Background The tumor-associated antigen 5T4 is expressed across a wide range of solid cancers. DuoBody-CD3x5T4 is a bispecific antibody (bsAb) that crosslinks CD3 on T cells with 5T4 on tumor cells, thereby inducing T-cell activation and T-cell mediated cytotoxicity in 5T4-expressing tumor cells. Here, we tested the capacity of DuoBody-CD3x5T4 to engage different T-cell subsets in vitro and investigated the mechanism of action (MoA) in vivo by combining preclinical efficacy studies with exploratory pharmacodynamic (PD) biomarker analyses.

Methods Immunohistochemistry was performed on patient-derived tumor tissue-microarrays using a commercial 5T4 monoclonal antibody (EPR5529). The capacity of DuoBody-CD3x5T4 to engage naïve and memory T-cell subsets was assessed in co-cultures of T cells and 5T4-positive tumor cells, using T-cell activation and T-cell mediated cytotoxicity as read-outs. Anti-tumor activity in vivo as well as peripheral and intratumoral PD biomarkers were investigated in humanized mice bearing 5T4-expressing cell line-derived xenograft (CDX) or patient-derived xenograft (PDX) tumor models.

Results High prevalence of 5T4 expression (in >86% of biopsies) was observed in NSCLC, SCCHN, TNBC, bladder, esophageal, prostate and uterine cancer. In co-cultures of 5T4+ tumor cells and T cells in vitro, DuoBody-CD3x5T4 induced dose-dependent cytotoxicity, associated with T-cell activation, proliferation, and cytokine, perforin and granzyme production. Crosslinking of T cells with 5T4-expressing tumor cells was essential as no cytotoxicity was observed in CRISPR-Cas9-generated 5T4-knockout tumor cells or with control bsAbs targeting only CD3 or 5T4. Importantly, naïve and memory CD4+ or CD8+ T-cell subsets had equal capacity to mediate DuoBody-CD3x5T4-induced cytotoxicity, although naïve T-cell subsets showed slower kinetics. DuoBody-CD3x5T4 (0.5–20 mg/kg) demonstrated anti-tumor activity in 5T4+ breast and prostate cancer CDX and lung cancer PDX models in humanized mice. Treatment with DuoBody-CD3x5T4 was associated with intratumoral and peripheral T-cell activation as well as elevated cytokine levels, including IFNγ, IL-6 and IL-8, in peripheral blood.

Conclusions DuoBody-CD3x5T4 induced T-cell mediated cytotoxicity in 5T4-expressing tumor cells, associated with T-cell activation and cytokine production in vitro. DuoBody-CD3x5T4 efficiently engaged naïve and memory T cells within both CD4+ and CD8+ T-cell populations to induce T-cell mediated cytotoxicity in 5T4+ tumor cells. In humanized CDX and PDX mouse models, DuoBody-CD3x5T4 showed anti-tumor activity, in addition to PD biomarkers associated with T-cell activation in the tumor and periphery. Currently, DuoBody-CD3x5T4 is being investigated in a first-in-human clinical trial for the treatment of solid tumors (NCT04424641), in which exploratory biomarker analyses to study the clinical MoA and PD are included.

Ethics Approval All experimental animal studies were performed according to Codia BioSciences IACUC approved AUP CB2020-001.

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