Background Claudin 18.2 (CLDN18.2), a tetraspanin family member essential for the formation of tight junctions, is highly expressed in gastric, pancreatic and esophageal adenocarcinomas, with physiological expression restricted to gastric mucosal epithelial cells. CLDN18.2 is a validated therapeutic target; zolbetuximab, a CLDN18.2-directed monoclonal antibody, demonstrated clinical benefit in combination with chemotherapy in CLDN18.2-high gastric cancer. Similarly, CLDN18.2-targeting chimeric antigen receptor T cells and antibody-drug conjugates have shown efficacy in gastric cancers and other modalities, including T cell-engagers (TCE), are also in clinical development. Here we describe AZD5863, a CLDN18.2 and cluster of differentiation 3 (CD3)-targeting TCE designed with bivalent high-affinity binding to CLDN18.2 and monovalent low-affinity binding to CD3 to reduce class-associated toxicities, such as cytokine release syndrome, while maintaining potent and specific antitumor activity.

Methods Surface plasmon resonance and cell-binding assays were used to assess AZD5863 affinity and binding to CLDN18.2 and CD3. T cell-dependent cellular cytotoxicity (TDCC) assays were conducted by co-culturing human peripheral blood mononuclear cells (hPBMCs) with human tumor cell lines (hTCLs) expressing distinct levels of CLDN18.2. Bystander killing was determined by mixing CLDN18.2-high or CLDN18.2-knockout cells at different ratios as targets in TDCC assays. In vivo studies tested AZD5863 in both immunodeficient mice humanized with CD3/CD28-activated hPBMCs and implanted with hTCLs, and in immunocompetent human CD3 knock-in mice implanted with CLDN18.2-expressing MC38 cells. Cytokine release was assessed in supernatant and mouse plasma by enzyme-linked immunosorbent assay or multiplex immunoassays.

Results AZD5863 demonstrated specific binding to CLDN18.2 with high affinity, and to CD3 with low affinity. AZD5863-driven TDCC was demonstrated in vitro on a range of hTCLs, where EC50 values significantly correlated with levels of CLDN18.2 expression. Importantly, the cytotoxic activity of AZD5863 was associated with modest release of tumor necrosis factor alpha and interleukin 6. AZD5863 mediated concentration-dependent bystander killing of CLDN18.2-knockout cells at various CLDN18.2 +/- ratios. In humanized mouse models, AZD5863 significantly inhibited the growth of CLDN18.2-expressing gastric, pancreatic and esophageal tumors compared with isotype controls. AZD5863 also inhibited tumor growth in a human CD3 knock-in mouse model implanted with CLDN18.2-expressing MC38 tumor cells.

Conclusions AZD5863, an affinity-optimized TCE targeting CLDN18.2 and CD3, demonstrated potent preclinical antitumor activity both in vitro and in vivo, and induced bystander killing of CLDN18.2-negative tumor cells. A phase 1 trial evaluating AZD5863 in gastric, pancreatic and esophageal adenocarcinoma, is ongoing.

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Ethics Approval This study was conducted in accordance with international, national, and institutional guidelines as well as all relevant laws and regulations regarding research involving animals. Studies performed in mice received IACUC approval under protocol numbers AUP-22-17 and AUP-22-25.

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