An Oral Small Molecule Combination Therapy Targeting PD-L1, VISTA and Tim-3 Immune Inhibitory Checkpoints Exhibits Enhanced Anti-tumor Efficacy in Pre-clinical Models of Cancer

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Introduction

CA-170 is a small molecule, orally bioavailable antagonist of the VISTA/PD-1H and PD-L1 immune checkpoint pathways which is currently undergoing Phase I clinical testing. CA-327 is a small molecule, orally bioavailable antagonist of Tim-3 and PD-L1 checkpoint pathways which is in pre-clinical development. CA-170 and CA-327 were identified based on their ability to selectively antagonize *in vitro* immune inhibitory checkpoint mediated suppression of human and mouse effector T cell function (table below).

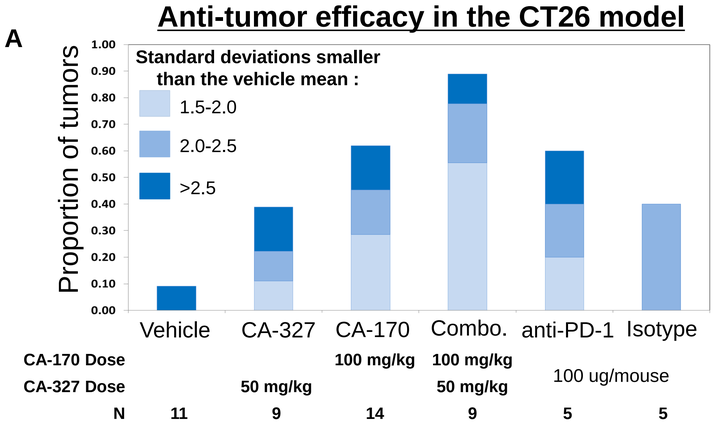
	Human PBMCs				Mouse Splenocytes		
	IFN-γ Rescue (*in vitro*) EC_{50} (nM)				IFN-γ Rescue (*in vitro*) EC_{50} (nM)		
Test Compound	PD-L1	PD-L2	VISTA/PD-1H	Tim-3	PD-L1	PD-L2	Tim-3
CA-170	**56.43**	**149.0**	**49.35**	**no rescue**	**33.79**	**54.98**	**no rescue**
CA-327	**107.9**	**56.06**	**no rescue**	**168.4**	**110.7**	**84.86**	**86.91**
Anti-PD-1 antibody clones: J116 (h) / J43(m)	44.17	93.52	N/T	N/T	69.96	89.88	N/T
Anti-VISTA antibody clone 730802(h)	N/T	N/T	25.82	N/T	N/T	N/T	N/T
Anti-Tim-3 antibody clones: F38-2E2(h) / 8B.2C12(m)	N/T	N/T	N/T	67.7	N/T	N/T	42.01

N/T = Not Tested

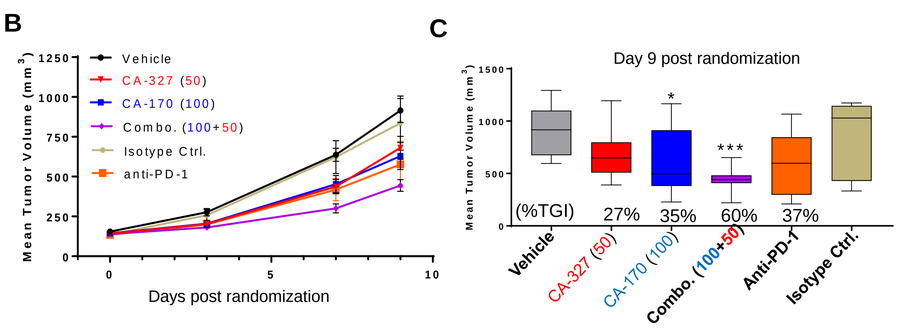
Increased activity of compensatory immune inhibitory checkpoint pathways is a key mechanism through which tumors escape targeted immune checkpoint inhibition therapy. Therapeutic approaches which target multiple functionally distinct immune inhibitory checkpoint pathways show substantially increased anti-tumor efficacy over mono-therapy approaches. Here we investigate the therapeutic potential of a CA-170/CA-327 oral combination therapy targeting the PD-L1, VISTA and Tim-3 pathways in non-clinical models of cancer.

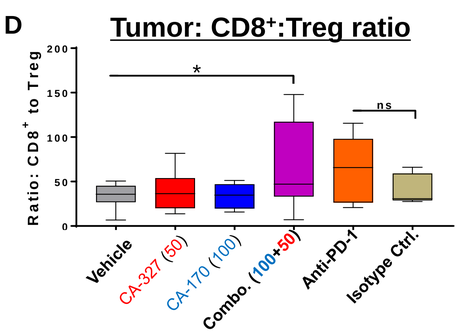
CA-170/CA-327 combination therapy shows increased anti-tumor efficacy, increased tumor growth inhibition and tumor immune modulation in the CT26 syngeneic mouse tumor model

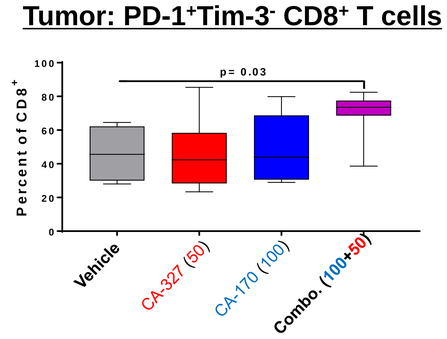
Anti-tumor efficacy in the CT26 model



Tumor growth inhibition in the CT26 model



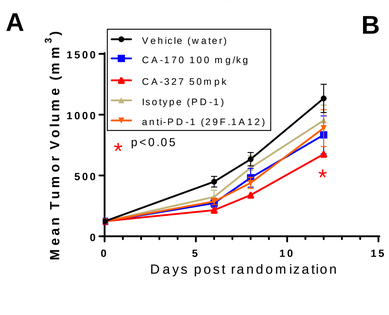


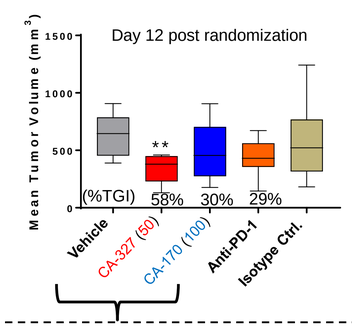


Mice were implanted with subcutaneous CT26 tumor cells, individuals with established tumors were randomized into treatment groups (at day 12) and treated as indicated. CA-170 and CA-327 were orally dosed once daily as single agents or in combination. The anti-PD-1 antibody (clone 29F.1A12) or isotype control were dosed via IP injection once every 3-4 days. Anti-tumor efficacy (**A**) was estimated by determining the proportion of tumors within each treatment group whose size is 1.5-2.0, 2.0-2.5, or >2.5 standard deviations smaller than the vehicle mean tumor size. Mean tumor growth curves and percent tumor growth inhibition (%TGI) are shown (**B** & **C**). Anti-PD-1 %TGI is relative to the isotype control. The relative ratio of intra-tumor $CD8^+$ T cells to regulatory $CD4^+$ T cells (**D**), and proportion of PD-1^+Tim-3^- $CD8^+$ T cells (**E**) was measured by flow cytometry. **C** and **D** were analyzed by one-way ANOVA with Dunnett's multiple comparisons test (* p>0.05; *** p>0.001). ns= not significant

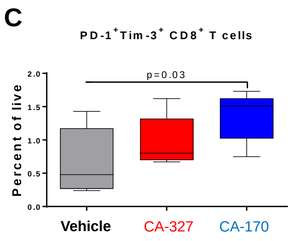
Tumor growth inhibition and modulation of the tumor immune response by CA-170 and CA-327 in the mouse CT26 model

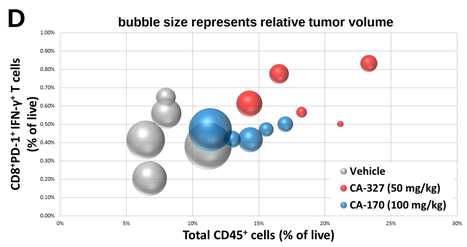
Tumor growth inhibition in the CT26 model





Evidence of increased Tim-3 mediated effector $CD8^+$ T cell suppression in CA-170 treated CT26 tumors

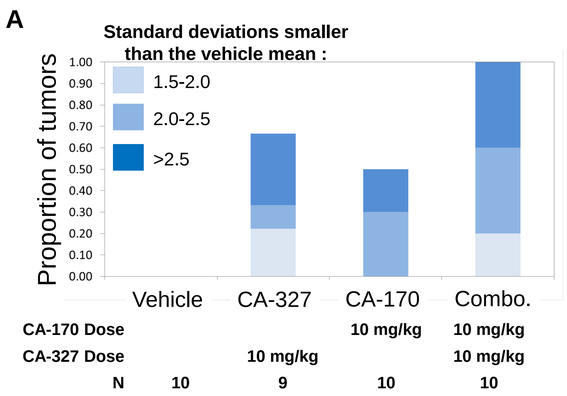




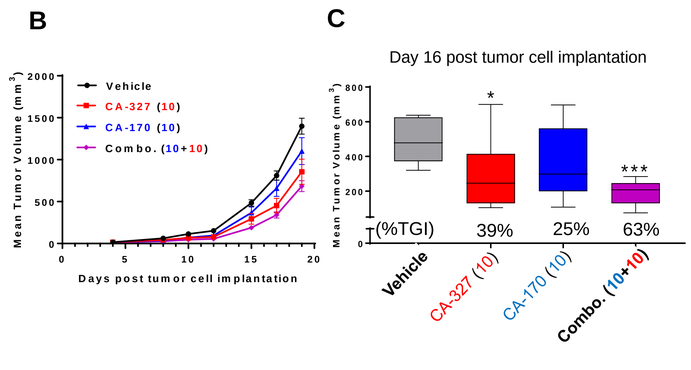
Mice were implanted with subcutaneous CT26 (**A** & **B**) tumor cells, individuals with established tumors were randomized into treatment groups (at day 12) and treated as indicated. CA-170 and CA-327 were orally dosed once daily at 50 mg/kg or 100 mg/kg, respectively. The anti-PD-1 antibody (clone 29F.1A12) or isotype control was dosed via IP injection (100 µg/mouse) once every 3-4 days. Mean tumor growth curves were plotted for each treatment group (**A**) and tumor growth inhibition (TGI) was calculated at the end of the study (**B**). Anti-PD-1 %TGI is relative to the isotype control. The number of intra-tumor PD-1^+Tim-3^+ $CD8^+$ T cells from **A** was measured by flow cytometry (**C**) and analyzed by unpaired t-test**.** The relationship between the number of total tumor $CD45^+$ cells, the number of $CD8^+$PD-1^+IFN-γ^+ T cells and the relative size of individual tumors is shown in **D**. **B** was analyzed by one-way ANOVA with Dunnett's multiple comparisons test (** p>0.01).

CA-170/CA-327 combination therapy anti-tumor efficacy, tumor growth inhibition and drug pharmacokinetics in the MC38 syngeneic mouse model

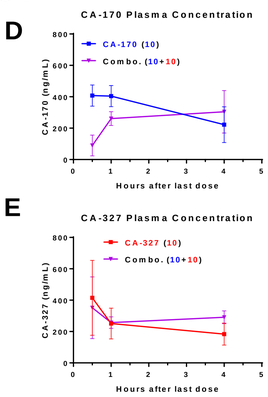
Anti-tumor efficacy in the MC38 model



Tumor growth inhibition in the MC38 model



Drug plasma concentration



Mice were implanted with subcutaneous MC38 tumor cells and were treated as indicated 4 days after tumor cell implantation. CA-170 and CA-327 were orally dosed once daily as single agents or in combination. Anti-tumor efficacy (**A**) was estimated by determining the proportion of tumors within each treatment group whose size is 1.5-2.0, 2.0-2.5, or >2.5 standard deviations smaller than the vehicle mean tumor size at day 16. Mean tumor growth curves and percent tumor growth inhibition (%TGI) are shown (**B** & **C**). Plasma concentrations of CA-170 (**D**) or CA-327 (**E**) were measured 0.5, 1 and 4 hours after the last dosing in this study. **C** was analyzed by one-way ANOVA with Dunnett's multiple comparisons test (* p>0.05; *** p>0.001).

Summary

- ❖ The Tim-3 inhibitory checkpoint may be an immune escape mechanism in mouse CT26 tumors treated with CA-170.
- ❖ Oral CA-170/CA-327 combination therapy significantly enhances anti-tumor efficacy and promotes the anti-tumor immune response in mouse cancer models.
- ❖ These pre-clinical data show that a combination therapy consisting of small molecule, oral, immune checkpoint antagonists may be used to treat cancer.

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