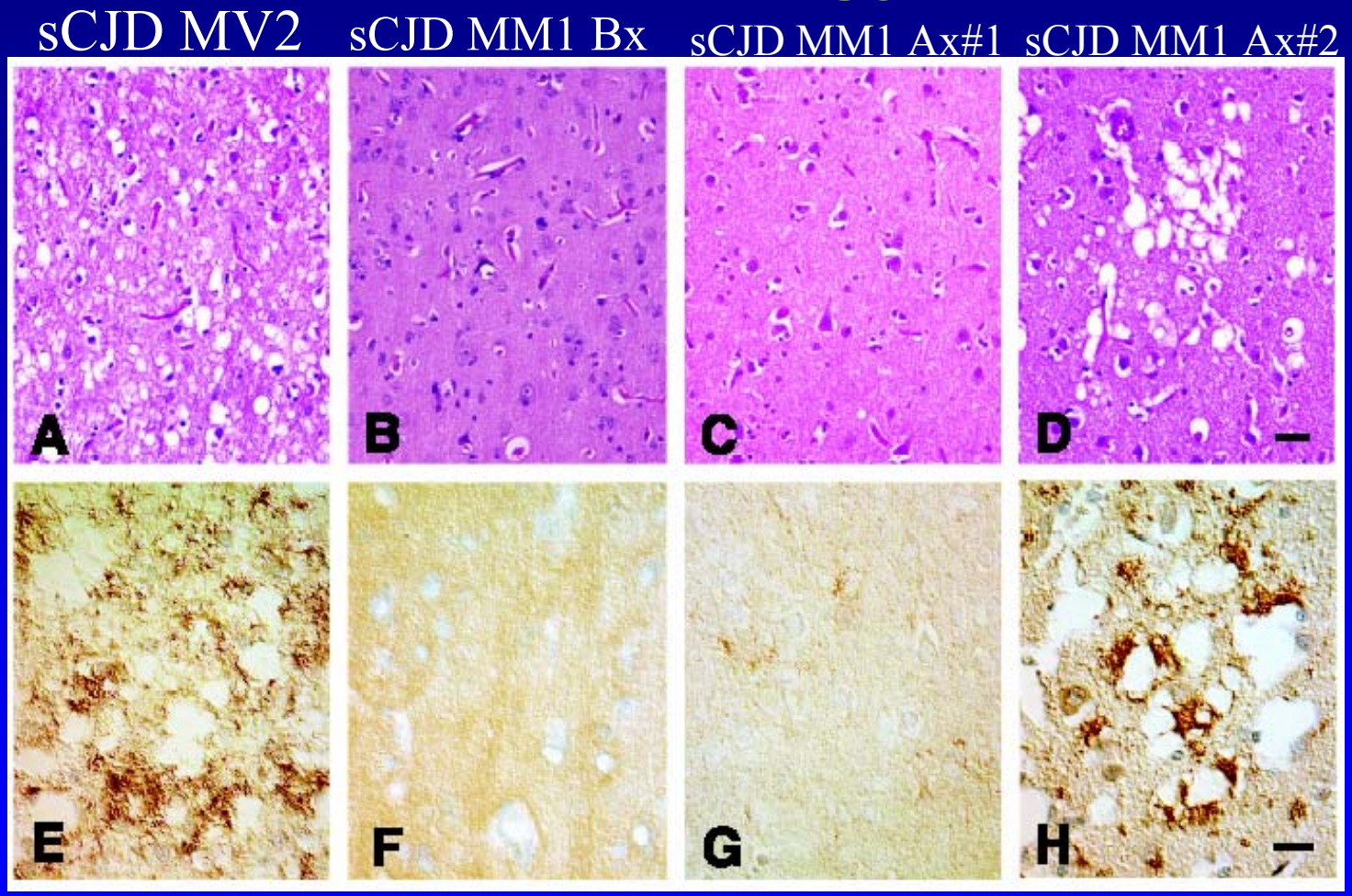

Safar et al PNAS 2005

# Distribution of $PrP^{Sc}$ in 24 anatomical areas of 24 sCJD brains determined by CDI




Safar et al PNAS 2005

# Imaging & CDI beat gold standard - pathology




Courtesy of Steve DeArmond

Safar et al PNAS 2005

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CA State Health Dept/CDC

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MAC RETREAT 2004