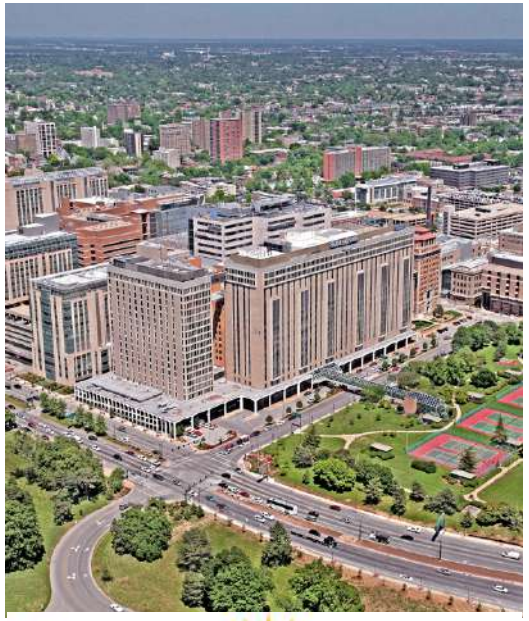
The Cutting Edge--A Surgeon's Perspective on Personalized Cancer Care



William G. Hawkins MD
Chief, Section of Hepatobiliary, Pancreatic, & Upper Gastrointestinal Surgery

Director, Washington University SPORE in Pancreatic Cancer

The Neidorff Family & Robert C. Packman, MD Professor of Surgery

BARNES-JEWISH Hospital
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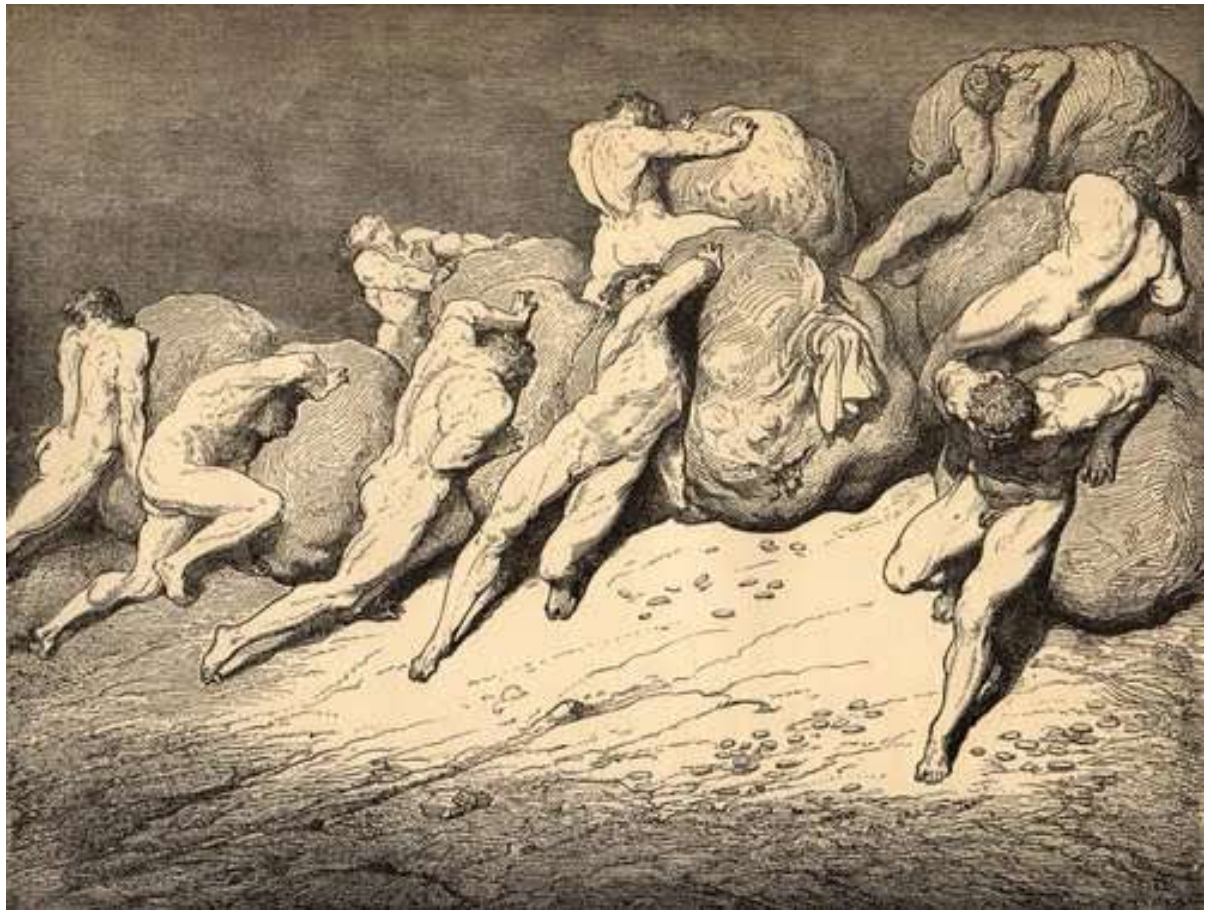
SITEMAN CANCER CENTER
A NATIONAL CANCER INSTITUTE COMPREHENSIVE CANCER CENTER

Washington University in St. Louis
SCHOOL OF MEDICINE

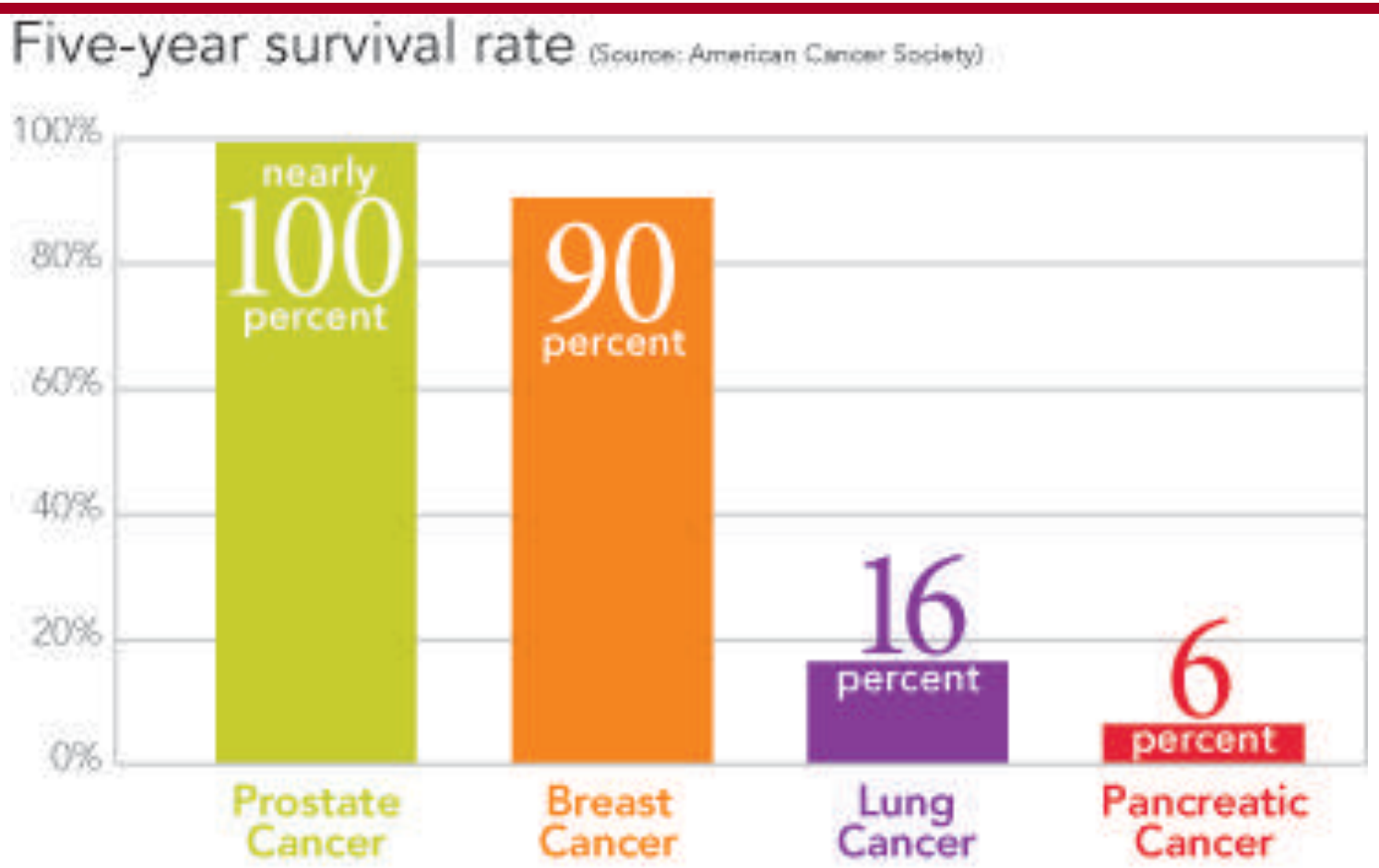
Disclosures- Potential Conflicts of Interest

- Accuronix Therapeutics*- Founder, BOD, and stockholder
- Sigma-2 Ligands- Patents and patents pending regarding chemical composition and potential uses
- Government sponsored research grants
- Foundation sponsored research grants

History of pancreatic cancer care



Adenocarcinoma of the Pancreas
Overall Survival



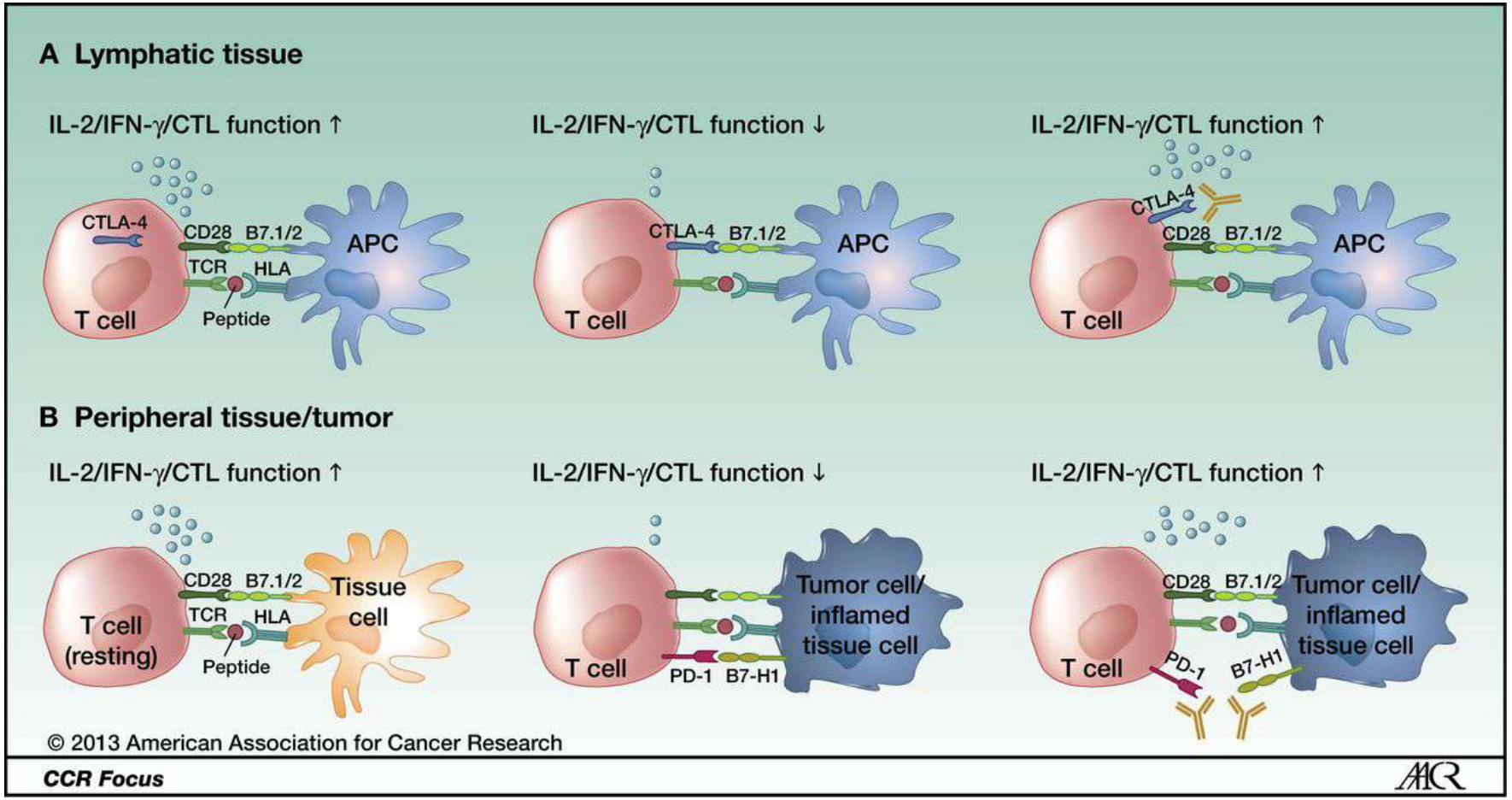
Former Pres. Jimmy Carter Says His L… No Sign of…

The 91-year-old… immunotherapy…

When th… Patient C… Mountai…

A gene therapy … Stage IV lung ca… peak in the Him…

Review: Checkpoint Therapy

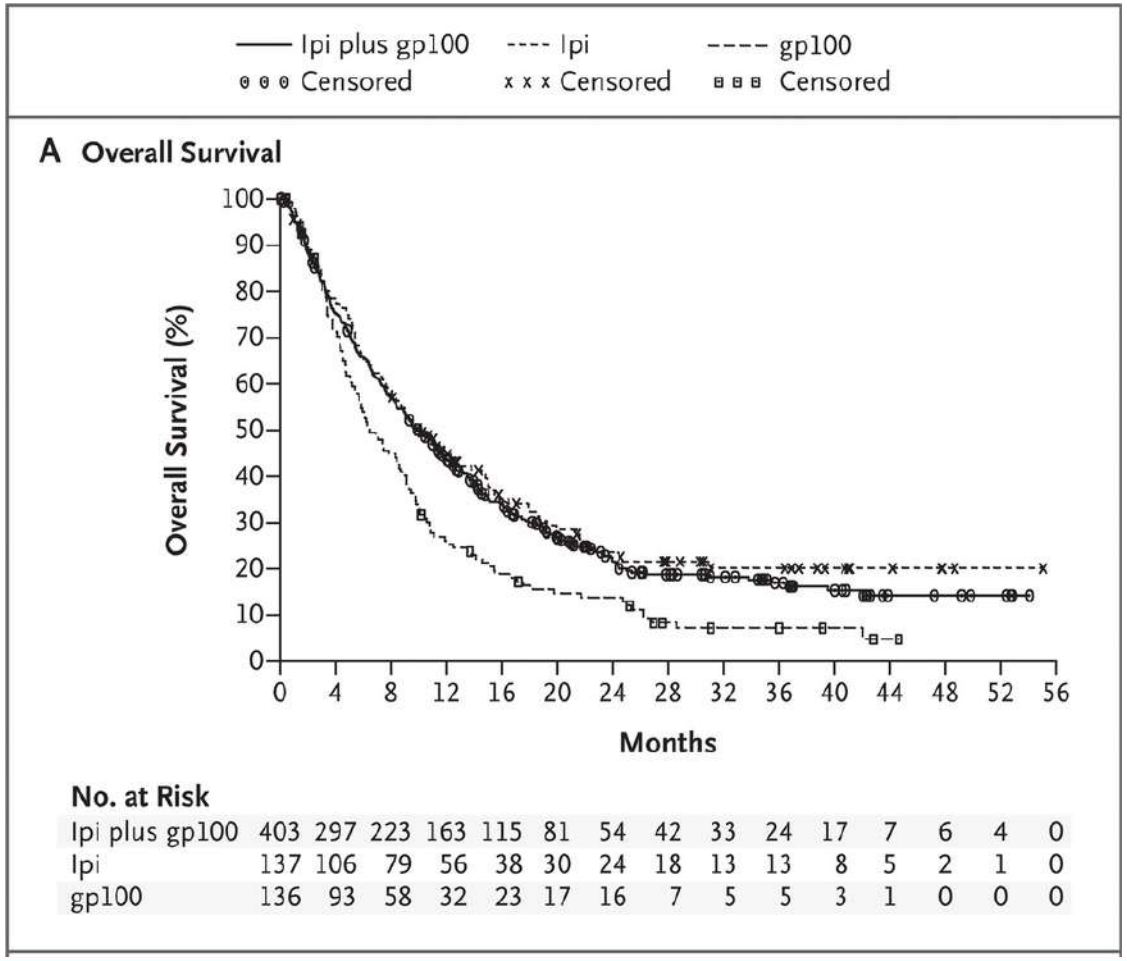


Ott, CCR, 2013

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 AUGUST 19, 2010 VOL. 363 NO. 8

Improved Survival with Ipilimumab in Patients with Metastatic Melanoma

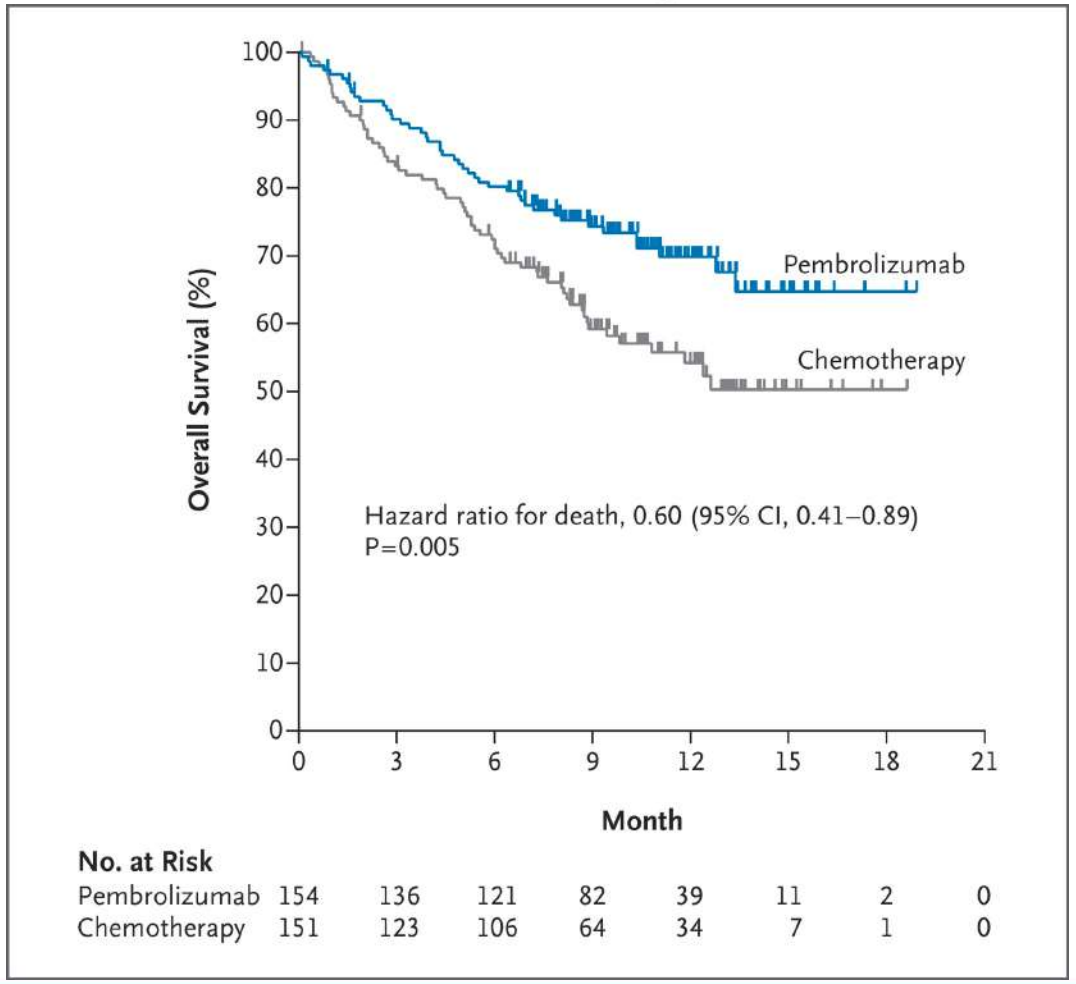


Hodi, NEJM, 2010

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 NOVEMBER 10, 2016 VOL. 375 NO. 19

Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer

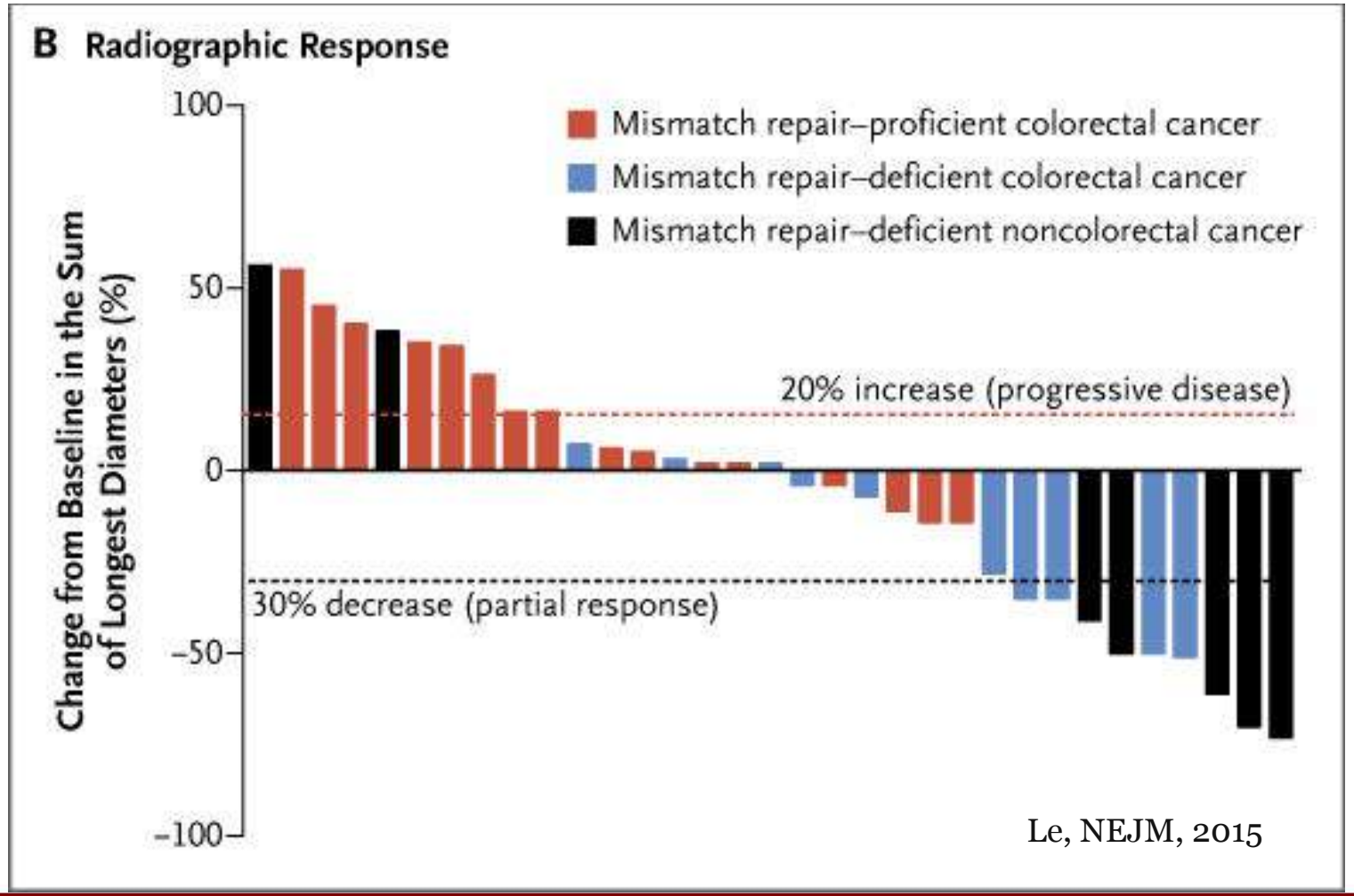


Reck, NEJM, 2016

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

PD-1 Blockade in Tumors with Mismatch-Repair Deficiency

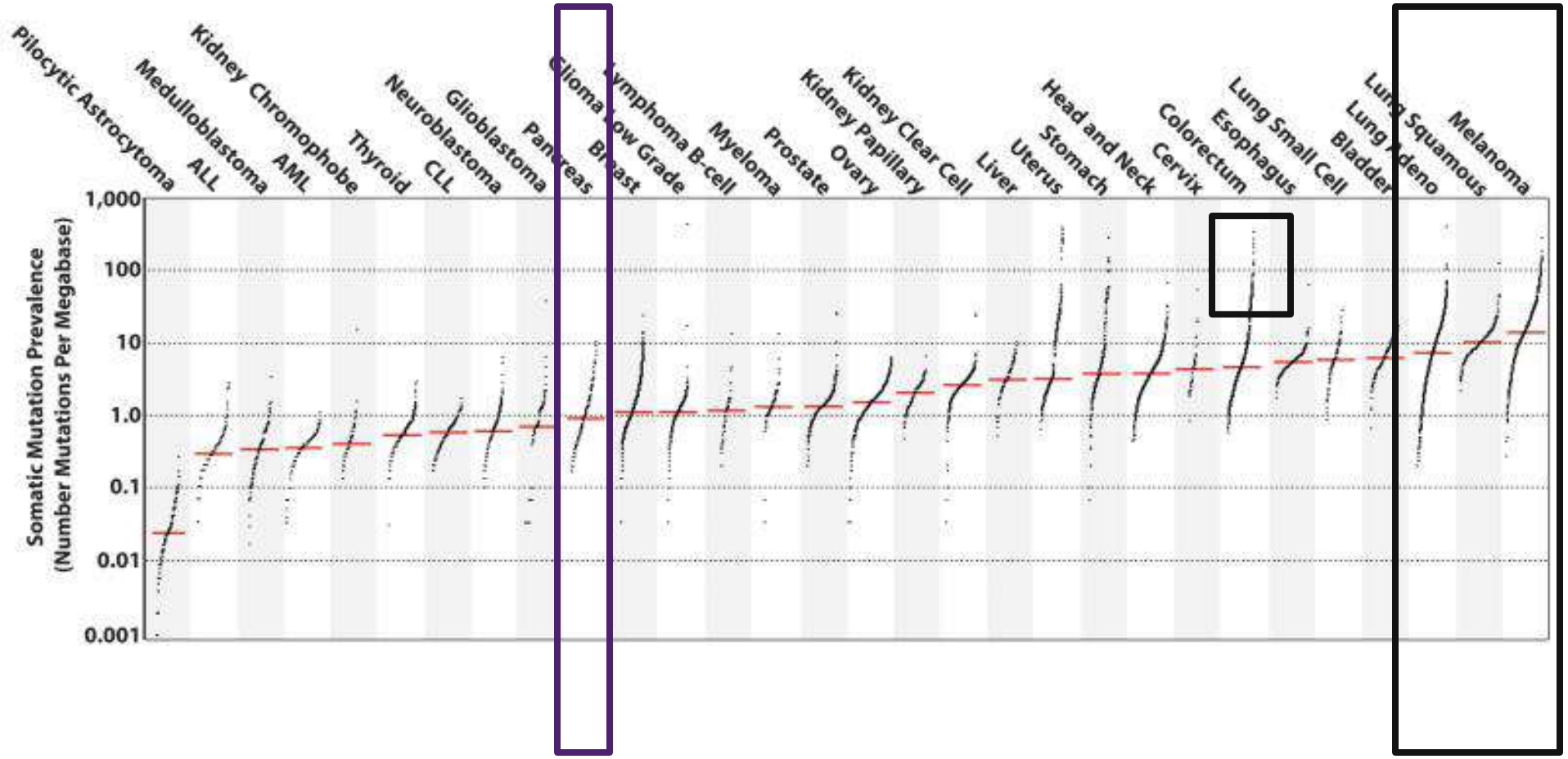


Le, NEJM, 2015

Immunotherapy has been successful

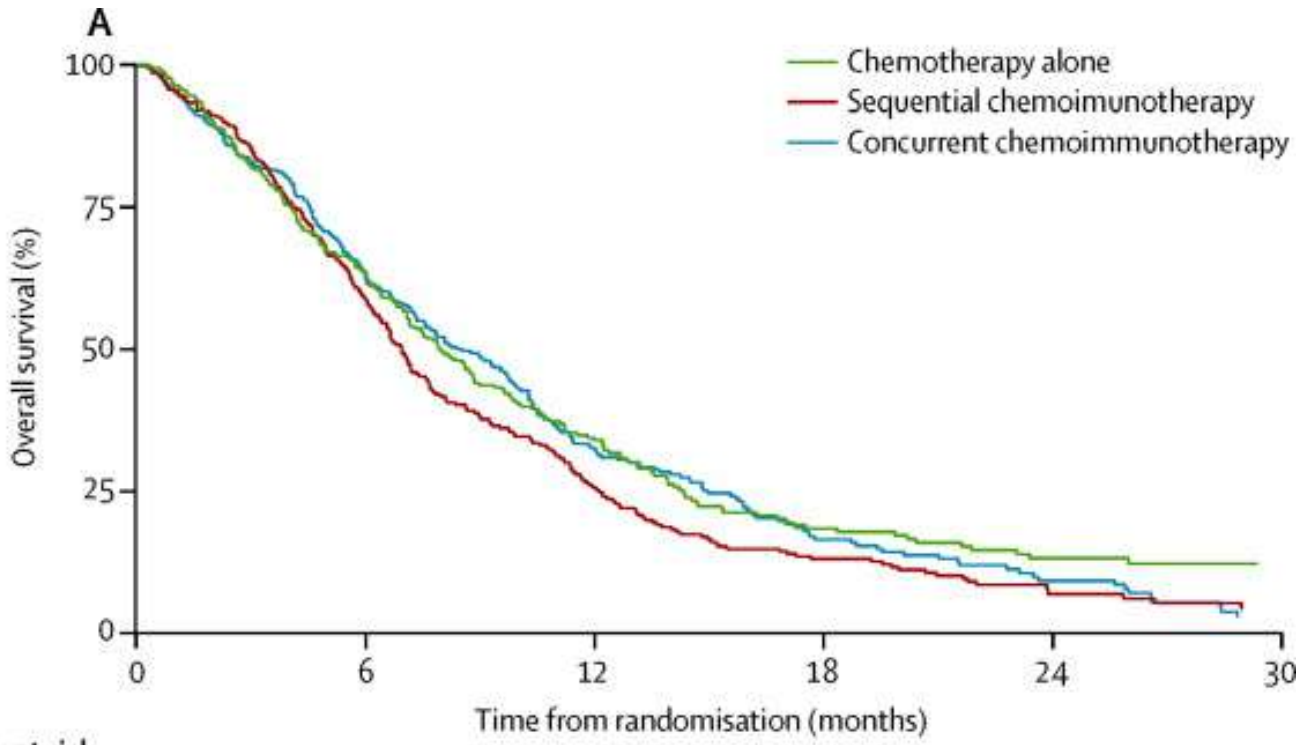
- Melanoma
- Non-small cell lung cancer
- Head and neck squamous cancer
- Mismatch repair deficient colon cancer

Cancer Neoantigen Frequency



Alexandrov, Nature, 2013

Pancreatic Cancer Immunotherapy Trials

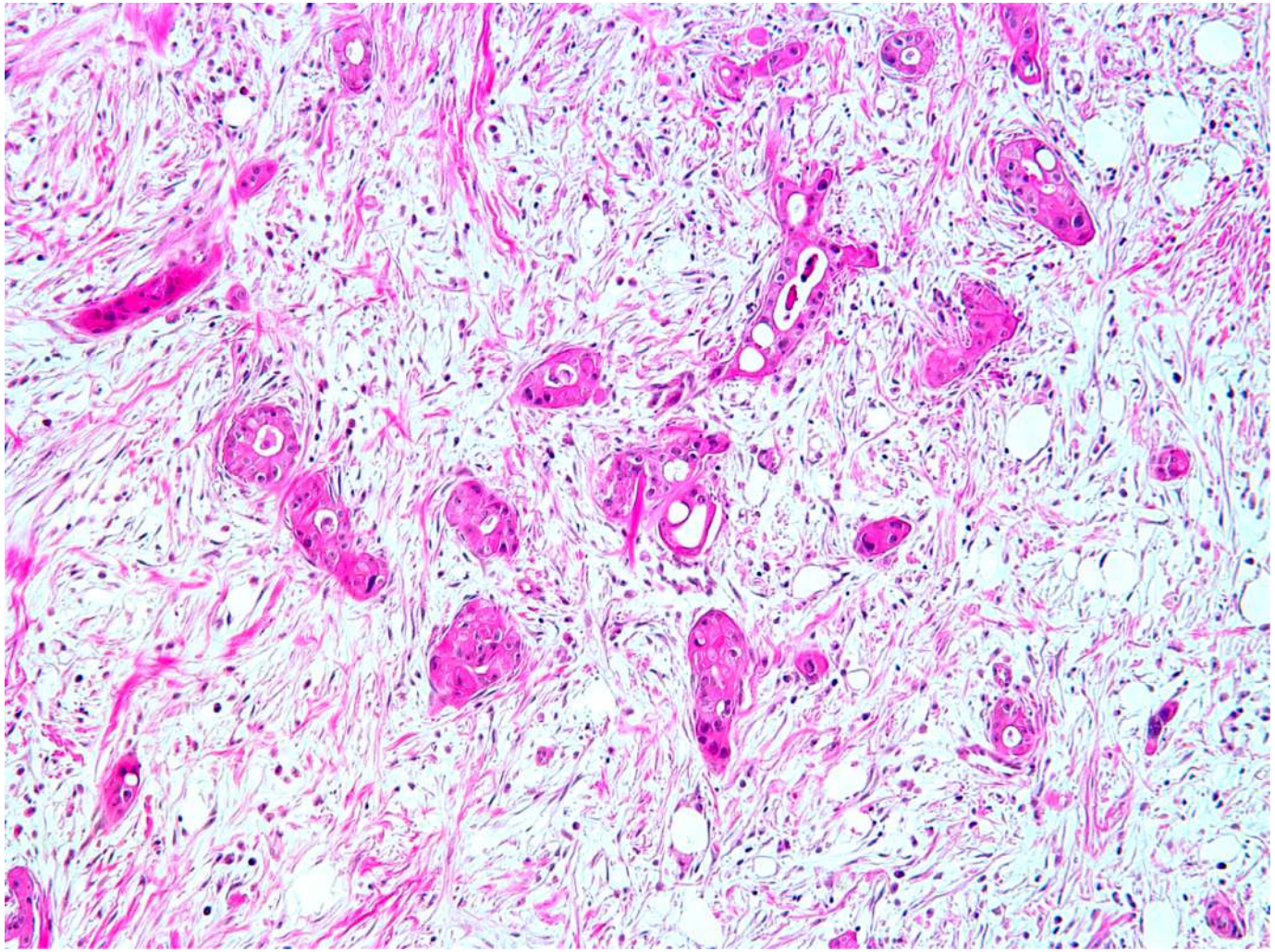


Number at risk						
Chemotherapy alone	358	190	76	31	18	7
Sequential chemoimmunotherapy	350	171	63	29	8	4
Concurrent chemoimmunotherapy	354	184	80	30	13	3

Middleton, Lancet Oncology, 2014

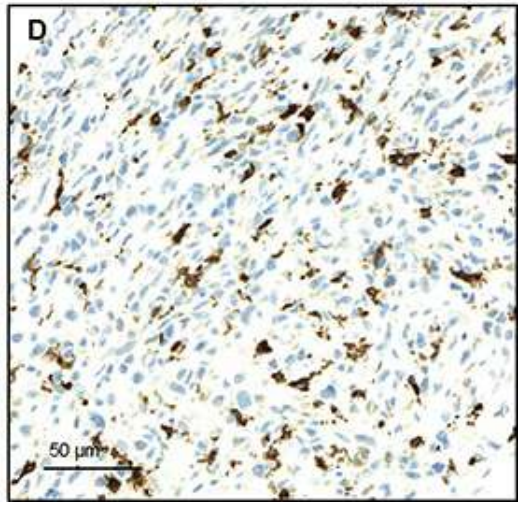
Clinical Observations

Pathologic Observations



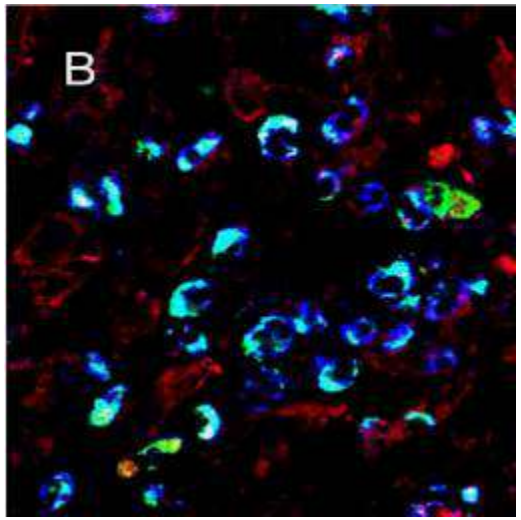
Immunosuppressive Microenvironment

Treg

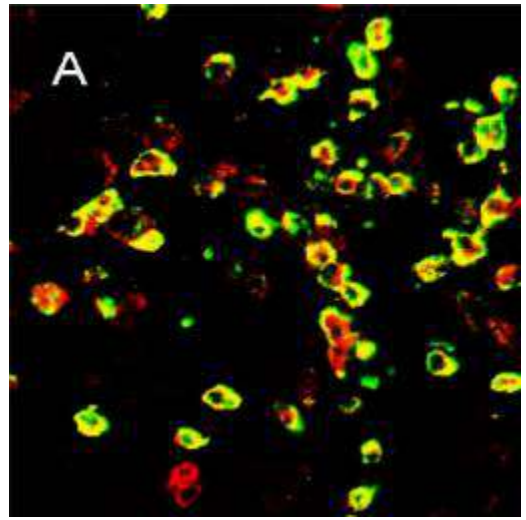


Wartenberg, Oncotarget, 2015

TAM

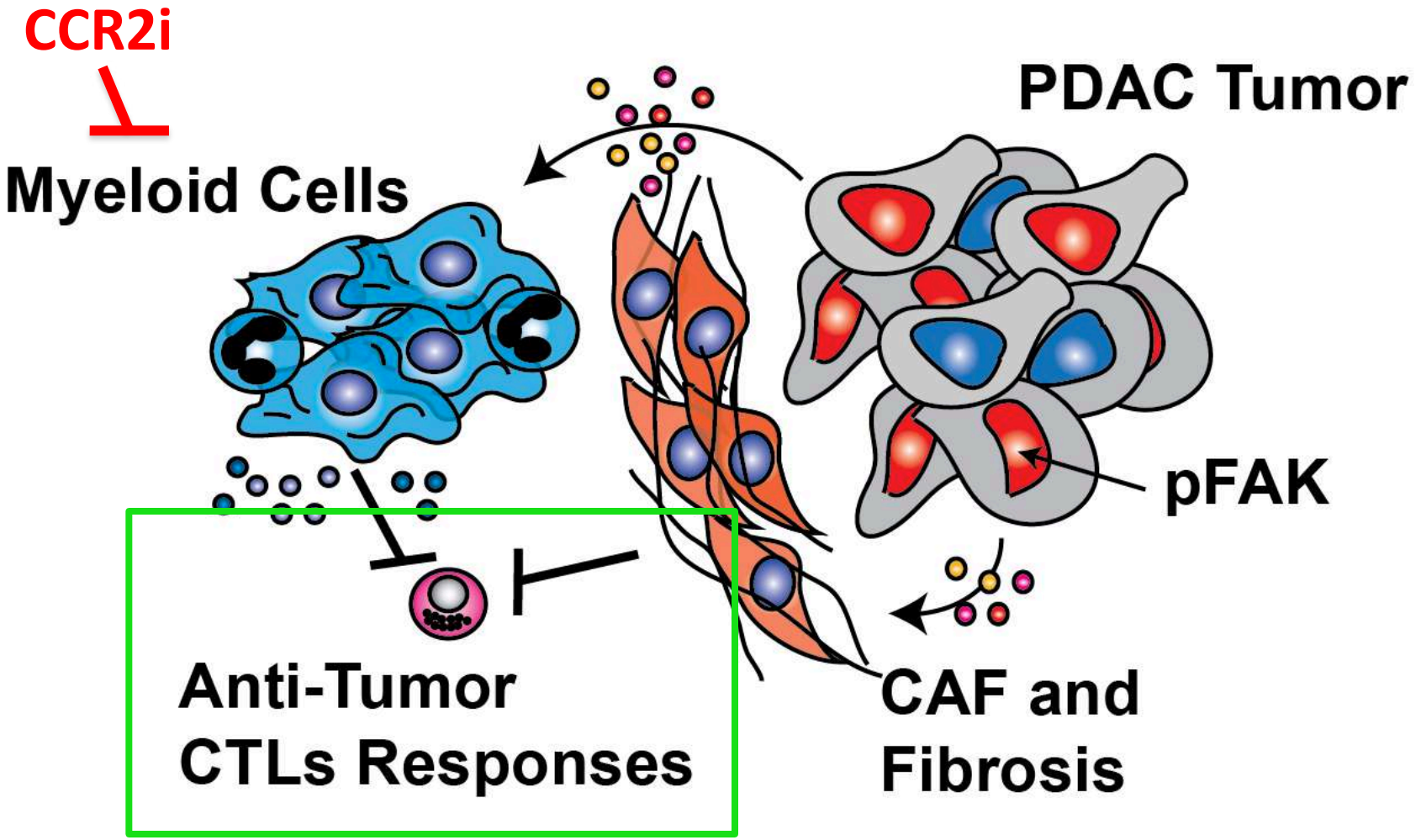


TAN

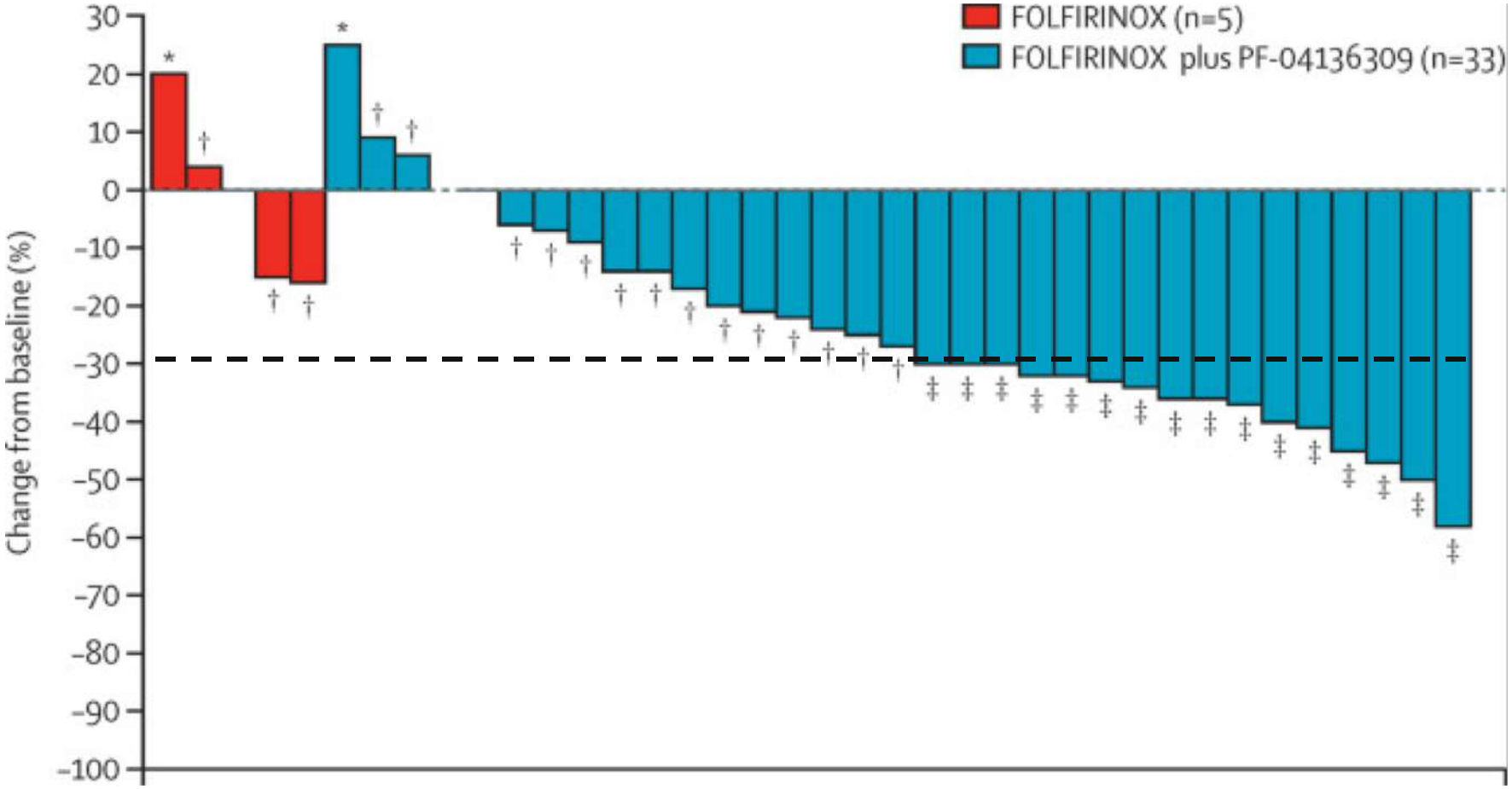


Goedegebuure, Curr Cancer Drug Targets, 2011

Targeting Immunosuppressive Microenvironment



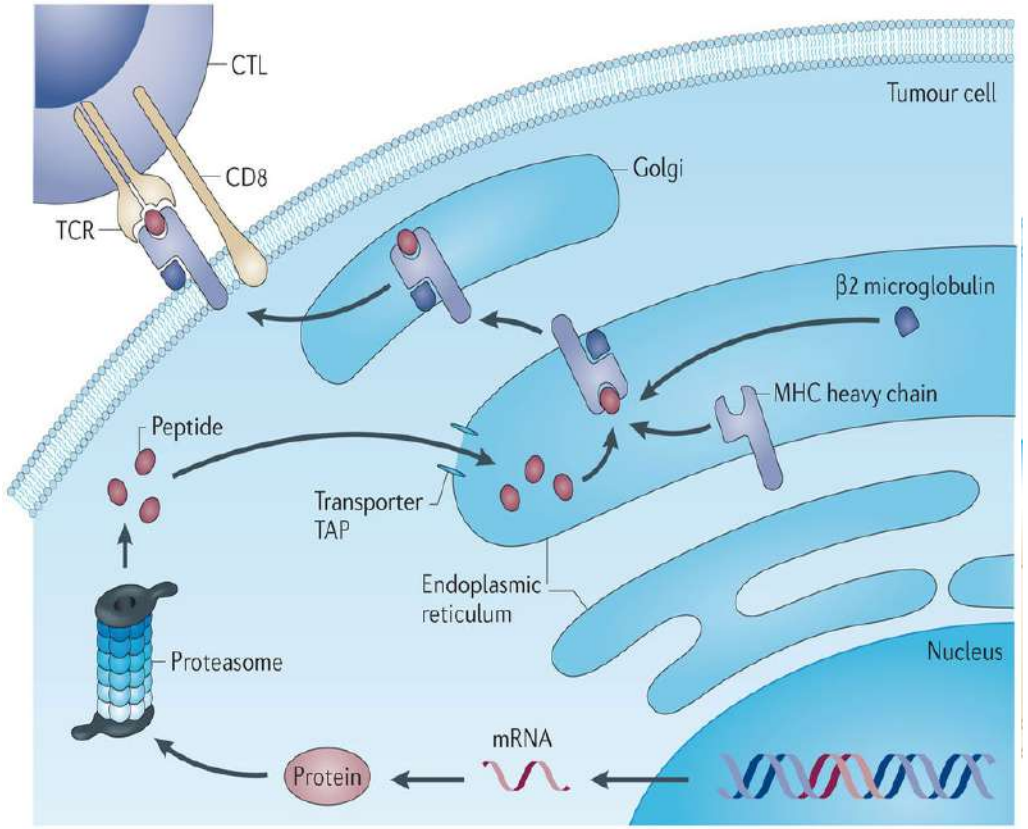
Targeting tumour-associated macrophages with CCR2 inhibition in combination with FOLFIRINOX in patients with borderline resectable and locally advanced pancreatic cancer: a single-centre, open-label, dose-finding, non-randomised, phase 1b trial



Nywening, Lancet Onc, 2016

Cancer Neoantigens

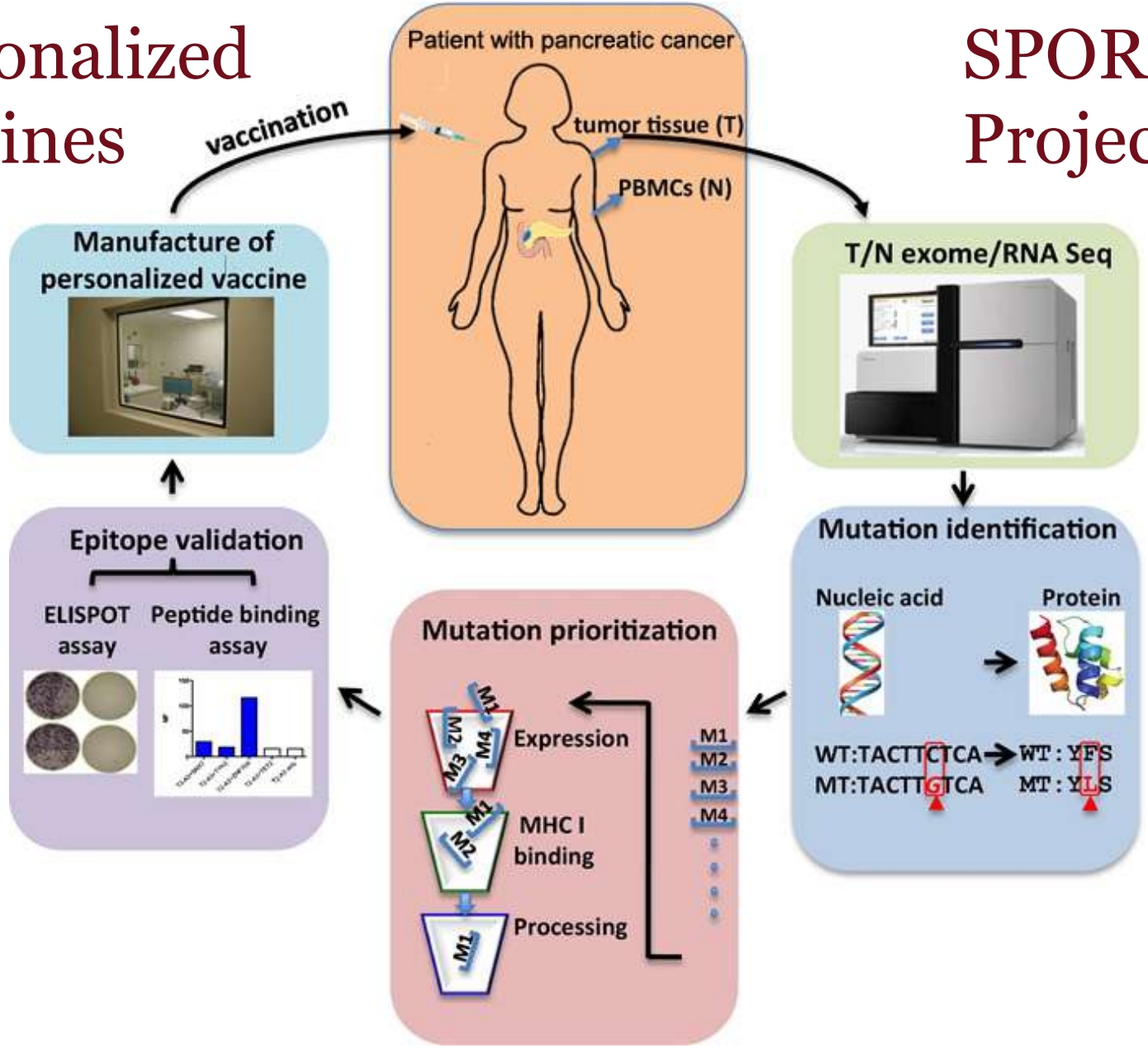
- Result from accumulated mutations to the host genome
- Less susceptible to tolerance
- SNV, In-Del, Frameshift



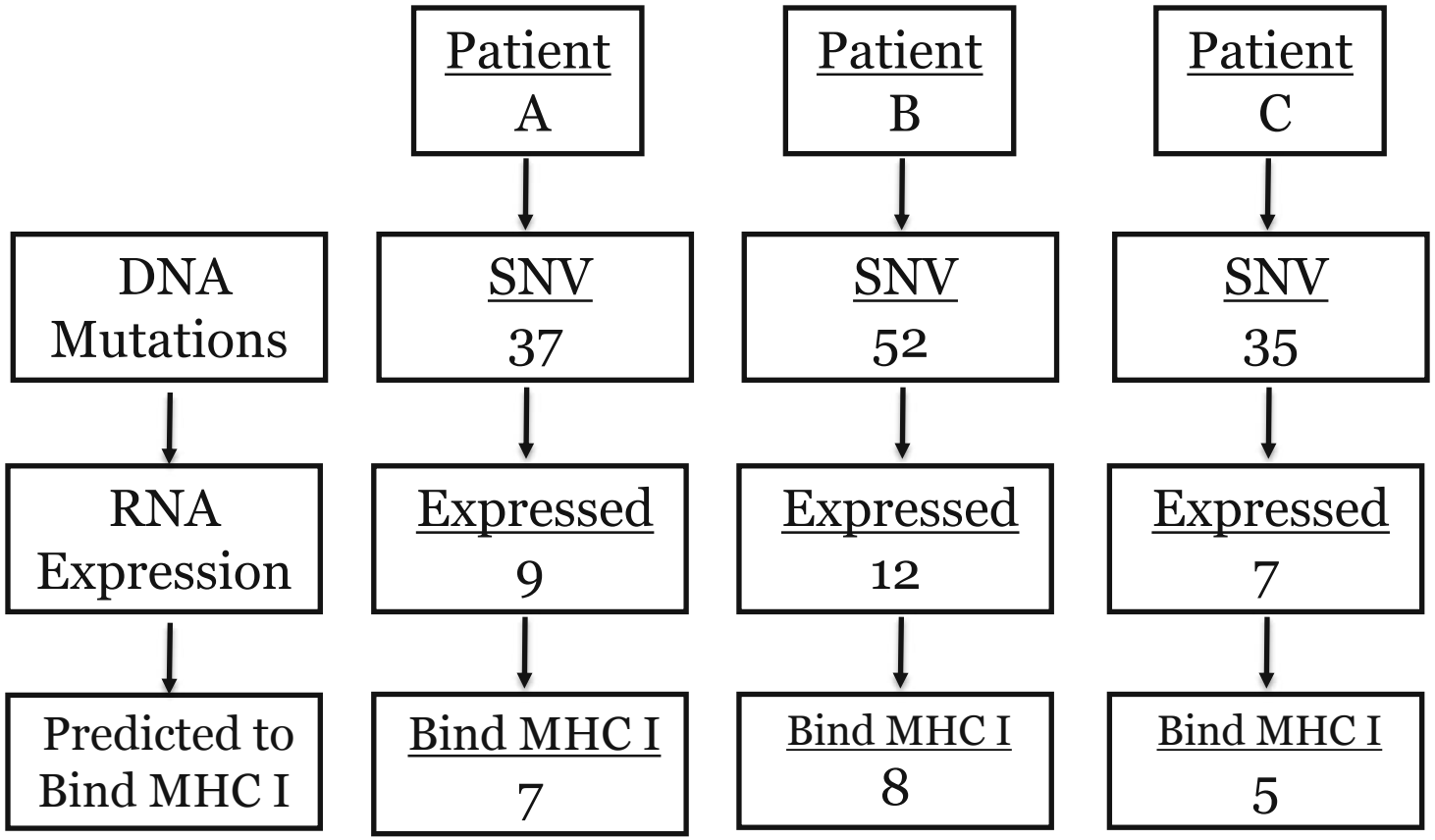
Coulie, Nature Reviews Cancer, 2014

Personalized Vaccines

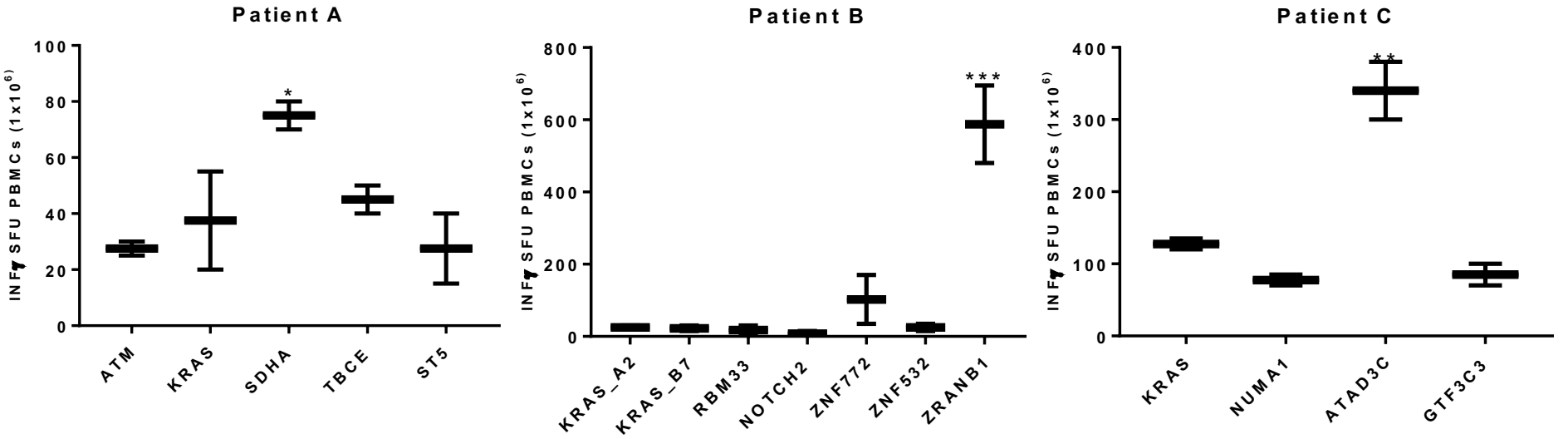
SPORE Project 3



Preliminary Data

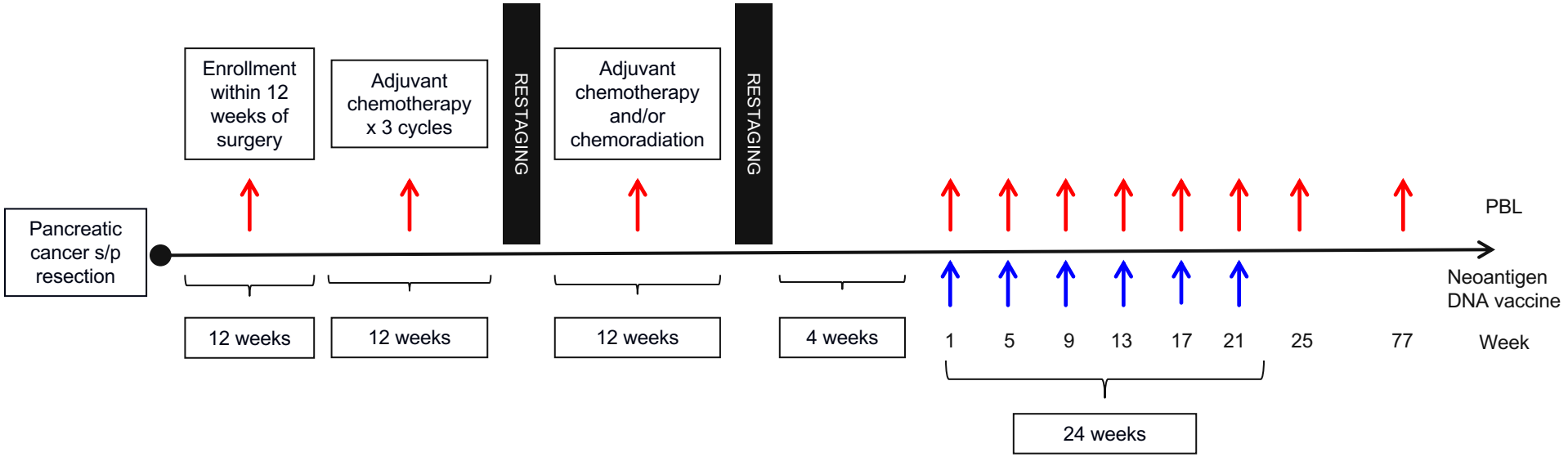


Patients can Recognize Neoantigens

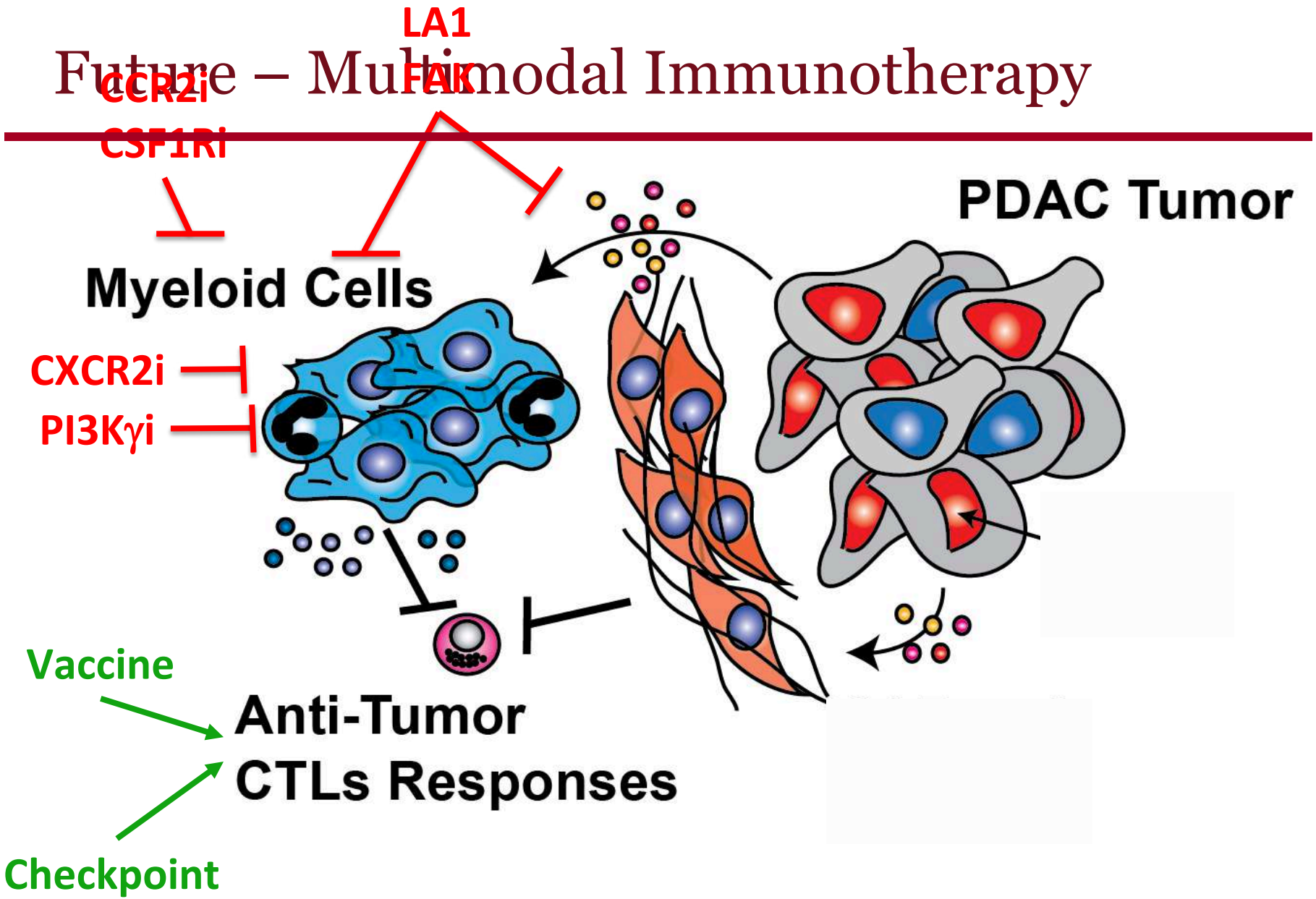


Patient	MHC Class I	MHC Class II	Unique Neoantigens
A	5	7	7
B	6	7	13
C	4	4	7

Clinical Trial Schema



Future – Multimodal Immunotherapy



Project Conclusions

- Immunotherapy is very promising but has yet to be effective in pancreatic cancer
- Will likely require reversal of myeloid derived immunosuppression combined with immunotherapy
- A personalized vaccine approach to each individual tumor may be required

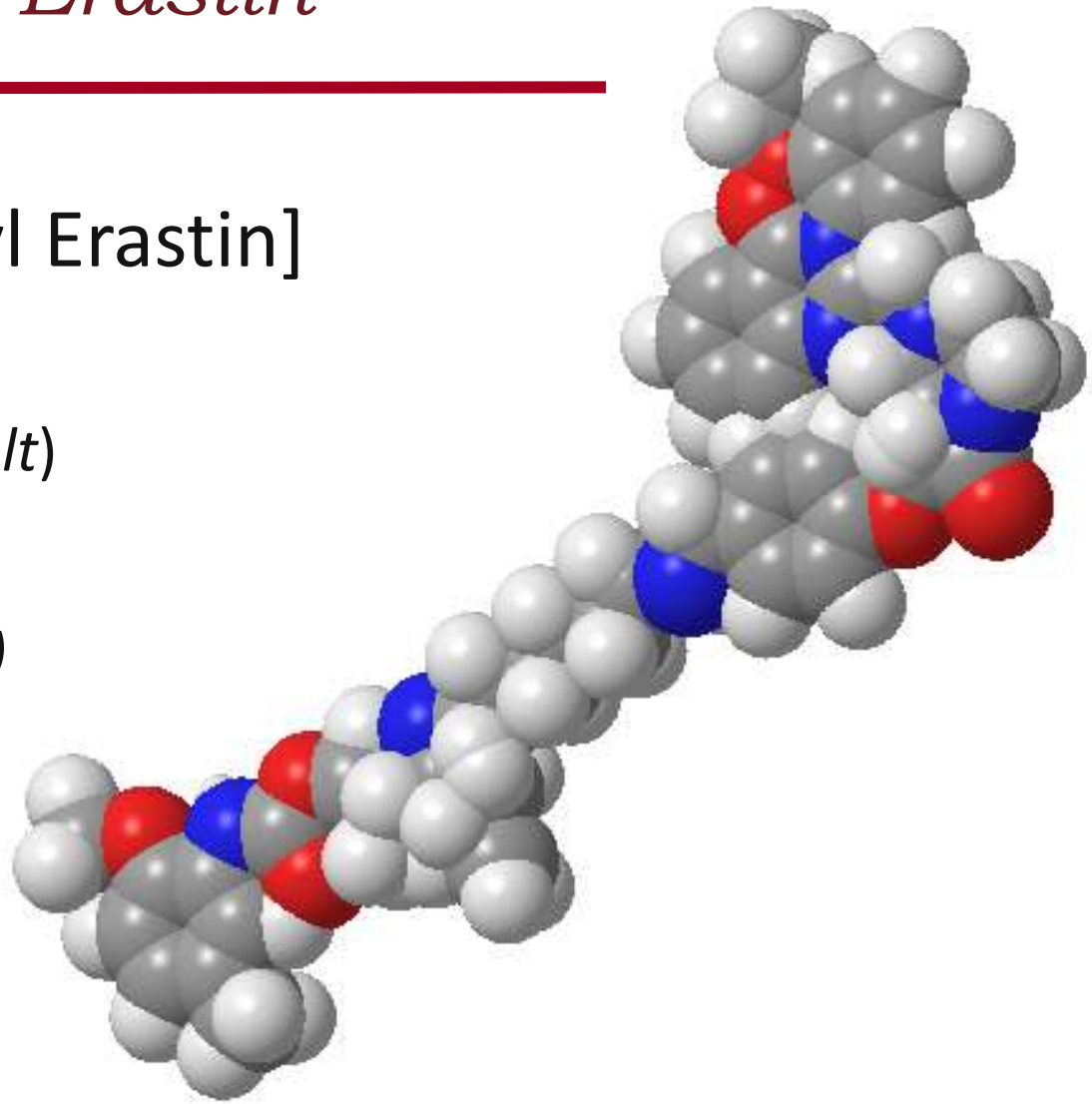
Sigma-2 Erastin

[SV119 Des-methyl Erastin]

SW V-49s (*Oxalate salt*)

&

ACXT 3102 *(HCL salt)*

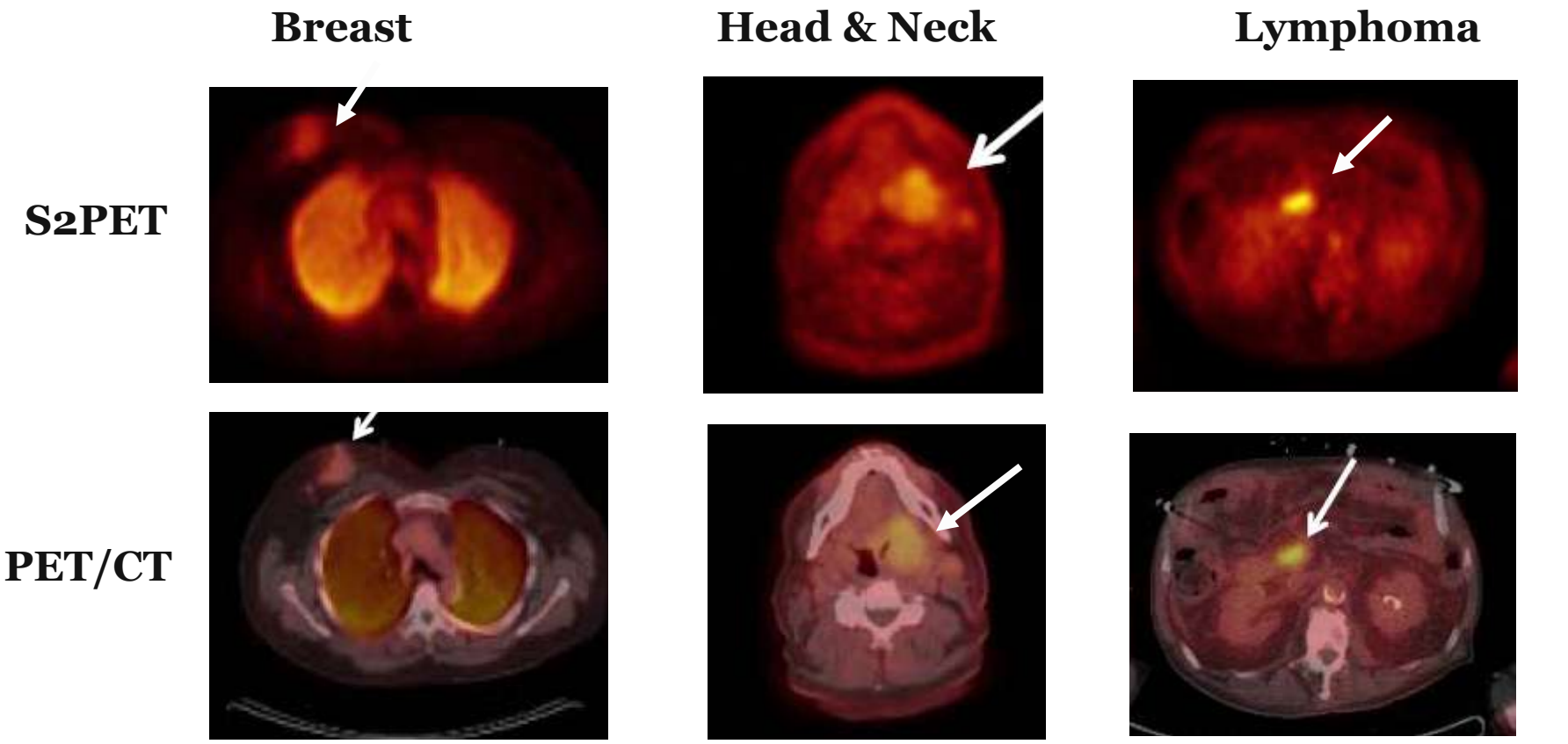


Background

- Sigma-2 receptors are over-expressed in proliferating tumor cells.
- Sigma-2 ligands are small molecules that bind these receptors with nanomolar affinity.
- At higher doses, sigma-2 ligands cause cell death in pancreatic cancer.
- Sigma-2 ligands rapidly enter the cancer cells, making them valuable tools for tumor imaging and drug delivery concepts.

Sigma-2 ligands localize to cancer

Clinical trial with a ^{18}F labeled Sigma-2 ligand



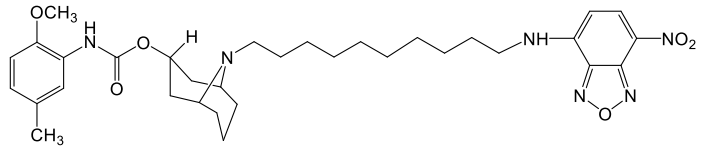
SUV = 3.5
T:M = 4.4

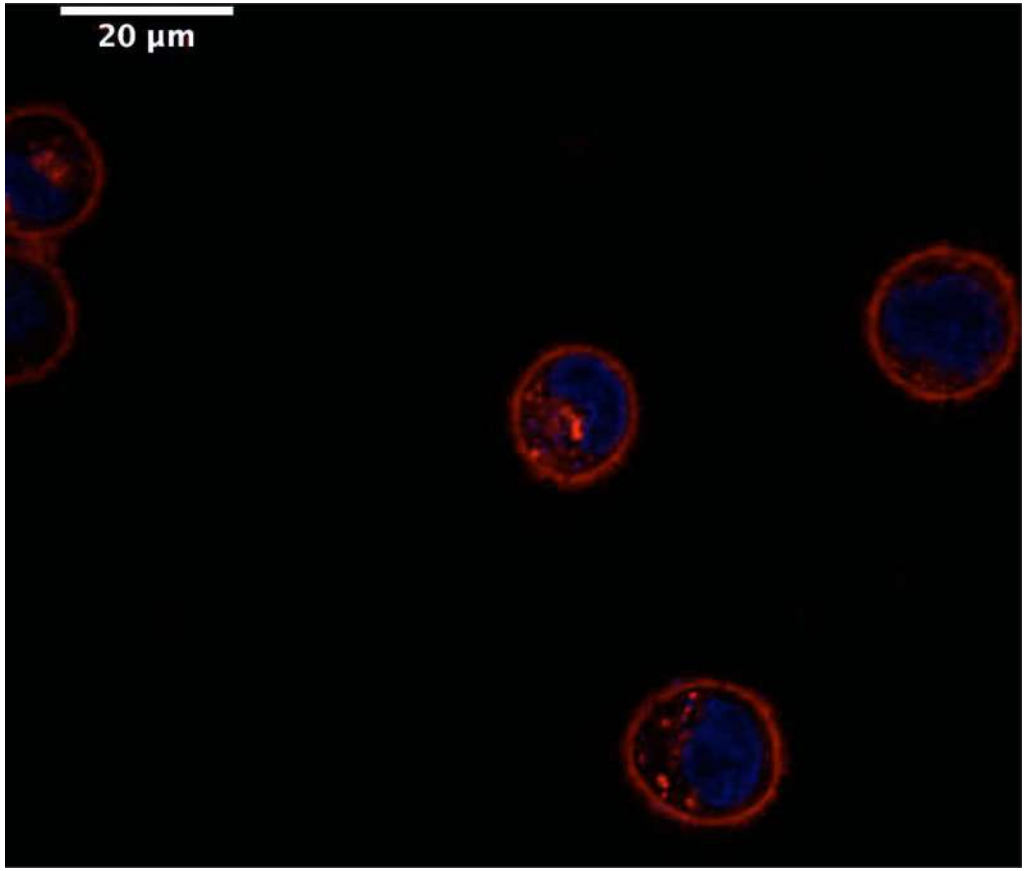
SUV = 2.9
T:M = 3.6

SUV = 11.1
T:M = 11.1

J Nucl Med 2013

Confocal Time Lapse Imaging of Cell Uptake



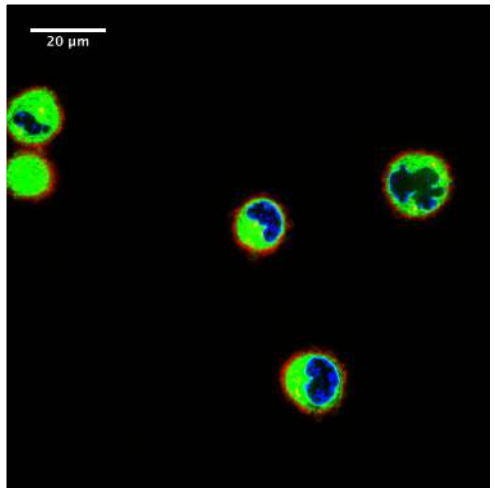


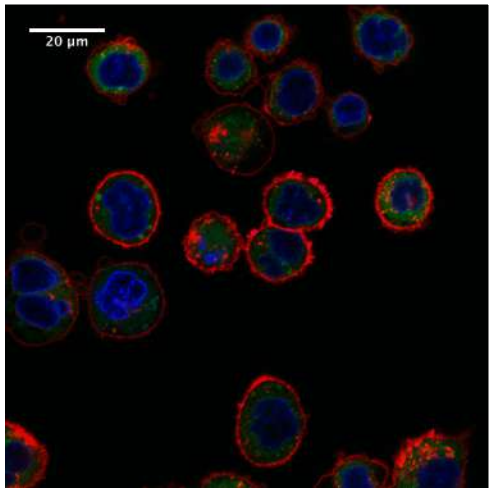
Blue – DAPI
Red – Cellmask Plasma Membrane
Green – SW120

S2 Uptake is Receptor Mediated

Comparison of SW120 uptake in ASPC-1

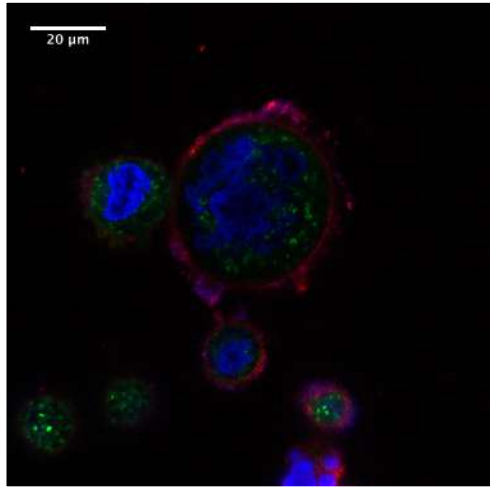
Control

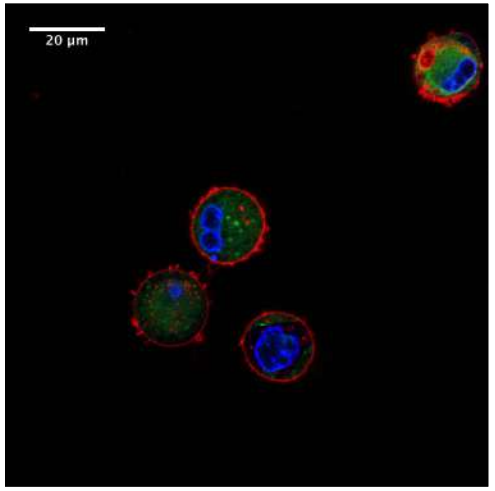




SW43

4 °C





Pitstop2

Using S2-ligands to deliver drugs

- Sigma-2 ligands as a drug platform to deliver death-inducing small molecule cargoes more efficiently to the cancer targets
  - Tumor-selective delivery of drugs to pancreatic cancer using Sigma-2 Ligands (SV119, SW43)
  - Tumor-selective death through delivery of death-inducing drug cargoes into the cancer cells (other small molecules)

Sigma-2 ligand	Cargo	Conjugate name	Publication date
SW43	none	N/A	2010/2012
SV119	none	N/A	2007/2009
SW43	NBD (fluorophore)	SW120	2007/2011
SV119	NBD (fluorophore)	K05-138	2007
RHM-1	none	N/A	2005/2011
ISO-1	none	N/A	2013
SV119	BH3-only Bim peptide	σ2/Bim	2012
SV119	CTMP-4 peptide	σ2/CTMP-4	2012
SV119	Rapamycin	σ2/Rapamycin	2012
SV119	SMAC mimetic	σ2/SMAC (SW III-123)	2013/2014
SW43	SMAC mimetic	σ2/SMAC (SW IV-134)	2014
SV119	dm-Erastin	σ2/dm-Erastin **(ACXT-3102)**	2016

Personalizing Cancer Metabolism

Dead

Alive

The Red Line

Cancer Engine

Targeted Metabolic Therapy

Normal Engine

Personalizing Cancer Metabolism

Dead

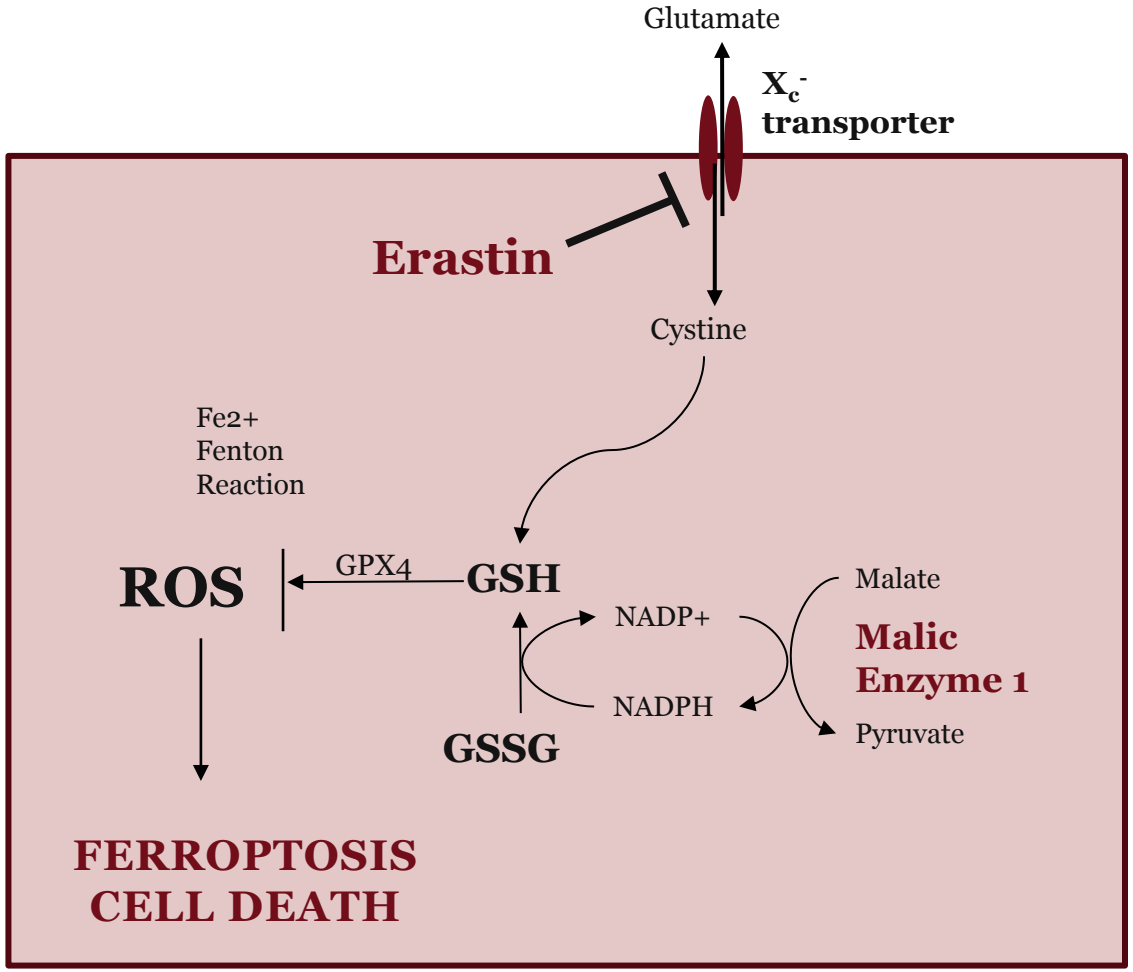
Alive

The Red Line

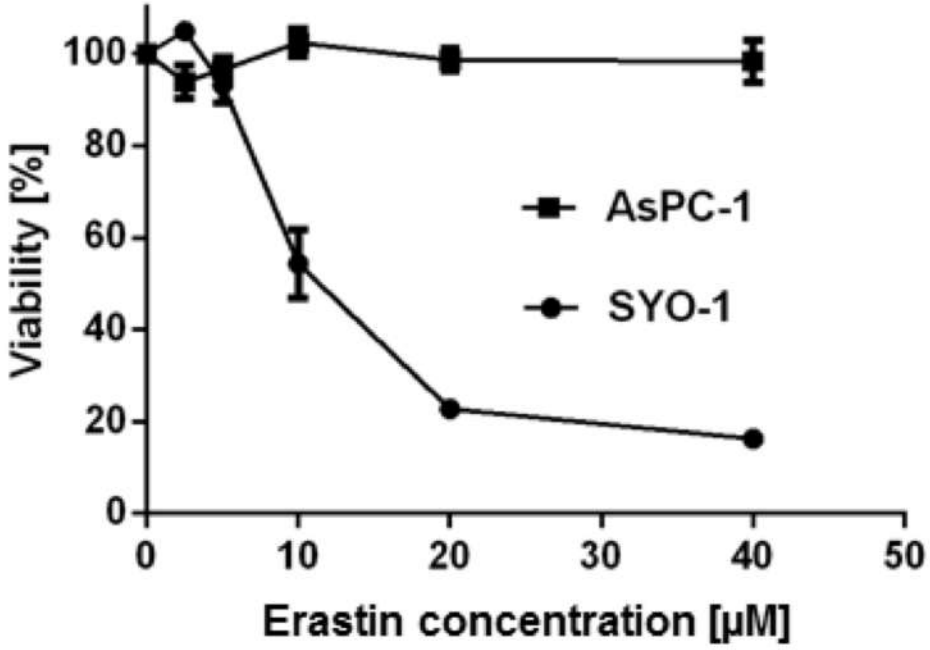
Cancer Engine

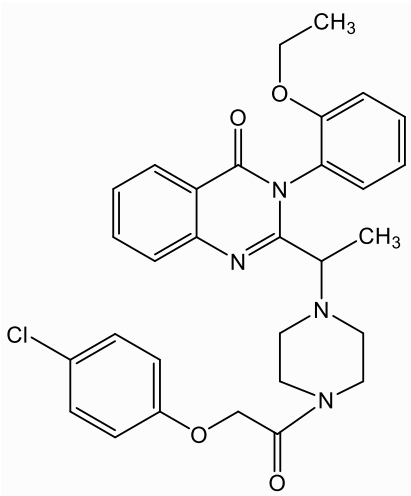
Normal Engine

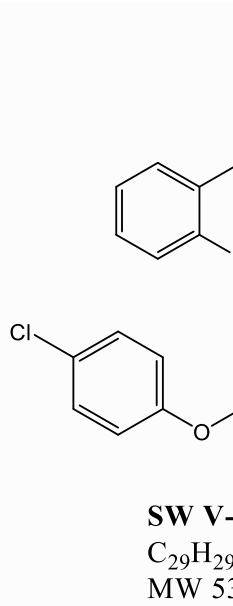
Erastin Causes Cell Death by Ferroptosis



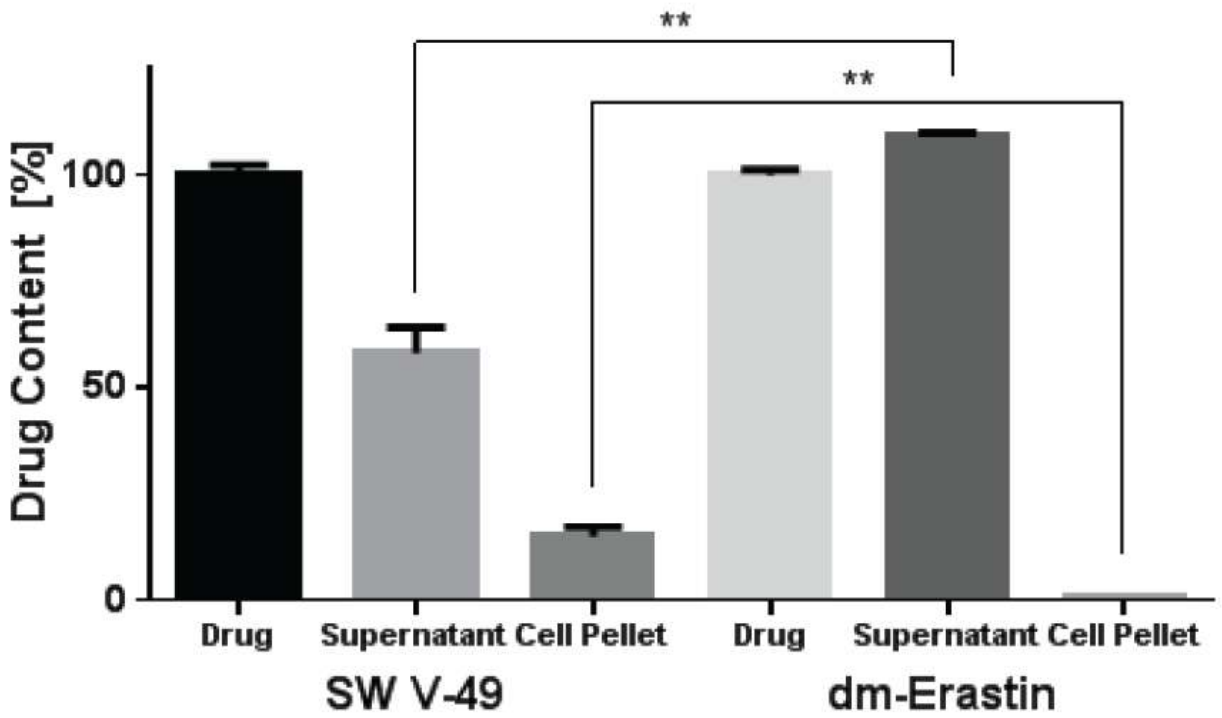
Non-targeted Erastin is unable to induce pancreatic cancer cell death







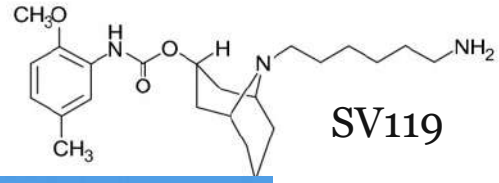
Non-targeted Erastin is not taken up by pancreatic cancer cells



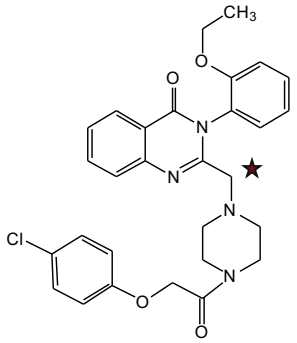
Ohman et al., Oncotarget 2016

SW V-49

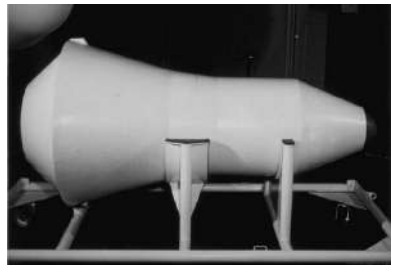
- Formed from conjugation of sigma-2 ligand SV119 and dm-Erastin

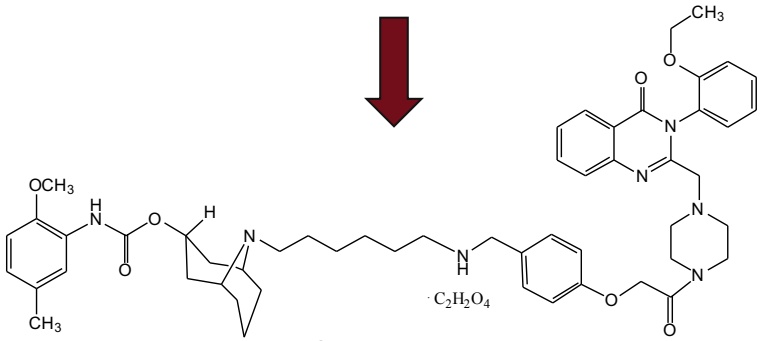


SV119



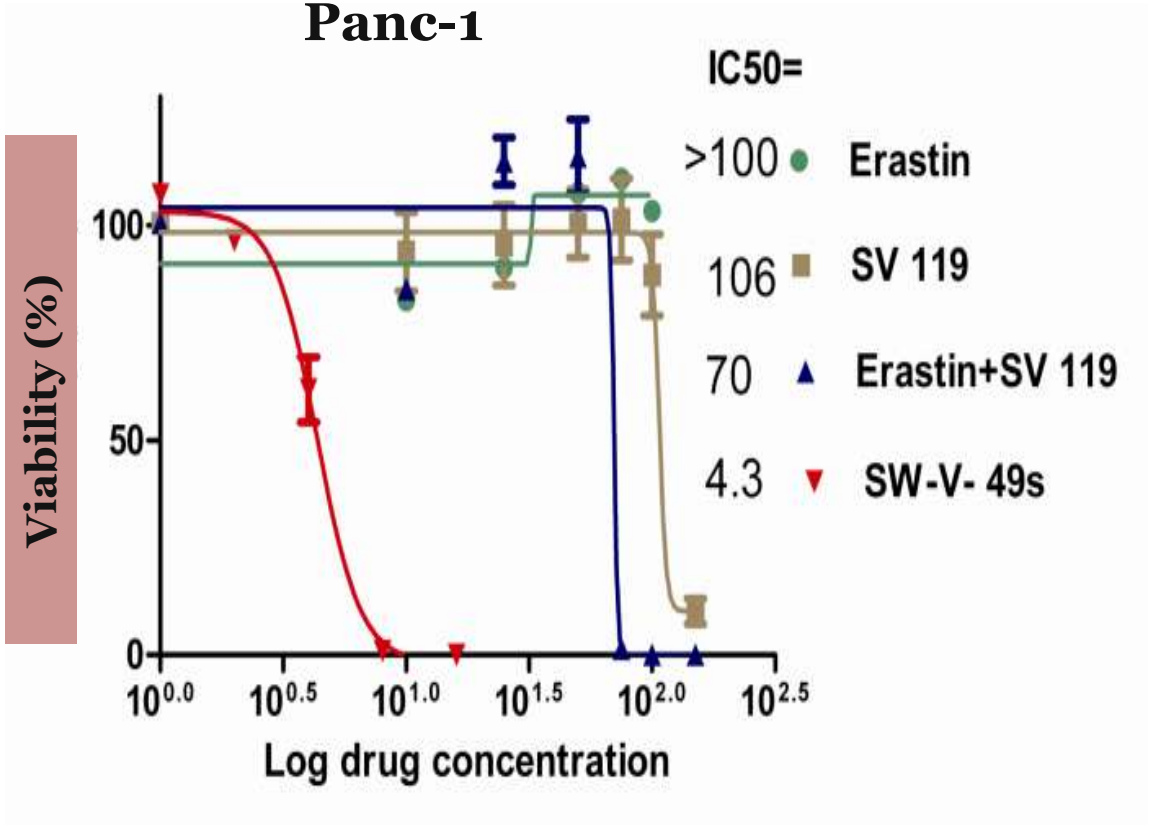
Des-methyl Erastin



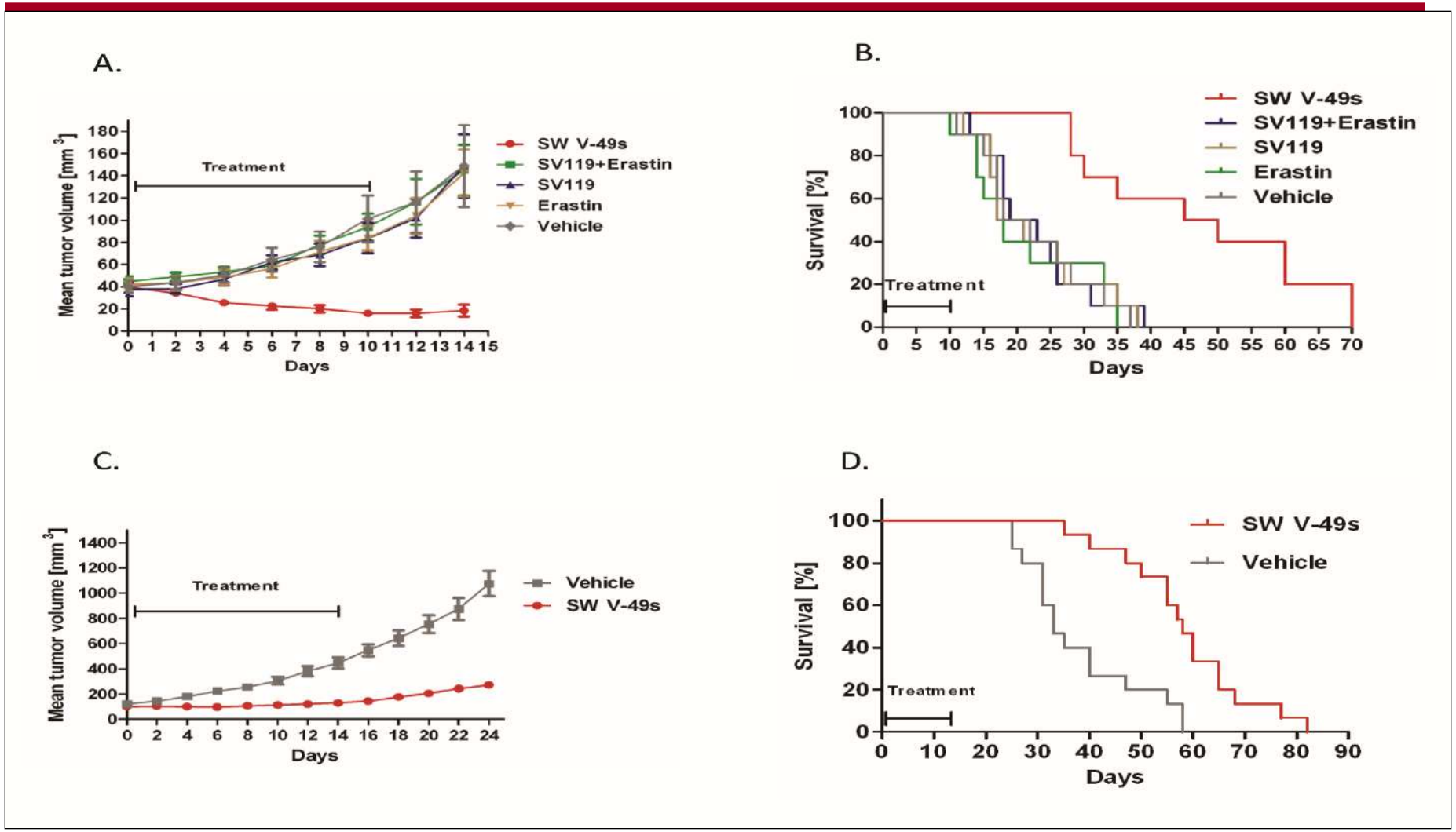


SW V-49

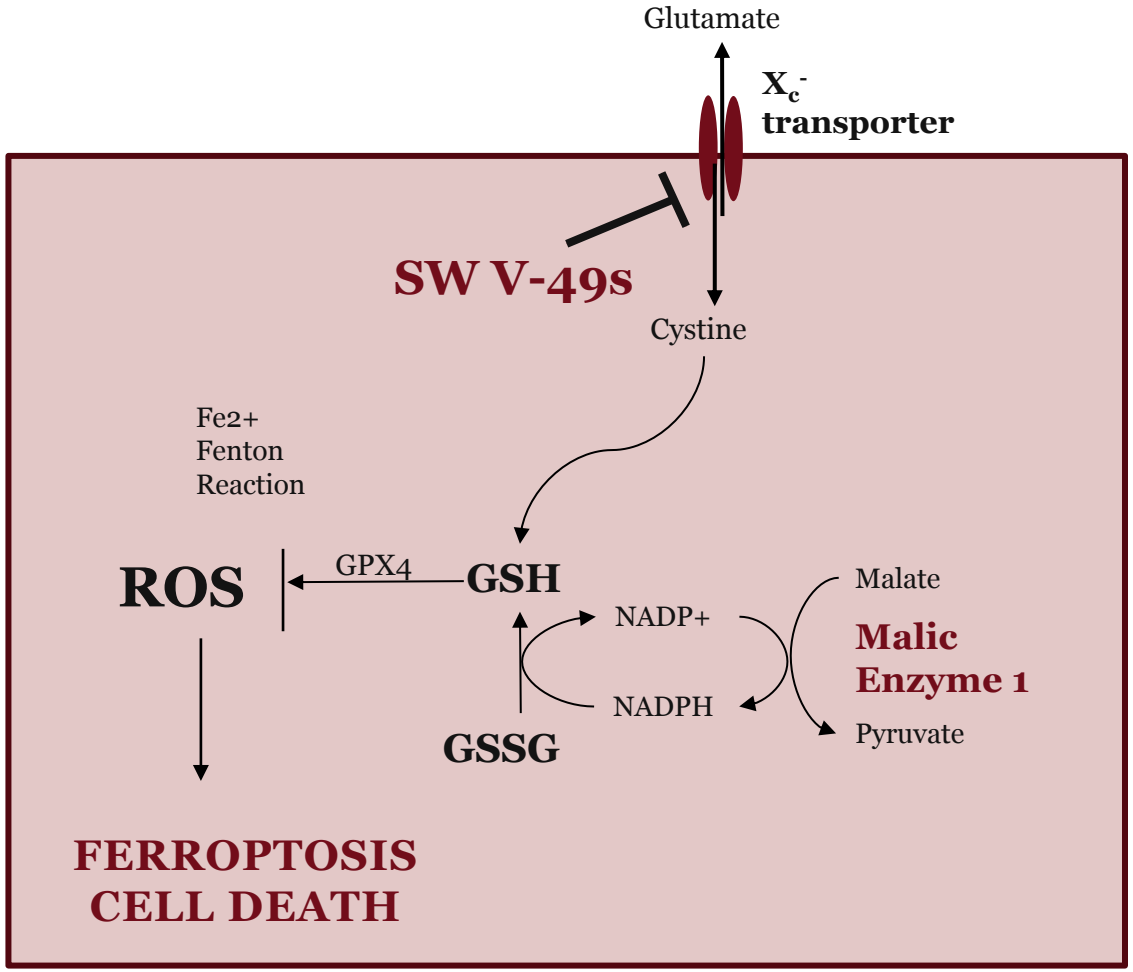
Conjugation allows SW V-49s to efficiently kill pancreas cancer cells



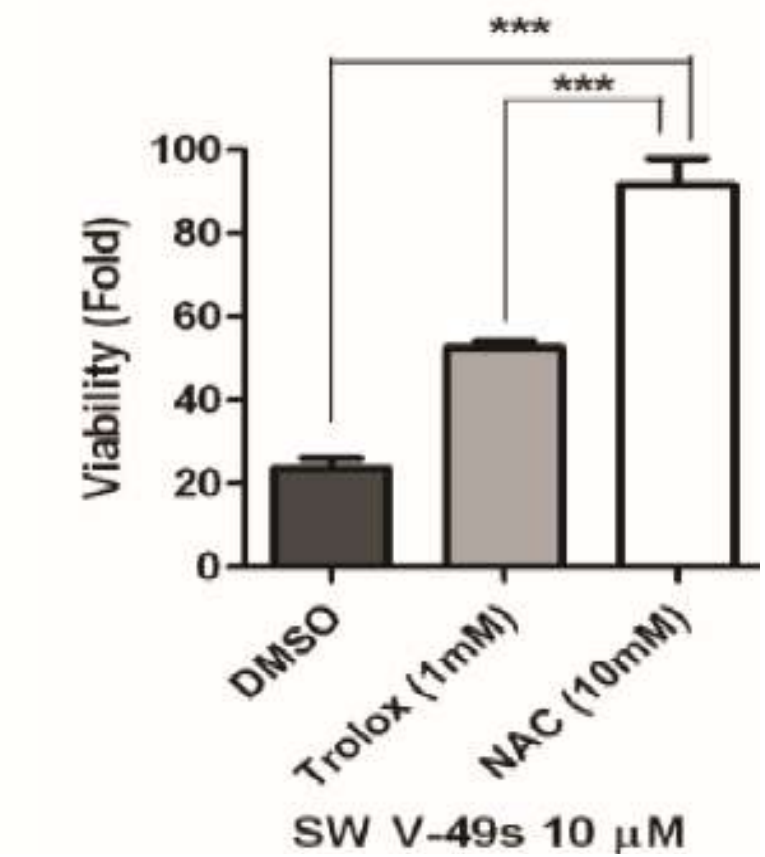
SW V-49s improves survival in pancreatic cancer



SW V-49s Drives Cell Death by Ferroptosis

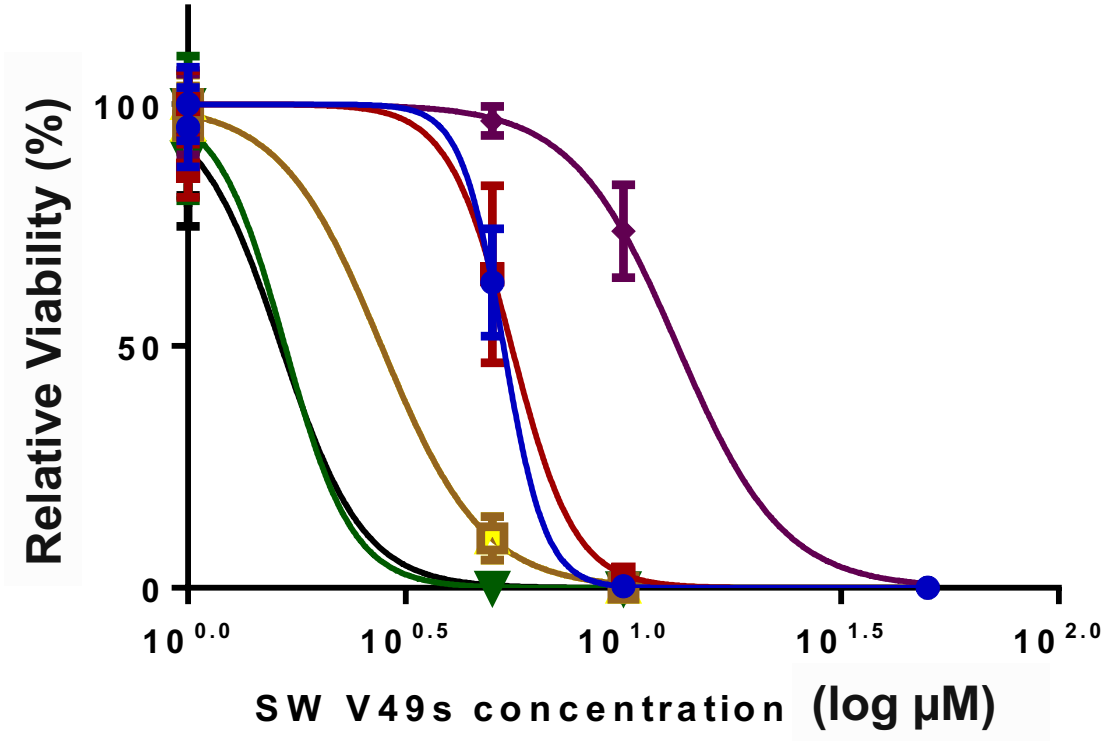


Antioxidants can block mechanism of action



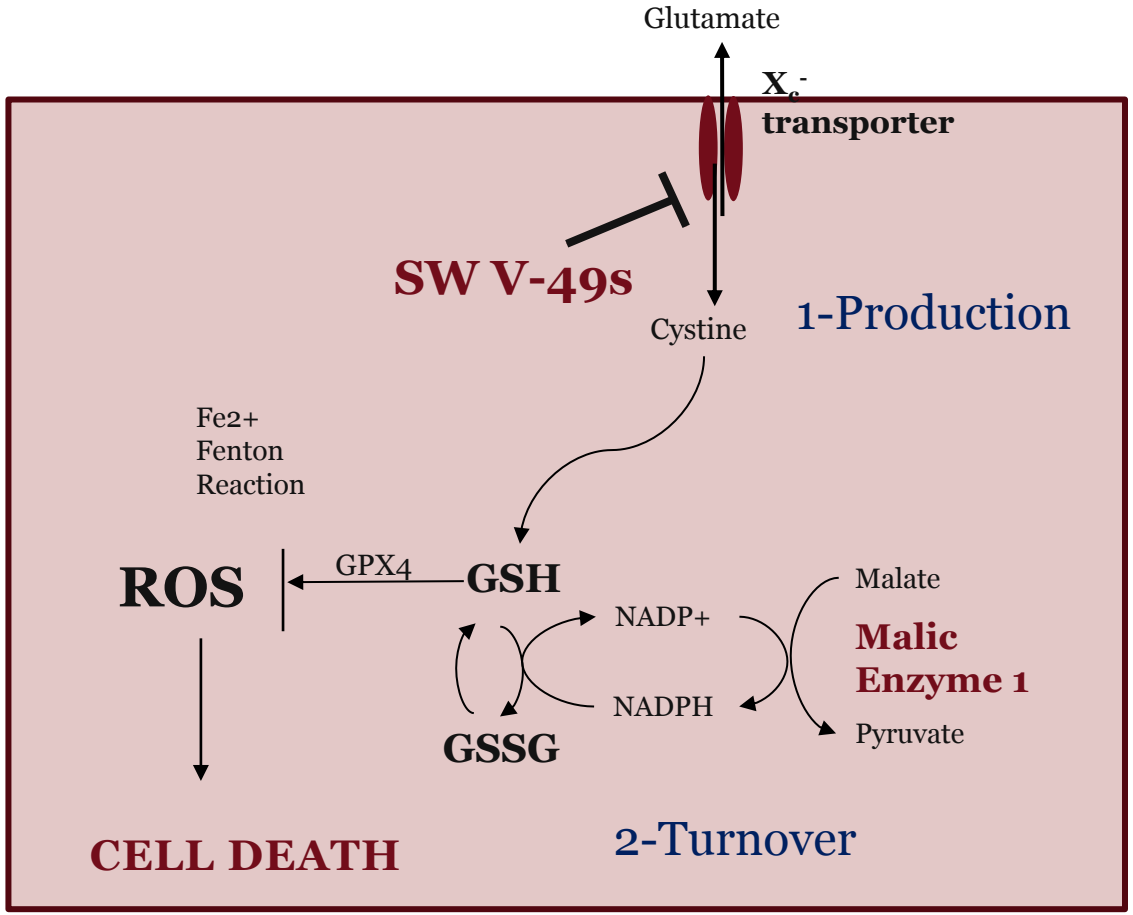
Ohman et al., Oncotarget 2016

SW V-49s is potent across cancer types

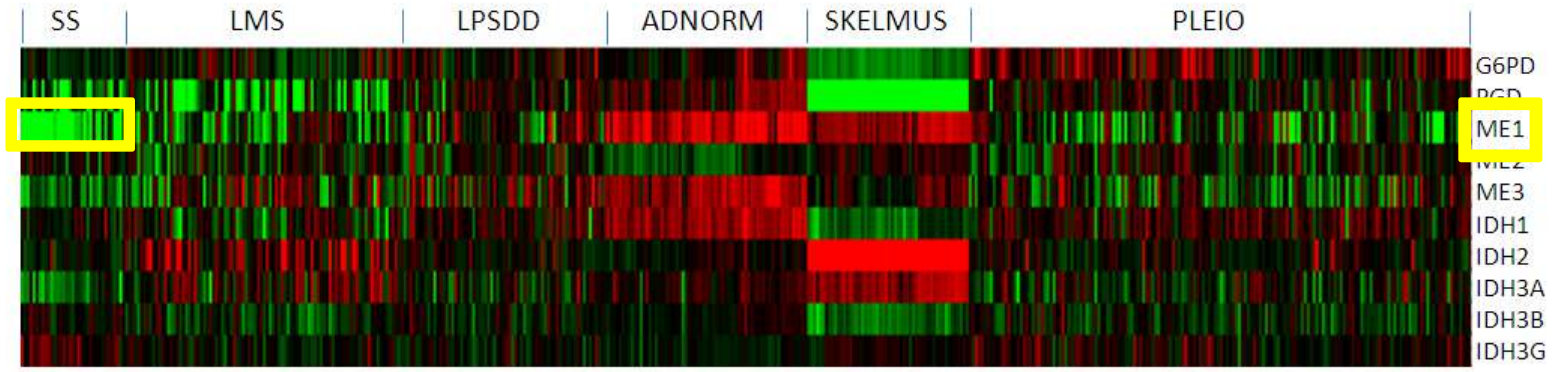


- DM6 (Melanoma)
- HCT116 (Colorectal)
- ASPC1 (Pancreas)
- Miapaca (Pancreas)
- PC3 (Prostate)
- MCF-7 (Breast)
- SYO-1 (Sarcoma)

Glutathione Production and Turnover



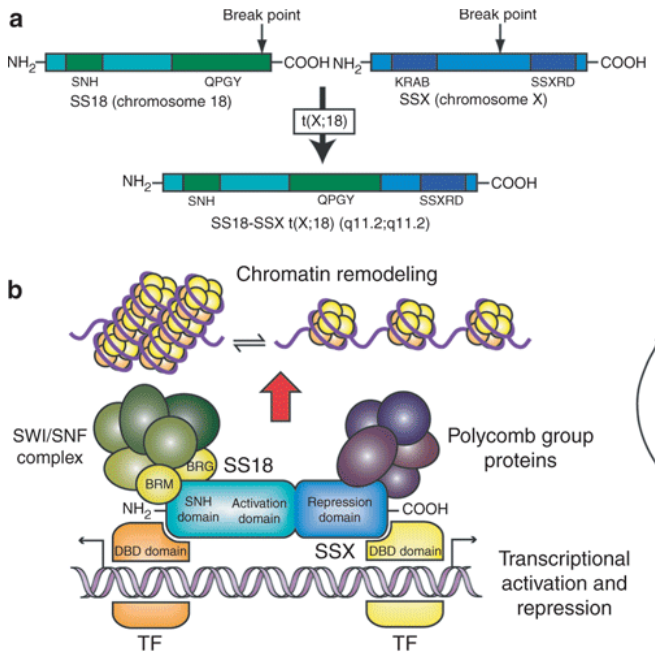
ME1 is a Newly Identified Metabolic Deficiency
Microarray of metabolite panel



-ME1 expression is absent

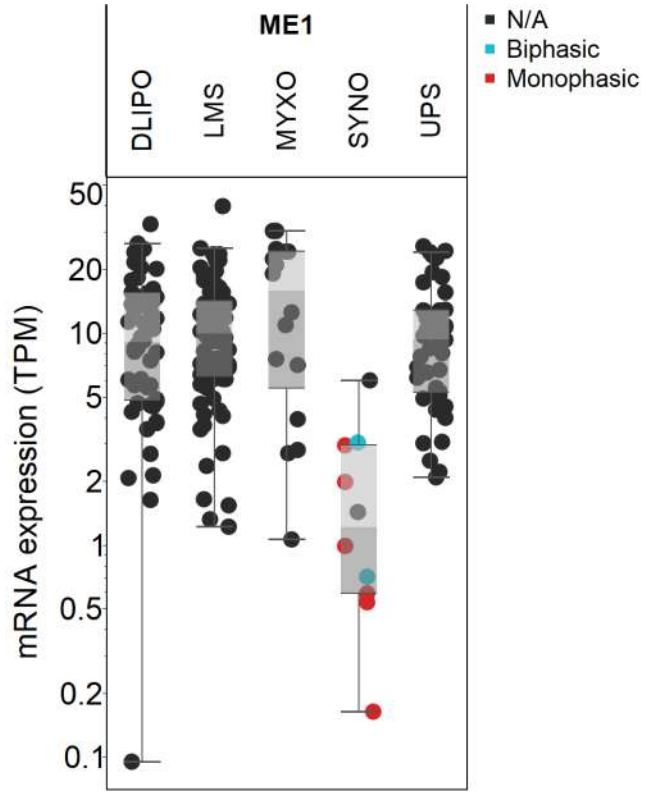
Synovial Sarcoma

- Synovial sarcoma (SS) is a lethal form of soft-tissue sarcoma with high metastatic potential
- Typically diagnosed in young adults between 15-40yo
- SS is associated with a gene fusion between transcription factors SYT and SSX, producing a hybrid transcription factor modulating SWI/SNF chromatin remodeling and gene expression
- No targeted chemotherapy has yet been developed for SS



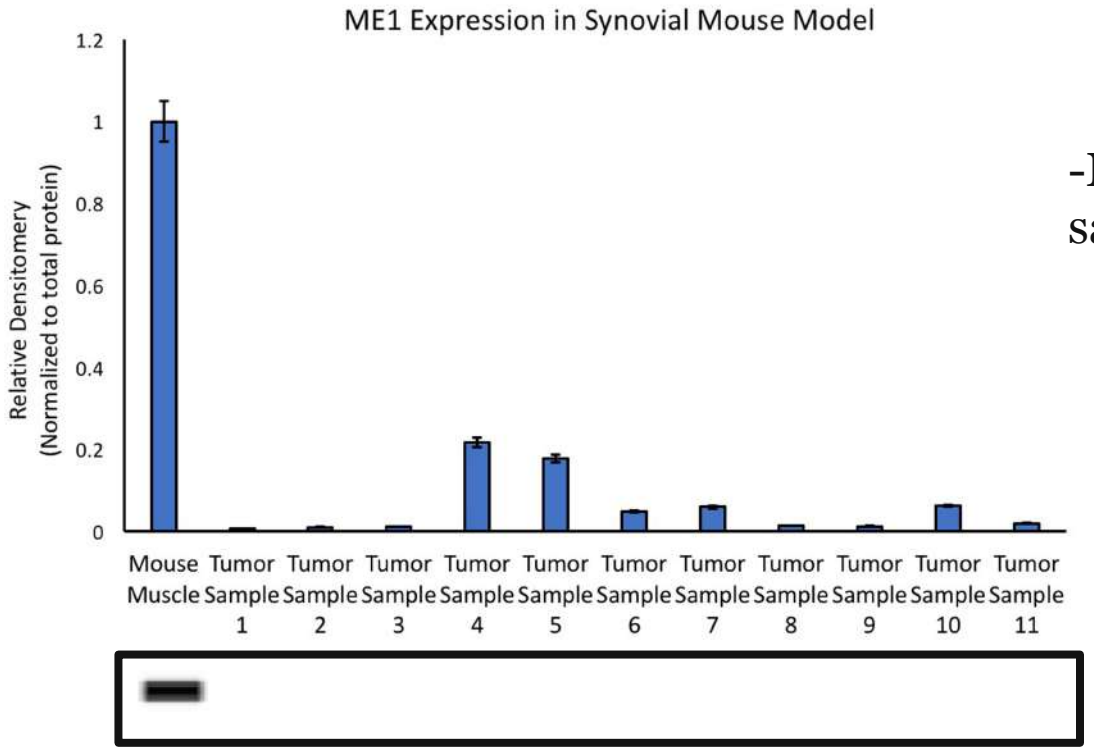
Pediatric Research (2012) 72, 112–121

TCGA – mRNA expression in cohort of Sarcoma



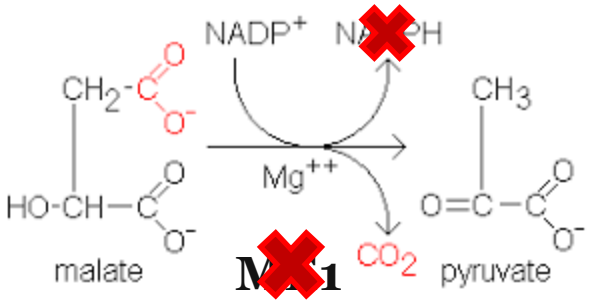
-Cohort of Sarcoma – Synovial Sarcoma have low ME1 expression

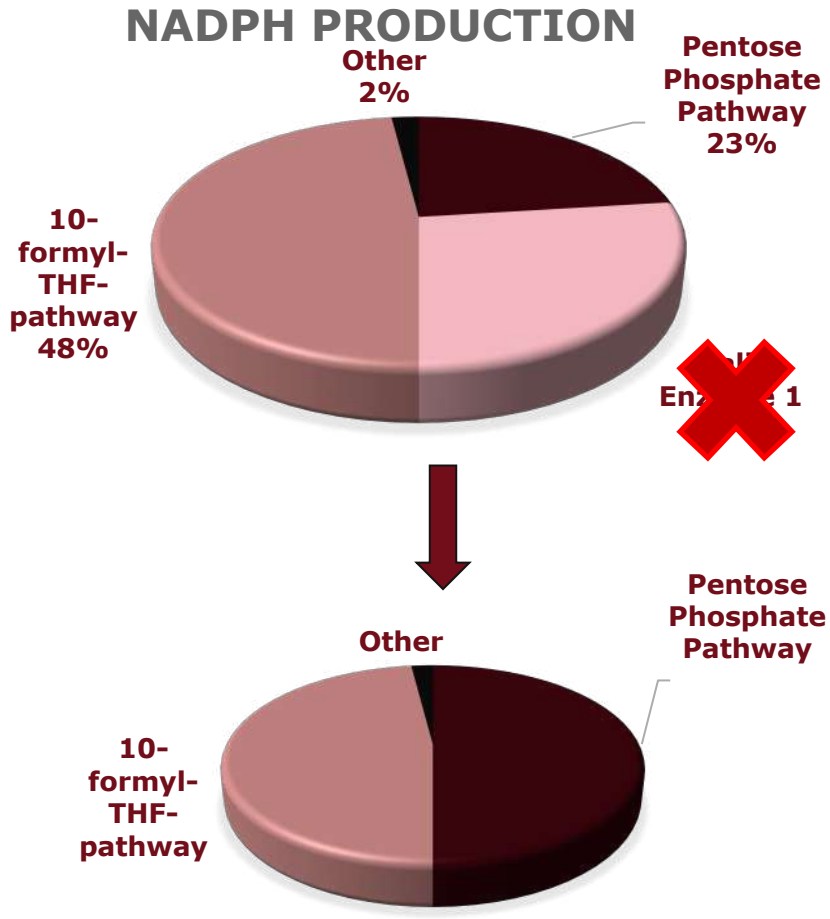
Protein Expression of ME1 in Tumor



-Mouse model of synovial sarcoma does not express ME1

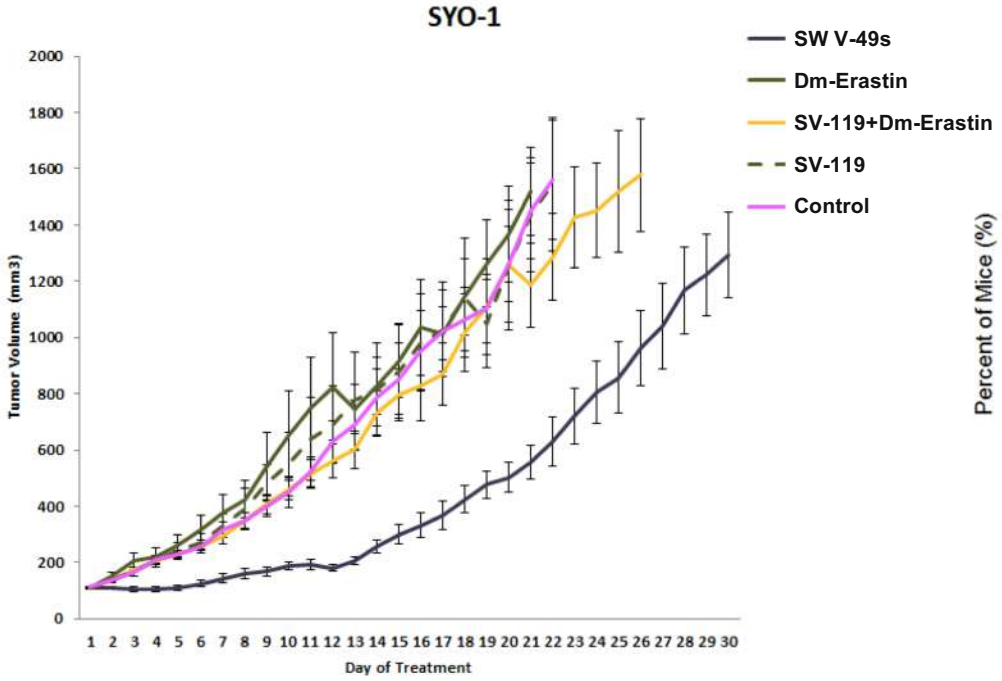
ME1 roles in a cell

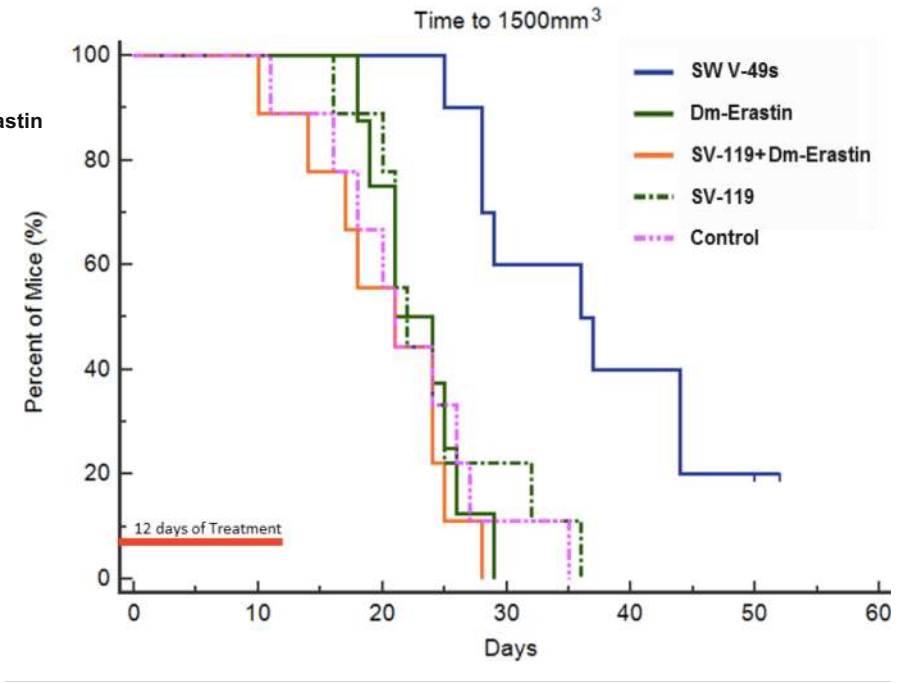




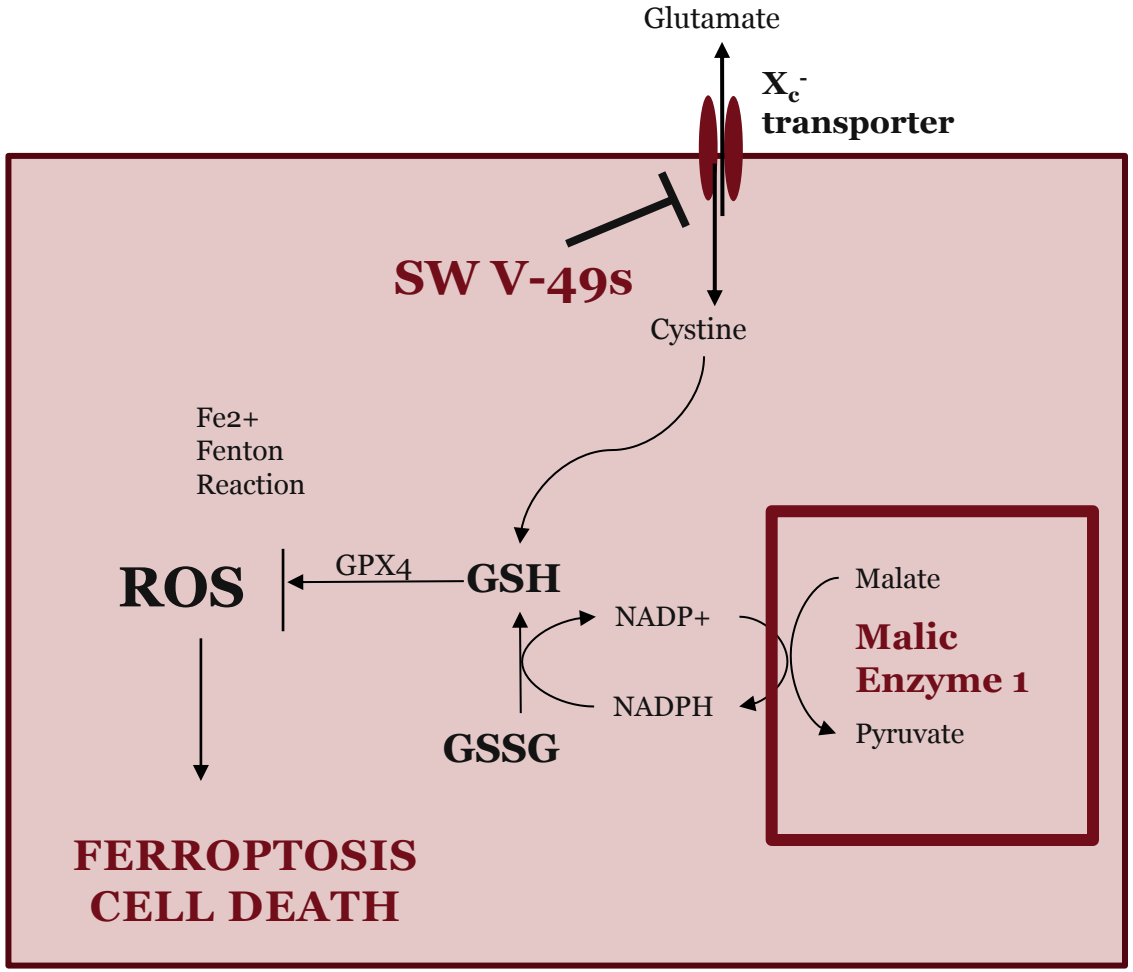
•*Fan et al. Nature* **volume 510**, pages 298–302 (12 June 2014)

Synovial Sarcoma
(potential surrogate for a biomarker)

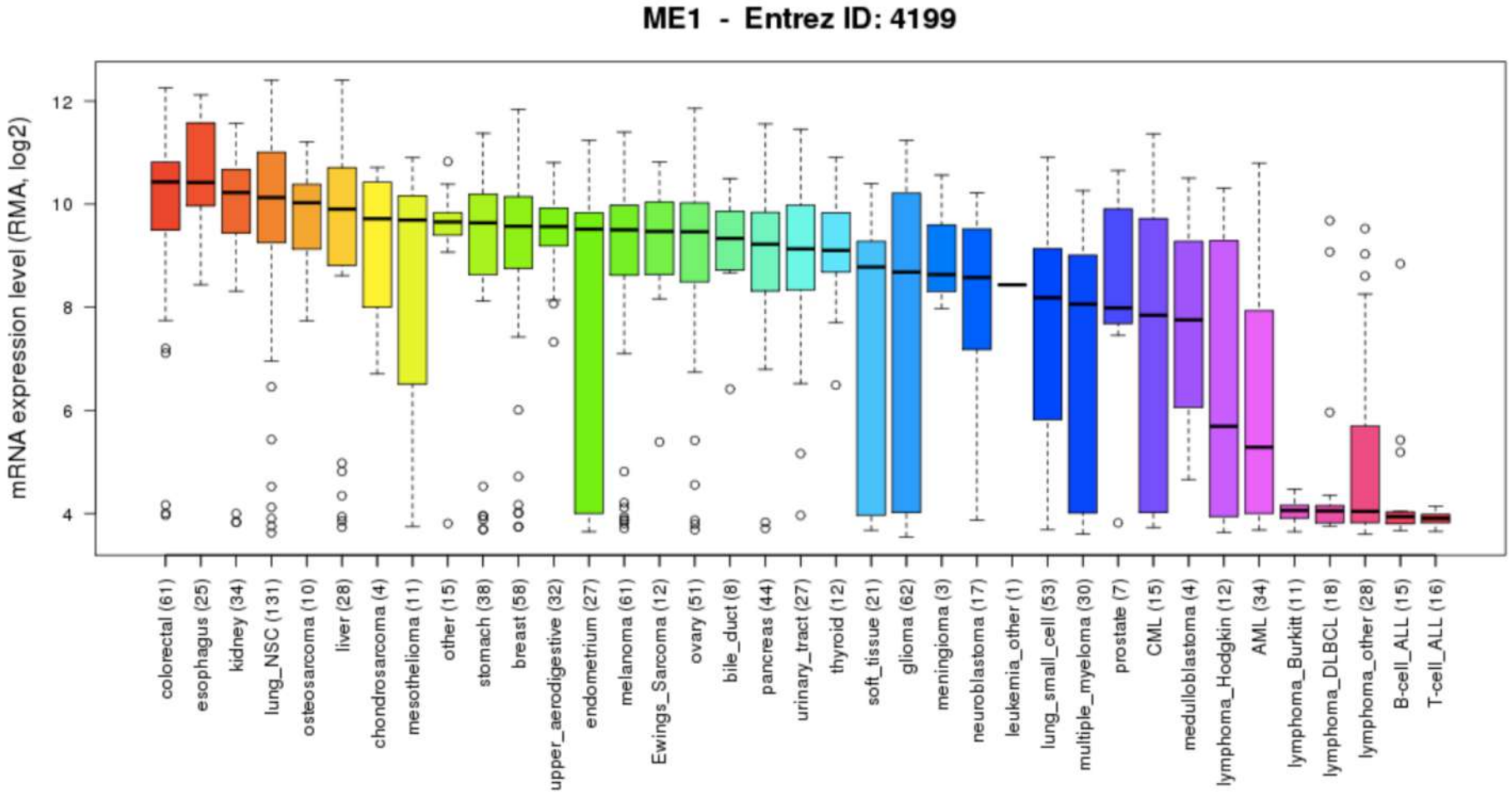




SW V-49s Drives Cell Death by Ferroptosis



Subsets of other tumors are ME-1 Deficient



Academic Summary: SW V-49s

- Sigma-2 ligands efficiently deliver drug cargo to solid tumors and cause internalization into tumor cells
- Sigma-2 conjugated Erastin (SW V-49s) shows promising efficacy in pancreatic and sarcoma models of cancer
- SW V-49s inhibits system xCT and causes ferroptotic ROS mediated cell death
- SW V-49s improves immune response in the tumor microenvironment

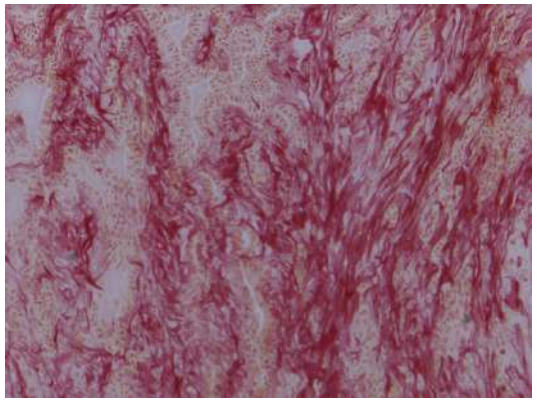
Translation to the clinic—ACXT 3102

- Accuronix Therapeutics was formed in 2016 with IP licensed from Washington University
- So far raised 1.7 million for de-risking experiments.
- Strengths include CMC progress, intellectual property position, market analysis.
- Challenges include (tight therapeutic window/ uncertain dosing)
- Currently looking for a Series A partner

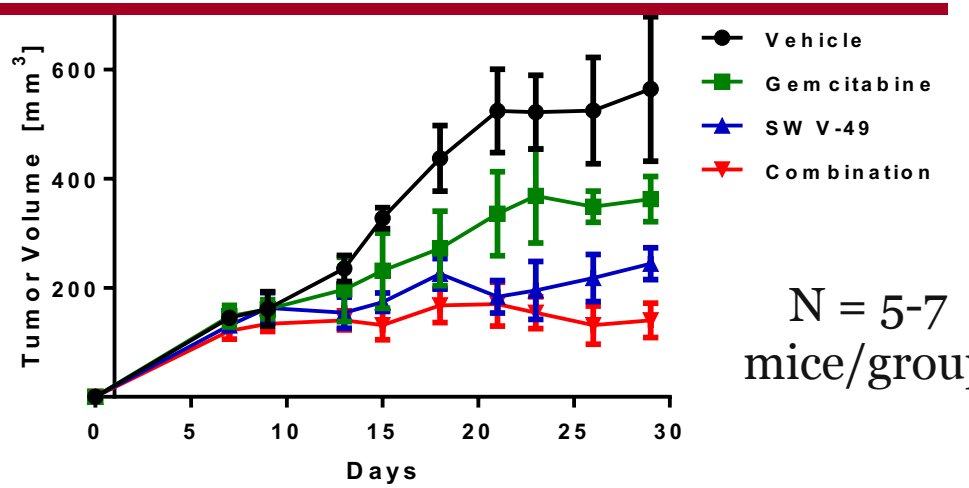
Combination therapy successful in stroma-dense models of pancreatic cancer

- KP-2 subcutaneous tumor model
- Treatment groups:
  - Vehicle
  - Gemcitabine
  - SW V-49
  - Combination

A

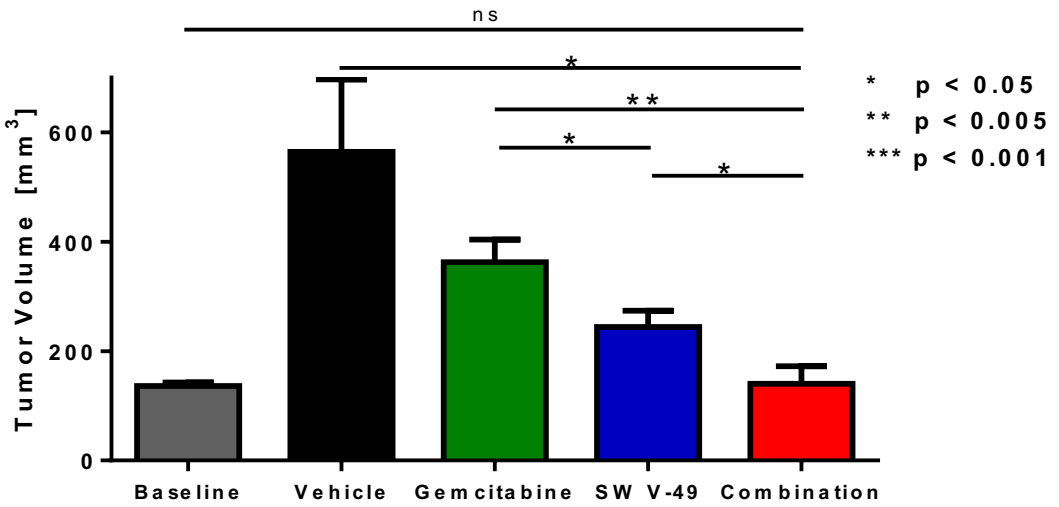


B

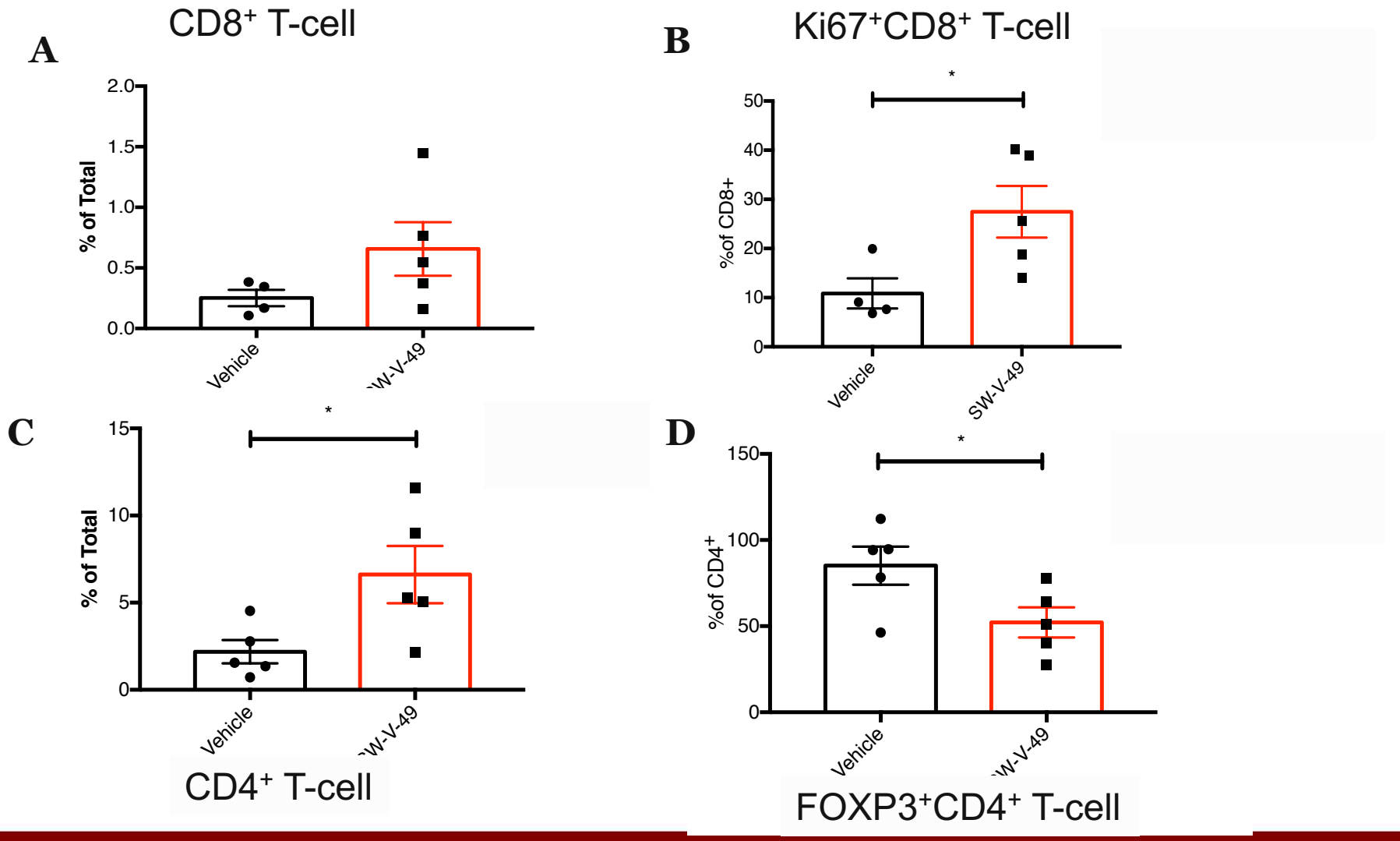


N = 5-7 mice/group

C



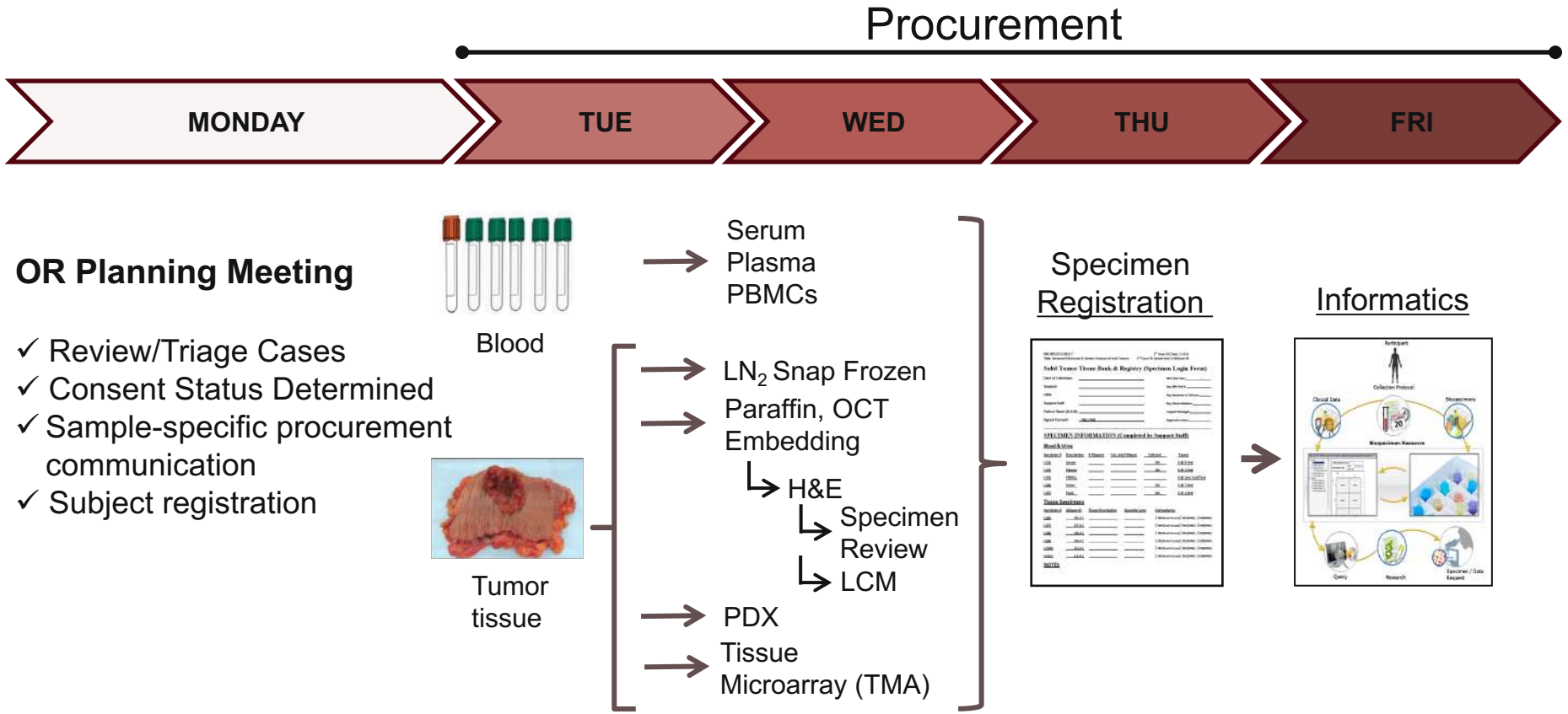
SW V-49s Improves the Immune Environment



Bench to the Bedside—CLINCAL TRIALS!

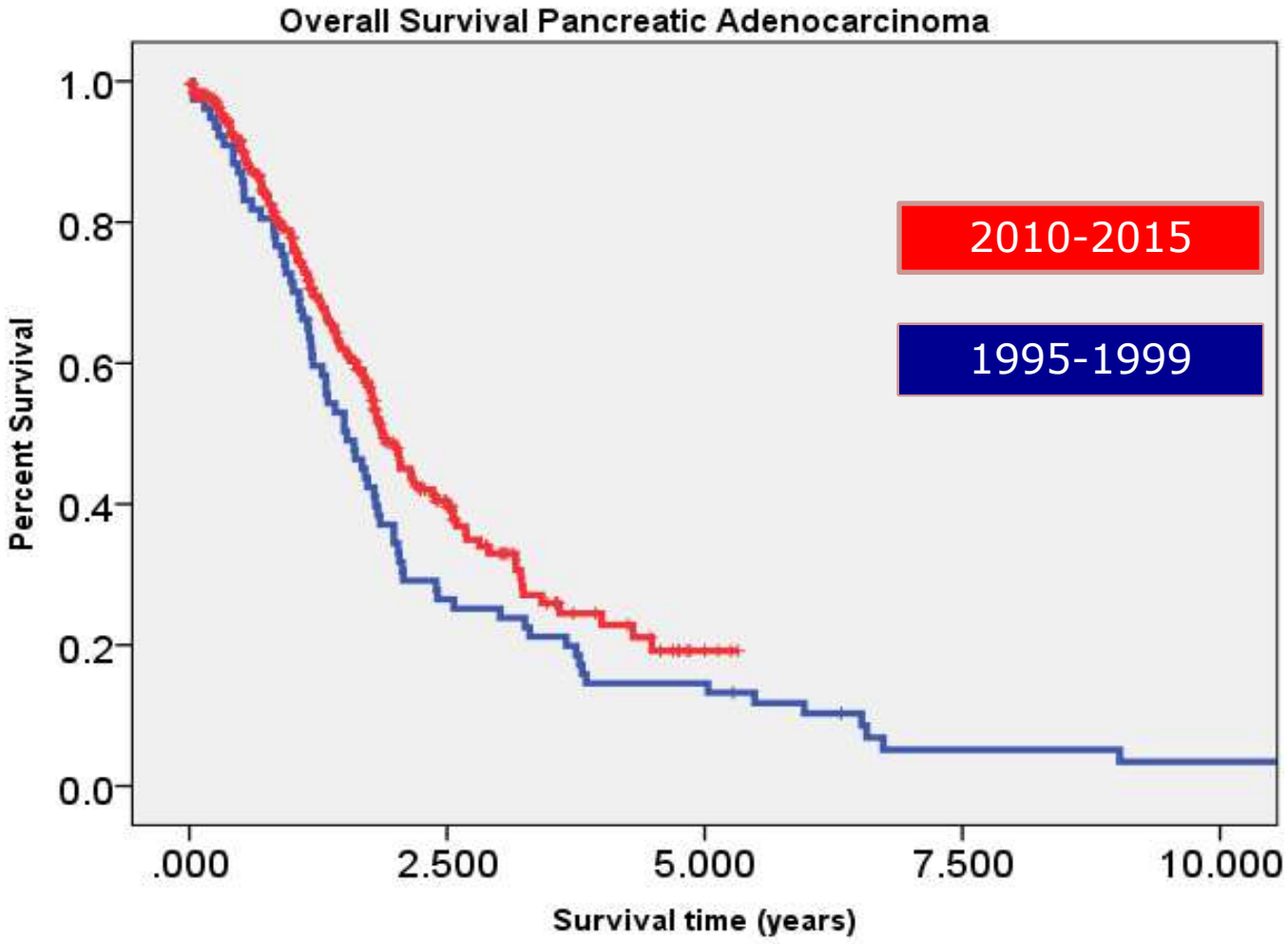
Short Title	Open To Accrual	Total Accrual Goal	Number Accrued	Phase	NCT Number
Gem/Abraxane/RT in pancreas	1/21/2015	35	26	I	NCT02283372
Indoximod+Gem+Abraxane in Metastatic Pancreatic Adeno	2/12/2015	20	19	I/II	NCT02077881
Maintenance Olaparib/Placebo in gBRCA+ Pancreatic	4/20/2015	2	1	III	NCT02184195
FOLFIRINOX + Ipi +/- Tumor Vaccine in Pancreatic	6/2/2015	25	17	II	NCT01896869
CL002-872	7/2/2015	20	17	I	NCT02345408
Tosedostat + Cape in Pancreatic	8/31/2015	36	16	I/II	NCT02352831
Palbociclib + Abraxane in Metastatic Pancreatic Ductal Adenocarcinoma	12/21/2015	15	10	I	NCT02501902
A Phase I Study of VS-4718, a Focal Adhesion Kinase Inhibitor, in Combination with Nab-paclitaxel and Gemcitabine in Subjects with Advanced Cancer	2/2/2016	15	4	I	NCT02651727
mFOLFIRINOX vs Gem/Abraxane in Resectable Pancreatic (CIRB S1505)	3/31/2016	6	1	II	NCT02562716
BVD-523 + Gem+Abraxane in Metastatic Pancreatic	6/6/2016	25	10	I	NCT02608229
FOLFIRI vs mFOLFIRI+Veliparib in Pancreatic (CIRB S1513)	1/11/2017	8	1	II	NCT02890355
BL-8040 + Pembro in Pancreatic (COMBAT)	2/14/2017	5	3	II	NCT02826486
BBI-608 + Abraxane/Gem in Pancreatic (CanStem111P)	8/17/2017	15	8	III	NCT02993731
Cabiralizumab +/- Chemo in Panc Cancer	12/15/2017	10		II	NCT03336216
Personalized DNA vaccine for pancreatic cancer	1/5/2018	15	1	I	NCT03122106

Back to the Bench-- Tissue Core



13,700 Specimens
1/2018

Adenocarcinoma of the Pancreas
Overall Survival



A Team Approach Creates Hope for Progress on Pancreatic Cancer

https://www.youtube.com/watch?v=Fl2ZrN-c410

Thank You's

Inspiring team's of people who make what seems impossible possible

Trainees are our Future Researchers

Immunology



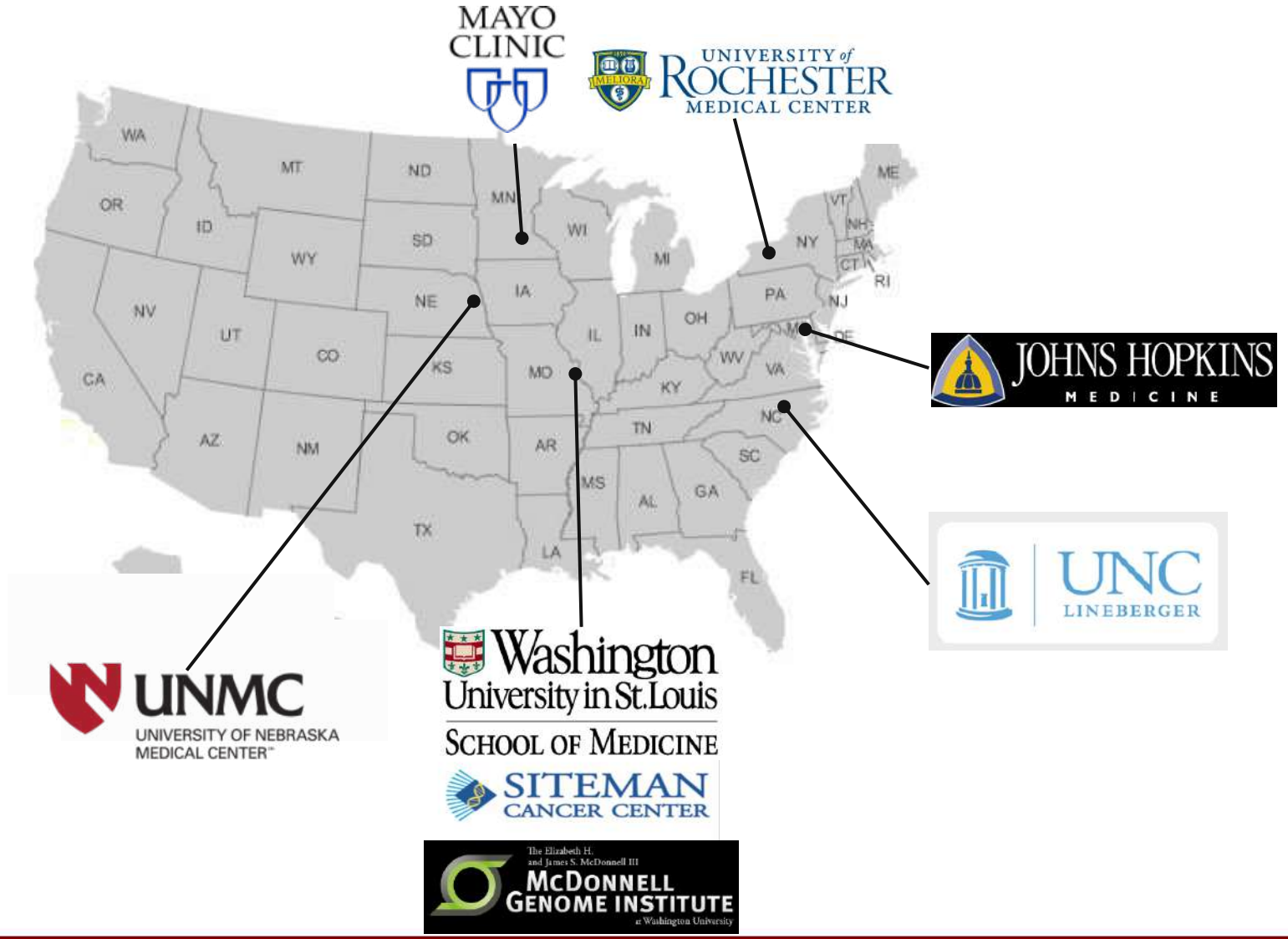
Clinical Outcomes

Drug Targeting

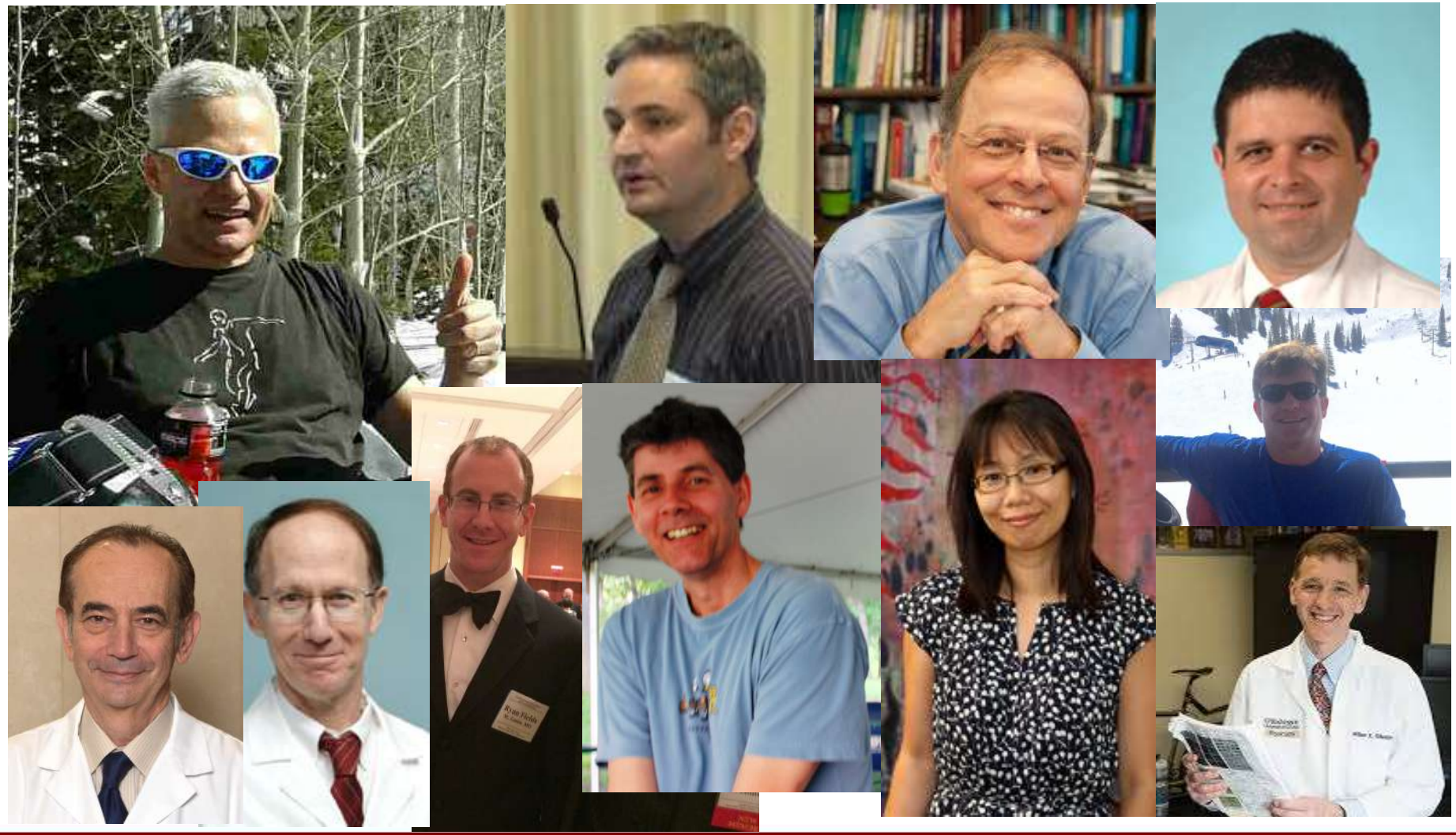



Oncology Basic Science
Training Grant
T32-CA009621-29

SPORE – Collaborators



Collaborative Teams



PEDAL THeCAUSE

SITEMAN CANCER CENTER
A NATIONAL CANCER INSTITUTE COMPREHENSIVE CANCER CENTER

PANCREATIC CANCER ACTION NETWORK
PanCAN
WORKING TOGETHER FOR A CURE

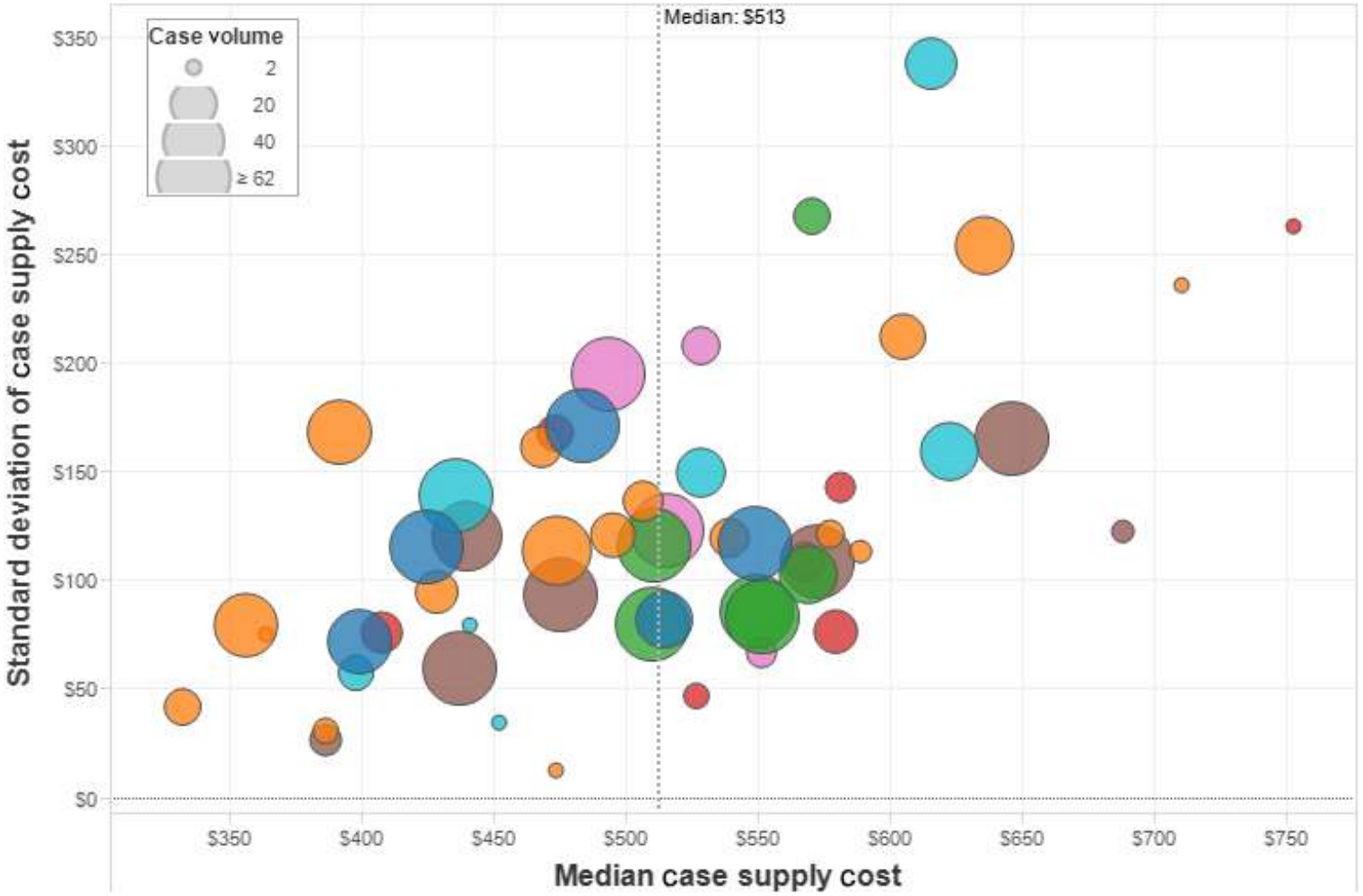
NIH National Institutes of Health
Turning Discovery Into Health

Department of Veterans Affairs

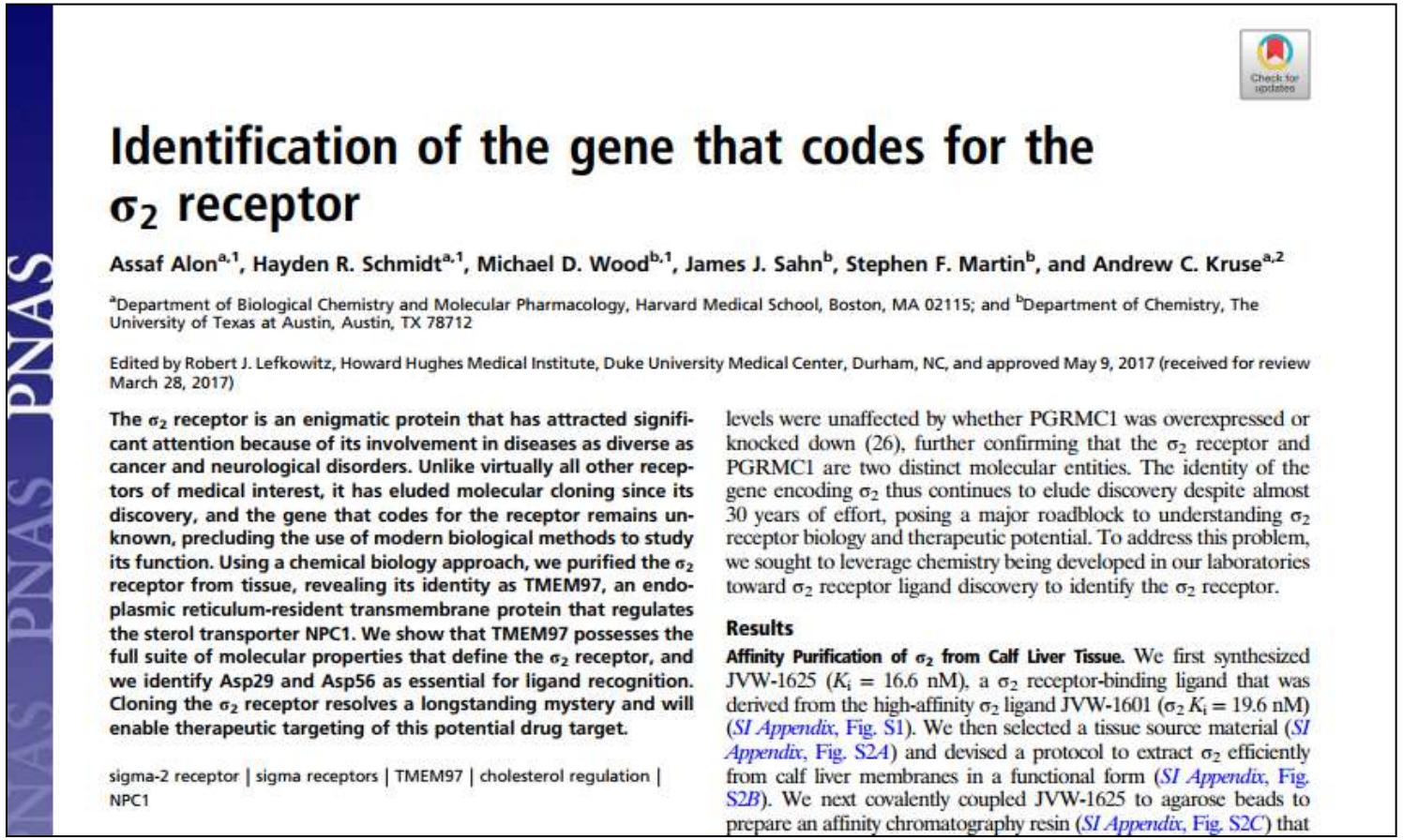
BARNES-JEWISH Hospital
BJC HealthCare

American Cancer Society

Patient Outcomes and Cost Effectiveness



Sigma-2 Receptor



PNAS

Identification of the gene that codes for the σ_2 receptor

Assaf Alon[a,1], Hayden R. Schmidt[a,1], Michael D. Wood[b,1], James J. Sahn[b], Stephen F. Martin[b], and Andrew C. Kruse[a,2]

[a]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115; and [b]Department of Chemistry, The University of Texas at Austin, Austin, TX 78712

Edited by Robert J. Lefkowitz, Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC, and approved May 9, 2017 (received for review March 28, 2017)

The σ_2 receptor is an enigmatic protein that has attracted significant attention because of its involvement in diseases as diverse as cancer and neurological disorders. Unlike virtually all other receptors of medical interest, it has eluded molecular cloning since its discovery, and the gene that codes for the receptor remains unknown, precluding the use of modern biological methods to study its function. Using a chemical biology approach, we purified the σ_2 receptor from tissue, revealing its identity as TMEM97, an endoplasmic reticulum-resident transmembrane protein that regulates the sterol transporter NPC1. We show that TMEM97 possesses the full suite of molecular properties that define the σ_2 receptor, and we identify Asp29 and Asp56 as essential for ligand recognition. Cloning the σ_2 receptor resolves a longstanding mystery and will enable therapeutic targeting of this potential drug target.

sigma-2 receptor | sigma receptors | TMEM97 | cholesterol regulation | NPC1

levels were unaffected by whether PGRMC1 was overexpressed or knocked down (26), further confirming that the σ_2 receptor and PGRMC1 are two distinct molecular entities. The identity of the gene encoding σ_2 thus continues to elude discovery despite almost 30 years of effort, posing a major roadblock to understanding σ_2 receptor biology and therapeutic potential. To address this problem, we sought to leverage chemistry being developed in our laboratories toward σ_2 receptor ligand discovery to identify the σ_2 receptor.

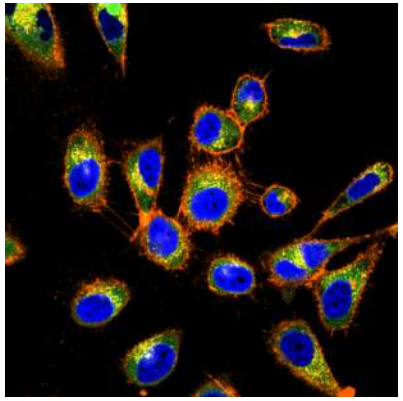
Results

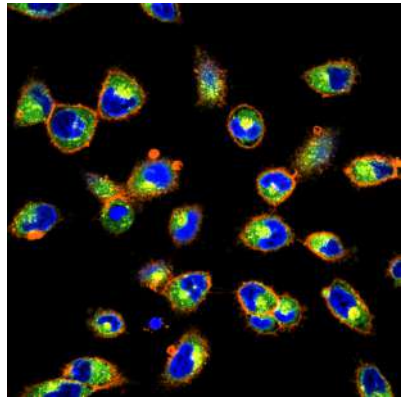
Affinity Purification of σ_2 from Calf Liver Tissue. We first synthesized JVW-1625 (K_i = 16.6 nM), a σ_2 receptor-binding ligand that was derived from the high-affinity σ_2 ligand JVW-1601 (σ_2 K_i = 19.6 nM) (*SI Appendix*, Fig. S1). We then selected a tissue source material (*SI Appendix*, Fig. S2*A*) and devised a protocol to extract σ_2 efficiently from calf liver membranes in a functional form (*SI Appendix*, Fig. S2*B*). We next covalently coupled JVW-1625 to agarose beads to prepare an affinity chromatography resin (*SI Appendix*, Fig. S2*C*) that

PNAS, vol. 114 no. 27, 7160–7165

Confocal imaging analysis shows unchanged uptake of fluorescently labeled S2 ligand (SW120) in TMEM97 and PGRMC1 knock out cell lines

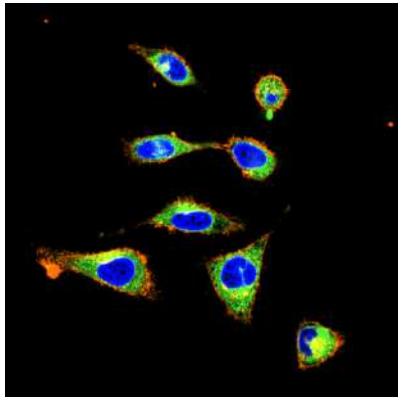
HELA/cas9

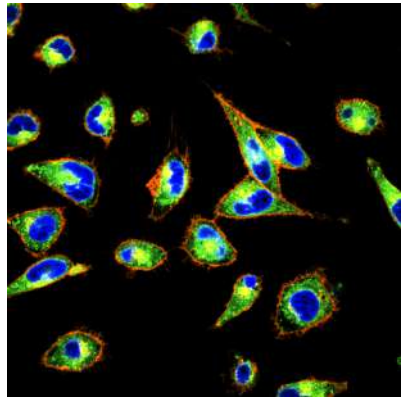




HELA/cas9
TMEM97 KO

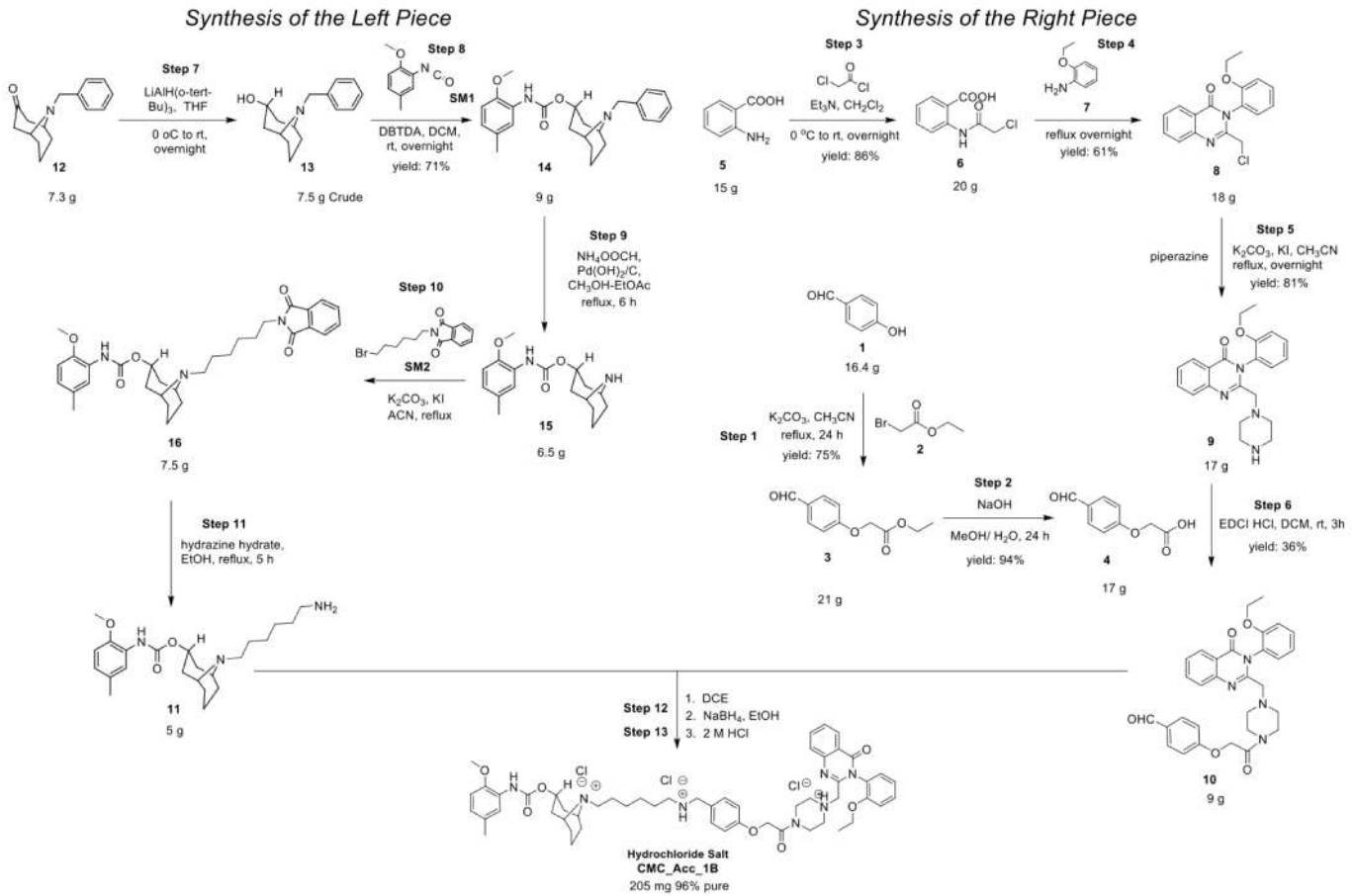
HELA/cas9
PGRMC1 KO





HELA/cas9 PGRMC1
TMEM97 double KO

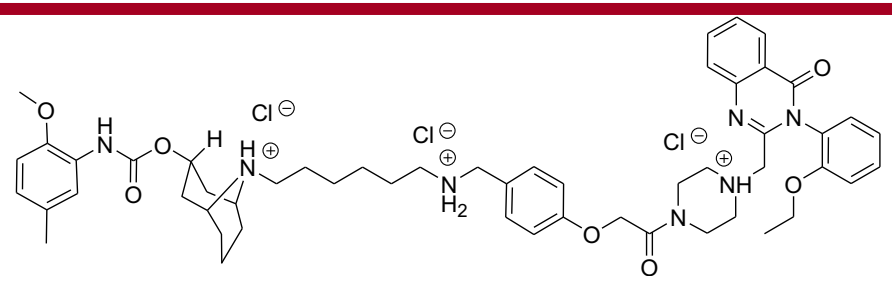
Accuronix CMC – Current Status



Convergent, multi-step synthesis, reproducible med chem process	Produced 5g in China and 25g in India, non-GMP, similar quality	ACXT-3102 HCl ~98%, amorphous solid-state, workable stability	Organic and inorganic impurities and residual solvents TBD

Acurronix- AXCT-3102 HCL Salt

$C_{53}H_{70}Cl_3N_7O_7$
Exact Mass: 1021.44
Mol. Wt.: 1023.52



Aqueous Solubility (Kinetic)	
pH 7.4 PBS	105 uM

Chemical Stability			
Parameter	pH 1.6 (FaSSGF)	pH 6.5 (FaSSIF)	pH 7.4 (PBS)
$t_{1/2}$ (min) Extrapolated	>120 750.6	>120 693.5	>120 1179.3
% Parent Remaining (at 60 min)	96.4	90.6	97.9

In Vitro Metabolic Stability						
	Liver Microsomes				Hepatocytes	
Parameter	Mouse	Rat	Dog	Human	Mouse	Human
Clearance (mL/min/Kg)	306.4	78.6	520.8	377.8	79.65	6.08
$t_{1/2}$ (min)	17.8	31.6	6.63	4.6	102.8	290.2
% Parent Remaining (at 45 min)	20.8	37.7	13.6	0.81	44.3 (120 min)	74.8 (120 min)

Plasma Stability			
Parameter	Mouse	Rat	Human
$t_{1/2}$ (min) Extrapolated	>120 150.7	>120 409.9	>120 735.0
% Parent Remaining (at 60 min)	78.7	89.1	94.1

Plasma Protein Binding			
	Mouse	Rat	Human
% Bound	98.4	98.9	99.9
% Unbound	1.6	1.9	0.1

Apparent Permeability			
	P_{app} (10^{-6} cm·s^{-1})		Efflux Ratio
	A to B	B to A	(P_{app}(B-A)/ P_{app}(A-B)
Caco2	<0.20	>13.93	>69.65
MDCK1-MDR1	<0.56	>15.82	>28.25

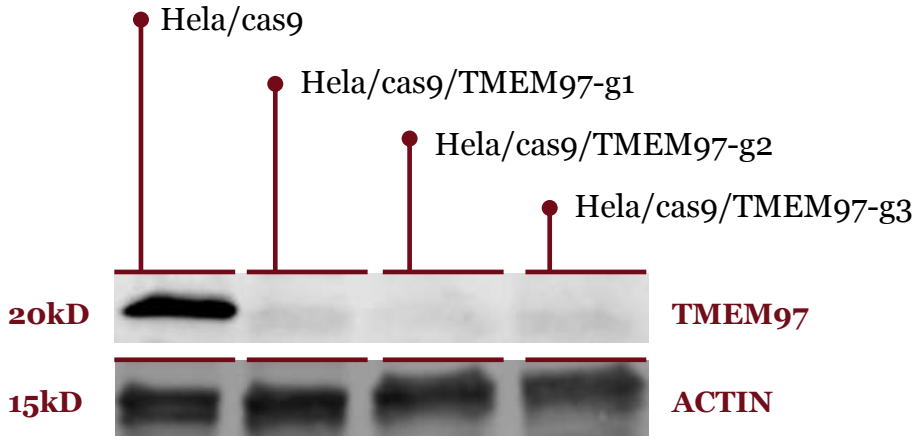
PK – (Non-GLP)								
Species	T½ (hrs)		C_{MAX}		AUC_{ALL}		Cl (mL/min/Kg	Volume of Dist (L/kg)
	IV	IP	IV	IP	IV	IP	IV	
MOUSE 1 mpk IV 2 mpk IP	4.04	2.02	162	131	199	296	63	19

MTT Cellular Cytotoxicity Data			
Compound	Cell Type	IC_{50} (μM)	
		24hr	72hr
ACC-1A (Oxalate Salt)	BxPC3	6.44	2.84
ACC-1B (HCl Salt)	BxPC3	6.67	3.00
ACC-1A (Oxalate Salt)	ASPC1	6.14	2.69
ACC-1B (HCl Salt)	ASPC1	6.18	3.00

Metabolite Profiles				
MET ID	Structural Location	~ % of parent per species (*In Vitro* liver microsome derived) (+ = <1%)		
		Mouse	Rat	Human
M1	Hydrolysis	+	+	+
M2	Hydroxylation	+	+	+
M3	Methylene to Ketone	+	+	+
M4	De-ethylation	3.20	6.82	5.01
M5	N-dalkylation (+2OH)	+	+	+
M6	Hydroxylation + desaturation	+	+	+
M7	Hydroxylation + desaturation	+	+	-
M8	Hydroxylation + desaturation	+	+	+

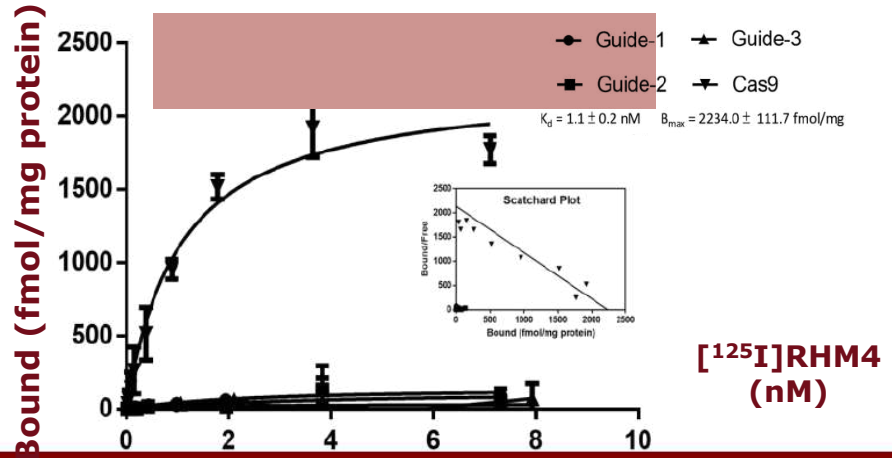
CRISPR Data Confirms Identification of σ-2 Receptor as TMEM97

A



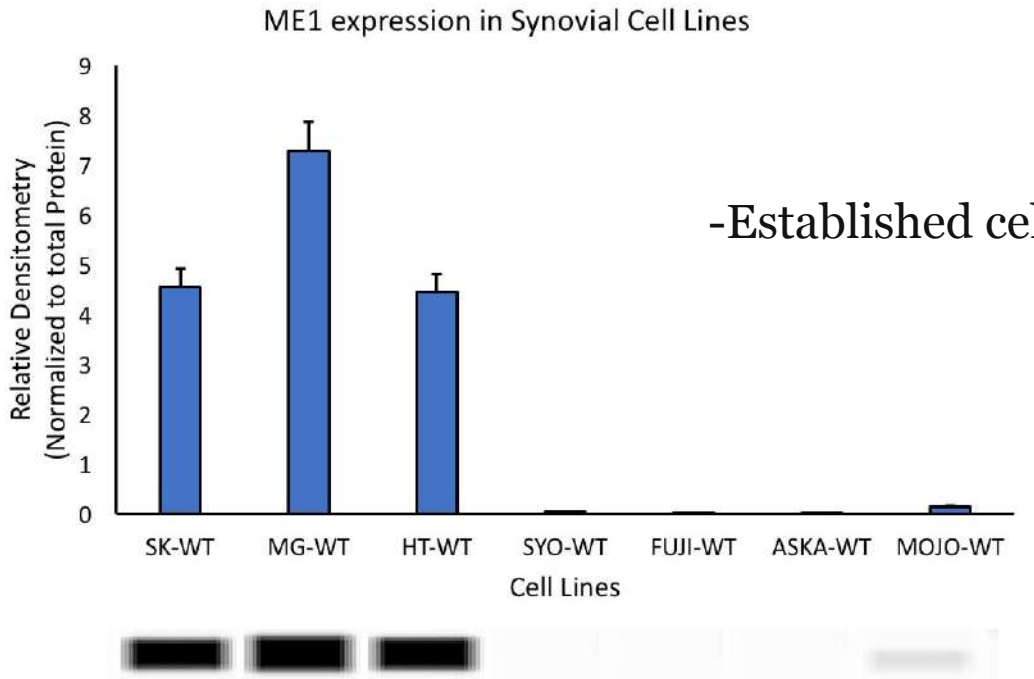
TMEM97 was knocked out using CRISPR/Cas9 technology using 3 different guide RNAs **(A)**

B



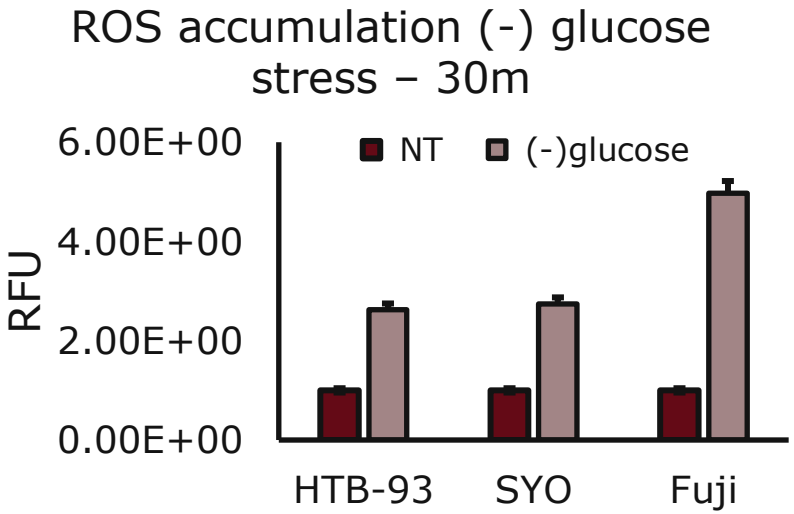
Knockout of TMEM97 resulted in a complete reduction in the specific binding of the s2-selective radioligand, [125I]RHM-4 **(B)**

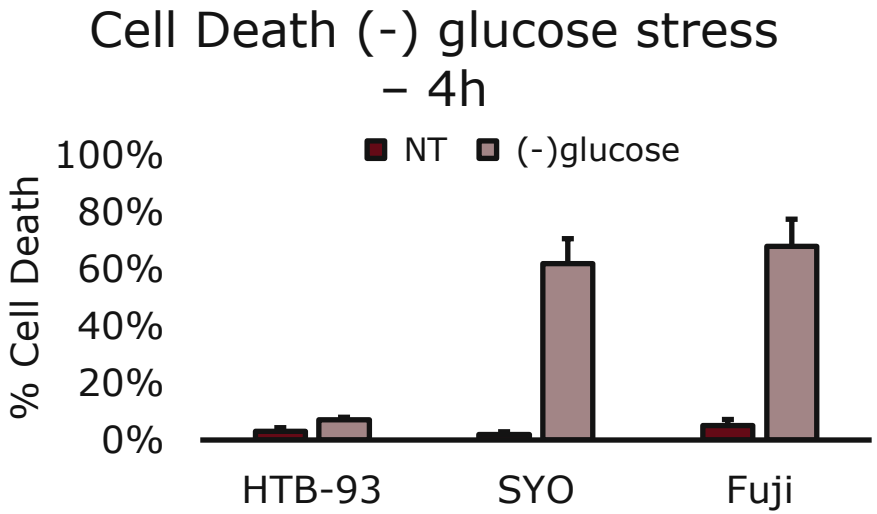
Expression of ME1 in cell lines



-Established cell lines do not express ME1

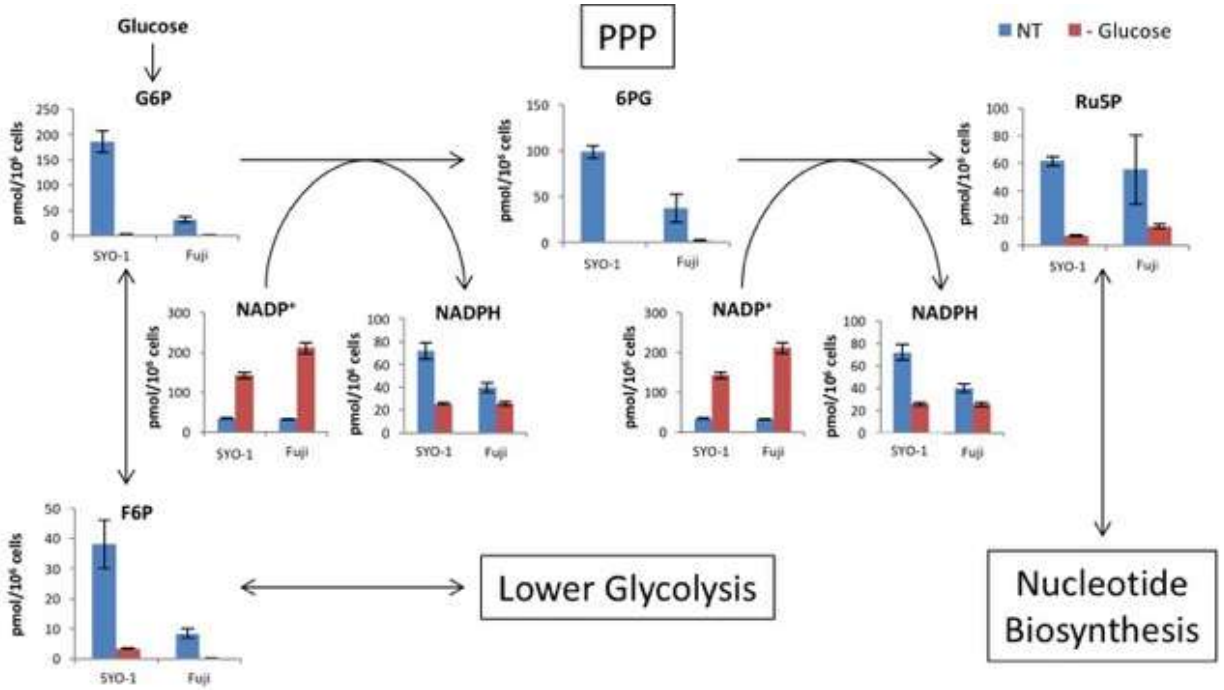
Increased ROS levels leads to Death





- With increased ROS levels and no expression of ME1 death is induced

Glucose Labeling – Tracer studies



-Glucose is shunted into the PPP for NADPH production
-Under glucose stress NADPH levels drop