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

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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 at doses that are not harmful to healthy 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+ 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 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 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 IFN??+ 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.

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Ethics Approval The University of Wisconsin-Madison Animal Care and Use Committee approved all protocols (M005915).

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561 DUOBODY®-PD-L1×4–1BB (GEN1046) INDUCES SUPERIOR IMMUNE-CELL ACTIVATION, CYTOKINE PRODUCTION AND CYTOTOXICITY BY COMBINING PD-L1 BLOCKADE WITH CONDITIONAL 4–1BB CO-STIMULATION

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Background Checkpoint inhibitors targeting the PD-1/PD-L1 axis (CPI) have changed the treatment paradigm and prognosis for patients with advanced solid tumors; however, many patients experience limited benefit due to treatment resistance. 4-1BB co-stimulation can activate cytotoxic T-cell- and NK-cell-mediated anti-tumor immunity and has been shown to synergize with CPI in preclinical models. DuoBody-PDL1×4-1BB is a first-in-class, Fc-silenced, bispecific next-generation checkpoint immunotherapy that activates T cells through PD-L1 blockade and simultaneous PD-L1-dependent 4-1BB co-stimulation. Here we present preclinical evidence for the mechanism of action of DuoBody-PD-L1×4-1BB, and proof-of-concept using mouse-reactive mbsAb-PD-L1×4-1BB in vivo.

Methods RNA sequencing analyses was performed on primary human CD8+ T cells that were co-cultured with PD-L1+ monocytes in the presence of anti-CD3/anti-CD28 and test compounds. T-cell proliferation and cytokine production were analyzed in primary human T-cell and mixed lymphocyte reaction (MLR) assays in vitro, and using patient-derived tumor-infiltrating lymphocytes (TILs). Cytotoxic activity was assessed in co-cultures of CLDN6+PD-L1+ MDA-MB-231 tumor cells and CLDN6-TCR+CD8+ T cells. Anti-tumor activity of mbsAb-PD-L1×4-1BB was tested in vivo using the CT26 mouse tumor model. Immunophenotyping of the tumor microenvironment (TME), tumor-draining lymph nodes (tdLNs) and peripheral blood was performed by flow cytometry.

Results DuoBody-PD-L1×4-1BB significantly induced expression of genes associated with immune cell proliferation, migration and cytokine production in activated CD8+ T cells, which were not altered by CPI. DuoBody-PD-L1×4-1BB dose-dependently enhanced expansion of human TILs ex vivo. DuoBody-PD-L1×4-1BB dose-dependently enhanced T-cell proliferation and pro-inflammatory cytokine production in vitro (e.g. IFNγ and TNFα; in polyclonal and antigen-specific T-cell proliferation assays and MLR), which was