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Abstract  

Introduction: We conducted a phase Ib proof of mechanism trial to determine whether bexarotene (Targretin) increases central nervous system (CNS) apolipoprotein E (apoE) levels and alters Aβ metabolism in normal healthy individuals with the APOE ε3/ε3 genotype.  

Methods: We used stable isotope labeling kinetics (SILK-ApoE and SILK-Aβ) to measure the effect of bexarotene on the turnover rate of apoE and Aβ peptides and stable isotope spike absolute quantitation (SISAQ) to quantify their concentrations in the cerebrospinal fluid (CSF). Normal subjects were treated for 3 days with bexarotene (n = 3 women, 3 men, average 32 years old) or placebo (n = 6 women, average 30.2 years old) before administration of C\textsuperscript{13}-leucine and collection of plasma and CSF over the next 48 hours. Bexarotene concentrations in plasma and CSF were also measured.  

Results: Oral administration of bexarotene resulted in plasma levels of 1 to 2 μM; however, only low nM levels were found in CSF. Bexarotene increased CSF apoE by 25% but had no effect on metabolism of Aβ peptides.  

Discussion: Bexarotene has poor CNS penetration in normal human subjects. Drug treatment resulted in a modest increase in CSF apoE levels. The absence of an effect on Aβ metabolism is likely reflective of the low CNS levels of bexarotene achieved. This study documents the utility of SILK-ApoE technology in measuring apoE kinetics in humans.  

Trial Registration: This trial is registered at clinicaltrials.gov (NCT02061878).  

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Keywords: Alzheimer’s disease; Apolipoprotein E; β amyloid; Retinoid X receptor; Bexarotene  

1. Introduction  

Alzheimer’s disease (AD) typically occurs late in life [1] and is associated with the impaired ability to clear amyloid-β (Aβ) from the brain [2,3]. Elevated levels of soluble forms of Aβ peptides are associated with the perturbation of synaptic function and neural network activity leading to the cognitive deficits observed in the disease [4].  

The most important genetic risk factor for sporadic AD is allelic variation in the Apolipoprotein E (APOE) gene, and possession of an APOE ε4 allele dramatically increases disease risk [5]. ApoE plays critical roles in the clearance and deposition of Aβ peptides [6]. ApoE scaffolds the formation of high-density lipoprotein (HDL) particles that traffic cholesterol and phospholipids throughout the brain. Cholesterol and phospholipids are transferred to apoE by...
the lipid transporter, ABCA1, to form HDL particles. Castellano et al. [7] reported that the apoE4 isoform slows Aβ clearance from brain interstitial fluid significantly more than the apoE2 and apoE3 isoforms in animals. Targeting Aβ clearance pathways have emerged as a promising therapeutic target.

We have demonstrated that apoE-containing HDL particles act to promote the proteolytic clearance of Aβ peptides from the brain of mouse models of AD. Significantly, the APOE ε4 gene product is impaired in this function [8]. In animal models, chronic induction of apoE and/or HDL expression in the brain is associated with reduced Aβ levels and improved cognitive function [8-12]. ApoE and its lipid transporters ABCA1 and ABCG1 are transcriptionally regulated by ligand-activated, type II nuclear receptors, most prominently liver X receptors (LXR) and peroxisome proliferator-activated receptor gamma (PPARγ), which form functional dimeric transcription factors through their interactions with retinoid X receptors (RXR) [13,14]. Importantly, oral administration of agonists of PPARγ and LXRs to animal models of AD results in the proteolytic degradation of soluble Aβ peptides in the interstitial fluid and by microglia [8,15,16].

Cramer et al. [17], and others [18,19], have reported that administration of the RXR agonist bexarotene increased brain apoE expression, elevated HDL levels, and enhanced normal Aβ clearance mechanisms. Bexarotene simultaneously activated the PPARs and LXRs in the brain [20,21] and reduced soluble Aβ levels in the brain and interstitial fluid [17-19,22]. The reduction in soluble Aβ peptide levels was associated with improved neural network activity and improved memory and cognition [17,18,23,24].

Bexarotene is a highly specific, Food and Drug Administration (FDA)-approved RXR agonist [25] with a favorable safety profile [26]. Bexarotene has been used clinically for the treatment of cutaneous T-cell lymphoma with chronic daily oral administration of the drug at doses of 300 mg/m²/d [27].

The objective of this proof of mechanism clinical trial was to determine whether the RXR agonist bexarotene acts in normal human subjects to increase the cerebrospinal fluid (CSF) levels of apoE and alter the clearance of Aβ. This study used stable isotope labeling kinetics (SILK) to evaluate the synthesis and clearance rate of apoE [28] and Aβ [2,7,29] in the CSF.

2. Methods

2.1. Study design

The trial was conducted as a double-blinded study, to measure the effect of bexarotene on the synthesis and clearance of Aβ peptides and production of apoE in the human brain. Compass Research, Tampa, Florida, enrolled the subjects and conducted the trial. The protocol was approved by Schulman Associates Institutional Review Board, Inc. All subjects signed informed consent forms before enrollment in the study. This study was conducted under Investigational New Drug Application 121548.

This study used SILK to measure production and clearance of Aβ peptides [29] and apoE [28,30] in the CSF of healthy volunteers. Subjects were administered 13C6-labeled leucine over 9 hours, and CSF samples were collected over the subsequent 48 hours. The incorporation of 13C6-labeled leucine into Aβ and apoE isolated from CSF was measured by mass spectrometry (MS), allowing determination of the biosynthesis and clearance of these biomolecules. Quantitation of the absolute levels of Aβ and apoE was achieved by adding Aβ and apoE internal standards to the CSF samples.

2.2. Subjects

The study population consisted of three men and nine women, aged 21 to 49 years all carrying the APOE ε3/ε3 genotype. The treatment, and average gender, age, weight, and body mass index of the subjects are detailed in Table 1. A sample size of six subjects per group allowed an 80% chance of observing a 47% change in Aβ AUC1-12 hours. There was a balanced randomization process in which subjects were randomized in a 1:1 ratio to either drug or placebo. Subjects were randomized into one of two groups based on an atmospheric (atmospheric noise) method for randomization. One group was assigned the active product, and the other group was assigned an identical placebo based on first subject received, first randomization assignment. Study subjects and researchers were blinded as to which product contained the active product or placebo.

2.3. Procedures

The subjects received either placebo or 225 mg of bexarotene (Targretin; Valeant Pharmaceuticals, Laval, Quebec) at 9 AM and 7 PM for 5 days (450 mg/d; 300 mg/m²/d). On the morning of the fourth day of dosing, subjects were admitted to the clinical research unit and researchers were blinded as to which product contained the active product or placebo.

Table 1: Subjects: The average age, weight, sex, and APOE genotype of the subjects enrolled in the study

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Bexarotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>30.2 ± 6.6</td>
<td>32 ± 9.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.9 ± 7.1</td>
<td>84.6 ± 23.2</td>
</tr>
<tr>
<td>Female sex (%)</td>
<td>6 (100)</td>
<td>3 (50)</td>
</tr>
<tr>
<td>APOE genotype</td>
<td>3/3</td>
<td>3/3</td>
</tr>
</tbody>
</table>

The subjects were randomized in a 1:1 ratio to either drug or placebo.

A lumbar catheter was inserted in the L3 to L4 interspace

Subjects signed informed consent forms before enrollment in the study.

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to acquire CSF samples. Blood was collected hourly through hour 15 of the study and then every other hour up to 48 hours. CSF samples were taken hourly until hour 36 and then every other hour until hour 48. No serious adverse events were reported during the study period. Three subjects had increased triglyceride levels greater than 200 mg/dL. One subject had increased cholesterol levels greater than 200 mg/dL. Two subjects had abnormal thyroid levels. Two subjects had abnormal aspartate aminotransferase/alanine aminotransferase levels. All abnormalities were resolved by the end of the study without treatment. Other AEs included headache (nine subjects), burping (one subject), rash (one subject), nausea (one subject), and bloating (one subject).

2.5. Analytical procedures

2.5.1. Bexarotene pharmacokinetics

Bexarotene concentration was measured in plasma and CSF samples using gas chromatography and/or MS. Briefly, a known amount of internal standard (13C4-labeled bexarotene) was added to each plasma and CSF sample. Bexarotene and 13C4-labeled bexarotene was extracted from the sample via liquid–liquid extraction [31]. Bexarotene and 13C4-labeled bexarotene were quantitated using selected-reaction monitoring MS. A standard curve consisting of samples containing 13C6-leucine–labeled, and the internal standard–labeled bexarotene at varying concentrations that covered the expected concentration range of the drug in the patient samples was constructed. The amount of endogenous bexarotene was quantified through integration of the product ion peaks and taking the ratio of the unlabeled and/or labeled bexarotene. Samples in which the amount of bexarotene measured in CSF was below the limit of quantitation (LOQ) were recorded as <0.021 μM.

2.5.2. Measurement of free plasma leucine levels

Plasma leucine concentrations were quantitated using Agilent 6890 gas chromatography–mass spectrometry (GC-MS) [32]. Selected ion monitoring was used to detect 13C6-leucine (m/z 349) and unlabeled (m/z 355) leucine. The molar enrichment of 13C6-leucine was determined using calibration curves prepared with isotopic standards (Cambridge Isotope Laboratories, Andover, MA, USA).

2.5.3. Quantitation and metabolism of Aβ40 and total Aβ in human CSF samples

CSF samples were combined with an internal standard and incubated with anti-Aβ (central domain) antibody-bound Sepharose beads [29]. After washing, the captured proteins were eluted from the antibody beads and digested with Lys-N. The resultant peptides were dried and resolubilized for MS and injected onto a nanoflow liquid chromatography (LC) reverse phase column coupled directly online with a triple quadrupole mass spectrometer (TSQ Vantage; Thermo Scientific, San Jose, CA, USA). Aβ40 [28–40] and total Aβ [16–27] peptides were monitored at 3 m/z ratios, corresponding to the unlabeled, the 13C6-leucine labeled, and the internal standard–labeled peptides. Each parent peptide was subjected to collision-induced dissociation (CID) and its product ions measured and their peak areas integrated.

Two standard curves and two sets of three quality control (QC) samples were analyzed concurrently with patient samples. One standard curve (SISAQ) consists of known concentrations of 15C6-leucine– and 15C6-leucine–labeled Aβ and is used for calculating the concentration of metabolically labeled and unlabeled Aβ in the sample. The other standard curve (SILK) contains Aβ that has been metabolically labeled with 13C6-leucine at various ratios and serves as a way to standardize the relative labeling data.

2.5.4. Quantitation and metabolism of apolipoprotein E in cerebrospinal fluid samples

A known amount of internal standard was added to each CSF sample. These samples, along with two standard curves and QC samples, were incubated with anti-apoE antibody–bound beads, washed, then eluted and digested with trypsin [29]. The resultant peptides were dried and injected using nanoflow LC onto a reverse phase column and separated and eluted into the mass spectrometer using a gradient of increasing organic mobile phase. ApoE (177–185) was monitored at 3 m/z ratios, corresponding to the unlabeled, the 13C6-leucine labeled, and the internal standard–labeled peptide. Each parent peptide was subjected to CID and its product ions measured and integrated. Quantitation was conducted by integrating the product ion peaks and taking the ratio of the endogenous apoE/internal standard apoE. The ratio of labeled to unlabeled apoE was plotted over 48 hr.

3. Results

3.1. Bexarotene plasma and CSF pharmacokinetics

The concentration of bexarotene was measured in the plasma and CSF of all subjects beginning on the fourth day of drug treatment over a period of 48 hours. Bexarotene was only detected in the plasma of subjects who were dosed with the drug, and all treated subjects showed similar pharmacokinetic profiles (Fig. 1). Average peak plasma concentrations were 1.46 ± 0.62 μM (average of four dosings per
subject) with a $t_{\text{max}} = 3.45 \pm 1.41$ hours and AUC (dose to dose) = 7.58 $\pm$ 3.80 $\mu$M. These data demonstrate plasma pharmacokinetics similar to that previously reported [31].

The CSF bexarotene levels were below the LOQs of the assay (0.021 $\mu$M) for $\geq$95% samples, regardless of subject treatment. In 5 of 6 bexarotene-treated subjects, at least one CSF sample with bexarotene levels higher than the LOQ was measured (Fig. 1). We estimated the plasma-to-CSF ratio of bexarotene concentration in this subset of samples by dividing the concentration of bexarotene in plasma by corresponding peak of bexarotene detected in the CSF (or LOQ). In samples in which bexarotene concentration was accurately measured, we estimated that the plasma-to-CSF ratio was approximately 85:1 (Fig. 1).

3.2. Effect of bexarotene on Apolipoprotein E

3.2.1. Stable isotope labeling kinetics

SILK was used to measure the fractional synthesis and clearance rates (FSR and FCR) of apoE in each subject by quantitating the amount of $^{13}$C$_6$-leucine–labeled and unlabeled apoE in CSF at each of the time points CSF was acquired. The SILK data are plotted as the normalized tracer-to-tracee ratio (TTR), which is the ratio of the amount of $^{13}$C$_6$-leucine–labeled apoE divided by the amount of unlabeled apoE in CSF. The mean normalized SILK-ApoE curves showed maximum stable isotope labeling of apoE occur at approximately 24 to 25 hours after initiation of $^{13}$C$_6$-leucine infusion (Fig. 2A). The mean FSR of apoE was measured between 6 and 17 hours (Fig. 2B) and the fractional clearance rate (FCR), determined over the period of 23 to 48 hours (Fig. 2C). There was no significant difference in the fractional synthesis ($P = .8578$) or clearance ($P = .4646$) of apoE in the CSF of subjects treated with bexarotene or placebo when measured after 3 days of drug treatment.

3.2.2. Stable isotope spike absolute quantitation

The absolute concentration of apoE in the CSF was calculated by adding the concentration values for the unlabeled and $^{13}$C$_6$-leucine–labeled apoE. The apoE concentrations of the individual subjects (Fig. 2D), and their average values ($\pm$95% CIs; Fig. 2E) were quantitated. There was a significant 25% increase ($P = .0367$, Glass’s delta effect size of 2.55) in the mean weighted area under the full concentration curves of apoE in the CSF of the bexarotene-treated subjects (Fig. 2F) compared with those treated with placebo.

This result suggests that bexarotene significantly increased total apoE concentration in the central nervous system (CNS) of normal healthy individuals with APOE ε3/ε3 genotype.

Using the concentration of labeled apoE and the average leucine TTR observed in each subject, the amount of newly generated apoE was calculated. The increase in newly generated CSF apoE approached but did not reach statistical significance between the two treatment groups ($P = .0538$; Fig. 2I) as indicated by the mean area under the concentration curve.
of newly generated apoE during the labeling period for the individual subjects (Fig. 2G) or group averaged values (Fig. 2H; ±95% CIs). These data suggest that there was no difference in the rates of apoE synthesis in the brain when measured 3 days after initiation of bexarotene treatment. The analysis of the lipidation status of apoE did not yield meaningful results owing to poor resolution of the analytic techniques.

3.3. CSF Aβ

3.3.1. Stable isotope labeling kinetics

The mean normalized SILK-Aβ total between placebo- and drug-treated subjects was compared and shown in Fig. 3A and Fig. 3B. The FSR (Fig. 3C) and FCR
Fig. 3. Stable isotope labeling kinetics (SILK) of total Aβ and of Aβ40 in cerebrospinal fluid (CSF). The synthesis and clearance rates of total Aβ were measured in CSF using an Aβ1-x capture antibody. The SILK data are plotted as the normalized tracer-to-tracee ratio (TTR), which is the amount of $^{13}$C6-Leu labeled Aβ divided by the amount of total unlabeled Aβ. (A) The values for the individual subject treated with placebo (blue) or bexarotene (red), and (B) the mean values are plotted ± 95% confidence intervals (CIs). (C) The fractional synthesis rates (FSRs), measured from 6 to 17 hours. (D) Fractional clearance rates (FCRs) determined from 23 to 48 hours were not significantly different between placebo- and drug-treated subjects. The SILK data are plotted as the normalized TTR, which is the amount of $^{13}$C6-Leu labeled Aβ40 divided by the amount of unlabeled Aβ40. (E) The values for the individual subject and (F) the mean values are plotted ± 95% CIs. (G) The FSRs, measured from 6 to 17 hours or (H) FCRs determined from 23 to 48 hours were not significantly different between placebo- and drug-treated subjects.
(Fig. 3D) of Aβ total did not show a significant difference between the placebo- and drug-treated groups. These results demonstrate that the fractional synthesis or clearance of Aβ total in CSF of normal healthy individuals treated with placebo was comparable to that of individuals treated for 3 days with bexarotene. Similarly, there was no difference observed in the turnover of Aβ40 between the bexarotene-and placebo-treated subjects at this time (Fig. 3E–3H).

3.3.2. Stable isotope spike absolute quantitation
Quantitation of the amount of Aβ total (Fig. 4A) and Aβ40 (Fig. 4D) in the CSF in the individual subjects or the mean values of Aβ total (Fig. 4B) or Aβ40 (Fig. 4E) demonstrated that there was no significant difference in the absolute levels of Aβ total (Fig. 4C) or Aβ40 (Fig. 4F) peptides between treatment groups. Evaluation of the concentration of newly generated (i.e., 13C6-leucine labeled) Aβ total (Fig. 4G) and Aβ40 (Fig. 4I) was measured. However, there was no difference in the amount of Aβ peptides synthesized (Fig. 4H and 4K) or cleared (Fig. 4I and 4L) between bexarotene- and placebo-treated subjects.

In summary, bexarotene treatment had no effect on the metabolism of Aβ peptides in normal human subjects with the current treatment regimen.

4. Discussion
Nuclear receptors are ligand-activated transcription factors that act broadly to regulate cellular metabolism [14]. In the brain, the principal type II nuclear receptors include the PPARs and LXRs, which form obligate heterodimers with RXRs to form a functional transcription factor [15,33]. The dimeric receptor binds to sequence-specific motifs in the promoter and enhancers of their target genes and on ligand binding induces gene expression [34]. These nuclear receptors are termed “permissive” as ligation of either member of the dimeric receptor is sufficient to stimulate gene expression. In the brain, the expression of the APOE gene is directly regulated by LXR:RXR, as are the lipid transporters ABCA1 and ABCG1, which act to transfer phospholipids and cholesterol to apoE. ApoE serves as a scaffold for the formation of HDL particles. PPARγ:RXR can act indirectly on the enhancers of these genes and through other mechanisms to promote apoE expression and elevate brain HDL levels [20]. The stimulation of brain HDL concentration results in the enhanced proteolytic degradation of soluble Aβ peptides and their clearance from the brain [8,17]. We reasoned that provision of an RXR agonist would provide an efficient means to drive apoE expression in the brain. Indeed, bexarotene treatment resulted in the rapid increase in apoE-based HDLs in the interstitial fluid of animal models, coincident with a decrease in soluble Aβ levels [17,18,22]. The reduction in brain soluble Aβ levels was associated with enhanced neural network activity and improved memory and cognition in murine models of AD [17,18,23,24]. The present study was a proof of mechanism trial to ascertain if bexarotene acts similarly in the normal human brain.

We report that bexarotene was poorly CNS penetrant in normal subjects, reflected by the low levels of drug found in the CSF. The bexarotene levels in CSF were below the LOQ of the assay in >95% of samples. However, five of the six subjects treated with the drug had levels of bexarotene above the LOQ in a small subset of samples. The ratio of peak plasma to CSF drug levels was approximately 85:1 with maximal bexarotene levels in the low nM range. The poor CNS exposure to bexarotene observed here was similar to that reported by Rotstein et al. [35] in the baboon where 18F-labeled bexarotene was found to rapidly enter the brain and distribute uniformly. However, the brain:tissue levels were on the order of 50:1. The detection of only low concentrations of bexarotene in the CSF was unexpected because in mice, bexarotene is freely CNS permeable [19,36,37]. The low CSF levels in humans may arise from its poor blood brain barrier and/or blood to CSF barrier permeability. Alternatively, bexarotene may be efficiently effluxed from the brain by p-glycoprotein or related transporters.

We observed that bexarotene-treated subjects had higher concentration of total apoE in CSF. The modest, but significant (P = .0367), 25% increase in apoE concentration in CSF reflected the weighted area under the full concentration curve over the interval from 72 to 120 hours. The elevated apoE concentration likely arises from a drug-induced increase in steady state mRNA levels achieved during the first 3 days of drug treatment. A similar effect was observed when the amount of newly synthesized apoE was quantitated; however, it approached, but did not reach, significance (P = .0538). There was no difference between treatment groups in the kinetics of apoE synthesis or clearance in CSF. The drug-induced change in steady state metabolism over several days of treatment measured by SILK is analogous to that previously reported by Sheline et al. [38] for Aβ.

The net increase in CSF apoE levels was achieved with levels of bexarotene estimated to be in the low nM range. The Kd of bexarotene for RXRs is approximately 20 nM; thus, we observed target engagement at modest levels of receptor occupancy. The magnitude of the increase in humans (25%) is significantly less than that elicited in the brain (>200%) [17], or interstitial fluid (250%) [22] of bexarotene-treated mice. In rats, LXR agonist treatment resulted in an approximate 200% increase in CSF apoE levels [12].

Metabolic curves for the synthesis and clearance of total Aβ and Aβ40 from the drug and placebo-treated subjects showed that bexarotene had no effect on the synthesis or clearance of Aβ in CSF, nor was there a difference in the total amount of the peptides in the CSF. The absence of any alteration of Aβ metabolism is likely reflective of the small changes in apoE levels we observed.

These data clearly demonstrate that bexarotene is poorly CNS penetrant in the normal human brain. However, it is
Fig. 4. Stable isotope spike absolute quantitation of total Aβ and Aβ40 and newly synthesized total Aβ and Aβ40 in cerebrospinal fluid (CSF). The absolute concentration of total Aβ and Aβ40 peptides in the CSF was calculated by adding the concentration values for the unlabeled and 13C6-labeled peptides using antibodies directed at Aβ1-42 or at the C-terminal Aβ40 epitope. (A) Total Aβ or (D) Aβ40 concentrations of the individual subjects treated with placebo (blue) or bexarotene (red) and (B, E) their average values (±95% confidence intervals [CIs]). There was no significant difference in the mean weighted area under the full concentration curves of (C) total Aβ or (F) Aβ40 in the CSF of the bexarotene-treated subjects. The absolute concentration of newly synthesized total Aβ and Aβ40 peptides in the CSF was calculated by adding the concentration values for the unlabeled and 13C6-labeled Aβ peptides at each time point. Quantitation of the mean amount of newly synthesized (G) total Aβ and (J) Aβ40 (±95% CIs) in placebo- and bexarotene-treated subjects revealed no difference in the amount of Aβ peptides (H, K) synthesized or (I, L) cleared in the placebo-treated compared with bexarotene-treated subjects. Abbreviation: AUC, area under the curve.
unknown if the drug is more permeant in the AD brain. Montagne et al. [39] have recently reported that the blood–brain barrier in the hippocampus was compromised in individuals with mild cognitive impairment. Thus, therapeutically relevant levels of the drug might be achieved in individuals even at the earliest stages of AD. Cummings and coworkers performed a randomized, placebo-controlled phase II trial of bexarotene in 20 mild-to-moderate AD patients. They reported that 30 days of bexarotene treatment (using a 30% lower dose) resulted in a statistically significant reduction in amyloid burden with a parallel increase in plasma 

APOE endangering Apolipoprotein E expression might have negative side effects. However, the work by Cummings et al. [40], and other clinical trials have provided no evidence for any negative CNS-based effects. Consistent with the findings of Cummings et al., Pierrot et al. [41] recently reported a case study demonstrating that 6 months of bexarotene treatment in an individual with mild AD resulted in improved cognition. Lerner et al. [42] reported that treatment of schizophrenic patients with bexarotene resulted in significant symptomatic improvement, arguing for biologically relevant levels of drug in the brain of these patients. Thus, it is possible that bexarotene’s poor CNS penetration may not be a barrier to its therapeutic use in AD. The utility of nuclear receptor agonists in the prevention or treatment of AD is supported by the recent report by Heneka et al. [43] who found that chronic administration of pioglitazone, an agonist of PPARγ:RXR, reduced the risk of dementia by 47% in a large population of elderly diabetics.

It is noteworthy that McFarland et al. [44] reported dramatic effects of bexarotene in a rodent model of Parkinson’s disease at bexarotene doses that were the equivalent of 1% of the FDA-approved dosage used in this study. The salutary effects of the drug were attributed to its activation of the nuclear receptor Nurr1 (NR4A2). This study argued that bexarotene exhibited neuroprotective and behavioral effects through Nurr1 target genes, including CREB and BDNF. This observation is consistent with recent work that has shown that ligation of PPARα:RXR induces BDNF expression and improved memory in a mouse model of AD [45]. Moreover, bexarotene has been reported to have direct effects on neurons in mouse models of AD [46] and aging [47] increasing expression of synaptic proteins and preservation of dendrites [48]. Similarly, in a mouse amyotrophic lateral sclerosis model, bexarotene treatment reduced neuronal death and increased survival [49].

The present study argues that treatment of individuals with an intact BBB are unlikely to benefit from bexarotene administration. However, it remains a formal possibility it may act through non-apoE–dependent mechanisms at low drug concentrations. The report by Cummings et al. [40] indicates that bexarotene has salutary effects in mild-to-moderate AD patients. Indeed, this study provides a clear rationale for a larger phase II/III trial over longer periods to test its efficacy on memory and cognition.

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