560 ALPHATOCOPHERLOXYACETIC ACID INDUCES APOPTOSIS OF MURINE RHABDOMYOSARCOMA IN VITRO WHILE MODULATING INNATE AND ADAPTIVE IMMUNE RESPONSES IN VIVO

Fernanda Szewc1, Longhen Song1, Sean Rinella1, Christopher Dubay1, Emmanuel Apoktasi2, William Redmond3, Christian Capitini3, University of Wisconsin-Madison School of Medicine and Public Health, Madison, WI, USA; 1Earle A. Chiles Research Institute, Portland, OR, USA; 2Vivana Therapeutics, Portland, OR, USA

Background Relapsed pediatric sarcomas have a poor prognosis with no available curative options. Alpha-Tocopherolxyacetic acid (a-TEA) is a redox-silent analog of alphatocopherol that induces apoptotic and immunogenic cell death of tumor cells at doses that are not harmful to normal 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 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+) and live cell imaging (Annexin V+ cells). In vivo studies involved orthotopic implantation of luciferase+ M3-9-M tumor cells into syngeneic C57BL/6 recipients. Once tumors were palpable, 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-infiltrating and splenic T cells were analyzed by bulk RNA-Seq and flow cytometry respectively.

Results 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) as measured by flow cytometry and live cell imaging. In vivo studies with the M3-9-M RMS mouse model showed that recipients of a-TEA treated-chow had 30–40% reduced tumor growth (p<0.01) and bioluminescence (p<0.05), leading to prolonged survival (> 4 weeks) compared to recipients of matched control chow (p<0.05). Spleen cells isolated from a-TEA-fed tumor-bearing mice demonstrated increased levels of IFNα/γ cells, CD4+ T-cells, Ki-67 proliferation, and decrease in splenic CD11b+ arginase-1+ (p<0.01) and PD-L1+ cells (p<0.05) compared to their counterparts on the control diet. Gene set enrichment analyses of excised RMS tumors after a-TEA treatment revealed increased gene expression of CD24, EP300, CXCR4, and c-Jun as compared to tumors from mice fed control chow.

Conclusions These data indicate that a-TEA mediates apoptosis of RMS in vitro and suppresses in vivo tumor growth, leading to prolonged survival likely via enhanced activation of adaptive immunity through CD4+ T cells and suppression of innate immunity through regulation of myeloid cell subsets. Furthermore, a-TEA may have direct effects on tumor cell proliferation through EP300 and c-Jun as well as indirect effects on tumor growth by regulation of immune cell recruitment through CD24 and CXCR4 gene expression. Administration of a-TEA as a potential salvage treatment for RMS is warranted.

Acknowledgements This study was supported by NIH TL1 TR002375 (FS), St. Baldrick’s Stand up to Cancer (SU2C) Pediatric Dream Team Translational Research Grant SU2C-AACR-DT-27-17, NIH/NCI R01 CA215461, American Cancer Society Research Scholar Grant RSG-18-104-01-LIB, and the Midwest Athletes Against Childhood Cancer (MACC) Fund (CMC). SU2C is a division of the Entertainment Industry Foundation. Research grants are administered by the American Association for Cancer Research, the scientific partner of SU2C. The contents of this article do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US government.

Ethics Approval The University of Wisconsin-Madison Animal Care and Use Committee approved all protocols (M005915).
SO-C101 INDUCES NK CELL CYTOTOXICITY AND ANTI-TUMOR ACTIVITY

Background SO-C101 is a superagonist fusion protein of inter-leukin (IL)-15 and the IL-15 receptor α (IL-15Rα) sushii+ domain, representing a promising clinical candidate for the treatment of cancer. SO-C101 specifically stimulates natural killer (NK) cells and memory CD8+ T cells with no significant expansion and activation of regulatory T cell compartment.

Methods Human NK cell proliferation, the expression of NK cell receptors and ADCC activity of human PBMC after stimulation with SO-C101 in vitro in combination with monoclonal antibodies were detected by flow cytometry. The anti-tumor efficacy of SO-C101 in combination with Daratumumab was assessed in a multiple myeloma SCID xenograft mouse model in vivo.

Results In this study, we show that SO-C101 induced proliferation and expansion of both major subsets of human NK cells, CD56brightCD16- and CD56dimCD16+. Furthermore, SO-C101 induced expression of the cytotoxic receptors Nkp30 and NKG2D whereas no upregulation of the inhibitory receptors CD158a, CD158b and NKG2A was detected. Both NK cell subsets activated by SO-C101 exhibited cytotoxicity towards cancer cells in vitro. Using human PBMCs, we show that SO-C101 potentiated killing of tumor cells induced by several clinically approved therapeutic monoclonal antibodies such as Cetuximab, Daratumumab and Obinutzumab in vitro. SO-C101 and Daratumumab mono-therapy treatment inhibited tumor growth in vivo, however, their combination showed the strongest anti-tumor efficacy. Specifically, sequential administration of Daratumumab, followed by SO-C101 promoted complete tumor regression, compared to partial anti-tumor responses induced by the respective monotherapies.

Conclusions SO-C101 augments the anti-tumor activity of therapeutic antibodies by increasing NK cells mediated antibody-dependent cell cytotoxicity. These results support the evaluation of SO-C101 in combination with monoclonal therapeutic antibodies in clinical studies.

Ethics Approval The anti-tumor efficacy studies in mice were approved by the internal ethics board of the respective contract research organization (CRO).

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0561