Comment on "ApoE-Directed Therapeutics Rapidly Clear β-Amyloid and Reverse Deficits in AD Mouse Models"

Ashleigh R. Price,* Guilian Xu,* Zoe B. Siemienski, Lisa A. Smithson, David R. Borchelt, Todd E. Golde, Kevin M. Felsenstein†

Cramer *et al*. (Reports, 23 March 2012, p. 1503; published online 9 February 2012) demonstrates short-term bexarotene treatment clearing preexisting β-amyloid deposits from the brains of APP/PS1ΔE9 mice with low amyloid burden, providing a rationale for repurposing this anticancer agent as an Alzheimer's disease (AD) therapeutic. Using a nearly identical treatment regimen, we were unable to detect any evidence of drug efficacy despite demonstration of target engagement.

Cramer *et al*. (*1*) report that bexarotene, a retinoid X receptor (RXR) agonist used primarily as a treatment for cutaneous T-cell lymphoma (*2*), demonstrated the ability to clear preexisting Alzheimer's disease (AD)–like β-amyloid (Aβ) pathology in cohorts of 6-month-old APPswe/PS1Δe9 (line 85 or APP/PS1) (*3*) mice after 3 to 7 days of treatment. The unexpected and remarkable finding raised hope for a therapeutic utility in the treatment of Alzheimer's disease (AD). A great deal of evidence indicates that accumulation of Aβ aggregates in the brain trigger a complex neurodegenerative cascade leading to AD. Thus, strategies to promote the clearance of Aβ have been and are being evaluated as potential therapeutics for AD (*4*). Agonism of the peroxisome proliferator-activated receptor (PPAR-γ), LXR (liver X receptor), and RXR nuclear hormone receptors have all been suggested as possible strategies for enhancing clearance of Aβ, but agonism of these receptors may attenuate AD pathologies in mice through other mechanisms as well (*5*, *6*).

To further assess bexarotene's therapeutic potential, and replicate the findings of Cramer *et al*. (*1*), we examined the effects of orally administering a soluble preparation of 100 mg per kg of weight (mg/kg) bexarotene to two cohorts of APP/PS1 mice aged 7 and 11 months. The size of the cohorts used in this study was identical to those reported by Cramer *et al*. (*1*). Bexarotene (free acid form) was purchased from LC Laboratories (Woburn, Massachusetts). As it is insoluble in water, and to achieve a uniform formulation for administration, bexarotene powder was initially dissolved in dimethyl sulfoxide (DMSO) (not to exceed 4.5% in the final mixture) and then combined with polyethylene glycol (15)-hydroxystearate (Solutol), ethanol, and water at a ratio of (15:10:75). To this mixture, one molar equivalent of sodium hydroxide was added. This pH-neutral formulation resulted in the total solvation of the compound. The selected vehicle is used extensively in the pharmaceutical industry to obtain aqueous preparations for safe and efficient oral or parenteral delivery in rodent models (*7*). Because the effects described by Cramer *et al*. (*1*, *8*) were relatively acute, we strove to achieve efficient delivery of fully solubilized compound. With a compound that is >99% plasma protein bound, it is essential to achieve an optimal formulation for absorption to obtain sufficient exposure at the target. Cramer *et al*. (*1*) administered a micronized suspension with excipients to the mice, which is usually used when no suitable aqueous formulation is available; the formulation described above overcomes this limitation and should result in similar, if not superior, pharmacokinetic properties. Final delivery of the preparation was via oral gavage, once a day for 7 days.

At the end of the dosing regimen, the cortex and hippocampus were examined for Aβ loads by both biochemical and histological means, as previously described (*6*). To examine soluble Aβ as well as the insoluble fractions consisting of oligomeric and plaque Aβ, brain samples were sequentially extracted with radioimmunoprecipitation assay (RIPA) buffer followed by SDS and then formic acid (*9*). Within both age groups, no statistically significant difference in Aβ load was noted between the bexarotene- and vehicle-treated groups (n = 5 to 6 mice) (Fig. 1A). Examination of plaque burden and by thioflavin-S staining and immunocytochemistry (Fig. 1, B and C) revealed no statistically significant reductions in plaque numbers or plaque area within the treated groups as compared with their vehicle control (Fig. 1D).

It has been observed that in this strain of transgenic mice, there is considerable variability in the Aβ load within the individual younger animals (6 to 7 months) that will tend to normalize as the animals age (*10*). Plaque numbers within the animals used in this study were similar to those previously reported for this strain (*10*). Although this is the identical strain and group sizes used by Cramer *et al*. (*1*), our experience with this strain suggests that larger group sizes may be needed to detect small reductions in amyloid load. The group size used here is sufficient to detect a 40% reduction. (*10*).

Although no significant effects were detected on Aβ load as determined by both biochemical and histological means, three lines of evidence indicated that the drug engaged the target. First, we observed a change in liver weights by 1.75 ± 0.2 fold ($P < 0.01$) (Fig. 2), which indirectly confirms delivery and bioavailabilty of the drug; findings are consistent with known peripheral side effects of bexarotene administration—for example, dyslipidemia and increases in liver metabolism. Both LXR and RXR agonists have previously been shown to modulate the expression of genes involved in lipid metabolism, including the ATP-binding cassette transporter (ABCA1), one of the major regulators of cellular cholesterol homeostasis, and apoE. Immunoblot analysis of brain protein lysates following RIPA buffer extraction of brain sections for apoE and ABCA1 expression revealed statistically significant increases for both apoE [1.6-fold increase ± 0.05 ($P < 0.01$)] and ABCA1 [~2-fold increase ± 0.05 ($P < 0.001$)] in the 11-month-old animals (Fig. 2), with similar results seen for the 7-month-old animals (data not shown).

With confirmation of target engagement by bexarotene in the APP/PS1 mice and no change in Aβ load, these data suggest that the reported effect of bexarotene in clearing Aβ deposits is not easily reproducible. Indeed, true clearance of Aβ pathology in mice is very challenging to observe experimentally, and when it is observed, it is often limited to diffuse plaques (*11*–*14*). Given the mechanism-based toxicities associated with bexarotene administration to humans and our inability to replicate efficacy against a key endpoint used to justify human studies, we suggest that further validation studies are warranted before considering human AD bexarotene clinical trials. The potential "off-label" use of this drug for AD treatment needs to be tempered by compelling and reproducible preclinical data.

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References and Notes

1. P. E. Cramer *et al*., *Science* **335**, 1503 (2012).
2. C. Querfeld, L. V. Nagelli, S. T. Rosen, T. M. Kuzel, J. Guitart, *Expert Opin. Pharmacother.* **7**, 907 (2006).
3. A. Savonenko *et al*., *Neurobiol. Dis.* **18**, 602 (2005).
4. T. E. Golde, L. Petrucelli, J. Lewis, *Exp. Neurol.* **223**, 252 (2010).
5. S. Mandrekar-Colucci, G. E. Landreth, *Expert Opin. Ther. Targets* **15**, 1085 (2011).
6. R. Koldamova, I. Lefterov, *Curr. Alzheimer Res.* **4**, 171 (2007).
7. B. Bittner *et al*., *Arzneimittelforschung* **52**, 684 (2002).
8. V. A. Miller *et al*., *J. Clin. Oncol.* **15**, 790 (1997).

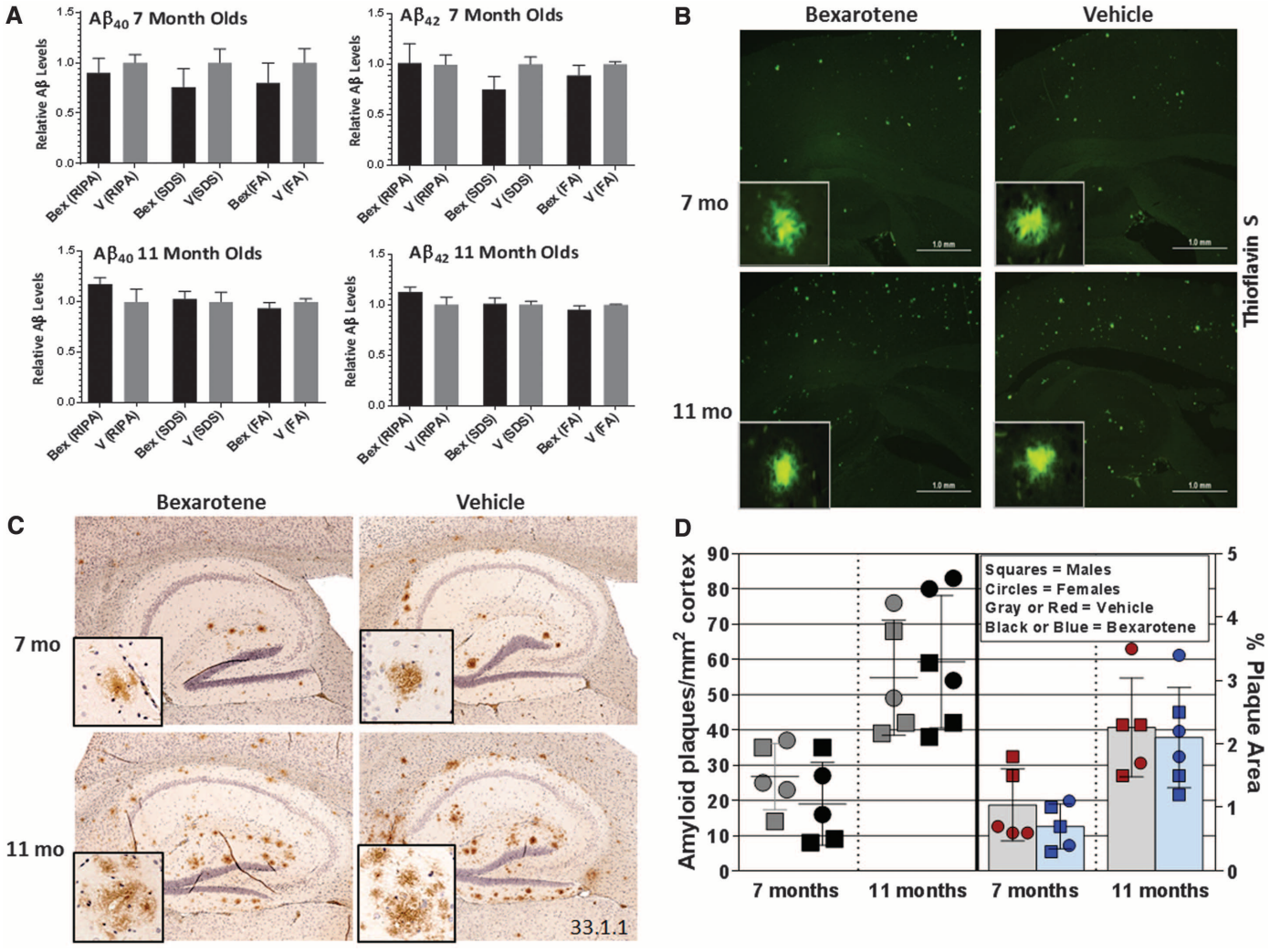

Fig. 1. Aβ levels and plaque burden are unchanged by bexarotene treatment. APP/PS1ΔE9 mice (7 and 11 months old, including both male and female; n = 5 to 6 per treatment group) were dosed with bexarotene (100 mg/kg per day) or vehicle by oral gavage for 7 days. Upon harvest, one hemibrain was frozen in liquid nitrogen, with the other fixed in 4% paraformaldehyde with 0.1 M phosphate buffer, pH 7.6, for histological analysis. (**A**) The frozen hemibrains were sequentially extracted essentially as previously described (*9*), using RIPA detergent followed by 2% SDS followed by 70% formic acid. The resulting extracts were analyzed for soluble and insoluble $A\beta_{40}$ and $A\beta_{42}$ by differential enzyme-linked immunosorbent assay. Bexarotene and vehicle treatment is shown by the black and gray bars, respectively. (**B**) Representative cortical sections of bexarotene- or vehicle-treated mice stained with thioflavin S (inset represents magnified image of a typical plaque). (**C**) Images of sections immunostained with panAβ1-16 antibody 33.1.1 (*15*); similar results are also seen by silver staining, antibody 6E10, or an $A\beta_{42}$-specific antibody (data not shown). (**D**) Quantitation of the plaque numbers. The fluorescent images were scanned using a ScanScope FL (Aperio Technologies). The cortex was outlined, and the numbers of amyloid plaques per mm^2 of the cortex were manually counted by an observer blinded to treatment. 7-month-old vehicle-treated = 26.8 ± 4.2 plaques; 7-month-old bexarotene-treated = 19 ± 5.6; 11-month vehicle-treated = 54.8 ± 7.3; 11-month bexarotene-treated = 59.3 ± 7.7; error bars, mean ± SEM; no statistical significance between the groups by Student's t test. The Aβ burden as percentage of total area was determined using the ImageScope program and Positive Pixel Count Algorithm (Aperio Technologies) from antibody 33.1.1 stained sections (using the average of three sections per brain 30 μm apart), with no statistical significance between the groups. 7-month-old vehicle-treated = 1.0 ± 0.6; 7-month-old bexarotene-treated = 0.7 ± 0.35; 11-month-old vehicle-treated = 2.3 ± 0.8; 11-month-old bexarotene-treated = 2.1 ± 0.8.

9. T. Kawarabayashi *et al.*, *J. Neurosci.* **21**, 372 (2001).
10. G. Xu, C. C. Green, S. E. Fromholt, D. R. Borchelt, *Alz. Res. Ther.* **4**, 12 (2012).
11. A. Wang, P. Das, R. C. Switzer 3rd, T. E. Golde, J. L. Jankowsky, *J. Neurosci.* **31**, 4124 (2011).
12. Y. Levites *et al.*, *J. Clin. Invest.* **116**, 193 (2006).
13. D. M. Wilcock *et al.*, *J. Neurosci.* **26**, 5340 (2006).
14. B. J. Bacskai *et al.*, *J. Neurosci.* **22**, 7873 (2002).
15. P. Chakrabarty *et al.*, *FASEB J.* **24**, 548 (2010).

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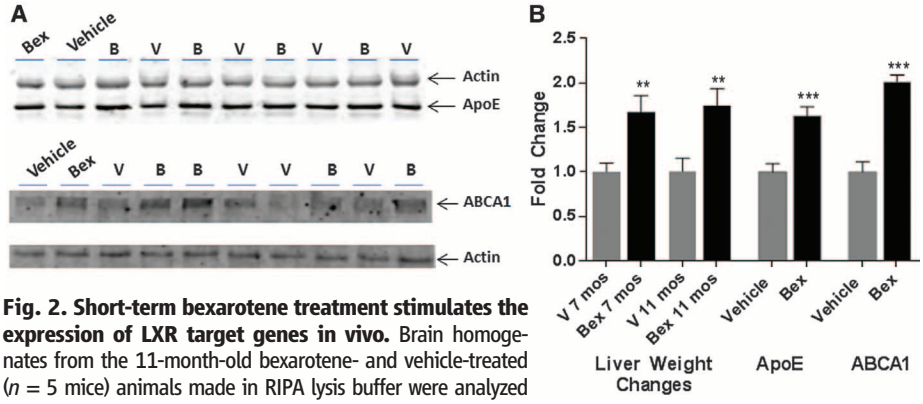

Fig. 2. Short-term bexarotene treatment stimulates the expression of LXR target genes in vivo. Brain homogenates from the 11-month-old bexarotene- and vehicle-treated (n = 5 mice) animals made in RIPA lysis buffer were analyzed on Western blot and probed with an antibody specific either for apoE or for ABCA1, along with an internal control for actin. Proteins were quantitated and normalized to actin using an Odyssey LiCor Imager, with results summarized in the accompanying graph. Also shown are the change in liver weights of the two groups (Student's t test; error bars, mean ± SEM, $^{**}P < 0.01$, $^{***}P < 0.001$). Similar results were seen with the 7-month-old animals; data not shown.

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