Supplemental Figure S1: IACS-8802/8803 show significantly higher potency than 2’3’cGAMP. THP-1 dual-reporter cells (Invivogen) were treated with the indicated STING agonist and NFkB activity was measured after 1 hour while IRF3 activity was measured after 16 hours using SEAP and Luciferase detection reagents respectively. Data shown are triplicates with error bars showing SEM but in some cases are not visible due to minimal variation.
Supplemental Figure S2: STING activation reduces the suppressive capacity of bone marrow-derived MDSC. (A) Generation of BM-MDSCs and repolarization protocol. Bone marrow harvested from WT C57BL/6 mice was cultured in the presence of GM-CSF and IL-6 for 4 days to induce differentiation of suppressive BM-MDSCs. Flow cytometric analysis of untreated BM-MDSC reveals ~95% expression of CD11b, and a 2:1 ratio of Ly6G⁺Ly6C<sup>mid</sup> PMN-MDSC (49.4%) to Ly6C⁺Ly6G⁻ MO-MDSC (24.0%). (B) Evaluation of MDSC suppression by CD8 T cell suppression assay following MDSC repolarization using indicated concentration of 8803. (C) Evaluation of MDSC suppression by CD8 T cell suppression assay following repolarization with 2.5μg/mL indicated CDN. Data are representative of at least two independent experiments. Statistical significance was calculated using Student’s T test. ns = not significant, * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001.
Supplemental Figure S3: Synthetic STING agonists reduce MDSC proliferation. Bone marrow derived granulocytic MDSC were labelled with CFSE and incubated with the indicated drug at the concentration shown (μg/mL) for 48 hours and then CFSE expression levels were measured using flow cytometry. Proliferation index was then calculated as 

$$PI = \frac{\sum_{i=0}^{i} (i * N_i^2)}{\sum_{i=0}^{i} N_i^2}$$

where $i =$ number of cell division peak as determined by CFSE dilution, $N_i =$ number of cells in that division peak. Data shown represents 2 independent experiments with each group in triplicate repeats. Statistical significance was calculated using ANOVA with ns = not significant, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$. 

Supplemental material

BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)

J Immunother Cancer

doi: 10.1136/jitc-2021-003246

Supplemental Figure S4: STING activation phenotypically repolarizes human M2c macrophages. (A) Protocol for generation of M2c polarized macrophages from CD14+ PBMC-derived monocytes and subsequent CDN stimulation, as detailed in methods. (B) Baseline phenotyping of unstimulated M2c macrophages by flow cytometry. Data shows percent positivity relative to isotype staining. (C) Rough estimation of macrophage morphology following 3-day CDN stimulation by flow cytometry. (D) Representative images of morphological changes in M2c macrophages following 3-day exposure to indicated CDNs. All images were recorded under 20X magnification using a light microscope. Data are cumulative of two independent experiments with 3 unique donors each. Statistical significance was calculated using Student’s T test. ns = not significant, * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001.
Supplemental Figure S5: Mutational analysis and tumor growth kinetics of mT4 PDAC model.

(A) Whole genome sequencing was performed on parental mT4-2D. Identified exonic nonsynonymous mutations were analyzed for predicted neoantigen epitopes using the NetMHC 4.0 algorithm. (B) Comparison of nonsynonymous mutational burden of mT4-2D relative to PancO2 (data adapted from Bhadury et al 2013) and B16-F10 (data adapted from Castle et al 2012). (C) Subcutaneous tumor growth kinetics and survival following orthotopic implantation of mT4-LA and mT4-LS cells. For subcutaneous implantation, 2.5x10^6 cells were implanted in 3 mice each. For orthotopic implantation, 0.35x10^6 cells were implanted in 30% matrigel v/v in the head of the pancreas by survival surgery in 10 mice each. Survival endpoint indicates death from tumor burden or observed overt morbidity.
Supplemental Figure S6

A

Supplemental Table S3

B

SubQ Tumor Growth Curve

C

% Moribund from SubQ Ulceration

D

E

% Moribund From D22 Surgery

Supplemental Table S4

Supplemental Figure S6: Extended data on combination 8803 and checkpoint blockade in mT4-LA PDAC. (A) Longitudinal IVIS imaging for mice treated as described in Fig 5A. (B) Growth kinetics of subcutaneous mT4-LA tumors in mice shown in (A) and treated as described in Fig 16A. (C) Enumeration of mice deemed moribund due to ulceration >5mm in diameter at the uninjected subcutaneous tumor in the experiment shown in (A). (D) Survival of mice shown in Fig 16C in control groups consisting of Gem/nP and 8803 or Gem/nP and CTLA-4/PD-1 compared to relevant controls. (E) Percent of mice that failed to recover from final survival surgery on day 22 in experiments shown in Fig 16C. Statistical significance was calculated using the Log-rank Mantel-Cox test. ns = not significant, * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001.
Supplemental Figure S7

A

Supplemental Figure S7

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance
placed on this supplemental material which has been supplied by the author(s)

Ager CR

doi: 10.1136/jitc-2021-003246

J Immunother Cancer

Supplemental Figure S7: Extended data on 30-parameter flow cytometric analysis of PDAC tumors following combination 8803 and αCTLA-4/αPD-1 therapy. (A) Timeline and treatment schedule. Mice in untreated group received reduced mT4-LA cell number at implantation (0.1x10^5) so that a majority would survive to the day of tumor harvesting. (B) Tumor mass at the time of harvesting and proportion of CD45^+ cells as a percentage of all single cells from processed tumor suspensions. (C) Orthotopic mT4-LA tumors were treated with the combination of intratumoral IACS-8803 and i.p. αCTLA-4/αPD-1 as in Figure 6. CD8 T cells, CD4 T cells or NK cells were depleted using the indicated antibody via i.p. injection every 3 days through day 26. (D) Median expression intensities of each marker expressed visually by normalized heat mapping overlayed on the master concatenated tSNE plot. Data includes 5-10 mice per group and is representative of two independent experiments. Statistical significance was calculated using Student’s T test for all except (C) Log-rank Mantel-Cox test. ns = not significant, * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001.