

¹AstraZeneca, Cambridge, UK; ²AstraZeneca, Gaithersburg, MD, USA; ³Discovery, Harbour BioMed, Shanghai, China; ⁴AstraZeneca, Waltham, MA, USA; ⁵AstraZeneca, Royston, UK.

Background

Diagram illustrating the mechanism of AZD5863 in T cell-mediated tumor killing:

- Direct T cell mediated CLDN18.2⁺ tumor cell killing:** The T cell interacts directly with the CLDN18.2⁺ tumor cell.
- Low CD3 and high CLDN18.2 affinity to minimize toxicities while maintaining potent activity:** AZD5863 binds to the CD3 receptor on the T cell, ensuring high affinity for CLDN18.2 while minimizing toxicity.
- Cytokine-driven bystander effect on CLDN18.2⁻ tumor cells:** The T cell releases IFN- γ , which acts on the CLDN18.2⁻ tumor cell, leading to its killing.

Results

Figure 1 consists of three line graphs showing the binding of AZD5863 to HEK293 cells expressing human CLDN18.2 and CLDN18.1, and Jurkat cells. The y-axis represents Mean Fluorescence Intensity (MFI) from 0 to 5000, and the x-axis represents Antibody concentration (nM) on a log scale from 0.001 to 1000. The legend indicates that blue circles represent AZD5863 and black squares represent the Isotype control.

- HEK293 Human CLDN18.2:** AZD5863 shows a dose-dependent increase in MFI, reaching a plateau around 4000 MFI at 1000 nM. The isotype control remains at a low MFI near zero.
- HEK293 Human CLDN18.1:** AZD5863 shows no significant binding, with MFI remaining near zero across all concentrations. The isotype control remains at a low MFI near zero.
- Jurkat:** AZD5863 shows no significant binding, with MFI remaining near zero across all concentrations. The isotype control remains at a low MFI near zero.

Figure 1: AZD5863 inhibits cell growth in a dose-dependent manner in pancreatic, gastric, and pancreatic adenocarcinoma cell lines.

The figure displays four graphs showing the cytotoxicity (%) of AZD5863 (blue diamonds) and an isotype control (black circles) across different antibody concentrations (pM) for four cell lines: PaTu-8988s HS (Pancreatic adenocarcinoma), SNU601 (Gastric adenocarcinoma), NUGC-4 (Gastric adenocarcinoma), and PANC05.04 (Pancreatic adenocarcinoma). The x-axis represents Antibody concentration (pM) on a logarithmic scale (0.01 to 1,000,000). The y-axis represents Cytotoxicity (%) on a linear scale (-25 to 125). A dotted line at 0% indicates no cytotoxicity.

PaTu-8988s HS (Pancreatic adenocarcinoma): Receptor density: 45339. AZD5863 shows a dose-dependent increase in cytotoxicity, reaching approximately 100% at 1,000,000 pM. The isotype control remains near 0%.

SNU601 (Gastric adenocarcinoma): Receptor density: 9992. AZD5863 shows a dose-dependent increase in cytotoxicity, reaching approximately 50% at 1,000,000 pM. The isotype control remains near 0%.

NUGC-4 (Gastric adenocarcinoma): Receptor density: 1591. AZD5863 shows a dose-dependent increase in cytotoxicity, reaching approximately 50% at 1,000,000 pM. The isotype control remains near 0%.

PANC05.04 (Pancreatic adenocarcinoma): Receptor density: 656. AZD5863 shows a dose-dependent increase in cytotoxicity, reaching approximately 50% at 1,000,000 pM. The isotype control remains near 0%.

Legend: Blue diamond: AZD5863; Black circle: Isotype control.

EC50 (TDCC) vs CLDN18.2 receptor density

Pearson's correlation:
 $R^2=0.82$
 $p<0.0001$

Emax (TDCC) vs CLDN18.2 receptor density

In vitro TDC on NUGC4

% cytotoxicity

Antibody concentration (pM)

HTA-TCE (black circles) AZD5863 (blue diamonds)

EC50 (pM)
AZD5863 21.5
HTA-TCE 40.9

In vitro cytokine secretion

Cytokine concentration (pg/ml)

Antibody concentration (pM)

AZD5863 (blue triangles) TNFα (blue circles)
HTA-TCE (black inverted triangles) TNFα (black diamonds)
IL6 (blue circles) IL6 (black diamonds)

In vivo cytokine secretion

IL-6

Cytokine concentration (pg/ml)

Isotype control HTA-TCE AZD5863

TNFα

Cytokine concentration (pg/ml)

Isotype control HTA-TCE AZD5863

Bystander killing TDCC assay

Flow cytometry endpoint:
% dead **CLDN18.2 KO**

TDCC on PaTu8988s CLDN18.2 KO

| AZD5863 concentration (nM) | 20% : 80% | 30% : 70% | 50% : 50% | 70% : 30% |
|----------------------------|-----------|-----------|-----------|-----------|
| 0.0001 | ~10 | ~15 | ~15 | ~15 |
| 0.001 | ~10 | ~15 | ~15 | ~15 |
| 0.01 | ~10 | ~15 | ~15 | ~15 |
| 0.1 | ~10 | ~60 | ~65 | ~65 |
| 1 | ~10 | ~65 | ~70 | ~80 |
| 10 | ~10 | ~65 | ~70 | ~80 |
| 100 | ~10 | ~65 | ~70 | ~80 |

Figure 3: AZD5863 treatment of syngeneic mouse models of gastric, pancreatic, and colorectal cancer.

Top Left: NUGC-4 Gastric cancer model (1e6 PBMCs admix)

Y-axis: Tumor volume ($\text{mm}^3 \pm \text{SEM}$)

X-axis: Days post tumor cell implantation

Legend:

- Isotype 2mg/kg (black circles)
- AZD5863 0.02mg/kg (light blue squares)
- AZD5863 2mg/kg (dark blue triangles)

Significance: *** p < 0.001

Top Right: PaTu8988 HS Pancreatic cancer model

Y-axis: Tumor volume ($\text{mm}^3 \pm \text{SEM}$)

X-axis: Days post tumor cell implantation

Legend:

- Isotype 2mg/kg (black circles)
- AZD5863 0.02mg/kg (light blue squares)
- AZD5863 0.2mg/kg (dark blue triangles)

Significance: ** p < 0.01, *** p < 0.001

Bottom Left: OE19 Oesophageal cancer model

Y-axis: Tumor volume ($\text{mm}^3 \pm \text{SEM}$)

X-axis: Days post tumor cell implantation

Legend:

- Isotype 2mg/kg (black circles)
- AZD5863 0.2mg/kg (light blue squares)
- AZD5863 2mg/kg (dark blue triangles)

Significance: *** p < 0.001

Bottom Right: MC38-Cldn18.2high syngeneic Colorectal cancer model (huCD3scγ knock-in)

Y-axis: Tumor volume ($\text{mm}^3 \pm \text{SEM}$)

X-axis: Days post tumor cell implantation

Legend:

- Isotype 10mg/kg (black circles)
- AZD5863 1 mg/kg (light blue squares)
- AZD5863 5 mg/kg (dark blue triangles)

Significance: * p < 0.05

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