Abstract

Dementia in general and Alzheimer’s disease in particular is increasingly seen in association with autoimmunity being causatively or supportively involved in the pathogenesis. Besides classic autoantibodies (AABs) present in dementia patients, there is the new autoantibody class called functional autoantibodies, which is directed against G-protein coupled receptors (GPCRs; GPCR-AABs) and are seen as pathogenic players. However, less is known about dementia patients’ burden with functional autoantibodies. We present here for the first time a study analyzing the prevalence of GPCR-AABs in patients with different dementia forms such as unclassified, Lewy body, vascular and Alzheimer’s dementia. We identified the GPCR-AABs’ specific targets on the receptors and introduced a neutralization strategy for GPCR-AABs. Patients with Alzheimer’s and vascular dementia carried GPCR-AABs targeting the first loop of the alpha1- and the second loop of the beta2-adrenergic receptors (α1-AABs; β2-AABs). Nearly all vascular dementia patients also carry autoantibodies targeting the endothelin A receptor (ETA-AABs). The majority of patients with Lewy body dementia lacked any of the GPCR-AABs. In vitro, the function of the dementia-associated GPCR-AABs could be neutralized by the aptamer BC007. Due to the presence of GPCR-AABs in dementia patients mainly in those suffering from Alzheimer’s and vascular dementia, the orchestra of immune players in these dementia forms, so far preferentially represented by the classic autoantibodies, should be supplemented by functional autoantibodies. As dementia-associated functional autoantibodies could be neutralized by the aptamer BC007, the first step was taken for a new in vivo treatment option in dementia patients who were positive for GPCR-AABs.

Introduction

Dementia in general and particularly Alzheimer’s disease are seen increasingly in association with an autoimmune background that could be causatively or supportively involved in the pathogenesis. In addition to the variety of autoantibodies (AABs) detected in patients with dementia and suggested to be pathogenic players, biomarkers and treatment targets such as those summarized in [1,2], there is a new class of autoantibodies, the so-called functional autoantibodies that are directed against G-protein coupled receptors (GPCRs; GPCR-AABs) which...
are increasingly seen as pathogenic players. For GPCR-AABs and the related diseases, which can be named "functional autoantibody disease", basics, diagnostics and treatment strategies are summarized in [3,4,5,6]. In patients with dementia, GPCR-AABs targeting α1- and β2-adrenergic receptors (α1-AABs; β2-AABs) [7,8], as well as the angiotensin 2 type 1 receptor (AT1-AABs) [9], have already been demonstrated, which possibly links dementia to the specific autoimmune background of functional autoantibody disease. However, data related to the different dementia forms are missing for these GPCR-AABs and for further vasoactive GPCR-AABs, specifically those directed against the endothelin A receptor (ETA-R, ETA-AABs), which could additionally affect dementia patients. Here, we present for the first time a study analyzing the GPCR-AAB prevalence in patients with different forms of dementia. We found significantly higher frequencies for α1-, β2- and ETA-AABs in patient with vascular dementia compared to patients with Alzheimer’s disease and even more with unclassified dementia, where ETA-AABs were widely missed. Patients with Lewy body dementia lacked GPCR-AABs in a very high percentage. AT1-AABs were absent in all patient groups. Additionally, we present the GPCR-AABs’ target regions on the receptors as well as the possibility to neutralize dementia-associated GPCR-AABs by the aptamer BC007 [10].

Material and methods

Patients

Sera were primarily sampled for the study “High prevalence of NMDA receptor IgA/IgM antibodies in different dementia types” [11]. For this retrospective descriptive subgroup analysis to analyze the prevalence of GPCR-AABs in patients with different forms of dementia, serum were used (based on the availability in quantities necessary for the GPCR-AAB analysis) of patients with unclassified, Lewy body, vascular, and Alzheimer’s dementia attending the Department of Neurology, Charité—Universitätsmedizin Berlin. For patients’ basic data, group composition, comorbidities, and medication, see Table 1 in results. The study was approved by the institutional Review Board of Charité—Universitätsmedizin Berlin; written informed consent was obtained from patients or their representatives.

GPCR-AAB analytics

To identify and quantify the GPCR-AABs, a bioassay established by Wallukat and Wollenberger was used [12], which was modified and standardized as described in [13,14]. In this bioassay, the chronotropic response of spontaneously beating cultured neonatal rat cardiomyocytes to patients’ IgG-containing GPCR-AABs was recorded.

Bioassay of spontaneously beating cultured neonatal rat cardiomyocytes. As schematically illustrated in Fig 1, to investigate GPCR-AABs, IgG was isolated from patient serum, which is the sample material required for the bioassay of spontaneously beating cultured neonatal rat cardiomyocytes. This bioassay measured the functional activity of the GPCRs via the cells’ chronotropic response after addition of the GPCR-AAB-containing IgG. Depending upon either the positive or negative chronotropic activity of the GPCR-AABs, the increase and decrease, respectively, of the cells’ beat frequency is monitored.

With the intelligent use of blockers and competitors, the GPCR-AABs are specified for their targeted receptors, extracellular binding sites and specific receptor epitopes, as well as to analyze any functionality activity change of the GPCR-AABs.

Sample preparation. After whole blood collection, serum was prepared according to standardized procedures. For the IgG preparation, 1 ml of serum and 660 μl of saturated ammonium sulfate solution was mixed (final concentration: 40% ammonium sulfate) and incubated for 18 h at 4°C. After centrifugation for 15 min at 6,000 g, the pellet was re-suspended in 750 μl
PBS, mixed with 750 μl of saturated ammonium sulfate solution (final concentration: 50% ammonium sulfate), and centrifuged again. Thereafter, the pellet was suspended in 700 μl of PBS and dialyzed (VISKING cellulose, type 27/32, MW Cut off 14 kDa; Carl Roth, Germany) against the 100-fold volume of PBS for 3 days at 4˚C. The resulting IgG fraction was aliquotted and stored at −20˚C for at least a month without a loss of activity.

**Cardiomyocyte preparation and culturing.** Hearts of approximately twenty 1- to 3-day-old rats were removed under sterile conditions and transferred to PBS (4˚C; without Ca2+, Mg 2+; Biochrom, Berlin, Germany). The ventricle tissue was separated and dissected into small pieces of nearly of nearly 1 mm³ for washing twice with 10 ml of solution 1. After decanting the wash solution, the tissue was re-suspended in 10 ml of PBS containing 0.2% of crude trypsin, and incubated for 15 min at 37˚C under stirring; thereafter, the solution was treated with 10 ml of ice-cold heat-inactivated calf serum to stop the trypsination. The resulting suspension was centrifuged at 130 g for 6 min and the pellet was transferred to 20 ml of SM20-I medium (Biochrom GmbH, Berlin, Germany). For cell counting, 100 μl of this suspension was added to 100 Trypan blue solution. Then, 2.4×10⁶ cells in 2.0 mL of SM 20-I medium, which was equilibrated with humid air, were transferred to 12.5cm² Falcon flasks, and cultured as a monolayer for 4–8 days at 37˚C. The medium was renewed after 2 days. Cardiomyocytes spontaneously started beating after 2 days in culture.

**Assay procedure and standardization.** On the day of measurement, the culture flask was transferred onto a heated stage (37˚C) microscope and 6 fields with synchronous and rhythmic beating cardiomyocytes were marked on the flask bottom. Thereafter, the basal beating rate of the 6 fields was counted for 15 seconds and averaged. After the addition of 40 μl of the IgG preparation, the culture flasks were incubated for 40 to 60 min at 37˚C and the beating rate in the 6 fields was then counted for 15 sec and averaged.
For GPCR-AAB measurements, the bioassay has to fulfill the following quality criteria: 1) the basal beating rate must range between 120 and 160 beats/min; 2) cells stimulated for 5 min with isoprenaline (10 μM) must respond with a frequency increase of more than 45 beats/min; and 3) cells must respond to goat anti-ADRB1 (0.5 μg/ml, 1:100), EB07133, a commercial polyclonal antibody against the beta1-adrenergic receptor (Everest Biotech Ltd., Oxfordshire, United Kingdom), with an increase in frequency of more than 20 beats/min after incubation for 1 h. For the delta beating rate in the presence of isoprenaline and the commercial autoantibody, a day-to-day variation of ≤15% was estimated.

**Calculation of the GPCR-AAB activity.** One unit of GPCR-AAB activity corresponds to a 1 beat/min frequency change. The lower limits of detection (LLD) for positive and negative chronotropic activity were calculated as 4.0 U and -4.0 U, respectively. GPCR-AAB positivity was defined using cut-offs based on x ± 3 SD of the GPCR-AAB level of more than 100 healthy subjects. Results of ≥ 8.0 U for positive and ≤ -8.0 U for negative chronotropic GPCR-AAB activity were calculated. To access any integrated autoimmune activity for the several patient groups, we calculated a score based on the general GPCR-AAB composition in the groups for which the GPCR-AAB activities were summarized based on: 0 point = GPCR-AAB level < lower limit of detection (LLD); 1 point = GPCR-AAB level > LLD < cut off; and 2 points = GPCR-AAB level > cut off.

**GPCR-AAB differentiation related to their targeted receptors.** Through the use of specific blockers of GPCRs, the cells’ chronotropic response can be attributed to the related GPCR-AABs. Using this strategy, the bioassay was performed in the presence of specific blockers for the α1-adrenergic (0.1 μmol/l Prazosin), β2-adrenergic (0.1 μmol/l ICI 118.551), ETA receptor (0.1 μmol/l BQ 123) and AT1 receptors (0.1 μmol/l Losartan). Fig 2 illustrates this measurement strategy representatively for one patient positive for α1-, β2-, and ETA-AABs and negative for AT1-AABs. Due to the successive addition of receptor blockers, the change in the cells’ beating rate, which is the result of positive (α1-AABs, β2-AABs, AT1-AABs) and negative chronotropy (ETA-AABs), can be attributed to the individual GPCR-AABs (A). For independent confirmation, the IgGs were pre-treated separately with each blocker before the GPCR-AAB measurement (B).

**Localization of the extracellular receptor binding side of the GPCR-AABs.** To localize the extracellular binding side (loops), 50 μl of the GPCR-AAB-containing IgG preparation was pre-incubated for 30 min with 2 μl of solutions containing synthetic peptides (50 μmol/l) (Bio-syntan GmbH, Berlin-Buch, Germany) which represent the first, second and third extracellular loops of β2-adrenergic, α1-adrenergic and ETA receptors. Then, 40 ml of this mixture was added to the Bioassay for GPCR-AAB measurements. To localize the extracellular binding side of the ETA-AABs described here first, (134LP1NVFKLAGRWFDHNGVFVLCL160), (229FEYRGEQHKTCMLNATSKFMEYQDVKD256), and (329KKTWYMIDKNRCELLLSSFL348), respectively, were used which represent the first, second and third extracellular loop ETA receptors.

**Mapping of the specific epitopes targeted by the GPCR-AABs.** To map the specific epitope on the receptor loop targeted by the GPCR-AABs, the bioassay was performed after pre-treatment of the GPCR-AABs with an excess of synthetic peptides (Biosyntan GmbH, Berlin-Buch, Germany), which overlapped to represent the amino acid sequence of the receptor loops, first described for GPCR-AABs against the beta1-adrenergic receptor in [15]. For the mapping of the ETA-AAB epitope on the second extracellular loop demonstrated here, peptides were used, as follows: P1: FEYRGEQ, P2: EQHKTCM, P3: MLNATSK, P4: SKFMEYQ, and P5: FYQDVKD. For this, 50 μl of the GPCR-AAB-containing IgG preparation was pre-incubated for 30 min with 2 μl of solutions containing the synthetic peptides (100 μmol/l)
(Biosyntan GmbH, Berlin-Buch, Germany). Then, 40 ml of this mixture was added to the Bioassay for GPCR-AAB measurement.

**Influence of the aptamer BC 007 on the activities of GPCR-AABs.** The aptamer BC 007 [10] is a single stranded 15 mer DNA oligonucleotide (5'-GGT TGG TGT GGT TGG-3') (BioSpring GmbH, Frankfurt/Main, Germany).

To analyze the potency of BC 007 for the neutralization of GPCR-AABs present in dementia patients, the activity of the GPCR-AABs was measured in the bioassay performed in the absence and presence of 0.1 μM BC 007. To demonstrate the BC 007’s specificity for GPCR-AAB neutralization, experiments were performed in the presence of a scrambled 15 mer DNA oligonucleotide BC 007 (BioSpring).
mer DNA aptamer (5’-GGT GGT GGT TGT GGT-3’) (BioTez Berlin-Buch GmbH, Germany).

**Statistics.** Statistical analysis was performed using the SPSS software package (SPSS Inc., Chicago, US) with Pearson chi-square test and Fisher’s exact tests for the comparison of binary variables. For the intergroup comparison of continuous data, the Kruskal-Wallis H-test combined with the Mann-Whitney U-test for post-hoc analysis was employed. For this, undetectable marker concentrations (<lower limit of detection, LLD) were numerically expressed as values representing one-half of the LLD. For the graphical representation of continuous patient data, box plots indicate the median and interquartile range (IQR; 25th and 75th percentiles), while whiskers with ends represent the largest and smallest values inside 1.5 times the IQR, outliers (open circles) representing values between 1.5 and 3 times the IQR, and extremes (stars) placed more than 3 times the IQR.

**Results**

**Patients’ basic data, group composition, comorbidities and medication**

There were no significant differences in the subgroup composition related to the patients’ age and gender (Table 1). In all groups, significantly (p < 0.001) more males vs. females were present. With respect to comorbidities and medication, significant differences between the dementia groups existed for the presence of hypertension and dementia drug medication. In detail, five patients with unclassified dementia had hypertension together with diabetes and coronary heart disease in two each. In Lewy body dementia, all patients presented with arterial hypertension, two had additionally coronary heart disease and two diabetes, one patient with coronary heart disease also had peripheral artery disease. In vascular dementia, all patients had hypertension, together with diabetes in 4 patients, coronary heart disease in 2 and peripheral artery disease in 2 patients. All patients with Alzheimer’s dementia presented with hypertension, 3 patients had additionally coronary heart disease and one patient peripheral artery disease. Patients with unclassified and vascular dementia did not receive any anti-dementia drugs, while it was prescribed for 5 patients with Lewy body dementia (Rivastigmine for 4 patients, Memantine for 1 patient) and 7 patients with Alzheimer’s dementia (Donepezil in 3 patients, Memantine in 3 patients, and Galantamine in 1 patient). Only two patients from the total cohort received immunotherapy at blood draw. Both patients had unclassified dementia and were taken oral prednisolone for suspected autoimmune contribution.

**The pattern of functional autoantibodies in patients with unclassified, Lewy body, vascular and Alzheimer’s dementia**

The patients’ serum negativity and positivity, respectively, are demonstrated in Table 2 for α1-AABs, β2-AABs, and ETA-AABs. Negative patients were those with undetectable GPCR-AAB activities (<LLD) or with detectable activities but below the cut-off for separating healthy and diseased subjects; positive patients had GPCR-AAB activities >cut-off. Additionally, a score was calculated for the integrated activity assessment of the three GPCR-AABs. For the score calculation, the GPCR-AAB activities were summarized based on: 0 point = GPCR-AAB level <lower limit of detection (LLD); 1 point = GPCR-AAB level > LLD < cut-off; 2 points = GPCR-AAB level > cut-off.

Positivity for α1-, β2- and/or ETA-AABs was found in each form of dementia, but with significantly different frequencies. However, in the Lewy body dementia group, only one patient was positive for GPCR-AABs, who presented all three autoantibodies. In contrast, none of the study patients was positive for AT1-AABs.
Using the Pearson Chi-square test, we calculated frequency differences between the patient groups for \( \alpha_1 \)-AABs (\( p < 0.001 \)) and ETA-AABs (\( p < 0.001 \)), whereas \( \beta_2 \)-AABs only tended (\( p = 0.07 \)) to be different between the dementia forms. By post hoc analysis with Fisher’s exact test, we attributed the frequency differences for \( \alpha_1 \)- and ETA-AABs specifically to the individual forms of dementia.

The majority of patients with vascular and Alzheimer’s dementia presented with \( \alpha_1 \)- and \( \beta_2 \)-AABs; the vascular group also presented with ETA-AABs. Consequently, with respect to \( \alpha_1 \)-AABs, significantly more patients were positive in the vascular (82%, \( p < 0.05 \)) and Alzheimer’s dementia (91%, \( p < 0.01 \)) groups than in the groups with unclassified (25%) and Lewy body dementia (17%). No significant differences existed between Alzheimer’s and vascular dementia or between unclassified and Lewy body dementia. ETA-AABs presented at a significantly higher frequency in patients with vascular dementia (91%) compared to those patients

---

Table 1. Patients with unclassified, Lewy body, vascular and Alzheimer’s dementia. Group composition, patients’ basic data, comorbidities, and medication are demonstrated. (D.m., Diabetes mellitus; CHD, coronary heart disease; PAD, peripheral artery disease).

| Dementia                          | un-classified (A) | Lewy body (B) | vascular (C) | Alzheimer’s (D) | Significance |
|-----------------------------------|-------------------|---------------|--------------|-----------------|-------------|
| Number (n)                        | 8                 | 6             | 11           | 11              |             |
| Age (yrs)                         | 63.5/77/45        | 70.5/78/67    | 72/81/55     | 70/87/40        |             |
| Male/Female (n/n)                 | 6/2               | 5/1           | 8/3          | 8/3             |             |
| Hypertension (+/-)                | 75/25             | 83/17         | 73/27        | 73/27           |             |
| Hypertension (n/n)                | 5/3               | 6/0           | 11/0         | 11/0            |             |
| Hypertension (%)                  | 62.5/37.5         | 100/0         | 100/0        | 100/0           |             |
| Diabetes (+/-)                    | 5/3               | 2/4           | 4/7          | 0/11            |             |
| Diabetes (%)                      | 62.5/37.5         | 33/67         | 36/44        | 0/100           |             |
| CAD (+/-)                         | 2/6               | 2/4           | 2/9          | 3/8             |             |
| CAD (%)                           | 25/75             | 33/67         | 18/82        | 27/73           |             |
| PAD (+/-)                         | 0/8               | 1/5           | 2/9          | 1/10            |             |
| PAD (%)                           | 0/100             | 20/80         | 18/82        | 10/90           |             |
| Medication                        |                   |               |              |                 |             |
| Antidepressive drugs (+/-)        |                   |               |              |                 |             |
| Antidepressive (n/n)              |                   |               |              |                 |             |
| Antidepressive (%)                |                   |               |              |                 |             |
| Immunosuppressives (+/-)          |                   |               |              |                 |             |
| Immunosuppressives (n/n)          |                   |               |              |                 |             |
| Immunosuppressives (%)            |                   |               |              |                 |             |

https://doi.org/10.1371/journal.pone.0192778.t001
Table 2. The pattern of functional autoantibodies in patients with unclassified, Lewy body, vascular and Alzheimer’s dementia. Serum positivity and negativity are demonstrated for autoantibodies directed against α1-adrenergic (α1-AABs), β2-adrenergic (β2-AABs) and endothelin A receptors (ETA-AABs); a score was calculated for the integrated activity assessment of the three autoantibodies directed against G-protein coupled receptors (GPCR-AABs). For the score calculation, the GPCR-AAB activities were summarized based on: 0 point = GPCR-AAB level < lower limit of detection (LLD); 1 point = GPCR-AAB level > LLD < cut-off; 2 points = GPCR-AAB level > cut-off.

| Form          | α1-AABs -/+ | β2-AABs -/+ | ETA-AABs -/+ | GPCR-AAB-Score (Median/Min/Max) |
|---------------|-------------|-------------|--------------|---------------------------------|
| Dementia      | (A)         | (B)         | (C)          | (D)                             |
|               | (n/n)       | (n/n)       | (n/n)        | (n/n)                           |
|               | (%/%)       | (%/%)       | (%/%)        | (%/%)                           |
|              |             |             |              |                                 |
| un-classified | 6/2         | 2/6         | 7/1          | 7/1                             |
|              | 75/25       | 25/75       | 88/12        | 88/12                           |
| Lewy body     | 5/1         | 5/1         | 5/1          | 5/1                             |
|              | 83/17       | 83/17       | 83/17        | 83/17                           |
| Vascular      | 2/9         | 3/8         | 1/10         | 4/7                             |
|              | 18/82       | 27/73       | 9/91         | 36/64                           |
| Alzheimer’s   | 1/10        | 3/8         | 9/91         | 8/3                             |
|              | 9/91        | 27/73       | 82/18        | 73/27                           |
| Significance  | (*) p < 0.05| (*) p < 0.01| (*) p < 0.001|                                 |
|              |             |             |              |                                 |
| Functional autoantibody pattern | Group diff. (***) | Group diff. (p = 0.07) | Group diff. (**) | Group diff. (**) |

Without reaching statistical significance, but numerically, the majority of patients with unclassified (75%), vascular (73%) and Alzheimer’s dementia (73%) presented with β2-AABs, whereas we found this autoantibody in only a minority of the patients with Lewy body dementia (17%).

To support any more pronounced autoimmune background in vascular and Alzheimer’s dementia compared with unclassified and Lewy body dementia, the patient cohort was analyzed for their general GPCR-AAB positivity as presented also in Table 1.
Significant group differences (Pearson Chi-square test) were calculated for the presence of at least one ($p<0.05$) or two ($p<0.001$) of the analyzed GPCR-AABs. In the case of the presence of three autoantibodies, a trend ($p = 0.07$) towards group differences was obvious. More detailed analyses (Fisher’s exact test) for the presence of at least one or any two of the GPCR-AABs, showed significantly more patients with vascular (91%, $p<0.05$; 91%, $p<0.001$) and Alzheimer’s dementia (91%, 0.05; 91%, $p<0.001$) to be positive compared with the patients suffering from Lewy body dementia (33%). Related to the patients’ positivity for any one of the GPCRs, unclassified dementia (75%) did not differ from the other dementia forms. However, in cases with the presence of any two GPCR-AABs, significantly fewer patients with unclassified dementia (25%) were affected compared with the vascular ($p<0.01$) and Alzheimer’s dementia ($p<0.05$) patients. The more prominent role of GPCR-AABs in Alzheimer’s dementia and even more in vascular dementia was clearly supported by the score that based on the following assumption: GPCR-AABs level $< LL = 0$ points; GPCR-AAB level $> LL < \text{cut off} = 1$ point; GPCR-AAB level $> \text{cut off} = 2$ points. The vascular dementia form presented with a score that was significantly higher than that calculated for unclassified ($p<0.05$), Lewy body (0.05) and Alzheimer’s dementia ($p<0.05$). Additionally, the score for Alzheimer’s dementia was significantly higher than that of Lewy body dementia ($p<0.05$).

We have also proven whether the GPCR-AAB pattern in the dementia patients was determined by their comorbidities, but we did not see a significant relationship for any of the three GPCR-AABs. However using the Pearson chi-square and Fisher’s exact test, we calculated for the presence of $\alpha_1$-AABs in hypertensive patients a p value of 0.051. Fig 3, presenting dementia group-related GPCR-AAB activities, substantiates the results of Table 1 and shows different $\alpha_1$- ($p<0.005$), $\beta_2$- ($p<0.05$) and ETA-AAB activities ($p<0.001$) between the patients groups. Following post hoc analysis, the $\alpha_1$-AAB activity was higher in patients with vascular dementia than in those with unclassified ($p<0.05$) and Lewy body dementia ($p<0.01$) as well as in patients with Alzheimer’s dementia compared to Lewy body dementia ($p<0.01$). Lewy body dementia patients also presented with lower $\beta_2$-AAB activity vs. patients with unclassified ($p<0.05$), vascular ($p<0.05$) and Alzheimer’s dementia ($p<0.05$). Significantly increased ETA-AAB activity was found exclusively in patients with vascular dementia vs. unclassified dementia ($p<0.05$), Lewy body dementia and Alzheimer’s dementia ($p<0.01$).

**Localization of the extracellular receptor binding side and mapping of the specific epitopes targeted by the GPCR-AABs**

As indicated in Table 3, $\alpha_1$-AABs and ETA-AABs targeted the second extracellular loop of their receptors and the specific epitopes ($^{169}\text{APEDET}^{174}$) and ($^{234}\text{EQHKCMLNATSK}^{246}$), respectively. $\beta_2$-AABs were directed against the first extracellular loop specifically targeting the epitope ($^{101}\text{FGNFWCE}^{107}$). Compared with the epitope of $\alpha_1$-AABs and $\beta_2$-AAB, the ETA-AAB epitope was clearly enlarged.

For the first described ETA-AABs, the *in vitro* experiments to localize their extracellular binding sites and the specific epitope are demonstrated in Figs 4 and 5.

**Influence of the aptamer BC 007 on the activity of $\alpha_1$-AABs, $\beta_2$-AABs and ETA-AABs of patients with dementia**

Fig 6A and 6B show representative results for 4 patients positive for $\alpha_1$-, $\beta_2$- and additionally ETA-AABs who suffered from vascular dementia and for 4 patients positive for $\alpha_1$- and $\beta_2$-AABs who suffered from Alzheimer’s dementia. Their GPCR-AAB activities in the absence and presence (0.1 $\mu$mol/l) of the aptamer BC 007 are demonstrated. For two patient, one with vascular dementia and another with Alzheimer’s dementia, the GPCR-AAB activity was
measured in the presence of the scrambled 15 mer aptamer. In the presence of BC 007, no GPCR-AAB activity could be measured. In contrast, the scrambled aptamer did not inhibit the activity of any of the dementia associated GPCR-AABs.

**Discussion**

A distinct proportion of patients with dementia are immunotherapy-responsive [16], which indicates a dementia-related autoimmune background. Target-destructing autoantibodies, many of which are documented in [1,2], were preferentially discussed in this context. However, there is a new class of functional autoantibodies which bind to GPCRs. Uncontrolled long-lasting receptor over-stimulation, which induces pathologically relevant disturbances in cell morphology and function, are the consequence as summarized in [3,4,5].

| GPCR-AABs | Extracellular receptor loop | Specific epitope       |
|-----------|-----------------------------|-----------------------|
| α1-AABs   | Loop II                     | APEDETE               |
| β2-AABs   | Loop I                      | FGNFWECE              |
| ETA-AABs  | Loop II                     | EQHKTCMLNATSK         |

Table 3. Dementia-associated autoantibodies directed against G-protein coupled receptors (GPCR-AABs) such as those directed against the α1-adrenergic (α1-AABs), β2-adrenergic (β2-AABs) and endothelin A receptor (ETA-AABs) related to their target (extracellular receptor loop) with the specific epitope.
Our study demonstrates for the first time that serum antibodies against the ETA-R are relatively common in patients with vascular dementia. ETA-AABs have already been found in patients with pulmonary hypertension [17], scleroderma [18], thromboangiitis obliterans [19] and benign prostate hyperplasia [20]. The ETA-AABs of patients with dementia targeted a more terminal epitope located on the second extracellular receptor loop, which is different from the epitope targeted by the ETA-AABs in benign prostate hyperplasia [20]. For the ETA-AABs' pathophysiologic function, their pathology driving or at least supporting role was intensively discussed for systemic scleroderma [18]. For brain pathology, the negative influence of endothelin was documented on neuron and blood-brain barrier integrity [21], synaptic plasticity [22], oxidative stress and apoptosis [23]. Following ETA-R blockade, protective effects such as lower degree of impaired learning and memory have been evidenced in animals [24,25]. The authors argued that ETA-R blockade diminishes the vasoactive effects of endothelin and this way in which ischemia damages the hippocampus, which is responsible for learning and memory. Considering the high interchangeability in the pathogenic effects of endothelin A and ETA-AABs, we assume that ETA-AABs in patients with vascular dementia could contribute to the pathogenesis whereby the ETA-AABs pathogenicity could exceed that of endothelin due to the absence of control mechanisms (receptor desensitization, receptor down regulation) to counteract over-boarding receptor stimulation. Only a minority of patients in the other groups presented with ETA-AABs. It remains speculative whether this indicates any non-recognized vascular background in these patients. Patients positive for ETA-AABs, independent of their clinically diagnosed dementia form, were always also positive.

[Fig 4. Autoantibodies directed against the endothelin A receptor (ETA-AABs) of patients with vascular dementia target the second extracellular receptor loop. Using the bioassay of spontaneously beating cultured neonatal rat cardiomycocytes, the chronotropic activity of the patients' IgG (n = 3), either untreated or pre-incubated with peptides representing the first (134LPINVFKLAGRVFPDNGVFLCKL160), second (128FEYRGEQHKTCMLNATKMEFQYQ9KD158), and third extracellular receptor loop (329KTVYNEIMPKNCCELLSSFL138), was measured. Values below the low limit of detection (LLD) were displayed as half range values. LLD = -4 beats/min; cut-off (separating healthy from disease subjects) = −8 beats/min.](https://doi.org/10.1371/journal.pone.0192778.g004)
for α1- and β2-AABs (except for one patient with Alzheimer’s dementia). Consequently, these patients presented with the typical vascular dementia GPCR-AAB pattern.

The presence of α1- and β1-AABs in patients with vascular and Alzheimer’s dementia confirmed previous studies [7,8]. Just as for the ETA-AABs, uncontrolled and over-boarding receptor stimulation were discussed as the key event for the autoantibodies’ pathogenic potency. However, as summarized in [26], the brain noradrenergic system plays a pivotal role in modulating cognitive activities. Consequently, increased agonist availability resulted in improved cognitive activities, whereas deficits in cases of low agonist levels have been documented. It is tempting to speculate that autoantibody-dependent over- and uncontrolled stimulation of the adrenergic system could disturb this highly regulated neurotransmission via the physiologic agonists leading to cognitive dysfunction.

Furthermore, β2-adrenergic receptor stimulation increased gamma-secretase activity for accelerated amyloid plaque formation, being one of the hallmarks in Alzheimer’s disease [27].

To attribute the place of α1- and β2-AABs in the orchestra of pathogenic players in vascular dementia, there are some points of discussion. The α1-AABs’ potency for inducing cellular remodeling processes has been demonstrated in rat heart [28] and vessels [29], (cardiomyocyte hypertrophy, aortic media thickening, collagen deposition in heart interstitium, mitochondria hyperplasia of vascular smooth muscle cells, increased expression of c-jun and matrix metalloproteinases). Many of these events were also accused of promoting vascular alterations in dementia patients [30,31]. Vascular defects in the brain have been evidenced by magnetic resonance imaging in rats immunized for the generation of α1-AAB [32]. Related to β2-AR
Fig 6. A and B. Influence of the aptamer BC 007 on the activity of autoantibodies directed against the G-protein coupled receptors (GPCR-AABs) specifically those to the β2-adrenergic (β2-AABs), α1-adrenergic (α1-AABs) and endothelin A receptor (ETA-AABs) in patients with (A) vascular (β2-AABs, α1-AABs, ETA-AABs) and (B) Alzheimer’s dementia (β2-AABs, α1-AABs). The total chronotropic activity of the patients’ IgG as well as the activities related to each autoantibody on spontaneously beating cultured neonatal rat cardiomyocytes isolated from the serum of all 4 patients in the absence (colored columns) and presence (0.1 μM) of BC 007 (grey columns) are demonstrated. For each one of the patients with vascular and Alzheimer’s dementia, the GPCR-AAB activity in the
stimulation, vascular pathology with enhanced vasoconstrictor response and increased vascular oxidative stress has been demonstrated, which might result in endothelial dysfunction [33]. The $\alpha_1$-AABs targeted the second, the $\beta_2$-AABs the first extracellular receptor loop of the related receptors. This—together with the identified epitopes—confirmed the data in [7] but was—concerning the receptor loop for $\alpha_1$-AABs—in contrast to the data in [8] where $\alpha_1$-AABs targeted the first loop. We cannot absolutely exclude first loop targeting $\alpha_1$-AABs in dementia patients, however, we haven’t seen such. In contrast to a recently published study, which used ELISA to demonstrate AT1-AAB positivity in Alzheimer’s patients [9]; the bioassay didn’t display functional active AT1-AABs in our patients. However, ELISA cannot distinguish between functional active and inactive GPCR-AABs and is therefore under criticism if not supplemented and validated with a functional assay such as the here used bioassay [4, 34, 35]. Although this criticism focused preferentially to ELISA vs. bioassay measurement of autoantibodies directed against the beta1-adrenergic receptor, the same problem exists in our view for all the other GPCR-AABs.

To connect serum GPCR-AABs with pathological processes in the brain, the AABs’ or related B-cells’ crossing of the blood-brain barrier would be prerequisite. As summarized in [36,37,38,39], there is no doubt that routes exist for the pathogenic autoantibodies and related B-cells to attack the “immune-privileged” central nervous system. Consequently, for dementia patients, treatment strategies (therapeutic plasma exchange (TPE), immunoabsorption) for GPCR-AAB removal from the patient’s circulation could be promising and have already tested as extensively reviewed for GPCR-AAB positive heart failure patients [5,6]. Recently, immunoabsorption was also tested in eight $\alpha_1$-AABs positive patients (5 patients were additionally positive for $\beta_1$-AABs) with vascular/Alzheimer’s dementia [40]. Patients who completed the aforementioned cycle immunoabsorption protocol, demonstrated nearly 100% $\alpha_1$-AAB removal; no autoantibody returns within the follow up period of 18 months (except one patient with GPCR-AAB return after 12 month) combined with the stabilizing of the Mini-Mental State Examination Score (MMES) at the level before treatment. In contrast, MMES deteriorated in the patients who interrupted the immunoabsorption protocol after the second and third run, respectively, therefore having incomplete $\alpha_1$-AAB removal and autoantibody return over time. Based on their findings, the authors hypothesized a substantial role of $\alpha_1$-AABs in the pathogenesis of dementia, specifically of Alzheimer’s and vascular dementia.

We are rather reserved to agree any exclusive or dominant pathogenic role specifically of $\alpha_1$-AABs in dementia. $\alpha_1$-AABs are typically associated to hypertension [41,42] and could therefore—as demonstrated for our dementia patient cohort—related to this frequent dementia comorbidity. However, the immunoabsorption technology used procided a design for the removal of the whole IgGs. Consequently, the patients’ blood was cleared from $\alpha_1$-AABs and all of the other possibly pathogenic IgG-associated AABs; in case of the dementia patients, therefore, also from $\beta_2$-AABs and ETA-AABs. Perhaps because of this, unspecific immunoabsorption for removal of all the GPCR-AABs in dementia patients should be a more hopeful treatment option than treatment concepts directed specifically to one of the GPCR-AABs. Unfortunately, cost factors, logistical problems and patient’s burden are associated with immunoabsorption, which form the main reasons for its restricted use. Treatment strategies for in vivo GPCR-AAB attack would minimize these problems and therefore be superior. Although already studied in dementia patients [43], intravenous IgG treatment (IVIG) and B-cell depletion were until now not applied specifically to attack the GPCR-AABs.
In the case of further manifestation of the GPCR-AABs pathogenic role in dementia patients, the *in vitro* neutralization of all three dementia-associated GPCR-AABs by the aptamer BC007 offers, as here demonstrated principally, a new treatment strategy. BC007 is a single stranded 15 mer DNA oligonucleotide (5’–GGT TGG TGT GGT TGG–3’) that was patented for the use as GPCR-AAB “broad spectrum neutralizer in diseases associated with GPCR-AABs. As we recently demonstrated [44], BC007 binds to the GPCR-AABs Fab fragments but clearly outside the complementarity-determining regions (CDRs) which explains the “surprising” potency of BC 007 for the neutralization of most several GPCR-AABs. Therefore most important, BC007 is able to also neutralize pathogenic GPCR-AABs directed to either other receptor loops or even other G-protein coupled receptors. This could be helpful for dementia patients suffering from comorbidities positive for other GP [10,44,45] CR-AABs (e.g. hypertension, diabetes mellitus) and interfering and aggravating dementia. In our view, this makes BC007 superior for treatment compared to compounds, which bind and affect the GPCR-AABs CDRs. To achieve such a concept, for each GPCR-AAB present in patients, a specific drug would be necessary. Irrespective of this disadvantage of CDR-binding compounds, a cyclic peptide [46] and an aptamer [47] were suggested for neutralization of the second loop targeting β1-AABs for the treatment of patients with DCM. However due to DCM patients with pathogenic β1-AABs directed against the first receptor loop and the frequent co-presentation of DCM patients with β1-AABs and GPCR-AABs directed against the muscarinic 2 receptor, α1-adrenergic and angiotensin 1 receptor type 2 in DCM patients, BC007 should be also superior in the treatment of these patients.

Translation of the aptamer-dependent concept of *in vivo* GPCR-AAB neutralization into clinical trials with GPCR-AAB-positive dementia patients might be a longer way. However related to autoimmunity-compromised heart failure patients, specifically those with DCM, promising steps such as animal studies [45], pre-clinical investigations as well as the phase I clinical trial [48] were already taken.

**Conclusion**

Patients with vascular and Alzheimer’s dementia, and to a lower frequency unclassified dementia, were carriers of α1- and β2-AABs. Patients suffering from vascular dementia carry additional ETA-AABs, which agrees with the strong vascular pathology in these patients. While α1- and ETA-AABs targeted specific epitopes on the second extracellular receptor loops, β1-AABs were directed against the first receptor loop. The majority of patients with Lewy body dementia were free of GPCR-AABs.

Due to the finding of GPCR-AABs in dementia patients, specifically in those suffering from Alzheimer’s and vascular dementia, the orchestra of players responsible for the autoimmune background in these dementia forms, which was so far preferentially represented by the classic autoantibodies, should be supplemented by functional autoantibodies. Because the functional autoantibodies found in dementia patients could be neutralized *in vitro* by the aptamer BC007, the first step was taken towards a new *in vivo* treatment option in GPCR-AAB-positive dementia patients.

**Supporting information**

S1 Table. Row data of Tables 1 and 2 and Fig 3.

(XLSX)

S2 Table. Row data of Fig 2.

(XLSX)
S3 Table. Row data of Figs 4 and 5.
(XLSX)

S4 Table. Row data of Fig 6A.
(XLSX)

S5 Table. Row data of Fig 6B.
(XLSX)

Author Contributions

Conceptualization: Gerd Wallukat, Harald Prüss, Johannes Müller, Ingolf Schimke.

Data curation: Gerd Wallukat, Harald Prüss, Ingolf Schimke.

Formal analysis: Gerd Wallukat, Johannes Müller, Ingolf Schimke.

Investigation: Harald Prüss, Ingolf Schimke.

Methodology: Gerd Wallukat, Ingolf Schimke.

Project administration: Johannes Müller, Ingolf Schimke.

Supervision: Ingolf Schimke.

Validation: Harald Prüss, Ingolf Schimke.

Writing – original draft: Ingolf Schimke.

References

1. Colasanti T, Barbati C, Rosano G, Malorni W, Ortona E (2010) Autoantibodies in patients with Alzheimer’s disease: pathogenetic role and potential use as biomarkers of disease progression. Autoimmun Rev 9: 807–811. Erratum in: Autoimmun Rev 11:374, https://doi.org/10.1016/j.autrev.2010.07.008 PMID: 20656067

2. Wu J, Li L (2016) Autoantibodies in Alzheimer’s disease: potential biomarkers, pathogenic roles, and therapeutic implications. J Biomed Res 30: 361–372. https://doi.org/10.7555/JBR.30.20150131 PMID: 27476881

3. Wallukat G, Schimke I (2014) Agonistic autoantibodies directed against G-protein-coupled receptors and their relationship to cardiovascular diseases. Semin Immunopathol 36: 351–363. https://doi.org/10.1007/s00281-014-0425-9 PMID: 24777744

4. Bornholz B, Wallukat G, Roggenbuck D, Schimke I. Autoantibodies against G-protein-coupled receptors in cardiovascular diseases: basics and diagnostics. In: Nussinovitch U, editor. The heart in rheumatologic, inflammatory and autoimmune diseases: pathophysiology, clinical aspects and therapeutic approaches. Amsterdam: Elsevier; 2017. pp 49–63. eBook ISBN: 9780128032688; Hardcover ISBN: 9780128032671

5. Müller J, Wallukat G, Schimke I. Autoantibody-directed therapy in cardiovascular diseases. In: Nussinovitch U, editor. The heart in rheumatologic, inflammatory and autoimmune diseases: pathophysiology, clinical aspects and therapeutic approaches. Amsterdam: Elsevier, 2017. pp 659–679. eBook ISBN: 9780128032688; Hardcover ISBN: 9780128032671

6. Becker NP, Müller J, Göttl P, Wallukat G, Schimke I (2017). Cardiomyopathy—An approach to the autoimmune background. Autoimmun Rev 16: 269–286. https://doi.org/10.1016/j.autrev.2017.01.012 PMID: 2816240

7. Kunze R, Wallukat G, Rosenthal P, Straube R. Peptides having binding affinity to an antibody which recognizes an epitope on an α1 loop 2 or β2 loop 1 of an adrenoceptor. WO 2009090227 A2; 2009. https://www.google.com/patents/WO2009090227A2

8. Karczewski P, Hempel P, Kunze R, Bimmler M (2012) Agonistic autoantibodies to the α(1) adrenergic receptor and the β(2)-adrenergic receptor in Alzheimer’s and vascular dementia. Scand J Immunol 75: 524–530. https://doi.org/10.1111/j.1365-3083.2012.02684.x PMID: 22860197
12. Wallukat G, Wollenberger A (1987) Effects of the serum gamma globulin fraction of patients with allergic asthma and dilated cardiomyopathy on chromotrope beta adrenoceptor function in cultured neonatal rat heart myocytes. Biomed Biochim Acta 46: 563–568. PMID: 2449194

13. Wallukat G, Muñoz Saravia SG, Haberland A, Bartel S, Araujo R, Valda G, et al. (2010) Distinct patterns of autoantibodies against G-protein-coupled receptors in Chagas’ cardiomyopathy and megacolon. Their potential impact for early risk assessment in asymptomatic Chagas’ patients. J Am Coll Cardiol 55: 463–468. https://doi.org/10.1016/j.jacc.2009.06.064 PMID: 20117461

14. Wenzel K, Schulze-Rothe S, Haberland A, Müller J, Wallukat G, Davideit H (2017) Performance and in-house validation of a bioassay for the determination of beta 1-autoantibodies found in patients with cardiomyopathy. Heliyon. 2017 Jul 31; 3(7):e00362. https://doi.org/10.1016/j.heliyon.2017.e00362 eCollection 2017 Jul. PMID: 28795160

15. Wallukat G, Wollenberger A, Morwinski R, Pitschner HF (1995) Anti-beta 1-adrenergic autoantibodies with chronotropic activity from the serum of patients with dilated cardiomyopathy: mapping of epitopes in the first and second extracellular loops. J Mol Cell Cardiol 27: 397–406. Erratum in: J Mol Cell Cardiol: 27: 2529. PMID: 7539084

16. Flanagan EP, McKean A, Lennon VA, Boeve BF, Trenery MR, Tan KM, et al. (2010) Autoimmune dementia: clinical course and predictors of immunotherapy response. Mayo Clin Proc 85: 881–897. https://doi.org/10.4065/mcp.2010.0326 PMID: 20884824

17. Wallukat G, Dandel M, Müller J, Bartel S, Schulze W, Hetzer R. (2007) Agonistic autoantibodies against angiotensin and endothelin receptors in the pathogenesis of systemic sclerosis. Autoimmun Rev. 2016; 15:690–694. https://doi.org/10.1016/j.autrev.2016.03.005 PMID: 26970493

18. Klein-Weigel PF, Bimmer M, Hempel P, Schöpp S, Dreusick S, Valierus J, et al. (2014) G-protein-coupled receptor auto-antibodies in thromboangiitis obliterans (Buerger’s disease) and their removal by immunoadsorption. Vasa 43: 347–352. https://doi.org/10.1024/0301-1526/a000372 PMID: 25147011

19. Wallukat G, Jandrig B, Kunze R, Wendler JJ, Müller J, Schostak M, et al. (2017) Autoantibodies directed against the endothelin A receptor in patients with benign prostatic hyperplasia. Prostate 77: 456–465. https://doi.org/10.1002/pros.23284 PMID: 27882567

20. Hung VK, Yeung PK, Lai AK, Ho MC, Lo AC, Chan KC, et al. (2015) Selective astrocyte-exaggerating astrocyte-derived amyloid secretion. J Cereb Blood Flow Metab 35: 1687–1696. https://doi.org/10.1038/jcbfm.2015.0004 PMID: 25436584

21. Drew GM, Coussens CM, Abraham WC (1998) Effects of endothelin-1 on hippocampal synaptic plasticity. Neuronpert 9: 1827–1830. PMID: 9865609

22. Koyama Y (2013) Endothelin systems in the brain: involvement in pathophysiological responses of damaged nerve tissues. Biomol Concepts 4: 335–347. https://doi.org/10.1002/bmc.2013-0004 PMID: 23863182

23. Singh G, Sharma B, Jaggi AS, Singh N (2014) Efficacy of bosentan, a dual ETA and ETB endothelin receptor antagonist, in experimental diabetes induced vascular endothelial dysfunction and associated dementia in rats. Pharmacol Biochem Behav 124: 27–35. https://doi.org/10.1016/j.pbb.2014.05.002 PMID: 24836182

24. Singh P, Gupta S, Sharma B (2016) Antagonism of endothelin (ETA and ETB) receptors during renovascular hypertension-induced vascular dementia improves cognition. Curr Neurovasc Res 13: 219–229. PMID: 27189349

25. Gannon M, Che P, Chen Y, Jiao K, Roberson ED, Wang Q (2015) Noradrenergic dysfunction in Alzheimer’s disease. Front Neurosci 9:220. https://doi.org/10.3389/fnins.2015.00220 eCollection 2015. PMID: 26136654

26. Ni Y, Zhao X, Bao G, Zou L, Teng L, Wang Z, et al. (2006) Activation of beta2-adrenergic receptor stimulates gamma-secretase activity and accelerates amyloid plaque formation. Nat Med 12: 1390–1396. https://doi.org/10.1038/nm1485 PMID: 17115048
28. Zhou Z, Liao YH, Wei Y, Wei F, Wang B, Li L, et al. (2005) Cardiac remodeling after long-term stimulation by antibodies against the alpha1-adrenergic receptor in rats. Clin Immunol 114: 164–173. https://doi.org/10.1016/j.clim.2004.09.011 PMID: 12358154

29. Zhou Z, Liao Y, Li L, Wei F, Wang B, Wei Y, et al. (2008) Vascular damages in rats immunized by alpha1-adrenergic receptor peptides. Cell Mol Immunol 5: 349–356. https://doi.org/10.1038/cmi.2008.43 PMID: 18954558

30. Di Marco LY, Venneri A, Farkas E, Evans PC, Marzo A, Frangi AF (2015) Vascular dysfunction in the pathogenesis of Alzheimer’s disease—A review of endothelium-mediated mechanisms and ensuing vicious circles. Neurobiol Dis 82: 593–606. https://doi.org/10.1016/j.nbd.2015.08.014 PMID: 26311408

31. Calabrese V, Giordano J, Signorile A, Laura Ontario M, Castorina S, De Pasquale C, et al. (2016) Major pathogenic mechanisms in vascular dementia: Roles of cellular stress response and hormesis in neuroprotection. J Neurosci Res 94: 1588–1603. https://doi.org/10.1002/jnr.23925 PMID: 27662637

32. Karczewska P, Pohlmann A, Wagenhans B, Wisbrun N, Hempel P, Lemke B, et al. Antibodies to the alpha1-adrenergic receptor cause vascular impairments in rat brain as demonstrated by magnetic resonance angiography. PLoS One 7(7): e41602. https://doi.org/10.1371/journal.pone.0041602 PMID: 22860001

33. Davel AP, Brum PC, Rossioni LV (2014) Isoproterenol induces vascular oxidative stress and endothelial dysfunction via a Gia-coupled β2-adrenoceptor signaling pathway. PLoS One 9(3): e91877. https://doi.org/10.1371/journal.pone.0091877 PMID: 24622771

34. Jahns R, Boege F (2015) Questionable validity of peptide-based ELISA strategies in the diagnostics of cardiopathogenic autoantibodies that activate G-protein-coupled receptors. Cardiology 131: 149–150. https://doi.org/10.1159/000376546 PMID: 25926265

35. Bornholz B, Hanel A, Reinke Y, Felix SB, Jahns R, Schimke I, et al. (2016) Detection of DCM-associated β1-adrenergic receptor autoantibodies requires functional readouts or native human β1-receptors as targets. Int J Cardiol 202: 728–730. https://doi.org/10.1016/j.ijcard.2015.10.068 PMID: 26461921

36. Diamond B, Honig G, Mader S, Brimberg L, Volpe BT (2013) Brain-reactive antibodies and disease. Annu Rev Immunol 31: 345–385. https://doi.org/10.1146/annurev-immunol-020711-075041 PMID: 23516983

37. Brimberg L, Mader S, Fujieda Y, Arinuma Y, Kowal C, Volpe BT, Diamond B (2015) Antibodies as Mediators of Brain Pathology. Trends Immunol 36: 709–724. https://doi.org/10.1016/j.it.2015.09.008 PMID: 26494046

38. Erdő F, Denes L, de Lange E (2017) Age-associated physiological and pathological changes at the blood-brain barrier: A review. J Cereb Blood Flow Metab 37: 4–24. https://doi.org/10.1177/0271678X16679420 PMID: 27837191

39. Schenk GJ, de Vries HE (2016) Altered blood-brain barrier transport in neuro-inflammatory disorders. Drug Discov Today Technol 20: 5–11. https://doi.org/10.1016/j.ddtec.2016.07.002 PMID: 27986224

40. Hempel P, Heinig B, Jerosch C, Decius I, Karczewska K, Kassner U, et al. (2016) Immunoadsorption of Antigenic Autoantibodies against α1-Adrenergic Receptors in Patients With Mild to Moderate Dementia. Ther Apher Dial 20: 523–529. https://doi.org/10.1111/1744-9987.12415 PMID: 27096216

41. Liao YH, Wei YM, Wang M, Wang ZH, Yuan HT, Cheng LX (2002) Autoantibodies against AT1-receptor and alpha1-adrenergic receptor in patients with hypertension. Hypertens Res 25: 641–646. PMID: 12358154

42. Wenzel K, Haase H, Wallukat G, Derer W, Bartel S, Homuth V, et al. (2008) Potential relevance of alpha(1)-adrenergic receptor autoantibodies in refractory hypertension. PLoS One 3(11): e3742. https://doi.org/10.1371/journal.pone.0003742 PMID: 19011682

43. St-Amour I, Cicchetti F, Calon F (2016) Immunotherapies in Alzheimer’s disease: Too much, too little, too late or off-target? Acta Neuropathol 131: 481–504. https://doi.org/10.1007/s00401-015-1518-9 PMID: 26689922

44. Haberland A, Holtzrueter M, Schlichtiger A, Bartel S, Schimke I, Muller J, et al. (2007) Aptamer BC 007 —A broad spectrum neutralizer of pathogenic autoantibodies against G-protein-coupled receptors. Eur J Pharmacol 789: 37–45. https://doi.org/10.1016/j.ejphar.2016.06.061 PMID: 27375076

45. Wallukat G, Muller J, Haberland A, Berg S, Schulz A, Freyse EJ, et al. (2016) Aptamer BC007 for neutralization of pathogenic autoantibodies directed against G-protein coupled receptors: A vision of future treatment of patients with cardiomyopathies and positivity for those autoantibodies. Atherosclerosis 244: 44–47. https://doi.org/10.1016/j.atherosclerosis.2015.11.001 PMID: 2684137

46. Jahns R, Jahns V, Lohse M, & Palm D. 2005. Means for the inhibition of anti 1-adrenergic receptor antibodies. WO 2006/103101 A2; 2005. http://www.google.com/patents/WO2006103101A2?cl=en
47. Schimke I, Haberland A, Kage A, Wallukat G, Dahmen C. Aptamers that inhibit interaction between antibody and 2nd extracellular loop of human beta1-adrenergic receptor. WO/2012/000889; 2012. https://www.bing.com/search?q=kanton+teissin&form=EDGEAR&qs=HS&cid=3cc69578c6e14592ba4f3d80c2547&cc=DE&setlang=de-DE

48. A study to investigate the safety, tolerability, pharmacokinetics and efficacy of BC 007 in healthy subjects. ClinicalTrials.gov Identifier NCT02955420; 2016. https://clinicaltrials.gov/ct2/show/NCT02955420