Minnelide: A Novel Therapy for Pancreatic Cancer

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Department of Surgery
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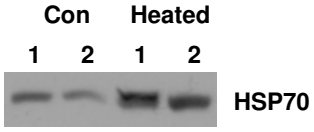
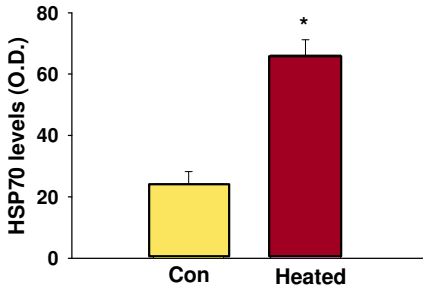
- Diseases of the *exocrine* pancreas cause considerable morbidity. In the US alone, *pancreatitis* causes **300,000** hospitalizations and **7,000** deaths, while *pancreatic cancer* claims another **38,000** deaths annually.
- Treatment is limited for both, likely reflecting an inadequate understanding of their etiological and pathophysiological mechanisms. The national economy suffers by **$3.5 billion** annually.

Heat Shock Proteins

- Discovered serendipitously by Ritossa in 1962.
- One of the mechanisms that have evolved to ensure the survival of living cells under stressful conditions.
- One of the most highly conserved mechanisms of cellular protection.

Thermal Stress Induces HSP70 Expression in the Pancreas





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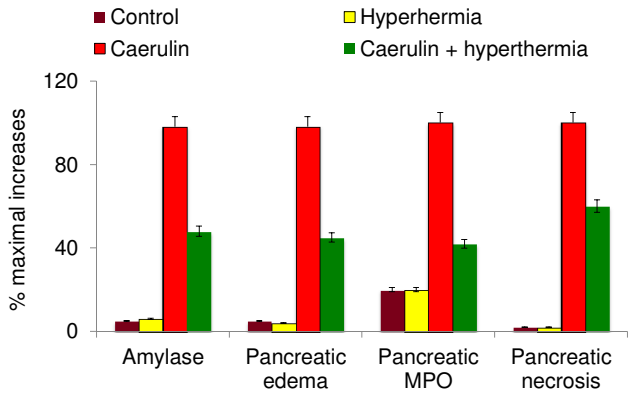
Effect of Prior Thermal Stress on Parameters Associated with Caerulein-Induced Pancreatitis



Frossard J-L…Saluja AK, *Gut* (2002)

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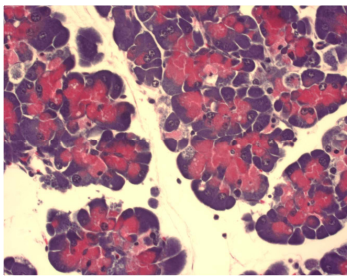
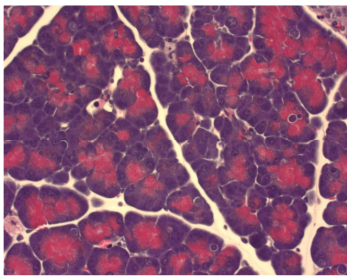
Transgenic Mice Over-expressing HSP70 are Protected Against Pancreatitis





Wild Type **HSP70-Transgenic**

HSP70 overexpressing mice were generated using a construct containing human HSP70 under control of the CMV promoter

Dawra R and Saluja AK, unpublished

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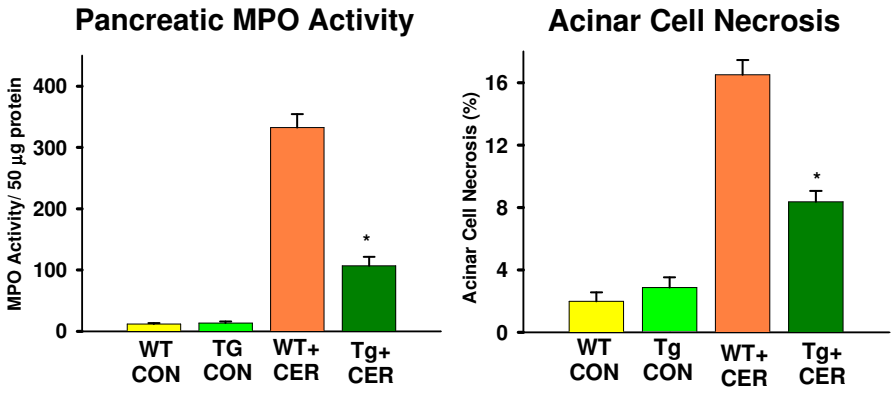
Effect of Caerulein Administration on the Parameters Associated with Pancreatitis in HSP70 Transgenic Mice



Dawra R and Saluja AK, unpublished

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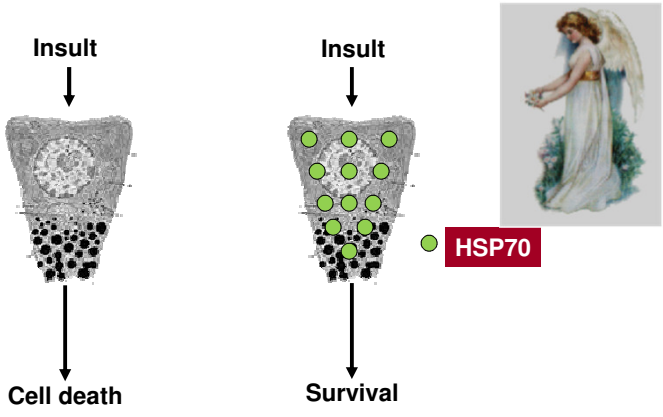
HSP70 and Pancreatitis



Bhagat et al., *Gastroenterology* (2002)
Frossard et al., *Gut* (2002)
Bhagat et al., *J. Clin. Invest.* (2000)

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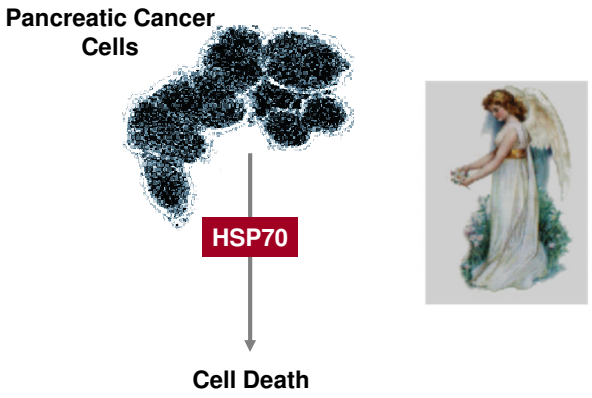
Pancreatic Cancer

- Pancreatic cancer is the most lethal cancer known to humankind.
- **45,000** Americans will be diagnosed with pancreatic cancer this year, and most everyone will succumb to the disease.
- Pancreatic cancer causes more than **250,000** deaths worldwide per year.

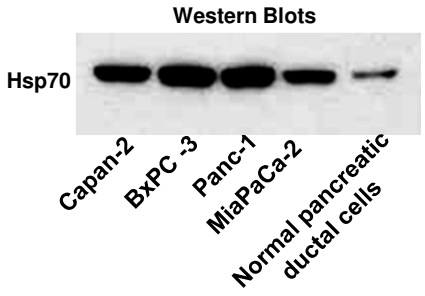
Current Treatments for Pancreatic Cancer

- Current therapies for pancreatic cancer are not very effective.
- **Gemcitabine**, approved more than 14 years ago, provides a 1.5 month survival advantage.
- **Tarceva** adds 10 more days.

HSP70 in Pancreatic Cancer

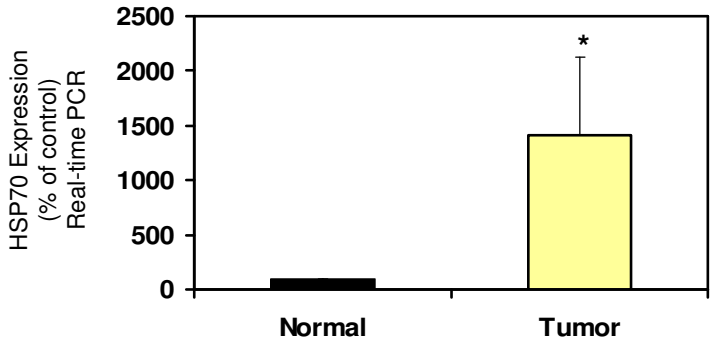


HSP70 is Overexpressed in Pancreatic Cancer Cells



Aghdassi A…Saluja AK, *Cancer Research* (2007)

HSP70 Expression in Pancreatic Cancer Patients



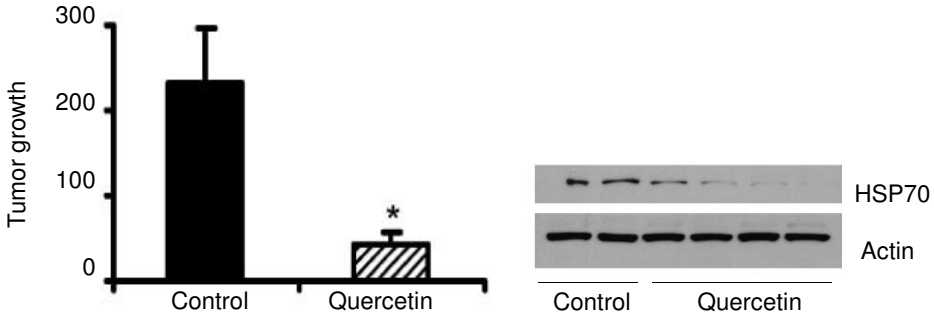
*p<0.002; n=7 patients

Aghdassi A…Saluja AK, *Cancer Research* (2007)

HSPs and Pancreatic Cancer Hypothesis

Blocking HSPs should increase apoptosis in cancer cells thereby slowing the growth and spreading of tumor cells.

Quercetin inhibited HSP70 and caused tumor regression



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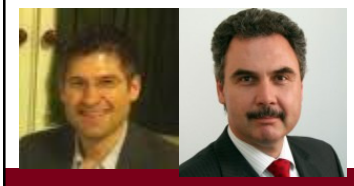
Heat Shock Protein 70 Increases Tumorigenicity and Inhibits Apoptosis in Pancreatic Adenocarcinoma

Ali Aghdassi, Phoebe Phillips, Vikas Dudeja, Dhara Dhaulakhandi, Rifat Sharif, Rajinder Dawra, Markus M. Lerch and Ashok Saluja

Cancer Research, Feb 2007



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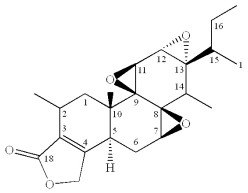
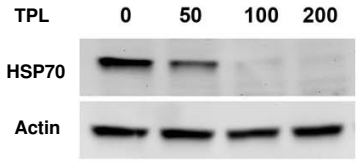
Triptolide – A Diterpenoid Triepoxide

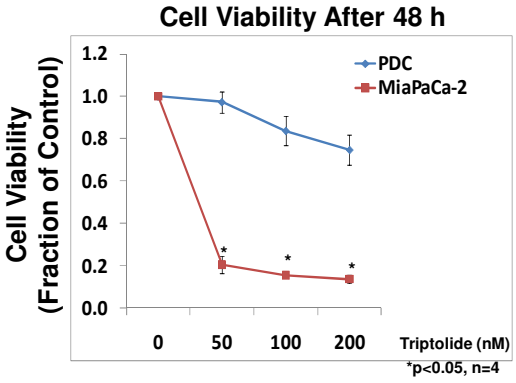


Has been used to treat inflammatory disorders such as rheumatoid arthritis.

tripterygium wilfordii Hook. f.
(‘ ‘Thunder God Vine’ ’)

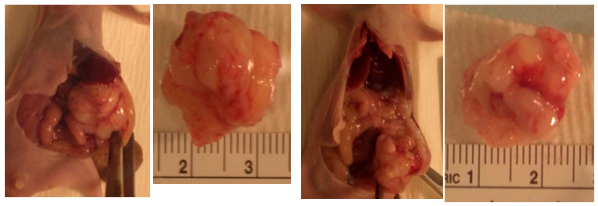
Triptolide Kills Pancreatic Cancer Cells *In Vitro*



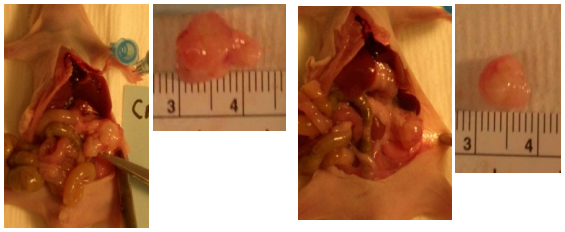


Phillips PA…Saluja AK, *Cancer Research* (2007)

Triptolide Reduces Pancreatic Tumor Growth *In Vivo*



Controls



Triptolide Treatment

Phillips PA…Saluja AK, *Cancer Research* (2007)

Research Article

Triptolide Induces Pancreatic Cancer Cell Death via Inhibition of Heat Shock Protein 70

Phoebe A. Phillips,[1] Vikas Dudeja,[1] Joshua A. McCarroll,[1] Daniel Borja-Cacho,[1] Rajinder K. Dawra,[1] William E. Grizzle,[2] Selwyn M. Vickers,[1] and Ashok K. Saluja[1]

[1]Department of Surgery, University of Minnesota, Minneapolis, Minnesota and [2]Department of Pathology, University of Alabama at Birmingham, Birmingham, Alabama

Cancer Res 2007; 67: (19). October 1, 2007

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Disadvantage of Triptolide

- Soluble only in organic solvent
- Limits clinical utility
- No IP (Patent protection)

Minnelide™: A Novel Prodrug of Triptolide

- In collaboration with Dr. Gunda Georg and Satish Patil, we have synthesized a highly water-soluble analog of triptolde.
- **Minnelide** is as effective as triptolide in killing tumor cells in both *in vitro* and *in vivo* models of pancreatic cancer.
- **Minnelide** is safe in mice at relatively high doses.
- A patent to protect intellectual property has been granted to UMN.

Disclosure

Dr. Saluja has a significant financial interest in and is the Chief Scientific Officer and a consultant for Minneamrita, a company which may commercially benefit from the results of this research. This relationship has been reviewed and managed by the University of Minnesota in accordance with its conflict of interest policies.

Effect of **Minnelide™** on Pancreatic Cancer Mortality in Mice

- MiaPaCa-2 cells were orthotopically implanted into pancreas.
- 10d post-implantation, **Minnelide™** was administered at doses indicated for 60d.
- Treatment was discontinued and mice sacrificed 30d after stopping treatment.

	Control	Treatment			
		0.1mg/kg	*0.15mg/kg/bid*	*0.3mg/kg*	*0.6mg/kg*
No of mice at beginning	10	10	10	9	10
No of mice alive at day 95	1	9	10	9	10

Chugh, Sangwan… Saluja (**Sc Tran Med Oct. 2012**)

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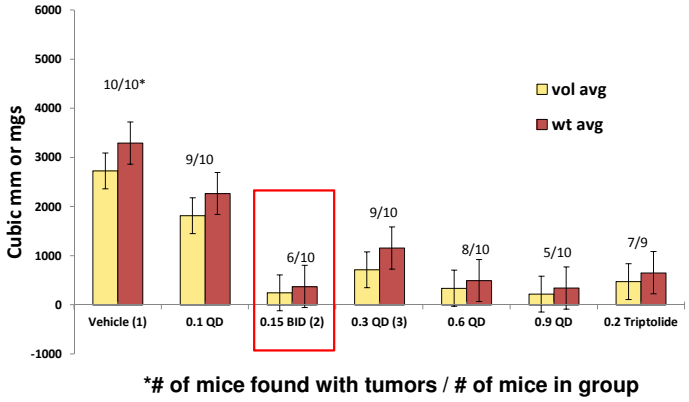
MiaPaca-2 cell-induced orthotopic tumors in nude mice - Average Tumor Size On Day of Death



*# of mice found with tumors / # of mice in group

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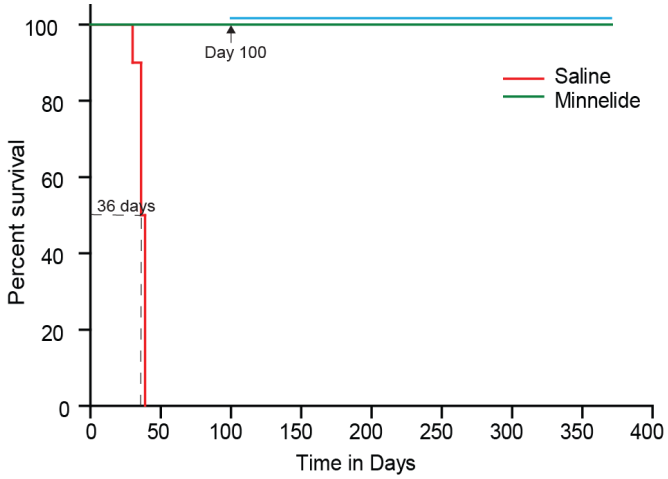
Minnelide Increases Survival in an Orthotopic Pancreatic Cancer Model



Aspc-1 (Ascites)

Day 1: Aspc-1 (2X 10^5) implanted into pancreas (n=20)
Day 7: 0.42 mg/kg Minnelide or saline IP begins (n=10)
Day 100: Minnelide treatment stopped (n=5)

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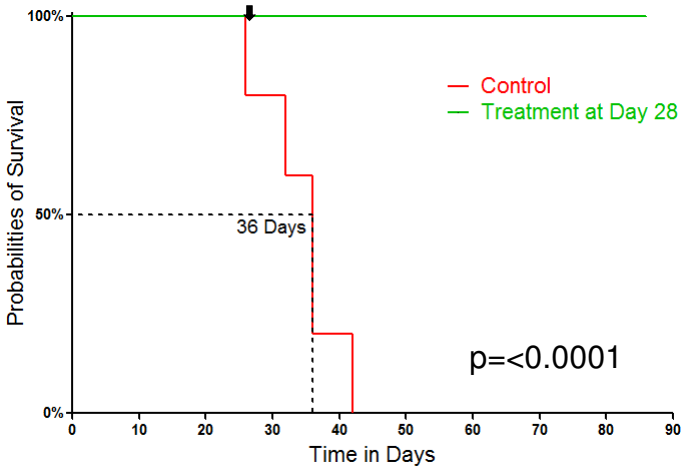
Minnelide Causes Tumor Regression in an Aspc-1 Induced Orthotopic Pancreatic Model



Aspc-1
(Ascites)

Day 1: Aspc-1 (2X 10^5) implanted
Day 28: Minnelide treatment
Day 85: Experiment terminated

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Minnelide™ causes tumor regression in S2-013 cell-induced orthotopic pancreatic tumors

S2-013 is one of the most aggressive pancreatic tumor-derived cell lines.

Saline

Minnelide™

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Minnelide™ decreases tumor metastatis in mice bearing S2-013-induced orthotopic tumors

ORGAN	CONTROL	MINNELIDE
Liver	5/8	0/10
Kidney	5/8	0/10
Abdominal Wall	8/8	2/10
Diaphragm	8/8	0/10
Spleen	8/8	1/10
Ascites	8/8	0/10

Minnelide™ significantly decreased the tumor metastasis.

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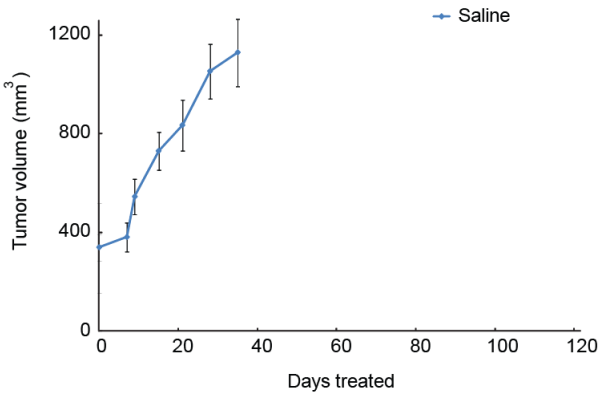
Minnelide decreases tumor burden in a human xenograft



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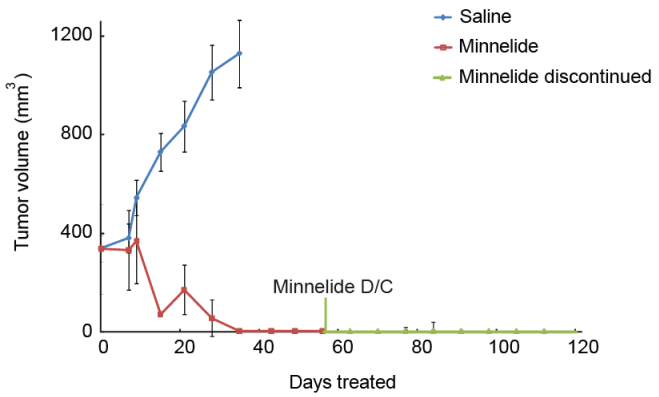
Minnelide decreases tumor burden in a human xenograft



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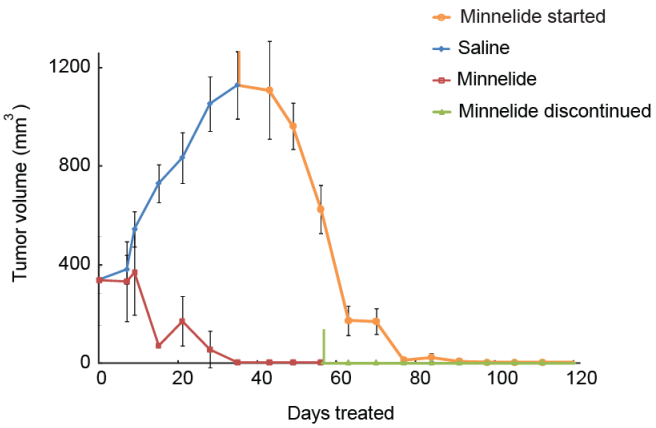
Minnelide decreases tumor burden in a human xenograft



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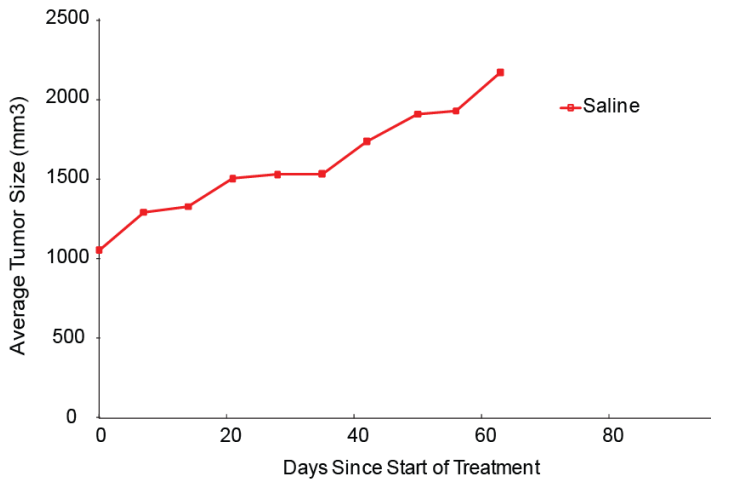
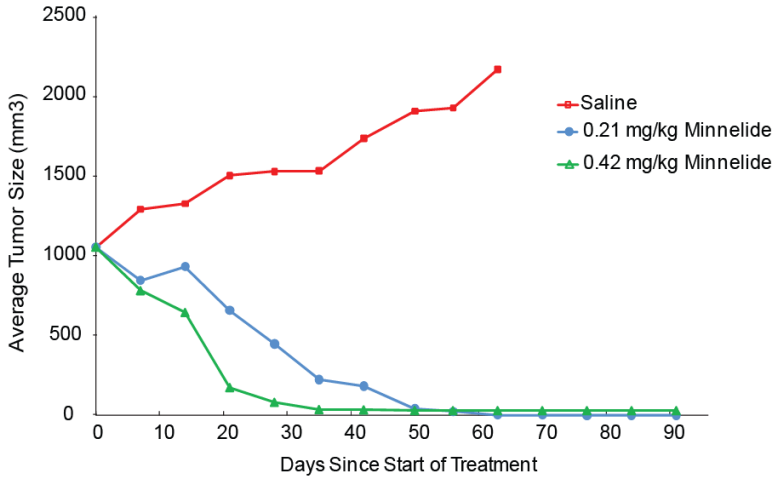
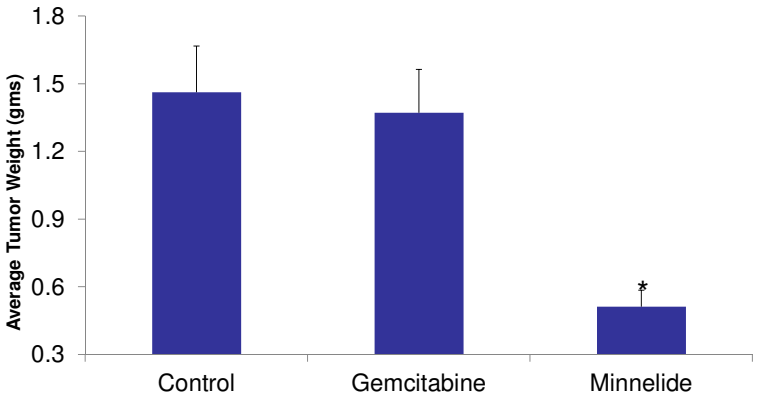
Minnelide causes tumor regression in a human xenograft



Minnelide causes tumor regression in a human xenograft



Orthotopic Pancreatic Cancer Model



Chugh R…Saluja AK, Sci Trans Med,2012

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Neuroblastoma

- One of the common pediatric tumors
- Advanced stage cases highly aggressive
  - Resistant to conventional chemotherapy
  - 5-year survival is 30-40%

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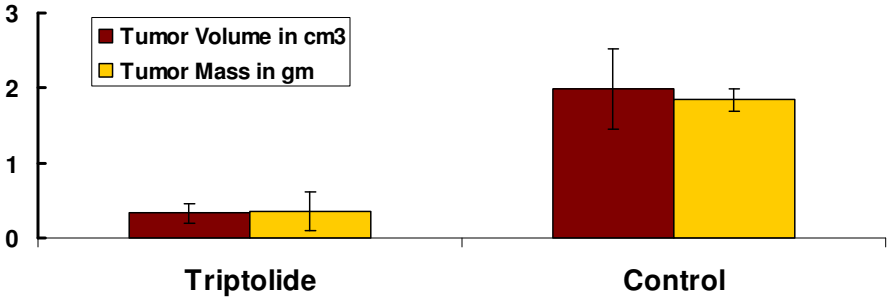
Results: Tumor Growth *In Vivo*



Antanoff M…Saluja AK, *J. Surgical Research* (2010)

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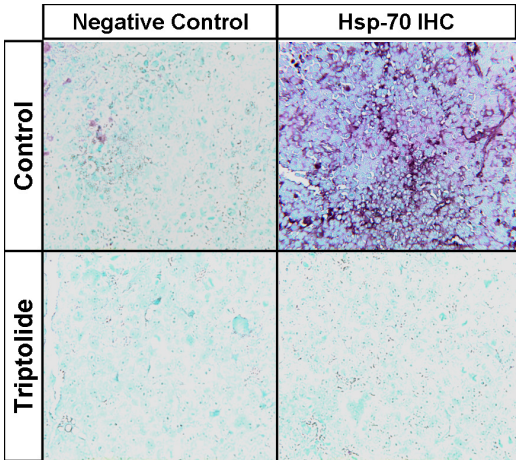
Hsp-70 in Residual Tumors



Antanoff M…Saluja AK, *J. Surgical Research* (2010)

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Surgery (2009)

Triptolide therapy for neuroblastoma decreases cell viability in vitro and inhibits tumor growth in vivo

Antonoff MB, Chugh R, Borja-Cacho D, Dudeja V, Clawson KA, Skube SJ, Sorenson BS, Saltzman DA, Vickers SM, Saluja AK

Journal of Surgical Research, in press

Role of HSP70 in Triptolide-Mediated Cell Death of Neuroblastoma

Antonoff MB, Chugh R, Borja-Cacho D, Dudeja V, Clawson KA, Skube SJ, Sorenson BS, Saltzman DA, Vickers SM, Saluja AK

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Minnelide is Also Effective in Colon, Ovarian, Osteosarcoma and Several Other Cancers

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Minnelide: A Prodrug of Triptolide is Soluble in Water

RESEARCH ARTICLE

PANCREATIC CANCER

A Preclinical Evaluation of Minnelide as a Therapeutic Agent Against Pancreatic Cancer

Rohit Chugh,[1]* Veena Sangwan,[1]* Satish P. Patil,[2] Vikas Dudeja,[1] Rajinder K. Dawra,[1] Sulagna Banerjee,[1] Robert J. Schumacher,[3] Bruce R. Blazar,[3] Gunda I. Georg,[4] Selwyn M. Vickers,[1] Ashok K. Saluja[1]†

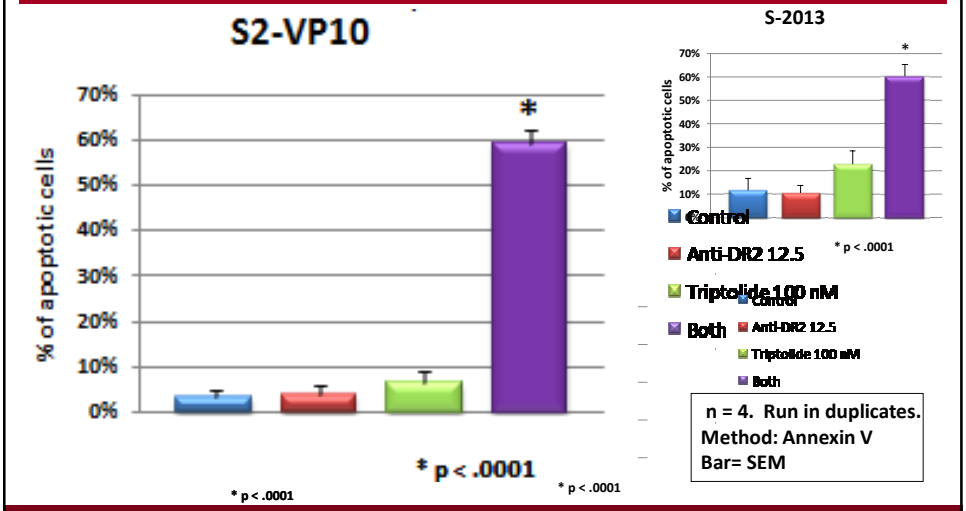
Pancreatic cancer is one of the most lethal human malignancies with an all-stage 5-year survival frequency of <5%, which highlights the urgent need for more effective therapeutic strategies. We have previously shown that triptolide, a diterpenoid, is effective against pancreatic cancer cells in vitro as well as in vivo. However, triptolide is poorly soluble in water, limiting its clinical use. We therefore synthesized a water-soluble analog of triptolide, named

Science Translational Medicine
AAAS

Minnelide in combination with death receptor therapy

- Pancreatic cancer is highly resistant to monotherapy.
- Death receptor therapy is not very effective against pancreatic cancer

Effect of triptolide and Anti-DR5 Ab on apoptosis of pancreatic cancer cells after 24 hrs of exposure



Borja-Cacho D…Saluja A and Vickers SM, J. Gastrointest. Surg. (2010)
Chen Z……..Saluja A (Cancer Lett. 2014)

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Pancreatic cancer stem cells and Minnelide

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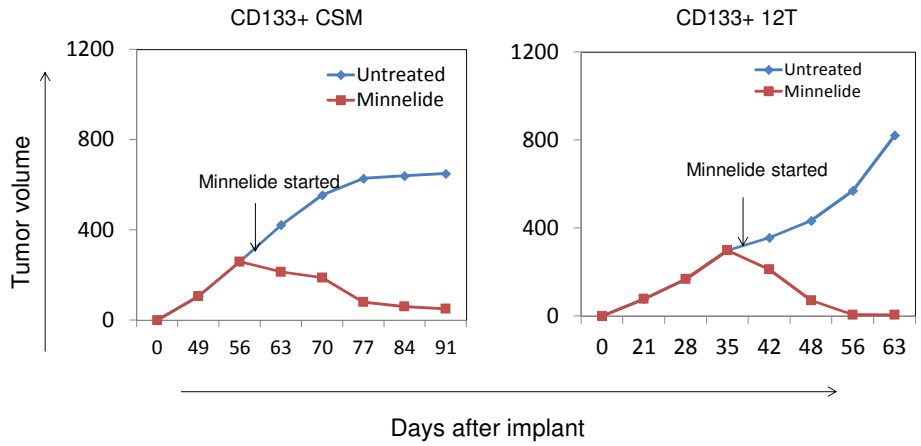
CD133+ tumors responded to therapeutic dose of Minnelide *in vivo*



Banerjee S……..Saluja A, unpublished
Banerjee S…… Saluja A ,Clin Can Res, 2014

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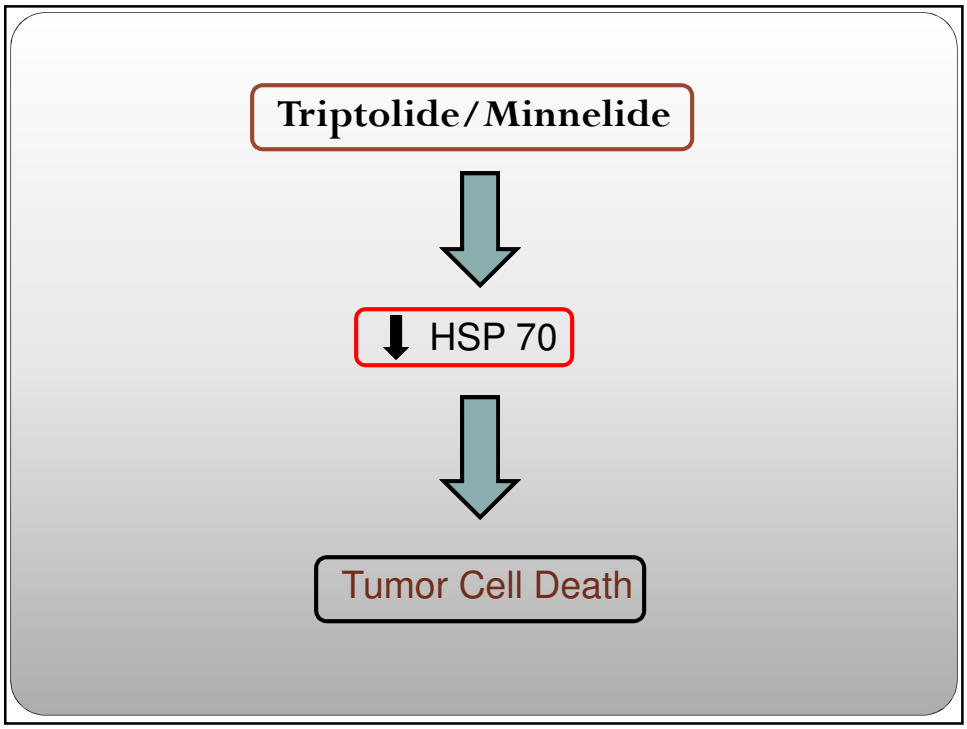

How does HSP70 Inhibition Results in Tumor Cell Death

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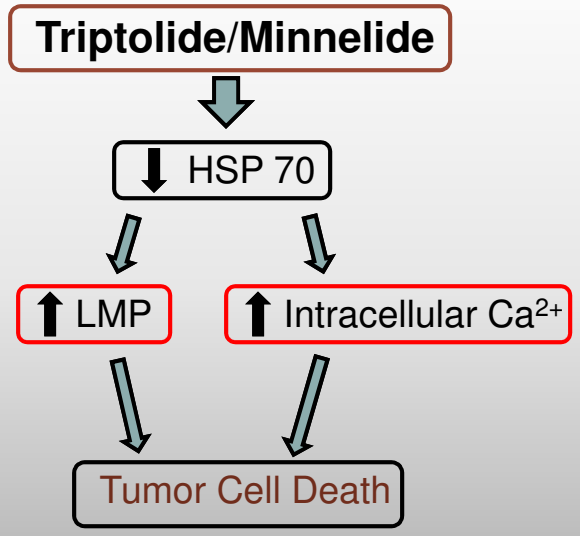
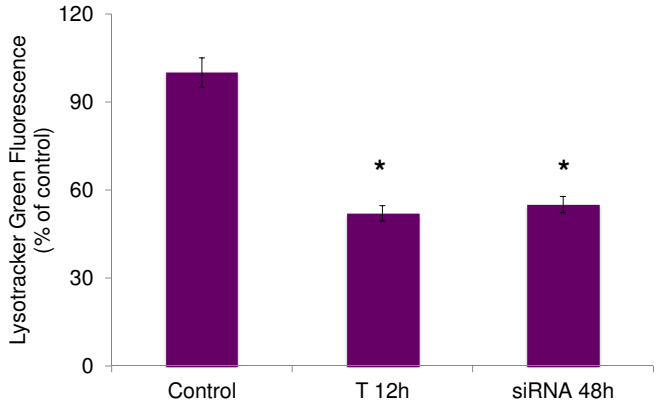


HSP70 Stabilizes Lysosomes in Pancreatic Cancer Cells



T = Triptolide 0.2 μM

** p ≤ 0.05, n=3*

Dudeja V…Saluja AK, Gastroenterology (2009)

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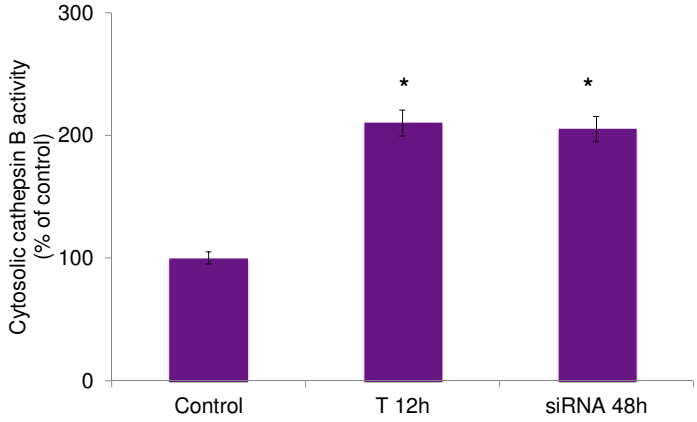
Inhibition of HSP70 results in lysosomal permeabilization



T = Triptolide 0.2 μM

** p ≤ 0.05, n=3*

Dudeja V…Saluja AK, Gastroenterology (2009)

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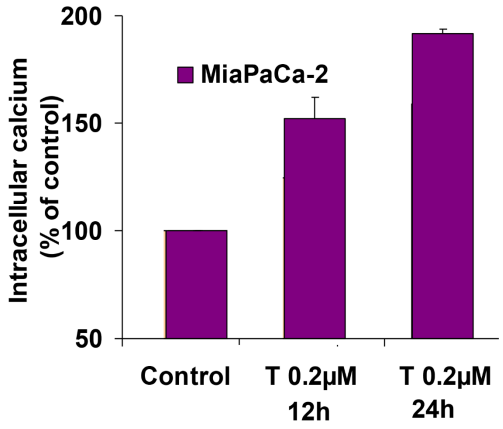
HSP70 Stabilizes Lysosomes in Pancreatic Cancer Cells

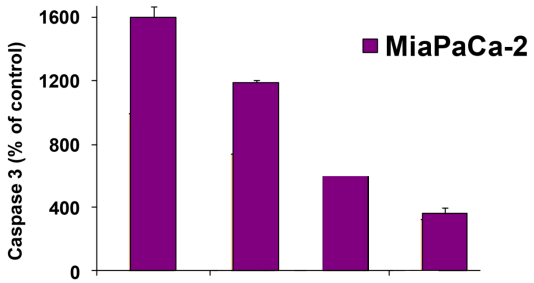
Inhibition of HSP70 Resulted in an Increase in Cytosolic Ca^{2+}



Dudeja V…Saluja AK, Gastroenterology (2009)

HSP70 Attenuates Intracellular Calcium

Effect of Cathepsin B Inhibition and Cytosolic Ca^{2+} Chelation on Cell Death After HSP70 Downregulation



Triptolide 0.2uM	+	+	+	+
BAPTA 10uM	-	+	-	+
CA074me 10uM	-	-	+	+

Dudeja V…Saluja AK, Gastroenterology (2009)

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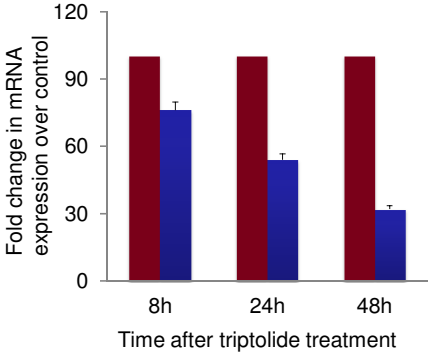
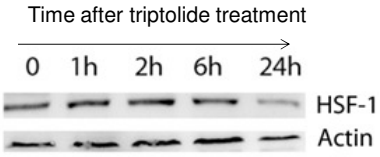
Mechanisms by Which Triptolide Downregulates Heat Shock Proteins

Triptolide Downregulated HSF1 Expression





- Untreated
- 100nM triptolide

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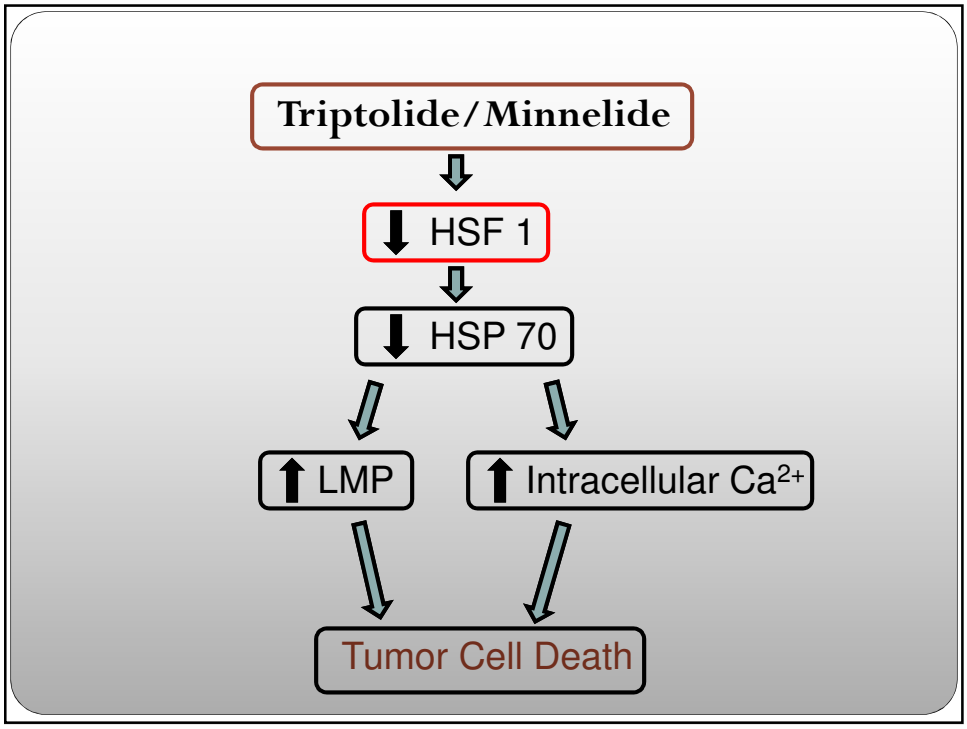

Heat Shock Protein 70 Inhibits Apoptosis in Cancer Cells through Simultaneous and Independent Mechanisms

Dudeja V, Mujumdar N, Phillips P, Chugh R, Borja-Cacho D, Dawra RK, Vickers SM, Saluja AK

Gastroenterology, 2009, May

Pro-survival Role of Heat Shock Factor 1 In the Pathogenesis of PancreatoBiliary tumors

Dudeja V, Chugh R, Sangwan V, Skube S, Mujumdar N, Borja-Cacho D, Antonoff M, Dawra R, Vickers SM, Saluja AK

Amer J Physiol, 2011

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Clinical Trials for Minnelide

A Phase 1, Multi-Center, Open-Label, Dose-Escalation, Safety, Pharmacokinetic, and Pharmacodynamic Study of Minnelide™ Given Daily for 21 Days Followed by 7 Days Off Schedule in Patients With Advanced GI Tumors.

As of this week fourteen patients have been enrolled

Trial Period: August 2013 - August 2015

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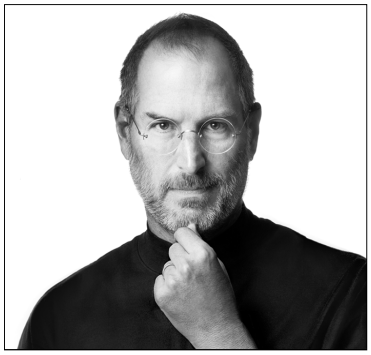
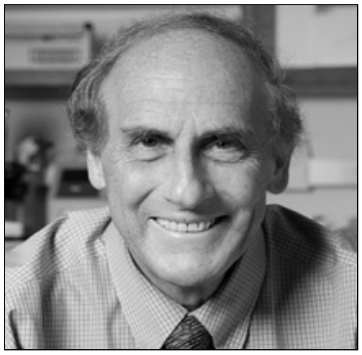


Steve Jobs
1955 - 2011



Ralph Steinman
1943 - 2011