



# AZD5863, a CLDN18.2 and CD3 binding T-cell engager, establishes a multi-faceted antitumour immune response and combines effectively with chemo-immunotherapy regimens



Abstract Number: 1073

Marina Natoli<sup>1</sup>, Sharif Rahmy<sup>2</sup>, Fabien Garçon<sup>1</sup>, Rodrigo Aguilera Olvera<sup>2</sup>, Chelsea Gerada<sup>1</sup>, Grace Opoku-Ansah<sup>1</sup>, Laura Dallaway<sup>1</sup>, Lisa Chinello<sup>1</sup>, Nicolas Giraldo<sup>2</sup>, Miranda Clements<sup>2</sup>, Sandra Margielewska-Davies<sup>3</sup>, Hayden Pearce<sup>3</sup>, Keith J Roberts<sup>3</sup>, Paul Moss<sup>3</sup>, Jim Eyles<sup>1</sup>, Kristen Pollizzi<sup>2</sup>, Kathy Mulgrew<sup>2</sup>, Miguel Gaspar<sup>1</sup>, Scott A Hammond<sup>2</sup>, Bala NN Rao Attili<sup>1</sup>, Maria AS Broggi<sup>2</sup>, Gayle Pouliot<sup>4</sup>, Jonathan Fitzgerald<sup>4</sup>

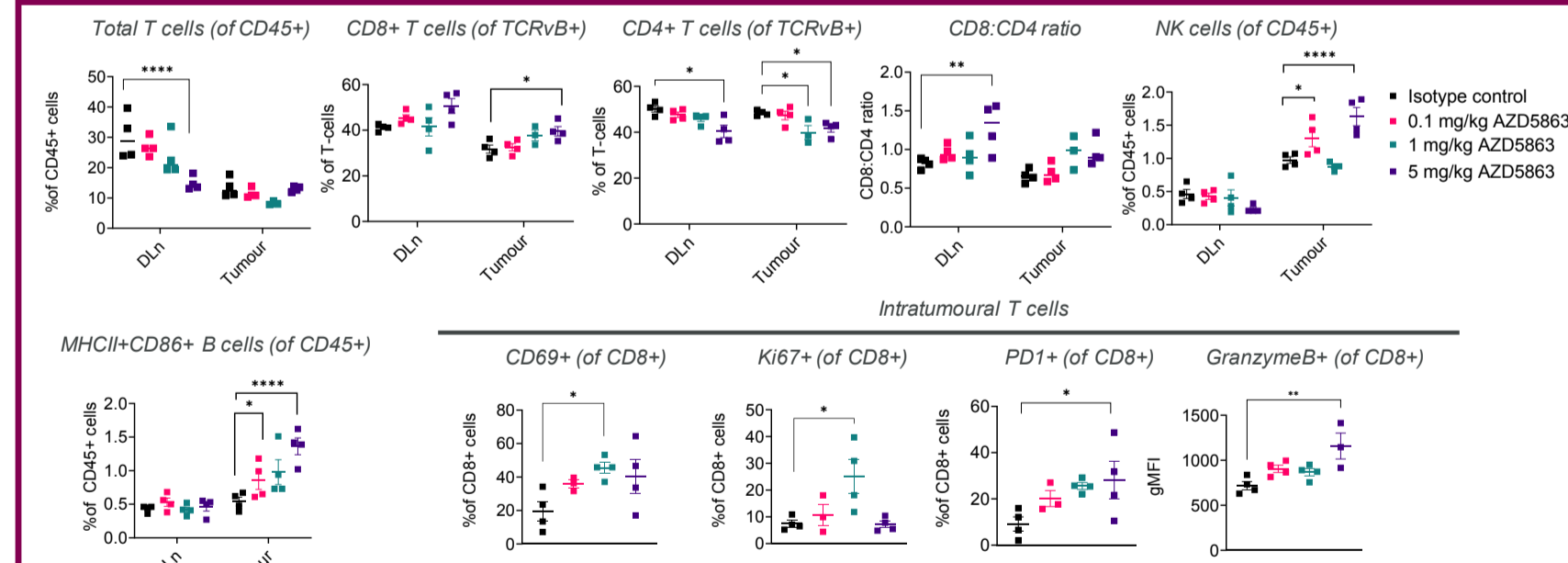
<sup>1</sup>AstraZeneca, Cambridge, UK; <sup>2</sup>AstraZeneca, Gaithersburg, MD, USA; <sup>3</sup>University of Birmingham, Birmingham, UK ; <sup>4</sup>AstraZeneca, Waltham, MA, USA.

## Background

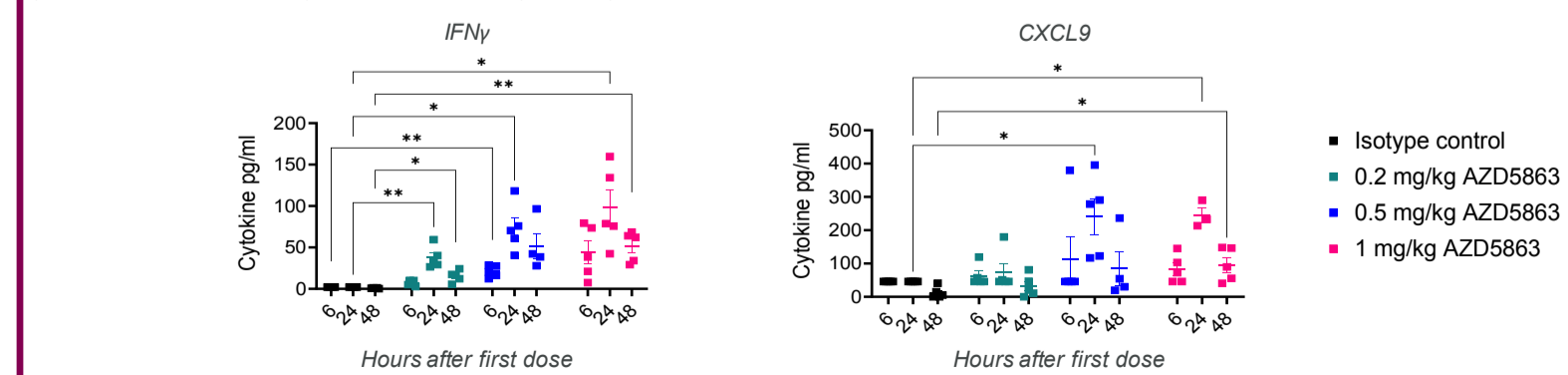
CLDN18.2 is a tetraspanin, highly expressed in gastric and pancreatic cancers. A CLDN18.2-targeting monoclonal antibody approach as monotherapy or in combination with chemotherapies has demonstrated benefit in gastric cancer [1,2]. AZD5863 is a CLDN18.2 and CD3-targeting T-cell engager (TCE), designed with high-affinity to human, cynomolgus monkey and mouse CLDN18.2 and low-affinity to human CD3. Gaining mechanistic insights into the mode of action of novel TCEs is critical to further their advancement into the clinic. Here, we investigated the pharmacodynamics of AZD5863 in preclinical models and assessed its combinability with chemo-immunotherapy regimens, relevant to the treatment of gastric cancer.

## Results

**AZD5863 treatment leads to cellular changes in the draining lymph nodes and tumour microenvironment paired with cytokine and chemokine changes in the serum of hCD3-transgenic mice bearing MC38-mCLDN18.2 tumours**

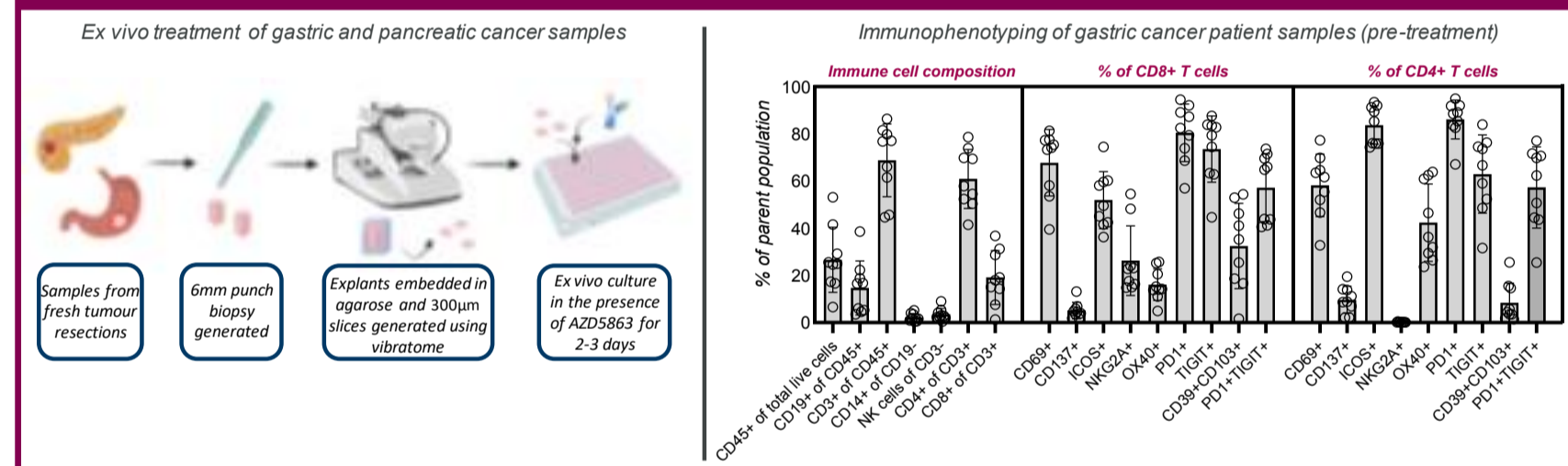


**Figure 1: Immunophenotyping of MC38-mCLDN18.2 tumours or tumour draining lymph nodes (DLN) from human CD3 (hCD3) transgenic mice (Genoway) at day 24 following tumour implantation, 72 hours after the second dose of AZD5863 or control treatments. p\*\*\*\*<0.0001; p\*\*<0.01; p\*<0.05 (One or Two-way ANOVA with multiple comparisons).**

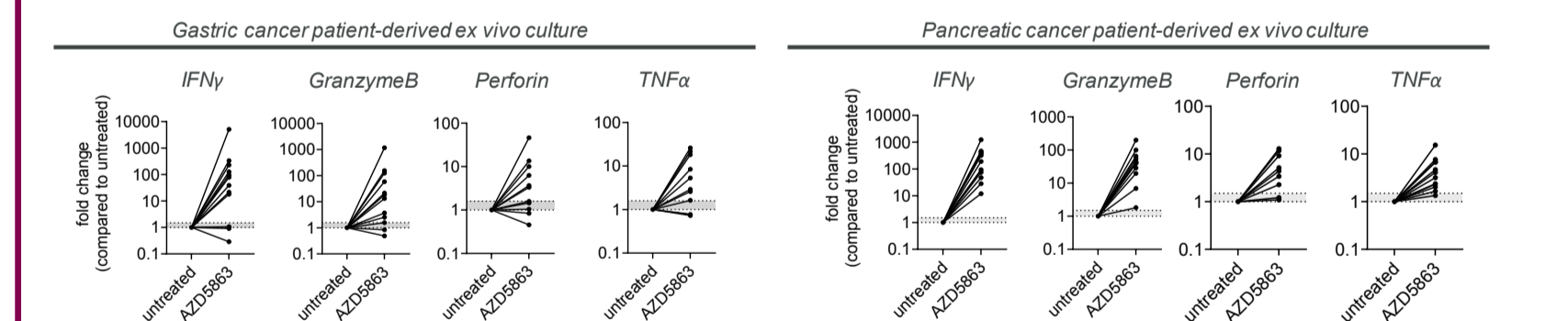


**Figure 2: Serum cytokine and chemokine changes in hCD3 transgenic mice (Genoway) bearing MC38-mCLDN18.2 tumours following one dose of AZD5863 (day 14 after tumour implantation). p\*\*\*<0.001; p\*\*<0.01; p\*<0.05 (Two-way ANOVA with multiple comparisons).**

## AZD5863 causes increased effector cytokine secretion from pancreatic and gastric cancer patient-derived tumour explants treated ex vivo

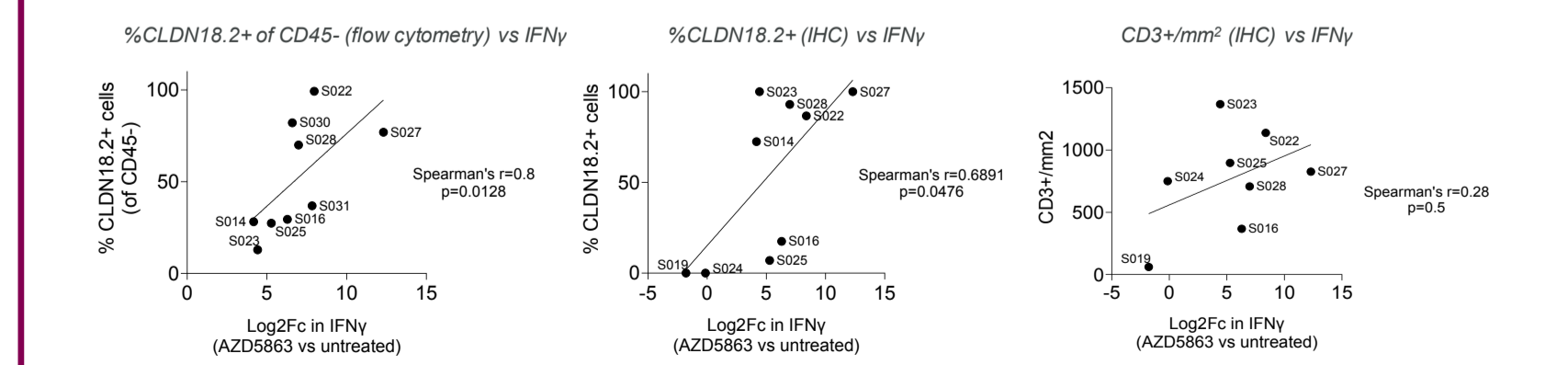


**Figure 3: Processing of gastric and pancreatic cancer samples for ex vivo culture and immunophenotyping of n=9 gastric cancer samples at baseline, i.e. before treatment, by flow cytometry. Right: each dot represents a single patient. Graphics created with BioRender.**



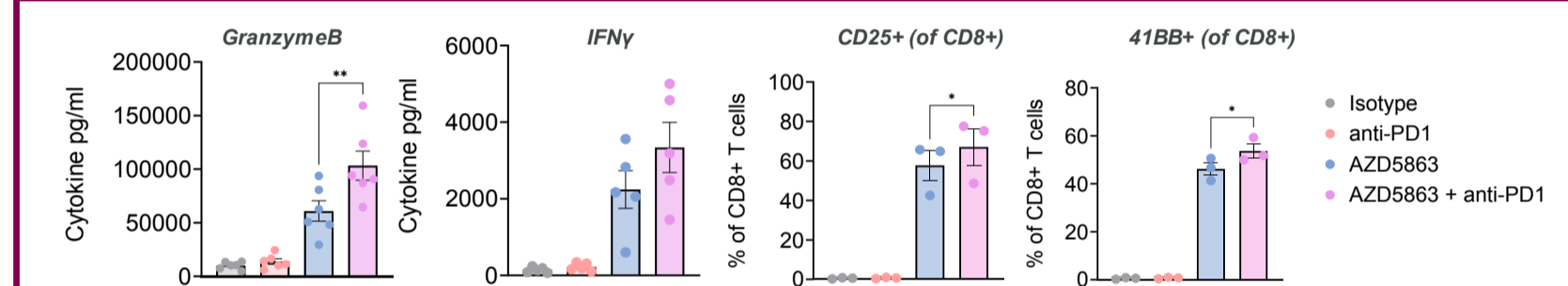
**Figure 4: Cytokine secretion from n=12 gastric and n=12 pancreatic cancer patient samples processed into 300µm thin slices and treated ex vivo with AZD5863 for 2 or 3 days. Line graphs displaying production of indicated analytes as fold change of AZD5863-treated over untreated condition for individual donors. Each dot represents average from 3-4 slices per patient sample. Shaded area indicates fold change <1.5.**

## Baseline CLDN18.2 expression - but not baseline CD3+ T cell content - significantly correlates with AZD5863-induced secretion of IFNγ from gastric cancer patient-derived explants



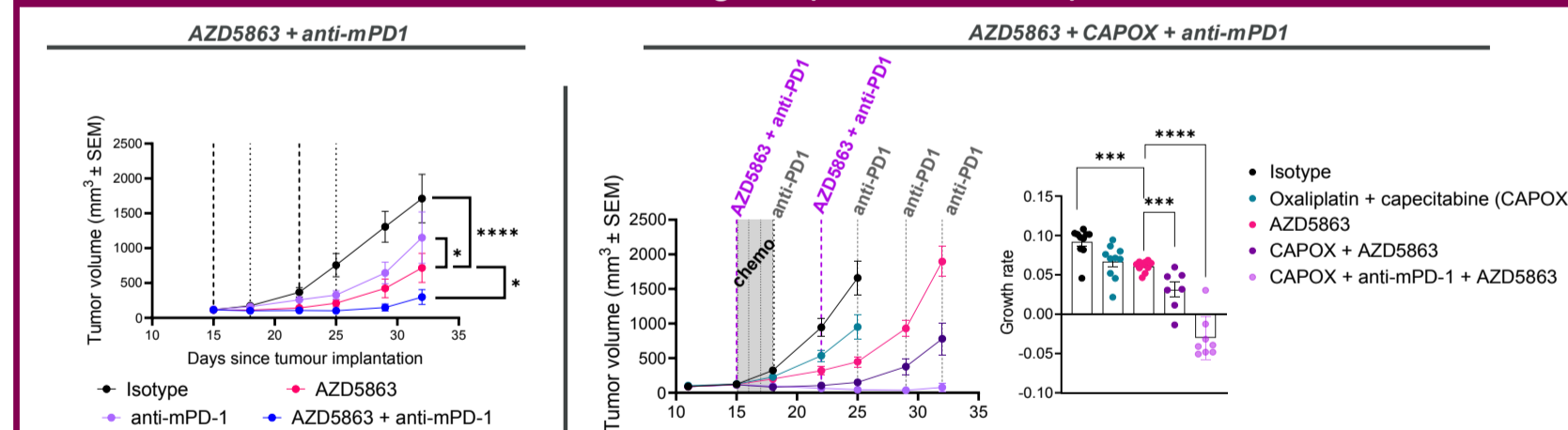
**Figure 5: Correlations of log2 fold change in IFNγ (comparing AZD5863 to untreated condition) with CLDN18.2+ cells measured by flow cytometry (left, n=9) or IHC (middle, n=9) and with CD3+ T cell counts/mm² measured with IHC (right, n=8), in gastric cancer patient-derived explants. Each dot represents average from 3-4 slices per patient sample; Spearman's correlation coefficient (r) and p values are indicated.**

## AZD5863 combination with anti-PD1 leads to increased cytokine secretion and T cell activation in vitro



**Figure 6: Cytokine secretion and marker expression on CD8+ T cells from in vitro co-culture of PBMCs with NUGC4 gastric cancer cell line, treated with controls, AZD5863 or AZD5863 in combination with anti-PD1 monoclonal antibody. PBMCs were pre-activated by co-culture with NUGC4 tumour cells at 4:1 E:T ratio in the presence of AZD5863; after 7 days, activated PBMCs were collected and reseeded onto fresh NUGC4 cells for 48h in the presence of treatments. Data shown from 48h timepoint as mean +/- SEM. Each dot is an individual PBMC donor. p\*\*<0.01; p\* <0.05 (Paired T test).**

## AZD5863 leads to improved tumour growth inhibition when combined with anti-PD1 or with gastric cancer standard of care regimen (CAPOX + anti-PD1) in vivo



**Figure 7: Tumour growth inhibition driven by AZD5863 combined with anti-PD1 or with CAPOX + anti-PD1. Tumour volume vs time plots (left and middle; n=10 mice per group, mean +/- SEM) in hCD3deg mice implanted with 1.5 x 10<sup>6</sup> MC38-mCLDN18.2 cells. Tumour growth rates (right) estimated based on fitting each tumour's growth curve to an exponential model; p values obtained with Two-way ANOVA (tumour volumes) or with Mann-Whitney t-Test (growth rate). p\*\*\*\*<0.0001; p\*\*\*<0.001; p\*\*<0.01; p\* <0.05. Groups were removed from graph when ≤50% animals remained in the study.**

## Conclusions

- AZD5863 treatment of human CD3-transgenic mice bearing MC38-CLDN18.2 tumours induced a decrease in total T cells in the tumour draining lymph nodes, paired with an increase in intratumoural activated CD8+ T cells, NK cells and activated B cells.
- AZD5863 ex vivo treatment of pancreatic and gastric cancer patient-derived tumour explants resulted in increased effector cytokine secretion, with AZD5863-induced IFNγ correlating with baseline CLDN18.2 expression but not with baseline CD3+ T cell content.
- In vivo, combination of AZD5863 with chemotherapy and anti-PD1 significantly reduced tumour growth rates compared to monotherapy AZD5863.

**Summary: AZD5863, currently in a Phase 1 clinical trial for gastric, pancreatic and oesophageal adenocarcinoma (NCT06005493), induced an antitumour immune response in preclinical in vivo models, showed potent activity in patient-derived explants and combined effectively with chemo-immunotherapy in vitro and in vivo.**