**EVALUATION OF THE TIGIT+ IMMUNE SUBSET DEPLETION EFFECT OF THE ANTI-TIGIT ANTIBODY M6223**

Cunxiao Xu*, Sireesha Yalavarthi, Clotilde Bourin, Jacques Moisan, Andree Blaukat, Laura Helming. EMD Serono, Billerica, MA, United States; The Healthcare Business of Merck KGaA, Darmstadt, Germany

**Background**

T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains (TIGIT) is an inhibitory receptor expressed on lymphocytes and has recently emerged as a target in cancer immunotherapy. M6223 is a fully human antagonistic anti-TIGIT antibody in immunoglobulin (Ig) G1 format with Fc-mediated effector function. Preclinical studies demonstrated that M6223 can induce an anti-tumor immune response by complementary mechanisms, including but not limited to: direct blockade of the TIGIT pathway; stimulation of costimulatory receptor CD226 dimerization/activation; and depletion of TIGIT+ immune subsets by Fc-mediated effector function. This study was designed to understand the mechanism of action of the depletion effect.

**Methods**

The TIGIT+ immune subset depletion effect of M6223 in the tumor microenvironment (TME) was investigated in humanized TIGIT knock-in mice using a flow cytometry assay. The anti-tumor efficacy of two chimeric M6223 antibodies, one with an effector competent mouse IgG2c constant region (M6223-muIgG2c) and the other with an effector null mouse IgG1-D256A constant region (M6223-muIgG1), was investigated in syngeneic tumor models in humanized TIGIT knock-in mice.

**Results**

M6223 dose-dependently depleted TIGIT+ immune subsets in the TME at day 1 post-treatment, but had no significant effect on total infiltrated CD8+ T cells and natural killer (NK) cells. The depletion effect gradually weakened over time but remained at day 7 and day 14. M6223 treatment stimulated CD8+ T cell and NK cell infiltration and boosted CD226 expression at day 7 and day 14 post treatment. The ratio of CD226 to TIGIT was significantly increased in immune subsets at all time points tested. Further studies demonstrated that only M6223 surrogate with effector function could deplete TIGIT+ immune subsets at day 1; the effector null version of M6223 slightly decreased TIGIT-expressing immune subsets on day 7 and day 14. In addition, this effector null surrogate version of M6223 did not have anti-tumor efficacy in syngeneic tumor models in humanized TIGIT knock-in mice.

**Conclusions**

The results demonstrate that innate depletion of TIGIT+ immune subsets by Fc-mediated effector function plays an important role in anti-tumor immunity and suggest that immune pharmacodynamics in clinical trials should be closely monitored at early time points. Currently, M6223 is being evaluated in combination with avelumab as first-line maintenance therapy for advanced urothelial carcinoma in the phase 2 JAVELIN Bladder Medley umbrella trial (NCT05327530).