ABvac40 induces anti-Aβx-40 plasma specific antibodies that bind with Aβ vascular deposits in brain slices from humans with cerebral amyloid angiopathy

Montañés M[1], Canudas J[1], Martínez I[1], Lacosta AM[1], Pascual-Lucas M[1], **Terencio J[1,2]**

[1] Araclon Biotech-Grifols, Zaragoza, Spain. [2] Grifols, Barcelona, Spain.

BACKGROUND

ABvac40 was designed as the **first active immunotherapy against the C-terminal end of amyloid-beta 40** (Aβ40). With established good tolerability profile and robust immune response in AB1601 phase 2 clinical trial[1], characterizing the binding profile of the antibodies elicited by ABvac40 is pivotal for mechanistic insights and specificity. To this end, in this study we comprehensively investigated **the ability of the antibodies raised by ABvac40** in the AB1601 study **to target various Aβ species and aggregation forms.** Additionally, we assessed **their reactivity against vascular amyloid deposition in human brains with cerebral amyloid angiopathy (CAA),** which is highly prevalent among Alzheimer's disease patients[2].

MATERIAL AND METHODS

Dot blot assays were conducted using synthetic Aβ peptides of different lengths, truncated at both the N-terminal and C-terminal ends. Nitrocellulose membranes were probed with pre-immune and post-immune (after five ABvac40 or placebo inoculations) plasma samples from three patients from placebo arm and six patients from ABvac40 arm (study details were previously presented[1]).

Western blot assays were performed using in vitro-generated oligomers, composed of either Aβ40, Aβ42, or a combination of Aβ40/Aβ42 (in a ratio 9/1)[3]. The membranes were incubated with plasma samples from three ABvac40-treated patients (pre-immune and after seven doses of ABvac40).

Immunohistochemistry analysis were carried out on post-mortem human brain paraffin-embedded sections from three patients diagnosed with CAA and three healthy controls (provided by CIEN Foundation Tissue Bank, Madrid). These histological sections were incubated with three post-immune plasma samples (after seven doses of ABvac40), using pre-immune plasma of the same patients as negative control and pre-adsorbed post-immune plasma as a control of specificity.

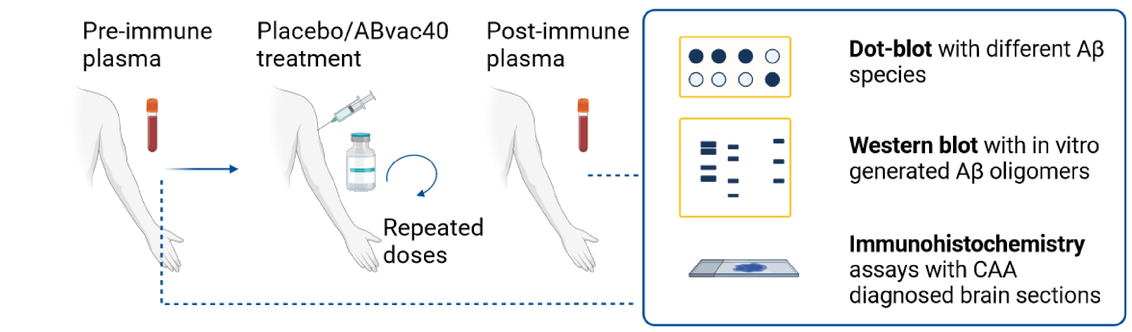

Fig.1- Schematic representation of sample sources and testing procedures in the study. Figure created by Biorender.

RESULTS

Plasma samples from patients treated with ABvac40 exhibited specific **dot-blot immunoreactivity against Aβx-40 peptides**, whereas they **did not bind to amyloid peptides ending at amino acids 38, 42 or 43.** Neither the plasma collected before treatment nor the plasma from patients who received placebo demonstrated recognition of any of the peptides examined in the study.

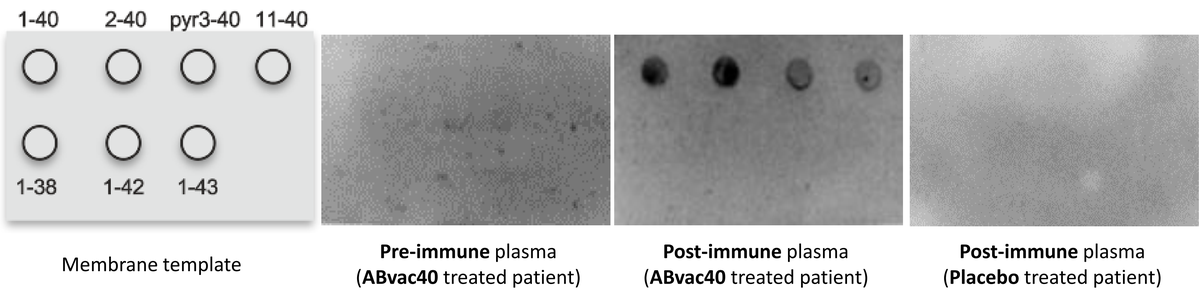

Fig. 2- Dot blot analysis of AB1601 plasma samples with different amyloid peptides sequences.

Western blot analysis of oligomers of **Aβ40 (oAβ40), Aβ42 (oAβ42) and a combination of Aβ40 and Aβ42 (oAβ40/42)** revealed that antibodies in plasma samples from ABvac40-treated patients recognized **Aβ40** in various forms, including **monomers, dimers, trimers and higher oligomers**. However, these antibodies **did not exhibit any reactivity towards Aβ42,** either in its monomeric form or aggregated states.

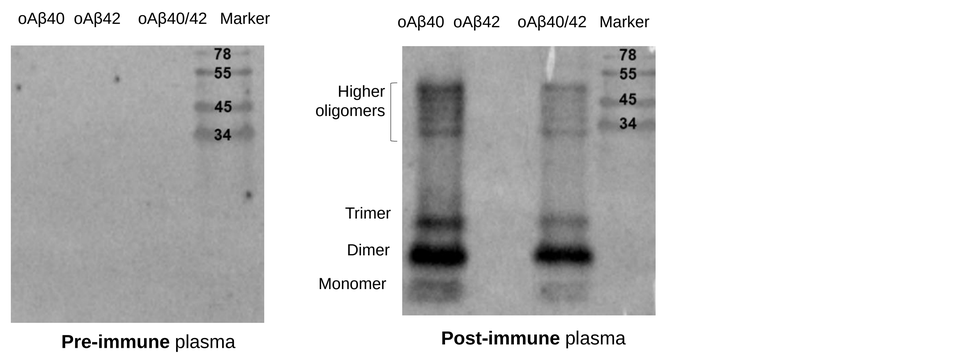

Fig. 3- Western blot analysis of pre-immune and post-immune plasma from a patient of the ABvac40-treated arm. Molecular weights are expressed in kDa.

Staining of occipital **brain tissue sections** from the three neuropathologically diagnosed CAA patients **with all ABvac40-treated plasma samples tested** revealed strong immunoreactivity **against Aβ vascular deposits** in all types of vessels (**arteries, arterioles and capillaries**). Additionally, **some senile plaques** were also recognized (Fig.4). These signals were absent when using plasma samples obtained prior immunization. The staining specificity was confirmed through pre-adsorption of post-immune plasma with the specific antigen (Fig.5).

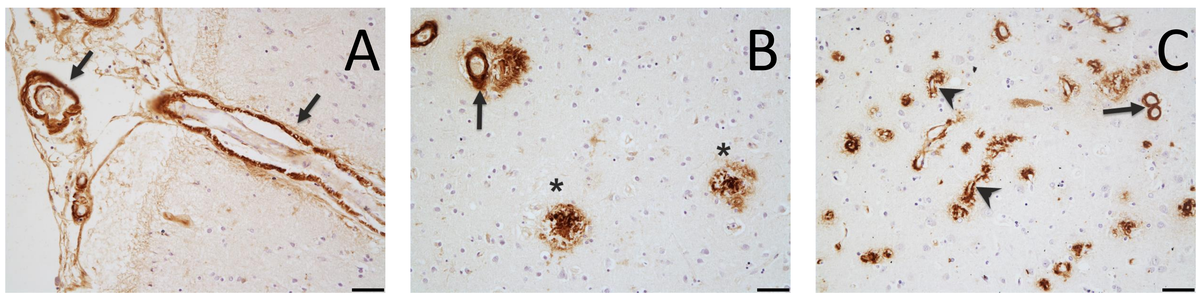

Fig.4- Plasma immunostaining includes all categories of vessels: leptomeningeal and penetrating arteries (arrows in A), arterioles (arrows in B and C) and capillaries (arrowheads in C). Also, some senile plaques are recognized (asterisks in B). Scale bar = 50 µm.

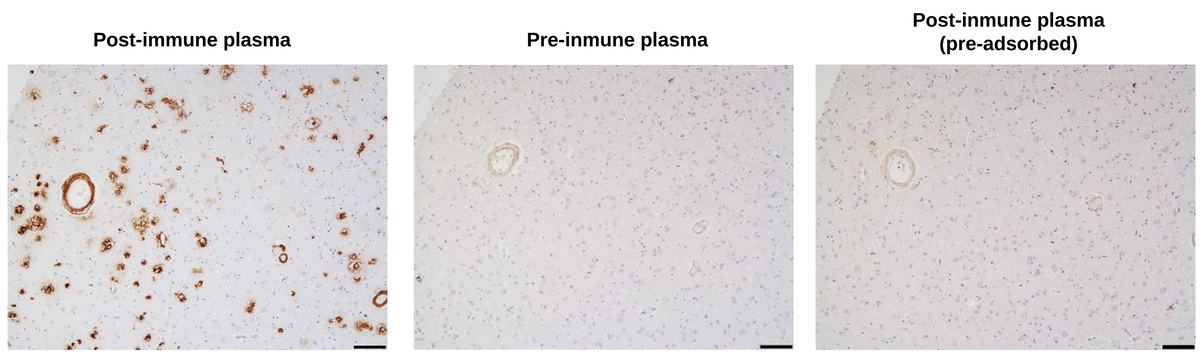

Fig.5- The same region is showed for post-immune, pre-immune and pre-adsorbed post-immune plasma staining. Scale bar= 100 µm.

CONCLUSIONS

This study demonstrates the **specificity of plasma antibodies triggered by ABvac40**, which recognize the **C-terminal region of Aβx-40** peptides in different aggregated states (monomeric, oligomeric, and brain deposits). Binding of ABvac40-elicited antibodies **to vascular and parenchymal amyloid brain deposits** could have an important role in the mechanism of action of ABvac40. We hypothesize that **the interaction of anti-Aβ40 antibodies with vascular amyloid could lead to a reduction of amyloid deposition in cerebral vessels.** This **could enhance the clearance of brain waste** through perivascular spaces, potentially **contributing to cognitive improvement**, **while maintaining an excellent safety profile as demonstrated previously.** However, additional studies are needed to better understand the mechanism of action of ABvac40.

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Contact: Jose Terencio jose.terencio@grifols.com ; María Montañés mmontanes@araclon.com

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