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Binding profiles of BAN2401 and aducanumab to different A β species

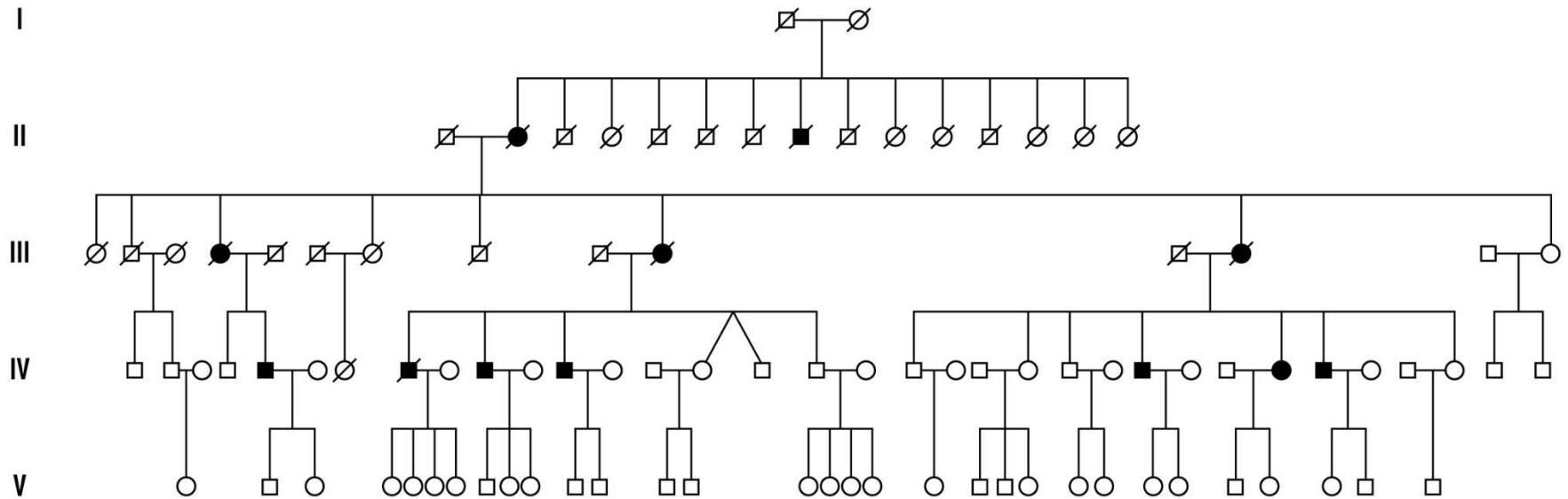
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Disclaimer: Co-founder of BioArctic AB

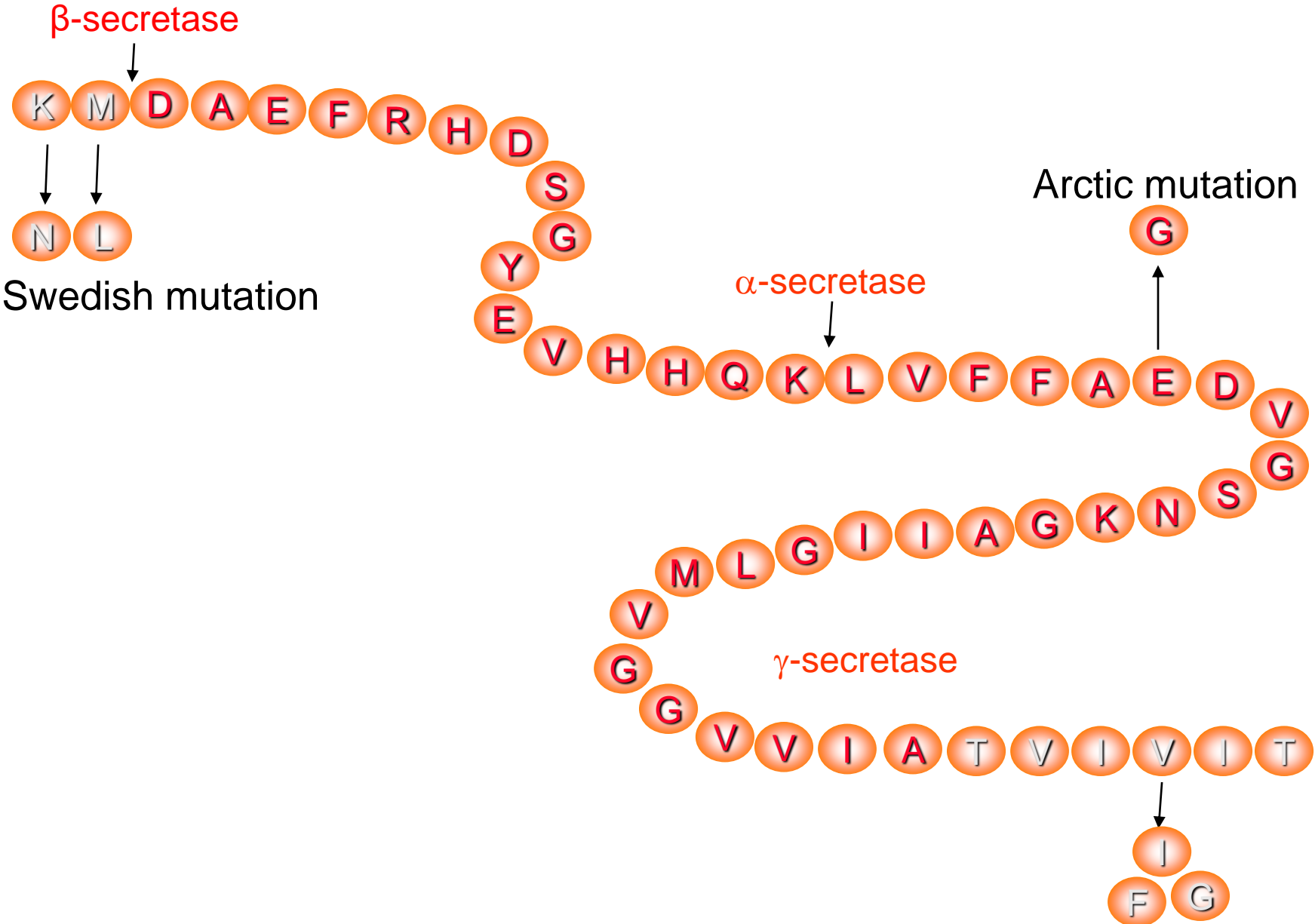
The Arctic mutation family



Originates from northern Sweden
Autosomal dominant Alzheimer's disease
No signs of cerebral haemorrhage
Age of onset: 57 ± 3 years
A lod score of 3.66

(Nilsberth et al. Nature Neurosci 2001)

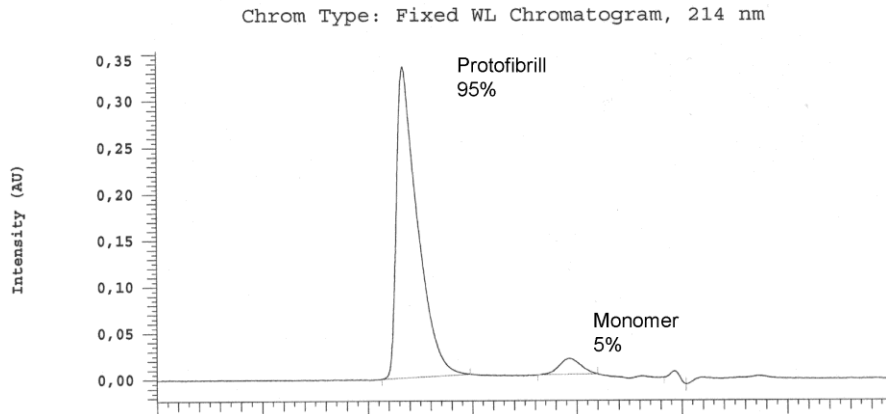
The Arctic mutation is inside Aβ (E22G)



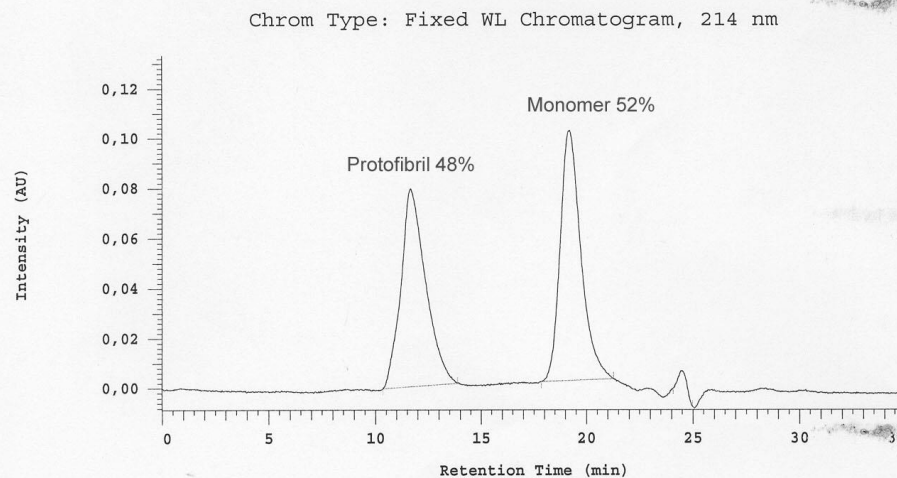
Accelerated protofibril formation of Arctic A β (A β 1-42E22G)

Size Exclusion Chromatography on a Superdex 75 column

Arc



Wt



A β 1-42Arc

Our definition of protofibrils:
soluble aggregated A β eluting
in the void volume of a Superdex
75 column, estimated size
> 75 kDa

Structure by AFM

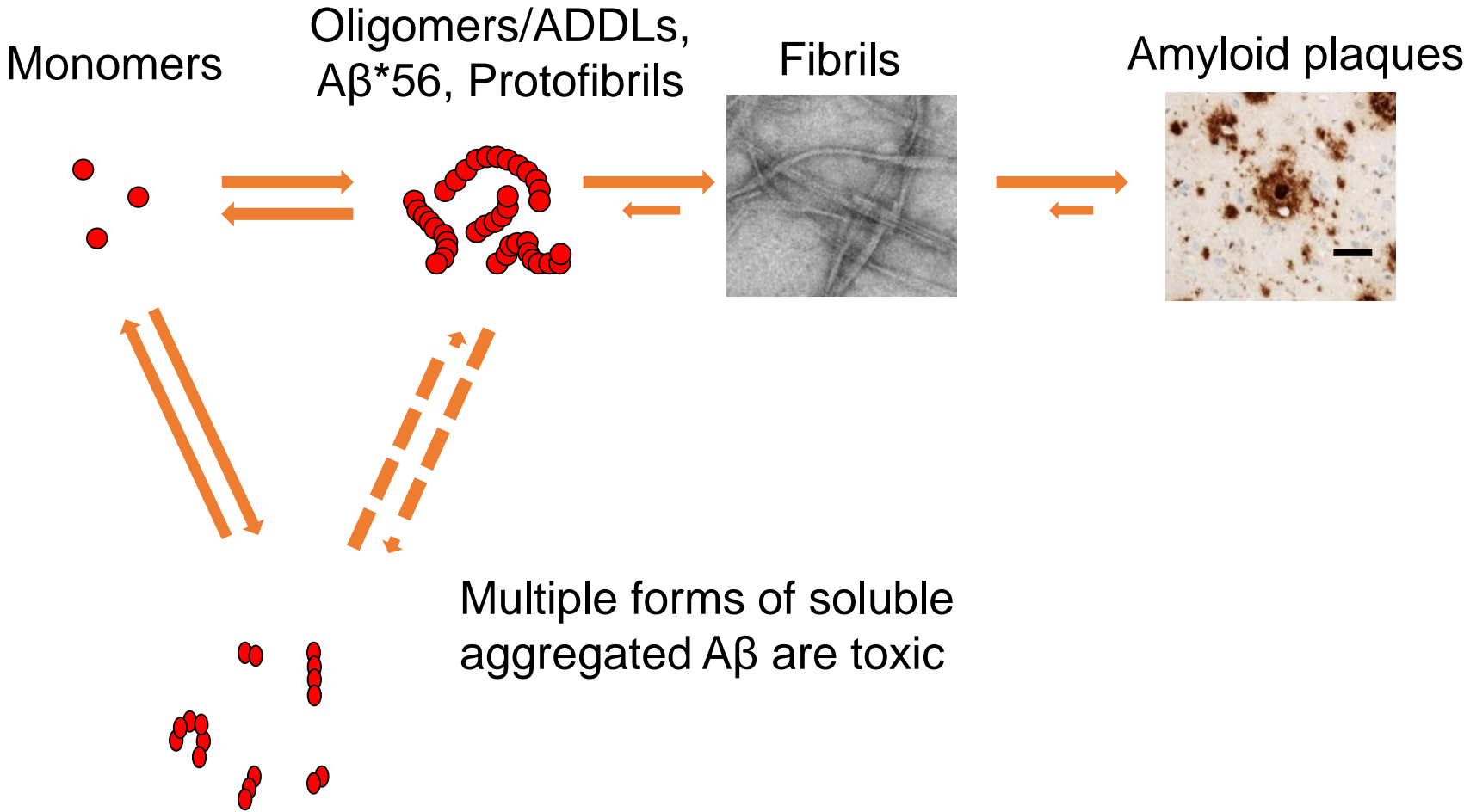
A β 1-42wt

Protofibrils are found in all AD cases but
are more prominent with the Arctic mutation

Nilsberth et al. 2001 Nat Neurosci
Johansson et al. 2006 FEBS J

Alzheimer brain contains a distribution of A β assemblies, - a fraction of these are toxic

"Protofibrils": most forms of A β that are not monomers or end-stage fibrils

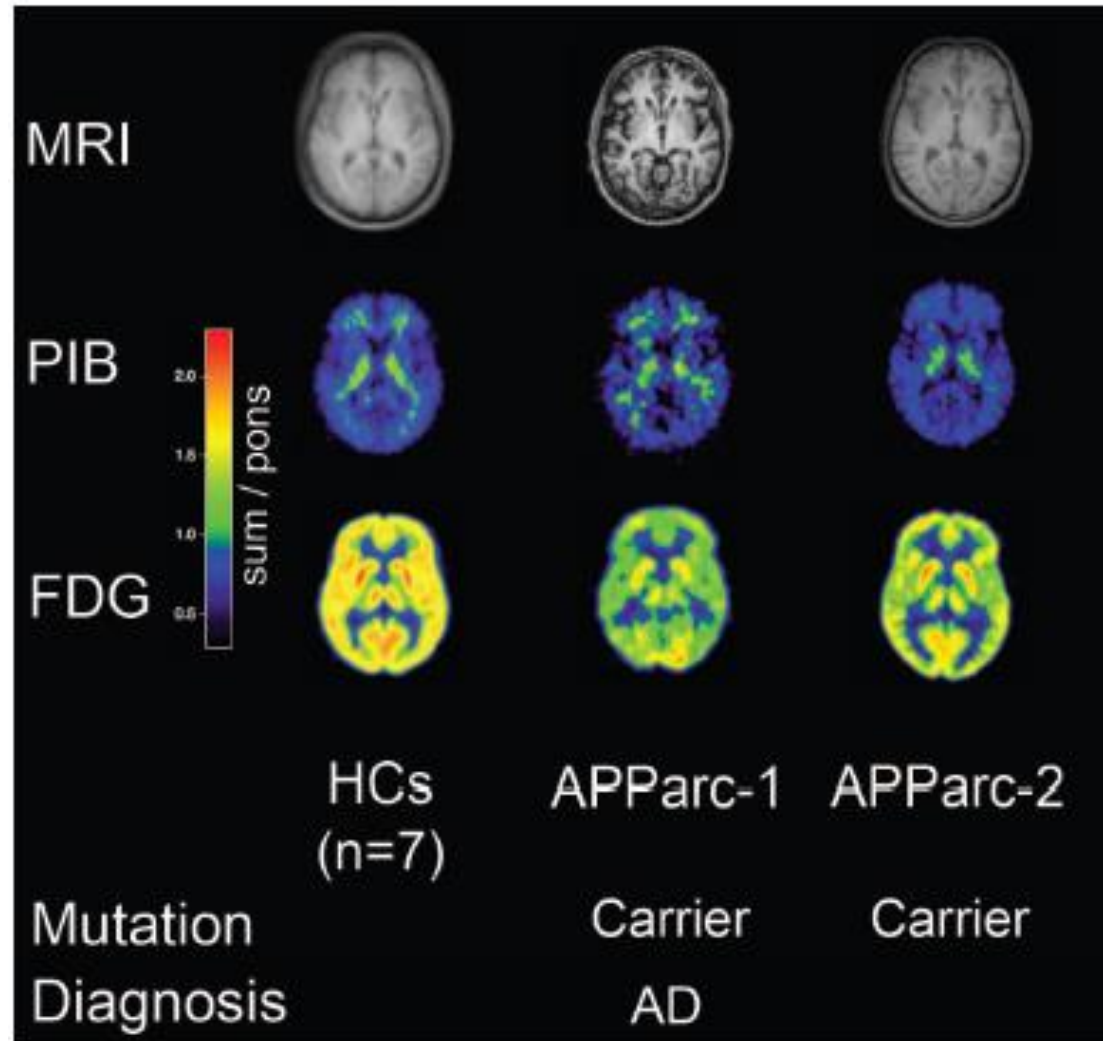


Courtesy, Dominic Walsh

No A β positive plaques in the Arctic mutation family with PET PIB

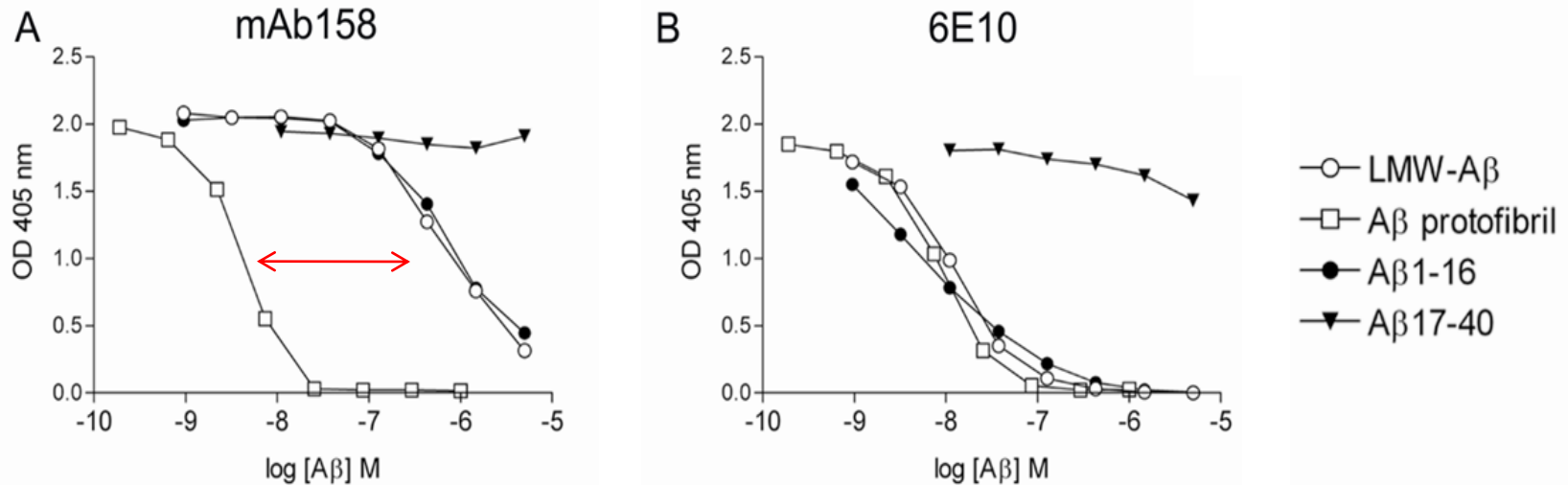
APParc-1 and 2: very low cortical PIB retention, APParc-1 had decreased glucose metabolism and atrophy, and APParc-2 regionally decreased glucose metabolism

Conclusion: toxicity in the AD brain can also be mediated by A β species not detected by amyloid PET



mAb158, precursor to BAN2401, binds A β protofibrils selectively

Inhibition ELISA



The antibody interacted in solution with A β monomers or A β protofibrils in increasing concentrations. The mix was added to a microtiter plate precoated with A β protofibrils. If the antibody binds to A β in the pre-incubation step, fewer antibodies will bind to the immobilized A β on the microtiter plate.

mAb158: Strong binding to A β protofibrils, weak binding to A β monomers

Rationale to target A β protofibrils

- First observed in 1997, defined by size and structure
- A β protofibrils are toxic
- Observed in the Arctic mutation family
- Relevant for all forms of AD
- Minimal interference with normally occurring monomeric A β and no sequestering of antibody in the periphery
- Low binding to A β fibrils – risk for side-effects, ARIA-E?

Walsh et al. 1997 J Biol Chem

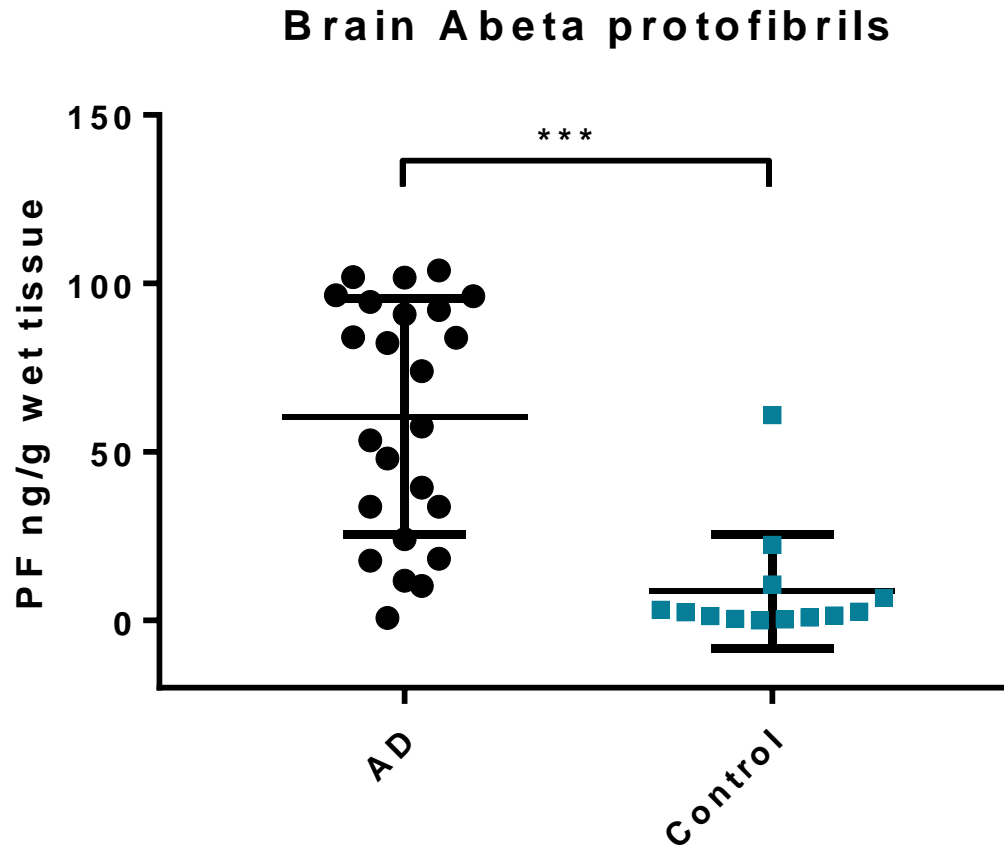
Harper et al. 1997 Chem Biol

Nilsberth et al. 2001 Nat Genet

Lannfelt et al. 2013 J Intern Med

Lannfelt et al. 2014 Alz Res Ther

Significantly higher A β protofibril levels in AD brain compared to control brain



Soluble aggregated A β , protofibrils/oligomers, measured by mAb158 (precursor to BAN2401), immunoprecipitation and ELISA.

Collaboration with Eisai on antibody for Alzheimer's disease



2005-2007	Research collaboration
2007	License agreement: Bring BAN2401 to the world-market for AD
2010	Clinical development, phase 1 started
2013	Phase 2b started
2018	Positive 18 months results, 856 early AD patients included
2019	Phase 3 started

BAN2401 – clinical development

- Phase 2b study in 856 patients: dose-dependent slower decline on cognition and function at 18 months on clinical scales, from 26-47% less decline on top dose, effect already at 6 months and increasing with time
- Effects on down-stream CSF biomarkers: p-tau, NfL and neurogranin
- Dose-dependent, statistically significant reduction in brain amyloid, 93% converted to amyloid negative on top dose
- No titration required, ARIA-E < 10% at all doses
- A confirmatory phase 3 study was initiated Q1 2019, results expected 2022, 1566 patients, highest dose from 2b/placebo 50/50
- Secondary prevention studies (“A3 and A45”) by Alzheimer’s Clinical Trial Consortium (ACTC) and Eisai, to start 2020

Similarities between BAN2401 and aducanumab

Both antibodies:

- N-terminal epitope
- Avidity is important for binding strength
- Very weak binding to A β monomers
- IgG1 subclass
- Clears amyloid-beta from brain with high efficiency

Comparing BAN2401 with aducanumab

- We examined the binding characteristics of BAN2401 and aducanumab to better understand the differences in clinical response
- Inhibition ELISA and Surface Plasmon Resonance (SPR, Biacore) were used to investigate binding of BAN2401 and aducanumab to monomers, small and large A β protofibrils and to A β fibrils
- Immunoprecipitation was used to deplete A β from TBS extracts from AD brain tissue
- Aducanumab was generated through screening healthy old people's blood for naturally occurring A β antibodies, and selecting with TAPIR assay

Like aducanumab, BAN2401 is also highly selective for A β aggregates

Monovalent binding to soluble monomeric A β (Biacore)

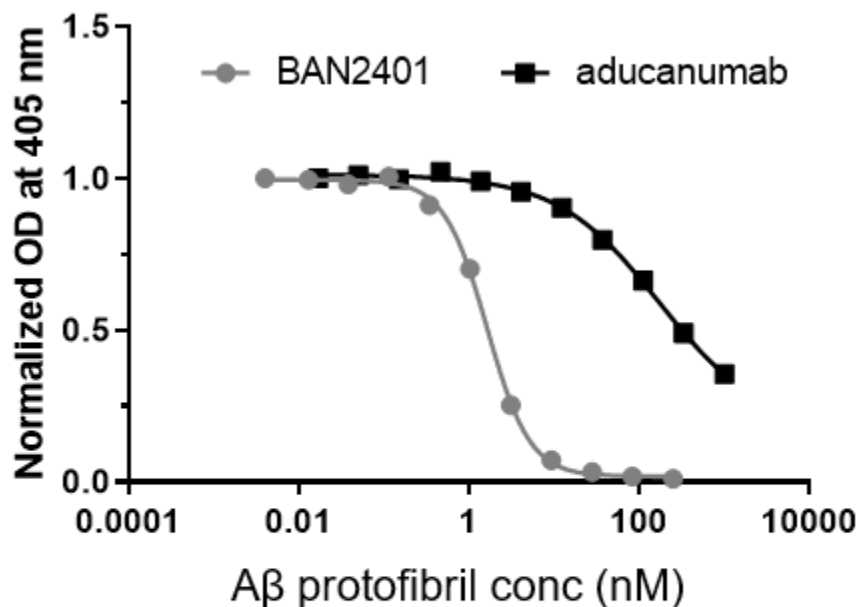
Fab fragment	Association rate, k_a (M ⁻¹ s ⁻¹)	Dissociation rate, k_d (s ⁻¹)	Equilibrium Affinity, K_D (nM)
aducanumab (mIgG chimer)	--	>1	~9,000
BAN2401 (hIgG1)	~10 ⁵	0.16	~2,000
gantenerumab (mIgG chimer)	6.6 x10 ⁵	1.5 x10 ⁻²	23
crenezumab (human IgG1 analog)	3.3 x 10 ⁵	0.0026	8
bapineuzumab (mouse 3D6)	7.2 x10 ⁵	7.9 x10 ⁻⁴	1.1
solanezumab (mouse m266)	2.1 x10 ⁴	5.8 x10 ⁻⁵	2.7

- BAN2401 binds soluble A β monomers with a slightly higher affinity than aducanumab
- Reflected in the observed plasma A β “spike” upon treatment with BAN2401 but not with aducanumab

Courtesy to Al Sandrock and the Biogen team

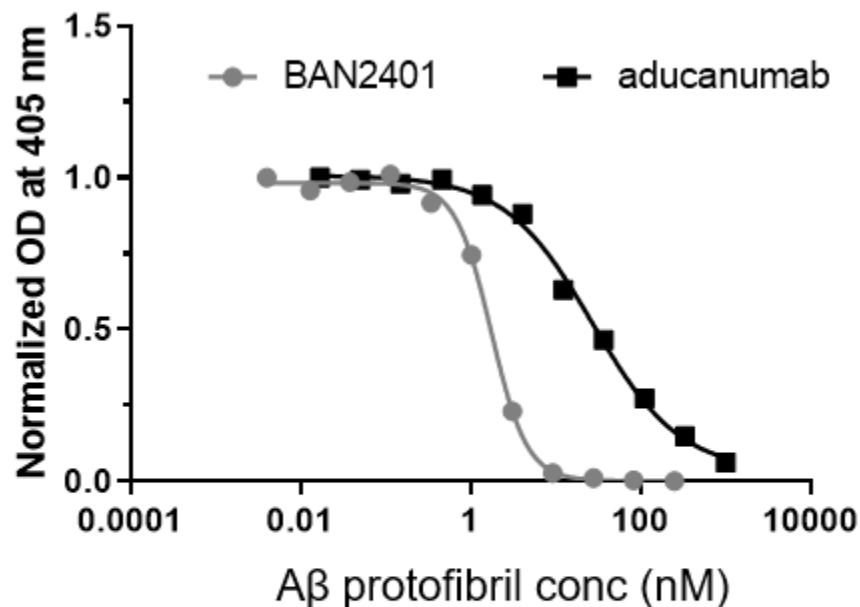
BAN2401 showed stronger binding than aducanumab to both small and large protofibrils by inhibition ELISA

Binding to small protofibrils



Antibody	IC50 (nM)
BAN2401	1.6
aducanumab	171.1
Fold difference	≈100

Binding to large protofibrils

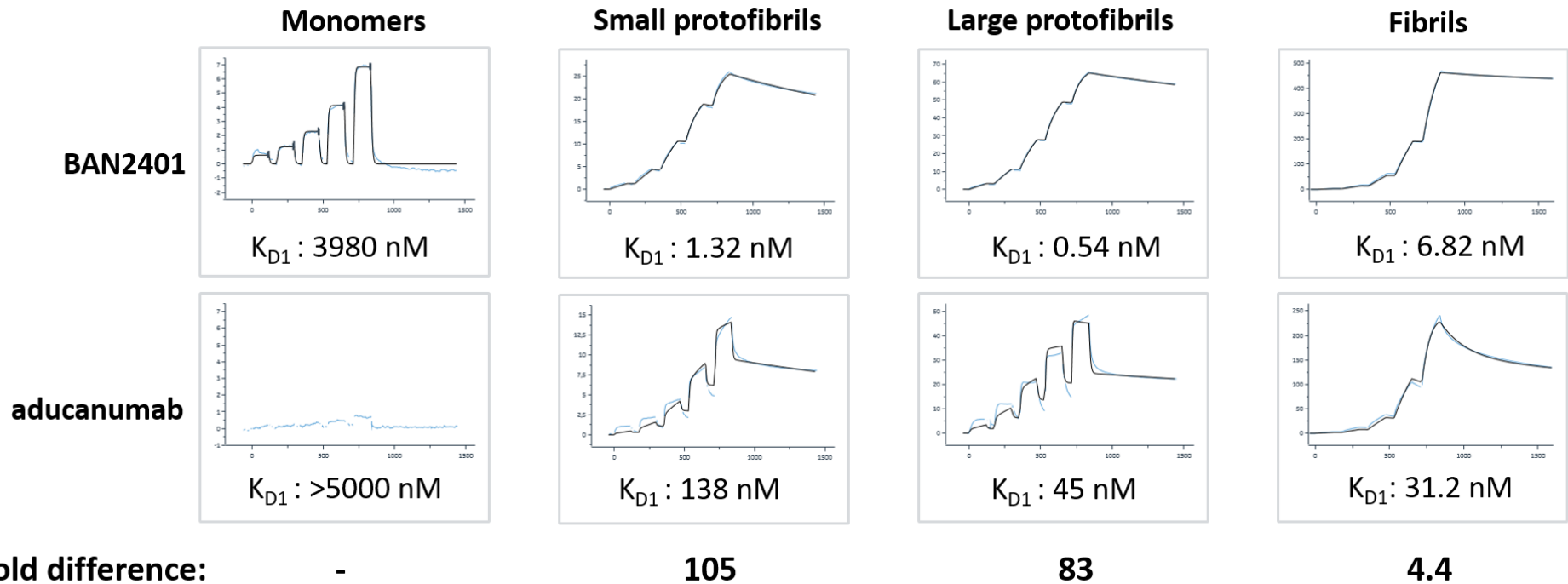


Antibody	IC50 (nM)
BAN2401	1.7
aducanumab	27.2
Fold difference	16

BAN2401 had similar binding strength to both small (IC50=1.6 nM) and large (IC50=1.7 nM) protofibrils. Aducanumab's binding was significantly weaker (IC50=171 nM and 27 nM, resp.)

BAN2401 showed stronger binding than aducanumab to all A β species, especially to protofibrils, by SPR

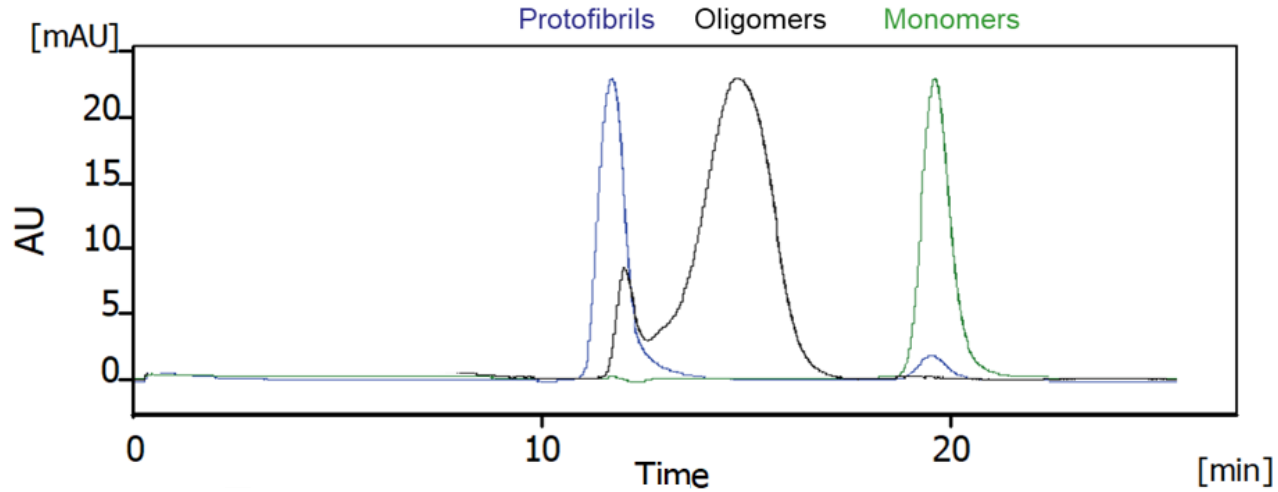
Surface Plasmon Resonance confirmed data from inhibition ELISA



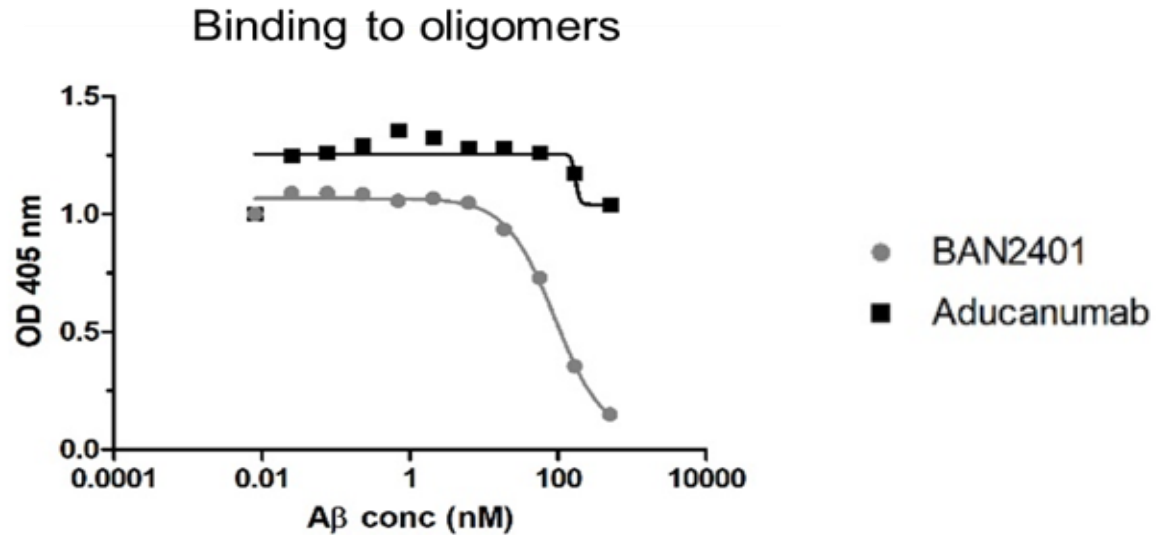
In SPR, the monomer affinities were determined by injecting monomers over captured antibodies. For protofibrils and fibrils, the antibodies were injected over immobilized A β species.

BAN2401 demonstrated stronger binding than aducanumab to cross-linked A β oligomers by inhibition ELISA

SEC

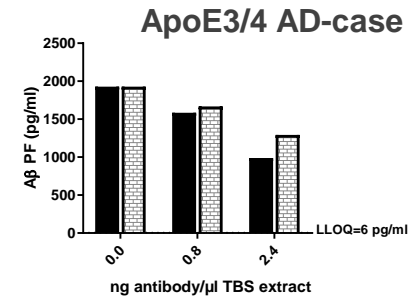
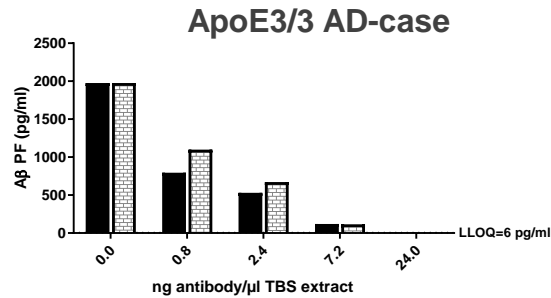
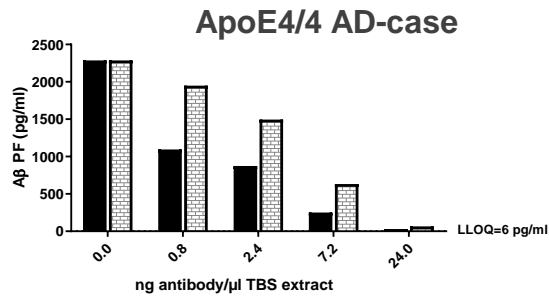


Inhibition ELISA



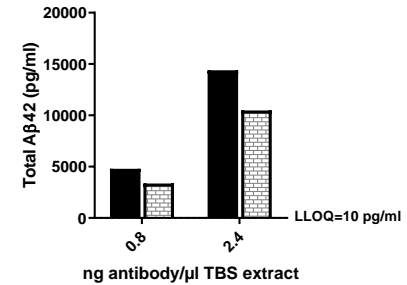
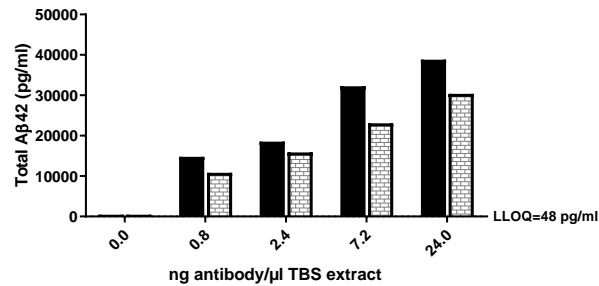
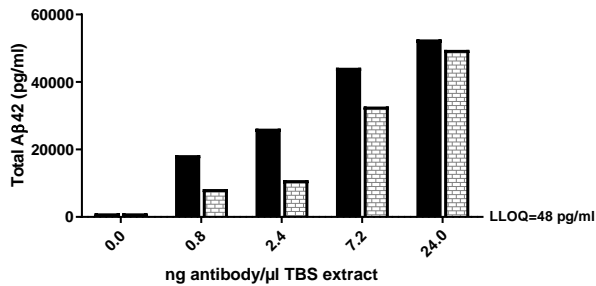
BAN2401 showed slightly more efficient immunodepletion of brain A β protofibrils

A β protofibril levels in TBS-extract after immunodepletion with BAN2401 or aducanumab



Total A β 42 levels in BAN2401 or aducanumab immunoprecipitate

■ BAN2401
▨ aducanumab



A dose-dependent decrease of soluble aggregated A β species, including protofibrils, in supernatant and increase of total A β 42 in immunoprecipitated was seen. Representative of four experiments.

Conclusion, BAN2401 vs. aducanumab

- BAN2401 showed 16 - 100 times stronger *in vitro* binding to A β protofibrils than aducanumab
- BAN2401 was more selective for oligomers and protofibrils as compared to fibrils
- BAN2401 showed stronger binding to A β oligomers
- BAN2401 showed slightly more efficient immunodepletion of brain A β protofibrils
- The stronger binding of BAN2401 to soluble aggregated A β species may mediate the differences in clinical profile observed between the two antibodies

Apparent distinguishing features in the clinic for BAN2401:

- ARIA-E in 1 of 10 patients, of those only 10% symptomatic
- No titration needed
- Clinical endpoints show benefit already after 6 months



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