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AZD5863: a specific, potent, affinity-optimized Claudin 18.2 and CD3 binding T cell-engager that elicits low cytokine release and is capable of bystander killing

AstraZeneca

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Background

Claudin 18.2 (CLDN18.2), an essential component of tight junctions in gastric epithelium, is highly expressed in gastric, pancreatic and esophageal adenocarcinoma [1] and is a validated therapeutic target in CLDN18.2-high gastric cancer [2,3]. Here we describe AZD5863, a CLDN18.2 and CD3-targeting T cell engager (TCE) designed with high CLDN18.2 and low CD3 affinity to reduce class-associated toxicities such as cytokine release syndrome while maintaining potent antitumor activity on CLDN18.2-positive cells and bystander killing of CLDN18.2-negative cells (Figure 1).

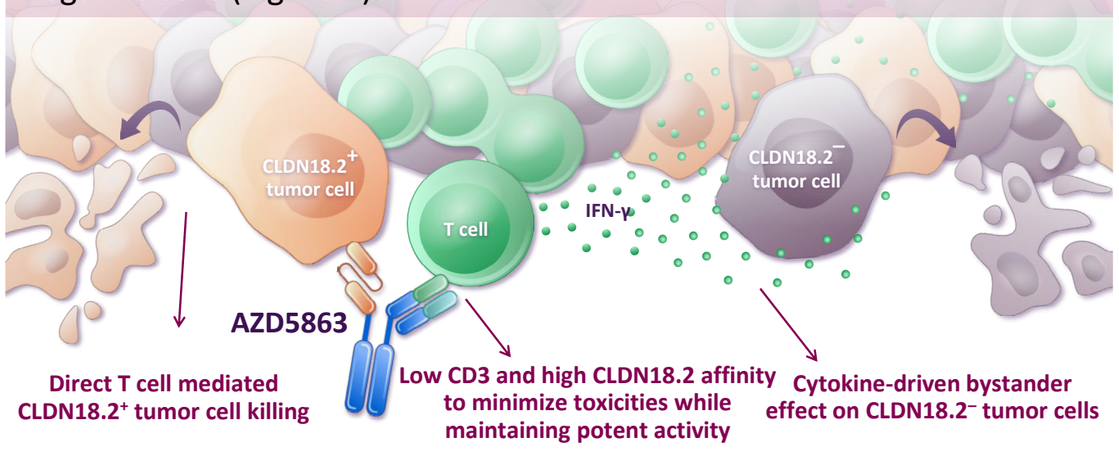

Figure 1: Proposed mechanism of action for AZD5863.

Results

AZD5863 is affinity-optimized and highly specific to CLDN18.2

In cell binding assays, AZD5863 showed specific binding to human CLDN18.2-expressing HEK293 cells and to CD3-expressing Jurkat cells, while it did not bind to HEK293 cells expressing CLDN18.1 (Figure 2).

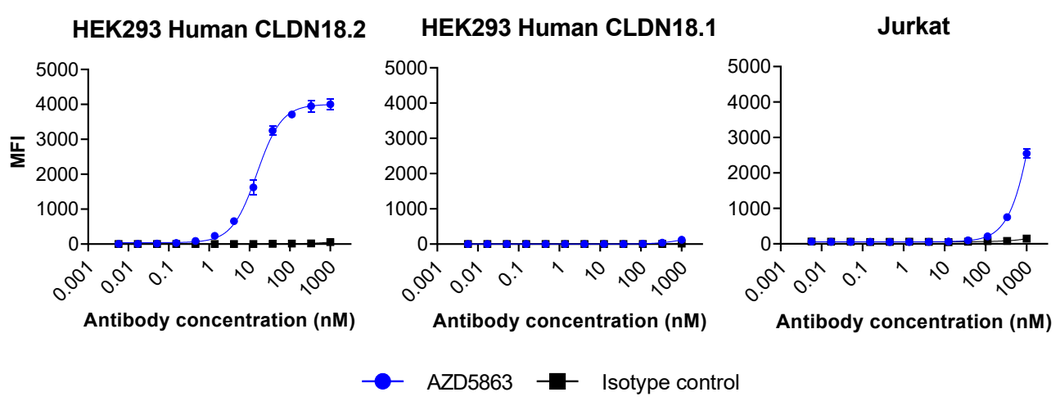

Figure 2: AZD5863 binding to human CLDN18.2-expressing HEK293 cells, CLDN18.1-expressing HEK293 cells and CD3-expressing Jurkat cells.

Surface plasmon resonance (SPR) experiments showed that AZD5863 binds to human CLDN18.2 with a KD of 0.1 ± 0.04 nM and to the human CD3δε heterodimer protein with an interaction affinity (KD) of 170 ± 7 nM.

AZD5863-driven T-cell dependent cellular cytotoxicity (TDCC) EC50s strongly correlate with levels of CLDN18.2 expression on a panel of human tumor cell lines

AZD5863-driven TDCC was demonstrated in vitro by co-culturing PBMCs from three donors with a panel (n=18) of human tumor cell lines. AZD5863-driven TDCC EC50 values were observed to significantly inversely correlate to levels of CLDN18.2 expression (measured by an antibody binding capacity [ABC] assay), indicative of potent and highly target-mediated AZD5863 activity (Figures 3-4). TDCC activity was consistently observed at > 1500 ABC/cell (Figures 3-4).

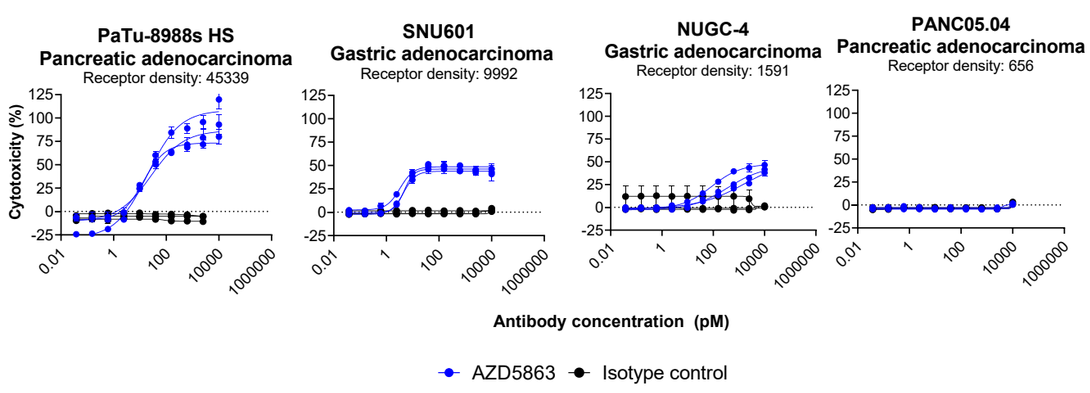

Figure 3: TDCC driven by AZD5863 using four representative human tumor cell lines with distinct CLDN18.2 receptor density (n=3 PBMC donors per treatment; E:T 4:1; 48 hours incubation).

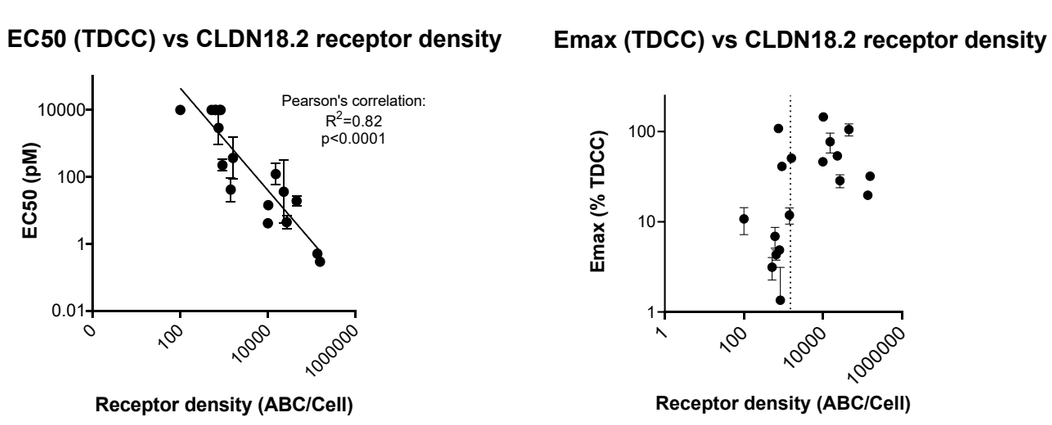

Figure 4: EC50s (pM) of AZD5863-mediated TDCC strongly correlate with tumor CLDN18.2 receptor density (n=18 cell lines tested, each with n=3 PBMC donors); TDCC activity is consistently observed at a receptor density > 1500 ABC/cell (indicated by dotted line).

AZD5863 maintains low secretion of IL-6 and TNF-α

AZD5863 was shown to be more potent than a TCE with high affinity for both CLDN18.2 and CD3 in an in vitro TDCC assay using NUGC4. However, AZD5863 triggered lower secretion of IL-6 and TNFα in vitro as well as in vivo, 4 hours post IV dosing in a NCG (NOD-*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}/NjuCrl) model humanized with PBMCs (Figure 5).

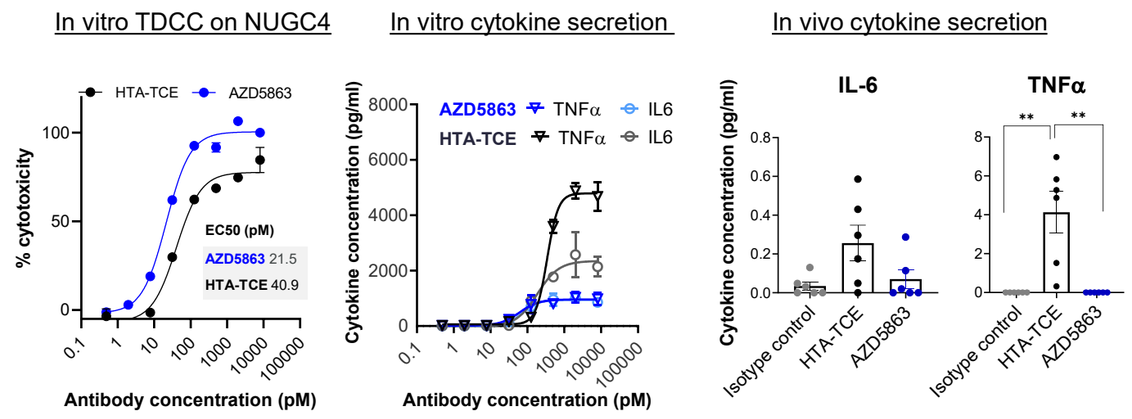

Figure 5: AZD5863 is more potent than a High T cell affinity CD3xCLDN18.2 TCE (HTA-TCE) while triggering lower secretion of IL-6 and TNFα, in vitro and in vivo.

AZD5863 mediates bystander killing of CLDN18.2-negative cells

AZD5863 mediated bystander killing of PaTu8988s CLDN18.2-knockout (KO) cells only when these were co-cultured (for two days) with CLDN18.2 positive PaTu8988s (WT, sorted for high CLDN18.2 expression), a pancreatic cancer cell line, and PBMCs (Figure 6).

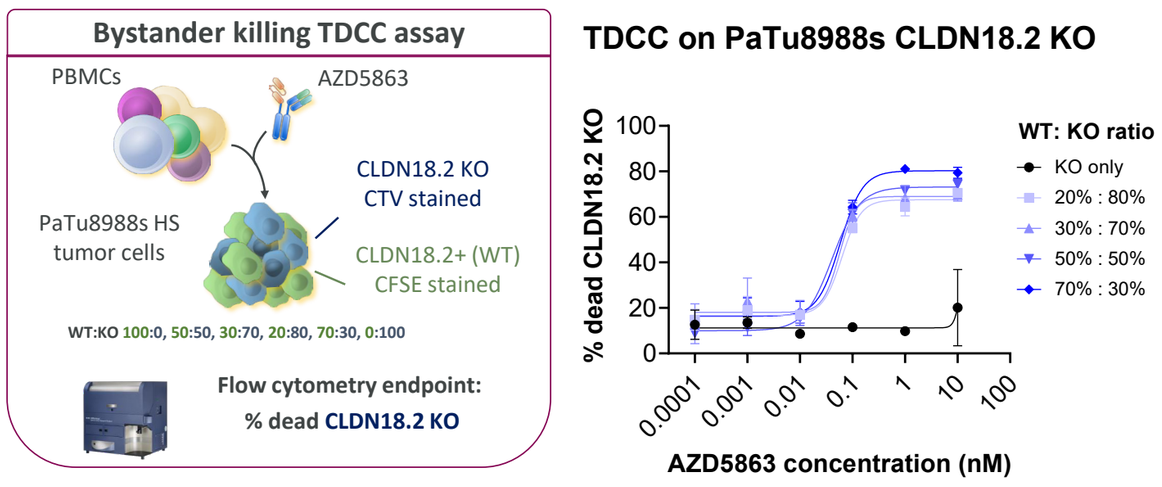

Figure 6: AZD5863 shows potent bystander killing of PaTu8988s CLDN18.2 KO cells when these are co-cultured with CLDN18.2+ cells (PaTu8988s CLDN18-2-high sorted [HS]).

AZD5863 induces tumor growth inhibition in humanized mouse models for gastric, pancreatic and esophageal tumors as well as in a human CD3 knock-in mouse model bearing moCLDN18.2-expressing MC38

In vivo, IP administration of AZD5863 mediated potent tumor growth inhibition in immunodeficient (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ, i.e. NSG) mice implanted with different human tumor cell lines - humanized with either CD3/CD28-activated hPBMCs or using unstimulated PBMCs in an admix model (NUGC4) - as well as in immunocompetent human CD3 knock-in mice implanted with murine CLDN18.2-expressing MC38 cells.

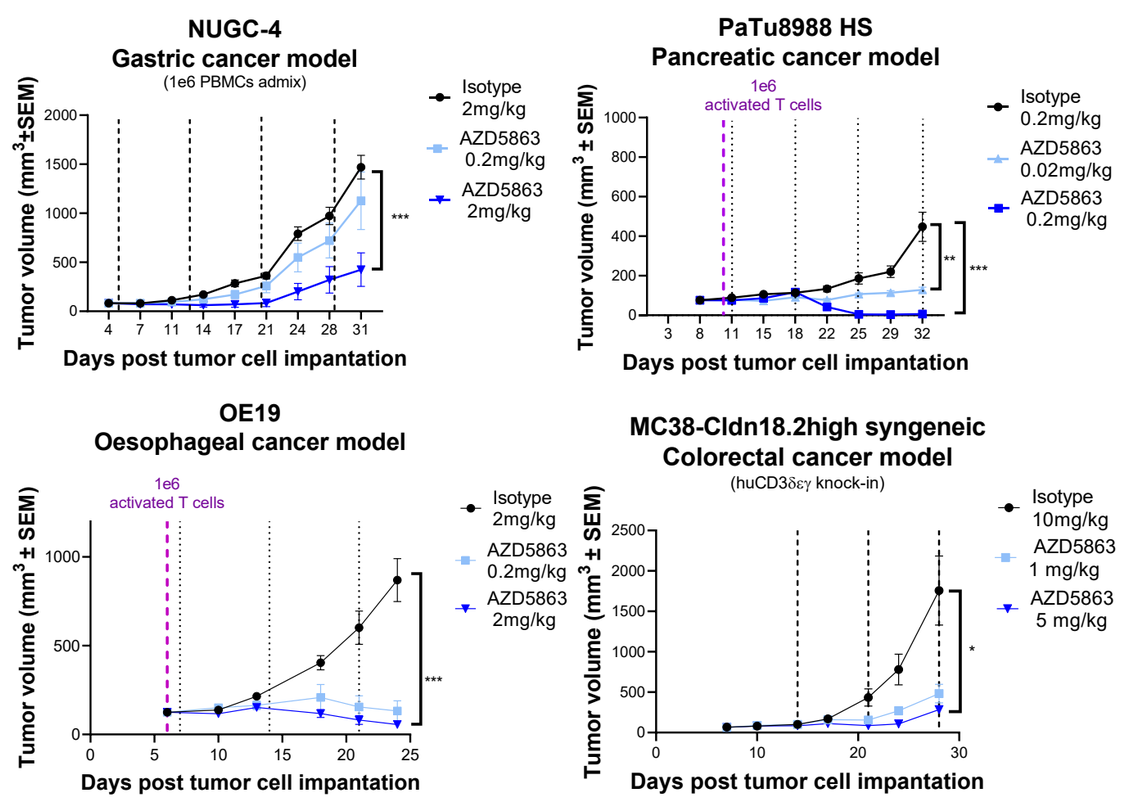

Figure 7: AZD5863 mediates tumor growth inhibition in humanized models and in syngeneic human CD3 knock-in mice implanted with CLDN18.2-expressing MC38 cells.

Conclusions

- AZD5863, an affinity-optimized TCE targeting CLDN18.2 and CD3, demonstrated potent and specific pre-clinical antitumor activity both in vitro and in vivo, induced bystander killing of CLDN18.2-negative tumor cells and low secretion of IL-6 and TNFα.
- A phase 1 trial testing AZD5863 in gastric, pancreatic and esophageal adenocarcinoma, is ongoing (NCT06005493).

References

1. Sahin et al. Clin Cancer Res (2008) 2. Shitara et al. J Clin Oncol. (2023) 3. Xu et al. J Clin Oncol. (2023)