Binding profiles of BAN2401 and aducanumab to different Aβ species

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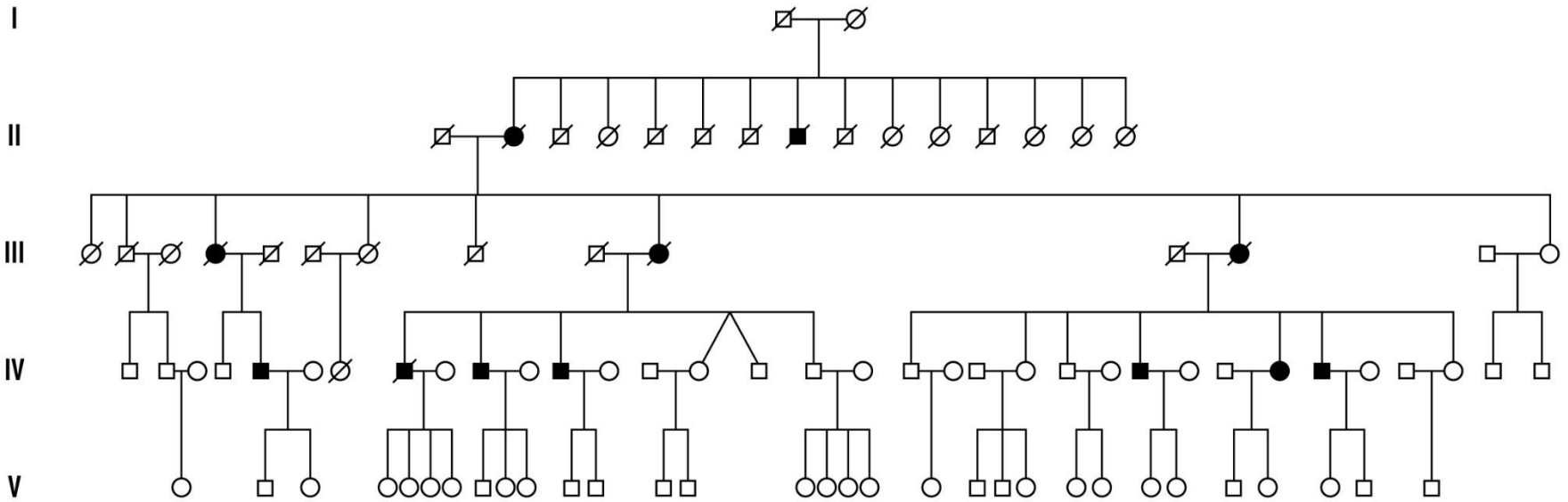
Dec 7, 2019 CTAD, San Diego

Disclaimer: Co-founder of BioArctic AB

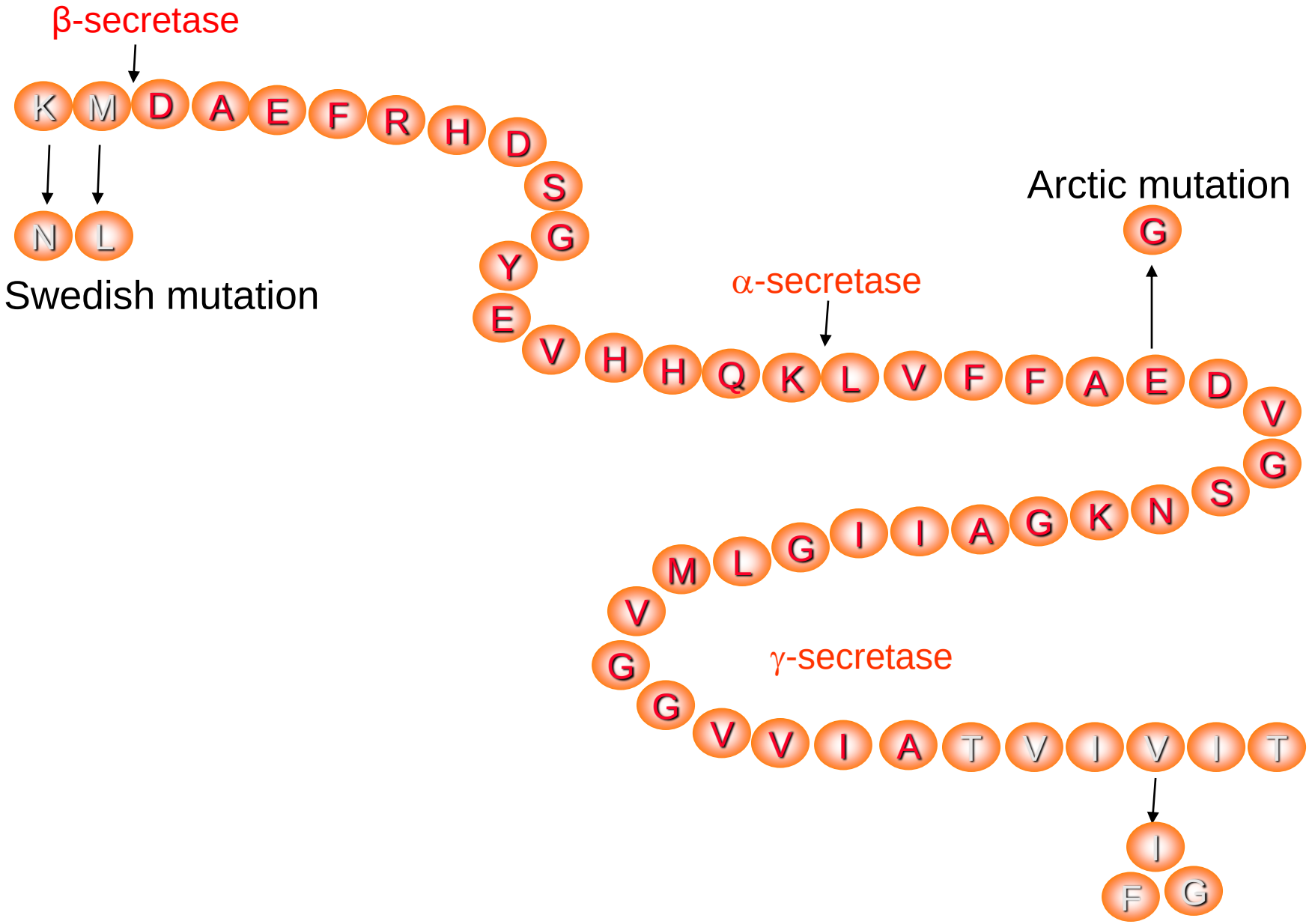
The Arctic mutation family



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)

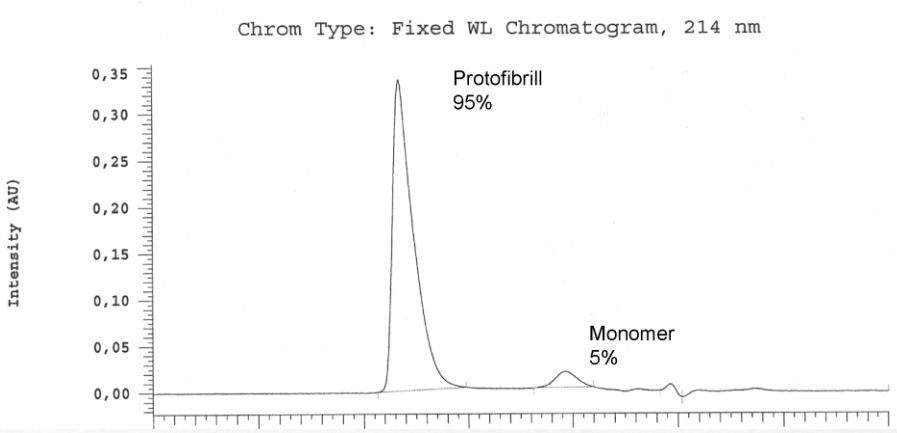
The Arctic mutation is inside Aβ (E22G)



Accelerated protofibril formation of Arctic Aβ (Aβ1-42E22G)

Size Exclusion Chromatography on a Superdex 75 column

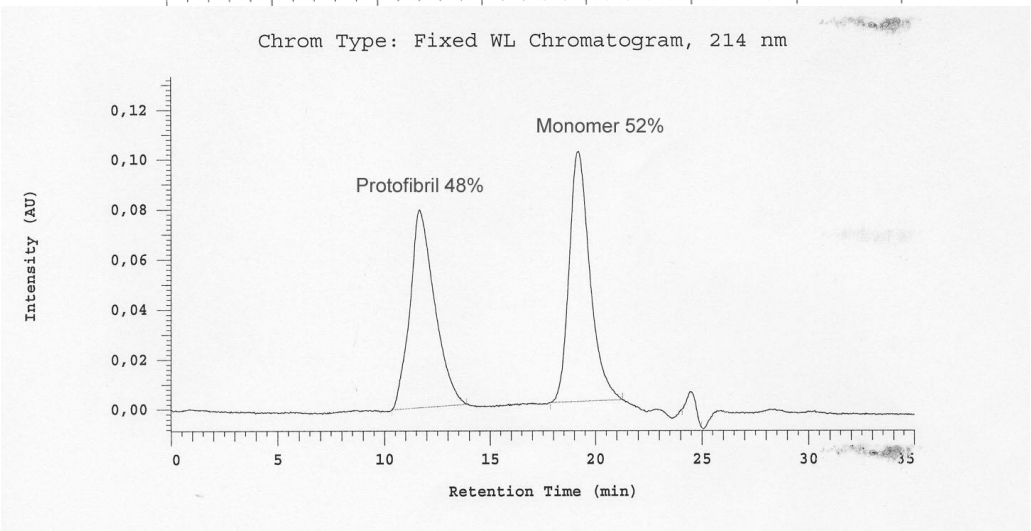
Arc



Aβ1-42Arc

Our definition of protofibrils:
soluble aggregated Aβ eluting in the void volume of a Superdex 75 column, estimated size
> 75 kDa
Structure by AFM

Wt



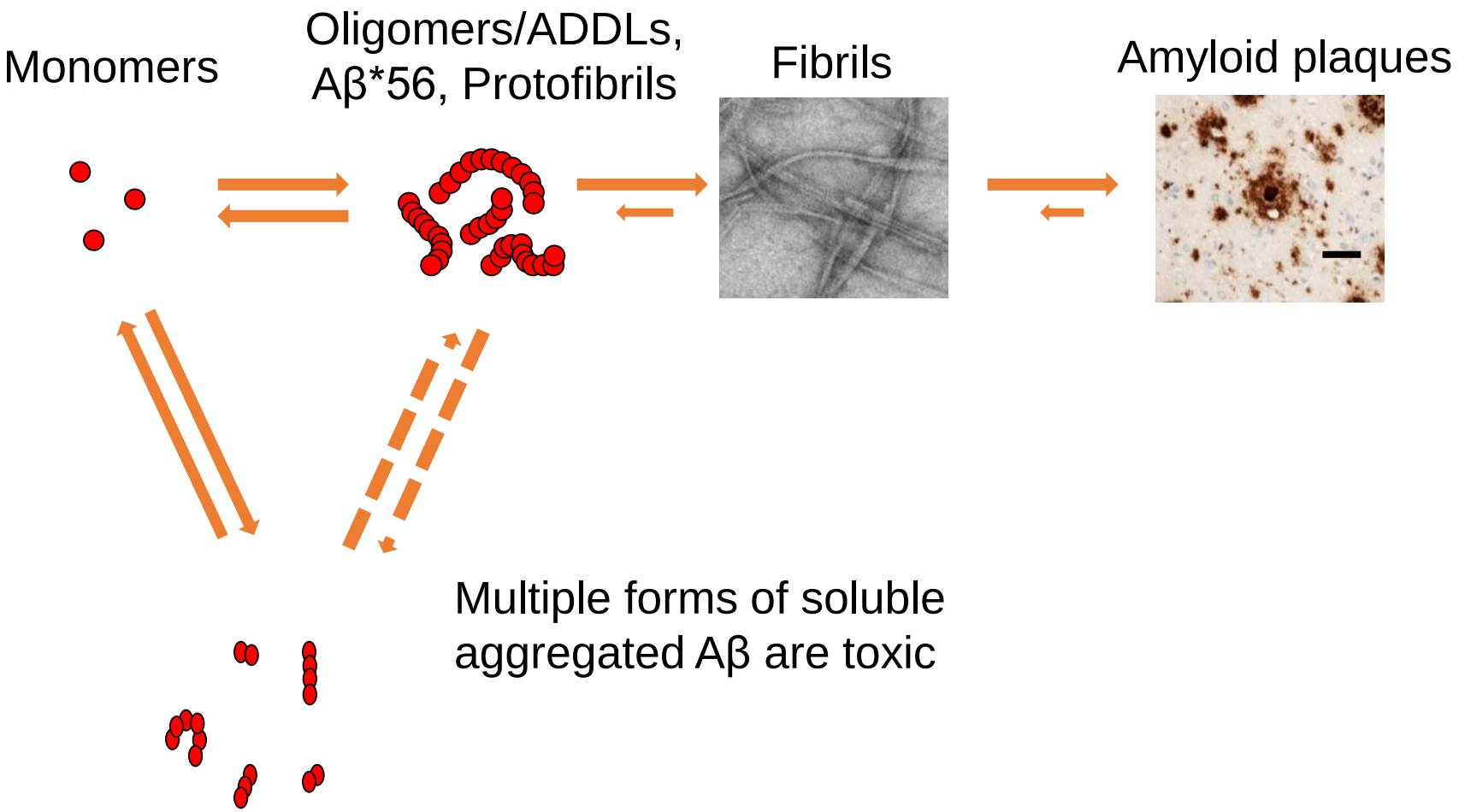
Aβ1-42wt

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 Aβ assemblies, - a fraction of these are toxic

”Protofibrils”: most forms of Aβ that are not monomers or end-stage fibrils

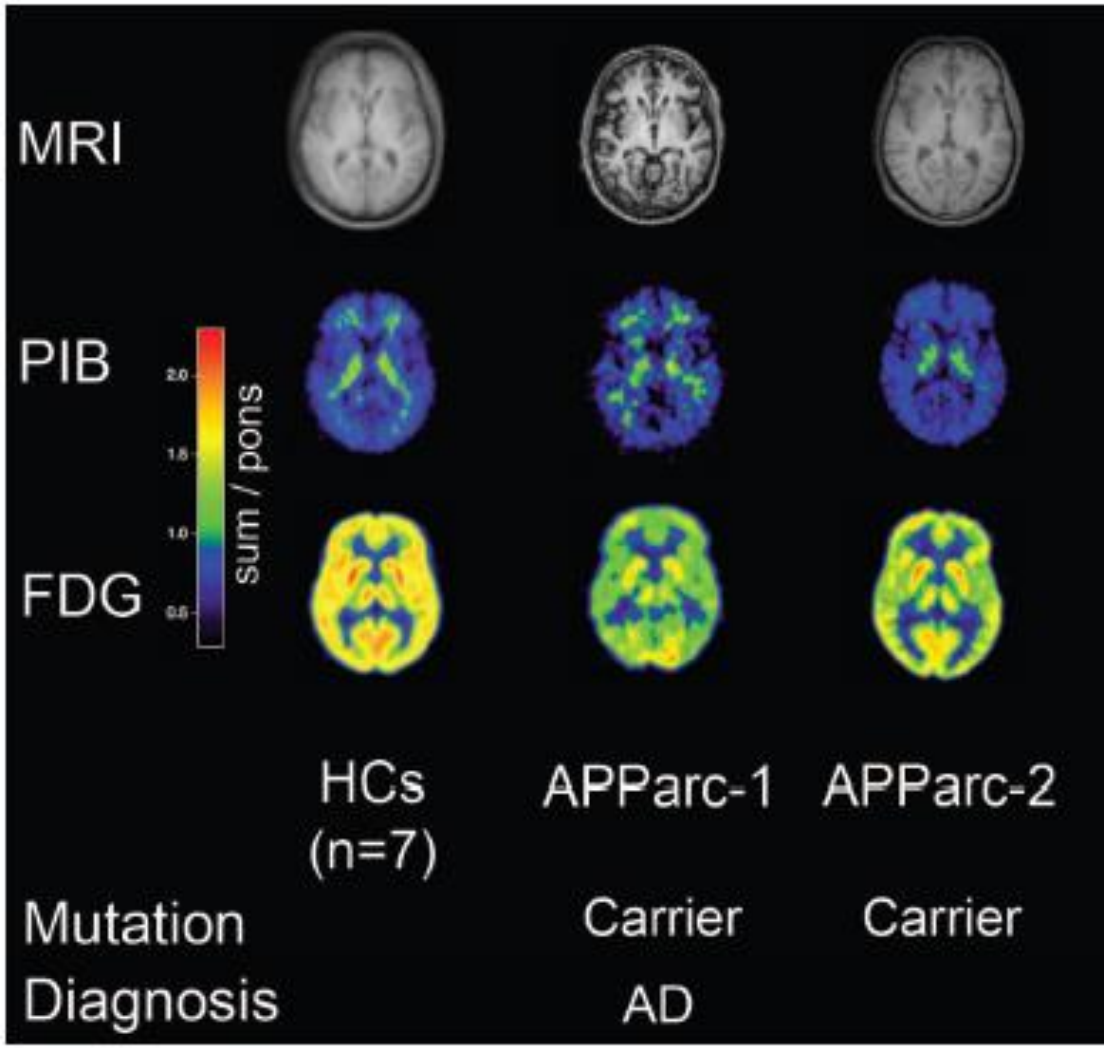


Courtesy, Dominic Walsh

No Aβ 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

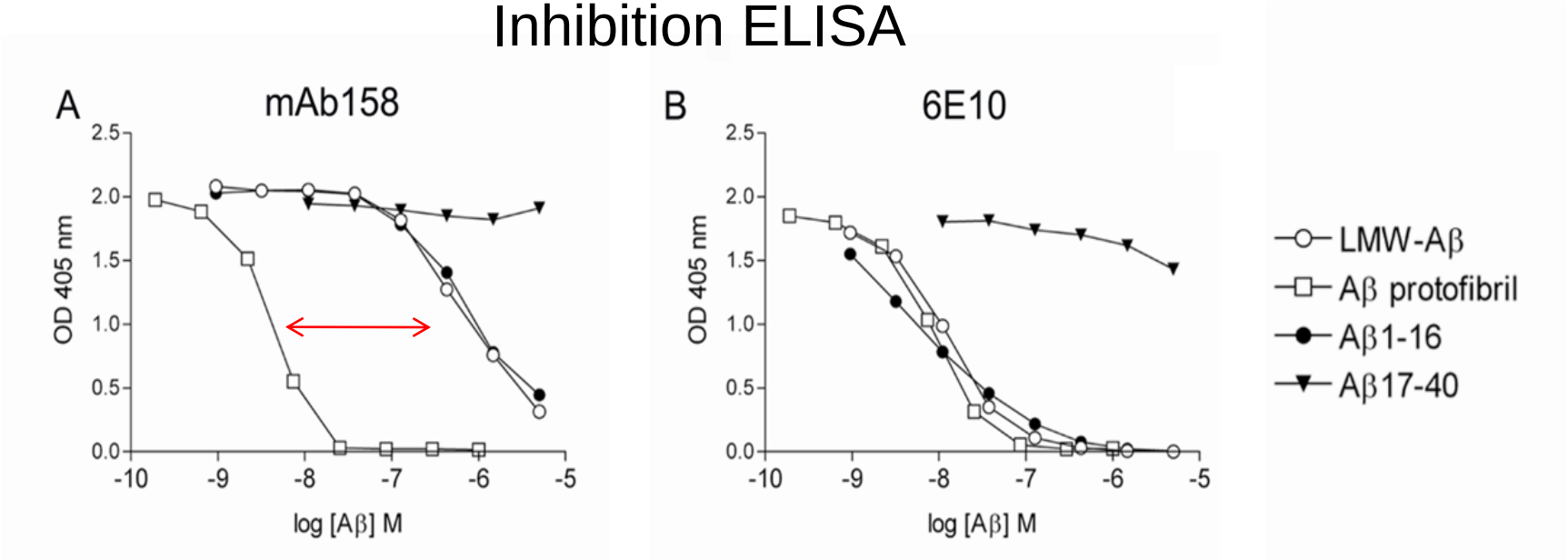
Conclusion: toxicity in the AD brain can also be mediated by Aβ species not detected by amyloid PET



Schöll et al. 2012 Neurology

mAb158, precursor to BAN2401, binds Aβ protofibrils selectively

Inhibition ELISA



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

mAb158: Strong binding to Aβ protofibrils, weak binding to Aβ monomers

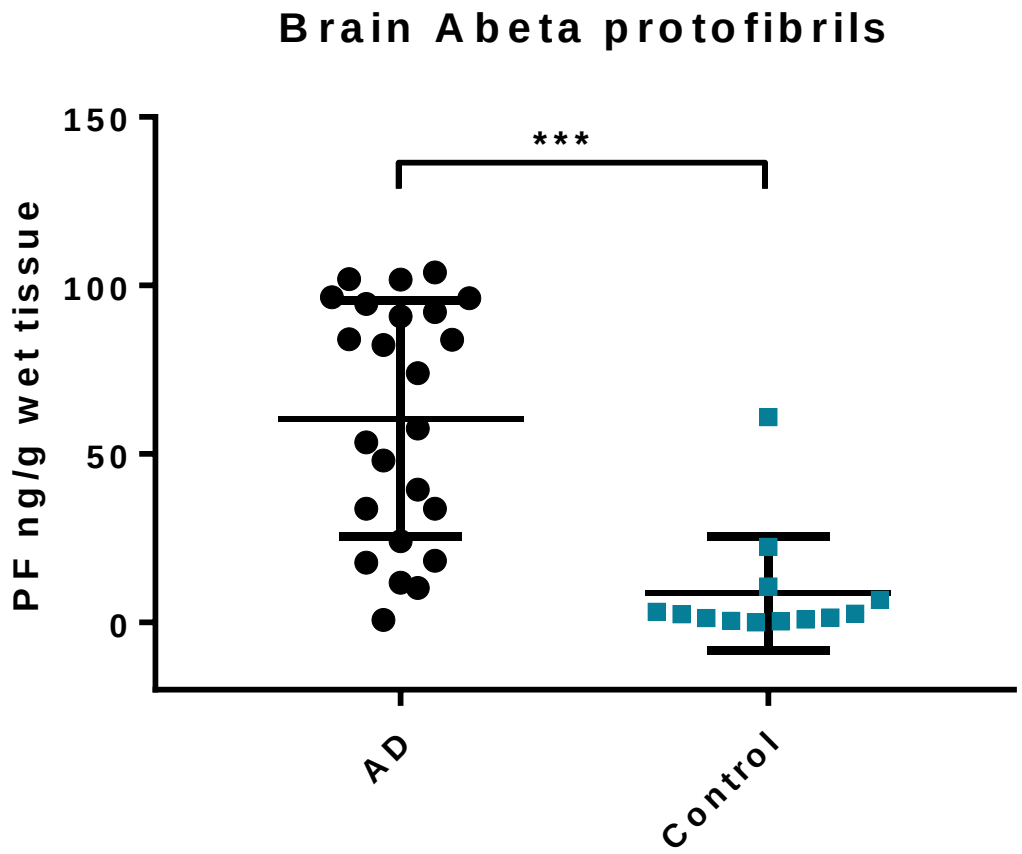
Englund et al. 2007 J Neurochem

Rationale to target Aβ protofibrils

- First observed in 1997, defined by size and structure
- Aβ protofibrils are toxic
- Observed in the Arctic mutation family
- Relevant for all forms of AD
- Minimal interference with normally occurring monomeric Aβ and no sequestering of antibody in the periphery
- Low binding to Aβ 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 Aβ protofibril levels in AD brain compared to control brain



Soluble aggregated Aβ, 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 Aβ 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 Aβ protofibrils and to Aβ fibrils
- Immunoprecipitation was used to deplete Aβ from TBS extracts from AD brain tissue
- Aducanumab was generated through screening healthy old people's blood for naturally occurring Aβ antibodies, and selecting with TAPIR assay

Like aducanumab, BAN2401 is also highly selective for Aβ aggregates

Monovalent binding to soluble monomeric Aβ (Biacore)

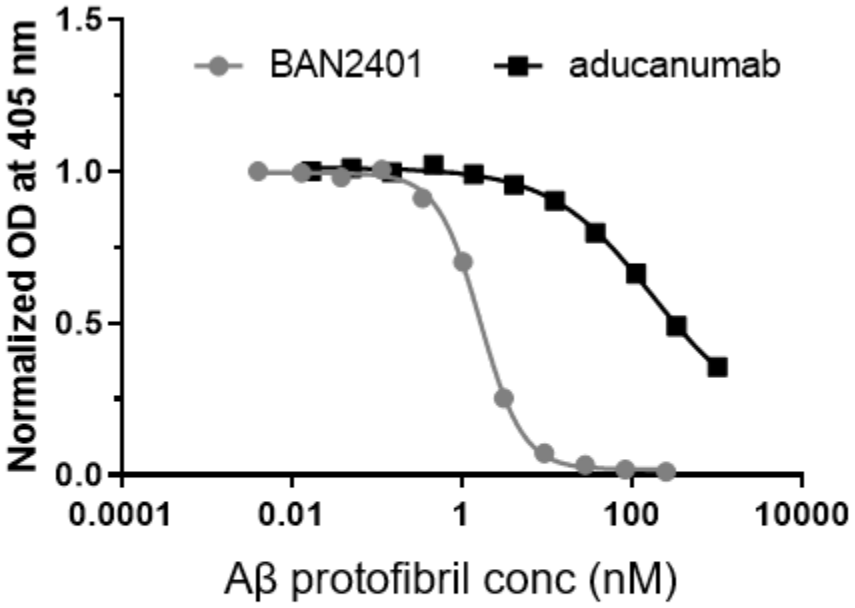
Fab fragment	Association rate, k_a (M^{-1} s^{-1})	Dissociation rate, k_d (s^{-1})	Equilibrium Affinity, K_D, (nM)
aducanumab (mIgG chimer)	--	>1	**~9,000**
BAN2401 (hIgG1)	~10^5	0.16	**~2,000**
gantenerumab (mIgG chimer)	6.6 x10^5	1.5 x10^{-2}	23
crenezumab (human IgG1 analog)	3.3 x 10^5	0.0026	8
bapineuzumab (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 Aβ monomers with a slightly higher affinity than aducanumab
- Reflected in the observed plasma Aβ "spike" upon treatment with BAN2401 but not with aducanumab

Courtesy to Al Sandrock and the Biogen team

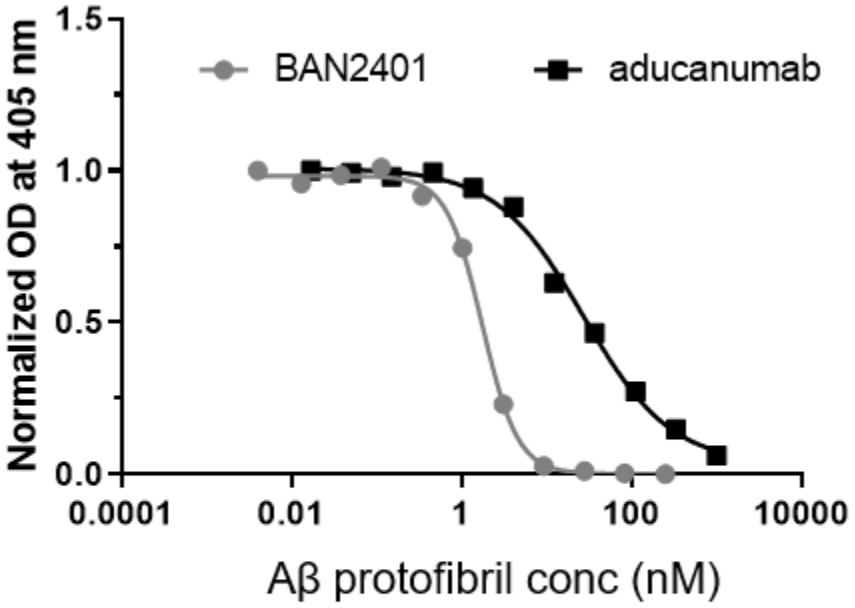
BAN2401 showed stronger binding than aducanumab to both small and large protofibrils by inhibition ELISA

Binding to small protofibrils



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

Binding to large protofibrils

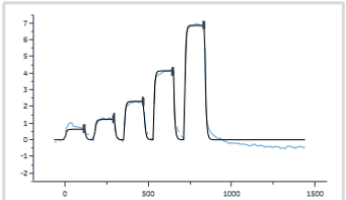
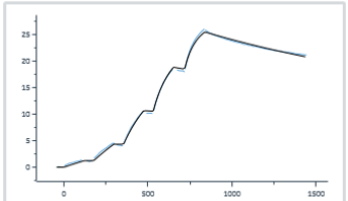
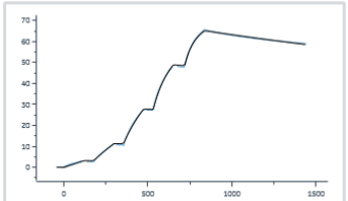
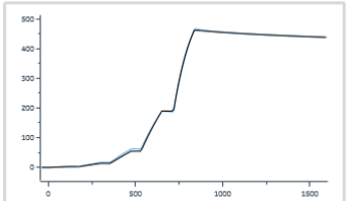
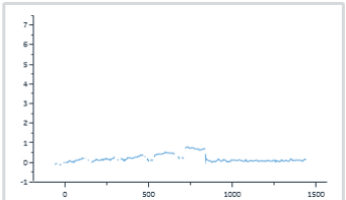
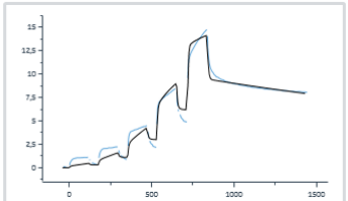
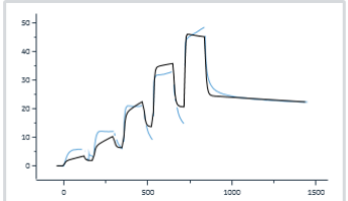
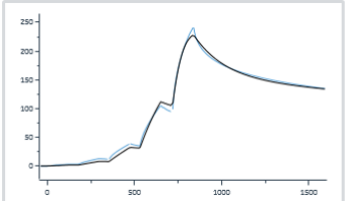


Antibody	IC50 (nM)
BAN2401	1.7
aducanumab	27.2
Fold difference	**16**

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 Aβ species, especially to protofibrils, by SPR

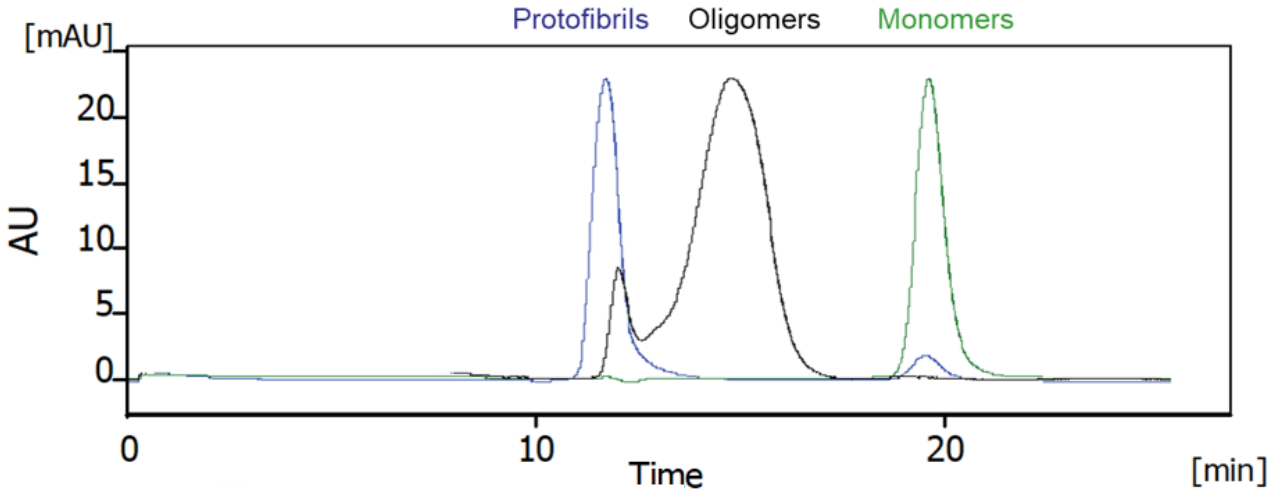
Surface Plasmon Resonance confirmed data from inhibition ELISA

	Monomers	Small protofibrils	Large protofibrils	Fibrils
BAN2401	 K_{D1} : 3980 nM	 K_{D1} : 1.32 nM	 K_{D1} : 0.54 nM	 K_{D1} : 6.82 nM
aducanumab	 K_{D1} : >5000 nM	 K_{D1} : 138 nM	 K_{D1} : 45 nM	 K_{D1}: 31.2 nM
Fold difference:	-	**105**	**83**	**4.4**

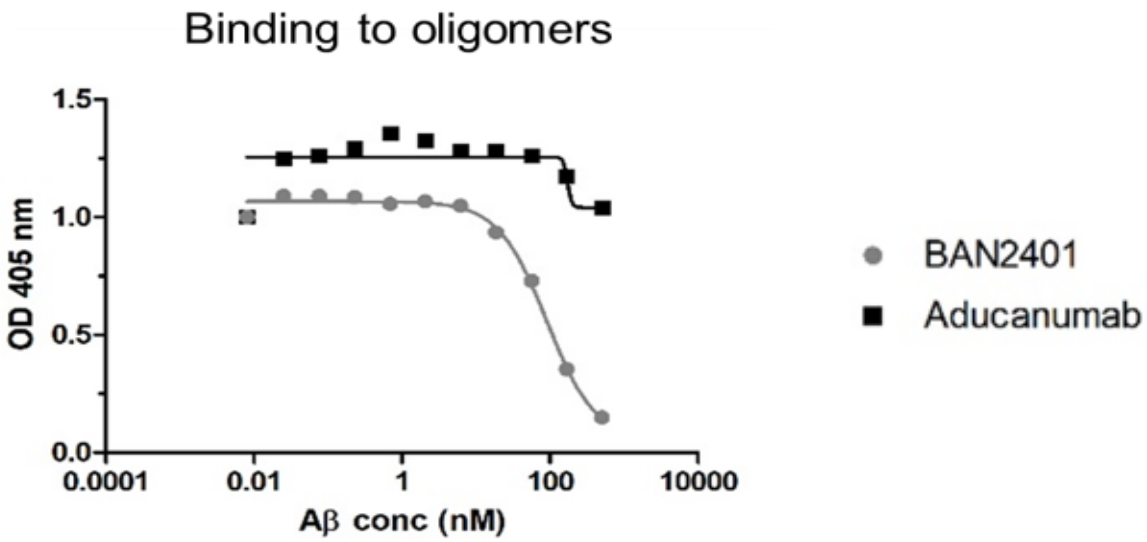
In SPR, the monomer affinities were determined by injecting monomers over captured antibodies. For protofibrils and fibrils, the antibodies were injected over immobilized Aβ species.

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

SEC

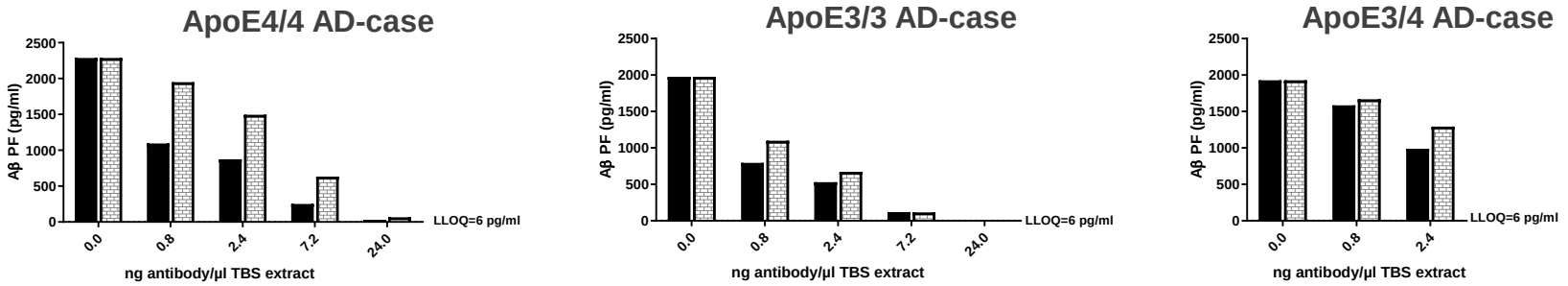


Inhibition ELISA



BAN2401 showed slightly more efficient immunodepletion of brain Aβ protofibrils

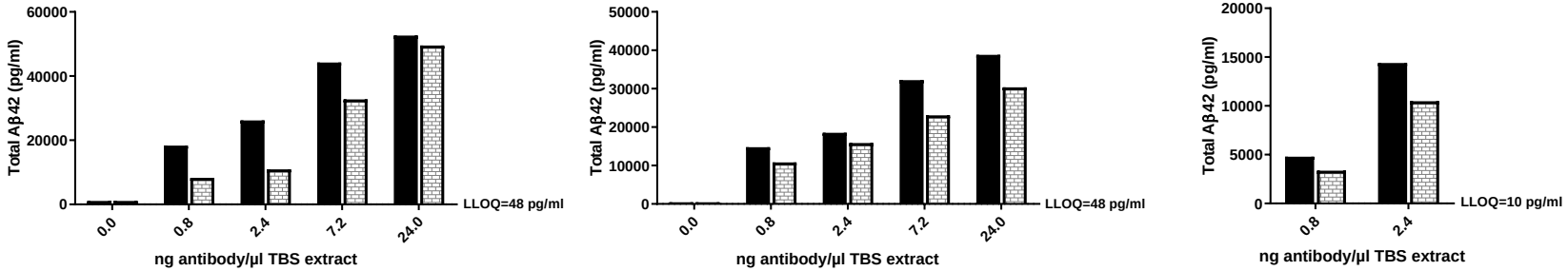
Aβ protofibril levels in TBS-extract after immunodepletion with BAN2401 or aducanumab



BAN2401

aducanumab

Total Aβ42 levels in BAN2401 or aducanumab immunoprecipitate



A dose-dependent decrease of soluble aggregated Aβ species, including protofibrils, in supernatant and increase of total Aβ42 in immunoprecipitated was seen. Representative of four experiments.

Conclusion, BAN2401 vs. aducanumab

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

<u>Apparent distinguishing features in the clinic for BAN2401</u>:

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

Thanks to:

BioArctic
Linda Söderberg
Malin Johannesson
Patrik Nygren
Charlotte Sahlin
Hanna Laudon
Johanna Fälting
Fredrik Eriksson
Christer Möller
Tomas Odergren
Gunilla Osswald
Hans Basun
Pär Gellerfors
Tomas Odergren
and many others

Eisai
Chad Swanson
Akihiko Koyama
Robert Lai
June Kaplow
Robert Gordon
Lynn Kramer
Teiji Kimura
Michael Irizarry
Harald Hampel

Molecular Geriatrics Uppsala University
Martin Ingelsson
Dag Sehlin
Joakim Bergström
Anna Erlandsson
Linn Gallasch
Tobias Gustavsson

Memory Disorder Unit
Lena Kilander
RoseMarie Brundin
Eva-Lis Lundberg
Ylva Cedervall
Malin Degerman
Gunnarsson
Lisa Henley
Lena Propst

Forskarpatent i Uppsala
Pär Svanström

Mabtech AB
Staffan Paulie

Former lab members
Lars Nilsson
Frida Ekholm Pettersson
Anna Lord
Hillevi Englund
Greta Hultqvist
Ola Philipsson
Kristina Magnusson
Astrid Gumicio
Elisabeth Nikitidou
Sofia Söllvander
Stina Tucker
Barbro Simu
Ann-Sofi Johansson
Camilla Nilsberth
Jan Näslund
Anita Westlind-Danielsson
Lena Lilius
Charlotte Forsell
Karin Axelman