alzheimer's research&therapy

COMMENTARY

# When the dust settles: what did we learn from the bexarotene discussion?

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**Abstract**

With 27 million people affected by Alzheimer's disease (AD), any proposal of a novel avenue for drug development is hot news. When Cramer and colleagues proposed last year that they could tackle AD pathology in an AD mouse model with bexarotene, a drug already in use in the clinic for other diseases, the news was covered worldwide by the popular press. Apolipoprotein E4 is the strongest genetic risk factor for AD and bexarotene appeared to exert spectacular effects on AD pathology when tested in APP/PS1 transgenic mice. One year later the slumbering discussion on the use of bexarotene in AD exploded in a flurry of papers. Four papers question the initial optimistic claims, while two others can only partially support the original work. We summarize here the available data and try to make sense out of the controversy. The major question is what we can learn from the experiments and what these studies imply for the further development of bexarotene in the clinic.

Apolipoprotein E4 (apoE4) is the most important genetic risk factor for sporadic Alzheimer's disease (AD) and therefore an interesting drug target *per se*. Despite numerous studies trying to address by which mechanism apoE4 affects the risk for AD, a definitive answer has not yet emerged. Both the ideas that apoE4 accelerates and fails to protect against disease have been proposed [1-3]. These opposite views have important implications when devising therapeutic strategies for AD. If apoE4 accelerates the disease, then inhibition of apoE4, or the toxic pathways in which it plays a role, would be required. Practically this would imply, for instance, inhibiting the cleavage of apoE4 into toxic fragments or blocking apoE4's effects on amyloid beta (Aβ) oligomerization [2,3]. If apoE4 fails to protect against AD, however, then potentiation of the function of wild-type apoE would be desirable - for instance, by generating apoE3 mimetics, which could improve the brain clearance of Aβ that may be affected in APOE4 carriers [1,3].

The paper by Cramer and colleagues [4] fitted nicely into the second strategy and showed that upregulation of apoE and apoE-related pathways could improve amyloid plaque accumulation and behavior in a mouse model of AD. Moreover, it demonstrated that this was possible by administering the drug bexarotene, which was already approved by the US Food and Drug Administration (FDA) for use in humans [5]. The excitement that this possibility could rapidly become tested in real, sporadic AD patients propelled the publication into the popular press.

Bexarotene is a retinoid X receptor (RXR) agonist that induces transcription of the ATP-binding cassette transporter gene A1 (*ABCA1*), as well as apoE and other genes involved in lipid metabolism through obligatory heterodimerization of RXR with liver X receptor and peroxisome proliferator-activated receptor-γ. As a result, bexarotene induces accumulation of triglycerides *in vivo*, which can result in liver steatosis and hypertriglyceridemia [6]. ApoE and ABCA1 are expressed in the central nervous system and in the periphery and measuring their expression should give a good indication of target engagement by bexarotene in both the central nervous system and the periphery.

Given the spectacular results of Cramer and colleagues [4], it is not surprising that many research groups worldwide attempted to replicate the original study. The data are summarized in Table 1. The major claim of the original study was a rapid clearance of Aβ deposits from the brains of AD model mice [4]. This claim could not be confirmed in five independent follow-up studies [7-11], despite target engagement. Interestingly, and in

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**Table 1 Overview of drug formulation and experimental outcomes of Alzheimer's disease model mice treated with Bexarotene**

| | | | Experimental claims | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Research group** | **Drug formulation** | **Duration of treatment** | **Target engagement (ApoE/ ABCA1)** | **Decrease of soluble Aβ** | **Clearance of deposited Aβ/ plaques** | **Behavioral improvements** | ***In vivo* microglia activation** | **Mechanism-based toxicity** |
| Cramer *et al.* [4] | Bexarotene/ Targretin in DMSO or micronized in water | 3 to 14 days and 3 months | Increase | Decrease of Aβ40 or Aβ42 >25% | Repeated dosing of 3 to 14 days decreases Aβ deposits by 30 to 75%; 3-month treatment had no effects | Improved context-dependent fear memory. Improved spatial memory. Improved social behavior | Unclear whether different from control | NR |
| Price *et al.* [9] | Bexarotene in solutol:ethanol: water (15:10:75) | 3 to 7 days | Increase | No effect | No effect | NR | NR | Increased liver weight |
| Fitz *et al.* [11] | Targretin in glycerol | 15 days | Increase | Decrease of ISF Aβ40 and Aβ42 by 23 to 26%; no effect on extracted soluble Aβ | No effect | Improved spatial memory. Improved long-term memory | NR | NR |
| Veeraraghavalu *et al.* [7] | Targretin in DMSO:ethanol: sunflower oil (6.6%:4%:89.6%) | 7 days | Increase | Small effects | No effect | NR | No effect | NR |
| Tesseur *et al.* [8] | Bexarotene in Captisol and HP-β-CD/ Tween | 19 days | Increase | No effect | No effect | Unclear effect on social recognition memory and fear memory | NR. | Loss of body weight, irritation and breathing problems, increased grooming |
| LaClair *et al.* [10] | Bexarotene in DMSO or corn oil | 3 to 14 days | Increase | NR | No effect | No effect on context- or conditioned stimulus-dependent fear memory | No effect | NR |
| Ulrich *et al.* [12] | Targretin in water | 36 hours | Increase | Decrease of ISF Aβ40 by 45% | NR | NR | NR | NR |

Aβ, amyloid beta; ApoE, apolipoprotein E; DMSO, dimethyl sulfoxide; HP-β-CD, 2-hydroxypropyl-β-cyclodextrin; ISF, interstitial fluid; NR, not reported.

contrast to the bulk of their results, Cramer and colleagues reported that, after chronic daily bexarotene treatment for 3 months, they could not find a decrease in Aβ deposits, despite reduced soluble Aβ levels and target engagement [4]. This result is puzzling compared to the rest of their results and no clear explanation for this discrepancy is given. The other claims of the publication were, optimistically speaking, confirmed by some groups and not by others. For instance, three groups found lowering of soluble Aβ levels in interstitial fluid [11,12] or in brain extracts [7], whereas three groups found no effects [8,9,11]. From Table 1 it becomes clear that the formulation of bexarotene might be very critical in this regard. The groups who observed an effect used Targretin for their mice. Targretin is the commercial formulation of bexarotene and contains a lot of additional 'stuff' (see detailed component list in [13]). This raises the question of whether those additional components could affect the results, especially since the bexarotene formulations used by the other groups resulted in efficient brain exposure as well, precluding the lack of target engagement as an explanation for this discrepancy. The original publication by Cramer and colleagues [4] mentioned the use of Targretin in some experiments and of bexarotene powder (in water or DMSO) in other experiments, explaining some of the confusion in the follow-up studies. The authors [14] indicated afterwards that they actually used Targretin for their dosing studies

[4] but it remains to be fully clarified what was exactly used in the different experiments of the original work [4].

The behavioral data analyzing the effects of bexarotene on cognition and memory, which are crucial for the clinic, are the most difficult and most controversial to interpret. Cramer and colleagues [4] claimed that bexarotene significantly improved spatial and fear memory in their mice. Improvement in spatial memory upon bexarotene treatment was reproduced in one follow-up study [11], but improvement of fear memory could not be reproduced [10] or was reported to be inconclusive in a third study [8]. The latter study, although inconclusive, is quite informative from another perspective. Indeed, these authors reported a series of confounding effects in the mice treated with the drug - that is, severe loss of total body weight, breathing problems, and increased grooming - indicating that the mice felt rather uncomfortable and might even suffer from the treatment [8]. Although the authors of the Cramer and colleagues' study interpreted the behavioral data of the latter study in their response-comment as confirmative of their original data [14], the authors of the latter study were much less positive and indicated that the animals might have been too sick to allow any confirmative conclusions [8]. In addition to these side effects, Price and colleagues [9] reported evidence that bexarotene significantly increased liver weight in treated mice, indicating liver steatosis.

FDA files report that bexarotene induces severe upregulation of triglyceride and total cholesterol levels in many patients, and high-density lipoprotein cholesterol is lowered in 25% of treated patients [5]. As the dyslipidemia is reversible after stopping the treatment, this may not pose major issues when treating cancer patients for a limited time. However, AD patients at risk might need treatment for many years, and in such cases the dyslipidemia would become chronic and could seriously compromise cardiovascular health.

Finally, the original publication of Cramer and colleagues [4] claimed a significant upregulation of microglial activation in bexarotene-treated animals. However, some control stainings and quantifications were lacking in the manuscript, making it difficult to ascertain how definitive these observations were. Indeed, two independent follow-up studies did not see effects on microglia activation [7,10].

## Conclusion

Collectively, the above summarized data neither support nor contradict the hypothesis that apoE4 fails to protect against AD and the question is still open. It is clear that the original data published by Cramer and colleagues [4] were interpreted with a lot of optimism, which is understandable given the huge unmet needs in the field. Given the central role of apoE4 in the risk for AD, the proposed approach remains potentially interesting. For the time being, however, we strongly suggest that researchers and clinicians interested in this work perform their own additional preclinical validation before starting clinical trials with bexarotene in humans. In addition, given the reported side effects and possible risks, 'off-counter' use of Targretin by desperate AD patients should be tempered until all the raised issues have been sufficiently clarified.

### Abbreviations

AD: Alzheimer's disease; apoE: Apolipoprotein E; Aβ: Amyloid beta; DMSO: Dimethyl sulfoxide; FDA: US Food and Drug Administration; RXR: Retinoid X receptor.

### Competing interests

BDS is consultant for Janssen Pharmaceutica, Remynd NV and Envivo Pharmaceuticals.

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