Background 4–1BB is a key costimulatory receptor that mediates robust and durable tumor control by providing a secondary signal to antigen-experienced effector T cells. First-generation 4–1BB agonistic antibodies activated 4–1BB in the presence of Fc receptors and showed signs of efficacy but triggered hepatotoxicity. The 4–1BB agonistic activity of CLN-418, a fully human bispecific antibody, is instead dependent on cross-linking by B7H4, a widely expressed tumor-associated antigen with high prevalence in solid tumors and minimal expression in normal tissues. Targeting B7H4, an immunosuppressive checkpoint that shows minimally overlapping expression with PD-L1, may potentially address patients resistant to PD1 blockade. Currently, CLN-418 is the only B7H4 x 4–1BB bispecific antibody in clinical development.

Methods Expression of B7H4, 4–1BB and immune correlates were examined in PBMC, cancer cell lines and solid tumors from patients using bioinformatics analysis of public datasets. CLN-418 binding to B7H4 and 4–1BB was evaluated using surface plasmon resonance, and simultaneous CLN-418 binding to 4–1BB and B7H4-expressing cells was assessed by flow cytometry. B7H4-dependent agonistic activity of CLN-418 was demonstrated by co-incubation with engineered 4–1BB reporter or primary T cells in the presence of B7H4-expressing cancer cells. CLN-418 efficacy was examined in a MC38-hB7H4 syngeneic tumor model in 4–1BB-humanized mice, as well as a human xenograft model in human PBMC-engrafted mice.

Results 4–1BB receptor expression was low or absent on resting PBMC but was induced on activated tumor antigen-specific T cells with levels increasing upon immune rechallenge. TCGA and CPTAC database mining revealed robust and consistent B7H4 RNA and protein expression across multiple solid cancers, including ovarian, endometrial and breast carcinoma. 4–1BB gene expression in solid tumors was associated with CD8 T cell gene signature. CLN-418 showed specific high-affinity binding to human B7H4 and 4–1BB proteins, as well as dual binding to 4–1BB and B7H4 expressing cells. CLN-418 binding to B7H4-positive cells promoted 4–1BB engagement and T cell activation, with costimulatory signal strength proportional to B7H4 levels. CLN-418 exhibited robust dose-dependent single agent efficacy against MC38-hB7H4 tumors, with 79% and 68% tumor growth inhibition at 3 and 0.5 mg/kg dose levels, respectively. CLN-418 monotherapy also controlled growth of a B7H4-positive human carcinoma in PBMC-engrafted mice.

Conclusions B7H4 and 4–1BB expression in cancer cells, PBMC and solid tumors, together with robust B7H4-dependent single agent activity of CLN-418 in preclinical tumor models, support the development of CLN-418 in patients with difficult-to-treat solid cancers. A Phase 1 dose-escalation trial of CLN-418 is ongoing (NCT05306444).

Ethics Approval The anti-tumor efficacy studies in mice were approved by the internal ethics board of the respective contract research organization (CRO).