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

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Director, Washington University SPORE in Pancreatic Cancer
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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

Five-year survival rate (Source: American Cancer Society)

- Prostate Cancer: nearly 100% survival
- Breast Cancer: 90% survival
- Lung Cancer: 16% survival
- Pancreatic Cancer: 6% survival
Former Pres. Jimmy Carter
Says His Latest Scan Shows
No Sign of Cancer
The 91-year-old was treated with new immunotherapy.

When the Lung Cancer Patient Climbs Mountains
A gene therapy clinical trial enabled a Stage IV lung cancer patient to summit a peak in the Himalayas.
Review: Checkpoint Therapy

A Lymphatic tissue

IL-2/IFN-γ/CTL function ↑

B Peripheral tissue/tumor

IL-2/IFN-γ/CTL function ↑

© 2013 American Association for Cancer Research

CCR Focus

Ott, CCR, 2013
Improved Survival with Ipilimumab in Patients with Metastatic Melanoma

A Overall Survival

Hodi, NEJM, 2010
Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer

Hazard ratio for death, 0.60 (95% CI, 0.41–0.89)  
\( P = 0.005 \)

Reck, NEJM, 2016
PD-1 Blockade in Tumors with Mismatch-Repair Deficiency

B Radiographic Response

- Mismatch repair-proficient colorectal cancer
- Mismatch repair-deficient colorectal cancer
- Mismatch repair-deficient noncolorectal cancer

Change from Baseline in the Sum of Longest Diameters (%)

- 20% increase (progressive disease)
- 30% decrease (partial response)

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

Pancreatic Cancer Immunotherapy Trials

![Graph showing overall survival over time for different treatment groups.]

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

Goedegebuure, Curr Cancer Drug Targets, 2011

TAN
Targeting Immunosuppressive Microenvironment

CCR2i

Myeloid Cells

Anti-Tumor CTLs Responses

PDAC Tumor

CAF and Fibrosis

pFAK
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

Manufacture of personalized vaccine

Epitope validation
- ELISPOT assay
- Peptide binding assay

Mutation prioritization
- Expression
- MHC I binding
- Processing

Mutation identification
- Nucleic acid
- Protein

T/N exome/RNA Seq

Patient with pancreatic cancer

Tumor tissue (T)
PBMCS (N)
Preliminary Data

DNA Mutations

RNA Expression

Predicted to Bind MHC I

Patient A

SNV 37

Expressed 9

Bind MHC I 7

Patient B

SNV 52

Expressed 12

Bind MHC I 8

Patient C

SNV 35

Expressed 7

Bind MHC I 5

DNA Mutations

RNA Expression

Predicted to Bind MHC I
Patients can Recognize Neoantigens

<table>
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<tr>
<th>Patient</th>
<th>MHC Class I</th>
<th>MHC Class II</th>
<th>Unique Neoantigens</th>
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<tbody>
<tr>
<td>A</td>
<td>5</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>7</td>
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<tr>
<td>C</td>
<td>4</td>
<td>4</td>
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</table>
Clinical Trial Schema

Enrollment within 12 weeks of surgery

Adjuvant chemotherapy x 3 cycles

Adjuvant chemotherapy and/or chemoradiation

PBL Neoantigen DNA vaccine

Week

1 5 9 13 17 21 25 77

24 weeks
Future – Multimodal Immunotherapy

- CCR2i
- CSF1Ri
- CXCR2i
- PI3Kγi

Myeloid Cells

PDAC Tumor

Vaccine

Anti-Tumor

CTLs Responses

Checkpoint
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

- **Breast**
  - SUV = 3.5
  - T:M = 4.4

- **Head & Neck**
  - SUV = 2.9
  - T:M = 3.6

- **Lymphoma**
  - 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

Linda Jin
S2 Uptake is Receptor Mediated

Comparison of SW120 uptake in ASPC-1

Control

4 °C

SW43

Pitstop2

Washington University in St. Louis • School of Medicine
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)

<table>
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<tr>
<th>Sigma-2 ligand</th>
<th>Cargo</th>
<th>Conjugate name</th>
<th>Publication date</th>
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<td>SV119</td>
<td>dm-Erastin</td>
<td>σ2/dm-Erastin (ACXT-3102)</td>
<td>2016</td>
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Personalizing Cancer Metabolism

Dead

Alive

Cancer Engine

Targeted Metabolic Therapy

The Red Line

Normal Engine
Personalizing Cancer Metabolism

Dead

Alive

The Red Line

Cancer Engine

Normal Engine
Erastin Causes Cell Death by Ferroptosis

MALIC ENZYME 1

ROS

GSH

NADPH

NADP+

GSSG

Ferroptosis

Cell Death

Cystine

X_c

transporter

Fe^{2+}

Fenton Reaction

GPX4

Malate

Pyruvate

Malic

Enzyme 1

Washington University in St. Louis • School of Medicine
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

![Chemical structures of SV119, Des-methyl Erastin, and SW V-49](image-url)
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

Fe²⁺
Fenton Reaction

ROS

GSH

NADP+

GSSG

NADPH

Malic
Enzyme 1

Malate

Pyruvate

GSH

GPX4

Cystine

Glutamate

ε-transporter

SW V-49s

FERROPTOSIS
CELL DEATH

Washington University in St. Louis • School of Medicine
Antioxidants can block mechanism of action

Ohman et al., Oncotarget 2016
SW V-49s is potent across cancer types

Relative Viability (%) vs. SW V49s concentration (log µM)

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

Fe^{2+} Fenton Reaction

ROS

GSH

GSSG

NADP+

NADPH

Malate

Pyruvate

Malic Enzyme 1

Cystine

X^- transporter

Sw V-49s

1-Production

2-Turnover

CELL DEATH

Glutathione

GPX4

SW V-49s

Glutamate

Washington University in St. Louis • School of Medicine
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
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

Pentose Phosphate Pathway 23%
Malic Enzyme 1 27%
10-formyl-THF-pathway 48%
Other 2%

*NADPH PRODUCTION

Synovial Sarcoma
(potential surrogate for a biomarker)
SW V-49s Drives Cell Death by Ferroptosis

Fe$_2^+$
Fenton Reaction

ROS

GSH

GSSG

NADPH

NADP$^+$

GPX4

Cystine

Glutamate

$X_c^-$
transporter

Malate

Enzyme 1

Pyruvate

FERROPTOSIS
CELL DEATH
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

N = 5-7 mice/group
SW V-49s Improves the Immune Environment

A CD8+ T-cell

B Ki67+CD8+ T-cell

C CD4+ T-cell

D FOXP3+CD4+ T-cell

* p=0.0388

* p=0.0317

* p=0.0482
<table>
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<th>Short Title</th>
<th>Open To Accrual</th>
<th>Total Accrual Goal</th>
<th>Number Accrued</th>
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<td>FOLFIRINOX + Ipi +/- Tumor Vaccine in Pancreatic</td>
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<td>Tosedostat + Cape in Pancreatic</td>
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<td>Palbociclib + Abraxane in Metastatic Pancreatic Ductal Adenocarcinoma</td>
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<td>A Phase I Study of VS-4718, a Focal Adhesion Kinase Inhibitor, in Combination with Nab-paclitaxel and Gemcitabine in Subjects with Advanced Cancer</td>
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<td>mFOLFIRINOX vs Gem/Abraxane in Resectable Pancreatic (CIRB S1505)</td>
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<td>FOLFIRI vs mFOLFIRI+Veliparib in Pancreatic (CIRB S1513)</td>
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<td>Personalized DNA vaccine for pancreatic cancer</td>
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Back to the Bench-- Tissue Core

OR Planning Meeting
- Review/Triage Cases
- Consent Status Determined
- Sample-specific procurement communication
- Subject registration

Specimen Registration
- Serum
- Plasma
- PBMCs
- LN₂ Snap Frozen
- Paraffin, OCT
- Embedding
- H&E
- Specimen Review
- LCM
- PDX
- Tissue Microarray (TMA)

Informatics

Procurement

MONDAY
- Blood
- Tumor tissue

TUE

WED

THU

FRI

13,700 Specimens
1/2018
Adenocarcinoma of the Pancreas
Overall Survival

Overall Survival Pancreatic Adenocarcinoma

Percent Survival

Survival time (years)

2010-2015
1995-1999
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

Drug Targeting

Clinical Outcomes

Oncology Basic Science Training Grant
T32-CA009621-29
SPORE – Collaborators
Collaborative Teams
Patient Outcomes and Cost Effectiveness

[Diagram showing a scatter plot with case volume on the left and standard deviation of case supply cost on the right, with median case supply cost ranging from $350 to $750.]
Identification of the gene that codes for the $\sigma_2$ receptor

Assaf Alon$^{*1}$, Hayden R. Schmidt$^{*1}$, Michael D. Wood$^{*1}$, James J. Sahn$^{*}$, Stephen F. Martin$^{*}$, and Andrew C. Kruse$^{*2}$

$^*${Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115; and $^1$Department of Chemistry, The University of Texas at Austin, Austin, TX 78712

PNAS, vol. 114 no. 27, 7160–7165

The $\sigma_2$ 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 molecular methods to study its function. Using a chemical biology approach, we purified the $\sigma_2$ 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 $\sigma_2$ receptor, and we identify Asp29 and Asp56 as essential for ligand recognition. Cloning the $\sigma_2$ receptor resolves a longstanding mystery and will enable therapeutic targeting of this potential drug target.

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.
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

C₃₃H₇₃Cl₃N₇O₇
Exact Mass: 1021.44
Mol. Wt.: 1023.52
CRISPR Data Confirms Identification of σ-2 Receptor as TMEM97

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

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