PATENT LICENSING PROPOSAL

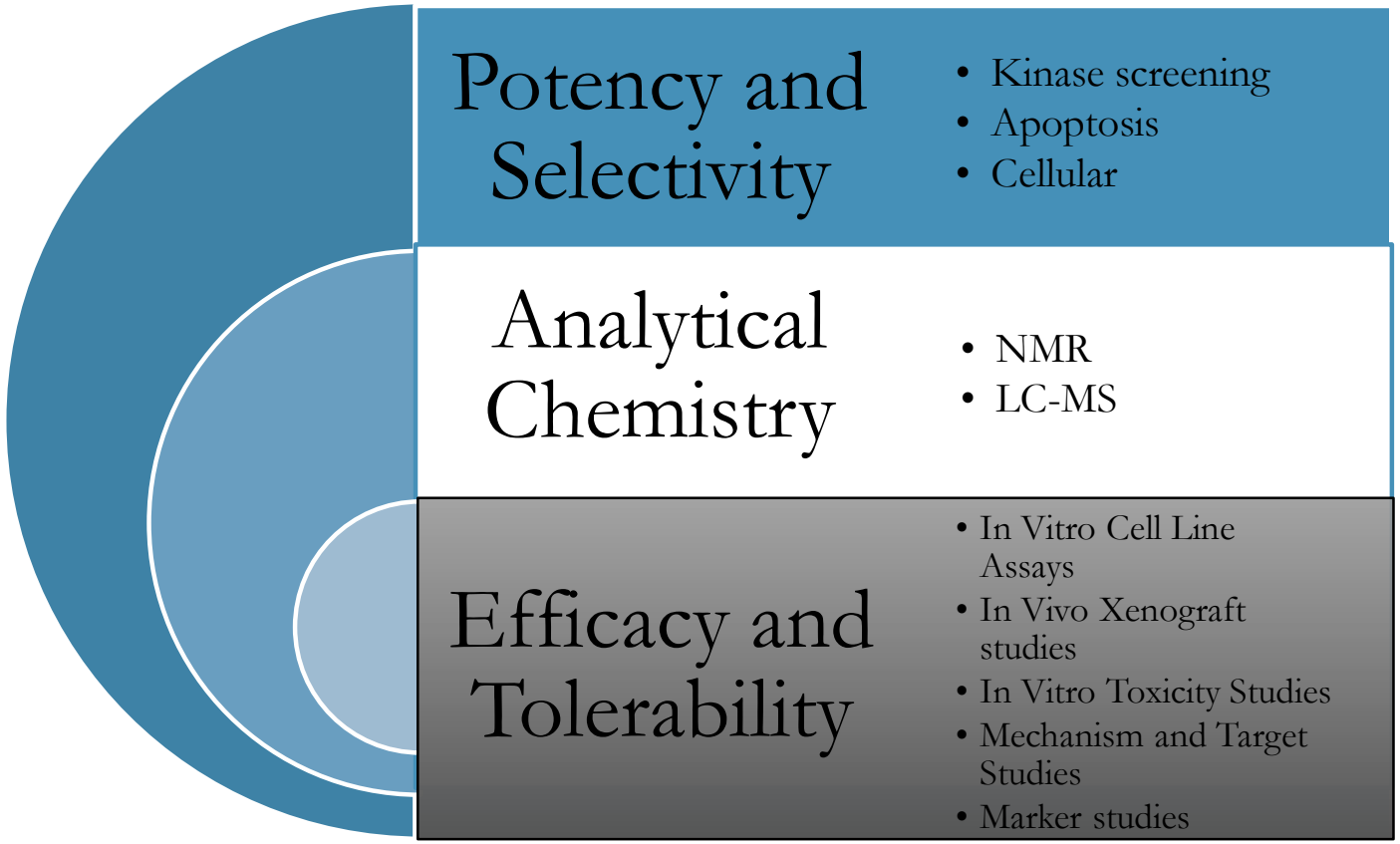
A COMPOUND FOR PANCREATIC CANCER

Inventor: Arora, Gurdial. S

INTRODUCTION

- The novel compound focuses on inhibiting tumor growth and synergy with chemo in select cancers by targeting Pancreatic Cancer Stem Cells (PanCSCs), which could be an ideal therapy to overcome chemoresistance. The compound tends to be a cutting edge science focused on large critical disease markets. This anti cancer compound invented by Dr. Gurdial Singh has the potential to be a life saving therapy for aggressive cancers.
- **A Proprietary Oncology Pipeline**
  - Program for multiple indications: Pancreatic cancer stem cells ,Pancreatic Adenocarcinoma, Lung Adenocarcinoma.
  - In Vivo studies: A549, PANCA, PANCSC, MIA-PA-CA2, ASPC1, xenograft models.
  - Biomarker Studies
  - Molecular Target studies
  - Small molecule for oral administration
- **Near Term, High Impact Milestones**
  - Pre Clinical candidate
  - Expansion of IP estate

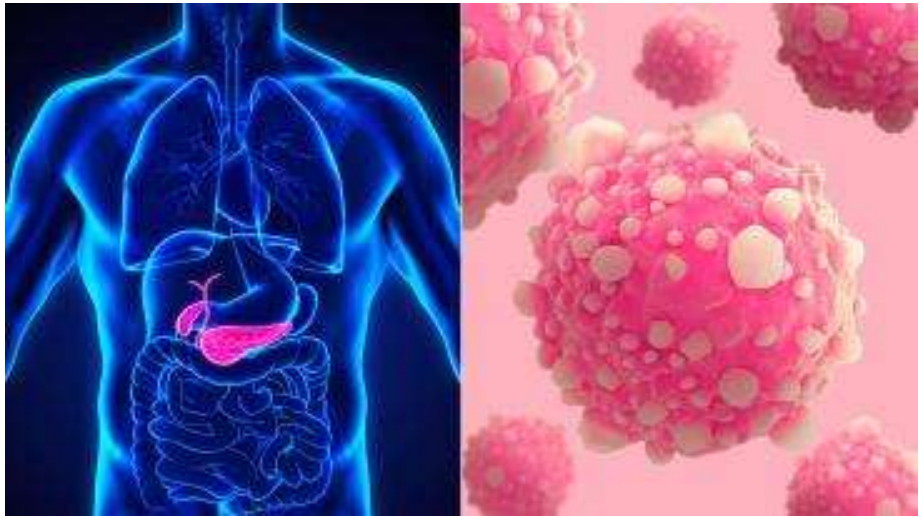
Developing Therapeutics to Inhibit the Tumor Growth and Prevent Metastasis



- Drugging the Undruggable: P53(Transcription Factors)
- Potent and selective Inhibitors: HER2
- EMT Markers: Increases E-Cadherin Expression and Prevents Metastasis
- Inhibits Stem Cell Markers: OCT4
- Pre clinical Development Stage
- Indication: Pancreatic Cancer, Pancreatic Stem Cells, Lung Adenocarcinoma, Prostate Cancer
- Tolerable
- Efficacious

Therapeutic efficacy of SACC against Pancreatic cancer

Global Pancreatic Cancer Therapeutics Market was worth **USD 2.41 billion** in 2018 and estimated to be growing at a CAGR of 7.54%, to reach **USD 3.47 billion** by 2023



SACC treatment dose-dependently inhibits the growth and induces apoptosis in human pancreatic cancer PANC1, ASPC1, cells. SACC is potential apoptosis inducing agent in PanCa cells. Pancreatic cancer stem cells (PanCSCs) are resistant to current chemotherapeutic drug of paclitaxel (PTX). This might be the reason of reoccurrence of pancreatic tumor after gemcitabine and paclitaxel therapy regimen. Thus, agents which target PanCSCs could be an ideal therapy to overcome chemoresistance. Interestingly, we observed that SACC also targets PanCSCs. We observed that SACC treatment inhibits the growth of PanCSCs followed by the inhibition of OCT 4 expression which is one of the cancer stem cells markers. We also observed SACC also targets cancer and based on these preliminary results, we hypothesize that SACC is a potential non-toxic therapeutic agent. SACC treatment of AsPC1 cells was resulted in a dose-dependent increase of PARP protein cleavage which is an early biomarker of apoptosis induction.

SACC induces apoptosis and inhibits the growth of PanCa cells

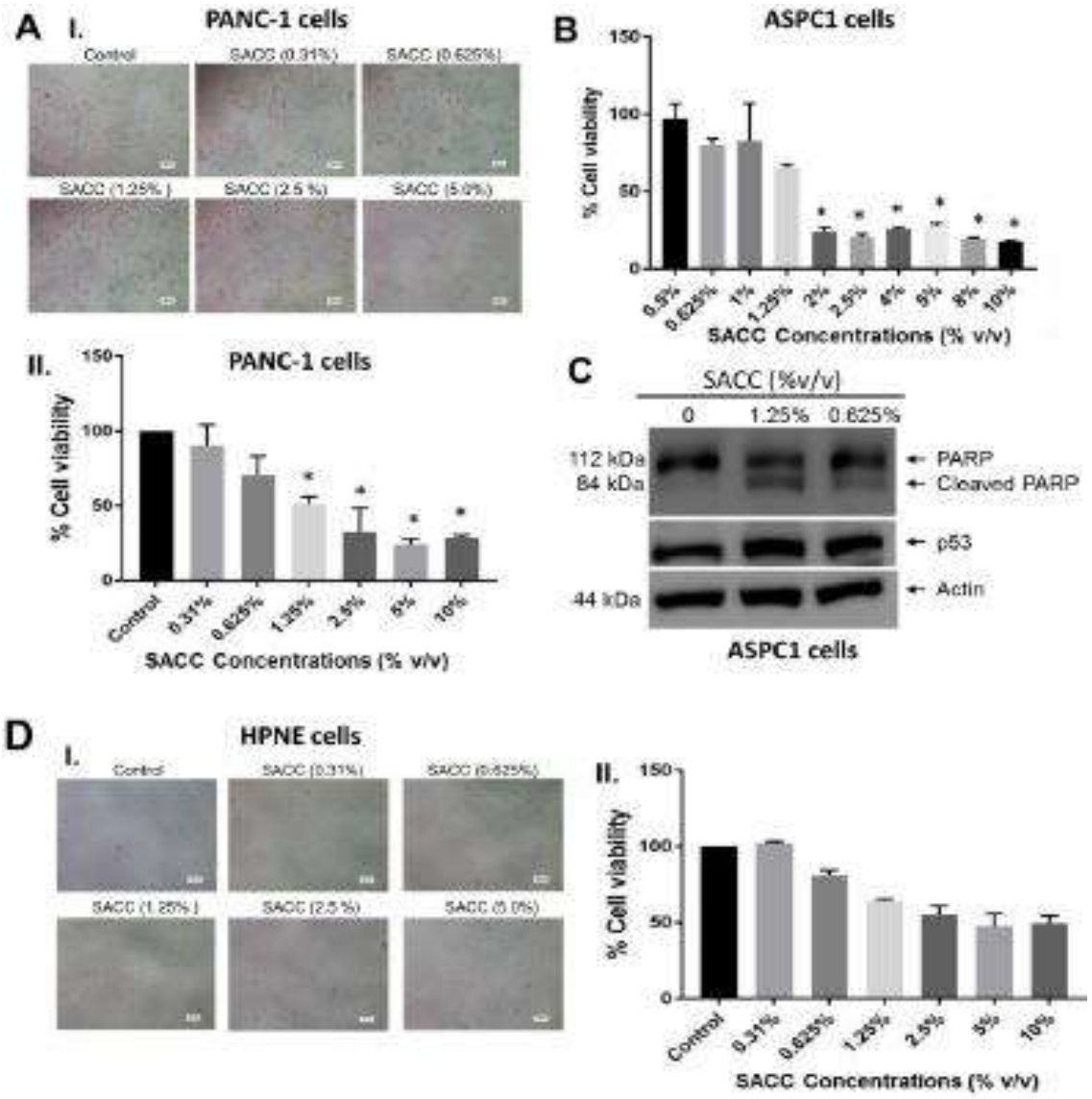
- **Figure: SACC induces apoptosis and inhibits the growth of PanCa cells.**

- **A-B:** Effect of SACC on growth of PANC1 and ASPC1 cells as determined by MTT assay. Representative images of PANC1 cells with the treatment of indicated concentrations of SACC after 24 hours, Bar graph indicating cell growth inhibitory effect of SACC in PANC1 cells and ASPC1 cells(B).

- **C:** Effect of SACC on the expression of apoptotic biomarker (cleaved PARP) and p53 in ASPC1 cells: Briefly, cells were treated with indicated concentrations of SACC for 24 hrs. Cell lysates were prepared for Western blot analysis. 40 microgram protein was Loaded in 10% tris glycine gel and blotted into nitrocellulose membrane.

- Protein levels of apoptotic biomarker (PARP protein cleavage) and expression of p53 was determined by probing the blots with specific antibodies. Equal loading of protein was determined in each lane by probing the blot with actin antibody. Results clearly indicating that SACC treatment induces apoptosis in pancreatic cancer cells and this effect may possibly due to induction of p53.

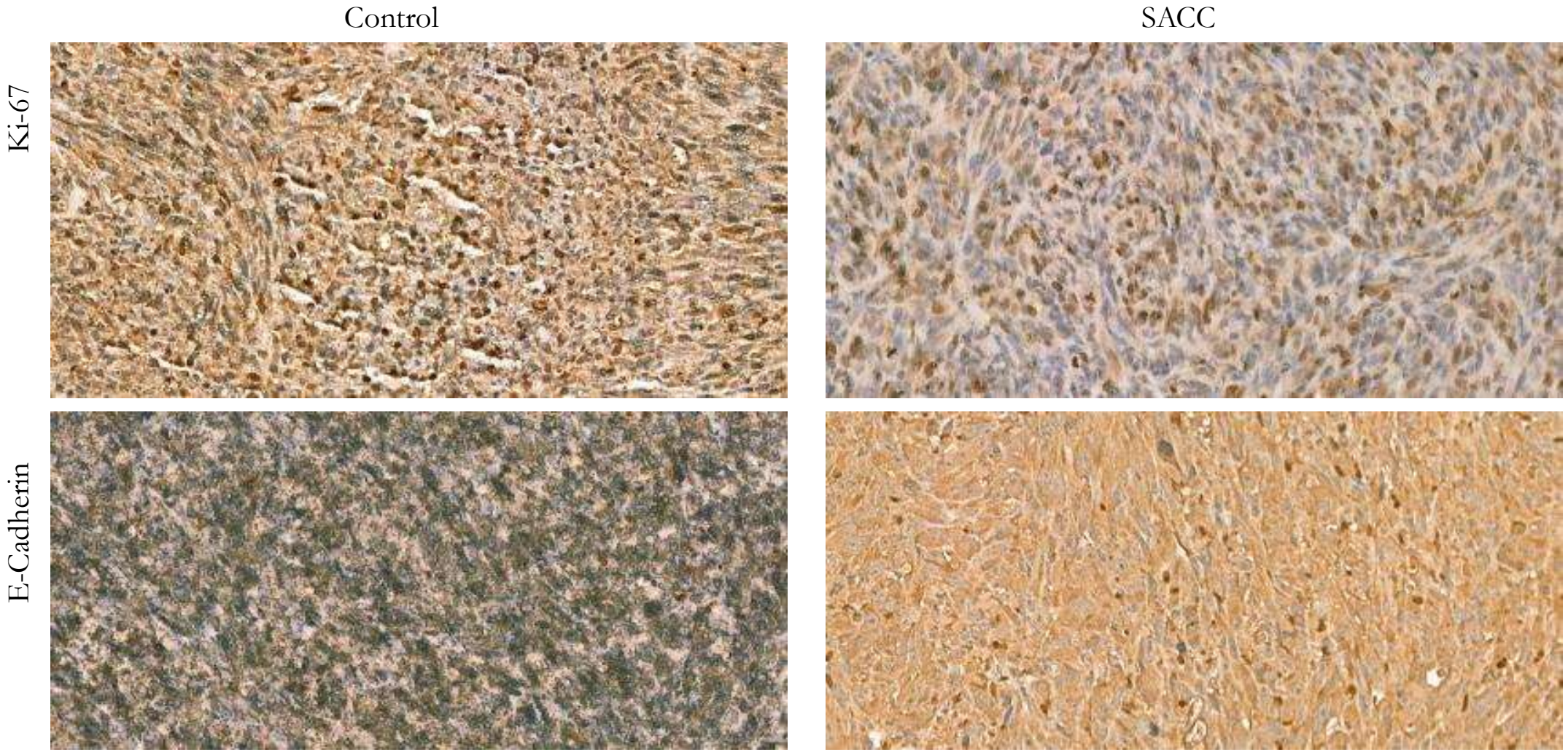
- **D:** Effect of SACC on the growth of normal human ductal epithelial cells (HPDE). **Di** Representative images of HPNE cells treated with various concentrations of SACC. **Dii.** Bar graph indicating comparatively less toxic effect of SACC on HPNE cells after 24 hrs treatment.



P53 and EMT Markers : Broad target validation/clinical impact in Multiple Cancers and Metastasis.

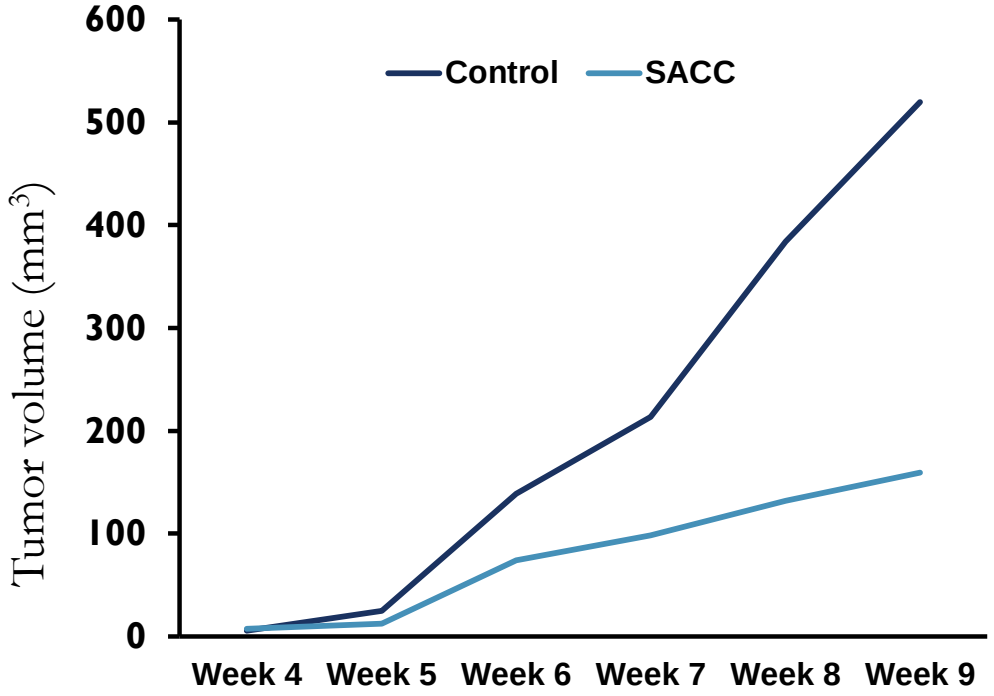
- TP53 (p53) is the single most frequently altered gene in human cancers, with mutations being present in approximately 50% of all invasive tumors. However, in some of the most difficult-to-treat cancers such as high-grade serous ovarian cancers, Pancreatic Cancers, triple-negative breast cancers, oeasophageal cancers, small-cell lung cancers and squamous cell lung cancers, p53 is mutated in at least 80% of samples. Clearly, therefore, mutant p53 protein is an important candidate target against which new anticancer treatments could be developed.
- E-Cadherin: Loss of E-cadherin function or expression has been implicated in cancer progression and metastasis. E-cadherin downregulation decreases the strength of cellular adhesion within a tissue, resulting in an increase in cellular motility. This in turn may allow cancer cells to cross the basement membrane and invade surrounding tissues
- E-cadherin plays a pivotal role in cancer progression, including the epithelial-mesenchymal transition (EMT) process and tumor metastasis. Loss of E-cadherin contributes to enhanced invasion and metastasis in human cancers. Therefore, restoring E-cadherin could be a potential approach for cancer therapy

Effect of SACC on ki-67 and E-Cadherin in pancreatic cancer stem cells xenograft tumor tissues



Results demonstrate that SACC significantly inhibits the nuclear expression of ki-67 protein and Increases E-cadherin expression. E-cadherin is involved in inhibition of epithelial to mesenchymal transition of cancer cells.

Therapeutic efficacy of SACC in human pancreatic cancer cells (AsPC1) ectopic xenograft mouse model



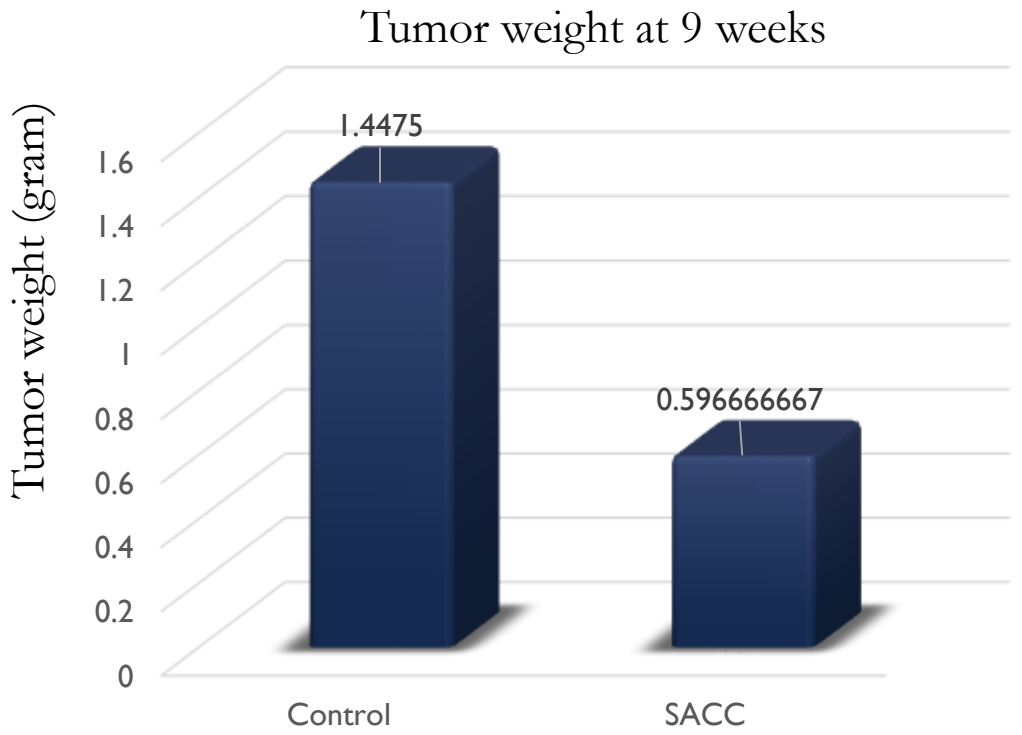
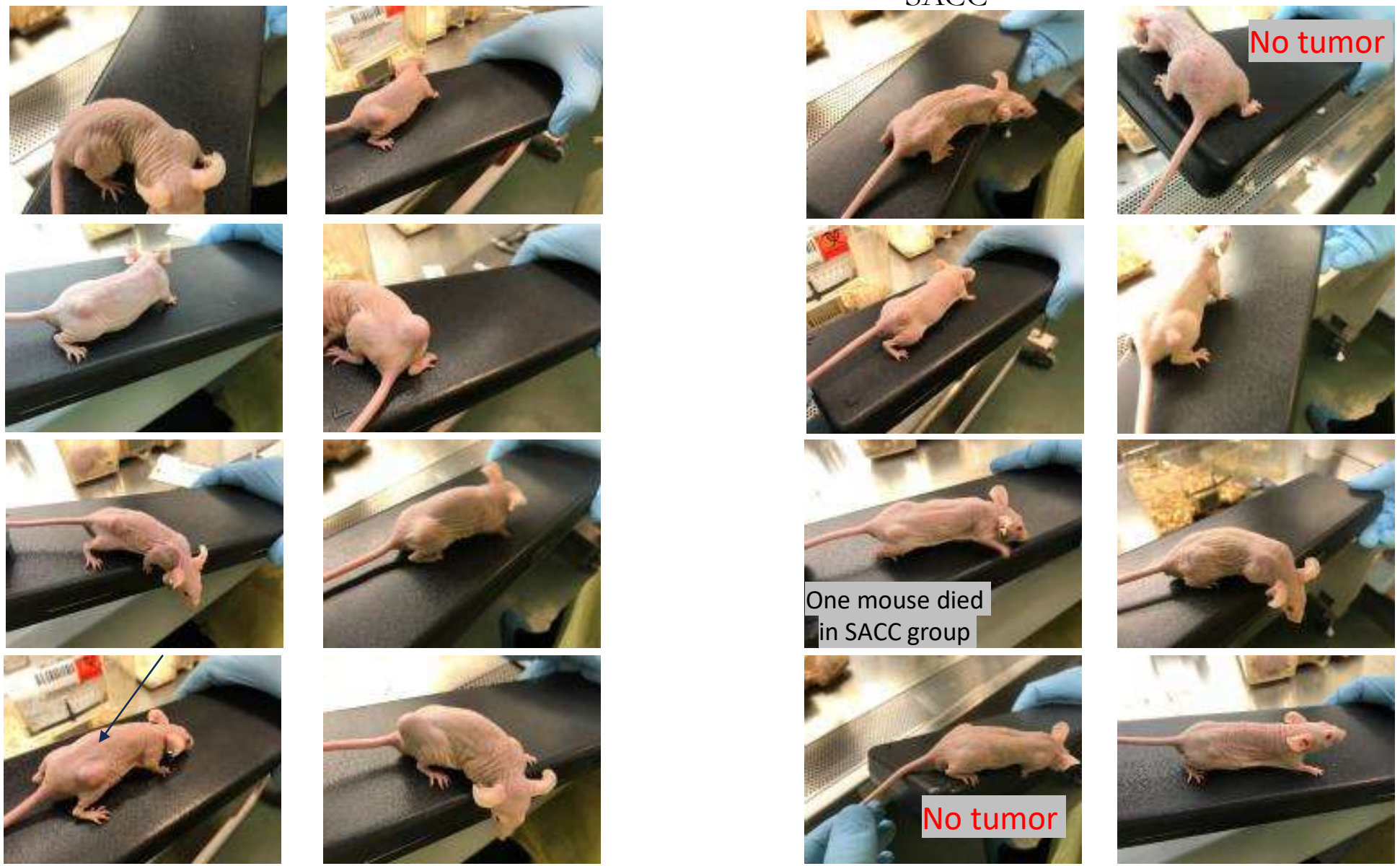


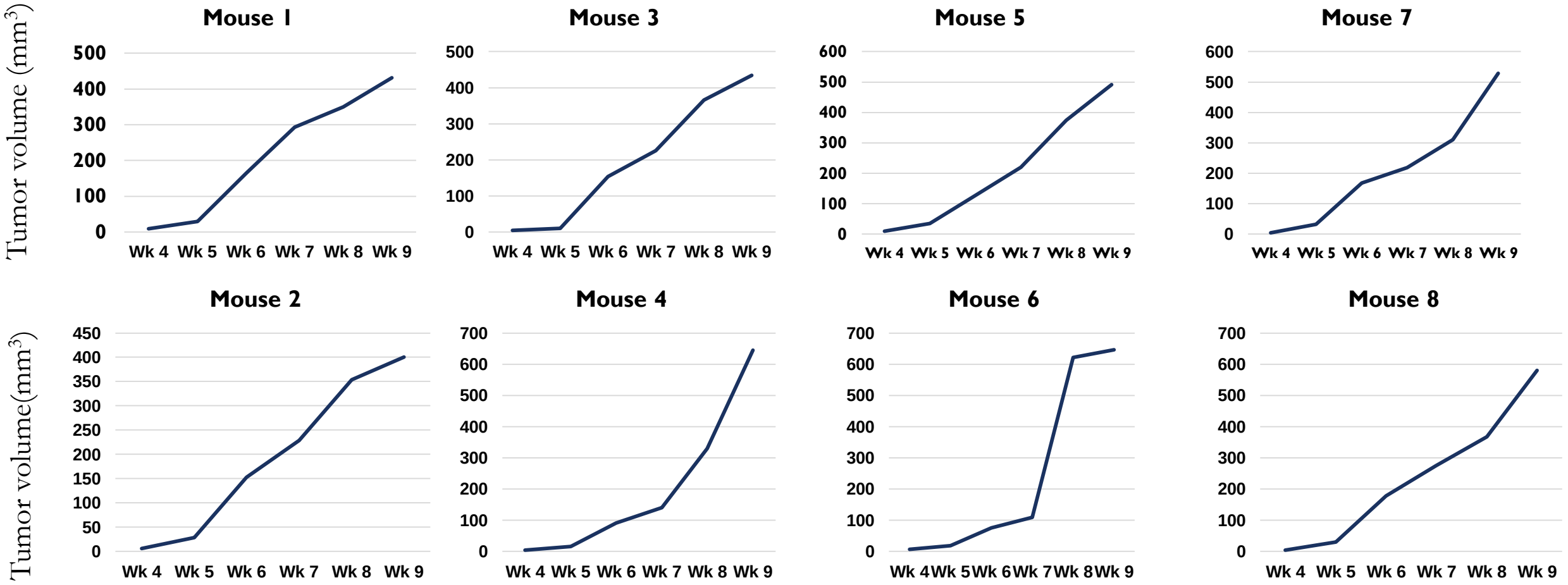
Figure Legend: SACC inhibits the growth of pancreatic tumors in xenograft mouse model of pancreatic cancer. SACC administration of 200 ul i.p significantly inhibited growth of AsPC1 cells derived xenograft tumors in athymic nude mice. **A.** Representative tumor graph illustrating tumor volume at indicated time point of control and SACC administered groups.
Value in the graphs are mean of n=8 mice in control and n=9 mice in SACC treated groups. SACC was administered 5 days/week for consecutively 6 weeks. **B.** Representative bar graph is showing tumor weight of control and SACC groups mice at 9 week. Values in the graph are mean of 8 and 9 mice of control and SACC treatment groups respectively.
Conclusion: SACC is a potential agent against pancreatic cancer.

N=8 **12 weeks study** **N=9**

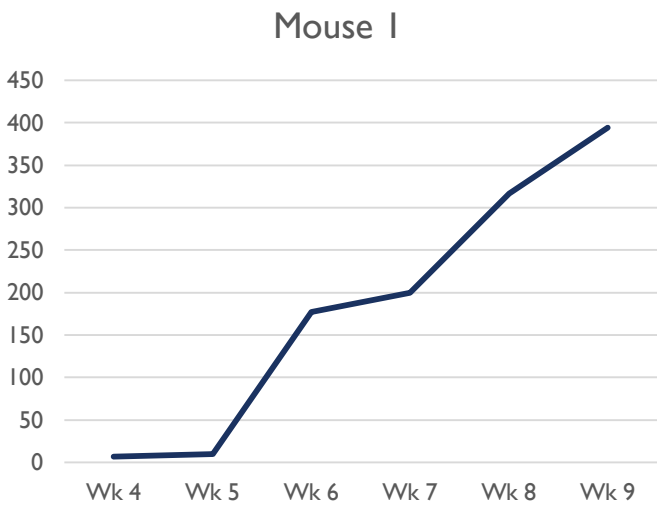
Control Control SACC SACC

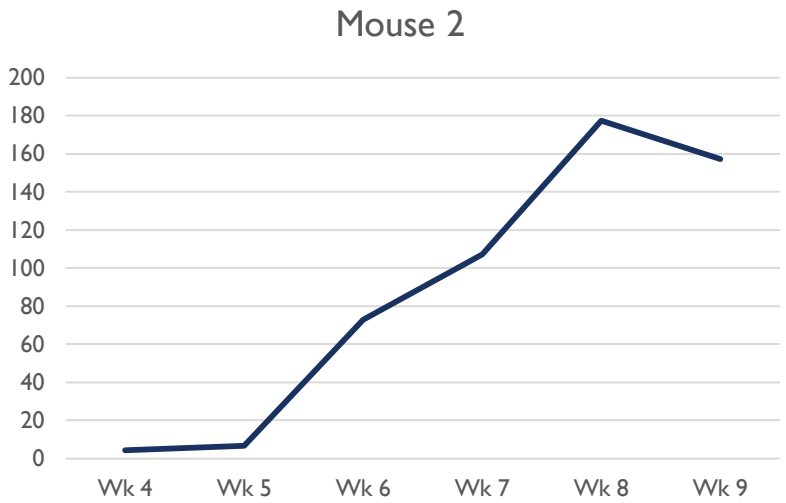


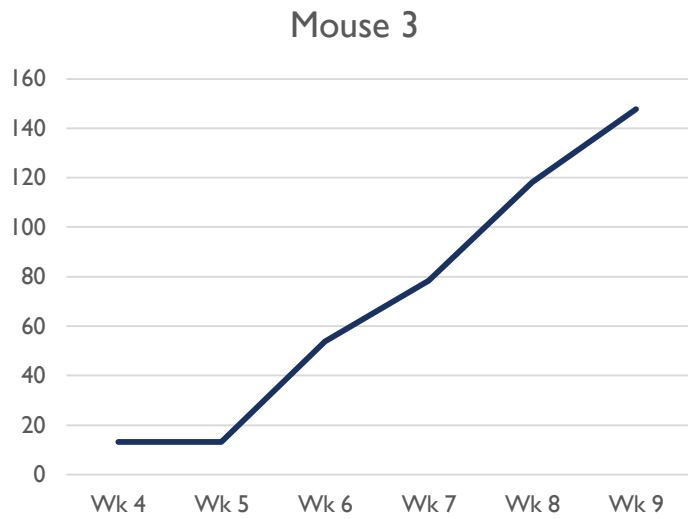
Control Group mice AsPC1 cells Xenograft Tumors

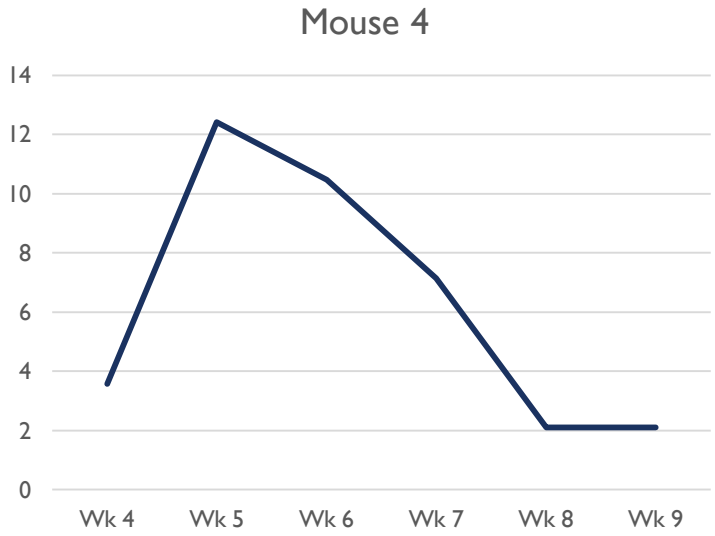


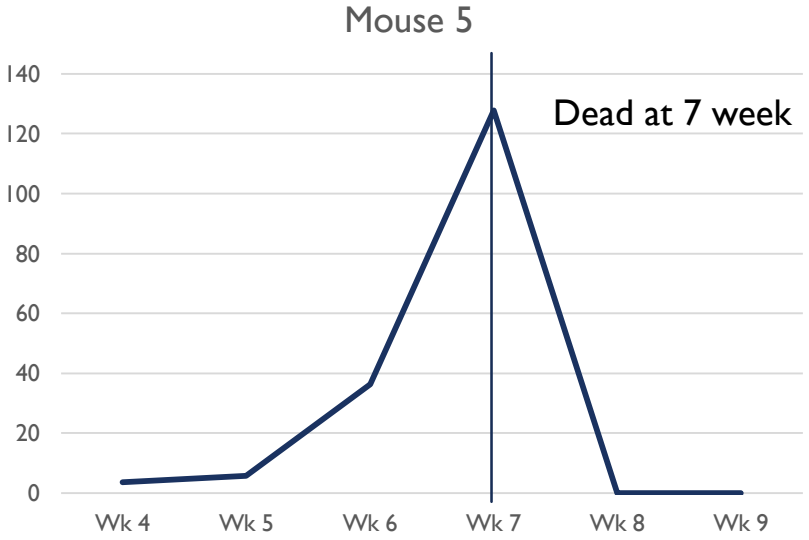
SACC Group mice AsPC1 cells Xenograft Tumors

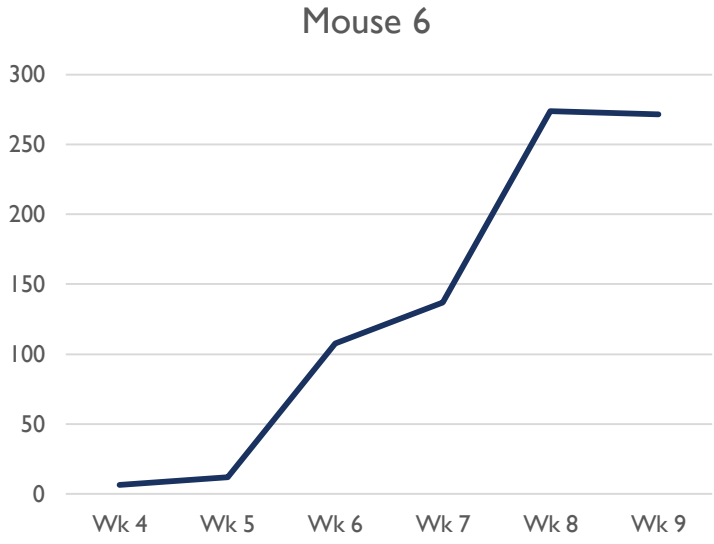




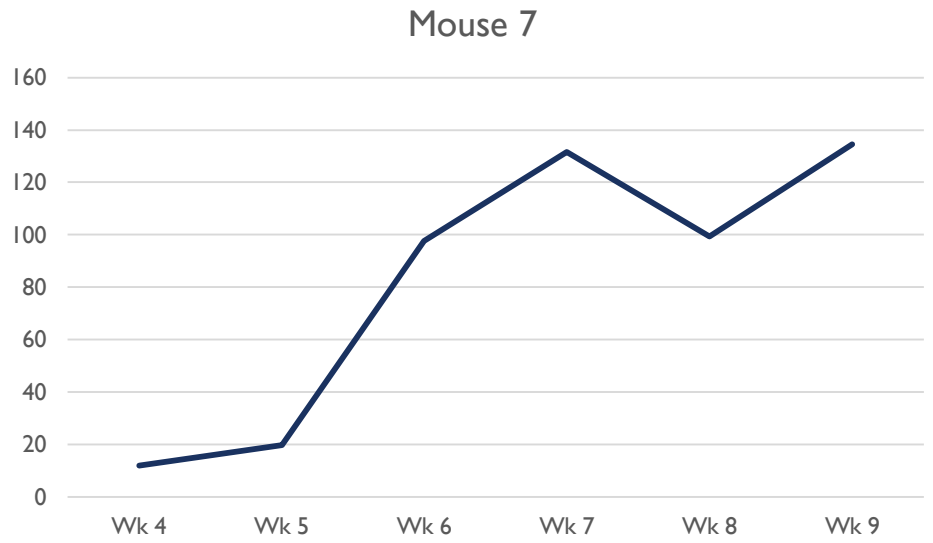


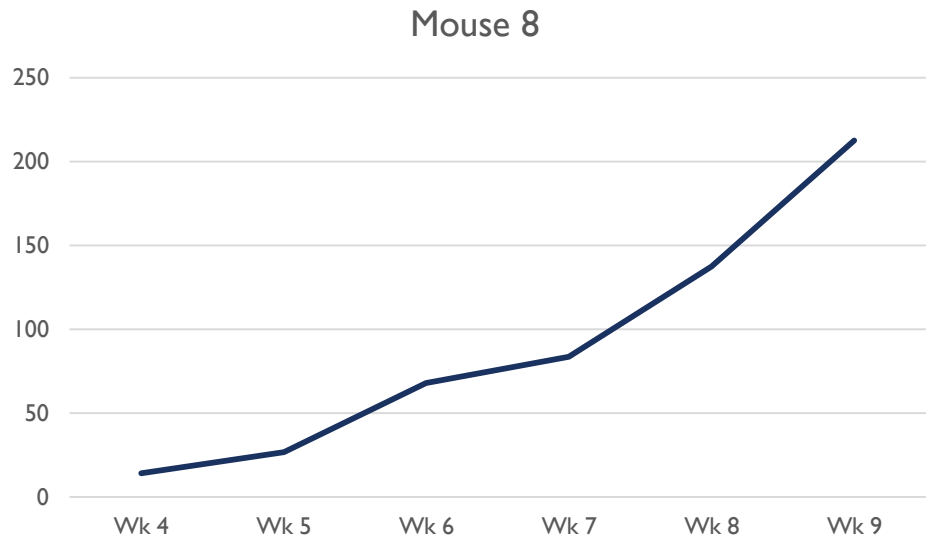


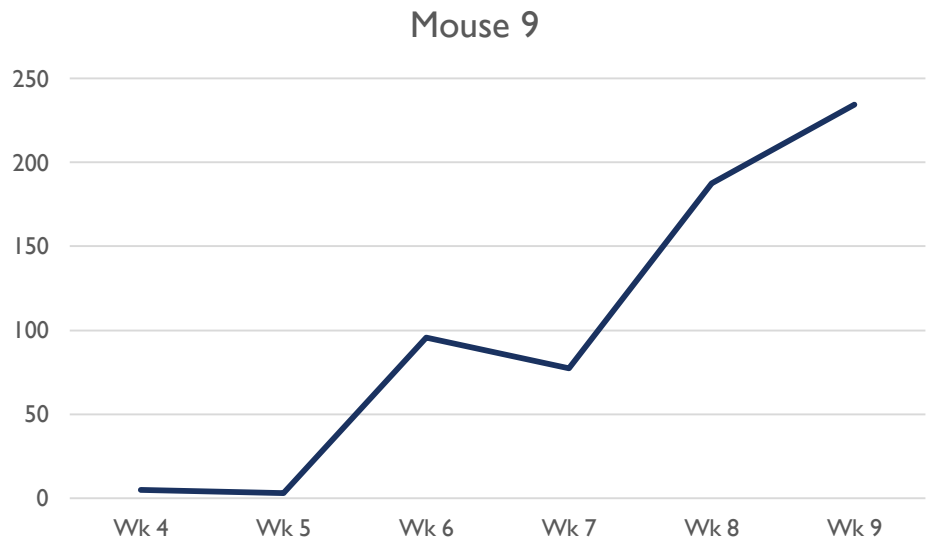




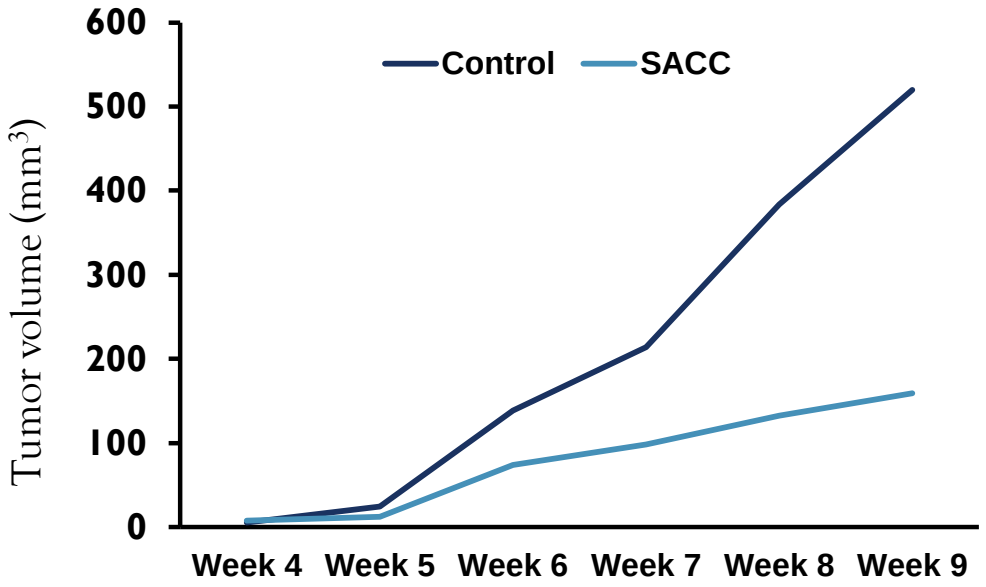
SACC Group mice AsPC1 cells Xenograft Tumors







Therapeutic efficacy of SACC in human pancreatic cancer cells (AsPC1) ectopic xenograft mouse model



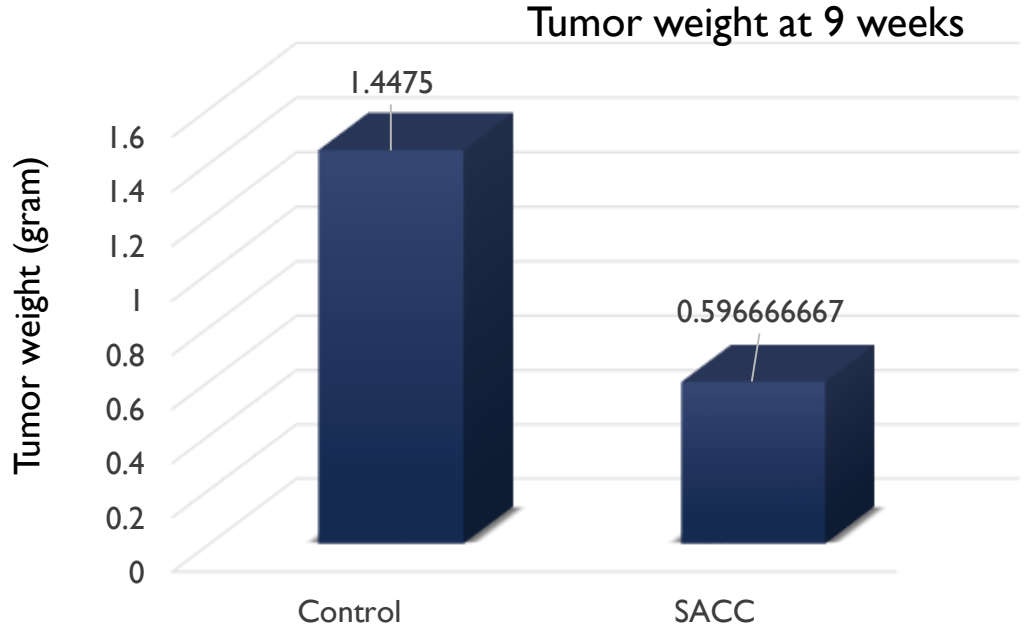


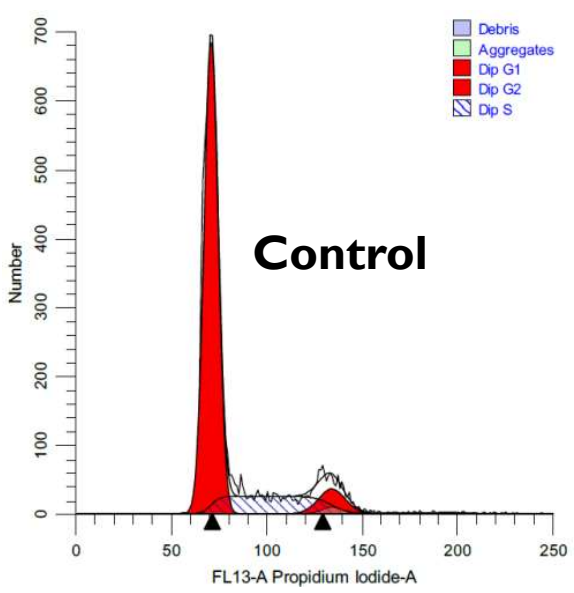
Figure Legend: SACC inhibits the growth of pancreatic tumors in xenograft mouse model of pancreatic cancer. SACC administration of 200 ul i.p significantly inhibited growth of AsPC1 cells derived xenograft tumors in athymic nude mice.

A. Representative tumor graph illustrating tumor volume at indicated time point of control and SACC administered groups. Value in the graphs are mean of n=8 mice in control and n=9 mice in SACC treated groups. SACC was administered 5 days/week for consecutively 6 weeks.

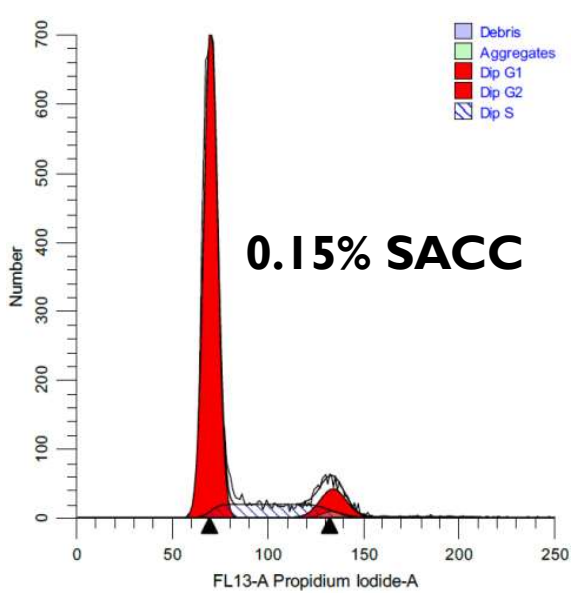
B. Representative bar graph is showing tumor weight of control and SACC groups mice at 9 week. Values in the graph are mean of 8 and 9 mice of control and SACC treatment groups respectively.

Conclusion: SACC is a potential agent against pancreatic cancer.

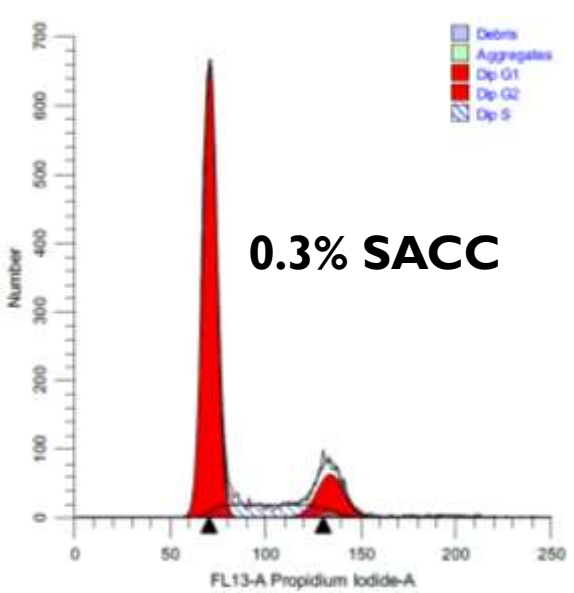
AsPC-1 cells treated with SACC for 24 hours for cell cycle.



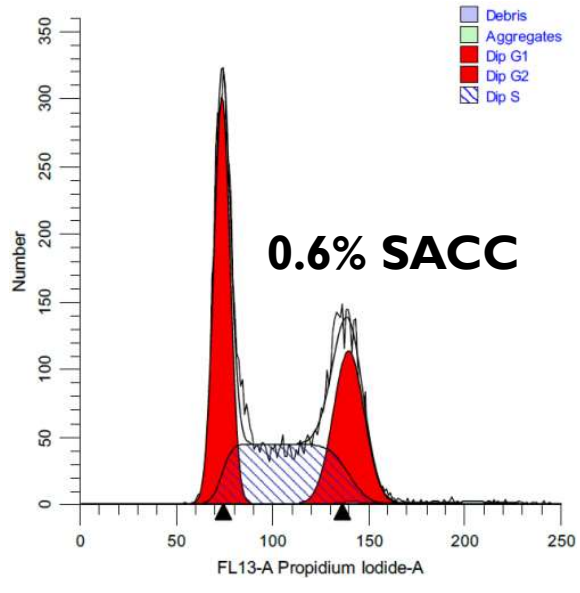
Diploid: 100.00 %
Dip G1: 74.24 % at 70.43
Dip G2: 7.34 % at 133.82
Dip S: 18.42 % G2/G1: 1.90



Diploid: 100.00 %
Dip G1: 77.21 % at 69.70
Dip G2: 8.47 % at 133.82
Dip S: 14.31 % G2/G1: 1.92



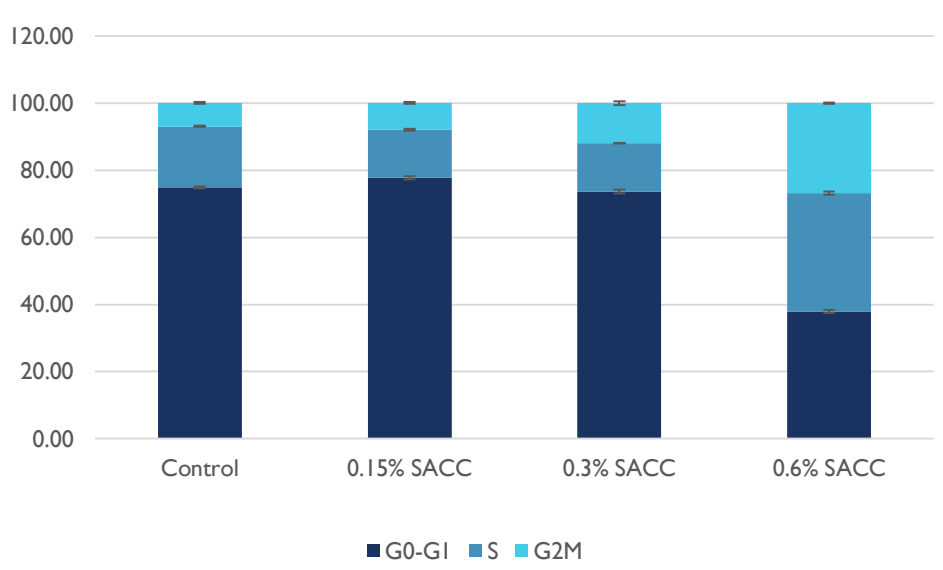
Diploid: 100.00 %
Dip G1: 72.92 % at 70.47
Dip G2: 12.96 % at 133.89
Dip S: 14.12 % G2/G1: 1.90



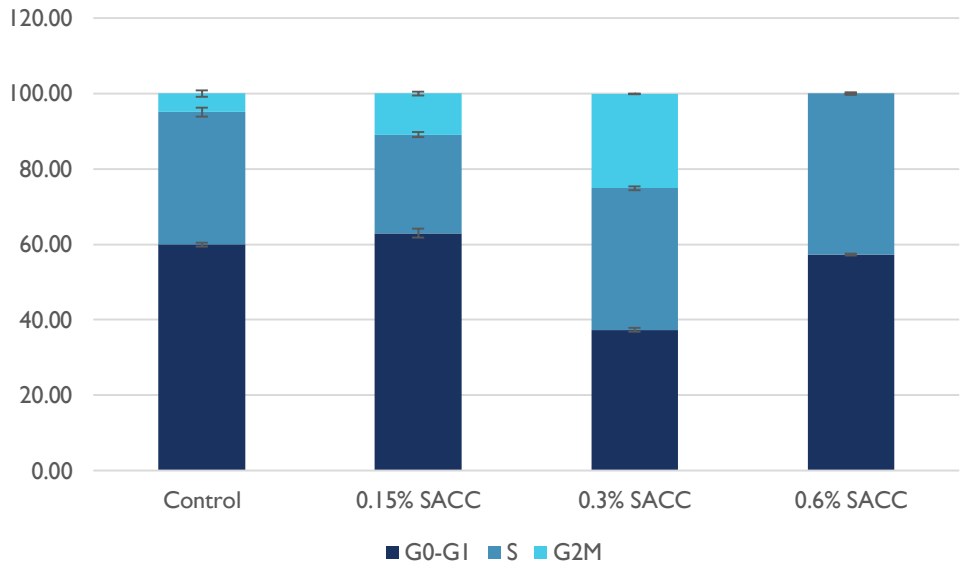
Diploid: 100.00 %
Dip G1: 38.08 % at 73.33
Dip G2: 27.10 % at 139.32
Dip S: 34.83 % G2/G1: 1.90

	Control	0.15% SACC	0.3% SACC	0.6% SACC
G0-G1	74.84 ± 0.30	77.77 ± 0.47	73.63 ± 0.49	37.92 ± 0.41
S	18.19 ± 0.18	14.31 ± 0.29	14.42 ± 0.17	35.30 ± 0.44
G2M	6.96 ± 0.25	7.92 ± 0.29	11.95 ± 0.57	26.78 ± 0.16

AsPC-1 cells treated with SACC for 24 hrs



MiaPaCa cells treated with SACC for 24 hrs

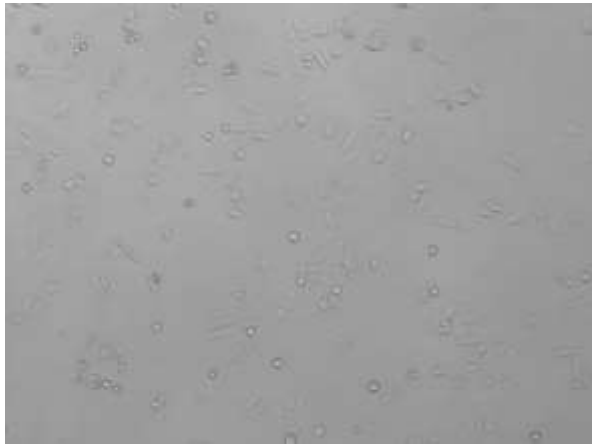


Average			
	G0-G1	S	G2M
Control	74.84	18.19	6.96
0.15% SACC	77.77	14.31	7.92
0.3% SACC	73.63	14.42	11.95
0.6% SACC	37.92	35.30	26.78

Average			
	G0-G1	S	G2M
Control	59.95	35.18	4.88
0.15% SACC	63.00	26.12	10.88
0.3% SACC	37.33	37.59	25.07
0.6% SACC	57.29	42.71	0.00

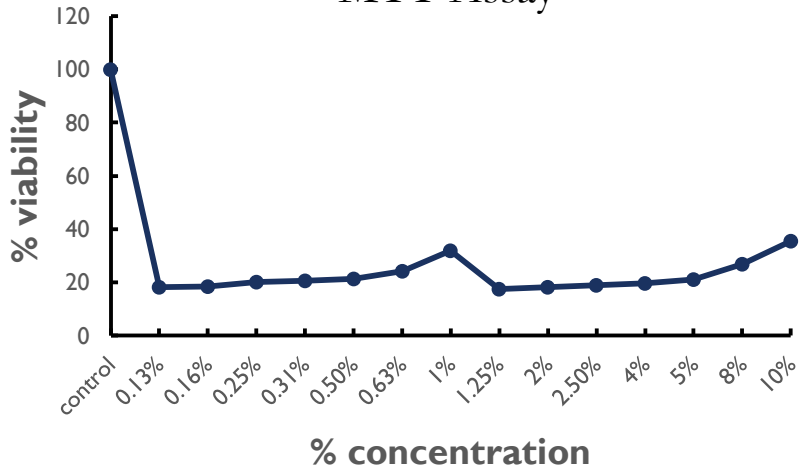
Pancreatic Cancer Stem Cells (PCSCs)

Control



0.13 % SACC

MTT Assay



Effect of drug on Pancreatic cancer Stem cells Cells:

- PCSCs were purchased from Cell Progen.
- 500 cells were seeded in each well of 96 well plate.
- After 25 hrs MTT assay was performed to determine the IC50 of SACC.
- Results demonstrate that SACC is a very potent agent which inhibits the growth of PCSCs at very low concentration.
- We are performing another experiment to confirm IC50.

SACC Suppresses Pancreatic Cancer Stem cells PanCSCs).

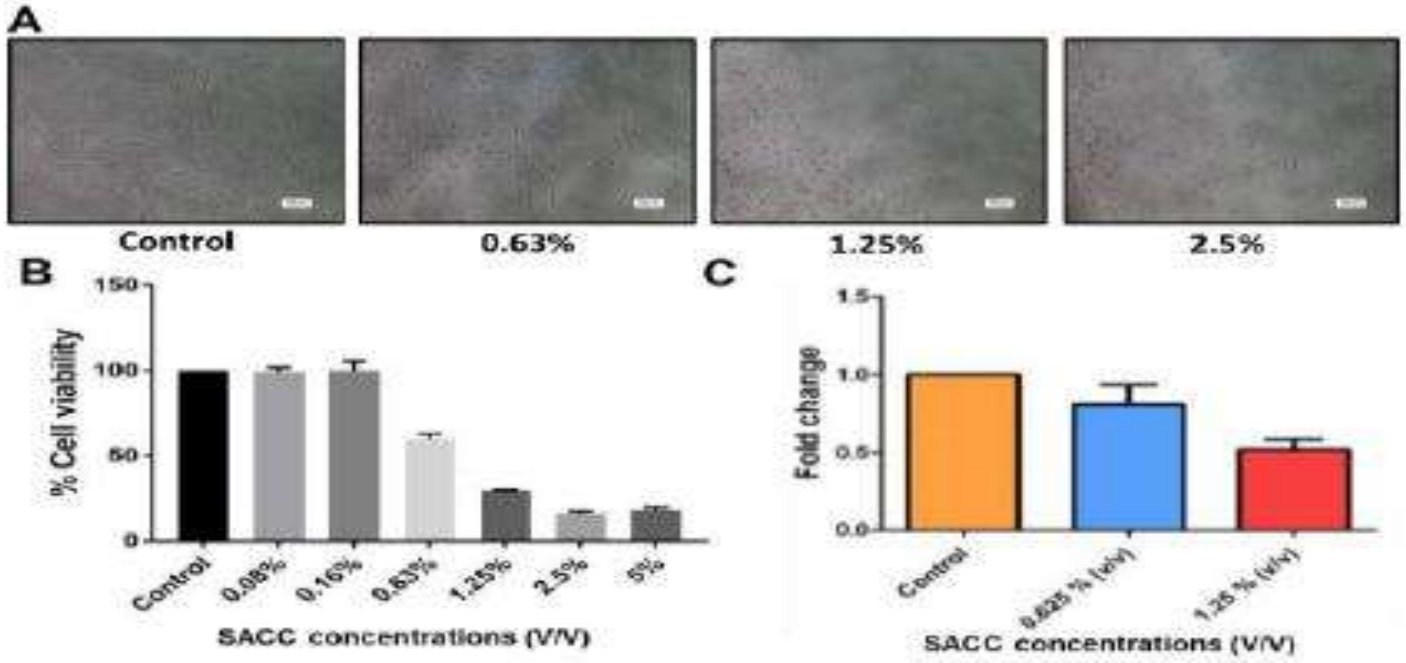


Figure 2: SACC suppresses pancreatic cancer stem cells (PanCSCs). A-B Effect of SACC treatment on growth of PanCSCs after 24 hrs treatment as determined by MTT assay.

A. Representative images of PanCSCs treated with indicated concentrations of SACC.

B. Bar graph representing growth inhibition of PanCSCs by SACC after 24 hrs treatment. Effect of SACC on the expression of OCT4 as determined by qPCR analysis.

Results demonstrates significant inhibition of OCT-4 expression in PanCSCs with the treatment of SACC.

SACC inhibits the growth of pancreatic cancer stem cells xenograft tumors in SCID mice.

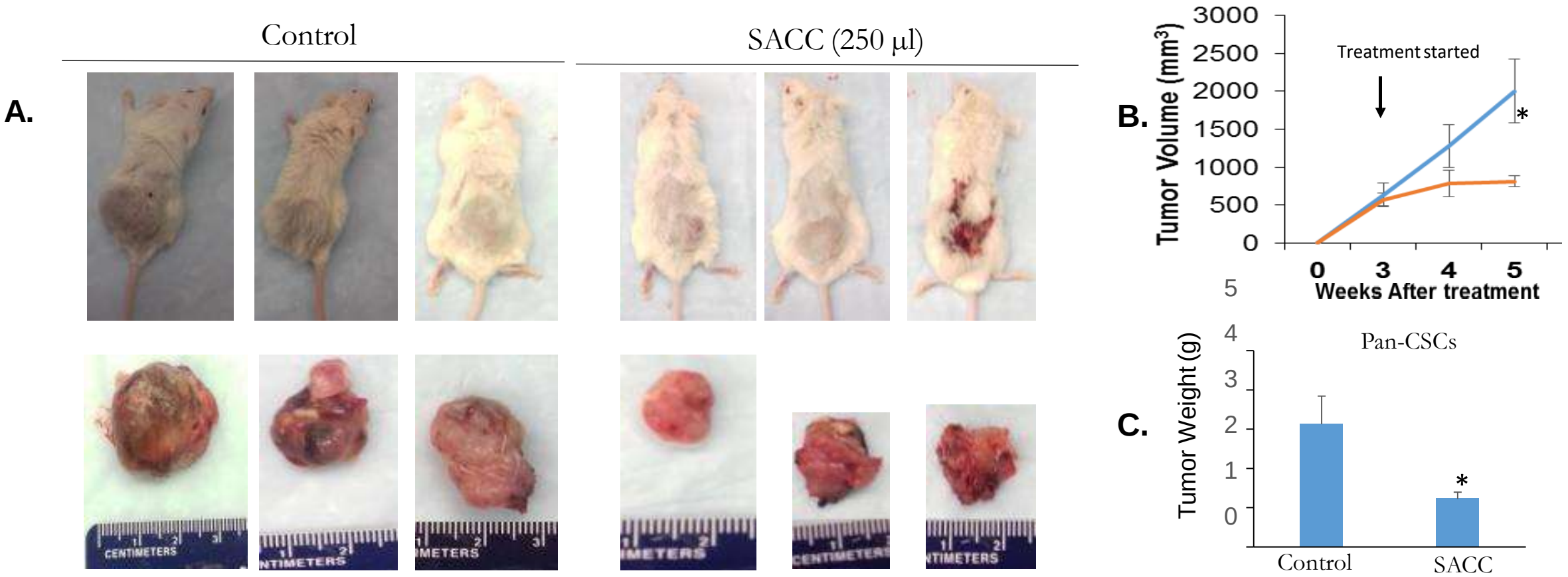


Figure Legend: Effect of SACC on pancreatic cancer stem cells xenograft in SCID mice. A total of eight mice were used in the study. Briefly, 100% viable 2000 cells in 100 µl volume (1:1 ratio of Culture media + Matrigel) were subcutaneously injected in SCID mice. Two weeks later, SACC (250 µl) was administered intraperitoneally 5 days in a week for two weeks. All of the mice were sacrificed when control mice tumors reach a targeted volume 1500 mm3.

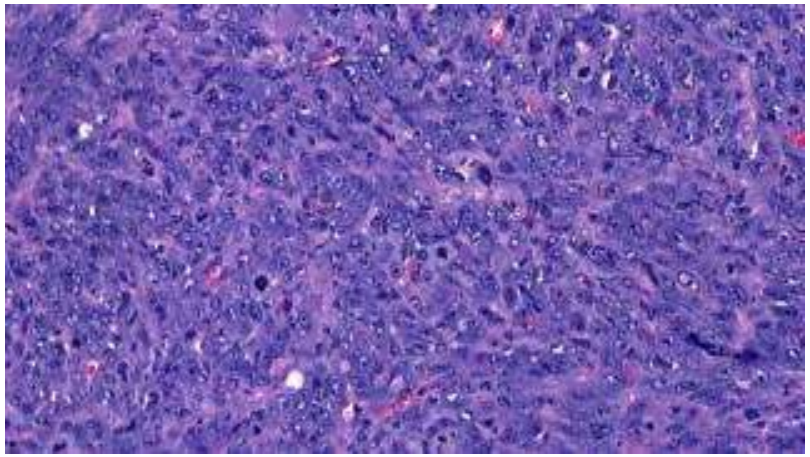
A. Representative images of control and SACC treated mice bearing xenograft tumors. Lower panel pictures indicate excised xenograft tumors of control and SACC treated mice. **B.** Tumor volume of xenograft tumors in control and SACC treatment at indicated week. **C.** Excised xenograft tumors weight of control and SACC treated mice at week 5.

Histopathology Of Pan-CSCs Derived Xenograft Tumors Of Control And SACC Treated Mice

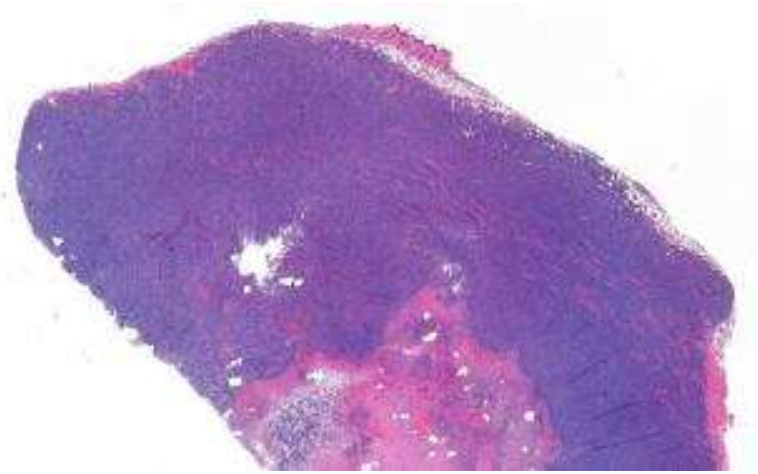
2X

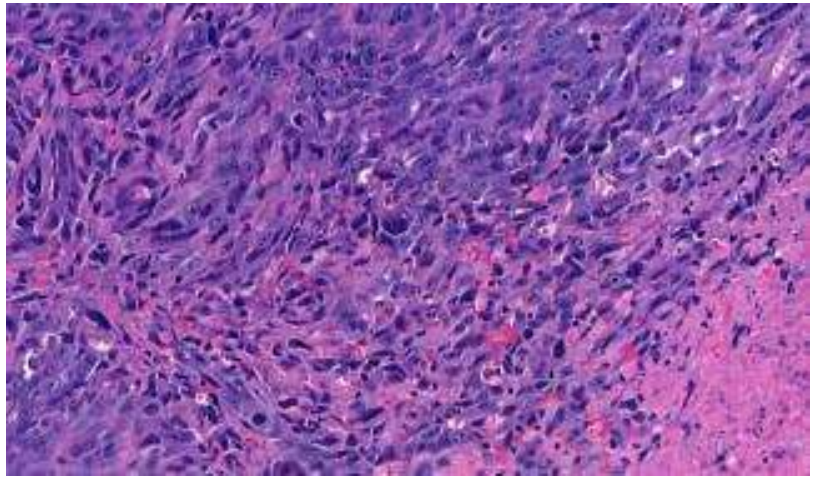
40X

Control

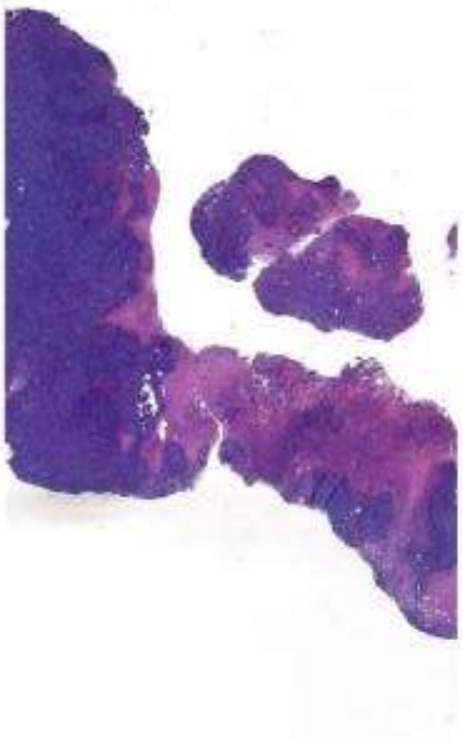


SACC

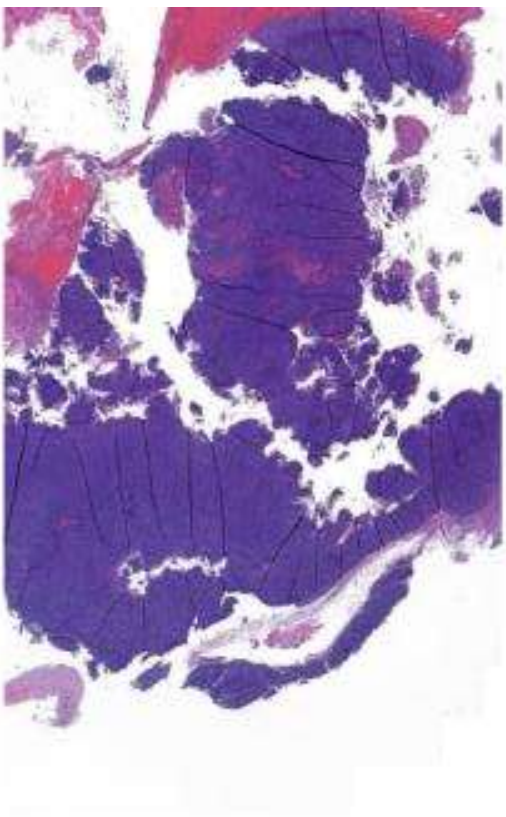


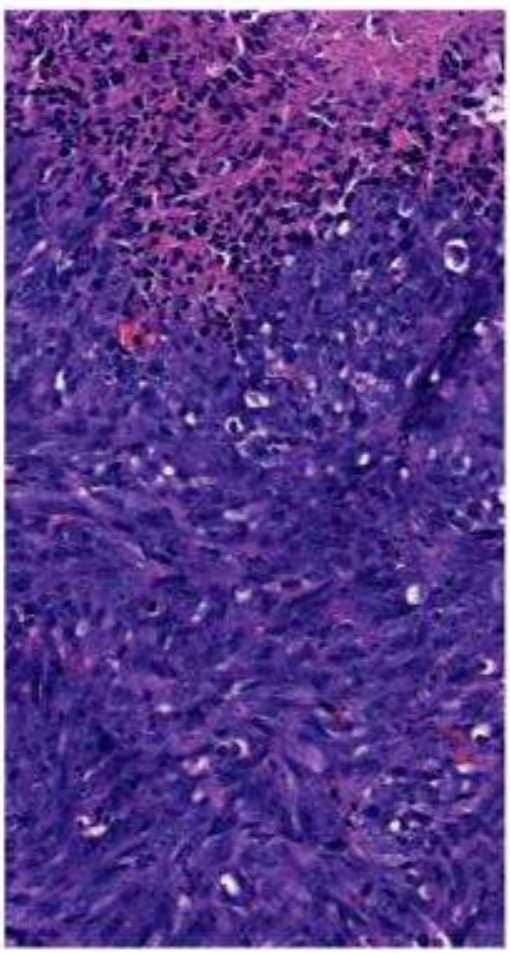


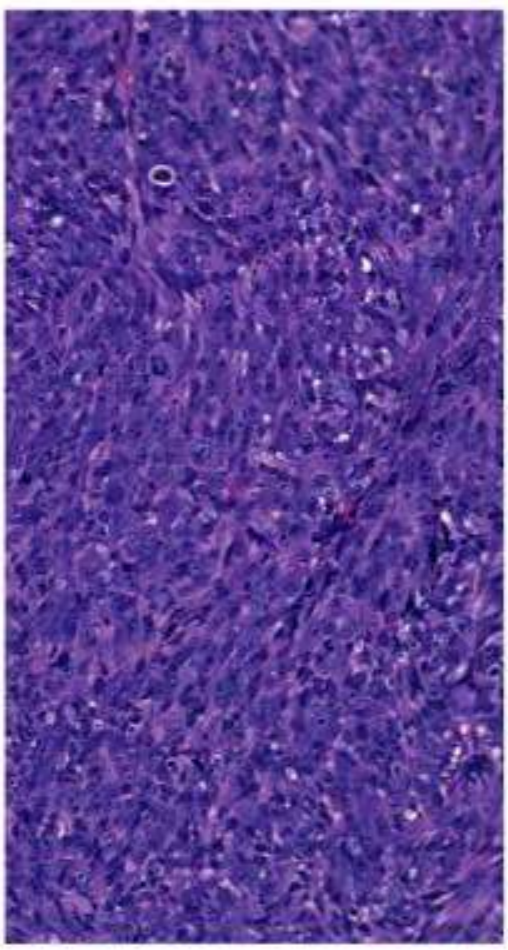
SACC

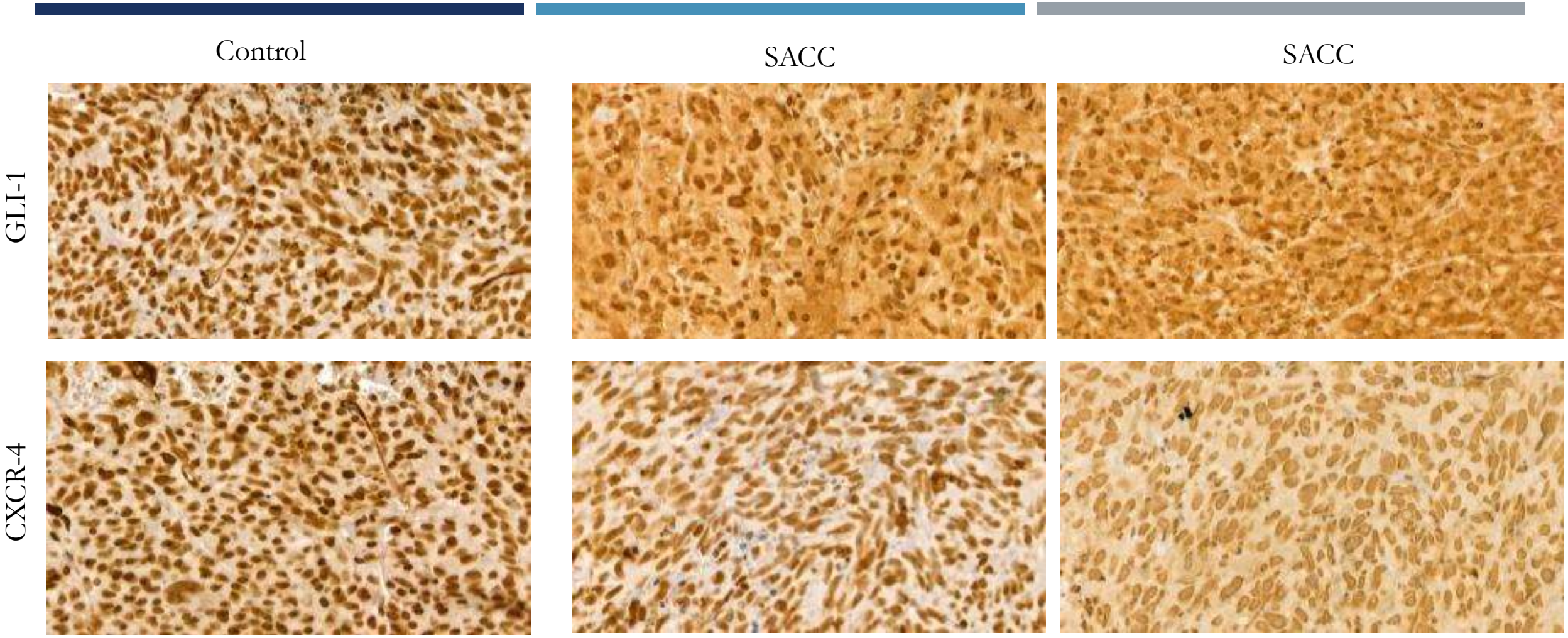


SACC





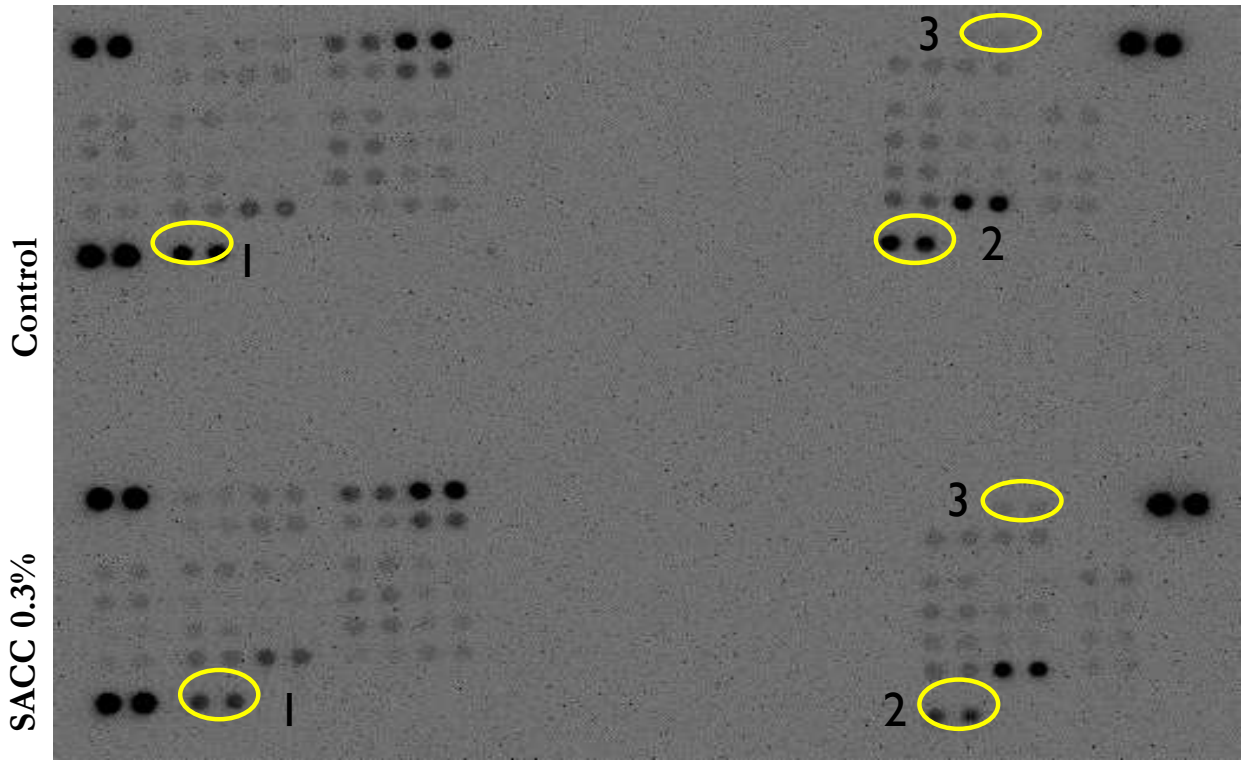




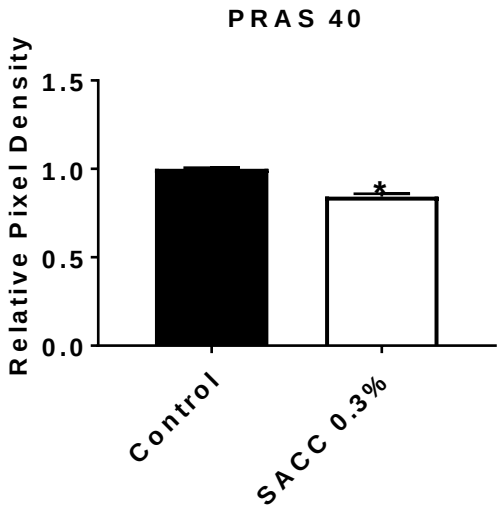
Effect of SACC on expression of transcription factors GLI-1 and chemokine receptor CXCR4. Representative Immunohistochemistry images of GLi-1 and CXCR4 expression of control and SACC xenograft tumors. Results indicate SACC partially inhibits both transcription factor Gli-1 and chemokine receptor CXCR4.

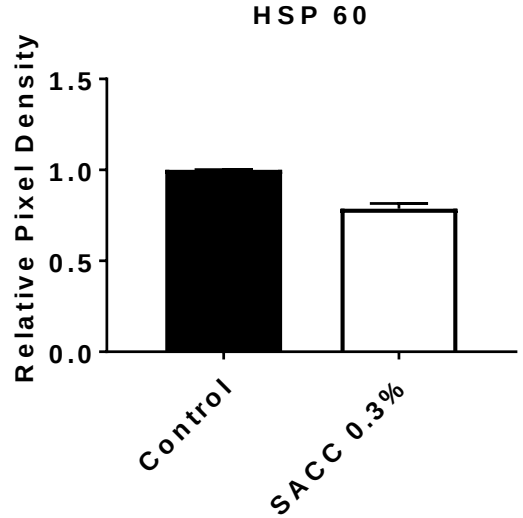
SACC treatment Inhibits PRAS40, HSP60 and Induces p53

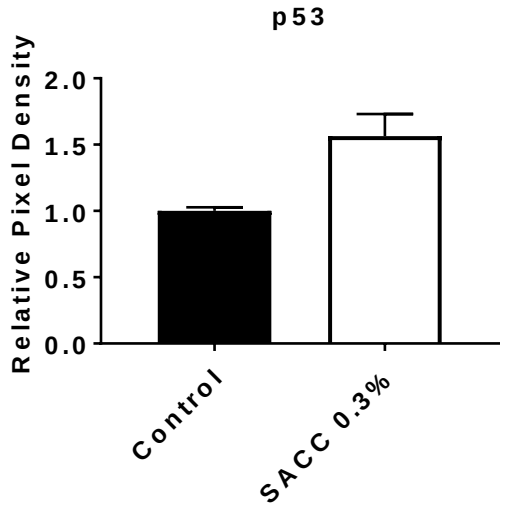
AsPC-1 cells



1 – PRAS 40
2 – HSP60
3 – p53

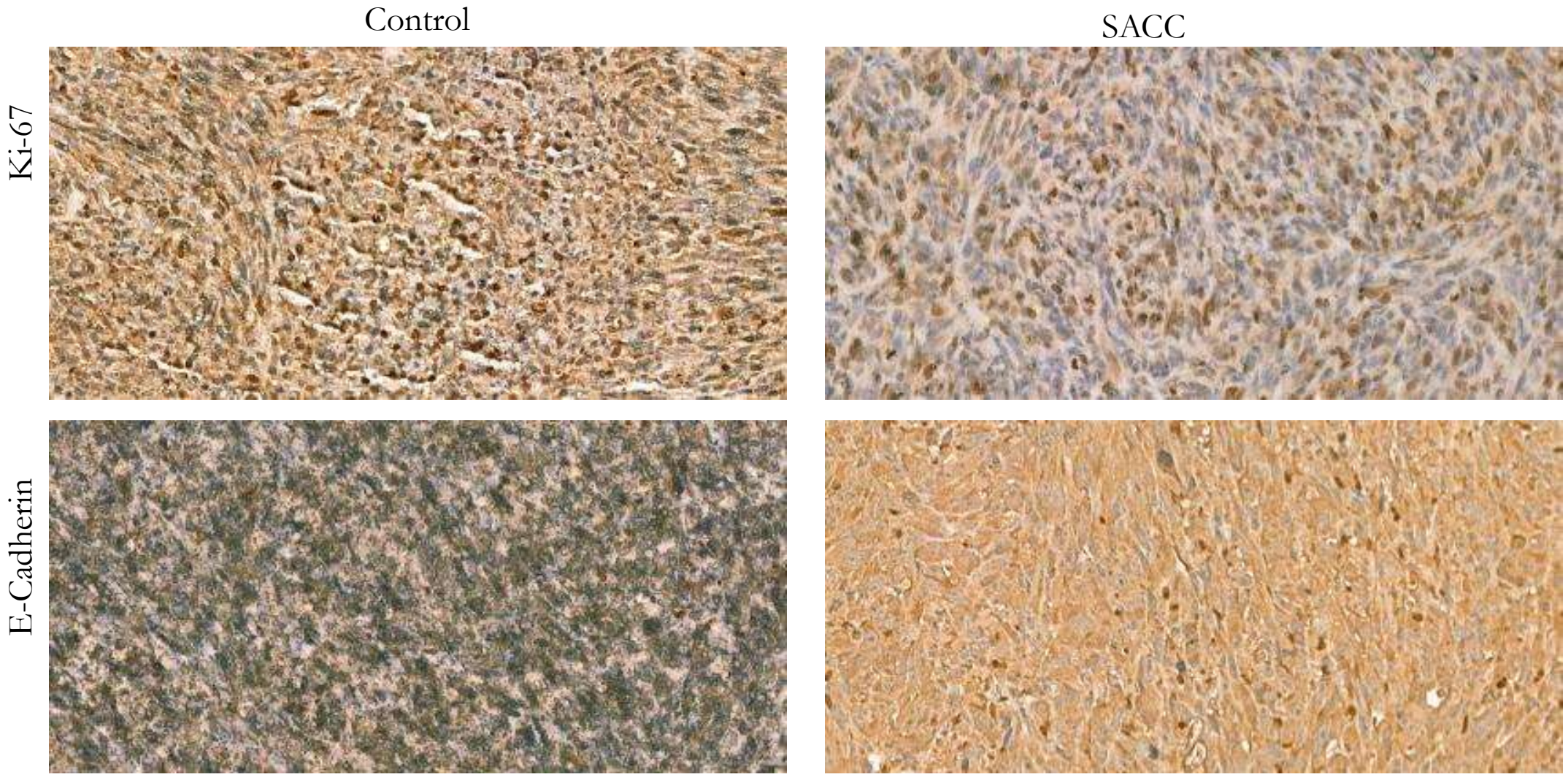






PRAS40: The proline-rich Akt substrate of 40 kDa
HSP 60: Heat scock Protein 60

Effect of SACC on ki-67 and E-Cadherin in pancreatic cancer stem cells xenograft tumor tissues



Results demonstrate that SACC significantly inhibits the nuclear expression of ki-67 protein and increases E-cadherin expression. E-cadherin is involved in inhibition of epithelial to mesenchymal transition of cancer cells.

Molecular Mechanisms of SACC to inhibit the growth of pancreatic cancer.

- SACC induces apoptosis and inhibits the growth of MiaPaCa-2 and AsPc-1 cells.
- We next evaluated the effect of SACC on apoptosis of PanCa cells.
- SACC (1.25 %v/v) treatment showed a significant apoptosis induction in MiaPaCa-2 cells.
- These results provide the evidence of SACC potential to kill the pancreatic cancer cells via inducing apoptosis.

SACC targets HER-2 and p53 signaling pathways in pancreatic cancer cells

- Our results revealed that SACC down-regulates the protein expression of HER-2 in both the cell lines in a dose-dependent manner.
- Interestingly, SACC treatment enhances the phosphorylation of HER-2 in both AsPC- 1 and MiaPaCa-2 cells at higher concentration (Fig.2).
- This effect was more in WT p53 expressing AsPC-1 cells compared to mutant p53 expressing MiaPaCa-2 cells. Our results are corroborate with a recently published study Figure 2: Effect of SACC on the expression of HER-2/p53 and apoptotic proteins in pancreatic cancer cells showed an enhanced phosphorylation of HER-2 in AsPC-1 with the treatment of chemotherapeutic drug gemcitabine

SACC modulates the expression of apoptotic proteins in pancreatic cancer cells.

- During apoptosis, cleavage of PARP has become a useful hallmark of this type of cell death. This cleavage is well studied and is generated by the caspases 3 and 7, proteases activated during apoptosis.
- Similarly, we also reported that the dose- dependent cytotoxicity of SACC was mediated by cleavage of PARP protein and subsequent apoptotic events in MiaPaCa-2 and AsPc- 1 cells. Most interestingly, SACC treatment resulted in a marked suppression of mutant p53 in MiaPaCa-2 cells, are known to possess mutant p53 (exon 3, 6, and, 7; R89W, R116W, R209W, R248W, C265T, C346T, C625T, and, C742T) and the existence of this mutant p53 has also been associated with the gemcitabine resistance in pancreatic cancer cells, particularly MiaPaCa-2.
- Based on these previous observations, the down-regulation of mutant p53 in MiaPaCa-2 cells in the current study signifies the antiproliferative efficacy of SACC. Interestingly, SACC treatment induced the expression of p53 in AsPC-1 cells.
- These results suggest SACC induces apoptosis mediated cell death of pancreatic cells via targeting mutant p53, WT p53 and HER-2. Similarly, over-expression of various p53-targeted genes i.e., p21 has also been linked to the tumor suppression and growth inhibition/cellular arrest.
- The findings from our study are very interesting as we reported an over-expression in p21 expression in AsPc-1 cells, which might be attributed to the induction of WT p53 by SACC treatment. Unlike AsPC-1 cells, the expression of p21 was unchanged in SACC treated MiaPaCa-2 cells.
- Moreover, the process of apoptosis is very complex and tightly regulated by coordination of distinct mediators including but not limited to Bcl-2 and Bcl-2-associated X, apoptosis regulator (BAX). BAX are known to trigger mitochondrial outer membrane permeabilization while Bcl-2 is plays a negative role in the phenomenon of apoptosis.
- In the same context, treatment with SACC markedly up-regulated the BAX/Bcl-2 ratio in both AsPc-1 and MiaPaCa-2 cells in a dose-dependent manner. These findings are clearly suggested that the SACC exerts anti proliferative potential against pancreatic cancer via targeting HER-2/p53/BAX-Bcl-2 axis.

Therapeutic efficacy of SACC against Mia-Pa-Ca2 pancreatic cancer

Treatment

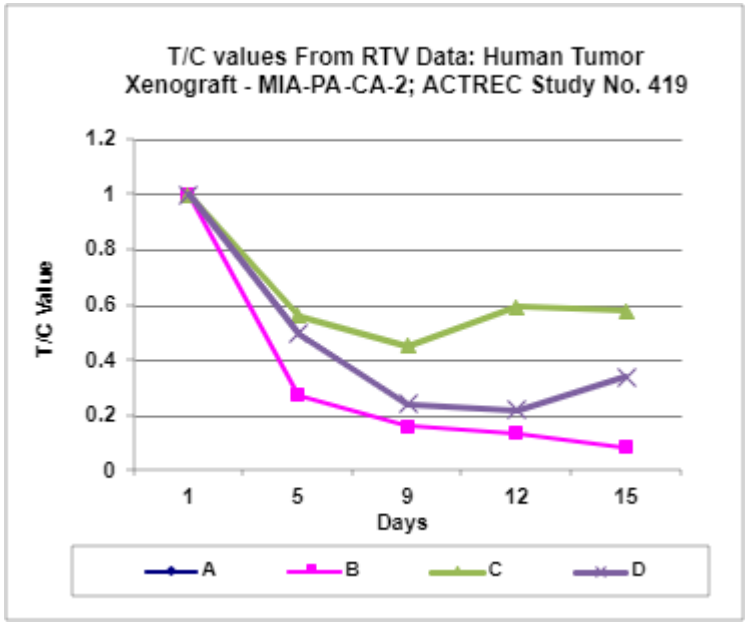
A	Control
B	Positive Control (ADR) 2.5 mg/kg i.v.injection on day 1,5,9
C	SACC- 4 mL /Kg Oral Twice a week for 4 Weeks
D	SACC 1.2 mL /Kg Oral daily 6 days/Week for 4 Weeks

Activity Criteria:

T/C ≤ 0.2 (highlighted yellow) is considered to demonstrate significant activity. T/C ≤ 0.42 (highlighted blue) is considered to demonstrate moderate activity.

Toxicity Criteria:

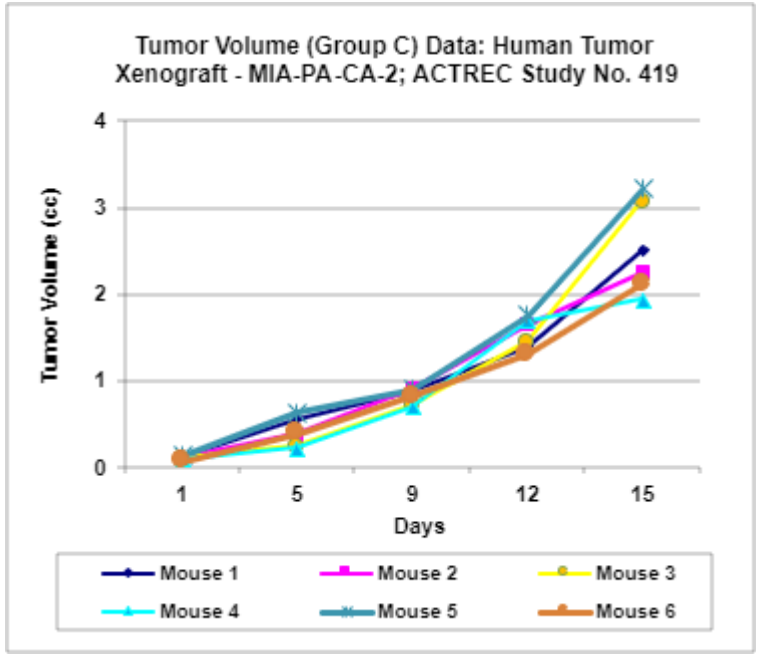
Mortality and weight loss ≥4 grams/ mouse are considered to indicate toxicity.



T/C (from RTV data)

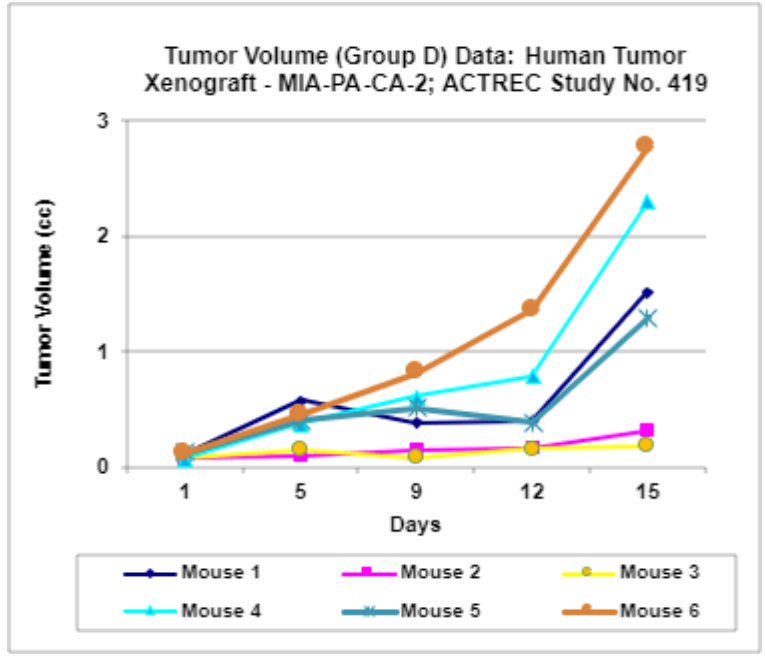
Weeks	Days	A	B	C	D
0.00	1		1.00	1.00	1.00
0.71	5		0.27	0.56	0.50
1.29	9		0.16	0.45	0.24
1.71	12		0.13	0.59	0.22
2.14	15		0.08	0.58	0.34

Tumor Volume Group C Data



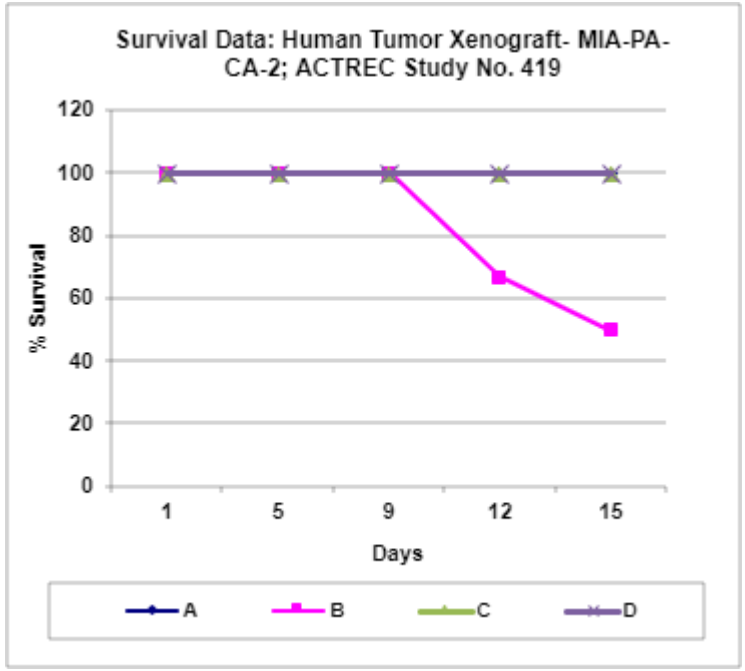
Week	Days	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6
0.00	1	0.12	0.12	0.11	0.10	0.15	0.08
0.71	5	0.56	0.39	0.26	0.23	0.64	0.41
1.29	9	0.87	0.92	0.73	0.70	0.91	0.83
1.71	12	1.40	1.65	1.46	1.70	1.76	1.31
2.14	15	2.51	2.25	3.07	1.94	3.22	2.13

Tumor Volume Group D Data



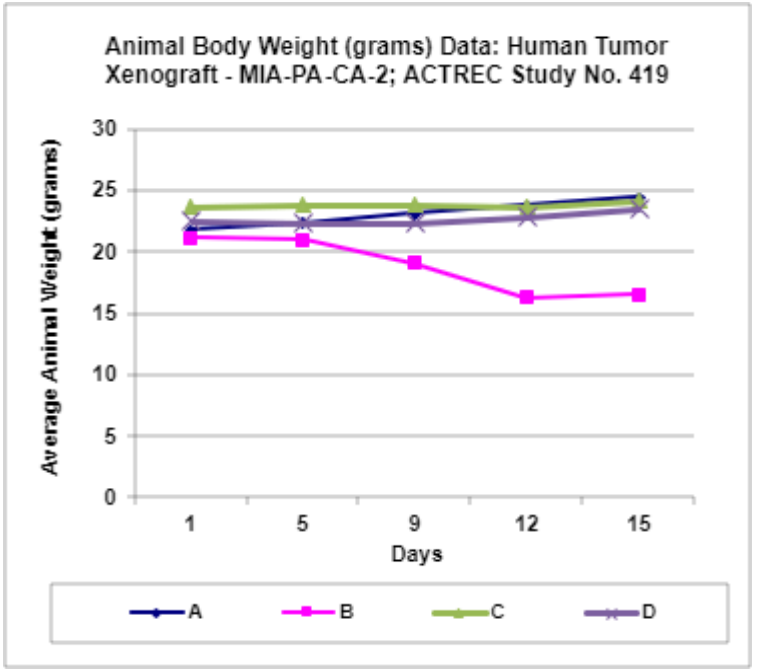
Week	Days	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6
0.00	1	0.11	0.08	0.09	0.08	0.13	0.13
0.71	5	0.59	0.11	0.15	0.38	0.41	0.47
1.29	9	0.39	0.15	0.08	0.61	0.53	0.83
1.71	12	0.41	0.17	0.16	0.80	0.40	1.37
2.14	15	1.53	0.32	0.19	2.31	1.31	2.79

% Survival



Weeks	Days	A	B	C	D
0.00	1	100	100	100	100
0.71	5	100	100	100	100
1.29	9	100	100	100	100
1.71	12	100	67	100	100
2.14	15	100	50	100	100

Animal Body Weight(Grams)



Weeks	Days	A	B	C	D
0.0	1	21.8	21.2	23.8	22.6
0.7	5	22.4	21.0	23.9	22.4
1.3	9	23.2	19.1	23.9	22.4
1.7	12	23.8	16.3	23.7	22.9
2.1	15	24.5	16.6	24.2	23.6

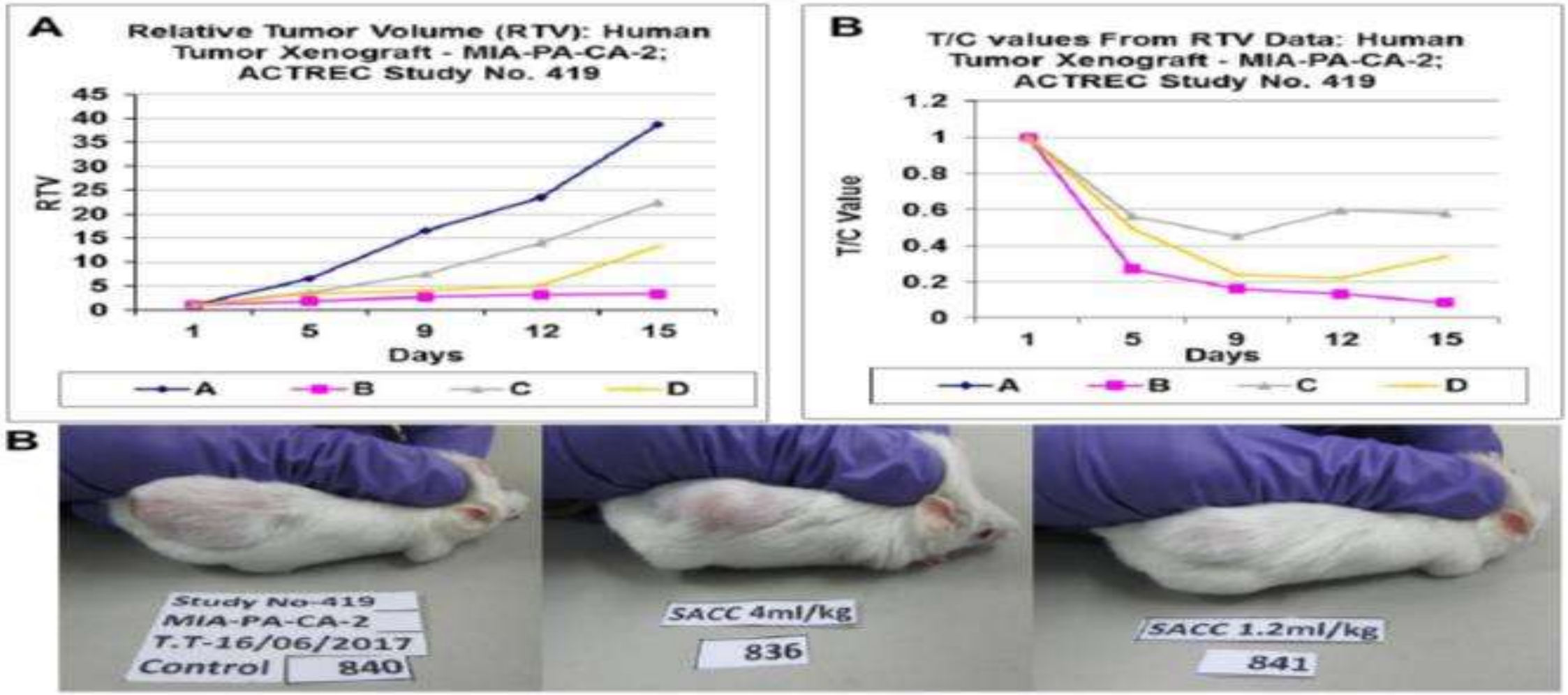
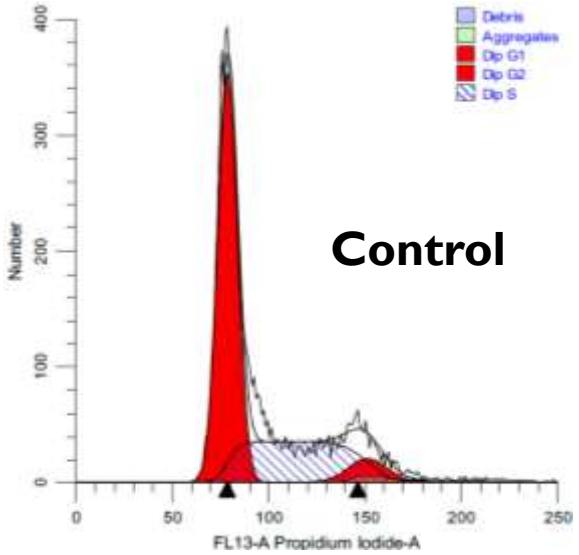
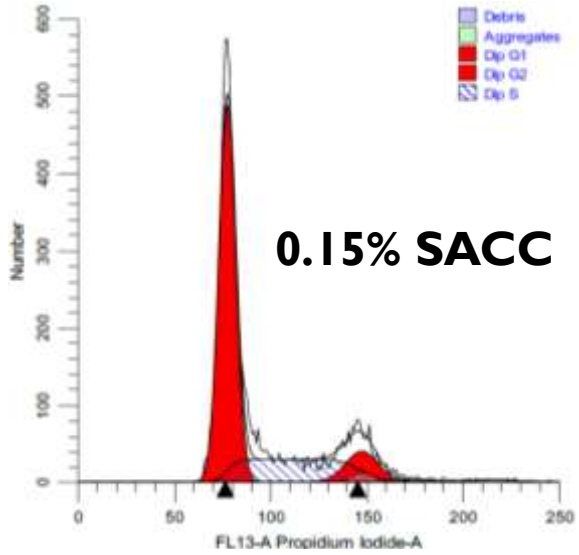


Figure 4: SACC inhibits growth of PanCa cells derived xenograft tumors in SCID mice. Briefly, MiaPaCa cells were subcutaneously injected in SCID mice. One week after mice were treated with indicated concentrations of SACC as Tumor growth was monitor weekly. A.-B. Bar graph representing relative tumor volume (RTV) at different time point. C. Representative pictures of tumor bearing mice treated and control group. **A** =Control, B =Positive Control (ADR) 2.5 mg/kg i.v. injection on day 1,5,9, **C** =SACC- 4 mL /Kg Oral Twice a week for 4 Weeks, and **D** =SACC 1.2 mL /Kg Oral daily 6 days/Week for 4 Weeks.

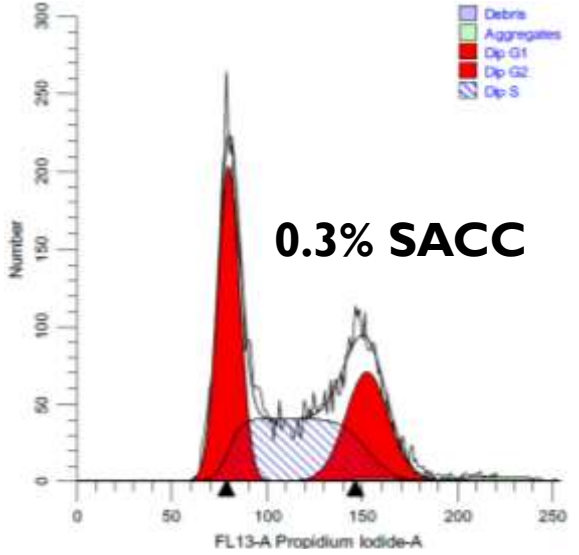
MiaPaCa cells treated with SACC for 24 hours for cell cycle.



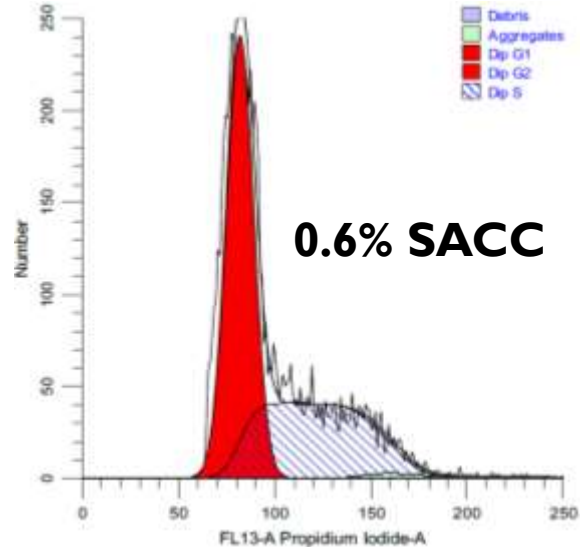
Diploid: 100.00 %
Dip G1: 60.22 % at 78.25
Dip G2: 6.62 % at 151.02
Dip S: 33.16 % G2/G1: 1.93



Diploid: 100.00 %
Dip G1: 65.16 % at 77.01
Dip G2: 10.06 % at 147.09
Dip S: 24.78 % G2/G1: 1.91



Diploid: 100.00 %
Dip G1: 37.81 % at 79.28
Dip G2: 25.03 % at 152.22
Dip S: 37.16 % G2/G1: 1.92



Diploid: 100.00 %
Dip G1: 57.59 % at 81.50
Dip G2: 0.00 % at 158.92
Dip S: 42.41 % G2/G1: 1.95

	Control	0.15% SACC	0.3% SACC	0.6% SACC
G0-G1	59.95 ± 0.52	63.00 ± 1.23	37.33 ± 0.47	57.29 ± 0.29
S	35.18 ± 1.22	26.12 ± 0.72	37.59 ± 0.54	42.71 ± 0.29
G2M	4.88 ± 0.89	10.88 ± 0.52	25.07 ± 0.09	0.00 ± 0.00

Summary of Preliminary Results:

- SACC inhibits the growth of PanCa cells.
- SACC induces apoptosis in PanCa cells.
- SACC induces cleavage in PARP protein in PanCa cells.
- SACC stabilizes p53 protein level in PanCa cells.
- SACC inhibits growth of pancreatic cancer stem cells (PanCSCs).
- SACC inhibits marker of PanCSCs (OCT4).
- SACC significantly inhibits the nuclear expression of ki-67 protein and increases E-cadherin expression.

- E-cadherin is involved in inhibition of epithelial to mesenchymal transition of cancer cells

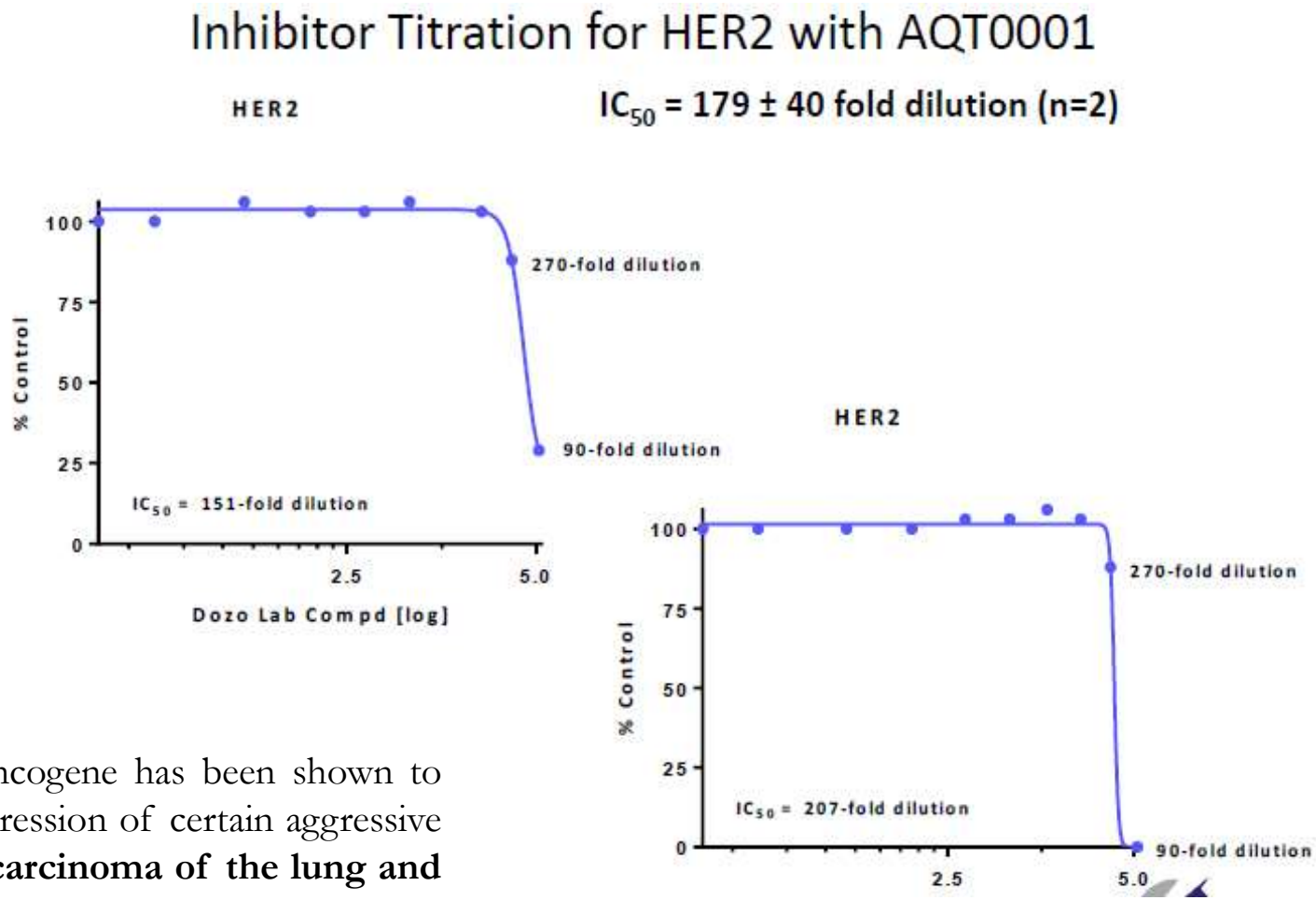
- SACC induces apoptosis and inhibits the growth of pancreatic cancer cells irrespective of mutant p53.
- SACC inhibits the expression of HER-2 but increases its phosphorylation at higher dose.
- SACC induces the expression of wild type p53 protein but degrades the level of mutant p53protein.
- SACC induces the expression of pro-apoptotic protein Bax and inhibits ant-apoptotic proteinBcl2. SACC induces the PARP protein cleavage

Therapeutic Efficacy Against Lung Adenocarcinoma

- Adenocarcinoma of the lung is **the most common type of lung cancer, and like other forms of lung cancer,** it is characterized by distinct cellular and molecular features.
- It is classified as one of several non-small cell lung cancers (NSCLC), to distinguish it from small cell lung cancer which has a different behavior and prognosis.
- The Global Lung Cancer Therapeutics Market was worth **$6.43 billion** in 2018 and estimated to be growing at a CAGR of 6.7%, to reach **$8.89 billion** by 2023.
- Lung cancer is the second most commonly detected and has the highest death rate of all cancers in both men and women.

A549 exhibits HER-2 gene amplification

HER2 Target Study



HER2 Amplification or over-expression of this oncogene has been shown to play an important role in the development and progression of certain aggressive types of **breast cancer, ovarian, stomach, adenocarcinoma of the lung and aggressive forms of uterine cancer, such as uterine serous endometrial carcinoma, gastric cancer**.

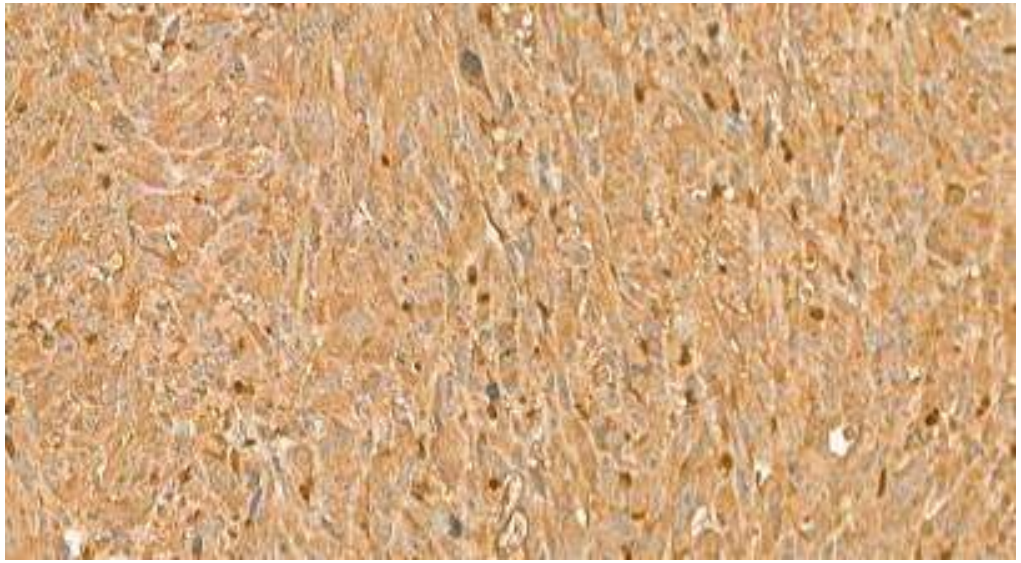
E-Cadherin repression increases amount of cancer stem cells in human A549 lung adenocarcinoma and stimulates tumor growth

[E-Cadherin repression increases amount of cancer stem cells in human A549 lung adenocarcinoma and stimulates tumor growth - PubMed (nih.gov)]

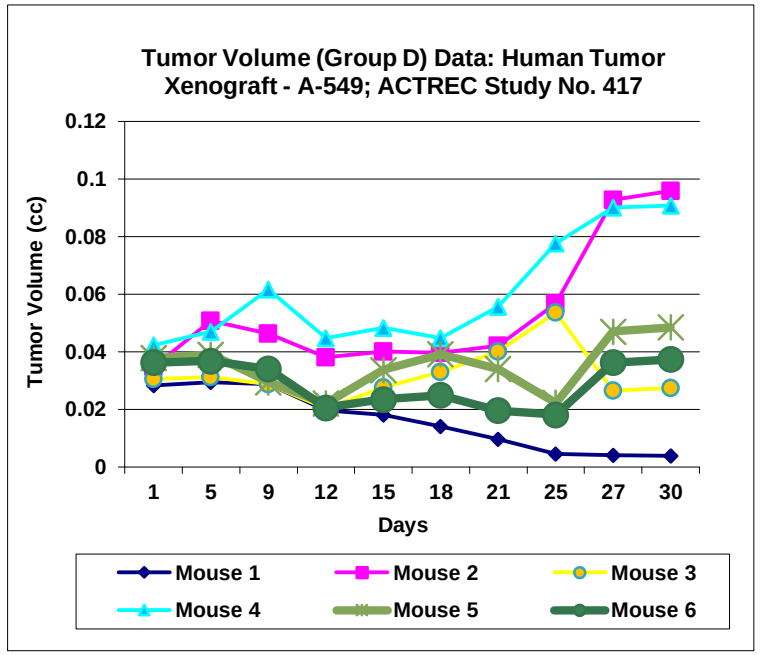
SACC increases E-cadherin expression

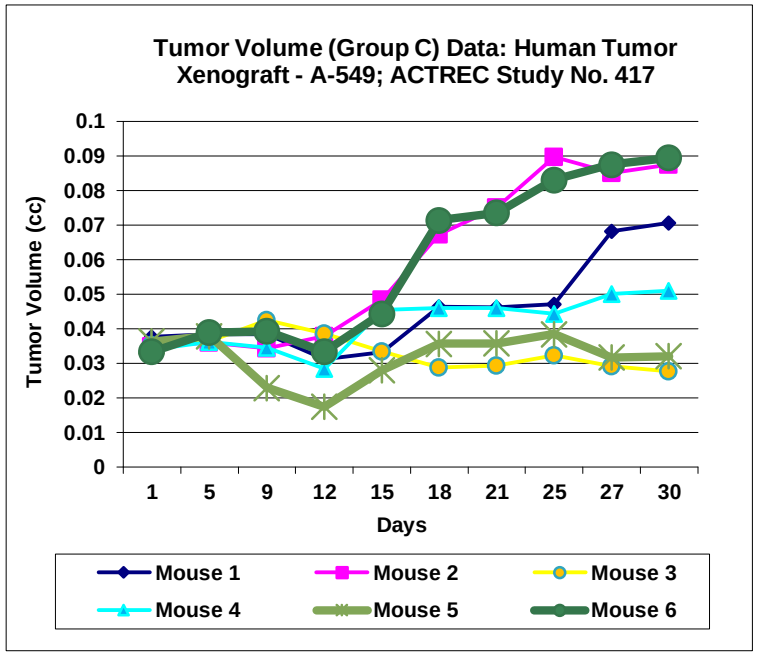
Control

SACC



Therapeutic efficacy of SACC against A549 Lung Adenocarcinoma



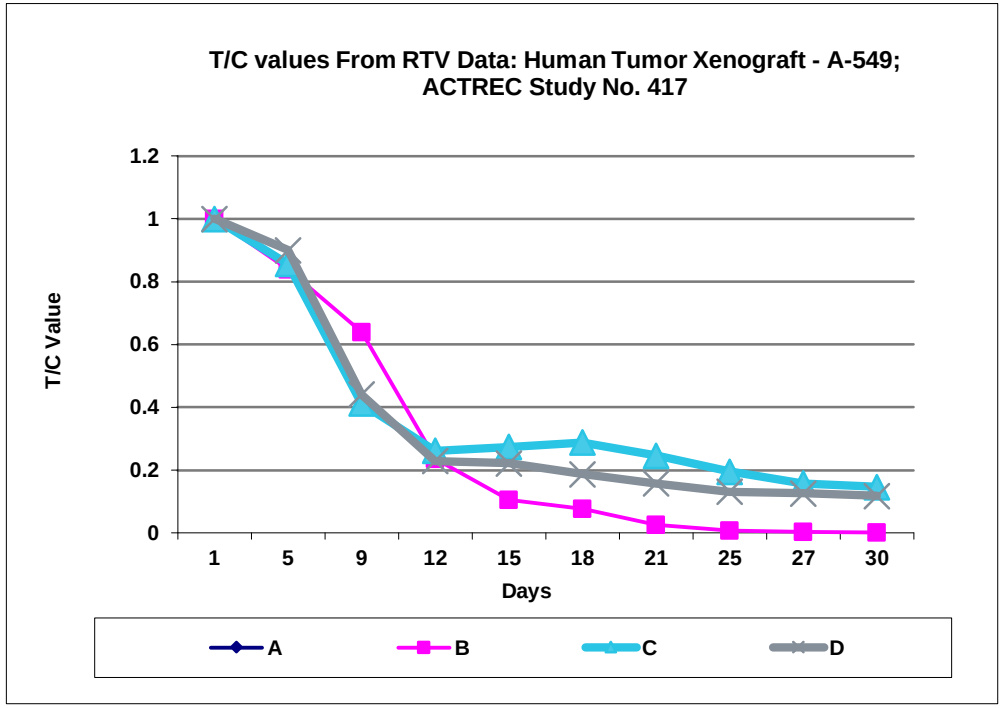


Tumor Volume Group D

	Week	Days	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6
	0.00	1	0.03	0.03	0.03	0.04	0.04	0.04
	0.71	5	0.03	0.05	0.03	0.05	0.04	0.04
	1.29	9	0.03	0.05	0.03	0.06	0.03	0.03
	1.71	12	0.02	0.04	0.02	0.04	0.02	0.02
	2.14	15	0.02	0.04	0.03	0.05	0.03	0.02
	2.57	18	0.01	0.04	0.03	0.04	0.04	0.02
	3.00	21	0.01	0.04	0.04	0.06	0.03	0.02
	3.43	25	0.00	0.06	0.05	0.08	0.02	0.02
	3.86	27	0.00	0.09	0.03	0.09	0.05	0.04
	4.29	30	0.00	0.10	0.03	0.09	0.05	0.04

Tumor Volume Group C

	Week	Days	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6
	0.00	1	0.04	0.03	0.04	0.03	0.04	0.03
	0.71	5	0.04	0.04	0.04	0.04	0.04	0.04
	1.29	9	0.04	0.03	0.04	0.03	0.02	0.04
	1.71	12	0.03	0.04	0.04	0.03	0.02	0.03
	2.14	15	0.03	0.05	0.03	0.05	0.03	0.04
	2.57	18	0.05	0.07	0.03	0.05	0.04	0.07
	3.00	21	0.05	0.08	0.03	0.05	0.04	0.07
	3.43	25	0.05	0.09	0.03	0.04	0.04	0.08
	3.86	27	0.07	0.09	0.03	0.05	0.03	0.09
	4.29	30	0.07	0.09	0.03	0.05	0.03	0.09



T/C (from RTV data)

Weeks	Days	A	B	C	D
0.00	1		1.00	1.00	1.00
0.71	5		0.84	0.86	0.90
1.29	9		0.64	0.41	0.44
1.71	12		0.24	0.26	0.23
2.14	15		0.11	0.27	0.22
2.57	18		0.08	0.29	0.19
3.00	21		0.03	0.25	0.16
3.43	25		0.01	0.20	0.13
3.86	27		0.00	0.16	0.13
4.29	30		0.00	0.15	0.12

C	SACC- 4 mL /Kg Oral Twice a week for 4 Weeks
D	SACC 1.2 mL /Kg Oral daily 6 days/Week for 4 Weeks

NOTES AND DEFINITIONS

- **Activity Criteria:** T/C ≤ 0.2 (highlighted yellow) is considered to demonstrate significant activity. T/C ≤ 0.42 (highlighted blue) is considered to demonstrate moderate activity.
- **Toxicity Criteria:** Mortality and weight loss ≥4 grams/ mouse are considered to indicate
- i.v. = intravenous;
- RTV = Relative Tumor Volume = Tumor Volume on day of measurement/ Tumor Volume on day 1

Toxicity Data

As recommended by Acute Oral Toxicity – Up-and-Down-Procedure (UDP), the maximum dose 2000 mg/Kg body weight for testing when there is no estimate of the substance's lethality. The range of selected doses included 175, 500, 2000 mg/Kg of body weight.

Mortality and LD50

No mortality was observed in any of the animals and at all the tested doses.

Histopathological findings

Observations were made on SAT 3-SAT 5 animals (SACC) ministered the assumed/ default LD50 for gross pathological changes. No evident changes for any severe toxicity was observed.

Body Weight

No significant change was observed in individual BW of animals administered the highest allowed dose (2000 mg/kg) for the test of formulation of SACC.

Treatment Group	LD 50 (mg/Kg BW)	Body Weight (Day 0) in grams	Body weight (day 14) in grams	Calculated t value at determined LD50	Remarks(S or NS)
SACC	2000	113.333±5.774	126.667±5.774	-2.828	S

Annexure : Effect of treatment on the body weight of rats determined after 14 days of administering the highest dose i.e. 2000 mg/Kg

[S- Significant, NS- Not Significant
*Power of performed test with alpha= 0.050:0.085]

Contact Us:

IIPRD Consulting
Email: licensing@iiprd.com
Tel: +91-(120) 4296878, 4909201, 4516201
Address: E-13, UPSIDC, Site-IV, Behind-Grand Venice, Kasna Road,
Greater Noida - 201310, UP, National Capital Region, India.