EVALUATION OF NOVEL ANTI-TIGIT ANTIBODY M6223 AS A SINGLE AGENT AND IN COMBINATION WITH AVELUMAB ON HUMAN NATURAL KILLER (NK) CELL CYTOTOXICITY

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**Background** M6223 is a fully human antagonistic anti-TIGIT immunoglobulin (Ig) G1 antibody with fragment crystallizable (Fc)-mediated effector function. Preclinical studies demonstrated that M6223 could induce an anti-tumor immune response through several mechanisms, including direct blockade of the TIGIT pathway, stimulation of CD226 dimerization/activation, and depletion of TIGIT+ immune subsets by Fc-mediated effector function. Avelumab is a human IgG1 anti–PD-L1 antibody with a wild-type Fc region that has been shown to induce antitumor activity in vitro via both adaptive effector cells (T cells) and innate immune effector cells (antibody-dependent cell-mediated cytotoxicity [ADCC] via NK cells). It is approved for urothelial carcinoma (UC), renal cell carcinoma, and Merkel cell carcinoma. We report an evaluation of the effects of M6223 as a single agent and in combination with avelumab on human NK cell cytotoxicity.

**Methods** NK cell anti-tumor cytotoxicity was measured against the cancer line MDA-MB-231 with beta-2 microglobulin (B2M) knockout using fluorescent live cell imaging. NK cells were treated with M6223 or its Fc effector null version PPB1791. Avelumab was also tested alone or at a single dose combination with M6223 to evaluate additive potential of these antibodies. A variety of CD155 (poliovirus receptor [PVR]) knockout and partial knockouts were generated to assess the dependence of anti-tumor cytotoxicity on expression of this ligand.

**Results** M6223 induced significant NK cell anti-tumor cytotoxicity in 4 different NK donors at doses above 0.3 mcg/mL (t-test: p<0.05) with an EC50 value of approximately 100 ng/mL. There was limited in vitro NK fratricide. An Fc-mutant version of M6223 had reduced anti-tumor cytotoxicity (at 0.3 mcg/mL: p=0.28). M6223 in combination with avelumab was more effective than either antibody alone, indicating that CD16-mediated ADCC is likely additive with TIGIT blockade. Anti-tumor cytotoxicity was retained with PVR (CD155) expression on target MDA-MB-231 cells at a wild-type level of 30–50% and was almost completely eliminated in CD155− cells. CD112 alone did not facilitate significant M6223-induced anti-tumor cytotoxicity, consistent with a weaker role of DNAX accessory molecule 1 (DNAM-1)-mediated recognition of this receptor.

**Conclusions** This study confirmed that NK cell cytotoxicity plays an important role in the anti-tumor activity of M6223 and demonstrated the additive effect of avelumab and M6223 in enhancing NK cell activation, especially in CD155+ human leukocyte antigen (HLA) class I-deficient target tumors. Currently, M6223 plus avelumab is being studied as first-line maintenance therapy for advanced UC in the phase 2 JAVE-LIN Bladder Medley umbrella trial (NCT05327530).