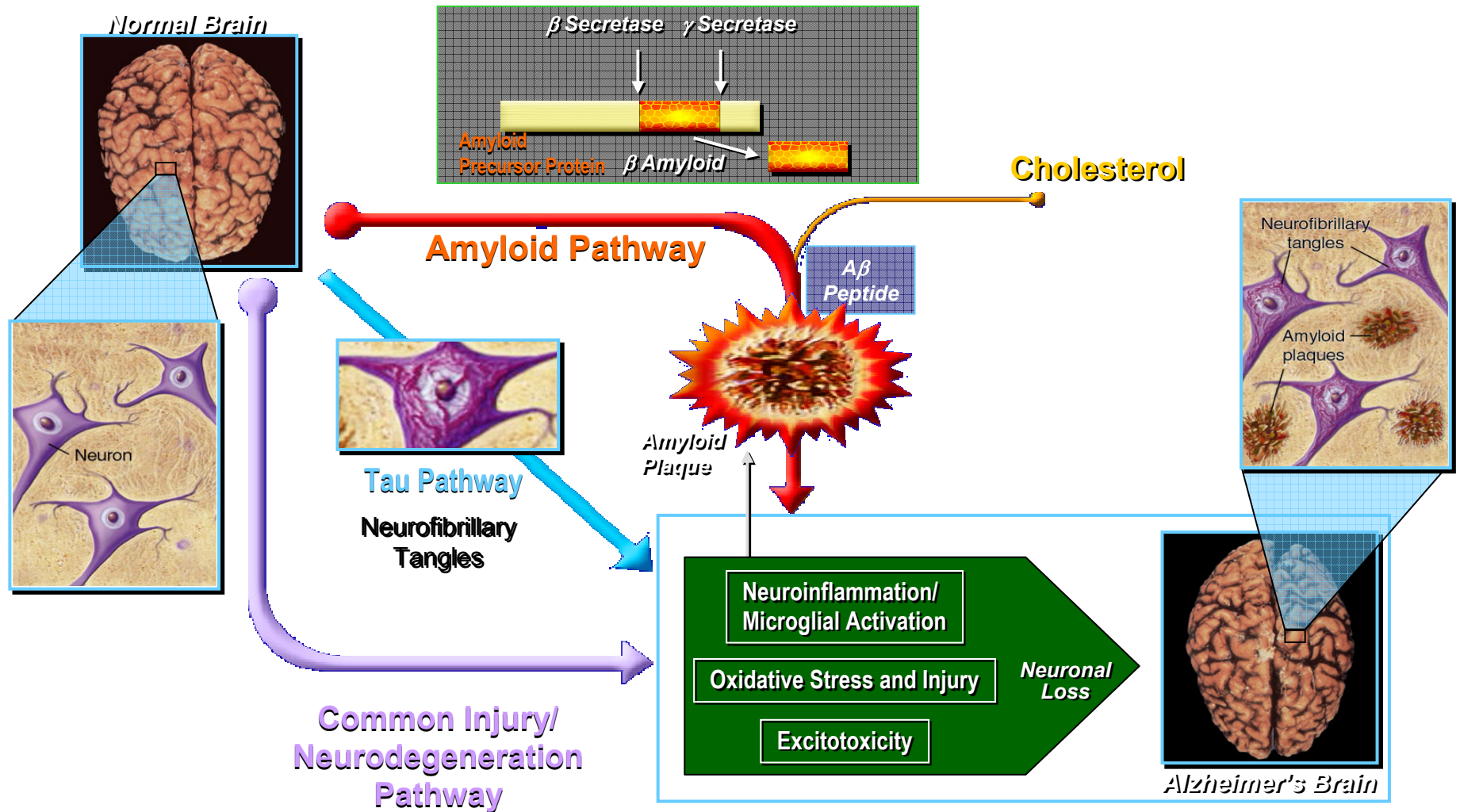

Target Validation in CNS Drug Discovery

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So many targets so little time!



But are these all good targets?

A good target is....

- /// Efficacious
- /// Safe
- /// Doable
 - Historical database of “doable” targets
 - > GPCR’s
 - > Antagonists
 - > Many enzymes

The impact of a modulator on a target can be measured by...

- /// Mechanism biomarkers
 - e.g. Lack of phosphorylation at a site of kinase action
- /// Outcome biomarkers
 - e.g. Slowing the loss of neurons in the AD brain
- /// Translation of preclinical biomarkers to clinical outcomes
 - e.g. Measurement of A β in CSF



Validation is the process that....

- /// increases our confidence in the relationship between target and disease, sometimes linking the disease and its symptoms and sometimes placing the target on the critical path for disease initiation and/or progression.
- /// helps us to explore whether modulation of the target will lead to mechanism-based side effects.
- /// How much do we need to know before venturing into human clinical trials?



**Even with our best preclinical
validation packages only clinical data
will be able to truly validate the target!**



Techniques used to validate a target

- /// No one answer
 - /// Gestalt of information from a wide variety of experiments
-

/// For example:

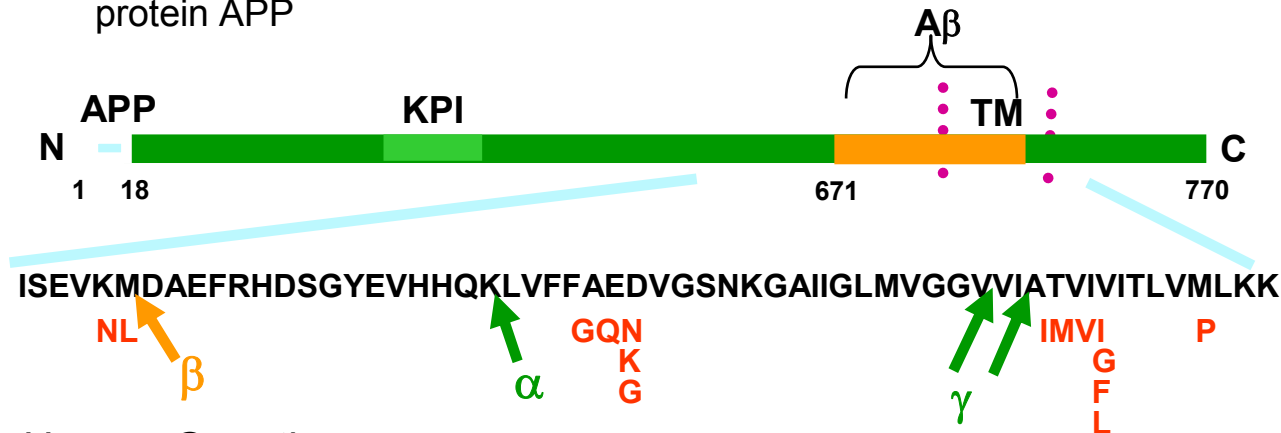
- Genetics
 - > SNPs
 - > Mutations
- Clinical data
 - > Disease database
- Expression patterns
 - > “Location, Location, Location”
 - > Disease modulation
 - > Microarray studies
- Pharmacological tools
- Cell culture models
 - > siRNA
 - > Cellular localization/co-localization
- Mammalian Animal models
 - > Induced by chemical
 - > Transgenic mice
 - > Knock-out or Null mice
 - > Behavioral Models
- Model organisms
 - > Drosophila
 - > Zebrafish
 - > *C. elegans*
- “Omics”
 - > Genomics
 - > Proteomics
 - > Transcriptomics



What are the data that supports validation of β - and γ -Secretases as good therapeutic targets?

Pathology

- Neuritic plaques, whose principal component is $A\beta$, are one of the pathological hallmarks of AD.
- Beta and gamma secretases are responsible for the formation of $A\beta$ from the precursor protein APP



Human Genetics

- APP mutations at BACE cleavage site increase the production of $A\beta$ and result in familial Alzheimer's disease (FAD)
- Mutations in the presenilin gene, the catalytic component of the multienzyme gamma secretase complex, result in FAD

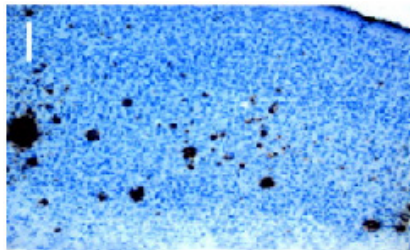
Biomarker

- Mechanism: $A\beta$ measurements in plasma and CSF are possible to follow in human clinical trials.
- Disease progression: imaging

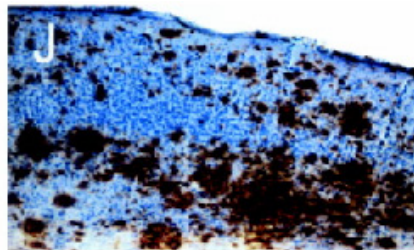


BACE: Large body of data supporting this target

- Increased expression and activity in the areas of the AD brain that are laden with amyloid plaques (Fukumoto, 2002; Holsinger, 2002; Yang, 2003)
 - Found in neurons of the temporal and frontal cortex but not the cerebellum (activity assays, immunohistochemistry)
- Expression of BACE in wild type mice increases the level of mouse A β (Bodendorf, 2002)
- BACE1/APP double transgenic mice
 - Accelerated amyloid plaque formation (Mohajeri, 2004; Willem, 2004)



16 mo APP Tg



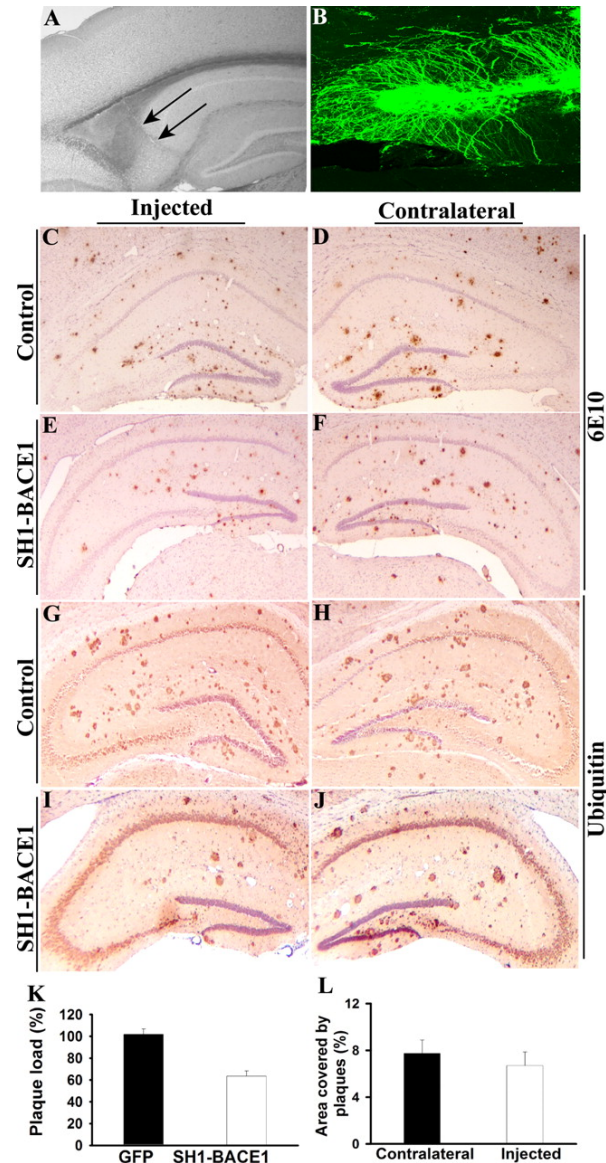
16 mo BACE1/APP dTg

(Immunohistochemistry, 3D6)
Willem et. al. Am. J. Path 165:1621-1631, 2004

- siRNA
 - BACE1 siRNA reduced A β and APP-CTFs from primary mouse cortical neurons (wild type and APP^{swe} transgenic mice) (Kao, 2004)
 - In neurons of the hippocampus and neocortex of APP transgenic mice, lentiviral BACE1 siRNA delivery reduced (Singer, 2005):
 - BACE1 expression
 - A β and APP CTFs
 - MAP-2 immunohistochemistry (a sign of neurodegeneration)
 - In APP^{swe}/PS1 Δ E9 mice, siRNA reduced amyloid burden in the hippocampus (Laird, 2005)



Silencing BACE1 through RNAi ameliorates A β amyloidosis in the hippocampus of APP^{swe}/PS1 Δ E9 mice



Laird, F. M. et al. J. Neurosci. 2005;25:11693-11709



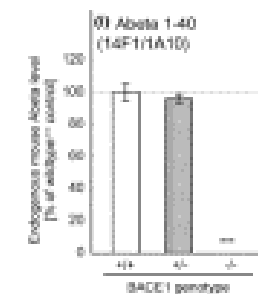
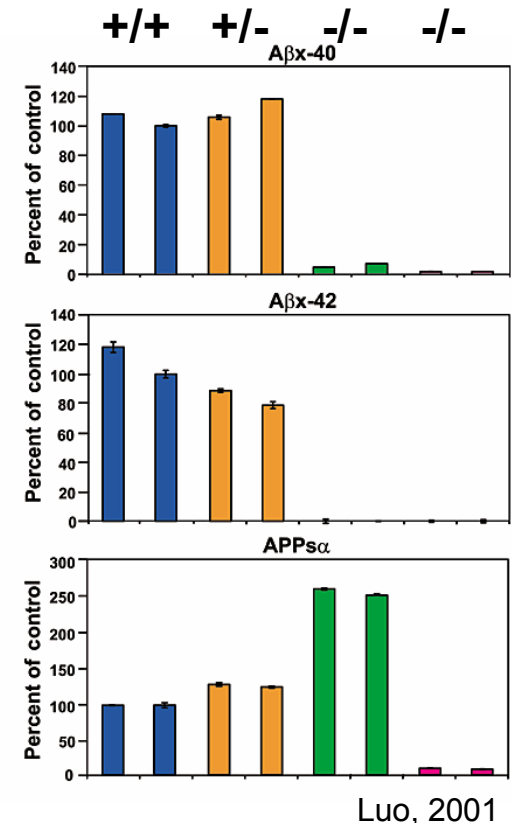
BACE

/// KO mice

- KO mouse is viable with no gross abnormalities (Roberds, 2001; Luo, 2001)
 - > With no BACE1, there is no A β produced. (Luo, 2001; Cai, 2001)
 - > Interestingly, in BACE1+/- mouse, there is no change in the level of brain A β
 - Wild type mice (Nishitomi, 2006)
 - PDAPP/BACE1+/- mice (Sinha, 2005)
 - > Rescues memory deficits in APP transgenic mice (Ohno, 2004)
 - > Modest behavioral alterations (Laird, 2005; Dominguez, 2005)

/// Potential biomarker

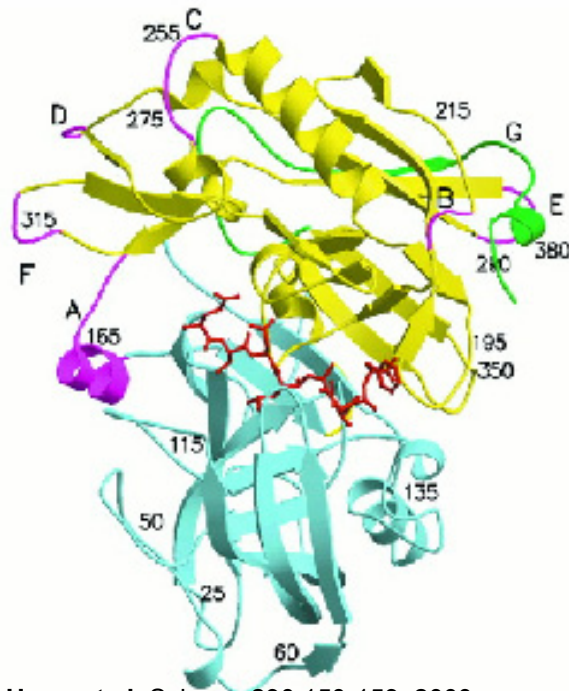
- Neuregulin recently identified as a BACE substrate (Willem, 2006).
 - > Null mice display hypomyelination of the sciatic nerve
 - > Suggests a that processing of neuregulin may be a biomarker for impact of BACE inhibitors *in vivo*
 - > BACE inhibitor in zebrafish confirmed impact on neuregulin processing



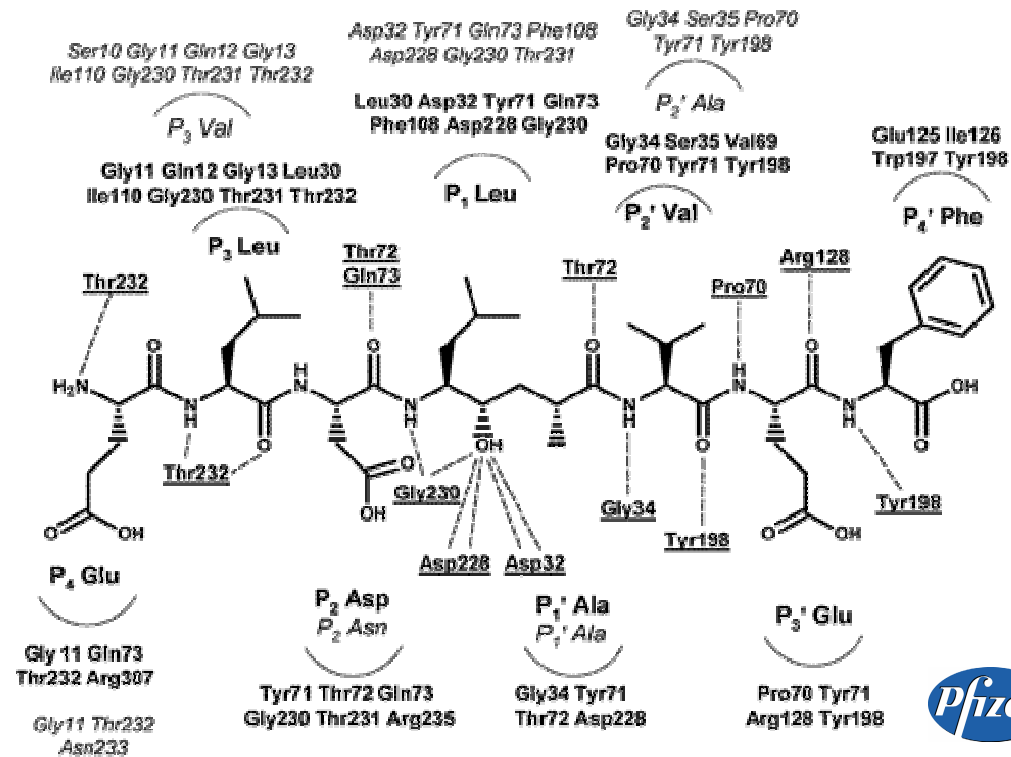
Nishitomi, 2006

Doability is the challenge for β -Secretase

- // Membrane tethered aspartyl protease
 - Active recombinant enzyme
 - Crystal structure to assist SAR
- // Very large active site as first demonstrated by J. Tang with complex interactions between high molecular weight peptidic inhibitor



Hong et al. Science 290:150-153, 2000



Hong et. al. Biochemistry 41:10963-10967, 2002



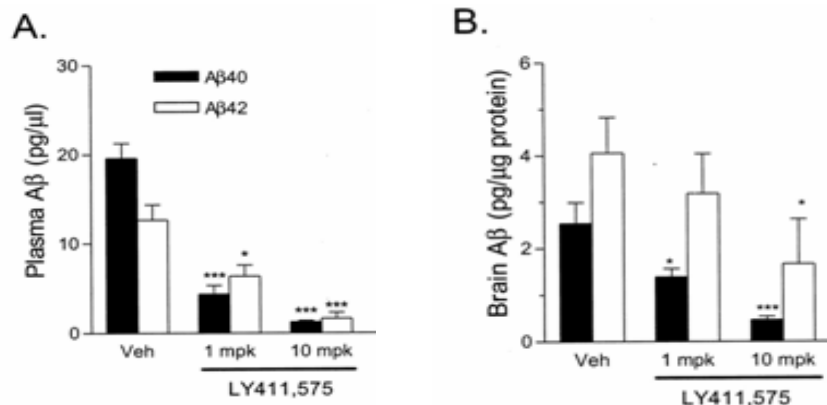
Doability is the challenge for β -Secretase

- /// 7+ years since its cloning and this high priority target has not reached clinical trials
- /// *In Vivo* activity is a challenge
 - Proof of concept achieved; however.....
 - Potent, peptidic inhibitors were transported into the brain covalently linked to a carrier peptide such as the Tat gene product. (Chang, 2004)
 - > $A\beta$ lowered in plasma and in the soluble fraction of brain $A\beta$
 - Potent, non-peptidic inhibitor lowers brain $A\beta$ under two conditions (Hussain, 2007)
 - > In the presence of p-glycoprotein inhibitor
 - > Upon repeated dosing over a 5 day period
 - Potent, small molecule inhibitor (Bard, 2006)
 - > 10nM *in vitro* potency
 - > BID, po, $A\beta_{40}$ in plasma and brain decreased after 7 days of dosing @30mpk and at 14 days of dosing @10mpk in APP transgenic mice.
 - Stereotaxic injection of 10 μ gs of Inhibitor IV (Stachel, Merck) into cerebral ventricles needed to obtain 25% reduction brain $A\beta_{40}$ (Nishitomi, 2006)

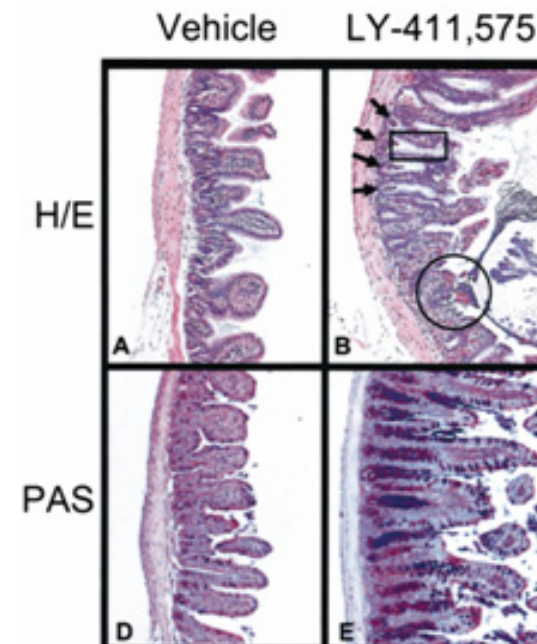


Safety is the challenge for γ -Secretase

- Large number of substrates for this enzyme complex and list continues to grow
 - Can inhibitors that are selective for a single substrate be identified?
- PS KO mice have an embryonic lethal phenotype that is identical to the phenotype of the Notch KO mouse (Wong, 1997)
 - These results focused safety concerns on Notch
- In animal models γ -secretase inhibitors cause unacceptable “side effects”
 - At the same concentrations that LY-411,575 reduces plasma and brain A β , it alters intestinal cell differentiation



Wong, G. T. et al. J. Biol. Chem. 279: 12876-12882, 2004.



Safety is the challenge for γ -Secretase - or is it?

- /// “The novel γ -secretase inhibitor MRK-560 reduces amyloid plaque deposition without evidence of Notch-related pathology in the Tg2576 mouse” (Best et.al. JPET 320:552-558, 2007)
 - Knowledge of the target drove drug discovery programs to find these molecules that would be substrate selective i.e. inhibitors affecting APP but not Notch.
- /// Clinical data will drive our understanding. Preclinical data can only guide us
 - Eli Lilly – LY450139
 - > 6 week study in AD patients – treatment well tolerated (Siemers, 2006)
 - > Phase II Safety and Tolerability Trial, 29 weeks, in progress
 - Merck – MK0752
 - > Phase Ib data reported on at ICAD Madrid (Rosen et al, Alzheimer’s & Dementia 2:S79, 2006)
 - > Decline in CSF $A\beta_{40}$ and generally well tolerated to 1000mg
- /// The answers await....



Would an MMP-9 be a good target for AD?

- /// MMP-9 expression is increased in AD and in APP transgenic mice
 - Plasma and CSF (activity assays) (Lorenzl, 2003; Adair, 2004)
 - Brain (immunohistochemistry and *in situ* hybridization) (Asahina, 2001; Backstrom, 1996)
 - > Particularly in hippocampal neurons
 - > In astrocytes in close proximity to amyloid plaques
 - > Thioflavin-S⁺ plaques
- /// Increased expression in plasma and brain is specific to AD
 - Not seen in vascular dementia (Adair, 2004; Asahina, 2001) (immunohistochemistry)
- /// MMP-9 degrades A β (Backstrom, 1996; Qiu, 1997; White, 2006; Yan, 2006; Yin, 2006)
 - Suggests that MMP-9 contributes to clearance of plaques.
 - Degrades both soluble A β and fibrillar A β *in vitro* and in transgenic mice (*in vitro* reconstitution, MALDI-TOF)
- /// β -amyloid induces secretion of several MMPs including MMP-9 *in vitro*.
 - Is this a response to increased local concentrations of A β ?
 - Stimulates matrix degradation in rat astrocytes (activity assays and western analysis). (Deb, 2005)
 - Human microglial cultures from post-mortem brain tissue (microarray studies followed by RT-PCR) (Walker, 2006)



Not if you are proposing to stimulate its activity!

- /// It has been observed that...
 - MMPs are elevated in AD and in APP transgenic mice
 - > Elevated expression is in areas consistent with a link to disease
 - > Elevated expression is specific to AD
 - MMPs may contribute to the catabolism of A β
 - > Demonstrated *in vitro*
- /// However...
 - elevated MMPs could negatively influence the stability of the extracellular matrix
- /// Elevation may be partly responsible for the degenerative process?
 - The levels of MMPs and their naturally occurring inhibitors (TIMPs) are very carefully balanced.

“Don’t mess with the matrix”

T.H. Vu, Nature Genetics 28;202-203, 2001



**Even with our best preclinical
validation packages only clinical data
will be able to truly validate the target!**



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