Alpha-tocopheryloxyacetic acid induces apoptosis of murine rhabdomyosarcoma in vitro while modulating innate and adaptive immune responses in vivo

Fernanda Szewc [1], M.Sc., Longzhen Song [1], Ph.D., Sean P. Rinella [1], M.P.H., C.C.R.P., Christopher Dubay [2], Ph.D., Emmanuel T. Akporiaye [3], Ph.D., William L. Redmond [2], Ph.D., Christian M. Capitini [1,4], M.D.
[1]Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA; [2]Earle A. Chiles Research Institute, Portland, OR, USA; [3]Veana Therapeutics, Portland, OR, USA; [4]Carbone Cancer Center, Madison, WI, USA

UW Health American Family Children's Hospital

Department of Pediatrics
UNIVERSITY OF WISCONSIN
SCHOOL OF MEDICINE AND PUBLIC HEALTH

BACKGROUND

- Relapsed pediatric sarcomas have a poor prognosis with no available curative options.
- Alpha-Tocopheryloxyacetic acid (a-TEA) is a redox-silent analog of alpha-tocopherol that induces apoptotic and immunogenic cell death of tumor cells.
- In a first-in-human clinical trial, a-TEA was well tolerated in adults with advanced solid tumors (NCT02192346),but has not yet been studied in pediatric sarcoma.
- We used a murine model of rhabdomyosarcoma (M3-9-M RMS) to assess the in vitro and in vivo anti-tumor effects of a-TEA.

METHODS

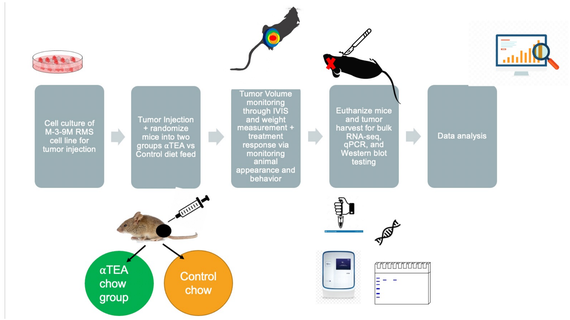

Figure 1. Experimental schematic of In-vivo experiments for M3-9-M tumor bearing mice randomized to alpha-TEA or control diet

- In vitro studies were performed on the M3-9-M RMS cell line to measure a-TEA-mediated apoptosis using flow cytometry (Annexin V+/7AAD+ cells) and live cell imaging (Annexin V+ cells).
- In vivo studies involved orthotopic implantation of luciferase+ M3-9-M tumor cells into syngeneic C57BL/6 (B6) recipients.
  - Mice were randomized to a control diet or a-TEA-supplemented chow for 21 days and evaluated for bioluminescence, tumor growth and overall survival.
- Gene expression of tumor and splenic T cells were analyzed by bulk RNA-Seq and flow cytometry respectively.
  - The top 50 increased and decreased genes were analyzed by Cytoscape FI Network analysis seeded with the human homologs. The depicted network has 84 genes due to the use of linker genes by Reactome

- **a-TEA, Alpha-Tocopheryloxyacetic acid (a-TEA)**, is a redox-silent analog of alpha-tocopherol that induces *apoptotic and immunogenic cell death* of tumor cells.
- a-TEA mediates *apoptosis of RMS* in vitro and *suppresses* in vivo *tumor growth*, leading to **prolonged survival**.
- Spleens from a-TEA treated mice showed **increased levels of IFN-gamma+, CD4+ T cells**, and decreased levels of *Arg1+ and PD-L1+ myeloid cells*.
- RNA-seq analysis of tumors from treated and control groups showed **gene expression changes** in *EP300, c-Jun, and CXCR4*.

RESULTS

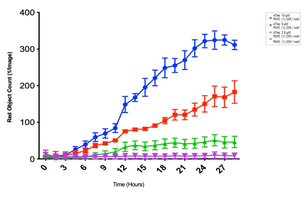

Figure 2. Live cell imaging of apoptosis after a-TEA. M3-9-M RMS treatment with 2.5-100 uM a-TEA induced apoptosis in a dose-dependent manner within 24 hours ($p < 0.05$).

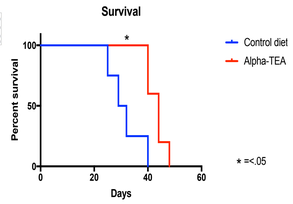

Figure 3. Overall survival of RMS-bearing mice after a-TEA. Prolonged survival (> 4 weeks) of recipients of a-TEA compared to recipients of matched control chow ($p<0.05$) treated for 3 weeks.

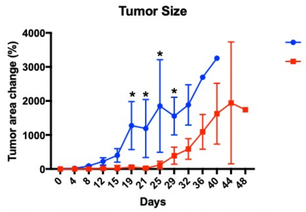

Figure 4. RMS tumor growth in mice after a-TEA. Recipients of a-TEA chow had 30-40 % reduced tumor growth ($p<0.01$)

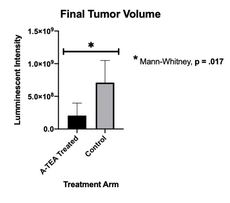

Figure 5. Bioluminescence of luciferase+ RMS-bearing mice after a-TEA. ($p<0.05$)

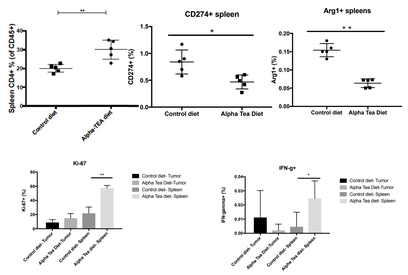

Figure 6. Immune modulation within splenocytes of RMS-bearing mice treated with a-TEA. B6 mice were injected with RMS M3-9-M and tumors were allowed to establish. Mice were then randomized to receive a control diet or a-TEA enriched diet for 3 weeks. Spleens were analyzed at the end of treatment. Increased levels of **(A)** CD4+ T-cells, **(B)** decreases in PD-L1+ (CD274+) cells ($p<0.05$) and **(C)** Arginase-1+ cells ($p<0.01$). **(D)** Increases in Ki-67 proliferation and in **(E)** IFN-gamma+ cells were also seen.

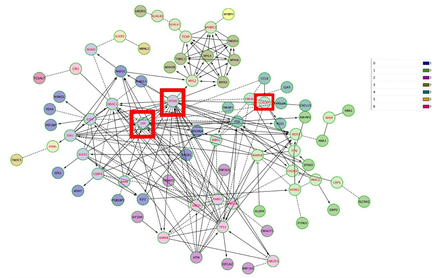

Figure 7. Gene set enrichment analyses of excised RMS tumors after a-TEA treatment. B6 mice were injected with RMS M3-9-M and tumors were allowed to establish. Mice were then randomized to receive a control diet or a-TEA enriched diet for 3 weeks. Tumors were analyzed 3 weeks post-treatment. Increased gene expression of EP300, CXCR4, and c-Jun as compared to RMS tumors from mice fed control chow.

CONCLUSIONS

- a-TEA mediates apoptosis of RMS in vitro and suppresses in vivo tumor growth, leading to prolonged survival
- Potential mechanisms may include direct apoptosis of RMS versus enhanced activation of adaptive immunity through IFN-gamma+ production by CD4+ T cells and/or suppression of Arg1+ PD-L1+ myeloid cells
- a-TEA may have direct effects on tumor cell proliferation through EP300 and c-Jun as well as indirect effects by immune cell recruitment through CXCR4 expression

ADDITIONAL KEY INFORMATION

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