Background The immune checkpoint protein B7H4 is expressed on malignant cells in various solid cancers, whereas expression is highly restricted in normal tissue. DuoBody-CD3xB7H4 (GEN1047) crosslinks CD3 on T cells with B7H4 on tumor cells, thereby inducing T-cell mediated cytotoxicity in B7H4-expressing tumor cells. Here, we evaluated the prevalence of B7H4 expression in solid cancers and investigated the preclinical mechanism of action (MoA) and pharmacodynamic (PD) markers associated with DuoBody-CD3xB7H4 biological activity in vitro and in vivo.

Methods B7H4 protein expression was determined by immunohistochemistry in human tumor biopsies, and by semi-quantitative flow cytometry for tumor cell lines. T-cell mediated cytotoxicity was assayed in vitro using co-cultures of B7H4+ tumor cells and healthy donor T cells. Antitumor activity in vivo was tested in a patient-derived xenograft (PDX) screen using several tumor models engrafted subcutaneously in mice with a humanized immune system (HIS). Antitumor activity and exploratory biomarkers were further evaluated in a follow-up study in ovarian cancer PDX HIS mice.

Results In tissue microarrays of primary NSCLC-SCC, ovarian, endometrial, and breast cancer, over 25% of the tumor biopsies contained at least 50% B7H4-positive tumor cells. In vitro, DuoBody-CD3xB7H4 induced target-specific, dose-dependent, and complete tumor cell kill in a panel of tumor cell lines expressing varying levels of B7H4, while B7H4 expression levels correlated with the EC50. DuoBody-CD3xB7H4 demonstrated antitumor activity in ovarian and breast cancer PDX models with high B7H4 expression. Antitumor activity of DuoBody-CD3xB7H4 in an ovarian cancer PDX model was confirmed in a dose-response study and was associated with the number of circulating human CD3+ T cells before randomization, an indicator of the humanization rate of the HIS mice. In the periphery, DuoBody-CD3xB7H4 treatment was associated with a transient decrease in circulating immune cells, increased T-cell activation, and elevated levels of granzyme B and cytokines. In the tumor, DuoBody-CD3xB7H4 treatment increased the number of tumor-infiltrating lymphocytes.

Conclusions High prevalence of B7H4 expression was observed in primary NSCLC-SCC, ovarian, endometrial, and breast cancer tissue. In vitro, DuoBody-CD3xB7H4 showed antitumor activity across a range of B7H4 expression levels. DuoBody-CD3xB7H4 showed dose-dependent antitumor activity in vivo, associated with increased numbers of intratumoral T cells and peripheral PD biomarkers of T-cell activation and cytokine production. Currently, DuoBody-CD3xB7H4 is being investigated in a first-in-human clinical trial for the treatment of solid tumors known to express B7H4 (NCT05180474), in which the MoA and PD, including biomarkers of response, will be clinically explored.

Ethics Approval Animal experiments were performed according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) and in accordance with the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).