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CORRESPONDENCE

Anti-Aβ antibody target engagement: a response to Siemers et al.

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We have read with interest the commentary by Siemers et al. [28] regarding our paper describing the ability of the three anti-Aβ antibodies, bapineuzumab, crenezumab and solanezumab to engage Aβ in both a synthetic and a biological setting. We appreciate the opportunity to clarify any misunderstandings and here provide a brief response to their concerns.

Siemers et al. [28] begin their commentary by stating that our findings led to the conclusion "that all three antibodies failed to engage the intended molecular targets". This statement is wrong; as clearly stated in the abstract of our paper, "Bapineuzumab demonstrated target engagement with brain Aβ, consistent with published clinical data". To reiterate, we reported data showing that bapineuzumab was capable of binding soluble Aβ with a low nanomolar affinity and demonstrated that the antibody could detect Aβ species in buffer and in brain homogenate and plasma from transgenic animal models of AD. Furthermore, in agreement with the Phase 3 clinical data [23], we demonstrated target engagement for bapineuzumab, where its target is brain-derived amyloid-β peptides associated with Alzheimer's pathology (see, Fig. 3a [28]). Our data also showed that neither solanezumab nor crenezumab effectively engaged this pool of Aβ, again consistent with the publicly available clinical data [9, 10].

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The first point raised by Siemers et al. is that the surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) "does not possess a dynamic range sufficient to measure endogenous Aβ peptides in murine and human plasma". This is incorrect. One of our investigations involved spiking diluted and denatured AD-affected plasma samples with $Aβ_{1–42}$ at equivalent levels to the Aβ levels reported in AD plasma [19, 21]. The resulting Aβ was clearly observable using this technique; however, no Aβ was observable in the non-spiked plasma. SELDI-TOF MS utilises antibody-based immunocapture coupled with the mass spectrometric detection of bound analytes. The advantages of MS-based systems are greater sensitivity and the ability to differentiate specific from non-specific interactions based on the molecular weight of the captured species. More specifically, SELDI-TOF MS is capable of binding soluble Aβ peptides at low femtomolar concentrations [4] and is able to resolve individual isoforms of Aβ. Any issues with dynamic range are more likely to be an issue at higher concentrations where saturation of the antibody occurs.

Furthermore and consistent with our SELDI-TOF MS findings, analysis of the AD plasma proteome with the three antibodies using LC–MS/MS on a LTQ Orbitrap Elite also failed to detect any Aβ. This high-resolution system has previously been used to characterise Aβ in biological matrices, both qualitatively and quantitatively [13] at levels consistent with those observed in AD-affected plasma [12, 21]. The results of this analysis were wholly consistent with the SELDI-TOF MS experiments.

The possible explanations for the lack of observable Aβ in AD-affected plasma include:

1. The Aβ is bound in higher molecular weight complexes with other plasma proteins. It should be noted that such interactions would also affect the signal observed in plasma from transgenic animal models of AD in addition to ELISA-based measures of Aβ in human samples.
2. The Aβ partitions into another blood fraction, as previously reported [26].
3. The Aβ levels as described by ELISA-based methodologies are overstated.

In their second point, Siemers et al. assert that the human brain homogenates utilised in our study "were prepared under conditions that yielded primarily aggregated/oligomer Aβ, a structural form of Aβ to which solanezumab does not bind". The preparation of the cortical homogenates in our investigation was kept purposefully minimalistic in an effort to reduce the induction of aggregates and other artefacts. We utilised detergents and denaturants that have previously been shown to have no effect on Aβ aggregation rates [22] and not to stabilise Aβ in solution [17]. However, our data clearly demonstrate that a subset of the observable Aβ in these samples was part of larger Aβ aggregates or oligomers, as shown by the N-terminally truncated Aβ species captured by bapineuzumab, which binds Aβ via the N-terminus [11, 20]. Siemers et al. also noted that "solanezumab is selective for soluble monomeric forms of Aβ, the Watt et al. brain extraction experiments were not properly designed to detect target engagement for this antibody". From this statement, are we to believe that Siemers et al. consider the AD-affected brain homogenate to contain no soluble monomeric Aβ? The lack of observable signal in the spectra indicates that either there is no soluble monomeric Aβ in the AD brain homogenate or that solanezumab is failing to bind Aβ in these samples due to cross-reactivity with more abundant proteins, a well-established short coming of antibodies raised against linear sequences, such as crenezumab and solanezumab [2, 3, 14, 16]. To reiterate, this technology is capable of detecting Aβ at femtomolar concentrations [4] and our previous work has demonstrated that the Aβ levels observed in these cortical homogenates using SELDI-TOF MS significantly correlate with the levels of soluble Aβ present in these samples [29]. We designed the experiments to perturb the AD-affected cortical tissue as little as possible, and under those conditions, solanezumab failed to effectively engage Aβ (see Figs. 3, 4 [28]).

In their third comment, Siemers et al. state that our immunoprecipitation (IP) pull-down experiments presented "no negative control experiments". This statement is wrong. To mitigate the "very high risk of unspecific binding to the beads used for immunoprecipitation", we performed IP experiments from plasma with Fab fragments from these antibodies, with the Fab derived from bapineuzumab and with the magnetic beads alone as negative controls (see final paragraph of Results [28]). Twelve of the proteins pulled down by solanezumab and crenezumab, but not bound by either bapineuzumab or the beads alone, proved to contain substantial sequence identity with the mid-region Aβ epitopes targeted by these antibodies. This highlighted the potential for cross-reactivity of these antibodies. The isolation of the interleukin-12 receptor protein was of particular interest given reports that modulation of this pathway alleviated cognitive symptoms in a number of AD models [25, 27].

In their concluding statement, Siemers et al. claim that the findings of our investigations were inconsistent with the wealth of published literature on the three antibodies; however, examination of the articles cited in their commentary reveals that our findings are largely consistent with the published literature. The paper by Legleiter et al. [18] reported that m266 was able to inhibit the formation of fibrils by synthetic Aβ whilst the articles by Seubert et al.

[24] and Gelfanova et al. [15] reported that m266 readily bound Aβ in human cerebrospinal fluid (CSF), a biological medium that contains tenfold more Aβ, but less than one-tenth of the total protein observed in human plasma. These findings were consistent with our analysis of synthetic Aβ using both SPR and SELDI-TOF MS which demonstrated that solanezumab had a sub-nanomolar affinity for Aβ in non-complex samples. Our findings also readily supported the studies by DeMattos et al. [5–8] and Bard et al. [1] who reported that solanezumab was readily able to bind Aβ in the cortical tissue and plasma of transgenic mouse models of AD. Furthermore, our findings remain consistent with the outcomes of the two Phase III clinical trials demonstrating that solanezumab was unable to modulate cortical Aβ levels, leading Doody et al. [9] to conclude that the "data from these two phase 3 solanezumab trials did not show efficacy of this monoclonal antibody".

To summarise, the findings of our recent paper remain consistent with the publicly available data generated by clinical trials investigating these three therapeutic antibodies. Bapineuzumab engages Aβ in AD-affected cortical tissue and remains the only antibody to definitively demonstrate target engagement through the significant reduction of cortical Aβ [23]. Clinical trials of bapineuzumab showed that if brain-derived Aβ is engaged, downstream perturbations in CSF tau levels will also be engaged. Solanezumab has a sub-nanomolar affinity for soluble Aβ, but has little-to-no effect on AD brain-derived Aβ [9]. Crenezumab has low nanomolar affinity for Aβ in simple samples; however, it is unable to readily engage brain-derived Aβ, consistent with its recent failure in two phase 2 clinical trials [10]. Our current findings coupled with the performance of these antibodies in their respective clinical trials indicate that a more thorough and open investigation of these therapeutic antibodies is warranted.

Conflict of interest None.

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