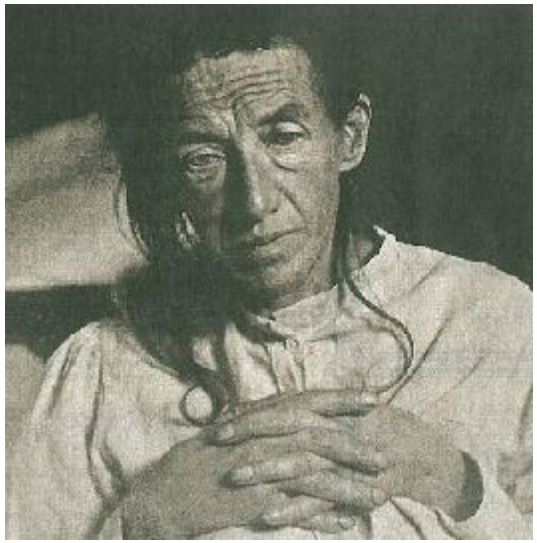
The Role of Amyloid in the Development and Treatment of Alzheimer's Disease

Dr. Charles Glabe
Department of Molecular Biology
and Biochemistry
University of California, Irvine
cglabe@uci.edu

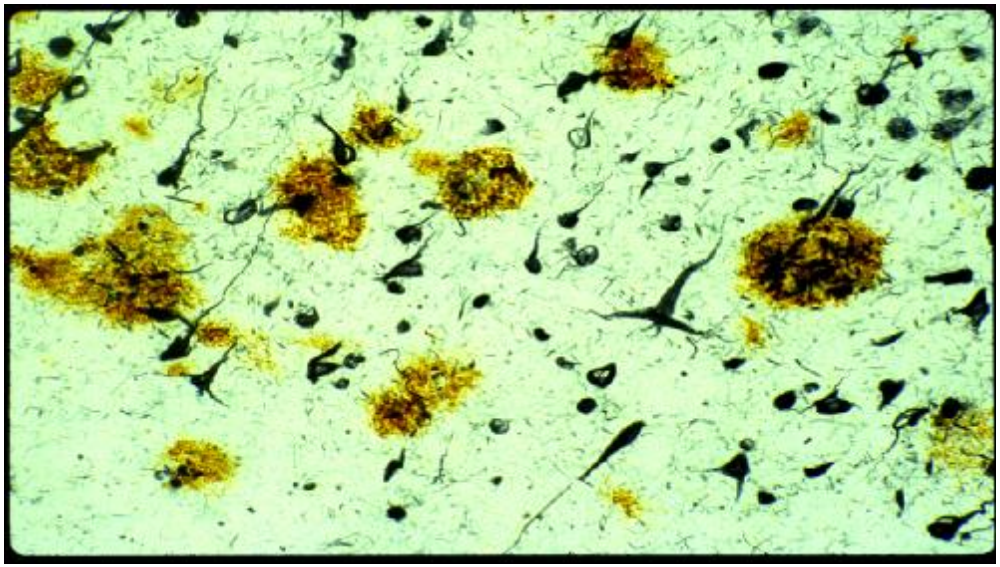
Overview

- A short history of AD and senile dementia.
- Familial AD and the amyloid cascade hypothesis.
- Therapeutic strategies based on the amyloid cascade hypothesis and their failure in human clinical trials.
- An alternative amyloid hypothesis based on intraneuronal amyloid accumulation leading to neuronal death and neuritic plaque formation.
- New potential therapeutic strategies.

Amyloid deposits in AD brain.



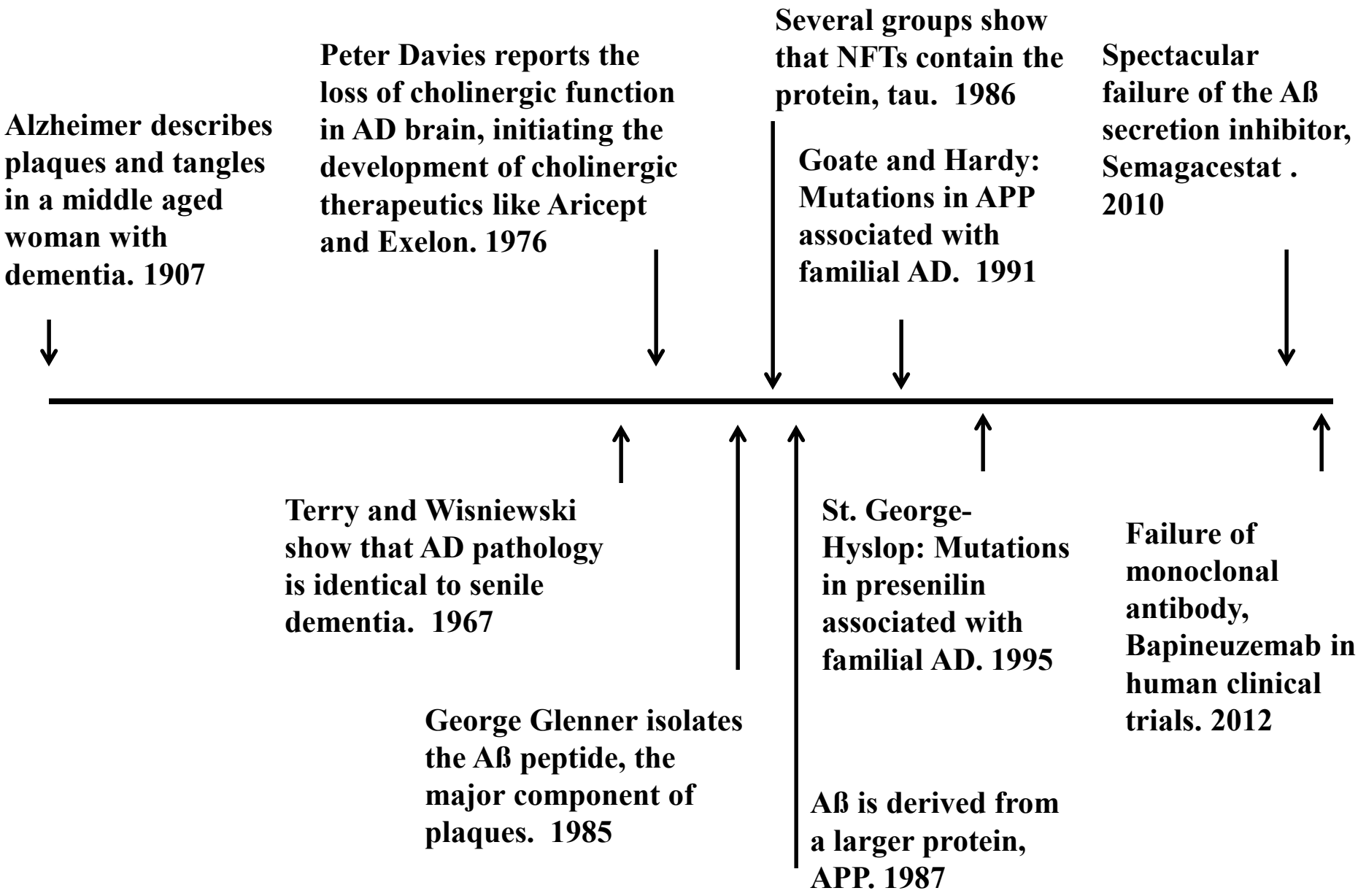
Auguste Deter, 1905. Alois Alzheimer's patient



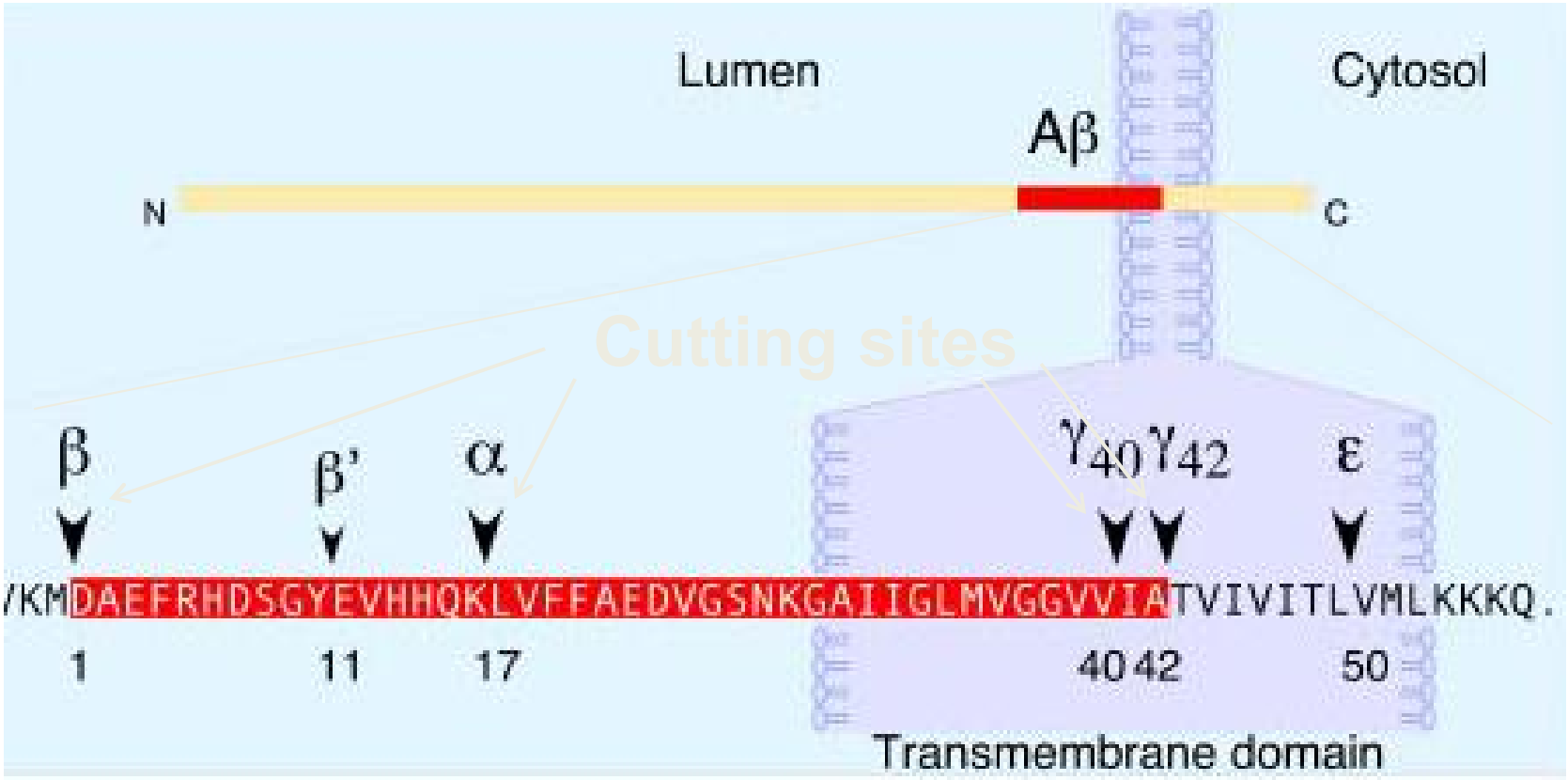
Brown: amyloid Aß, (senile plaques)

Black: Tau, (neurofibrillary tangles, NFT)

Timeline of Alzheimer's milestones



Aß is derived by proteolytic cutting of APP



Thinakaran and Koo, JBC 2008

Alzheimer's disease has a familial or inherited early onset form (FAD).

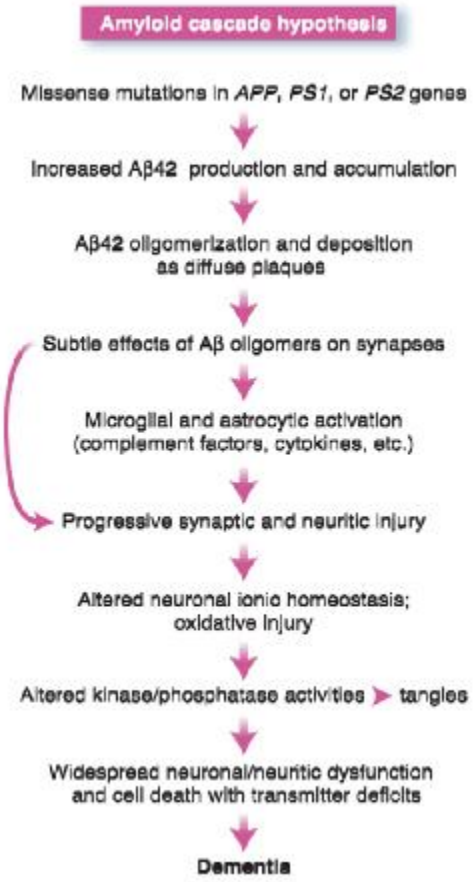
3 Genes are associated with FAD:

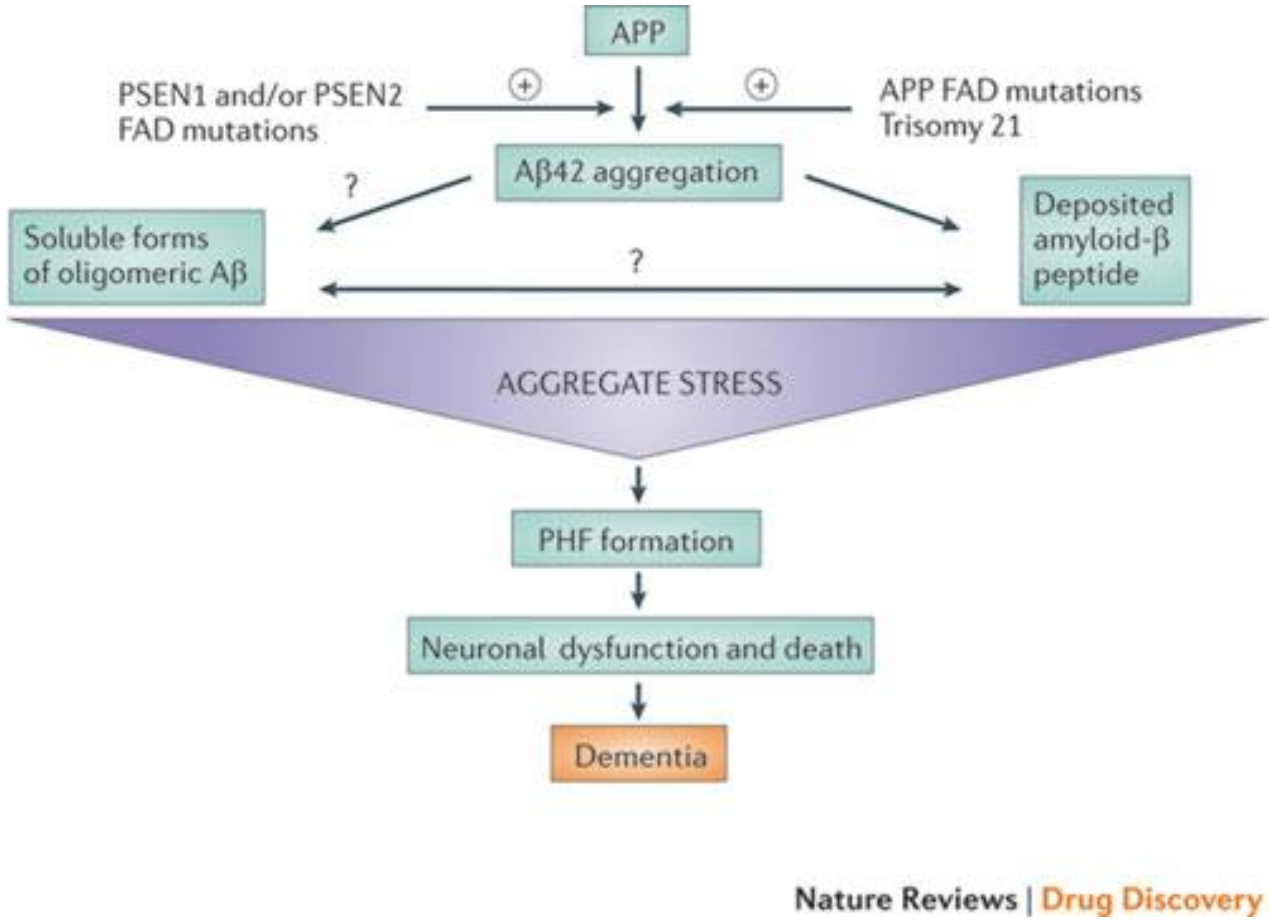
Amyloid precursor protein

Presenilin 1 (gamma secretase)

Presenilin 2 (gamma secretase)

FAD genetics supports the amyloid cascade hypothesis

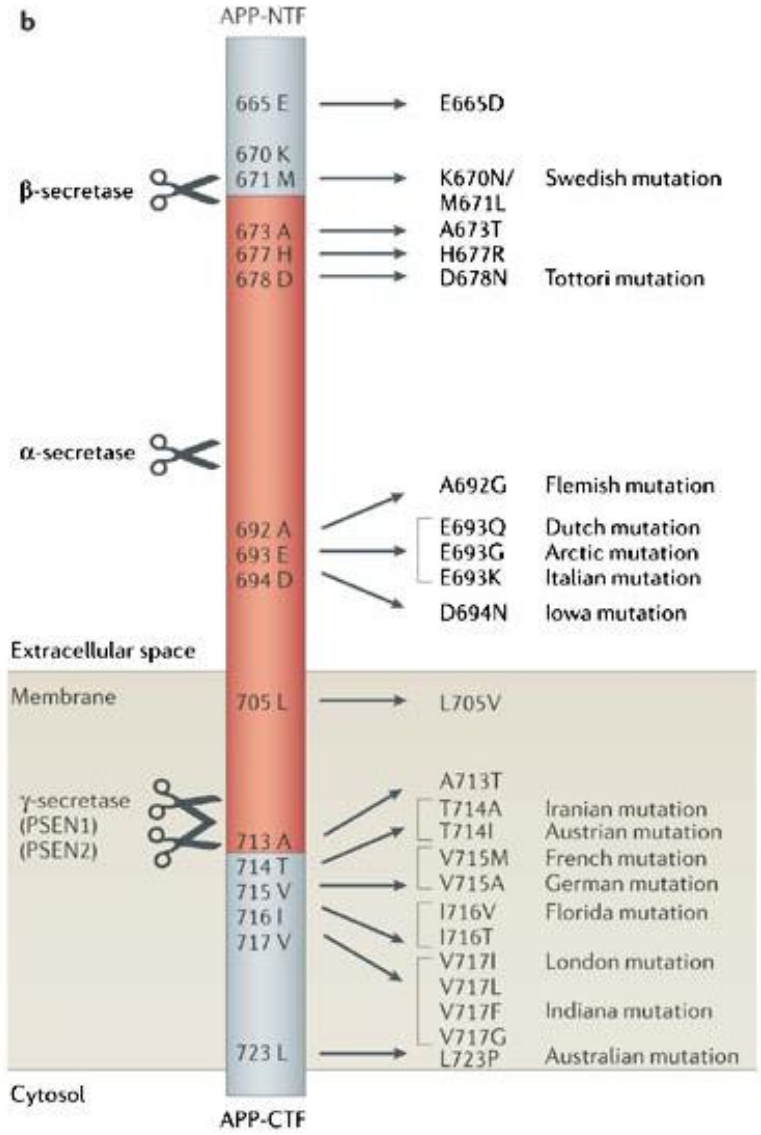




Hardy and Selkoe 2002

Karran, Mercken and De Strooper, Nature Reviews 2011

Effect of APP mutations on Aß



"Swedish" mutation increases ß-secretase cleavage and Aß production 5-8 fold.

Amino acid substitution mutations increase Aß aggregation and oligomerization.

Transmembrane domain substitution mutations increase production of Aß42 over Aß40. Aß42 aggregates much faster.

Presenilin mutations increase the ratio of Aß42/Aß40

Increased amyloid-β42(43) in brains of mice expressing mutant presenilin 1

Karen Duff*†, Chris Eckman†, Cindy Zehr*†, Xin Yu*, Cristian-Mihail Prada†, Jordi Perez-tur*†, Mike Hutton*†, Luc Buee‡, Yasuo Harigaya†, Debra Yager†, David Morgan§, Marcia N. Gordon§, Leigh Holcomb§, Lawrence Refolo†, Brenda Zenk†, John Hardy*† & Steven Younkin†

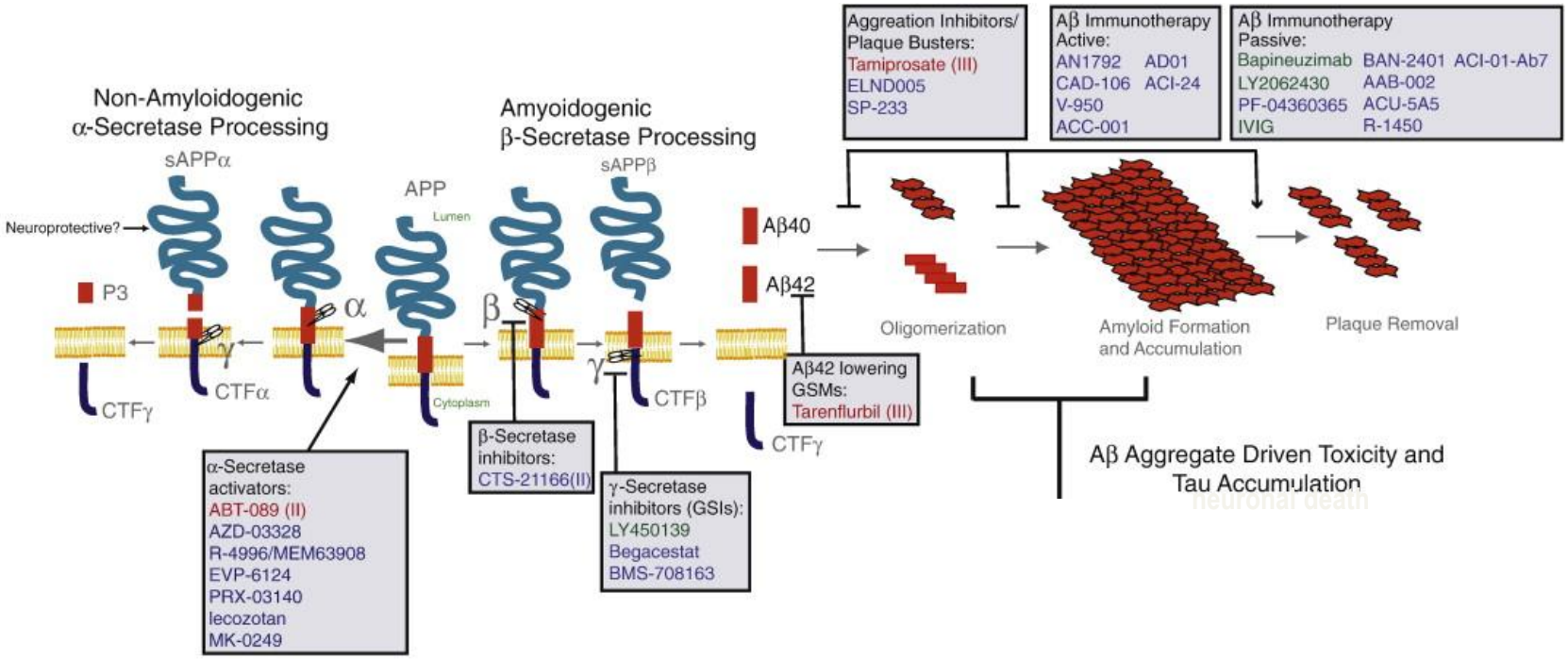
* Suncoast Alzheimer's Disease Laboratories, University of South Florida, Tampa, Florida 33612, USA
† Birdsall Building, Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville, Florida 32224, USA
‡ Unite 422 INSERM, Place de Verdun, 50945 Lille Cedex, France
§ Alzheimer's Research Laboratory, Department of Pharmacology, University of South Florida, Tampa, Florida 33613, USA

MUTATIONS in the genes encoding amyloid-β precursor protein (*APP*)[1], presenilin 1 (*PS1*)[2] and presenilin 2 (*PS2*)[3,4] are known to cause early-onset, autosomal dominant Alzheimer's disease. Studies of plasma and fibroblasts from subjects with these mutations have established that they all alter amyloid β-protein (βAPP) processing, which normally leads to the secretion of amyloid-β protein (relative molecular mass 4,000; M_r 4K; ~90%

NATURE · VOL 383 · 24 OCTOBER 1996

Presenilin mutations increase production of Aß42 over Aß40. Aß42 aggregates much faster.

Investigational new drugs based on the amyloid cascade hypothesis



Golde, T., et al., Exp. Neurol. 2010

Topics:Pipeline | R&D

Pharma counts just 3 Alzheimer's drug wins in 13 years (101 losses!)

September 14, 2012 | By Ryan McBride

Alzheimer's drug research has riddled biopharma with some of the worst odds of success in the already risky R&D game. … drug developers have scrapped or halted development of 101 meds for the complex disorder and brought to market only three treatments for symptoms of the disease, according to the Pharmaceutical Research and Manufacturers of America (PhRMA). This should come as no surprise to those who have followed the recent late-stage disasters of Johnson & Johnson ($JNJ) and Pfizer's ($PFE) bapineuzumab and Eli Lilly's ($LLY) solanezumab. Do the math on PhRMA's figures and from 1998 to 2011 you end up with a sad win-to-loss ratio of one to 34.

Semagacestat (gamma secretase inhibitor) actually made the treated group cognitively worse.

INDIANAPOLIS, Aug 17, 2010 /PRNewswire via COMTEX News Network/ --

Eli Lilly and Company (NYSE: LLY) will halt development of semagacestat, a gamma secretase inhibitor being studied as a potential treatment for Alzheimer's disease, because preliminary results from two ongoing long-term Phase III studies showed it did not slow disease progression and was associated with **worsening of clinical measures of cognition** and the ability to perform activities of daily living.

So why have all the new drugs based on the amyloid cascade hypothesis failed miserably?

1. **The amyloid cascade is wrong and Aß is not important.**

2. **We are not thinking about the amyloid cascade hypothesis correctly.**

Neuron
Perspective

The Transcellular Spread of Cytosolic Amyloids, Prions, and Prionoids

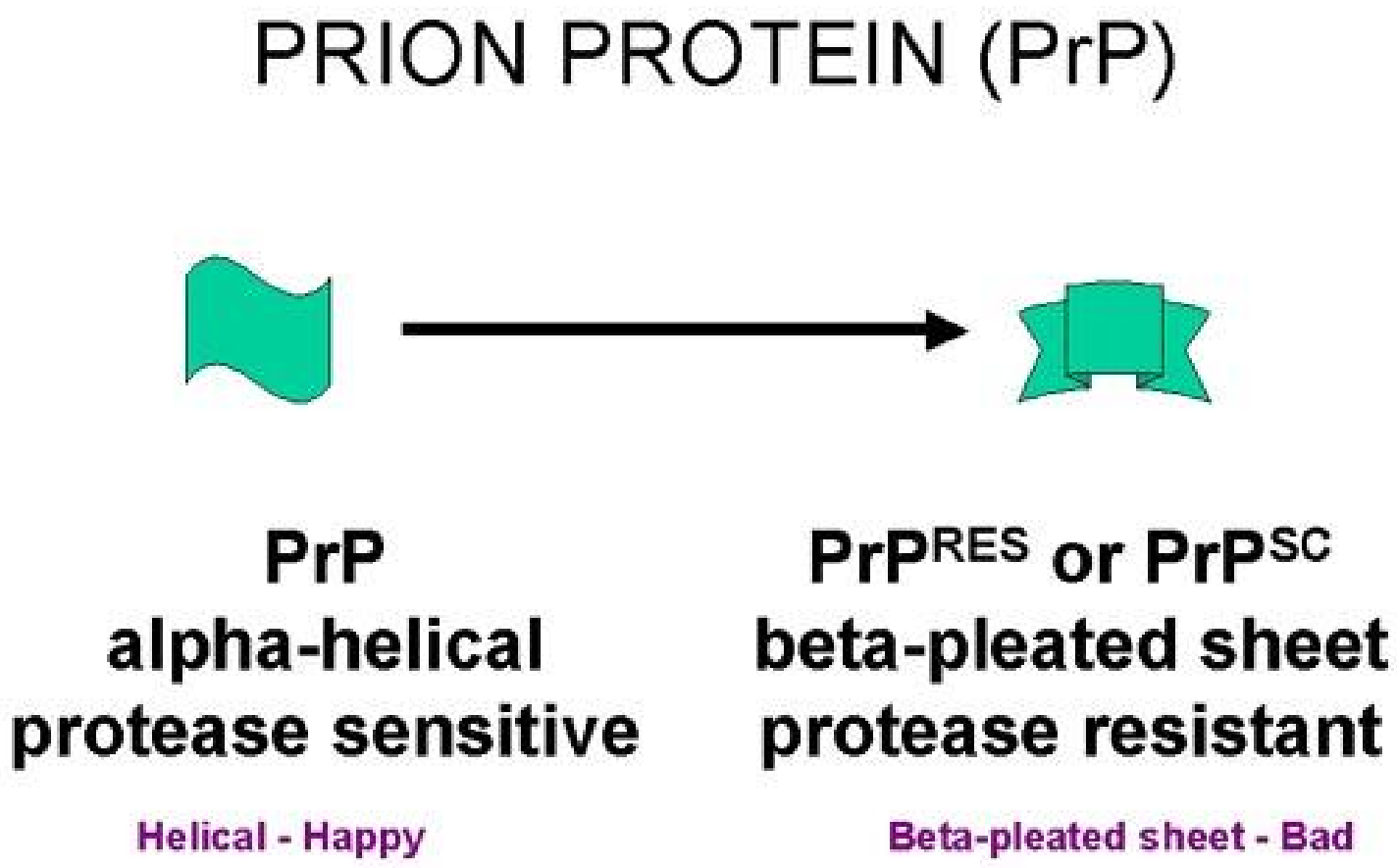
Adriano Aguzzi[1,*] and Lawrence Rajendran[2,*]
[1]Institute of Neuropathology, University Hospital of Zürich, Schmelzbergstrasse 12, CH-8091 Zürich, Switzerland
[2]Systems and Cell Biology of Neurodegeneration, Psychiatry Research, University of Zurich, CH-8008 Zürich, Switzerland
*Correspondence: adriano.aguzzi@usz.ch (A.A.), rajendran@bli.uzh.ch (L.R.)
DOI 10.1016/j.neuron.2009.12.016

Table 1. Potential Prionoids in Health and Disease (Adapted from Aguzzi, 2009)

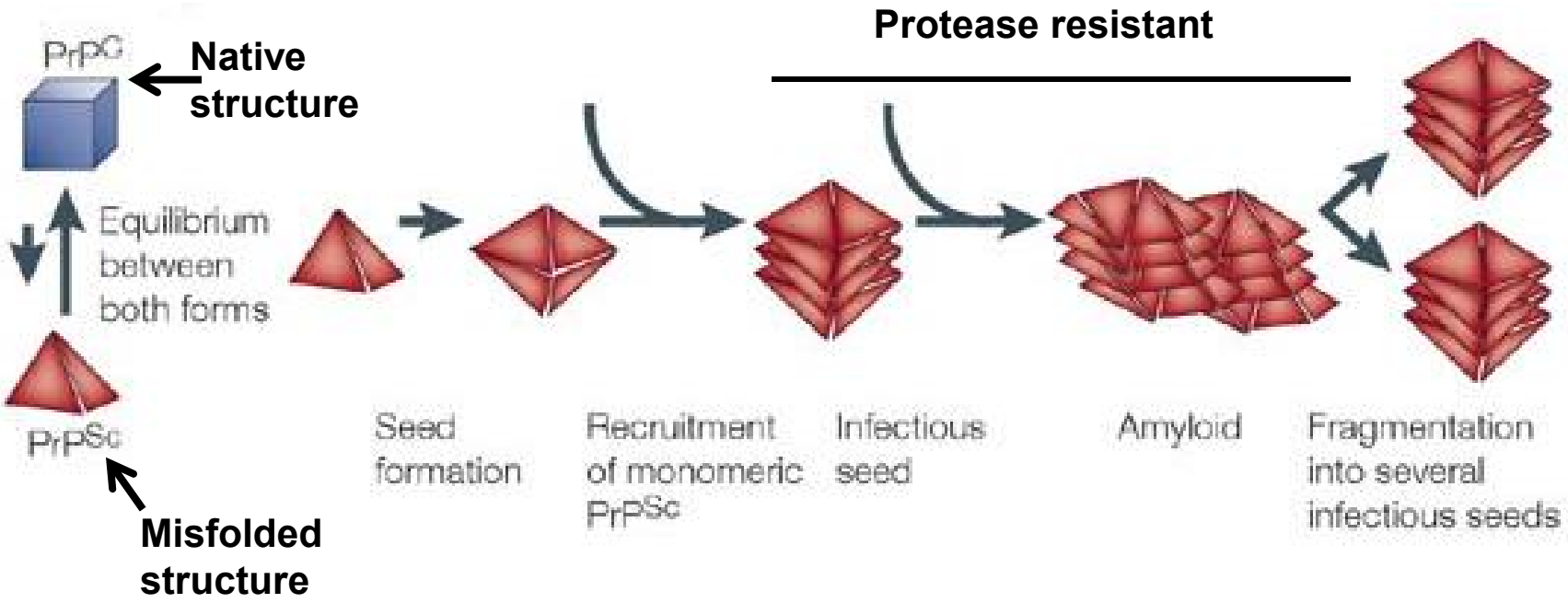
Phenotype/Function	Protein	Molecular Transmissibility	Bona Fide Infectivity
Prion diseases	PrP^{Sc} (luminal)	yes	yes
Alzheimer's disease	Aβ (luminal)	yes	in APP-overexpressing mice
Tauopathies	Tau (cytosolic)	possibly	not shown
Parkinson's disease	α-synuclein (cytosolic)	host-to-graft	not shown
AA amyloidosis	SAA (luminal)	yes	probable
Huntington's disease	PolyQ (nuclear)	yes	not shown
Suppressed translational termination (yeast)	Sup35	yes	limited
Biofilm production (bacteria)	bacterial curlin	yes	questionable
Heterkaryon incompatibility (fungi)	Het-s	yes	limited
Pituitary secretory granules	peptide hormones	not shown	not shown
Mammalian skin pigmentation	Pmel17	not shown	not shown

Neuron. 2009 Dec 24;64(6):783-90.

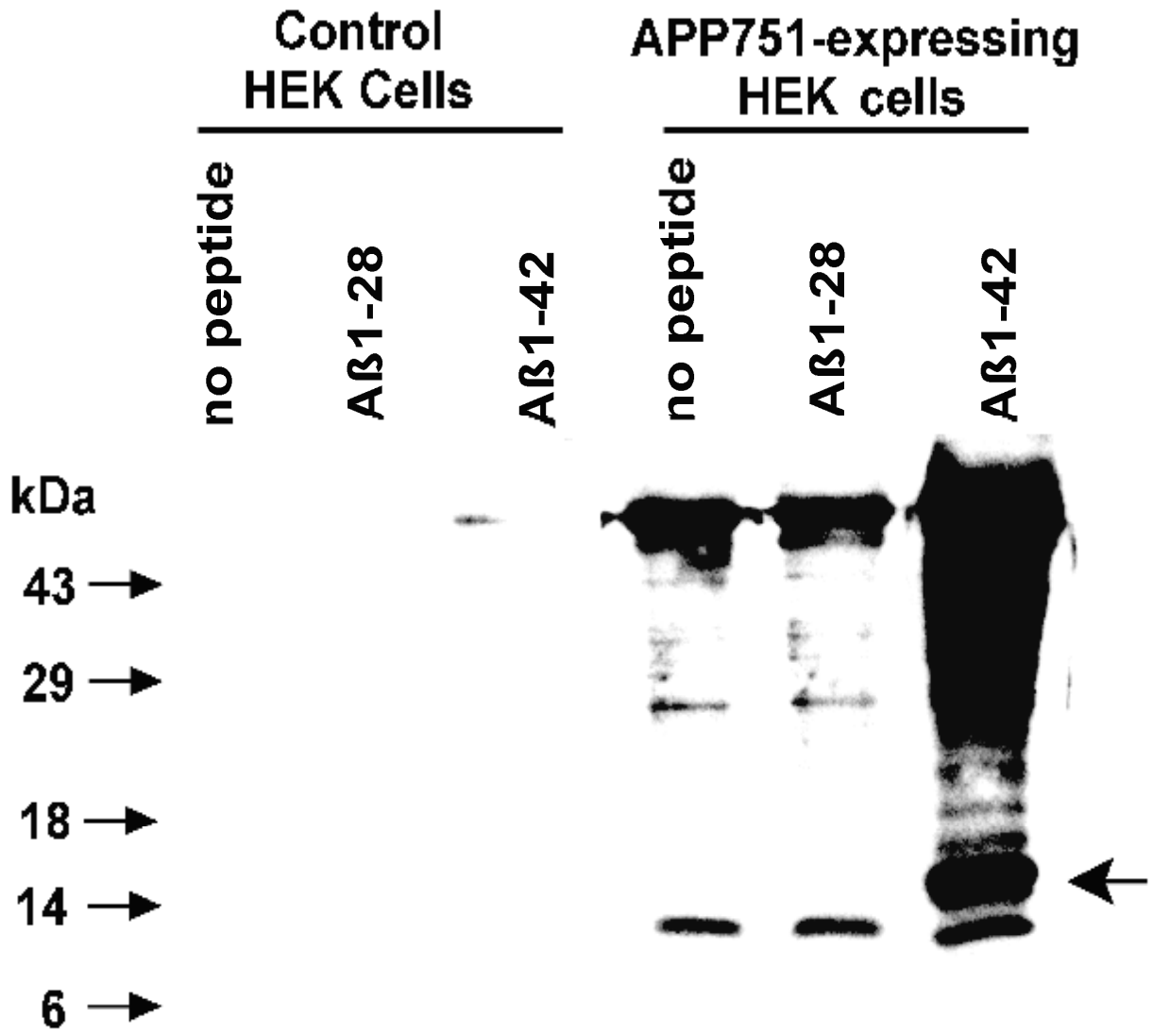
The fundamental mechanism of prion replication involves misfolding of PrP and its aggregation into a ß-sheet fibril where the amyloid domain is resistant to degradation.



Prion replication involves “seed” formation, elongation, fragmentation and transmission of seeds to other cells or individuals.



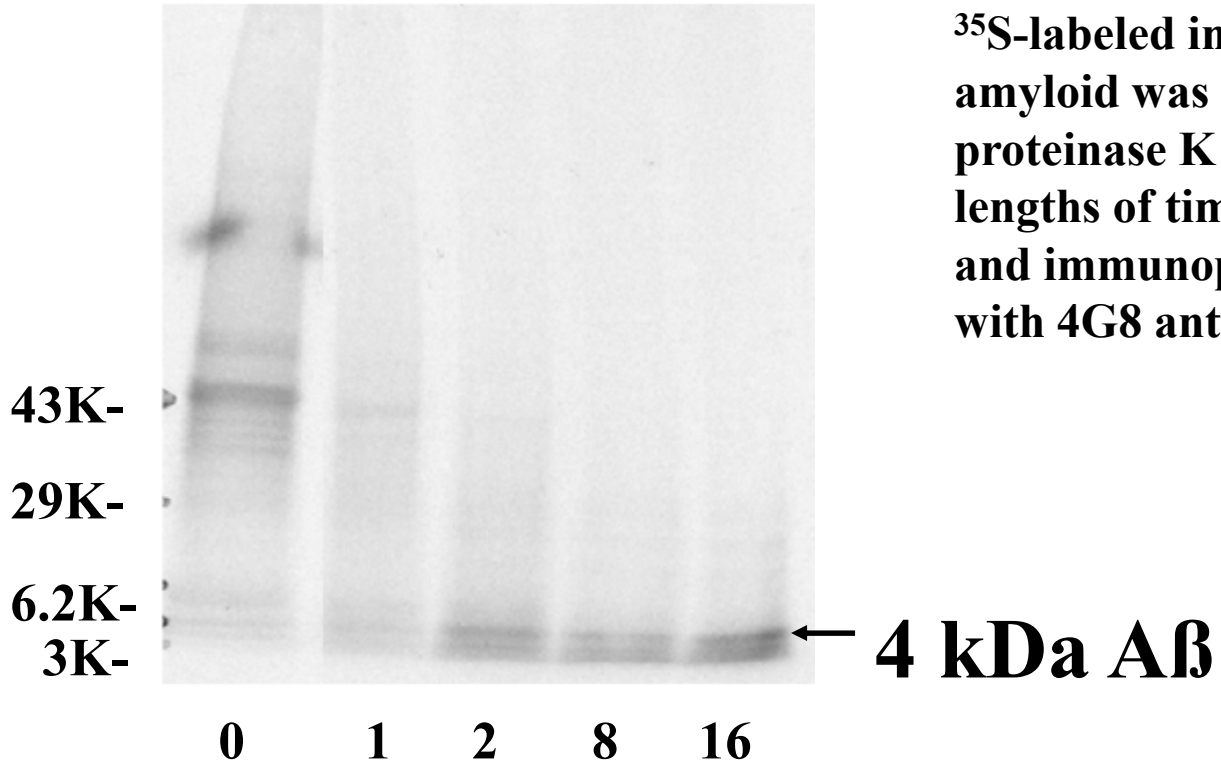
Uptake of Aß42 oligomers seeds the prion-like accumulation of insoluble, intracellular APP and amyloidogenic fragments of APP.



Yang, et al., 1995

JBC 270:14786-14792

Digestion of insoluble intracellular amyloid with Proteinase-K results in the accumulation of degradation-resistant Aß.



^{35}S-labeled intracellular amyloid was digested with proteinase K for varying lengths of time, solubilized and immunoprecipitated with 4G8 anti-Aß.

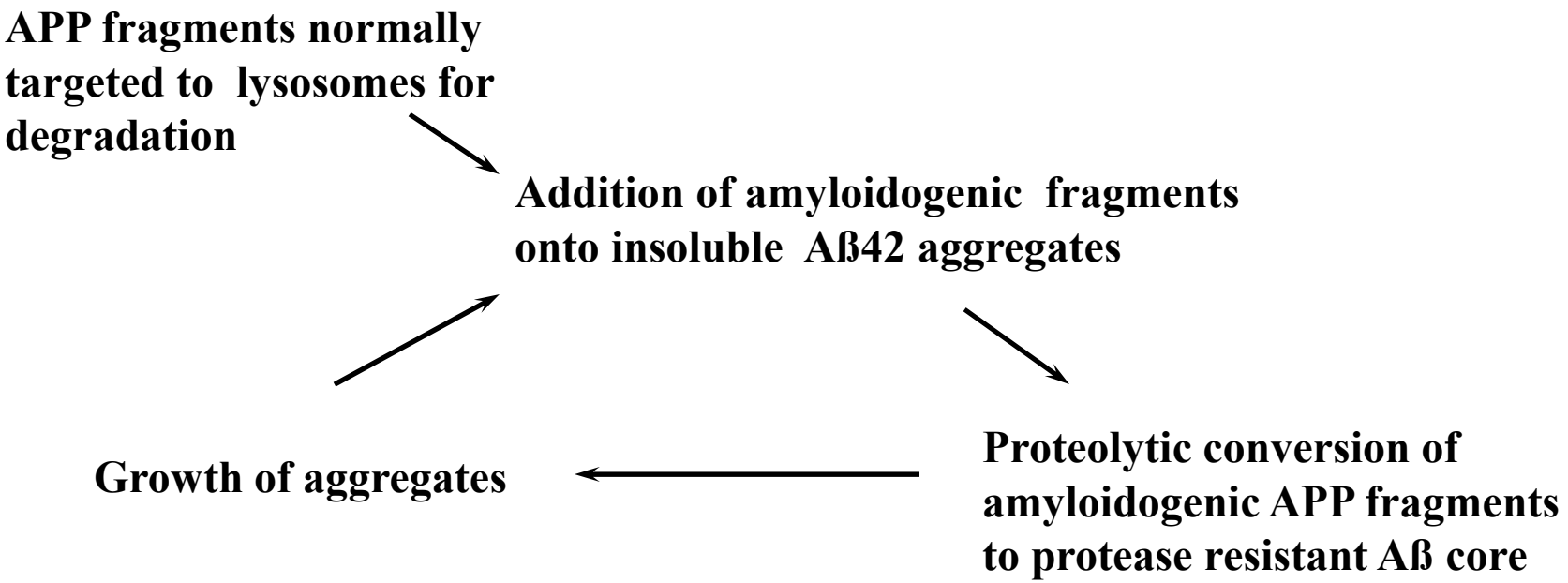
Yang, et al., 1999 JBC 274:20650-20656

Prion-like mechanism for Aß amyloid accumulation

Initiation:

Aggregation of Aß42 → Protease Resistance

Replication:



So is there any evidence for a prion-like mechanism for amyloid Aß accumulation in AD brain?

Conformation dependent fibril specific monoclonal antibodies

Antigen: Aß42 fibrils.

NZW rabbits boosted 6 times at monthly intervals.

Titer for Aß42 fibrils > 1:20,000

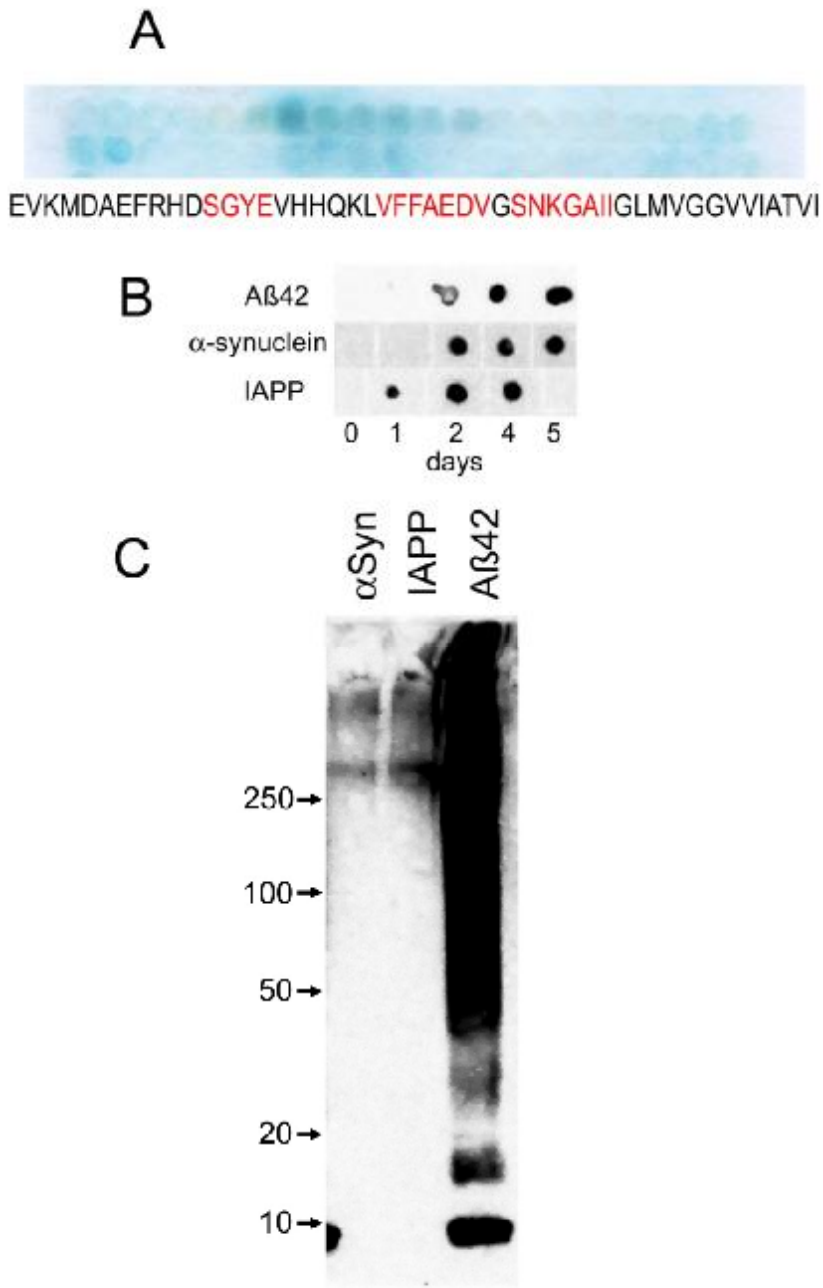
Primary screen: Aß42 fibrils, prefibrillar oligomers and monomer.

120 primary pools of hybridomas selected

Secondary Screen

- Medium density array of: Aß42, Aß40, IAPP, polyQ fibrils, monomers, oligomers. Overlapping linear Aß peptides: 1-15, 5-20 etc. Random sequence amyloid peptides from phage display.
- IHC on human AD brain.
- 24 clones selected based on differential reactivity on array and on IHC of AD brain.

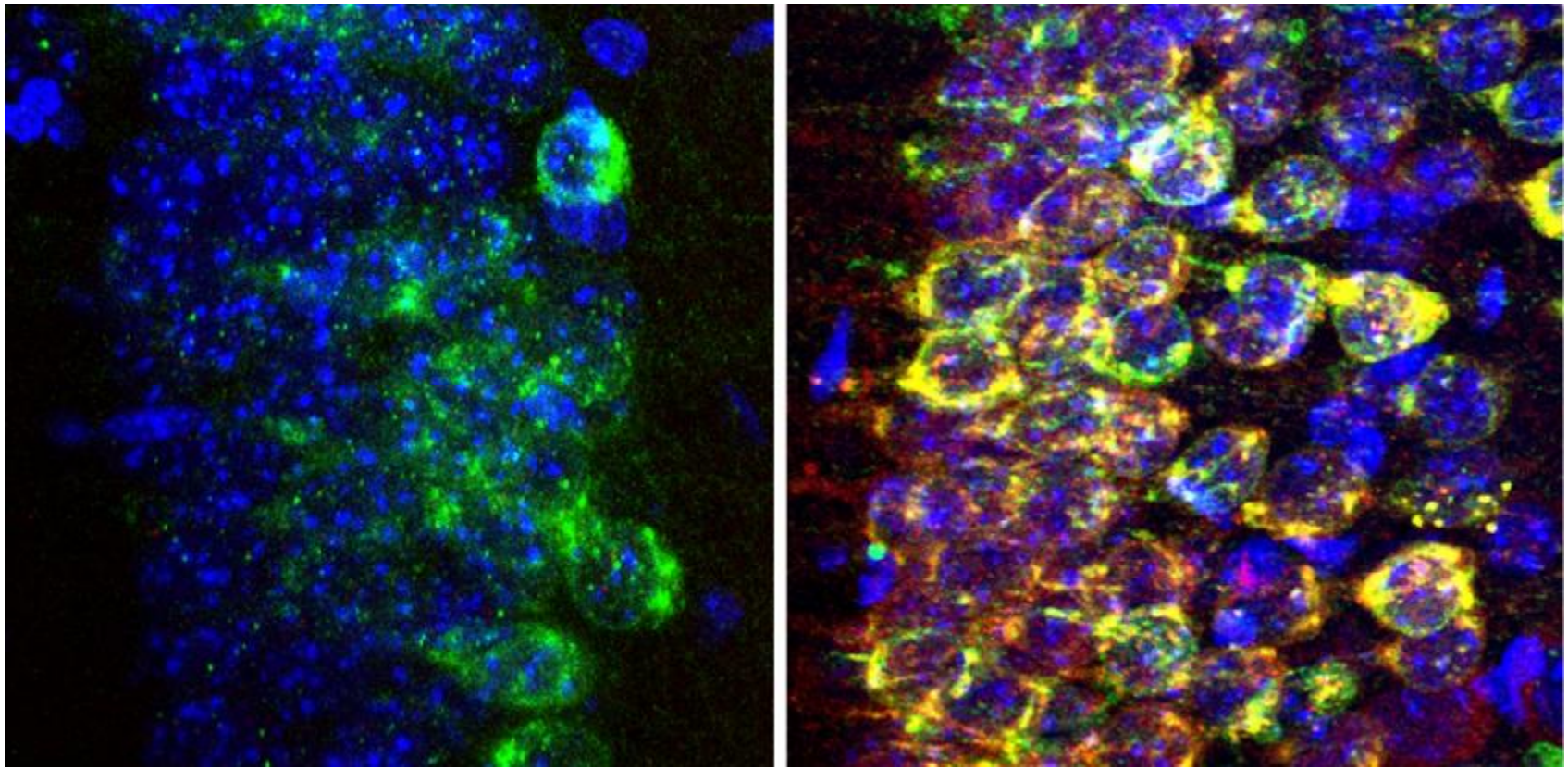
M78 recognizes a “generic” aggregation specific fibril epitope.



M78 recognizes a generic fibril epitope because it reacts with amyloid fibrils from Aß, synuclein and islet amyloid polypeptide. M78 does not recognize the monomeric or natively folded forms of these peptides and proteins.

M78 immunoreactivity first appears intracellularly in 3XTg-AD mice.

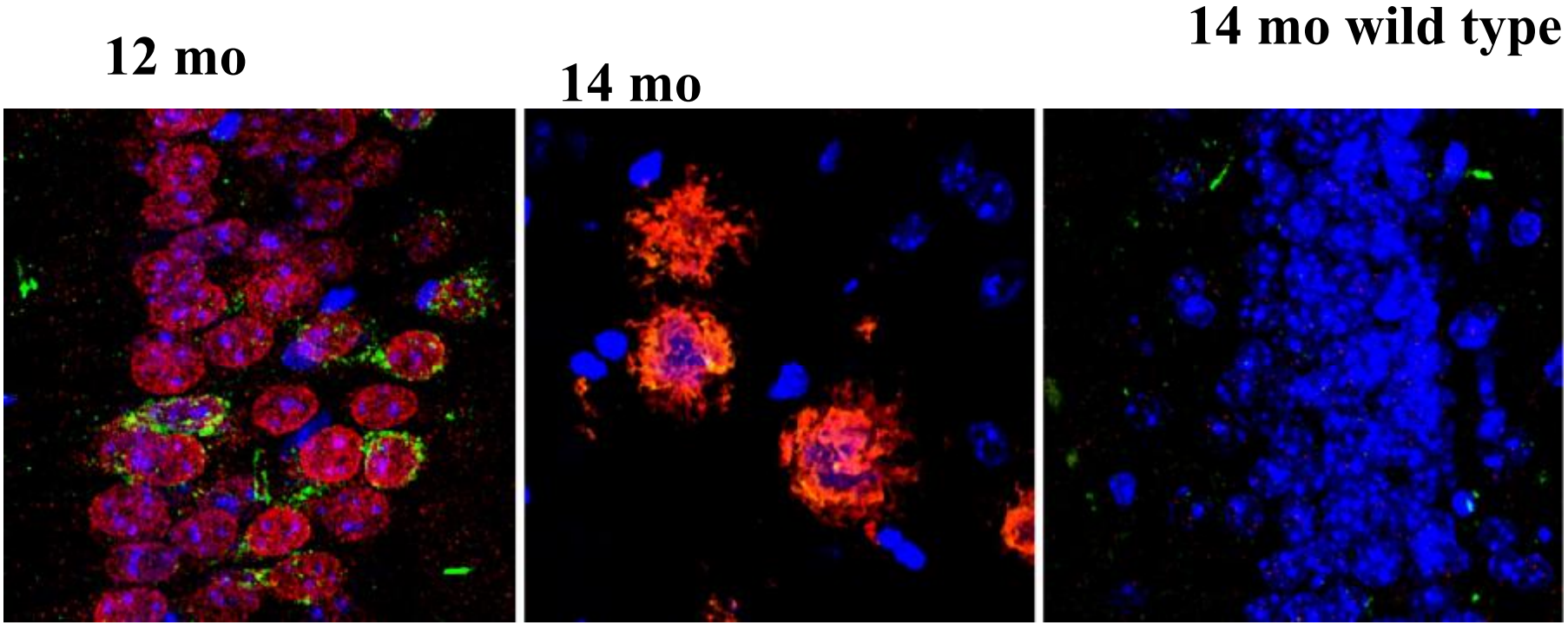
3 mo **10 mo**



3 mo: 6E10 is perinuclear in some neurons and and no M78 is observed.
12 mo: M78 colocalizes with perinuclear 6E10 and accumulates in nuclei

M78 – Red **6E10 (Aß) – Green** **DAPI (DNA) - Blue**

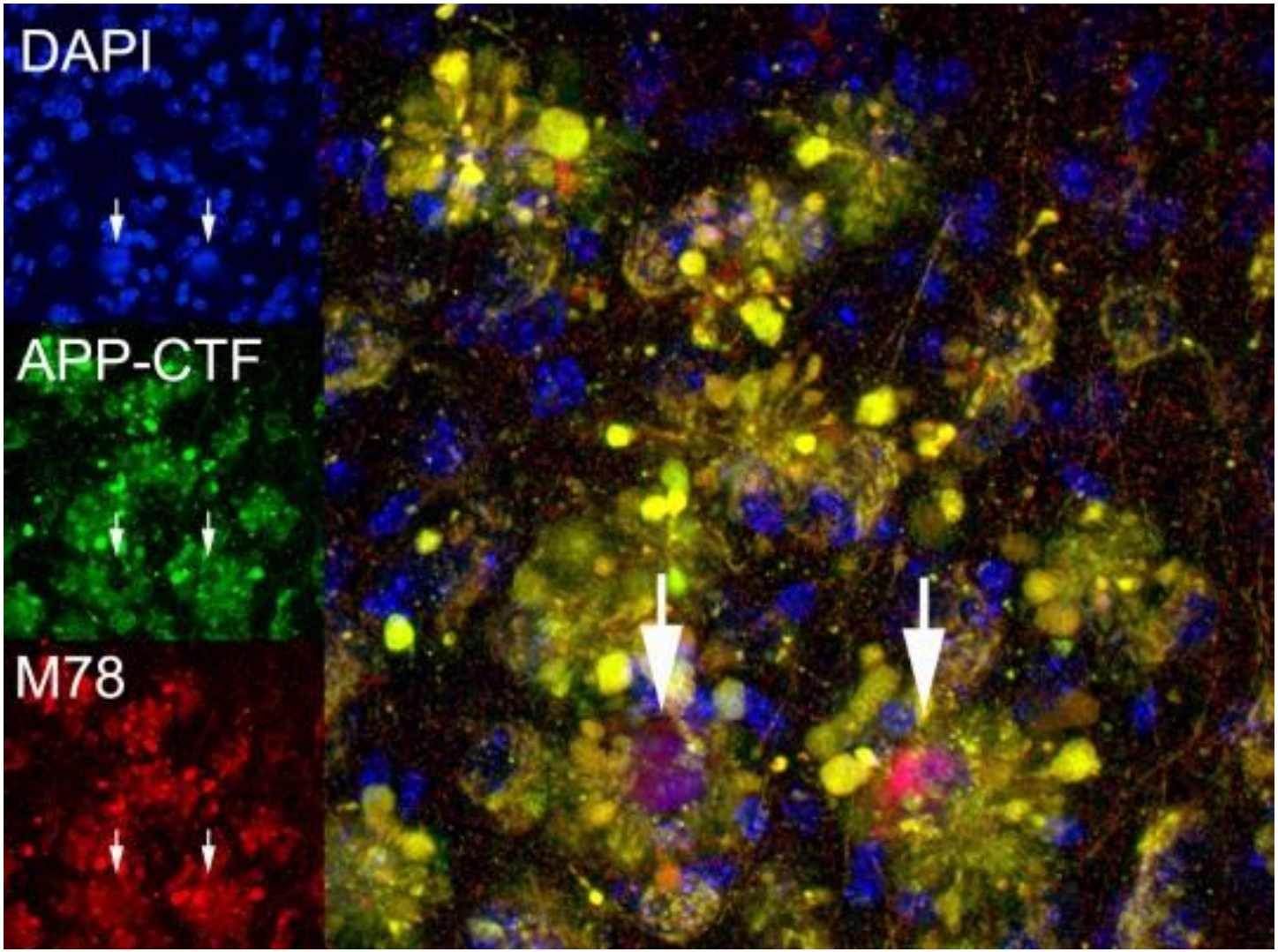
At 12 mo, M78 is predominantly nuclear while at 14 mo, it is primarily extracellular.



12 mo: M78 accumulates in nuclei with perinuclear 6E10 immunoreactivity.
14 mo: M78 stains only extracellular plaques.
14 mo wild type mice: No 6E10 or M78 staining is observed.

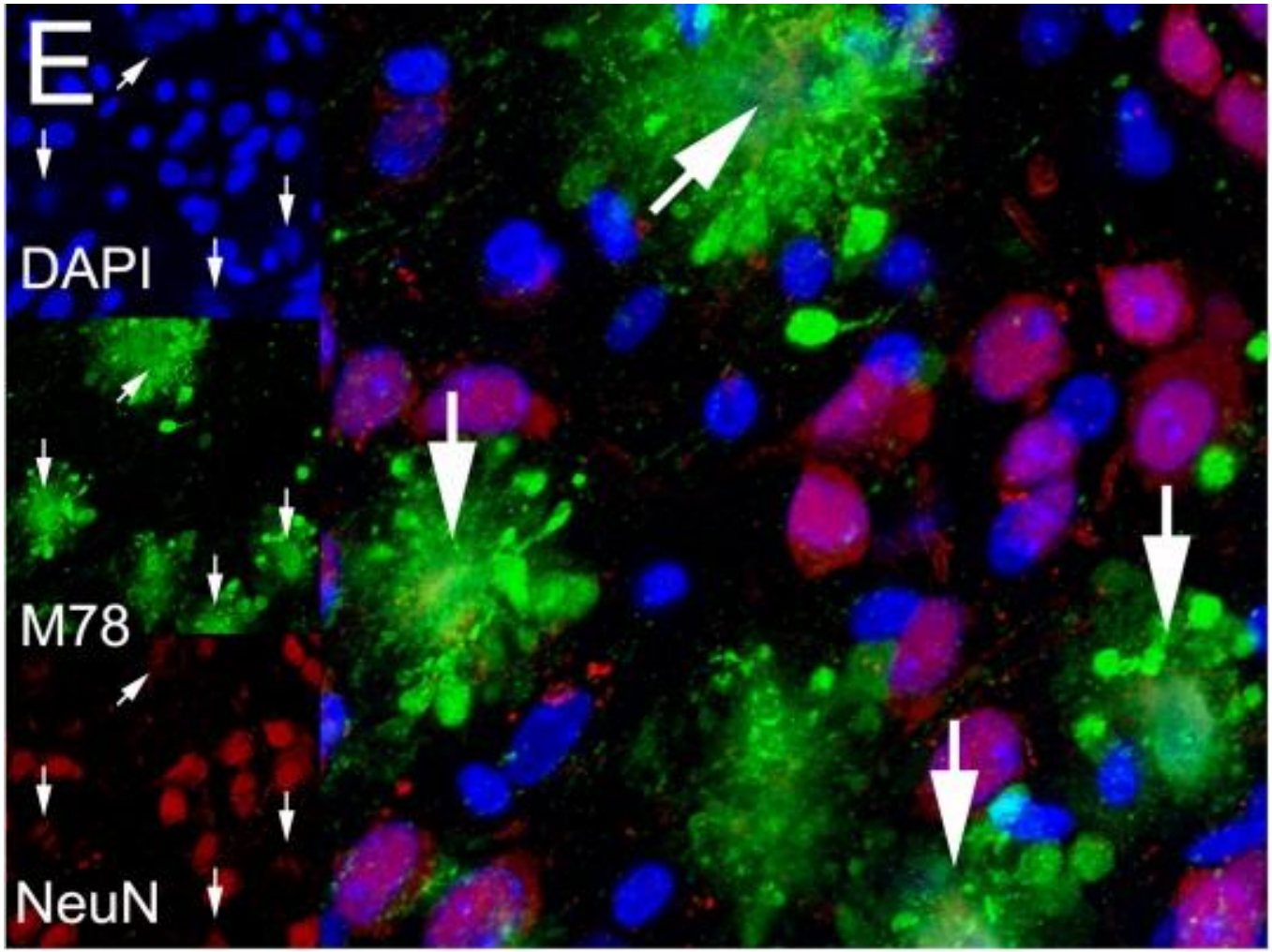
M78 – Red **6E10 (Aß) – Green** **DAPI (DNA) - Blue**

M78 and DAPI DNA fluorescence are found in the center of neuritic plaques in 12 mo 3XTG-AD mice



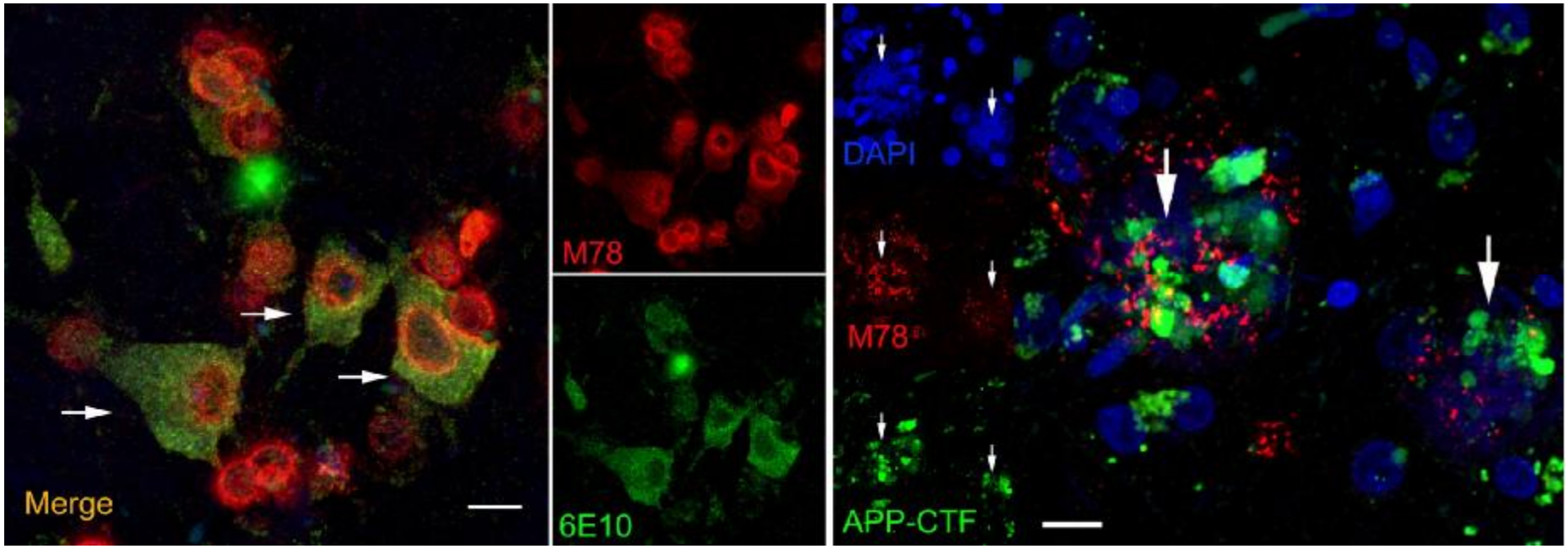
This indicates that neuritic plaques are derived from neurons with intranuclear M78 staining.

The core of neuritic plaques also contains NeuN immunoreactivity; a marker of neuronal nuclei.

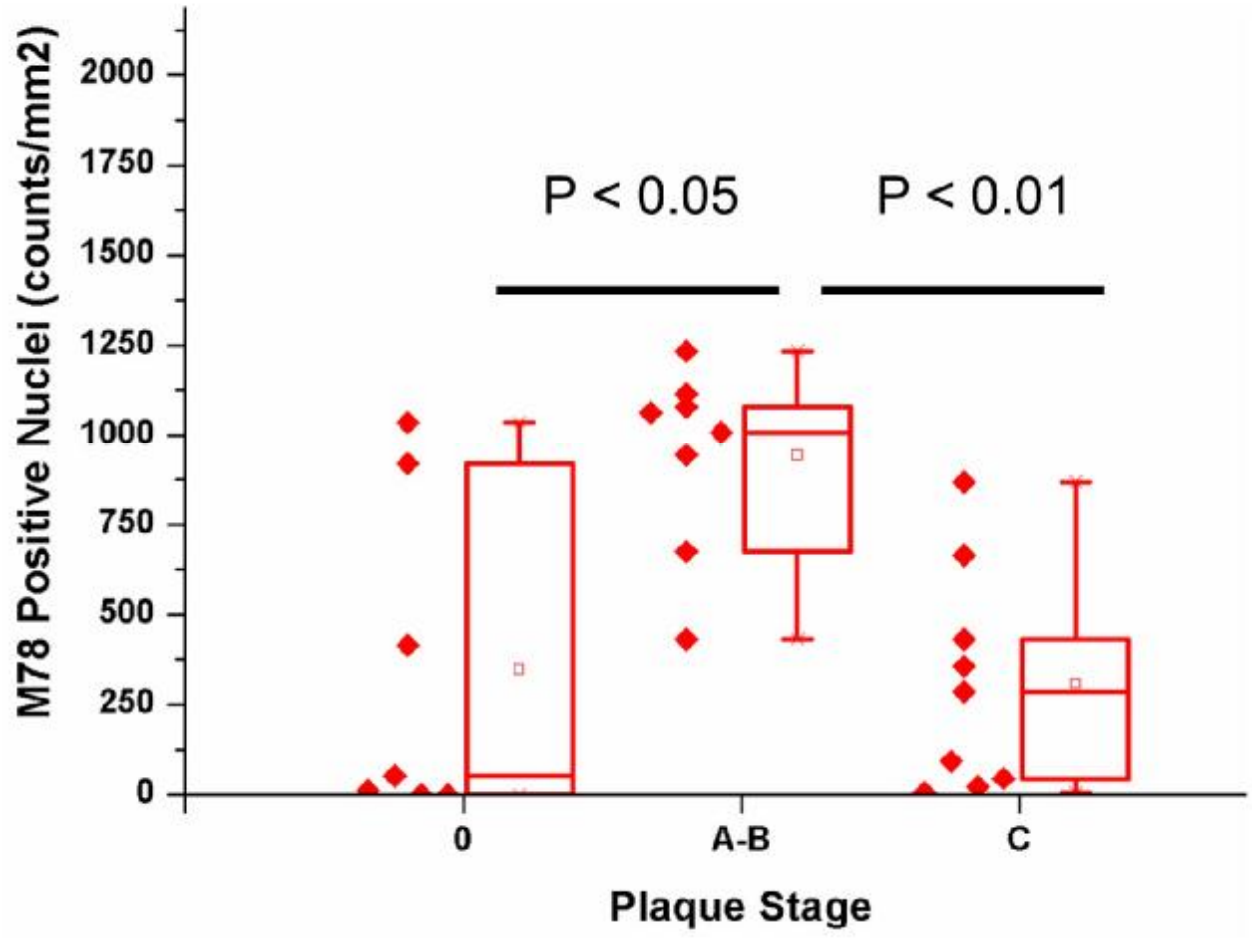


This is further evidence that neuritic plaques are derived from neurons with intracellular amyloid.

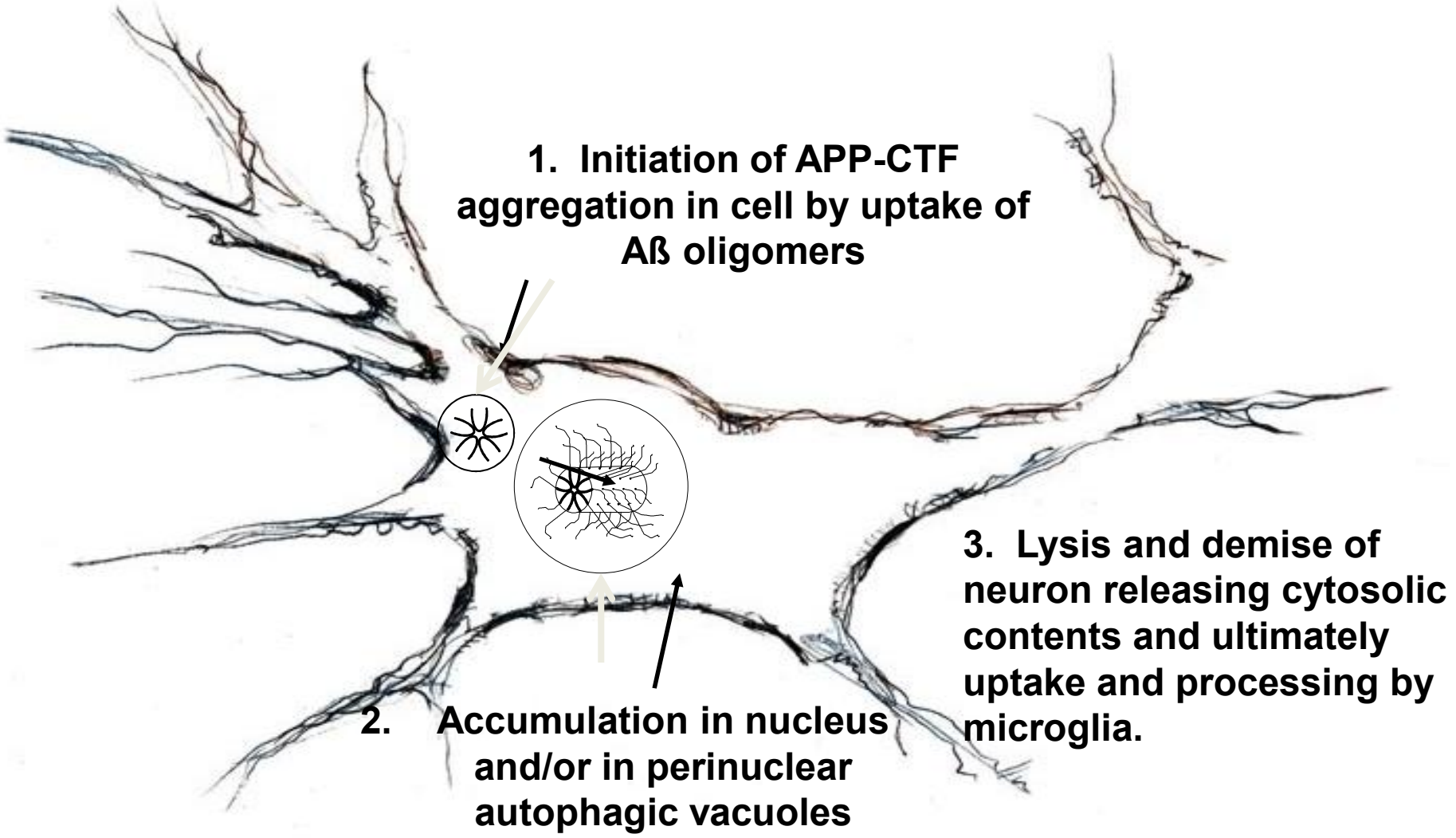
Perinuclear and nuclear M78 IR is also observed in human neurons and neuritic plaques.



M78 immunoreactive nuclei are specifically elevated in early AD.



- An alternative amyloid hypothesis based on intraneuronal amyloid accumulation leading to neuronal death and senile plaque formation.



Summary

- **The conformation dependent, generic fibril specific monoclonal antibody M78 stains nuclei in aged human brain is correlated with intracellular APP and Aß immunoreactivity.**
- **Nuclear M78 co-localizes with APP-CTF immunoreactivity in and around the nucleus.**
- **The same spatial relationship of the nucleus, M78 staining and APP-CTF immunoreactivity is observed in senile plaques, suggesting that senile plaques are initiated by the demise of these neurons.**

Implications: The alternative amyloid cascade hypothesis provides a facile explanation for FAD mutations and the results of the Semagacestat clinical trial.

OPEN ACCESS Freely available online

PLoS one

Alzheimer's Disease-Linked Mutations in *Presenilin-1* Result in a Drastic Loss of Activity in Purified γ-Secretase Complexes

Matthias Cacquevel, Lorène Aeschbach, Jemila Houacine, Patrick C. Fraering*

École Polytechnique Fédérale de Lausanne, Brain Mind Institute, Laboratory of Molecular and Cellular Biology of Alzheimer's Disease, Lausanne, Switzerland

Conclusion/Significance: Our data support the view that PS1 mutations lead to a strong γ-secretase loss-of-function phenotype and an increased Aβ1–42/Aβ1–40 ratio, two mechanisms that are potentially involved in the pathogenesis of Alzheimer's disease.

Both PS mutations and Semagacestat partially inhibit PS proteolytic function. They both increase the levels of ß-CTF which would be expected to drive its misfolding, aggregation and the alternative pathway leading to cognitive dysfunction.

Therapeutic implications

Intracellular amyloid accumulation begins early, before significant cognitive impairment is evident, so treatment must also begin early if it is going to arrest disease progression.

The same kind of strategies that have been tested in mild to moderate AD may also work if they are started early, like immunotherapy and beta secretase inhibitors and gamma secretase modulators.

Inhibitors of intracellular amyloid aggregation would need to gain access to the inside of the cell.

Extracellular aggregation inhibitors and immunotherapy may work by preventing the spreading of disease by cell to cell seeding.

Glabe Lab

- Dr. Suhail Rasool
- Dr. Anna Pensalfini
- Dr. Leonid Breydo
- Dr. Jessica Wu
- Hiromi Arai
- Asa Hatami
- Ricardo Albay III

- Collaborators:
- Dr. Carl Cotman
- Dr. Claudia Kawas
- Dr. Wayne Poon
- Dr. Ralf Langen
- Dr. Hudel Leucke

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