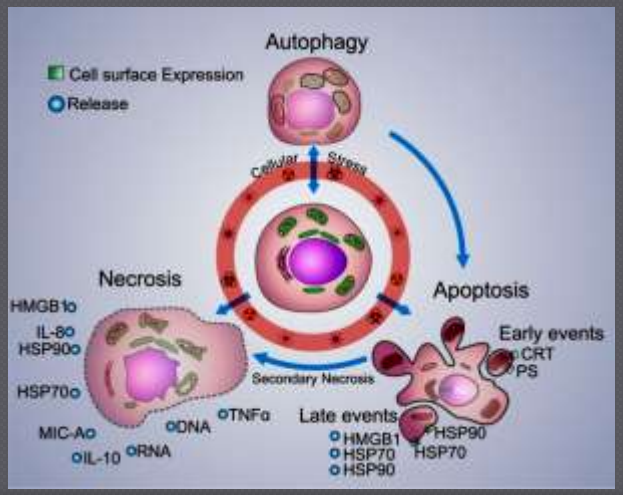
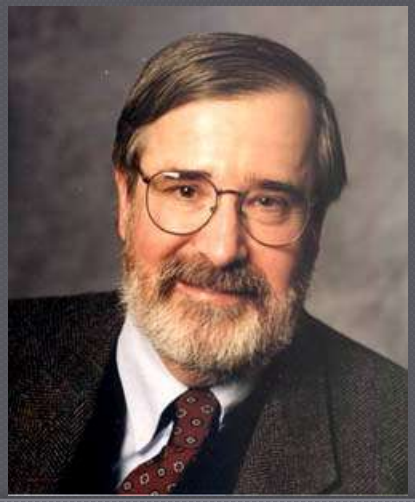


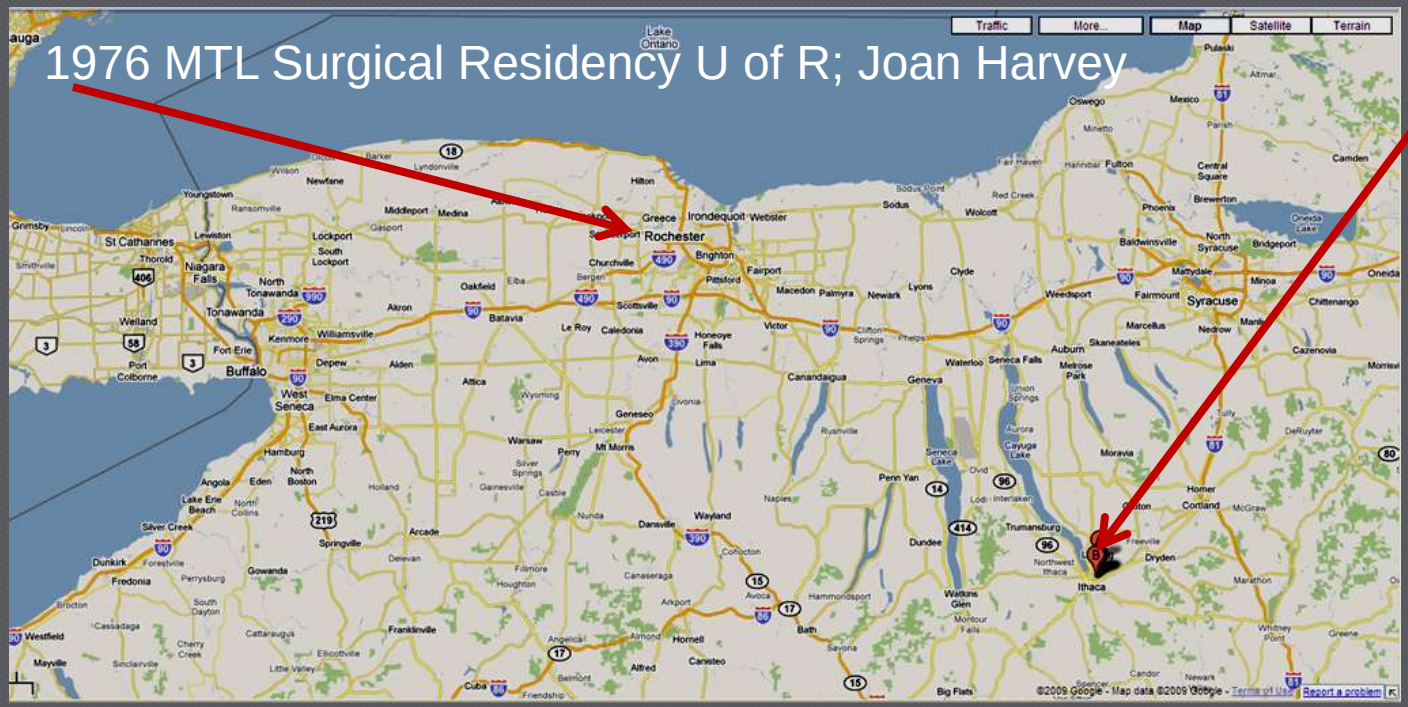


October 31, 2009

"When Models Go Awry: DAMPs and Autophagy in Pancreatic Cancer"

Michael T. Lotze, MD
Vice Chair Research
Department of Surgery
Assoc. Dir. Strategic Partnerships, University of Pittsburgh Cancer Institute
Asst. Vice Chancellor, UPSHS

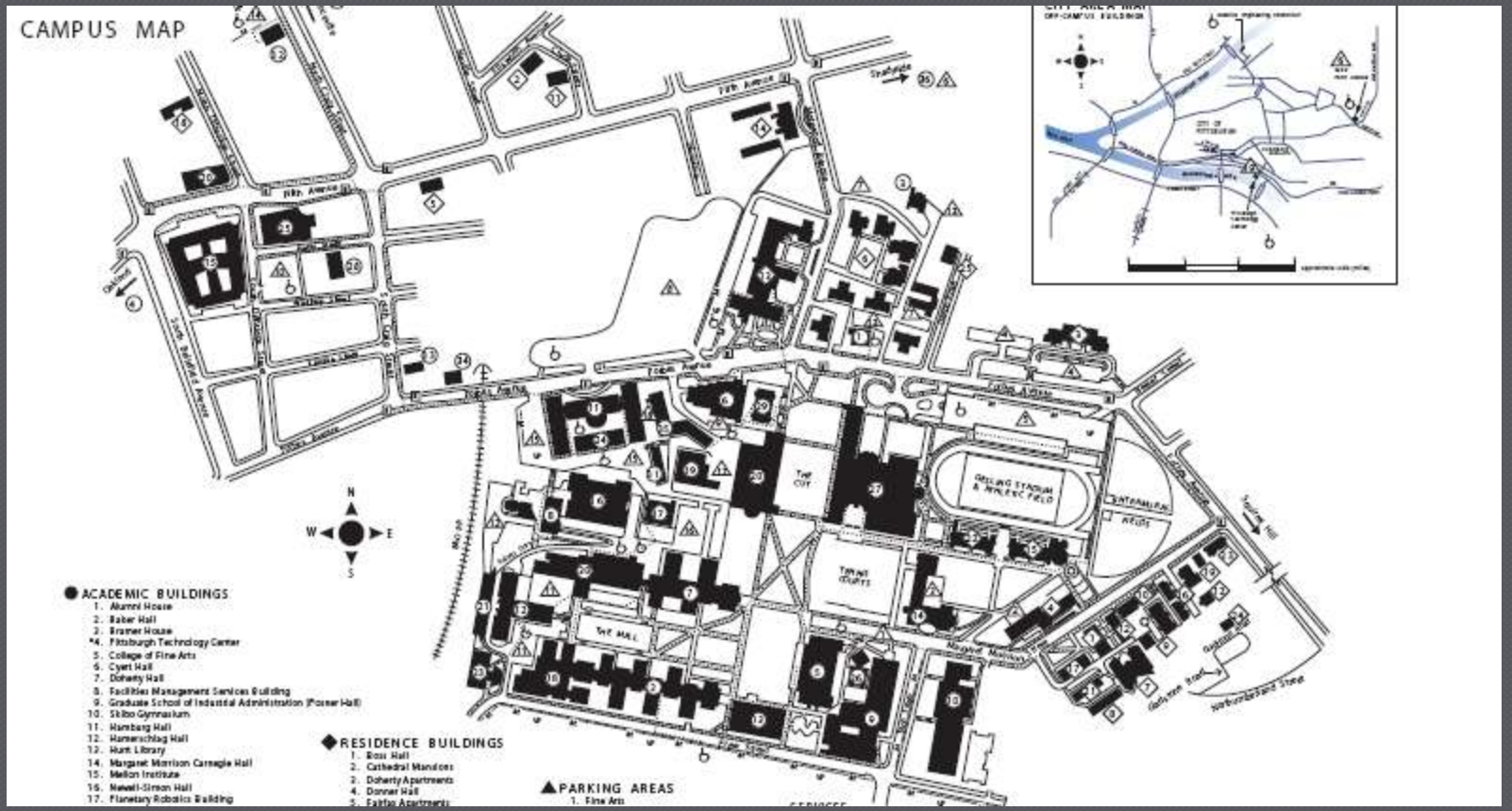
Close Encounters of the 1st Kind 1/3 of a Century Ago



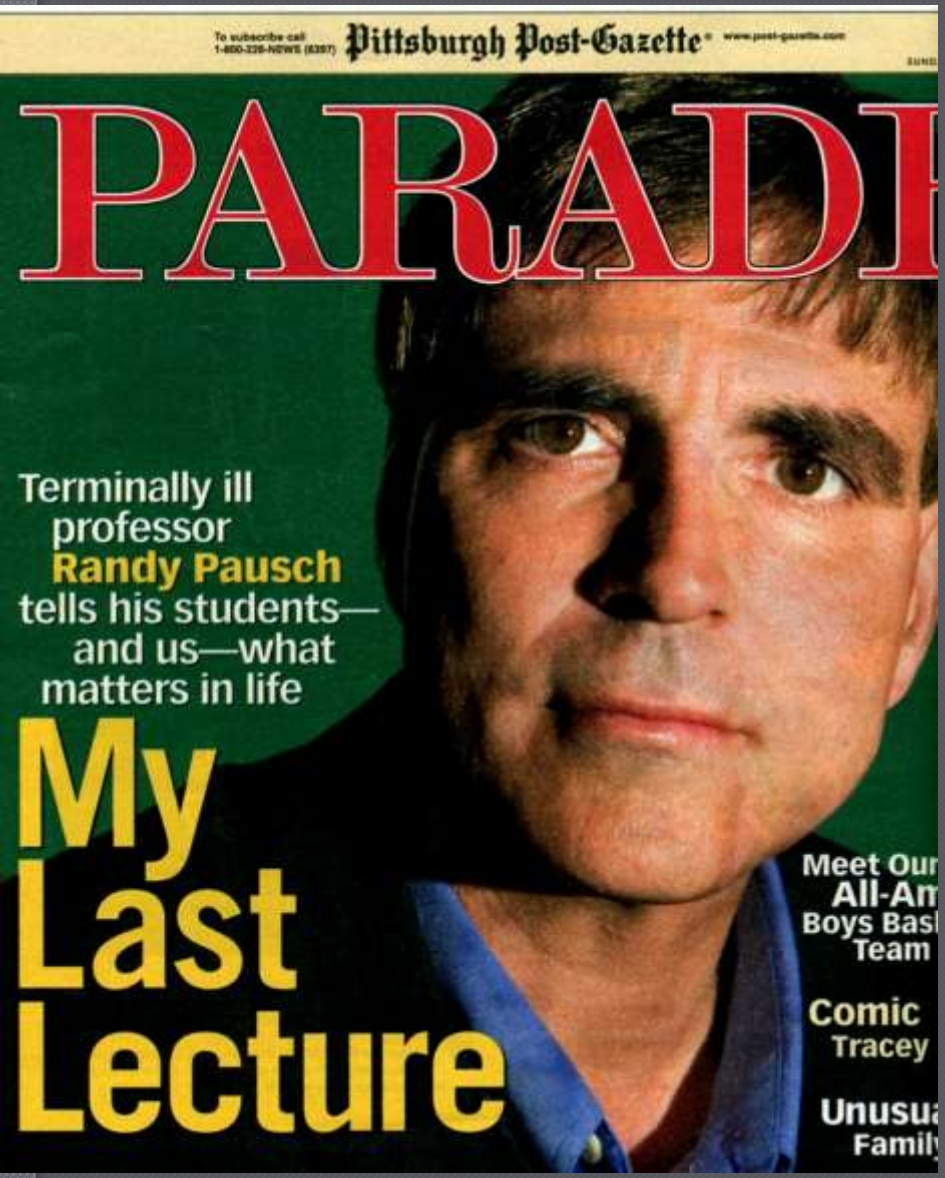
EMC Ph.D. in Computer Science From Cornell University Ithaca NY, in 1976

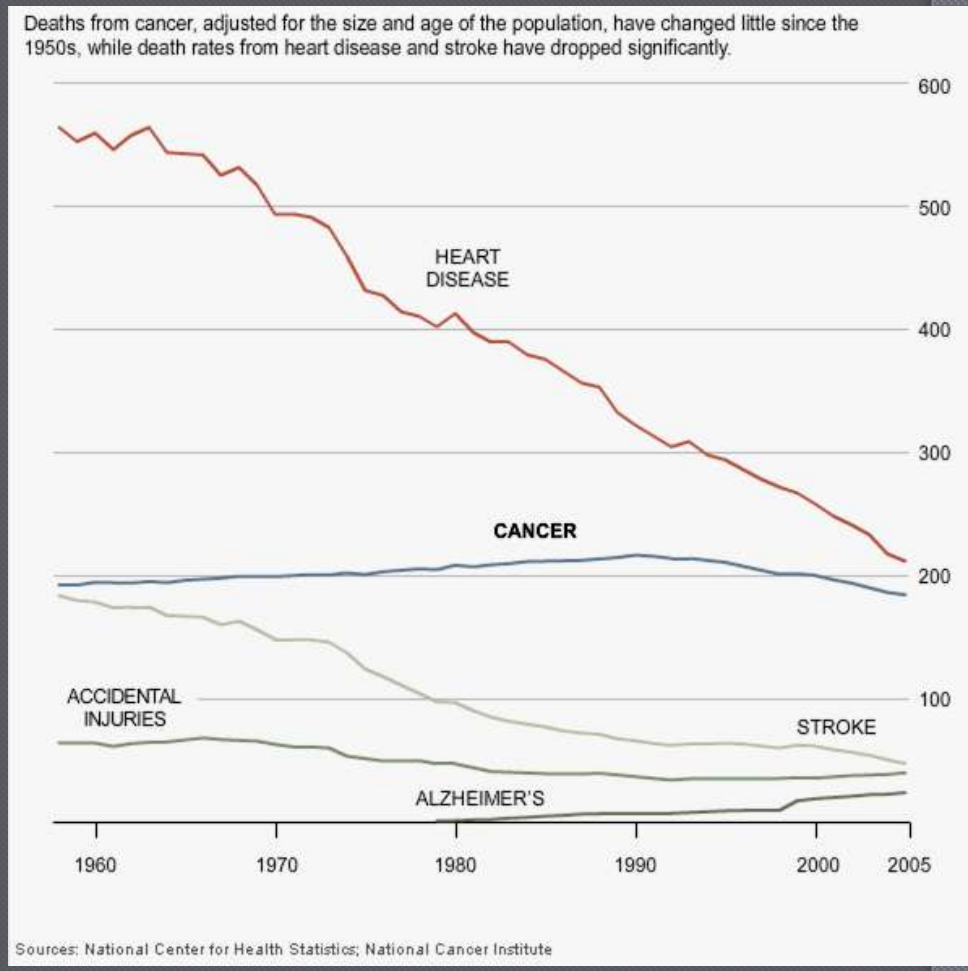
Model Checking as a verification technique for finite state concurrent systems. His research group pioneered the use of Model Checking for hardware verification. Symbolic Model Checking using BDDs was also developed by his group.
In addition, his resarch group developed the first parallel resolution theorem prover (**Parthenon)** and the first theorem prover to be based on a symbolic computation system (**Analytica)**.

Now – A Pausch Bridge

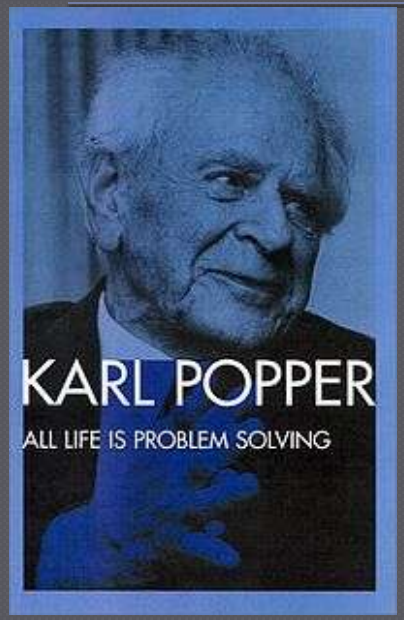


Deaths from Cancer





Cancer is a "Wicked Problem"



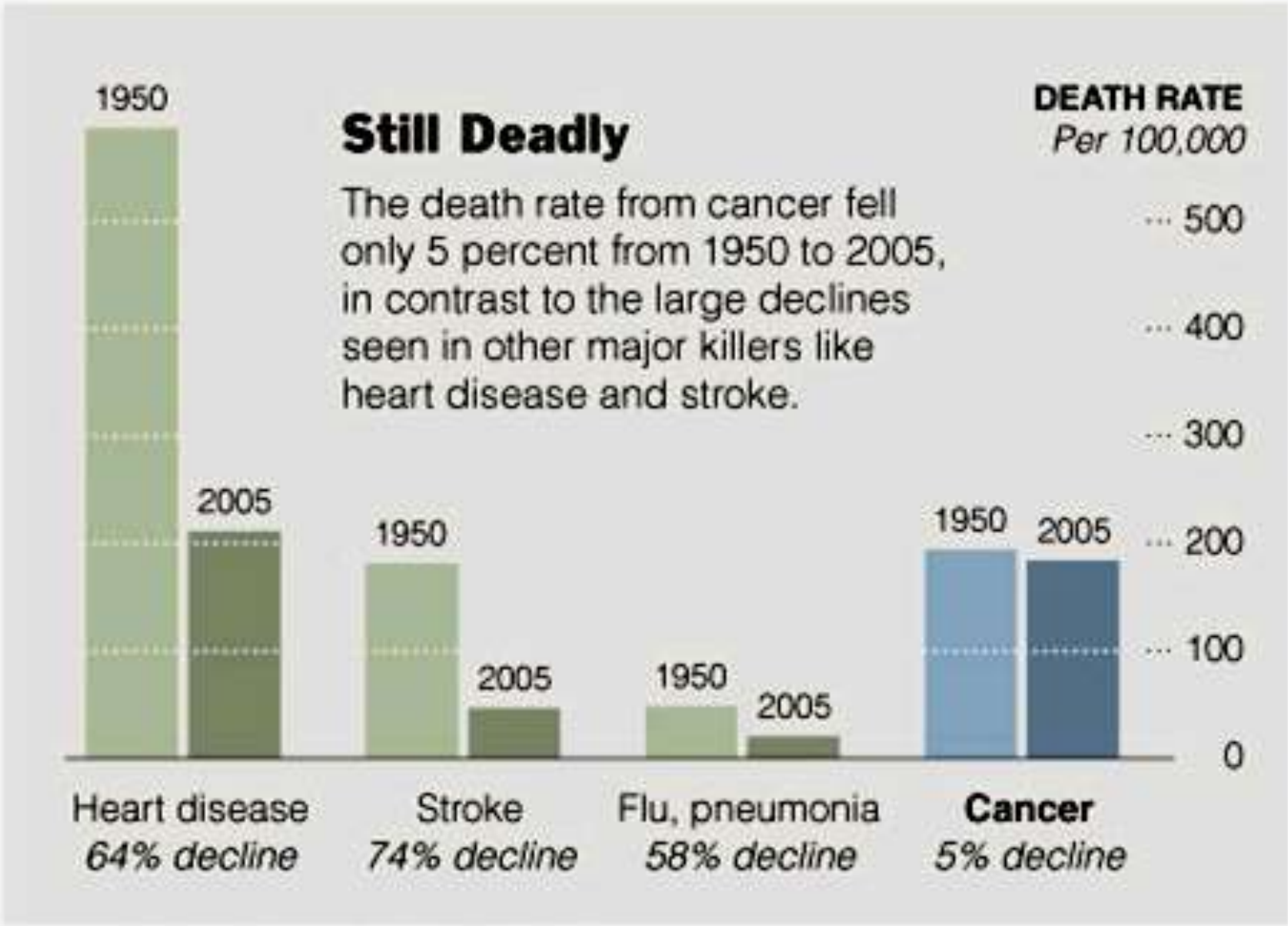
- Popper, Karl. The Null Theory. *The Two Fundamental Problems of the Theory of Knowledge*, 1930–33 (*Die beiden Grundprobleme der Erkenntnistheorie; The Logic of Scientific Discovery*, 1934 (as *Logik der Forschung*) 1999. *All Life is Problem Solving.*
- Horst Rittel and Melvin Webber [1973] contrasted "wicked" problems with relatively "tame," soluble problems in mathematics, chess, or puzzle solving.

Cancer is a "Wicked Problem"

- One that is difficult or impossible to solve because of incomplete, contradictory, and changing requirements that are often difficult to recognize.
- Because of complex interdependencies, the effort to solve one aspect of a wicked problem may reveal or create other problems.

The New York Times

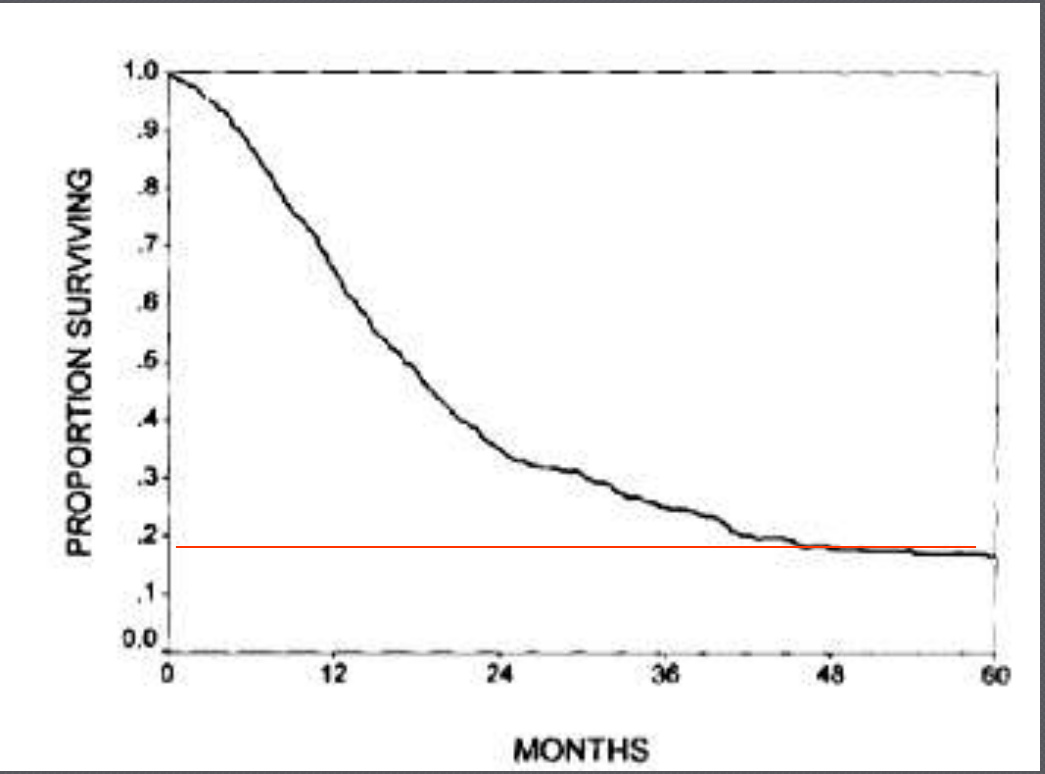
April 24, 2009



Source: National Center for Health Statistics

THE NEW YORK TIMES

Survival after Resection of Adenocarcinoma of the Pancreas



Actuarial 5yr =20%

Actual 5yr= 17%

616 pts. Sohn et al J. Gastrointestinal Surgery 4(6) 570-9 (2000)

Cancer and Inflammation

Rudolf Virchow suggested that the origin of cancer was in sites of chronic inflammation since 1863.

Table 1 | **Chronic inflammatory conditions associated with cancer**

Chronic inflammation	Associated cancer	Aetiological agent	Percent predisposed that progress to cancer
Bronchitis	Lung cancer	Tobacco smoke	11–24
Gastritis	Gastric cancer	*Helicobacter pylori*	1–3
Cervicitis	Cervical cancer	Human papillomavirus	<1
Warts	Non-melanoma skin cancer	Ultraviolet light, human papillomavirus	Varies with skin pigment and solar intensity
Asbestosis	Mesothelioma	Asbestos fibres	10–15
Inflammatory bowel disease	Colorectal cancer	Gut pathogens, altered gut permeability	1*
Pancreatitis	Pancreatic cancer	Tobacco, genetic factors	≤10%‡
Oesophagitis	Oesophageal cancer	Gastric acid, alcohol, tobacco	15
Sunburned skin	Melanoma, basal-cell carcinoma, squamous-cell carcinoma	Ultraviolet light	Varies with skin pigment and solar intensity, ≤9% of Caucasians
Hepatitis	Hepatocellular carcinoma	Hepatitis B virus, hepatitis C virus	10
Mononucleosis	Burkitt's lymphoma, Hodgkin's disease	Epstein–Barr virus	<1
Cholecystitis	Gall bladder cancer	Bacteria, gall bladder stones	1–2§
Cystitis	Bladder cancer	Gram-negative uropathogens, pelvic irradiation, carcinogens	<1

*Per year. ‡In susceptible populations. §At cholecystectomy.

Vakkila & Lotze, Nat Rev Immunol 2004

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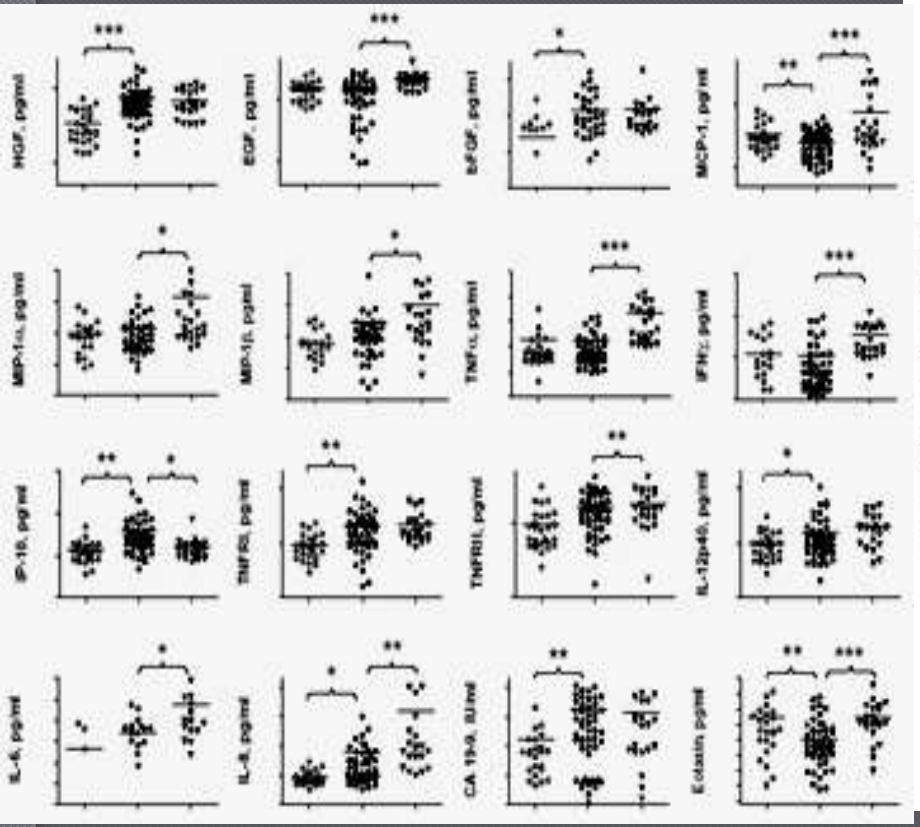
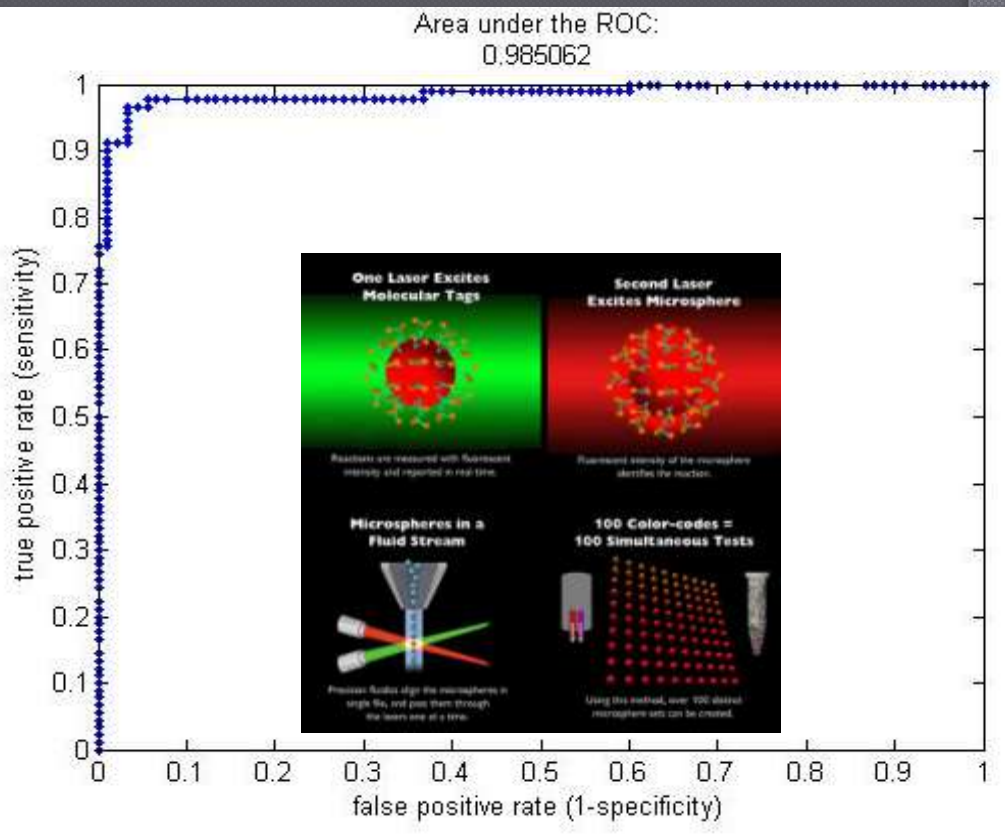
Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. *Nature Reviews Immunology* 4:641-647, 2004.

Multianalyte profiling of serum cytokines for detection of pancreatic cancer

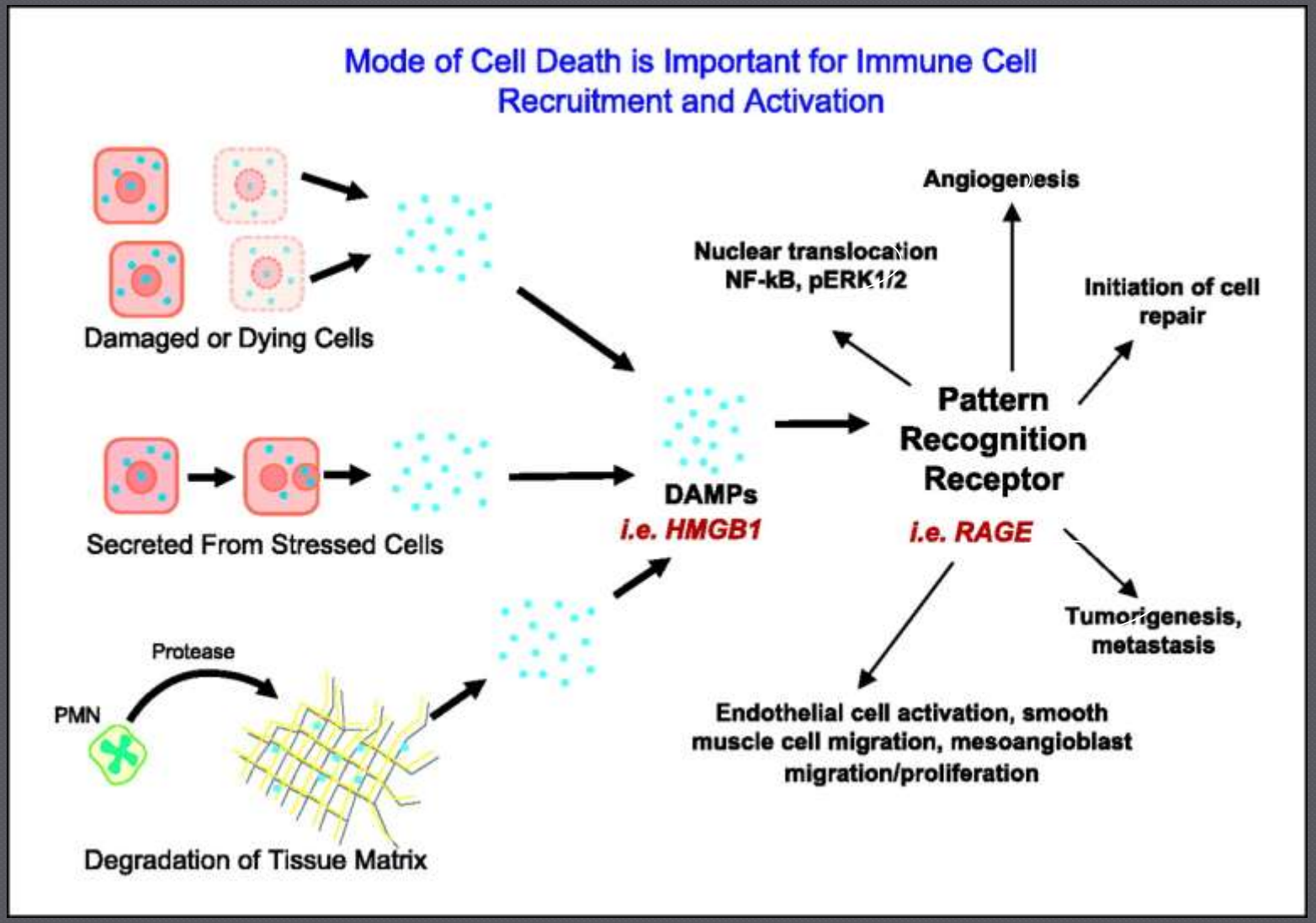
H.J. Zeh[a,b], S. Winikoff[a,b], D.P. Landsittel[c], E. Gorelik[b,d], A.M. Marrangoni[b], L. Velikokhatnaya[b], M.T. Winans[b], K. Lee[a], A. Moser[a], D. Bartlett[a,b], M.T. Lotze[a,b], J.M. Siegfried[b,h], D. Whitcomb[b,d], A. Slivka[b,e], W.L. Bigbee[b,f] and A.E. Lokshin[b,g,*]





Cancer Biomark. 2005;1(6):259-69.

Chronic Inflammation and Cancer



Ellerman, J. E. et al. Clin Cancer Res 2007;13:2836-2848

Classes of Molecules That Initiate The Innate Immune Response – Signal 0

Pathogen-associated Molecular Patterns (PAMPs):

Molecules expressed or released by invading microorganisms that are structurally unique to the pathogen.

Ruslan Medzhitov, 2000

Damage-associated Molecular Patterns (DAMPs):

Molecules expressed or released that are normally unavailable to the immune system but are released and recognized by immune cells following tissue injury [Danger].

Polly Matzinger, 1995

DAMPs -Chronic Tumor Lysis Syndrome

Cell Constituents:

HMGB1 – Cytochrome C

Heat shock proteins

Uric Acid, ATP, Adenosine; CpG DNA

s100 proteins

Hepatoma derived growth factor

LDH

DNA

Acute Tumor Lysis Syndrome

Secreted molecules:

Fibrinogen domain A

Surfactant protein A

Matrix elements:

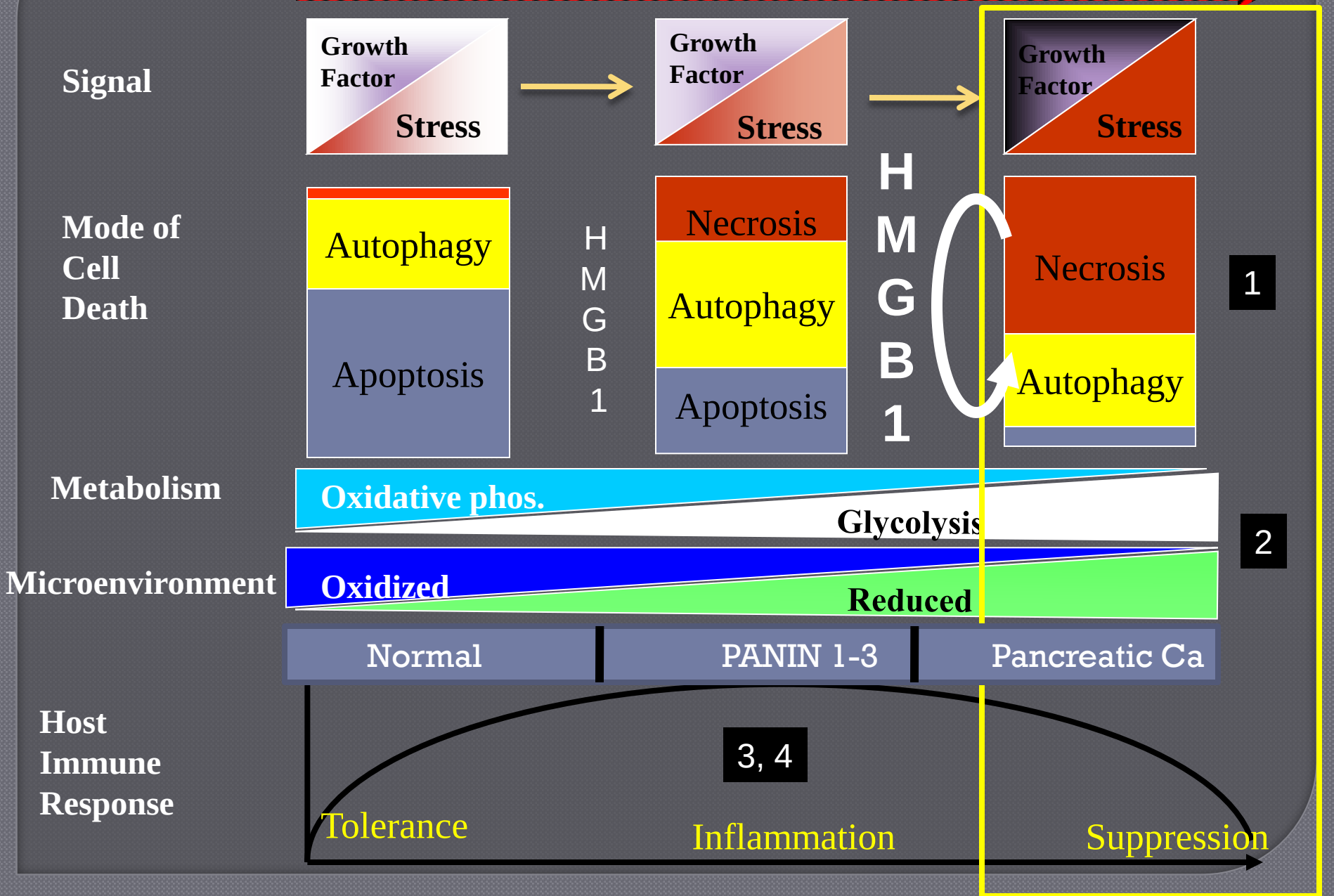
Heparan sulfate

Soluble hyluranan

Fibronectin

Lotze MT, Wang E, Marincola FM, .. Coukos G, .. Whiteside TL. Workshop on cancer biometrics: identifying biomarkers and surrogates of cancer in patients: a meeting held at the Masur Auditorium, National Institutes of Health. J Immunother 2005;28(2):79-119.8.

Pancreatic Tumor Progression



DAMPs in Pancreatic Cancer

- Death is a rather constant concomitant of Pancreatic Cancer
- Current clinical protocols UPCI [Zeh]
- Death Pathways [Apoptosis, Autophagy, Necrosis]
- DAMPs
- HMGB1
- Novel Therapeutic Strategies

Targeting Autophagy

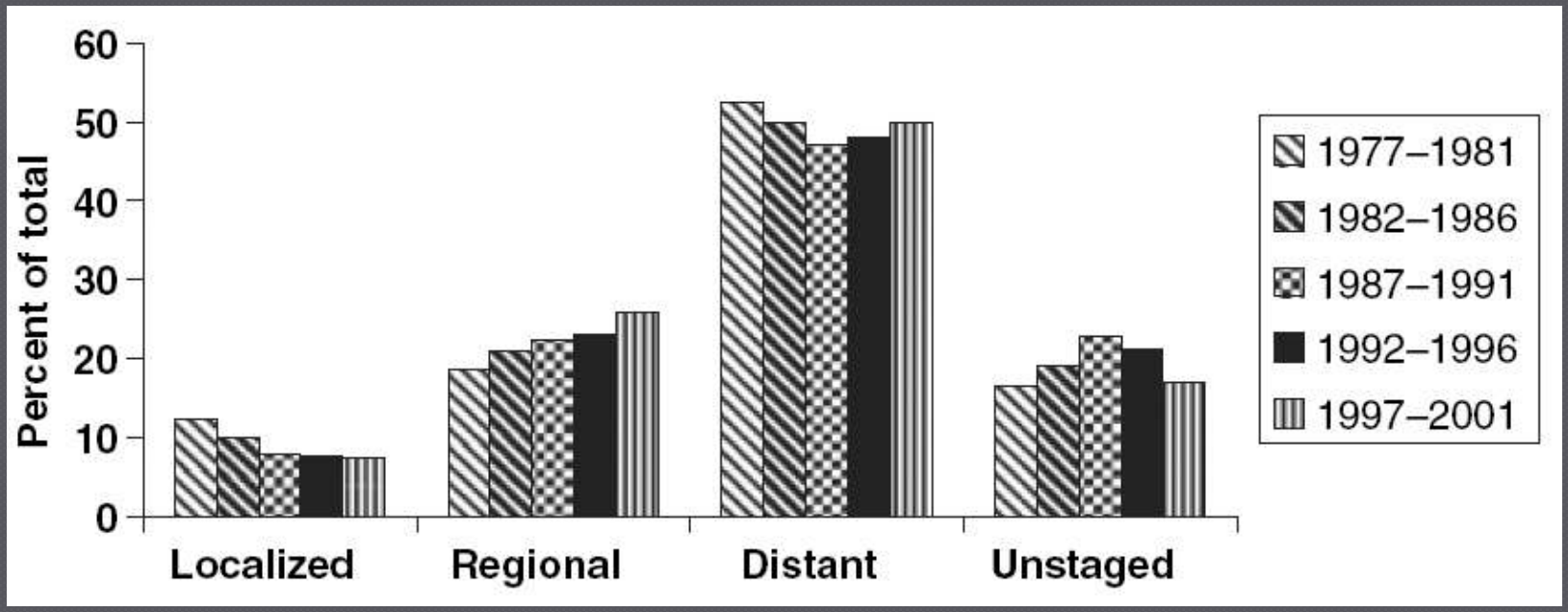
Resources in Pancreatic Cancer

- Clinical Program with 450 new cases/year of Pancreatic Cancer; Robotic Program [WTAE]
- Current clinical protocols UPCI [Zeh, Moser, Bahary, Lembersky, Lotze, Whitcomb, Normolle]

- Subcutaneous and Orthotopic Pancreatic Tumors
- PDX-cre Mutant Kras Spontaneous Tumor Model

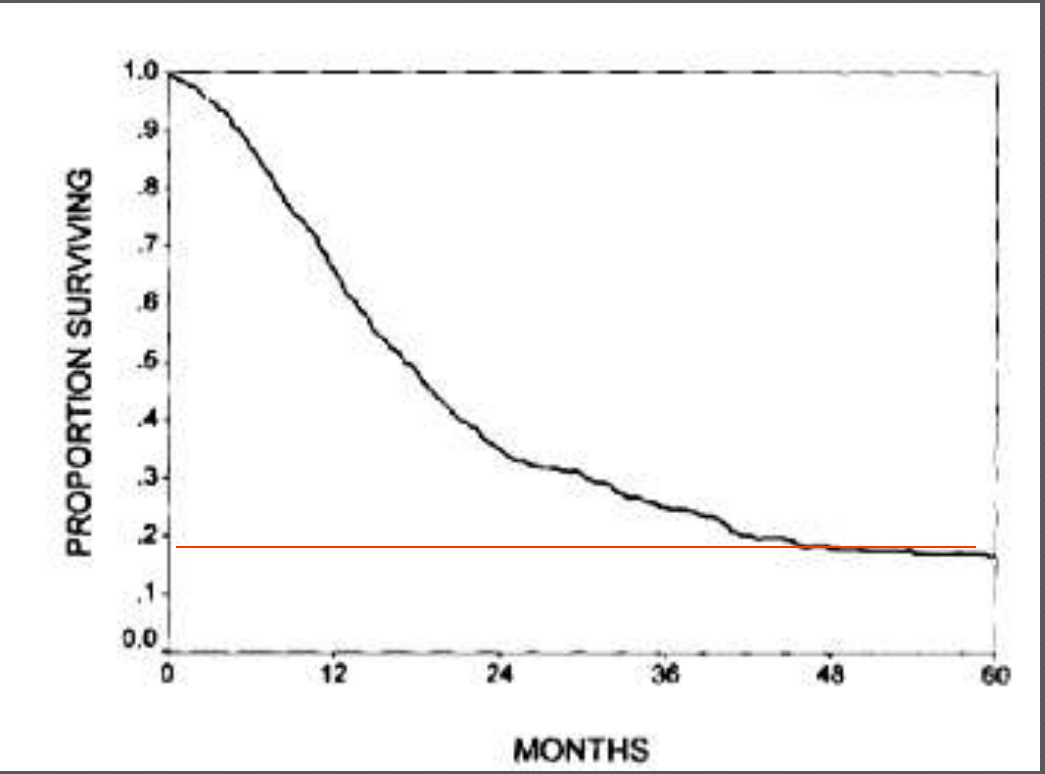
- Imaging and Flow Cytometers [Buchser]
- ForteBio Interferometer
- Seahorse Measures of OXPHOS and Glycolysis
- Targeting Autophagy
- miRNA, proteomics and transcriptomics

Extent of Disease at Diagnosis



Shaib et al, Aliment Pharmacol Ther 24, 87-94, 2006

Survival after Resection of Adenocarcinoma of the Pancreas

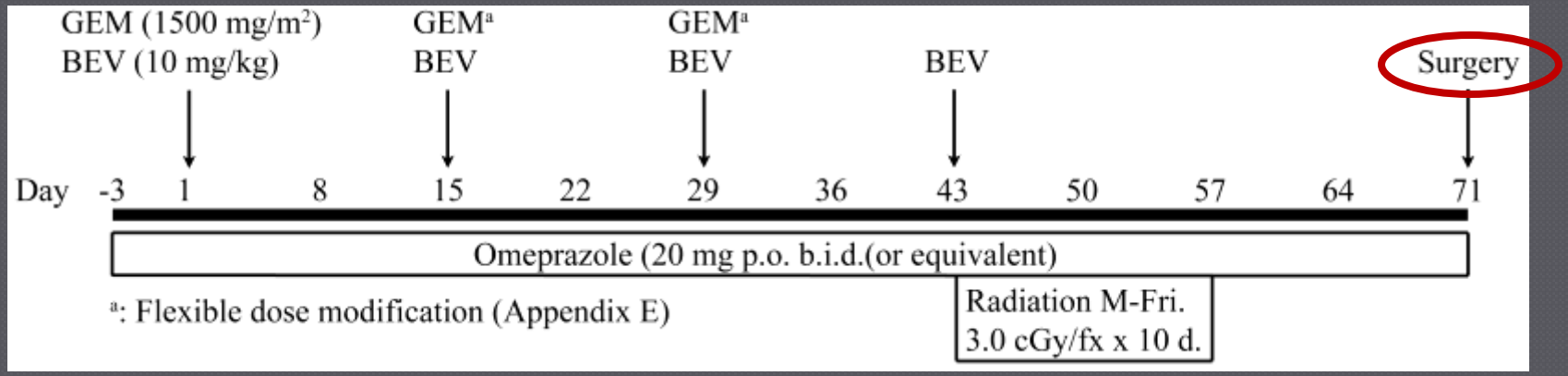


Actuarial 5yr =20%

Actual 5yr= 17%

616 pts. Sohn et al J. Gastrointestinal Surgery 4(6) 570-9 (2000)

Neoadjuvant Phase II Trial: UPCI 06-035



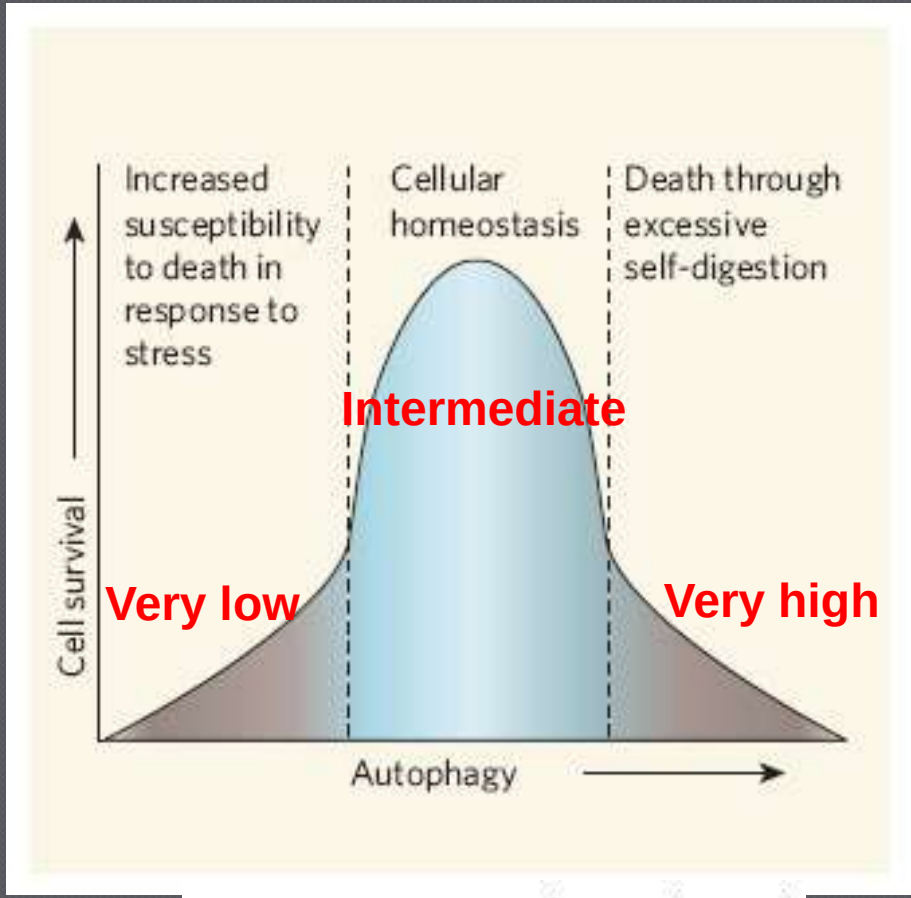
- Investigator-initiated (Genentech)
  - Largest trial of its kind in the world (n=60)
  - *Nationwide* prospects (25 centers interested)
- Dual primary (objective) endpoints:
  - Margin negative resection rate
  - Complete pathologic response rate
- Treated tissue is the key to future trial development
  - Pathology, cancer genetics, gene expression
  - 35/60 enrolled; opened 2/08

The Pancreatic Cancer Research Team (PCRT) is a collaboration of international researchers with the mission to organize and accelerate the clinical development of new agents for the treatment of patients with pancreatic cancer. PCRT offers a central resource for patients seeking the most up-to-date clinical trials. TGEN – Dan Von Hoff and Ramesh Ramanathan. **New U01 Funding with Tim Wang at Columbia and Jamie Lee at Scottsdale Mayo Clinic [$10M].**

Cancer Necrosis Correlates with Poor Prognosis

- Mesothelioma *(Edwards, 2003) p=0.008*
- Renal-clear cell carcinoma *(Cheville 2003; Tollefson 2007) p<.001*
- Colon carcinoma *(Hunter, 1983)*
- NSCLC *(Swinson, 2003) p=0.0016*
- Breast *(Gilchrist, 2003) p=0.0003; Kato, 2002) p=0.0068*
- Mucosal melanoma *(Prasod, 2002) p=0.007*
- Melanoma *(Balch, 2001)*
- Sarcoma *(Miyajima 2002; Gustafson 2003)*

Relationship Between The Levels Of Autophagy And Cell Death



Nature **446**, 745-747 (12 April 2007)

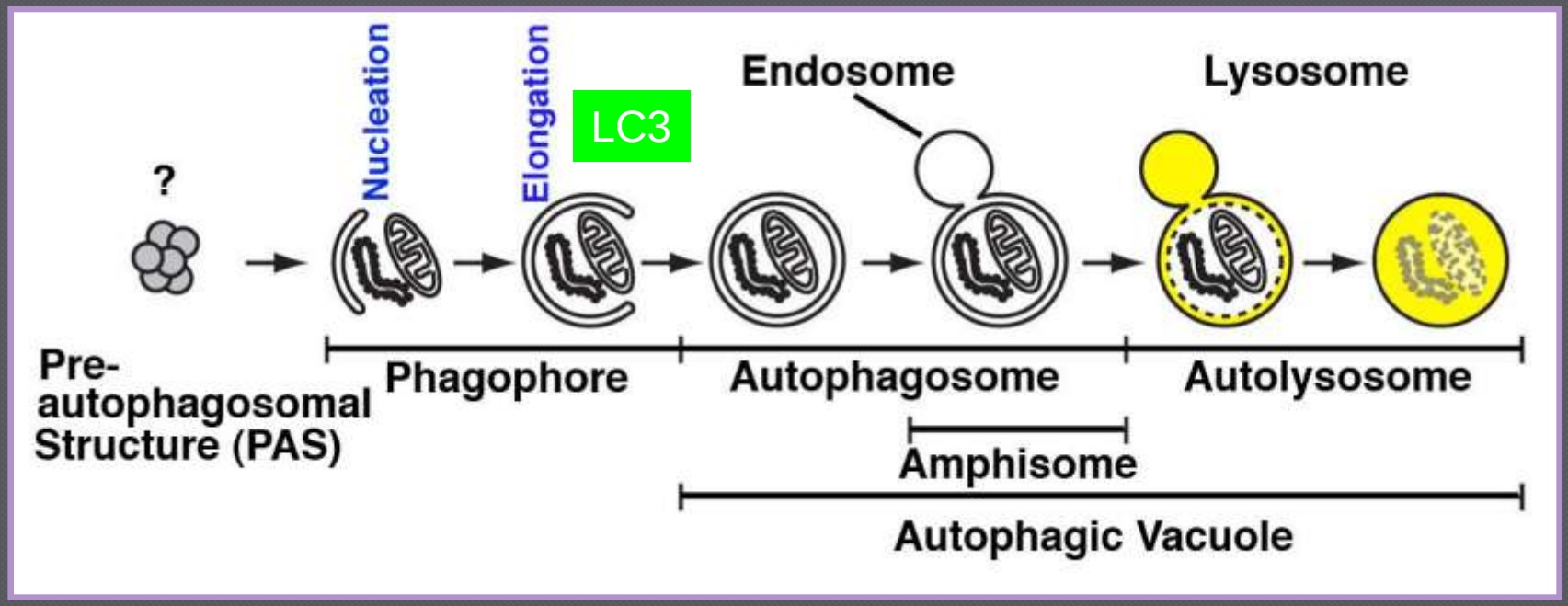
Very low -- The absence of autophagy increases cell death during metabolic stress and on treatment with cytotoxic chemotherapeutic agents.

Intermediate -- Physiological levels of autophagy are essential for normal cellular homeostasis.

Very high -- excessive levels of autophagy promote cell death.

Beth Levine

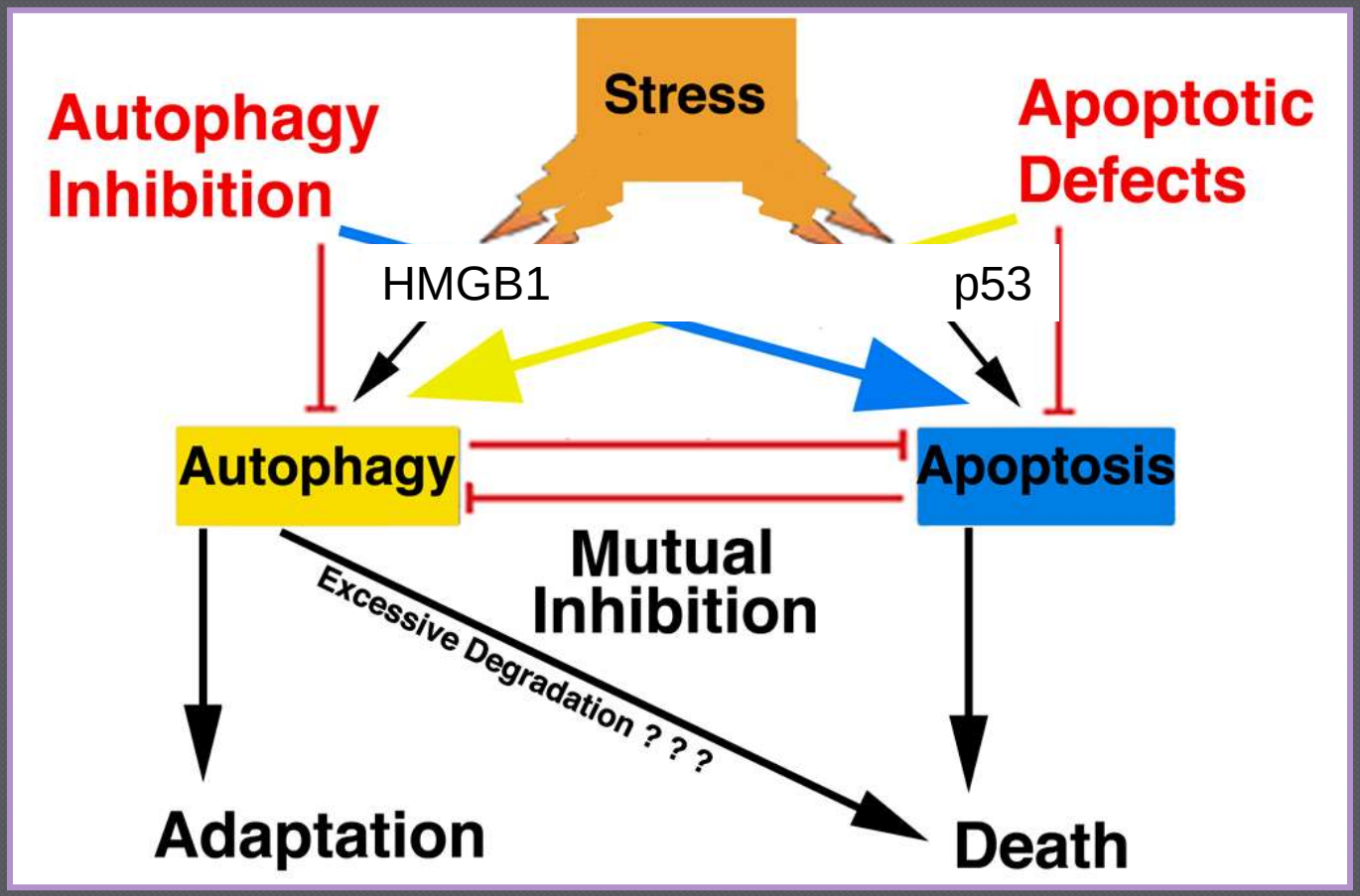
Mammalian Autophagy



Distinct autophagic phases:

I. Autophagosome formation — Sequestration
II. Degradation
III. Utilization — Provision of amino acids

Cross-Regulation Between Autophagy and Apoptosis



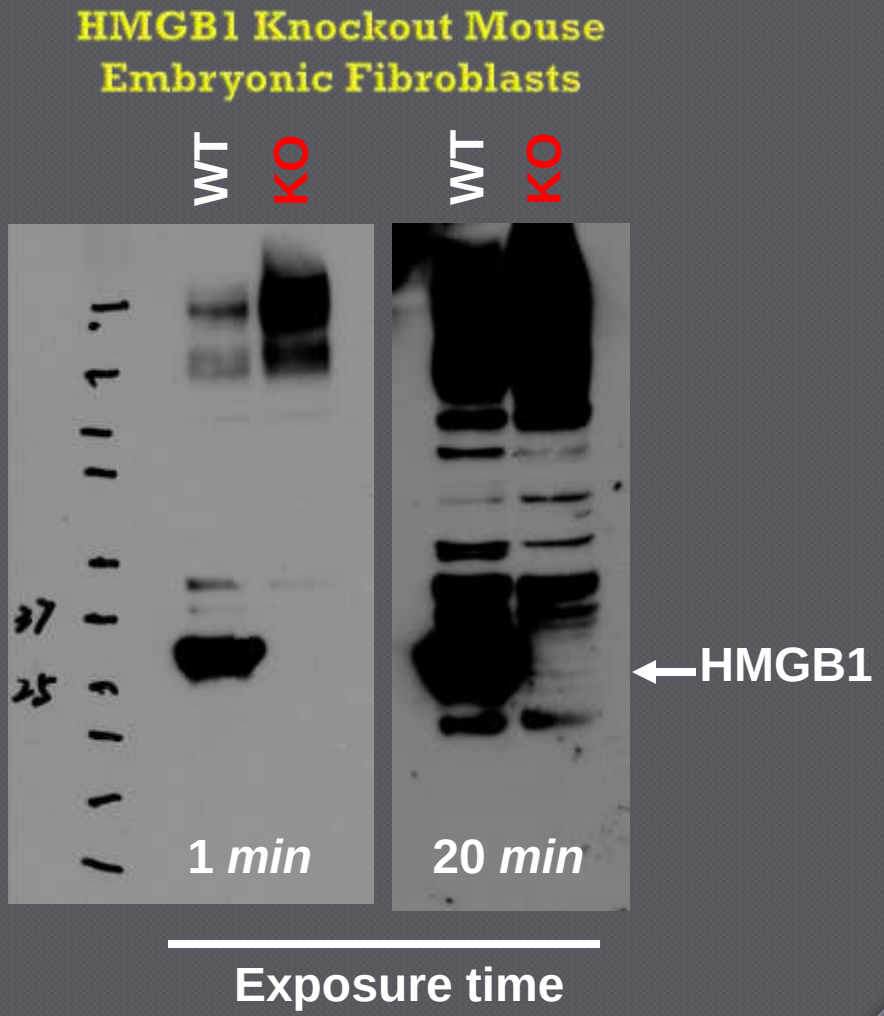
Modified from Nat Rev Mol Cell Biol. 8:741 (2007)

HMGB1 Knockout Mice And Cell Lines

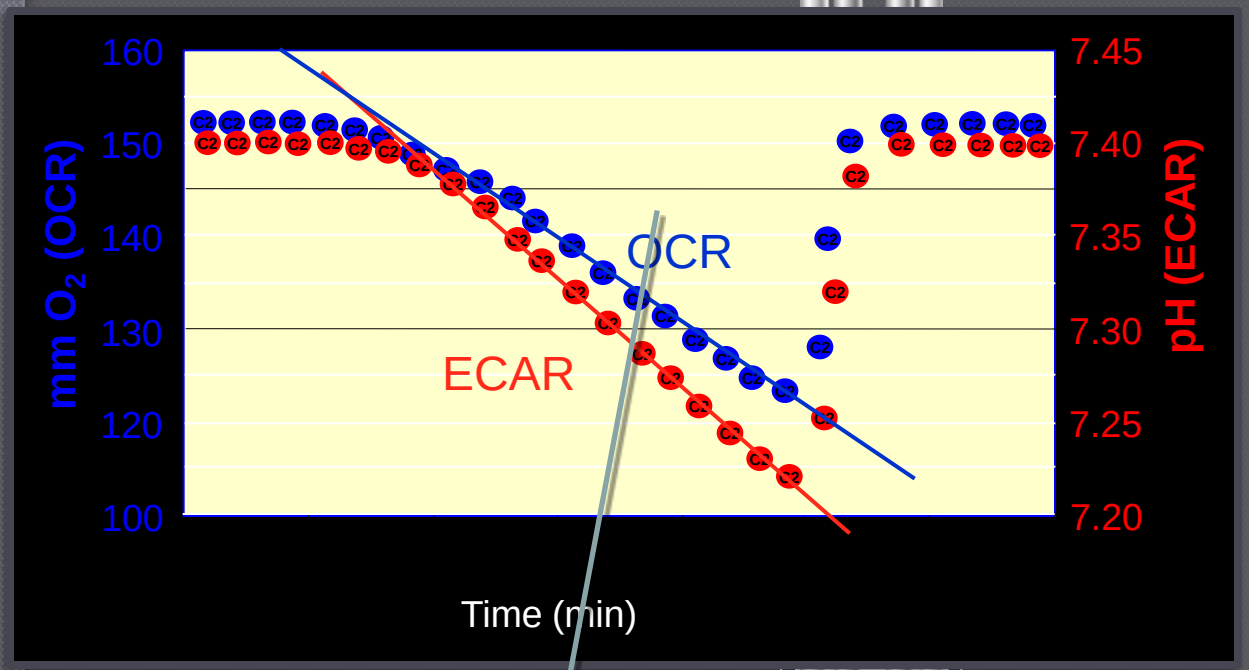
HMGB1 Knockout Mice die soon after birth as a result of lethal hypoglycemia

Nature Genetics **22, 276 - 280 (1999)**

Marco E. Bianchi Lab
bianchi.marco@hsr.it



Measuring Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR)



The rate is calculated from the slope

The bio-cartridge is raised, bringing the system back to baseline

micro-chamber is formed

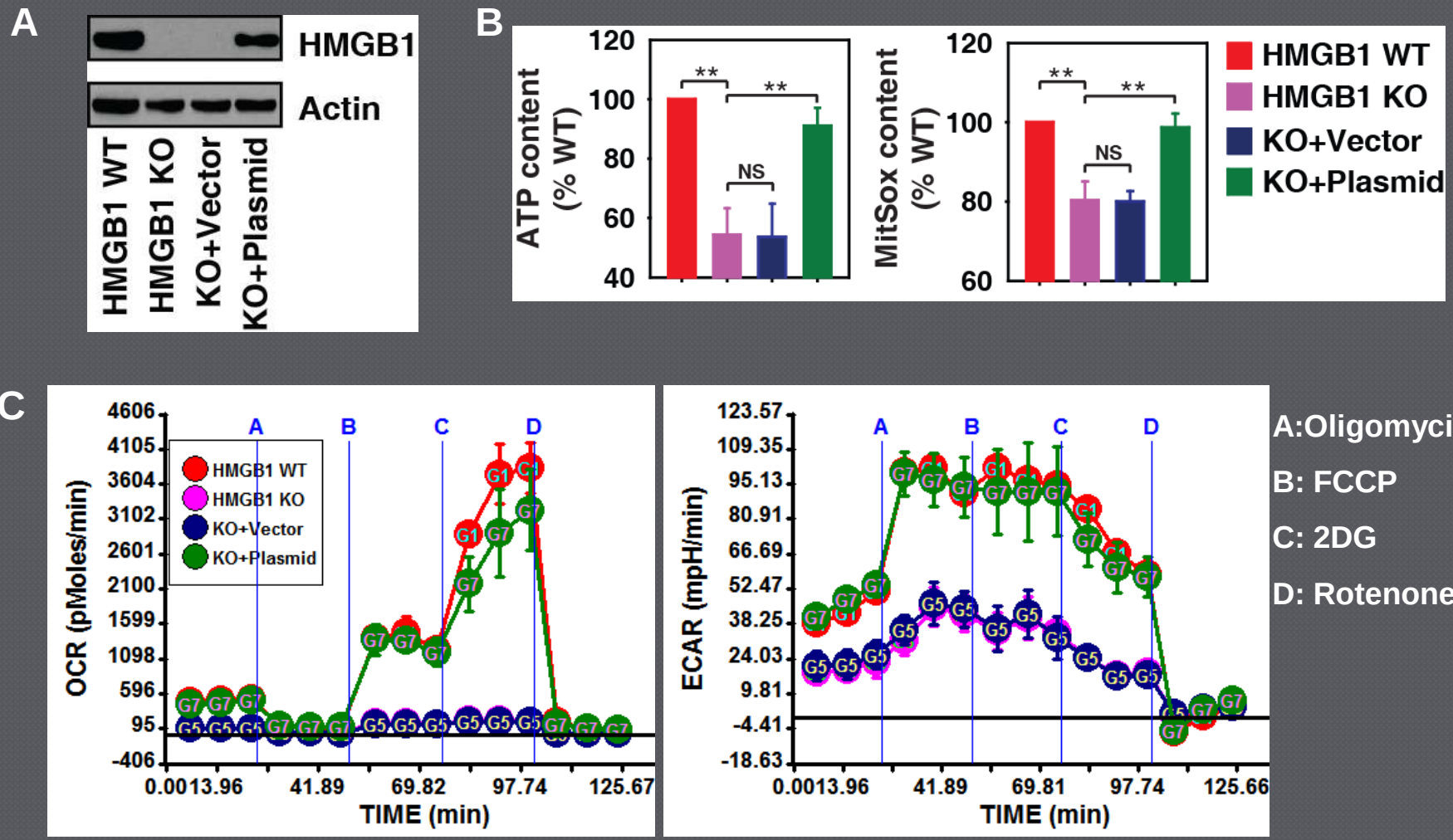
The bio-cartridge is raised, bringing the system back to baseline

Ben Van Houten

Well 1

Well 2

Knockout of HMGB1 Impairs Mitochondrial Function



Tumor Progression