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703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

**Development and Testing of a Glycoengineered Anti-ROR1 Antibody with Enhanced Capacity for Directing Antibody-Dependent Cellular Cytotoxicity (ADCC) of Chronic Lymphocytic Leukemia Cells**

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Receptor tyrosine kinase-like orphan receptor 1 (ROR1) is an oncoembryonic surface antigen expressed on the neoplastic cells of patients with chronic lymphocytic leukemia (CLL) or other types of cancer, but not on virtually all normal postpartum tissues (Fukuda T., et al., PNAS, **105**:3047, 2008). Zilovertamab (also known as UC-961 or cirmtuzumab) is a humanized anti-ROR1 monoclonal antibody (mAb) currently under clinical investigation; this mAb is capable of blocking ROR1-signaling, which can contribute to cancer-cell migration, proliferation, survival, and self-renewal. We generated a glycoengineered form of zilovertamab (GE- zilovertamab) and examined whether it could mediate enhanced antibody-dependent cellular cytotoxicity (ADCC) against neoplastic cells that express ROR1. Initial studies used Jurkat-Lucia™ NFAT-CD16 cells (InvivoGen, San Diego, CA, USA) which express high-affinity CD16A (FcγRIIIA, V158 allotype) as effector cells (EC), and the CLL-cell line MEC1, or MEC1 cells transduced to express ROR1 (MEC1-ROR1), as target cells (TC), which each also express CD20. Co-culture of Jurkat-Lucia cells for 6 hours with the anti-CD20 mAb rituximab and MEC1 or MEC1-ROR1 cells induced Jurkat-Lucia cells to express a luciferase reporter gene under the control of an ISG54 minimal promoter fused to six NFAT response elements; this endowed the Jurkat-Lucia cells with high luminescence activity that was not observed in co-cultures of EC and TC without added mAb. Neither zilovertamab nor GE-zilovertamab endowed Jurkat-Lucia cells with higher than background luminescence activity when co-cultured with MEC1 cells. However, GE-zilovertamab could induce high levels of luminescence activity in Jurkat-Lucia cells co-cultured with MEC1-ROR1 cells that was comparable to that of rituximab, and significantly greater than that of zilovertamab. We generated an orthogonal system with which to examine the relative ADCC activity of each of these mAb, using EC generated from the NK cell line, NK92, which we transduced with a lentivirus encoding a newly-generated high-affinity variant of CD16A (CD16v), which has been modified to block its cleavage by ADAM17. NK92 or NK92-CD16v were used as EC to effect cytotoxicity of Cr<sup>51</sup>-labeled MEC1, MEC1-ROR1, or primary CLL cells in a chromium-release assay. Addition of rituximab to co-cultures of EC with Cr<sup>51</sup>-labeled MEC1, MEC1-ROR1, or primary CLL caused mAb-dose-dependent ADCC of each of these TC by NK92-CD16v that was significantly greater than that by parental NK92 cells. Again, neither zilovertamab nor GE-zilovertamab could direct ADCC by NK92-CD16v or NK92 ADCC of MEC1 cells, which lack ROR1. However, GE-zilovertamab directed dose-dependent ADCC of MEC1-ROR1 or primary ROR1<sup>+</sup> CLL cells by either NK92-CD16v or NK92 at levels comparable to that directed by rituximab for either EC population; such levels were significantly greater than that directed by zilovertamab. ROR1<sup>+</sup> CLL cells harboring del(17p) or mutations in TP53 (del(17p)/m TP53) and/or that were resistant to targeted therapies (e.g., inhibitors of BTK or BCL2), were as susceptible to GE-zilovertamab-directed ADCC as were CLL cells without del(17p)/m TP53 from patients who had not had prior therapy. These data demonstrate that GE-zilovertamab can direct high-level ADCC lysis of ROR1-expressing neoplastic cells with greater activity than zilovertamab, encouraging development of clinical studies to evaluate GE-zilovertamab for therapy of patients with CLL or other ROR1-positive cancers.

**Disclosures Breitmeyer:** Oncternal Therapeutics Inc.: Current Employment, Current equity holder in publicly-traded company, Membership on an entity's Board of Directors or advisory committees. **Kipps:** Nexus Biopharma, inc.: Honoraria, Other: Travel; Johnson & Johnson: Honoraria, Other: Travel; Curio Bioscience: Honoraria, Other: Travel; Dava Oncology: Honoraria, Other: Travel; Genentech/Roche: Research Funding; Janssen: Honoraria, Other: Travel; Breast Cancer Research Foundation:

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