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

1Fernanda Szewc*, 1Longhen Song, 1Sean Rinella, 2Christopher Dubay, 3Emmanuel Akpodjja, 4William Redmond, 5Christian Capitelli, 6University of Wisconsin-Madison School of Medicine and Public Health, Madison, WI, USA; 7Earle A. Chiles Research Institute, Portland, OR, USA; 8Vicena Therapeutics, Portland, OR, USA

Background Relapsed pediatric sarcomas have a poor prognosis with no available curative options. Alpha-Tocopheryl oxycetic 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 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 μM 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 20–30% 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 IFNγ+ 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).

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

1Alexander Muik*, 2Ist Atinbas, 2Rachel Kosoff, 2Friederike Gieseke, 2Kristina Schödel, 2Theodora Salcido, 2Saska Burm, 2Avra Toker, 2Lena Kranz, 2Mathias Vormehr, 2David Eisel, 2Mark Fereshteh, 2Özlem Türeci, 2Esther Breij, 2Tahamtan Ahmadi, 2Ugur Sahin, 2Maria Jure-Kunkel. 1BioNTech SE, Mainz, Germany; 2Genmab A/S, Utrecht, Netherlands

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-PD-L1×4-1BB 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 DNA 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
SO-C101 is a superagonist fusion protein of inter-leukin (IL)-15 and the IL-15 receptor α (IL-15Rα) sushii+ domain. SO-C101 effectively stimulates natural killer (NK) cells and memory CD8+ T cells with no significant expansion and activation of regulatory T cells in vitro. SO-C101 potentiates antibody-dependent cell cytotoxicity. These results support the evaluation of SO-C101 in combination with monoclonal therapeutic antibodies in clinical studies.

Methods In this study, we investigated different administration schedules of SO-C101 in cynomolgus monkeys to assess its pharmacodynamics and pharmacokinetics properties using intravenous (IV) and subcutaneous (SC) routes of administration.

Results Subcutaneous administration of SO-C101 was more effective than IV administration in terms of activating target immune cells which was correlated to the differences in SO-C101 exposure. Repeated administration of SO-C101 over two weeks promoted an increase of absolute lymphocyte counts and of the circulating NK and CD8+ T cell numbers. Moreover, two administrations on consecutive days were optimal and comparable to four daily administrations. We further determined an optimal schedule for a repetitive SO-C101 SC administration to achieve a cycle-dependent stimulation of NK and CD8+ T cells over the course of 10 weeks. These studies allowed to correlate the concentration to response relationship in vitro with the relationship between Cmax following SC administration and the resulting NK and CD8+ T cell activation levels in vivo. These data were used to determine the starting dose and subsequent dose escalation steps of SO-C101 in an ongoing Phase I clinical trial in patients with advanced solid tumors.

Conclusions Since the potency of SO-C101 to activate NK and CD8+ T cells in vitro is equivalent between human and cynomolgus monkeys, these studies informed the dose and schedule selection for the ongoing Phase I clinical study (NCT04234113).

Ethics Approval Pharmacodynamics and pharmacokinetics studies in cynomolgus monkeys were approved by Ethics Board of an appropriate contract research organizations (CROs).