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### Authors

Knight, Elysse M

Kim, Soong Ho

Kottwitz, Jessica C

et al.

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# Effective anti-Alzheimer A $\beta$ therapy involves depletion of specific A $\beta$ oligomer subtypes

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Elysse M. Knight, PhD  
Soong Ho Kim, PhD  
Jessica C. Kottwitz, BS  
Asa Hatami, PhD  
Ricardo Albay, BS  
Akinobu Suzuki, PhD  
Alex Lublin, PhD  
Cristina M. Alberini, PhD  
William L. Klein, PhD  
Paul Szabo, PhD  
Norman R. Relkin, MD,  
PhD  
Michelle Ehrlich, MD  
Charles G. Glabe, PhD  
Sam Gandy, MD, PhD  
John W. Steele, PhD

Correspondence to  
Dr. Gandy:  
Samuel.Gandy@mssm.edu

## ABSTRACT

**Background:** Recent studies have implicated specific assembly subtypes of  $\beta$ -amyloid (A $\beta$ ) peptide, specifically soluble oligomers (soA $\beta$ ) as disease-relevant structures that may underlie memory loss in Alzheimer disease. Removing existing soluble and insoluble A $\beta$  assemblies is thought to be essential for any attempt at stabilizing brain function and slowing cognitive decline in Alzheimer disease. IV immunoglobulin (IVIg) therapies have been shown to contain naturally occurring polyclonal antibodies that recognize conformational neopeptides of soluble or insoluble A $\beta$  assemblies including soA $\beta$ . These naturally occurring polyclonal antibodies have been suggested to underlie the apparent clinical benefits of IVIg. However, direct evidence linking anti-A $\beta$  antibodies to the clinical bioactivity of IVIg has been lacking.

**Methods:** Five-month-old female Dutch APP E693Q mice were treated for 3 months with neat IVIg or with IVIg that had been affinity-depleted over immobilized A $\beta$  conformers in 1 of 2 assembly states. Memory was assessed in a battery of tests followed by quantification of brain soA $\beta$  levels using standard anti-soA $\beta$  antibodies.

**Results:** We provide evidence that NU4-type soA $\beta$  (NU4-soA $\beta$ ) assemblies accumulate in the brains of Dutch APP E693Q mice and are associated with defects in memory, even in the absence of insoluble A $\beta$  plaques. Memory benefits were associated with depletion from APP E693Q mouse brain of NU4-soA $\beta$  and A11-soA $\beta$  but not OC-type fibrillar A $\beta$  oligomers.

**Conclusions:** We propose that targeting of specific soA $\beta$  assembly subtypes may be an important consideration in the therapeutic and/or prophylactic benefit of anti-A $\beta$  antibody drugs. *Neurol Neuroimmunol Neuroinflamm* 2016;3:e237; doi: 10.1212/NXI.0000000000000237

## GLOSSARY

A $\beta$  =  $\beta$ -amyloid; AD = Alzheimer disease; EPM = elevated plus maze; FC = fear conditioning; IG = Immune Globulin; IVIg = IV immunoglobulin; nAb = naturally occurring antibody; NOR = novel object recognition; nTg = nontransgenic; oA $\beta$  = oligomeric  $\beta$ -amyloid; PBS = phosphate-buffered saline; SA = spontaneous alternation; soA $\beta$  = soluble oligomeric  $\beta$ -amyloid.

Alzheimer disease (AD), the most common form of dementia among the elderly, is attended by decades of accumulation of the neurotoxic  $\beta$ -amyloid (A $\beta$ ) peptide.<sup>1</sup> Removing existing soluble and insoluble A $\beta$  assemblies is thought to be essential for stabilizing brain function and slowing cognitive decline. While prior active or passive immunotherapies have been successful in AD mouse models, success in clinical trials has been elusive.<sup>2</sup>

IV immunoglobulin (IVIg) consists of purified plasma Ig pooled from thousands of healthy donors, is associated with reduced risk of developing AD,<sup>3</sup> and was shown to contain naturally

Supplemental data  
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occurring antibodies against A $\beta$  (nAbs-A $\beta$ ).<sup>4,5</sup> Such nAbs-A $\beta$  appear to be decreased in patients with AD, suggesting that some component(s) of IVIg may be useful for the treatment of early sporadic or familial forms of AD,<sup>4,6</sup> and an independent phase 3 trial of IVIg yielded benefit in patients with moderate-stage AD who carried an *APOE*  $\epsilon$ 4 allele.<sup>7</sup>

Immune Globulin (IG), an IVIg therapy developed by Baxter Pharmaceuticals, has shown benefit in AD models<sup>8,9</sup> and produced cognitive benefit in early trials.<sup>10,11</sup> IG contains nAbs that recognize conformational neopeptides on detergent-soluble and -insoluble A $\beta$  aggregates. However, direct evidence linking anti-A $\beta$  antibodies to the clinical bioactivity of IG has been lacking. The aim of this study was to test the effects of neat or A $\beta$ -affinity-depleted forms of IG on learning behaviors and pathology in Dutch APP E693Q<sup>12</sup> transgenic mice, and to determine whether improved learning behavior(s) might be associated with the depletion of specific soluble oligomeric A $\beta$  (soA $\beta$ ) immunosubtypes.

**METHODS** **Experimental animals.** Animal procedures were conducted in accordance with the NIH Guidelines for the Care and Use of Experimental Animals and were approved by the Institutional Animal Care and Use Committee at the Icahn School of Medicine at Mount Sinai. All mice were given ad libitum access to food and water, and housed in micro-isolator cages under a 12-hour light/dark cycle. Generation of Dutch (APP E693Q) and PS1 $\Delta$ E9 transgenic mouse lines have been described previously.<sup>12</sup> For baseline cued and contextual fear conditioning (FC) behavior, experimentally naive, 6-month-old male and female mice were used: nontransgenic (nTg; n = 8), Dutch (n = 9), and Dutch/PS1 $\Delta$ E9 (n = 13).

For IG drug-treatment experiments, 5-month-old female Dutch APP E693Q transgenic mice were given weekly subcutaneous injections of either saline (n = 11) or 2 g/kg neat Baxter IG (n = 12), 2 g/kg IG depleted of anti-fibril A $\beta$  antibodies (fibril A $\beta$ -affinity-depleted IG; n = 11), 2 g/kg IG depleted of anti-oligomer A $\beta$  antibodies (oligomer A $\beta$ -affinity-depleted IG; n = 11), or 2 g/kg IG depleted of both anti-oligomer and anti-fibril A $\beta$  antibodies (A $\beta$ -affinity-depleted IG; n = 11) for 3 months.

**Preparation of A $\beta$  monomers, oligomers, and fibrils for affinity depletion.** Resin bearing A $\beta$ <sub>42</sub> monomers coupled at either the N or C terminus was obtained from Alpha Diagnostic International (San Antonio, TX). A $\beta$  globular oligomers were prepared as previously described.<sup>13</sup> The resultant oligomers were characterized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and stored at 4°C for up to 2 weeks. Stabilized oligomers, for coupling to affinity columns, were cross-linked by treatment with 1 mM glutaraldehyde for 15 minutes at 20°C and terminated in 5 mM ethanolamine at 20°C. The ethanolamine was removed by ultrafiltration using a Vivaspin

centrifugal concentrator with a 10-kDa cutoff centrifugal concentrator (Sartorius Stedim, Bohemia, NY). A $\beta$  fibrils were prepared as described.<sup>14</sup> HFIP-treated A $\beta$ <sub>40</sub> monomer was dissolved in 2 mM NaOH, then centrifuged at 10,000g for 60 minutes to remove amyloid clumps. The supernatant was adjusted to 1 $\times$  phosphate-buffered saline (PBS) 0.05% NaAz and incubated with agitation at 37°C for 14 days. Fibril structure was confirmed by electron microscopy. Before coupling onto the affinity resin, the fibrils were sonicated as described.<sup>15</sup>

**Depletion of anti-A $\beta$  antibodies from Baxter IG by affinity chromatography.** Resins for the depletion of anti-A $\beta$  oligomer and anti-A $\beta$  fibril antibodies were prepared as described.<sup>16</sup> Baxter IG at 10 mg/mL in PBS was passed at least 5 times over columns at 1 to 2 mL/min to deplete either anti-oligomer or anti-fibril antibodies. To produce IG deficient in antibodies against all types of amyloid, the columns, including monomer amyloid columns, were run in series. Unbound IG from the various columns was concentrated to approximately 500 mg/mL by ultrafiltration through 30k molecular weight cutoff filters (Sartorius Stedim) and dialyzed against PBS before use in the animal studies.

**Behavioral testing.** Mice were placed in the testing room 1 hour before testing to acclimatize to the room. All equipment was cleaned between animals. Memory was assessed in the novel object recognition (NOR), Y-maze spontaneous alternation (SA), and contextual/cued FC tests and anxiety-like behavior assessed in the elevated plus maze (EPM) as previously described.<sup>17,18</sup>

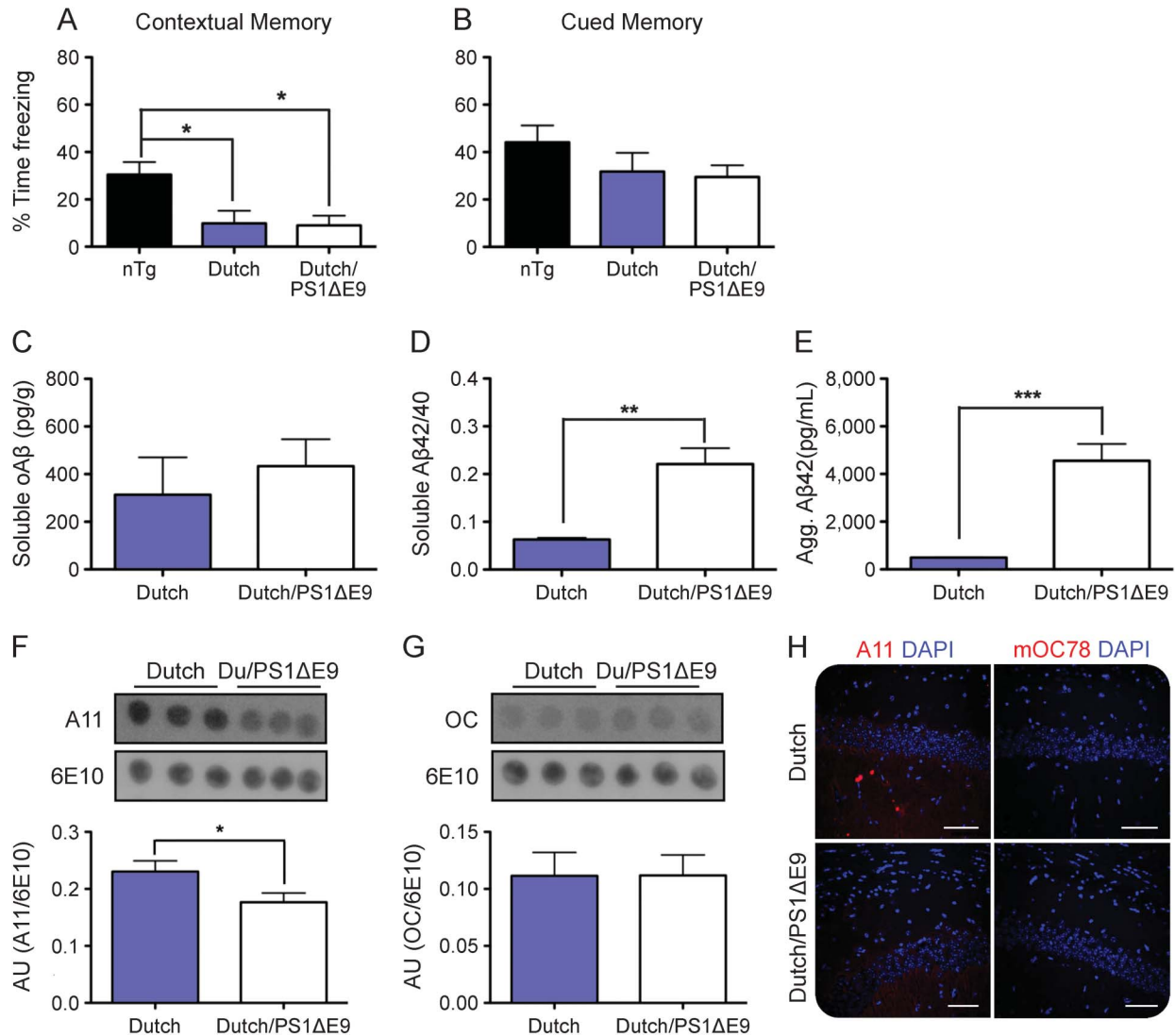
**Tissue preparation.** Animals were killed, perfused with ice-cold 1 $\times$  PBS, and brains were hemisected. One hemisphere was postfixed in 4% paraformaldehyde, stored at 4°C, and prepared for immunohistologic analyses. The remaining hemisphere was snap frozen and stored at –80°C before biochemical analysis. Brain samples were prepared for biochemical analysis as described previously.<sup>19</sup>

**Antibody-based assays.** For a list of antibodies used in the current study, or for immunofluorescence and immunohistology, Western blot, dot blot, and ELISA assays described here, see e-Methods at [Neurology.org/nn](http://Neurology.org/nn).

**Statistical analysis.** Independent-samples *t* tests were used to determine significant differences between 2 groups. One-way analyses of variance with Bonferroni post hoc analyses were used to compare 3 or more groups. Significance for *t* tests and analyses of variance are reported with a *p*  $\leq$  0.05 using 2-tailed tests with an  $\alpha$  level of 0.05. All statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA).

**RESULTS** **Impairment of hippocampus-dependent memory is evident at 6 months of age in Dutch and Dutch APP/PS1 $\Delta$ E9 mice.** Six-month-old nTg, Dutch APP, and Dutch APP/PS1 $\Delta$ E9 mice were trained and tested on the cued and contextual FC paradigm. By comparison to their nTg littermates, both Dutch APP and Dutch APP/PS1 $\Delta$ E9 mice displayed significant impairment of contextual fear memory, whereas no differences were observed among the 3 groups for cued fear memory (figure 1, A and B). No significant differences were observed between male and female mice for performance on cued or contextual FC tasks

**Figure 1** Impaired contextual memory is associated with levels of soluble fibrillary oligomeric A $\beta$  assemblies



Six-month-old mice were trained and tested for contextual (A) and cued (B) fear memory. Brain lysates were analyzed for levels of soA $\beta$  (C), soluble A $\beta_{42/40}$  (D), and insoluble aggregated A $\beta_{42}$  (E). Using conformation-specific antibodies, levels of soluble prefibrillar (F) or fibrillar (G) oA $\beta$  were analyzed from brain lysates. (H) Brain sections were stained for soluble prefibrillar oA $\beta$  assemblies (A11) or nuclear oligomeric assemblies (M78); scale bars = 40  $\mu$ m;  $\times$ 20 magnification, counterstain is DAPI. Data are representative littermates; histograms represent mean  $\pm$  SEM; \* $p$  < 0.05; \*\* $p$  < 0.01; \*\*\* $p$  < 0.001. A $\beta$  =  $\beta$ -amyloid; Agg. = aggregated; AU = arbitrary units; DAPI = 4',6-diamidino-2-phenylindole; nTg = nontransgenic; oA $\beta$  = oligomeric  $\beta$ -amyloid; soA $\beta$  = soluble oligomeric  $\beta$ -amyloid.

(data not shown), consistent with our prior observations using the Morris water maze task<sup>12</sup>; therefore, males and females were combined for analysis. Because both contextual and cued fear memory rely on an intact amygdala,<sup>20</sup> but only contextual memory relies additionally on an intact hippocampus, our data suggest that impaired contextual memory reflects a defect in hippocampus-dependent memory. This finding is consistent with prior studies of AD mouse models<sup>12,19,20</sup> and patients with AD.<sup>21</sup> No difference was observed between Dutch APP and Dutch/PS1ΔE9 mice for contextual memory, suggesting that the cause of cognitive defect may be common (i.e., related to APP E693Q transgene product)

among the 2 mouse lines and not related to the accumulation of amyloid plaques that occurs only in Dutch/PS1ΔE9 mice.

**Accumulation of soluble, but not insoluble, oligomeric A $\beta$  assemblies is a common feature among Dutch APP and Dutch APP/PS1ΔE9 mice.** Using a 7n22/7n22 duplicate-epitope sandwich immunoassay, we measured levels of total soA $\beta$  assemblies from both mouse lines. No differences were observed between Dutch APP and Dutch APP/PS1ΔE9 littermates for levels of soA $\beta$  assemblies (figure 1C). Next, we measured levels of soluble A $\beta_{40}$  and A $\beta_{42}$ , revealing a significant difference between Dutch APP and Dutch APP/

PS1 $\Delta$ E9 mice for A $\beta$ <sub>42</sub>/A $\beta$ <sub>40</sub> ratio (figure 1D), which is consistent with expression of the PS1 $\Delta$ E9 transgene.<sup>12,22</sup> We also observed a nearly 10-fold-higher accumulation of detergent-insoluble aggregated A $\beta$ <sub>42</sub> in the brains of Dutch APP/PS1 $\Delta$ E9 mice as compared to their Dutch APP littermates (figure 1E).

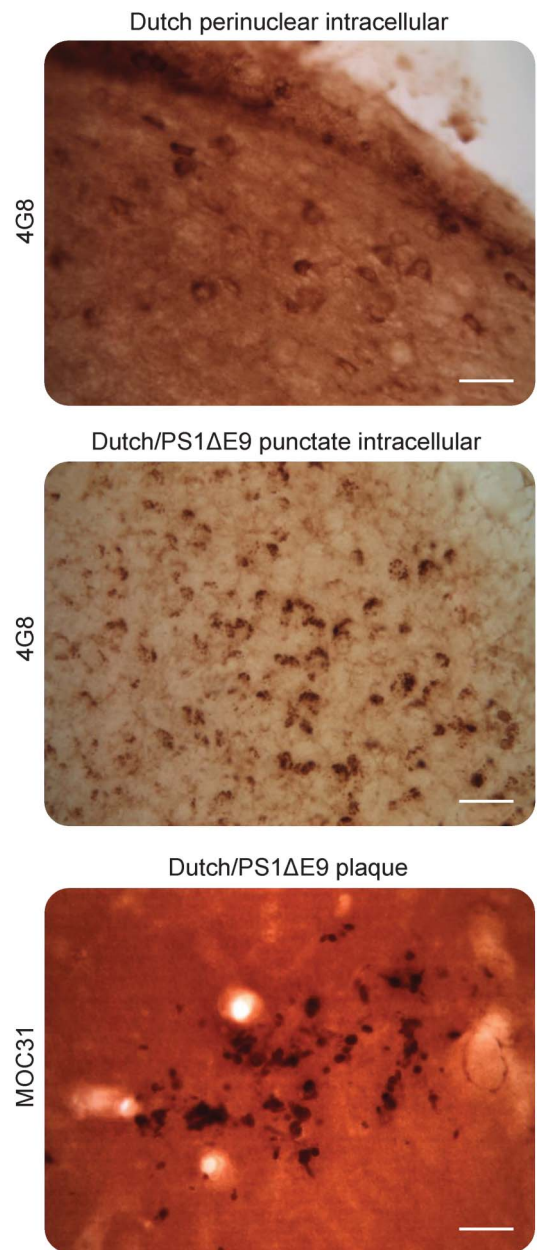
Based on the observation that both mouse lines accumulate the same amount of soA $\beta$  assemblies (figure 1C), we utilized conformation-specific antibodies to assess the native conformation of the soA $\beta$  assemblies. We noted a significant decrease in levels of A11-immunoreactive soluble prefibrillar oA $\beta$  assemblies among Dutch APP/PS1 $\Delta$ E9 mice when compared to their Dutch APP littermates (figure 1, F and H), whereas no difference was observed between the 2 genotypes for levels of OC-immunoreactive soluble fibrillar oA $\beta$  assemblies (figure 1G).

Previously, we reported marked intraneuronal accumulation of APP/A $\beta$  in the brains of both Dutch APP and Dutch APP/PS1 $\Delta$ E9 mice.<sup>12</sup> We further characterized this pathology, revealing subtle differences in intra- and extraneuronal pathology between the genotypes (figure 2). Whereas Dutch APP mice primarily exhibited perinuclear intracellular 4G8-immunoreactivity without plaque-like M31-immunoreactivity, Dutch APP/PS1 $\Delta$ E9 mice exhibited punctate intracellular 4G8-immunoreactivity and plaque-like M31-immunoreactivity (figure 2).

We analyzed brain lysates from nTg, Dutch APP, and Dutch APP/PS1 $\Delta$ E9 for markers of autophagic/lysosomal stasis including LC3, p62,  $\alpha$ -synuclein, and APP in order to determine whether autophagic/lysosomal failure was evident and/or associated with accumulation of soA $\beta$  assemblies or insoluble A $\beta$ <sub>42</sub>. No accumulation of soluble LC3, p62, or  $\alpha$ -synuclein was observed by comparison to nTg controls among either Dutch APP or Dutch APP/PS1 $\Delta$ E9 mice, suggesting that autophagic/lysosomal clearance may be intact at this stage of pathology (figure 3). However, we noted a significant accumulation of insoluble p62 among only Dutch APP/PS1 $\Delta$ E9 mice by comparison to their nTg and Dutch APP littermates (figure 3, A and C) and this effect was also observed to precede onset of autophagic/lysosomal failure in TgCRND8 mice.<sup>19</sup> Based on this result, it is tempting to speculate that the insoluble accumulation of A $\beta$ <sub>42</sub> and p62 may represent an early marker of toxicity to the autophagic/lysosomal pathway; however, further aging studies with these mouse lines will be necessary to determine whether autophagic/lysosomal failure occurs at any age.

**Treatment of Dutch mice with IG rescued learning behavior.** Next, we sought to determine whether antibodies against various forms of soluble or insoluble

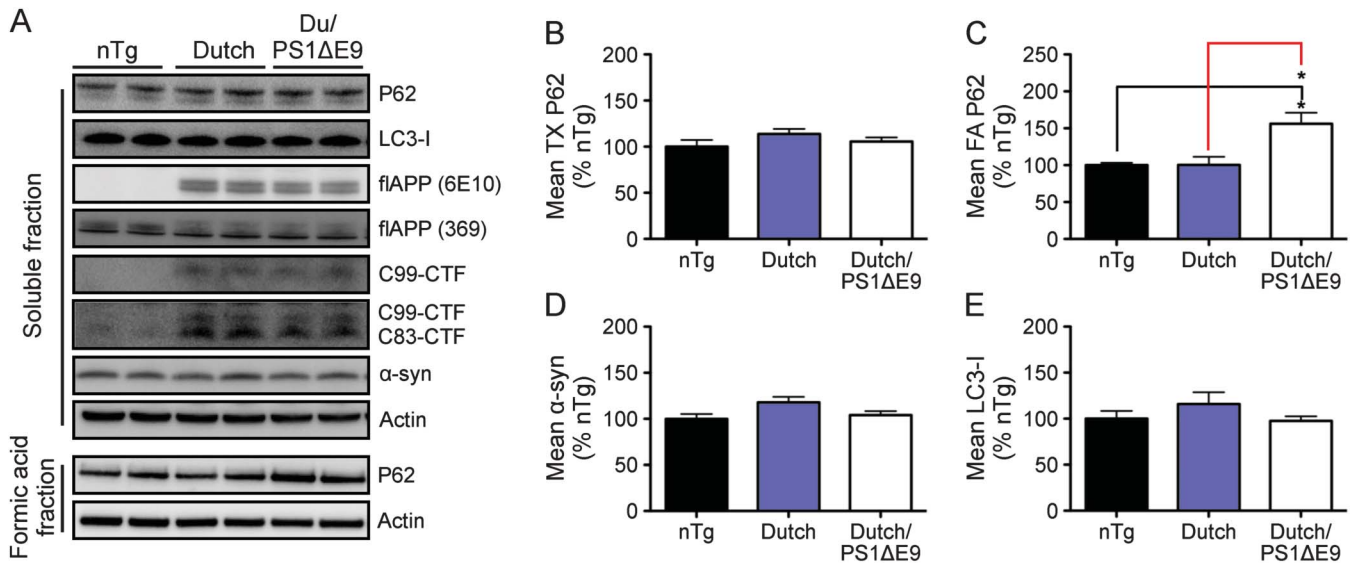
**Figure 2** Subtle differences in intra- and extraneuronal accumulation of A $\beta$  between Dutch and Dutch/PS1 $\Delta$ E9 mice



Brains were stained to analyze intracellular accumulation of APP/A $\beta$  (4G8) or extracellular accumulation of A $\beta$  (mOC31). Punctate immunoreactivity to 4G8 was typically observed in Dutch APP/PS1 $\Delta$ E9 mice, whereas Dutch APP mouse brains typically exhibited perinuclear immunoreactivity. Only Dutch APP/PS1 $\Delta$ E9 mice accumulated insoluble mOC31-immunoreactive extracellular plaques. Micrographs are representative littermates; scale bars = 10  $\mu$ m;  $\times$ 20 magnification. A $\beta$  =  $\beta$ -amyloid.

A $\beta$  assemblies may be responsible for the benefit provided by IG therapy. We previously reported deficits on hippocampus-dependent memory in the Morris water maze task and FC in the Dutch APP mouse line.<sup>12,23</sup> Here, we chose a battery of tasks that target

**Figure 3** Only Dutch APP/PS1ΔE9 mice accumulate insoluble p62



Brain lysates were analyzed for levels of soluble (LC3, p62, α-synuclein) and insoluble (p62) autophagic/lysosomal substrates and APP metabolites (A-E). No impairment of autophagic/lysosomal clearance was observed at this age; however, only Dutch/PS1ΔE9 mice accumulated insoluble p62, which we previously reported to occur before autophagic/lysosomal failure in the brains of TgCRND8 mice<sup>19</sup>. Western blots are representative littermates, histograms represent mean ± SEM; \**p* < 0.05; 1-way analysis of variance with Bonferroni post hoc analyses. nTg = nontransgenic.

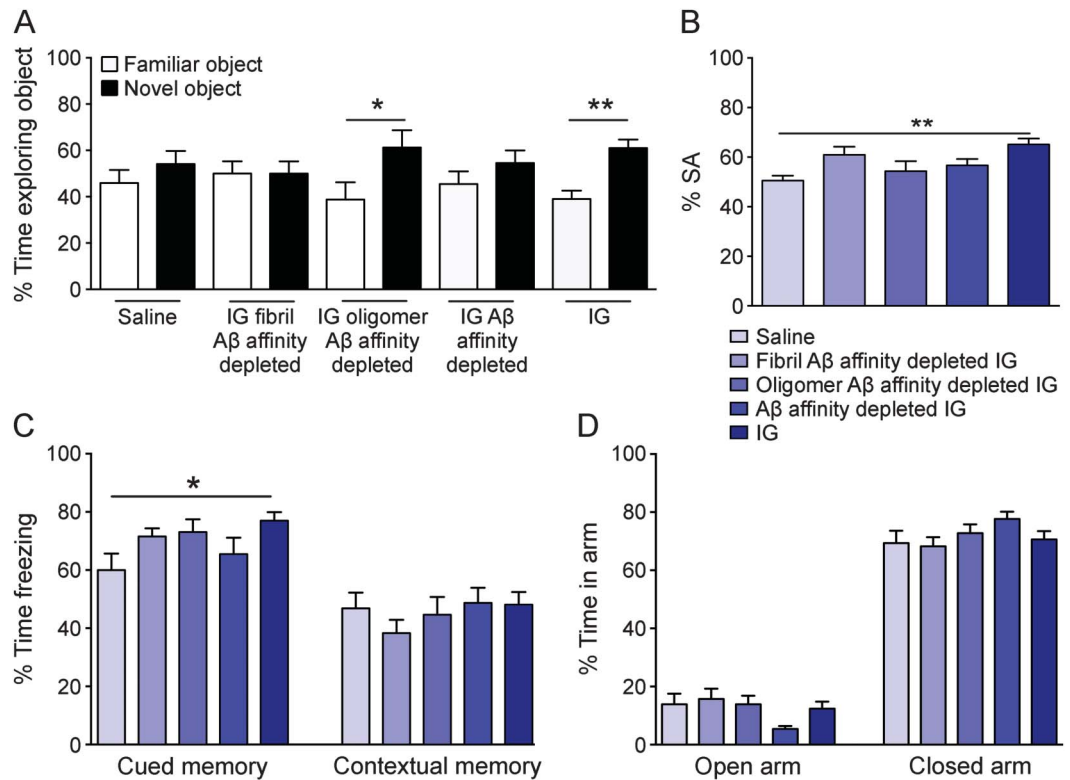
both hippocampus-dependent memory as well as the entorhinal cortex and amygdala to assess complete memory function; these include FC,<sup>17,23</sup> NOR,<sup>17,23</sup> and Y-maze SA (previously untested), or anxiety-like behaviors using the EPM (previously reported<sup>17,23</sup>). Three-month treatment of Dutch APP mice by once-weekly subcutaneous injection of neat IG prevented onset of NOR (figure 4A), SA (figure 4B), and FC (figure 4C) deficits, without affecting anxiety-like behavior in the EPM (figure 4D) in female Dutch APP mice. This behavioral protection was completely abrogated by treatment with either saline or fibrillar Aβ-affinity-depleted or total Aβ-affinity-depleted IG. However, a statistically significant benefit in learning behavior tasks (e.g., NOR) was observed among Dutch APP mice treated with oligomer Aβ-affinity-depleted IG (figure 4A).

Treatment of mice with neat IG did not affect brain levels of Aβ<sub>40</sub> (figure 5, A–D) or Aβ<sub>42</sub> (figure 5, E–H), or its ratio (figure 5, I–L), but rather reduced brain levels of both toxic prefibrillar A11-immunoreactive soAβ (figure 5M) and NU-4-immunoreactive assemblies (figure 5N) in Dutch APP mice. Levels of OC-immunoreactive soluble fibrillar oligomers were not affected following treatment with neat IG in Dutch mice (figure 5O). Treatment of mice with fibrillar Aβ-affinity-depleted IG apparently reduced brain levels of NU-4-immunoreactive (figure 5N) but not A11-immunoreactive (figure 5M) soAβ assemblies in Dutch APP mice. Thus, neat IG treatment was associated with clinical benefit and reduction of 2 immunosubtypes of soAβ assemblies

(i.e., both A11-type and NU-4-type oligomers). Among 3 immunoaffinity-depleted IG preparations, only one was clinically effective, and, unexpectedly, that bioactive fraction was the fibrillar Aβ-affinity-depleted IG that was associated with reduction in the content of NU-4-type soAβ assemblies. We cannot determine whether reduction in the content of A11-type soAβ assemblies might also be associated with clinical benefit. The relevance of this point is discussed in detail below.

**DISCUSSION** The current study indicates the utility of rodent models to study multiple stages of AD-related pathology, the relationship of Aβ pathology to cognitive status, and the ability to separate canonical Aβ plaque pathology from underlying toxic mechanisms. Here, we describe a hippocampus-dependent contextual fear memory deficit at 6 months of age in Dutch APP and Dutch APP/PS1ΔE9 mice, and the accumulation of cognitive deficit-associated soAβ assemblies that are common to both lines. Of note, prior studies indicate that A11-immunoreactive prefibrillar soAβ assemblies are associated with conversion from soluble low-n multimers to insoluble fibrillar assemblies, whereas OC-immunoreactive soluble fibrillar soAβ assemblies do not form fibrils and, rather, seed formation of more soluble fibrillar soAβ assemblies.<sup>24</sup> Moreover, levels of soAβ assemblies were recently found to be predictive of cognitive status at death among patients with AD,<sup>25</sup> validating our findings (as discussed above) and the utility of these mouse models of AD. Here, we noted a significant decrease in A11-immunoreactive soAβ assemblies among Dutch APP/PS1ΔE9 mice as

**Figure 4** Prevention of onset of behavioral deficits following 3-month treatment of Baxter IG in Dutch APP E693Q transgenic mice



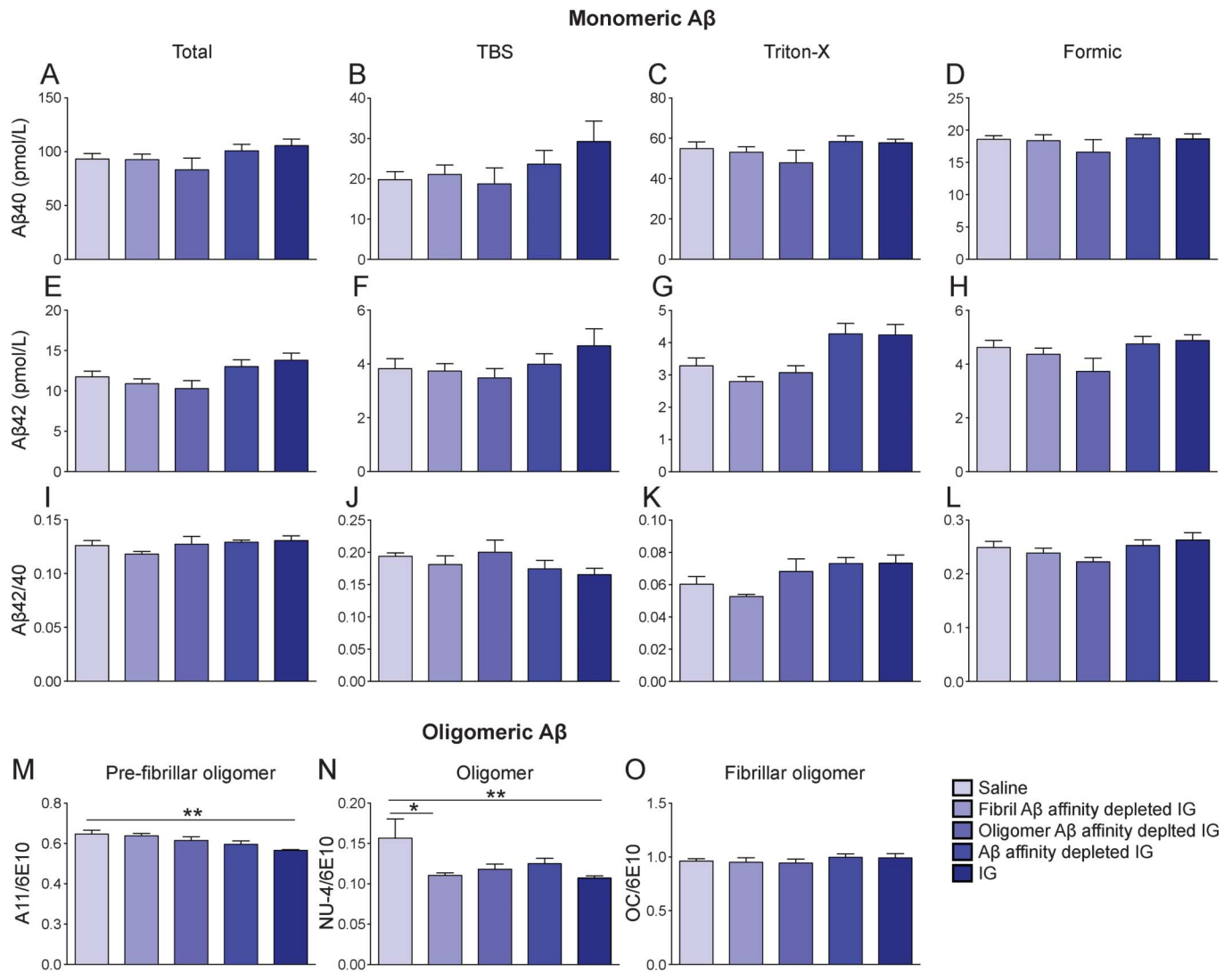
Five-month-old female Dutch APP E693Q transgenic mice were given weekly subcutaneous injections of either saline ( $n = 11$ ) or 2 g/kg neat Baxter IG ( $n = 12$ ), 2 g/kg IG depleted of anti-fibril A $\beta$  antibodies (fibril A $\beta$ -affinity-depleted IG;  $n = 11$ ), 2 g/kg IG depleted of anti-oligomer A $\beta$  antibodies (oligomer A $\beta$ -affinity-depleted IG;  $n = 11$ ), or 2 g/kg IG depleted of both anti-oligomer and anti-fibril A $\beta$  antibodies (A $\beta$ -affinity-depleted IG;  $n = 11$ ) for 3 months. Unlike treatment of saline, fibril A $\beta$ -affinity-depleted IG and A $\beta$ -affinity-depleted IG, treatment of IG prevented onset of behavioral deficits in novel object recognition (A), Y-maze spontaneous alternation (B), and contextual/cued fear conditioning (C) without affecting anxiety-like behavior in the elevated plus maze (D) in Dutch APP E693Q transgenic mice. Treatment of oligomer A $\beta$ -affinity-depleted IG prevented onset of behavioral deficit in novel object recognition (A) in Dutch APP E693Q transgenic mice. \* $p < 0.05$ ; \*\* $p < 0.01$ ; 1-way analysis of variance with Bonferroni post hoc analyses. Data expressed as mean  $\pm$  SEM. A $\beta$  =  $\beta$ -amyloid; IG = Immune Globulin.

compared to their Dutch APP littermates (figure 1, F–H; table). Our conclusions are supported by recent work by Liu et al.<sup>26</sup> who analyzed contributions of A11- and OC-immunoreactive soA $\beta$  assemblies to cognitive failure in Tg2576 mice and concluded that A11-immunoreactive, but not OC-immunoreactive, soA $\beta$  assemblies were associated with impaired memory. Based on these reports and our current results, we propose that A11- and/or NU-4-immunoreactive soA $\beta$  assemblies constitute key soA $\beta$  assemblies related to early hippocampal and entorhinal cortex-related memory failures in AD. Moreover, we propose that the Dutch APP E693Q mutation can support stabilization of these structures in a soluble state, perpetuating memory failure despite a failure to polymerize into insoluble fibrillar assemblies. A more dramatic example of this situation is exemplified by the Arctic APP E693G mutation wherein typical clinical AD develops despite the inability of the mutant peptide to form plaques, a property evidenced by the

failure of [<sup>11</sup>C] Pittsburgh compound B to detect cerebral amyloidosis at any stage of the Arctic APP E693G-related familial AD.<sup>27</sup>

Our group recently performed an integrative genomic analysis of the transcriptomes from human AD brain tissue or 2 independent mouse lines (including the Dutch APP mouse line) to identify altered molecules and pathways between wild-type and transgenic mice, and human patients with AD.<sup>28</sup> These studies revealed consistent alterations in APP/A $\beta$  metabolism, epigenetic control of neurogenesis, cytoskeletal organization, and extracellular matrix regulation. Comparison of these transcriptomic results with human AD postmortem gene expression data indicated significant similarities in pathway alterations between mouse models and patients with AD. In addition, it has been reported that changes in neuroinflammation-related gene regulation at early stages of AD was not related to neurofibrillary tangles, A $\beta$  plaque burden, total A $\beta$ <sub>40</sub> or A $\beta$ <sub>42</sub>, or membrane-bound

**Figure 5** Reduction of oligomeric A $\beta$  following 3-month treatment of Baxter IG in Dutch APP E693Q transgenic mice



Five-month-old female Dutch APP E693Q transgenic mice were given weekly subcutaneous injections of either saline (n = 11) or 2 g/kg neat Baxter IG (n = 12), 2 g/kg IG depleted of anti-23 fibril A $\beta$  antibodies (fibril A $\beta$ -affinity-depleted IG; n = 11), 2 g/kg IG depleted of anti-oligomer A $\beta$  antibodies (oligomer A $\beta$ -affinity-depleted IG; n = 11), or 2 g/kg IG depleted of both anti-oligomer and anti-fibril A $\beta$  antibodies (A $\beta$ -affinity-depleted IG; n = 11) for 3 months. A $\beta_{40}$  (A–D), A $\beta_{42}$  (E–H), or A $\beta_{42/40}$  ratio (I–L) were unaffected with all treatments. Treatment with IG reduced brain levels of prefibrillar oligomers (M) and oligomers (N) but not fibrillar oligomers (O) in Dutch APP E693Q transgenic mice. Treatment of fibril A $\beta$ -affinity-depleted IG reduced brain levels of oligomers in Dutch APP E693Q transgenic mice (N). (A, E, I) Total A $\beta$ . (B, F, J) TBS fraction. (C, G, H) Triton-X fraction. (D, H, I) Formic acid fraction. (M, N, O) TBS fraction (n = 5/group). \*p < 0.0; \*\*p < 0.01; 1-way analysis of variance with Bonferroni post hoc analyses. Data expressed as mean  $\pm$  SEM. A $\beta$  =  $\beta$ -amyloid; IG = Immune Globulin.

fibrillar A $\beta$ , but rather to increased levels of soA $\beta$ .<sup>29</sup> Taken together, these studies suggest that soA $\beta$  assemblies represent a critical driver of early-stage disease processes, leading to cognitive and cellular dysfunction observed at later stages of disease.

IVIg formulations have been previously assessed in other mouse models of AD, demonstrating that peripheral administration of IVIg crossed the blood–brain barrier and binding of A $\beta$  deposits in APP/PS-1 mice. In ex vivo APP/PS-1 brain sections, IVIg enhanced microglial-mediated A $\beta$  clearance and depletion of nAbs-A $\beta$  in IVIg significantly reduced clearance.<sup>8</sup> Treatment of TgCRND8 mice with nAbs-A $\beta$ s isolated from IVIg reduced plaque load

in mice treated at 3 months of age but not those treated later during disease progression, at 12 months of age.<sup>30</sup> When memory was assessed in Tg2576 mice, nAbs-A $\beta$  treatment improved object recognition.<sup>30</sup> In contrast, when APP/PS1 $\Delta$ E9 mice were treated with IVIg for 3 or 8 months, no evidence of reduced A $\beta$  was reported.<sup>9</sup> Instead, increased monomeric A $\beta_{40}$  and A $\beta_{42}$  levels, enhancement of neurogenesis, suppression of proinflammatory cytokine gene expression, and evidence of modulation of microglial activation was observed in mice treated for 8 months.<sup>9</sup> More recently, another study in Tg2576 mice saw an improvement in memory and synaptic plasticity following IVIg treatment that was not



**Table** Summary of A $\beta$  antibodies used in the current study

Antibody	Specificity	Preparation	Clonality	Primary characterization reference
mAb 6E10	flAPP, sAPP $\alpha$ , aa3-8 of A $\beta$ , A $\beta$ monomer, oligomer, fibrils, plaques	Purified from ascites	Monoclonal	Commercially available: Covance
OC	Soluble fibrillar oligomers, fibrils, plaques	Antiserum, purified on antigen	Polyclonal	25
mOC- (e.g., mOC31)	Polymorphic aggregation states, including fibrils	Family of monoclonals derived from OC	Monoclonal	38
NU-4	Soluble fibrillar oligomers, fibrils, plaques	Purified on protein G	Monoclonal	39
A11	Soluble prefibrillar oligomers	Antiserum, purified on antigen	Polyclonal	40
IG	Polymorphic aggregation states	Baxter IVIg preparation	Polyclonal	8
Fibril-depleted IG	Polymorphic aggregation states	A $\beta$ fibril affinity depletion	Polyclonal	Current study
Oligomer-depleted IG	Polymorphic aggregation states	A $\beta$ oligomer affinity depletion	Polyclonal	Current study
A $\beta$ -depleted IG	Monomeric A $\beta$ , polymorphic aggregation states	A $\beta$ monomer affinity depletion	Polyclonal	Current study

Abbreviations: A $\beta$  =  $\beta$ -amyloid; IG = Immune Globulin; IVIg = IV immunoglobulin.

Antibodies used in the current study are summarized to reflect known specificity, preparation, and clonality. IG antibodies were administered to Dutch APP E693Q mice before behavioral testing and biochemical studies. Soluble A $\beta$  assemblies recognized by NU-4 and A11 were reduced following treatment with neat IG, but neither oligomeric-A $\beta$ -depleted IG nor A $\beta$ -depleted IG. NU-4 epitopes were also reduced in the brains of mice treated with fibril-A $\beta$ -depleted IG, suggesting that the relevant soluble oligomeric A $\beta$  assemblies providing cognitive benefit from IG are not fibrils or fibril-like in structure. Our results suggest that IG may contain NU4-like and/or A11-like antibodies that contribute to therapeutic benefit in the Dutch APP E693Q mouse model.

linked to altered monomeric A $\beta$  levels, but rather by the activation of complement components.<sup>31</sup>

IVIg contains antibodies against both monomeric and soA $\beta$ .<sup>32</sup> In vitro studies in N2a neuroblastoma cells have revealed that IVIg can disaggregate A $\beta$  fibrils and promote A $\beta$  removal<sup>33</sup> while preventing neurotoxicity-associated soA $\beta$  assemblies.<sup>5</sup> Similarly, in cultured primary mouse hippocampal neurons, treatment with IVIg has been shown to reduce A $\beta$  fibril formation and reduce A $\beta$  neurotoxicity.<sup>8</sup> Many studies, including ours, revealed no detectable reduction in monomeric A $\beta$ . When IVIg was assessed in the 3xTgAD model of AD, an improvement in NOR, reduced immunologic CD4/CD8 blood ratio, reduced interleukin (IL)-5/IL-10 ratio in the cortex together with limited effects on tau but reduced A $\beta_{42/40}$  ratio and levels of soluble 56-kDA A $\beta$  oligomers were reported.<sup>34</sup> Our study is the first to focus on oligomer immunosubtypes post IVIg treatment. In the present study, IG protection was not associated with altered levels of monomeric A $\beta_{40}$  and A $\beta_{42}$  levels but rather with decreased levels of some but not all immunosubtypes of soluble prefibrillar oligomeric A $\beta$  assemblies. A11-immunosubtype and/or NU4-immunosubtype soA $\beta$  assemblies appear to be the key conformer(s) driving memory failure. This study suggests that IG may contain A11-like and NU4-like antibodies that confer some protection in the Dutch APP mouse line. An unexpected result of this study is the improvement in NOR in the oligomer A $\beta$ -affinity-depleted IG-treated mice with no change in A $\beta$  biochemistry. One explanation for this result is that the oligomer preparation used for fibrillar and oligomer A $\beta$  affinity depletion of IG may not

contain a complete and comprehensive representation of the structurally diverse spectrum of soluble A $\beta$  assemblies. In other words, these 2 affinity-depleted IG fractions designated “oligomeric” or “fibrillar” may represent an oversimplified binary classification. Another possibility is that the nAbs may contain catalytic antibodies that induce changes in the conformations of the soA $\beta$  assemblies as part of their therapeutic bioactivity. “Chaperone-like” activity has been reported for other antibodies<sup>35,36</sup> such that the antibodies induce changes in the conformation of soA $\beta$  assemblies as part of their therapeutic action and/or as a biochemical reaction in IG-treated brain homogenates during assays. We are currently characterizing the chaperone content of our IG fractions in order to assess this possibility properly.

Baxter IG has been proposed as a potential treatment for AD, especially at moderate stage and in carriers of *APOE*  $\epsilon$ 4 alleles.<sup>37</sup> IG contains natural human polyclonal IgG antibodies that recognize conformational neopeptides expressed on soluble and insoluble A $\beta$  assemblies. The current study demonstrates for the first time that 3 months of neat IG treatment of Dutch APP transgenic A $\beta$ -oligomer-forming mice is associated with prevention of memory deficits in NOR, SA, and FC that is abrogated by affinity depletion of IG using certain conformers of soluble A $\beta$  assemblies. These principles might well be kept in mind during the continued development and optimization of active and passive anti-A $\beta$  immunotherapies and during the continued early-stage work on creating therapeutic chaperones that have the capacity to stabilize soA $\beta$  assemblies in nontoxic conformations.

## AUTHOR CONTRIBUTIONS

E.M.K. designed the experiments with M.E., P.S., N.R.R., C.M.A., C.G.G., S.G., and J.W.S. E.M.K., S.H.K., J.C.K., A.H., R.A., A.L., A.S., and P.S. performed the experiments. E.M.K. and J.W.S. analyzed the data. E.M.K., P.S., N.R.R., S.G., and J.W.S. wrote the manuscript.

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## DISCLOSURE

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## POTENTIAL CONFLICT OF INTEREST STATEMENT

J.W.S. is a cofounder, shareholder, and member of the board of directors and scientific advisory board of OrPhi Therapeutics Inc. (Carlsbad, CA). Within the past 5 years, S.G. has held research grants from Amicus Therapeutics. S.G. is also a member of the Data and Safety Monitoring Board for the Pfizer-Janssen Alzheimer's Immunotherapy Alliance.

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