ALZ-201, a monoclonal antibody therapy for specific neutralisation of toxic amyloid-β in Alzheimer's disease

Anders Sandberg, Alzinova AB, Sweden

Introduction

The conspicuous plaques in Alzheimer' disease (AD) brains are mainly deposits of insoluble fibrillar assemblies of the peptide amyloid-β (Aβ). These assemblies are effectively targeted by monoclonal antibodies (mAb) exhibiting selectivity for aggregated forms of Aβ (aducanumab, lecanemab, and gantenerumab) or specificity for pyroglutamated forms (donanemab). Plaques are, however, not as toxic as soluble oligomeric forms of Aβ and clinical benefits of plaque reduction are indeed modest.

The oligomer-targeting vaccine candidate ALZ-101 (in clinical phase 1b; NCT05328115) was therefore used to develop a murine mAb, ALZ-201, as a potential therapy capable of specifically targeting toxic oligomeric Aβ thereby avoiding binding to plaques and non-aggregated Aβ altogether. ALZ-101 is based on the Aβ42CC oligomer-stabilising peptide technology (Sandberg et al., 2010).

Methods

Monoclonal murine ALZ-201 was developed using hybridoma technology on B-lymphocytes from mice immunised with ALZ-101. Conformational specificity for oligomeric Aβ was confirmed with ELISA against various non-aggregated and aggregated forms of the peptide. Non-reactivity towards plaques was demonstrated by IHC on human tissue sections (not shown here; Sandberg et al., 2022).

Therapeutic potential was investigated using human AD brain extracts and automated high content microscopy analysis of primary mouse neuron cultures *in vitro*, as previously reported (AAIC2021 Poster #53659; Sandberg et al., 2022). This strategy ensures that physiologically relevant Aβ is studied already in a non-clinical setting to increase the likelihood for translatability and efficacy in AD patients.

A chimeric antibody, chALZ-201, with backbones for human IgG1 was designed and binding specificity of chALZ-201 verified and benchmarked with biosimilar versions of aducanumab, lecanemab, and gantenerumab using ELISA against different aggregated forms of Aβ (Sandberg et al., 2022).

Further humanisation of ALZ-201 resulted in several promising candidates with >85% sequence identity to human antibodies. Biacore analysis was used to determine the binding affinity towards the stabilised oligomer antigen in vaccine ALZ-101.

References

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In vitro assessments of binding specificity

ALZ-201 was isolated as a murine IgG3 and found to be truly specific, not just selective, for the structured oligomers in vaccine ALZ-101. Murine ALZ-201 and chALZ-201 did exhibit a small yet statistically significant difference in EC50 for the oligomeric form by 29 ng/mL (95% CI, 22.3267 to 35.9192, p<0.0001), but did not bind any other conformations of the peptide. In contrast, aducanumab, lecanemab, and gantenerumab had similar affinities for all different conformations of the peptide, although gantenerumab seemed to slightly favour fibrils and monomers over oligomers in this assay.

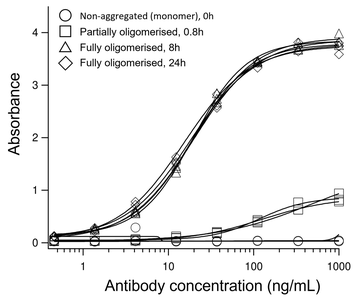

Figure 1. Antibody dose-response curves of ALZ-201 against non-aggregated (0h), partially aggregated (0.8h), and fully aggregated (8h and 24h) Aβ42CC peptides. The EC50 was taken from a 4-parameter logistic equation fitted to the experimental data. N=3 for each antigen. Control antibody 6E10 exhibited no preference for any form of Aβ (not shown). Adapted from Sandberg et al., 2022.

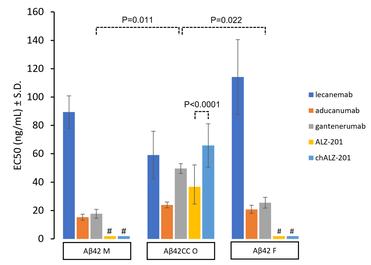

Figure 2. The EC50 (taken from a 4-parameter logistic equation fitted to the experimental data) from antibody-dose response curves against monomeric non-aggregated Aβ42 peptides ("M"), 702 ± 4 kDa Aβ42CC oligomers ("O"), and fibrillar forms of the Aβ42 peptide for which the Mw is unknown ("F") . #: No binding detected. The error is the standard deviation (SD). Adapted from Sandberg et al., 2022.

Biacore measurements demonstrated that chALZ-201 had high affinity for ALZ-101 oligomers, with a binding off-rate (k_{off}) of 4.67×10^{-4} s^{-1} and a binding constant of 1.56 nM (humanised variants had similar values; data not shown).

Very few species of Aβ are "ALZ-201 reactive". In actively aggregating Aβ42 they reach a maximum of 39.4 ± 5.6 ng/mL after 20 min, then slowly disappear as the rate of fibrillisation increases. This corresponded to 0.047% of all Aβ42 in the reaction mixture.

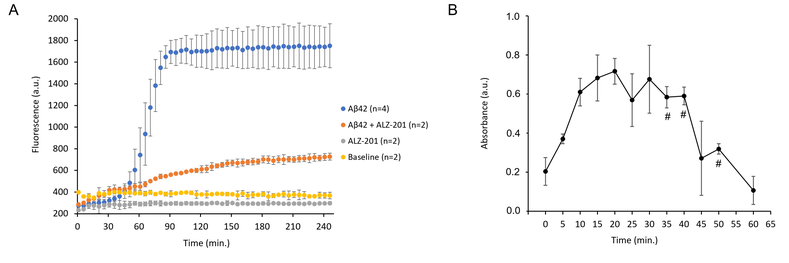

Figure 3. (A) Thioflavin-T binding assay to assess the effect of ALZ-201 on fibrillisation of the Aβ42 peptide. (B) ALZ-201/ALZ-201 sandwich ELISAs detecting ALZ-201-reactive oligomers formed during actively aggregating Aβ42. Standard curves were run in parallel for quantitation purposes (not shown). # N=2; all other data points are N=3; the standard deviation (SD) = the errors. Adapted from Sandberg et al., 2022.

Therapeutic potential using *ex vivo* tissues

Immunodepleting AD brain extracts with ALZ-201 did not significantly impact the measured amounts of Aβ. Remarkably, immunodepletion of AD brain extracts with ALZ-201 still neutralised the Aβ-mediated neurotoxicity in these extracts to a similar extent as 4G8 (removes all Aβ) despite the low abundance of "ALZ-201 reactive oligomers".

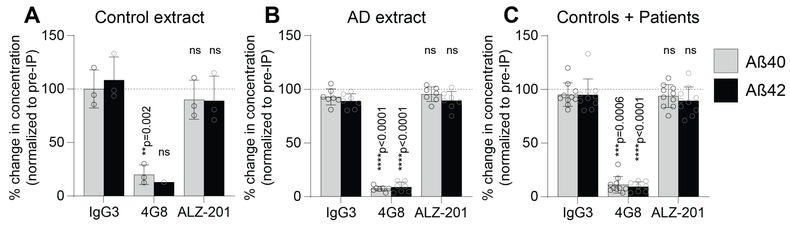

Figure 4. The percentage change of Aβ40 (grey) and Aβ42 (black) concentration to pre-immunodepleted (pre-IP) conditions (set to 100%) for (A) controls and (B) AD patients separately or (C) combined. Bar graphs show the mean ± SD, data points represent the individual values for control and/or AD patient brain extracts. N=3 controls; N=7 AD patients. Note that Aβ42 levels were above the detection limit for only one control case treated with 4G8, resulting in a N=1 sample size and a non-significant statistical difference. Adapted from Sandberg et al., 2022.

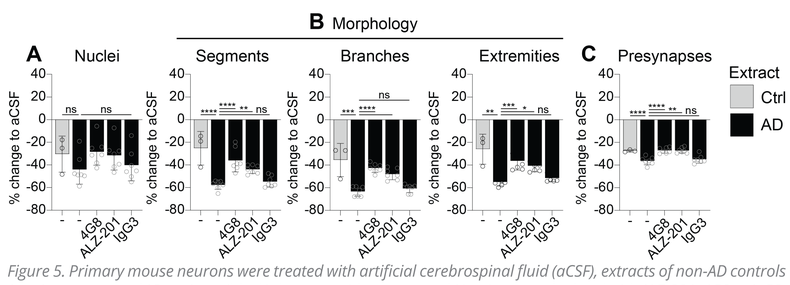

Figure 5. Primary mouse neurons were treated with artificial cerebrospinal fluid (aCSF), extracts of non-AD controls (grey), or extracts of AD patients (black) that were either untreated (-) or immunodepleted with ALZ-201, 4G8 or IgG3. Automated microscopy was employed for the quantification of (A) neuronal nuclei, (B) morphology (as determined by segments, branches and extremities), and, (C) number of vGlut1-positive presynapses. Values are displayed as the % change to cultures treated with aCSF. Bar graphs show the mean ± SD. Extracts are the same as in Figure 4. Adapted from Sandberg et al., 2022.

Conclusion

MAb ALZ-201 recognises a conformational epitope on a very low-abundant but highly toxic Aβ42 oligomer and has a therapeutic potential comparable to the 4G8 antibody that targets all Aβ. In contrast, aducanumab, lecanemab, and gantenerumab exhibit no conformational preference for Aβ, indicating that their weak binding to non-aggregated forms (their selectivity) stem almost exclusively from fast binding off-rates. Consequently, these antibodies target plaques with high functional affinity.

Given the modest clinical effect plaque reduction has on pathology, next generation anti-Aβ therapies ought to instead focus on antibodies that are truly specific for toxic forms of soluble oligomeric Aβ. To this end, a lead humanised candidate of ALZ-201 is now in preclinical development in preparation for clinical trials on AD patients.