Science Advances

AAAS

advances.sciencemag.org/cgi/content/full/3/6/e1700090/DC1

Supplementary Materials for

WNT antagonists exhibit unique combinatorial antitumor activity with taxanes by potentiating mitotic cell death

Marcus M. Fischer, Belinda Cancilla, V. Pete Yeung, Fiore Cattaruzza, Cecile Chartier, Christopher L. Murriel, Jennifer Cain, Raymond Tam, Chieh-Yang Cheng, James W. Evans, Gilbert O'Young, Xiaomei Song, John Lewicki, Ann M. Kapoun, Austin Gurney, Wan-Ching Yen, Timothy Hoey

Published 21 June 2017, *Sci. Adv.* **3**, e1700090 (2017)
DOI: 10.1126/sciadv.1700090

This PDF file includes:

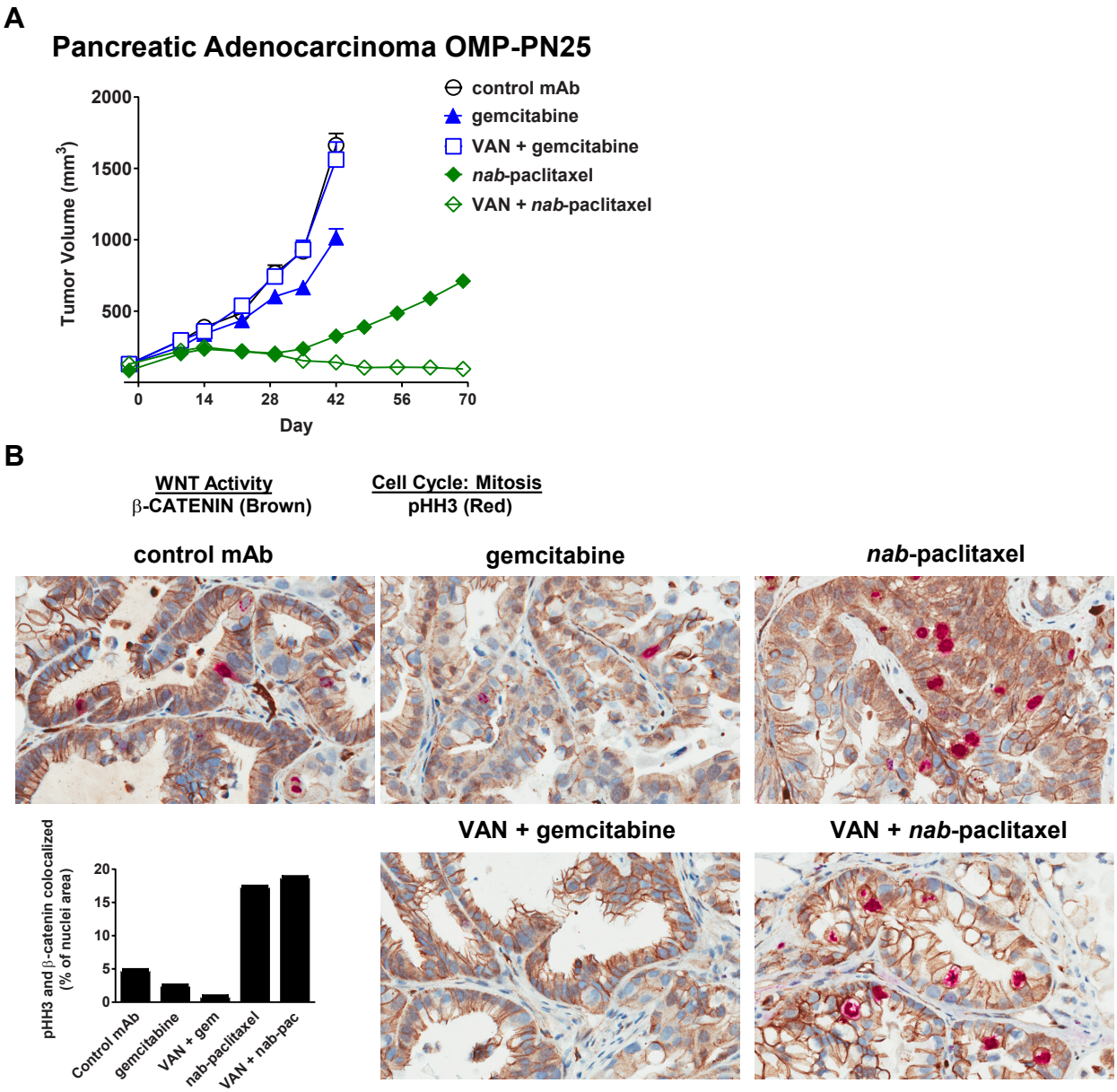


fig. S1. Nab-paclitaxel arrests cells at mitosis and synergizes with WNT antagonists. (**A**) Pancreatic adenocarcinoma OMP-PN25 was treated with VAN (25 mg/kg) every two weeks and with gemcitabine (20 mg/kg) or *nab*-paclitaxel (15 mg/kg) every week, n=5 to 10 per group. (**B**) Tumors on day 44 and 72 were preserved in FFPE and IHC was performed for pHH3 (red label) and β-CATENIN (brown label) with hematoxylin counterstain (blue nuclei). Digital scans performed

on an Aperio AT scanner and analyzed with Definiens Tissue Studio, 20x magnification. Quantification of mitotic cells with β-CATENIN expression by Definiens Architect. Data expressed as mean with two to three replicates per group.

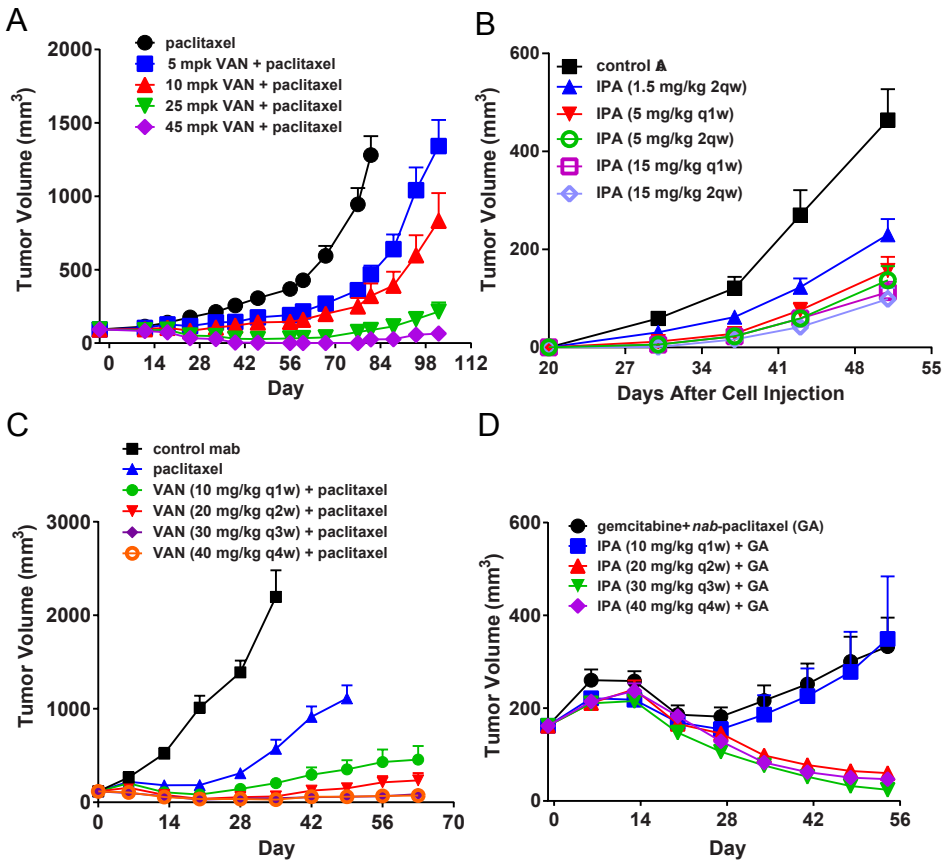


fig. S2. Higher doses of WNT antagonists administered infrequently are more active than corresponding dosages administered weekly. (**A**) A dose of 25 mg/kg or 45 mg/kg with weekly paclitaxel results in stable disease. Dose response study of vantictumab (VAN, every three weeks) with paclitaxel (10 mg/kg weekly) in a breast tumor PDX, UM-PE13. (**B**) Dose response study demonstrating the single agent activity of ipafricept in a colon tumor PDX, OMP-C28. Treatment with ipafricept (IPA) initiated one week after tumor cell implantation with weekly (q1w) or twice per week (2qw) dosing. (**C**) Dose partition study of VAN with weekly paclitaxel. Breast tumor OMP-B60 dosed with weekly paclitaxel (10 mg/kg) and VAN weekly (q1w), every other week (q2w), every three weeks (q3w), or every four weeks (q4w). (**D**) Dose partition study of

IPA with weekly gemcitabine and nab-paclitaxel (GA). Pancreatic tumor OMP-PN8 dosed with weekly gemcitabine (5 mg/kg) and *nab*-paclitaxel (15 mg/kg on weeks 1 and 2 then 7.5 mg/kg until study end) and IPA weekly (q1w), every other week (q2w), every three weeks (q3w), or every four weeks (q4w).

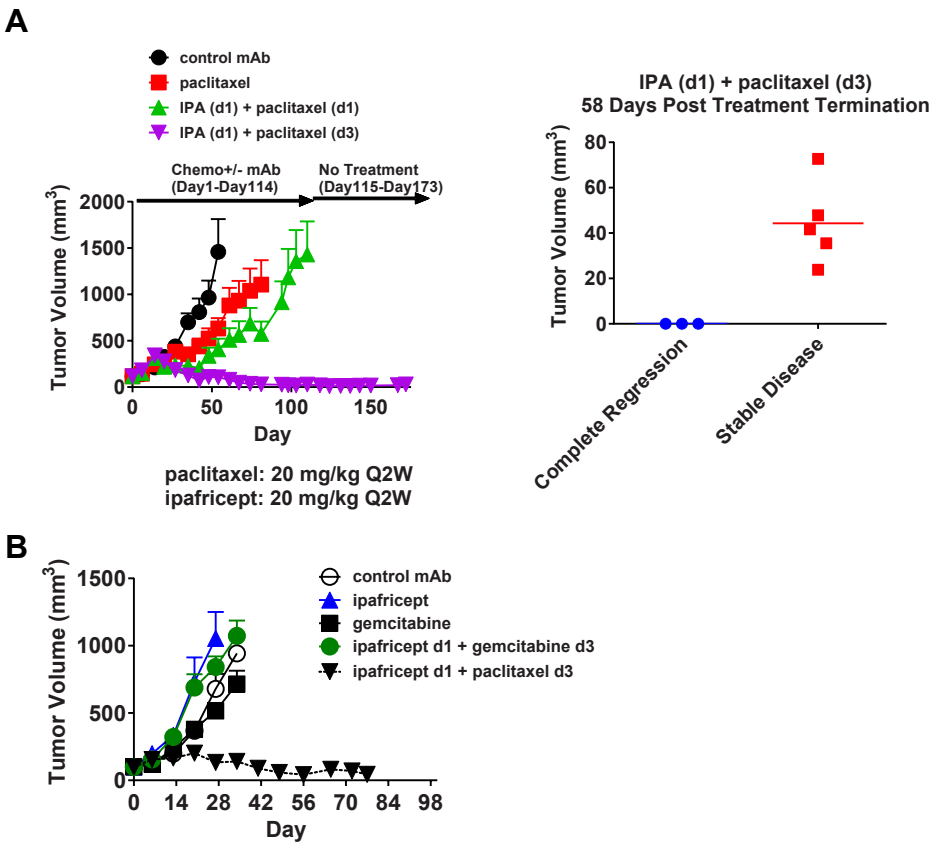
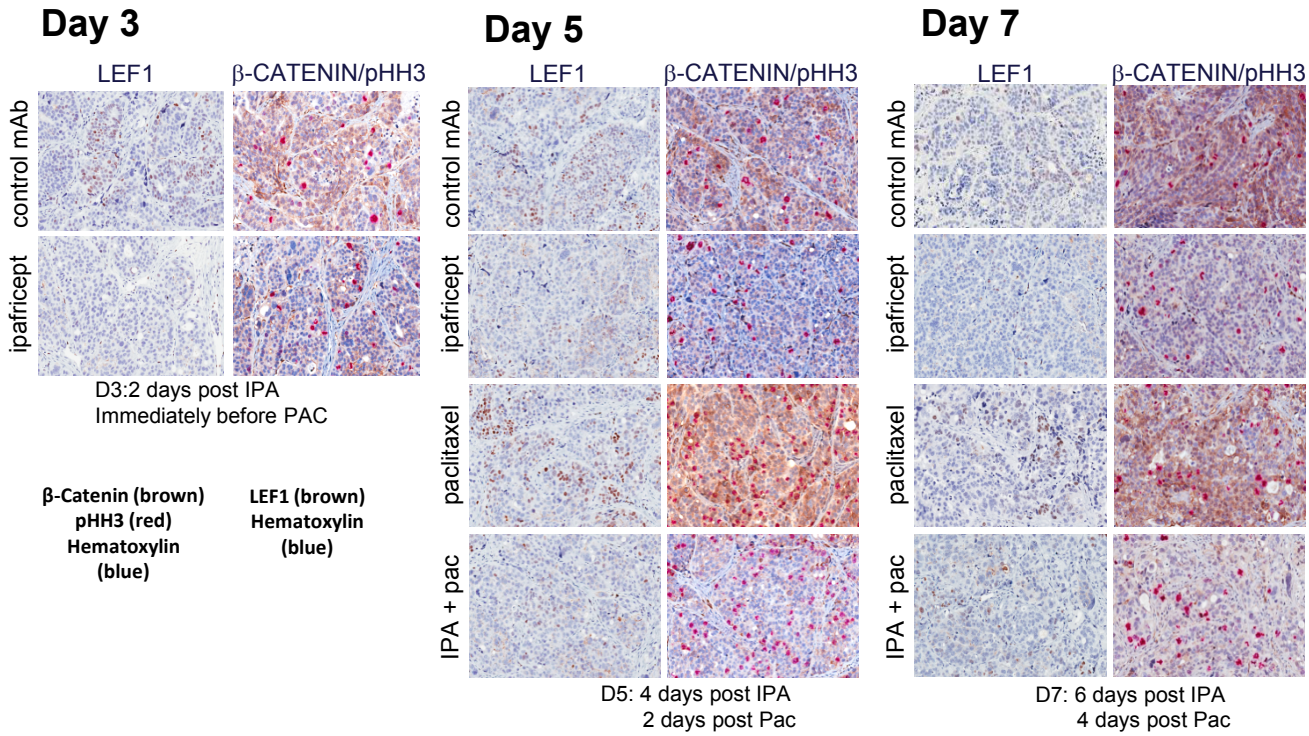


fig. S3. Ipafricept induces mitotic catastrophe when dosed sequentially before paclitaxel. Administration of vantictumab (VAN) or ipafricept (IPA) two days before paclitaxel is essential for blocking tumor growth. (**A**) OMP-OV19 treated with IPA on day 1 and paclitaxel (Pac) on day 1 or day 3 in two week cycles (from Figure 3C). Treatment terminated on Day 116 and incidence of recurrence monitored until day 173 *$P < 0.01$; **$P < 0.001$ combination versus chemotherapy. (**B**) OMP-OV38 treated with ipafricept (25 mg/kg) on days 1, 13, 27, 41, 55, and 78. Paclitaxel (20 mg/kg) and gemcitabine (40 mg/kg) were dosed every other week two days after ipafricept. Data expressed as Mean + SEM.


Day 3
LEF1
β-CATENIN/pHH3
control mAb
ipafricept
D3:2 days post IPA
Immediately before PAC
β-Catenin (brown)
pHH3 (red)
Hematoxylin
(blue)
LEF1 (brown)
Hematoxylin
(blue)
Day 5
LEF1
β-CATENIN/pHH3
control mAb
ipafricept
paclitaxel
IPA + pac
D5: 4 days post IPA
2 days post Pac
Day 7
LEF1
β-CATENIN/pHH3
control mAb
ipafricept
paclitaxel
IPA + pac
D7: 6 days post IPA
4 days post Pac

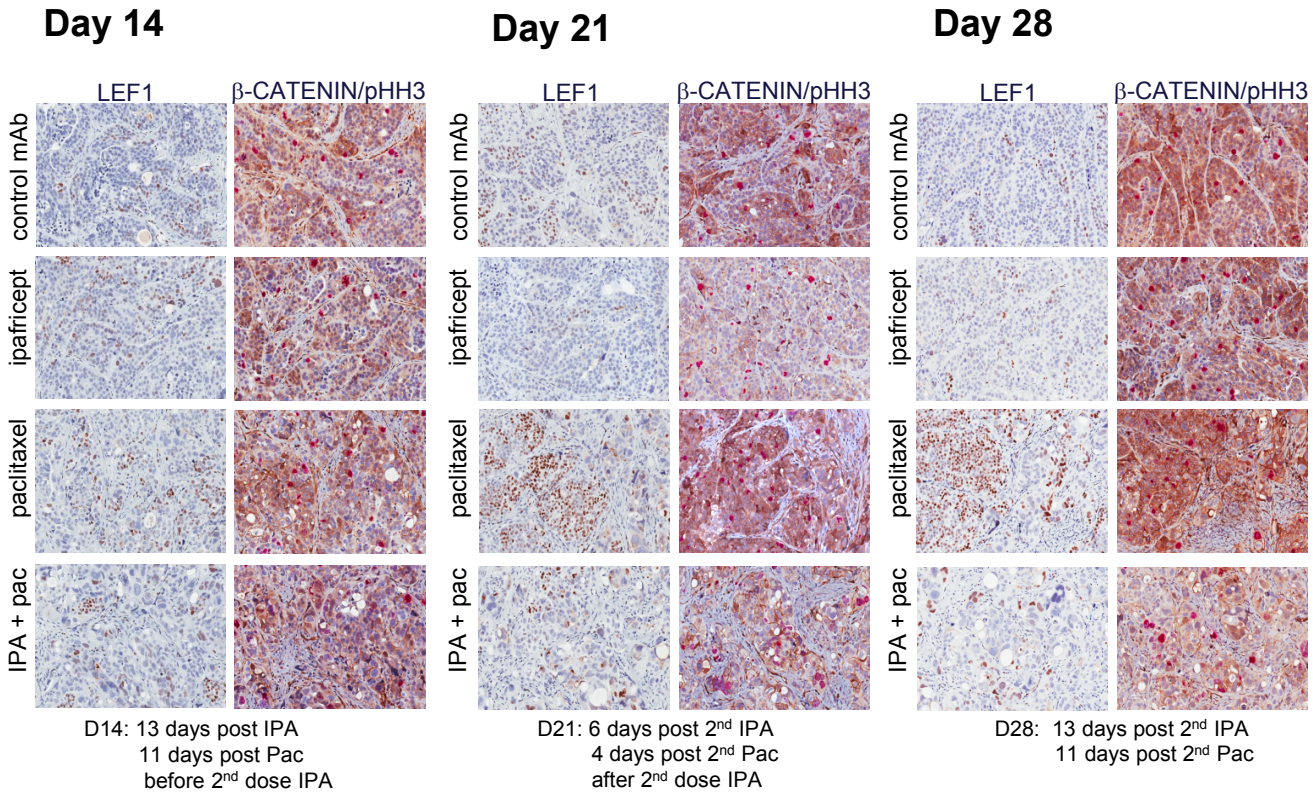

Day 14
LEF1
β-CATENIN/pHH3
control mAb
ipafricept
paclitaxel
IPA + pac
D14: 13 days post IPA
11 days post Pac
before 2nd dose IPA
Day 21
LEF1
β-CATENIN/pHH3
control mAb
ipafricept
paclitaxel
IPA + pac
D21: 6 days post 2nd IPA
4 days post 2nd Pac
after 2nd dose IPA
Day 28
LEF1
β-CATENIN/pHH3
control mAb
ipafricept
paclitaxel
IPA + pac
D28: 13 days post 2nd IPA
11 days post 2nd Pac

fig. S4. Ipafricept blocks selection of WNT-active cell types by paclitaxel in ovarian cancer. (**A**) From Figure 4B. Ipafricept dosed two days before paclitaxel, in two week cycles, prevents the accumulation of drug resistant WNT pathway active ovarian tumor cells. Representative images from time course study: 3-5 tumors per treatment group per time point, time points were from days 2, 3, 4, 5, 6, 7, 14, 21, 28 and 35, relative to first ipafricept dose on day 1. IHC performed from FFPE whole tumor sections for pHH3/β-CATENIN and LEF1. Digital scans were performed on an Aperio scanner.

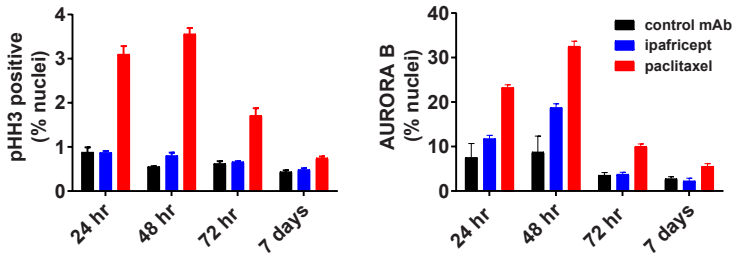


fig. S5. Paclitaxel up-regulates Aurora B and pHH3 by 24 hours up to 72 hours. Ipafricept (25 mg/kg) and paclitaxel (20 mg/kg) single agent time course study in OMP-OV38. IHC from FFPE whole tumor sections for pHH3 or AURORA B. Digital scans were performed on an Aperio scanner and analyzed on Definiens Architect with training for exclusion of mouse stromal cells, tumor capsule, and necrotic regions. Data normalized to control and expressed as Mean + SEM.

table S1. PDX tumors.

Patient Derived Xenograft (PDX) tumor models utilized in this report.
NDRI (National Disease Research Interchange), CHTN (Cooperative Human Tissue Network),
The MT Group (Thomas Moss, Los Angeles CA)

Tumor	Histopathology	Source
OMP-PN8	Pancreatic Adenocarcinoma, stage III (T3N0MX)	University of Michigan
OMP-PN9	Pancreatic Adenocarcinoma, stage III (T3N1MX)	University of Michigan
OMP-PN13	Pancreatic Adenocarcinoma, stage III (T3N1MX)	University of Michigan
OMP-PN25	Pancreatic Adenocarcinoma, stage IIA (T3N0M0)	The MT Group
UM-PE13	Breast Lobular Carcinoma; Luminal A. ER- PR- HER2- p120+	University of Michigan
OMP-B60	Breast Invasive Ductal Carcinoma; Basal. ER- PR- HER2-	University of California, San Francisco
OMP-B90	Breast Ductal Carcinoma; Luminal A/B. ER- PR- HER2-	Molecular Response
OMP-OV19	Serous Carcinoma, stage III (pT3N1M1)	NDRI
OMP-OV38	Serous Carcinoma, metastatic	CHTN-Midwestern
OMP-OV40	Serous Carcinoma, stage III (pT3cNxMx)	CHTN-Eastern
OMP-C28	Colon Adenocarcinoma, (T3N0Mx). CD133+	CHTN-Midwestern