Introduction: ST4 (trophoblast glycoprotein), an oncofetal antigen involved in embryo development, is expressed on the cell surface of multiple cancers. Several ST4-directed interventions have been reported, including a cancer vaccine and an antibody-drug conjugate. A superantigen-driven redirected cell killing modality was developed, and this strategy was applied to target 5T4-expressing tumors.

ST4 (trophoblast glycoprotein), an oncofetal antigen involved in embryo development, is expressed on the cell surface of multiple cancers. Several ST4-directed interventions have been evaluated, including a cancer vaccine and an antibody-drug conjugate. A superantigen-driven redirected cell killing modality was developed, and this strategy was applied to target 5T4-expressing tumors.

Methods: ST4 x CD3 DART, a humanized Fc-bearing DART molecule, was stable expressed in CHO cells and purified to homogeneity via a standard antibody-purification platform. In vitro characterization and functional studies were performed with ST4-positive tumor cell lines and human T cells. In vivo studies were performed in immune-deficient tumor-bearing mice co-implemented with activated human T cells or reconstituted with human peripheral blood mononuclear cells (PBMCs). Pharmacokinetic studies were performed in both human FcRn transgenic mice and cynomolgus monkeys.

Results: ST4 x CD3 DART demonstrated bispecific binding properties to both human and cynomolgus monkey antigens. In redirected cytolytic studies, ST4 x CD3 DART-mediated lysis of ST4-positive pancreatic, lung, renal, triple-negative breast, and ovarian cancer cell lines was observed. Tumor clearance studies in NOD/SCID mice implanted orthotopically with TNBC MB-231 cells established, were treated with ST4 x CD3 DART. Antitumor activity was observed at doses as low as 0.8 µg/kg, but not with a CD3-binding control DART protein. In addition, human PBMC-reconstituted NOD/SCID mice splenic lymphocytes were intradermally injected with renal and pancreatic tumor cell lines or orthotopically with a triple-negative breast cancer cell line. Tumor establishment, were treated with ST4 x CD3 DART. In vivo studies were performed in both human FcRn transgenic mice and cynomolgus monkeys. Tumor clearance studies in NOD/SCID mice implanted subcutaneously with 5T4-positive pancreatic, lung, renal, triple-negative breast cancer cell lines were performed with ST4 x CD3 DART. In redirected cytolysis studies, ST4 x CD3 DART-mediated CTL activity against tumor cell lines was observed. Tumor clearance studies in NOD/SCID mice implanted subcutaneously with and cynomolgus monkeys. Tumor clearance studies in NOD/SCID mice implanted subcutaneously with and cynomolgus monkeys. Tumor clearance studies in NOD/SCID mice implanted subcutaneously with and cynomolgus monkeys.

Conclusions: In summary, ST4 x CD3 DART displays robust antitumor activity against several cancer cell lines in vitro and in vivo together with a favorable pharmacokinetic profile and merits further consideration as a potential treatment for ST4-positive cancers.