Pancreatic Cancer Research

Jonathan Brody, Ph.D.
Co-director of the Jefferson Pancreatic, Biliary, and Related Cancer Center
Director of Surgical Research
Kimmel Cancer Center Member
Wallace Davis Professor of Surgery
Thomas Jefferson University

Pancreatic Cancer Action Network
Educational Session
04/14/2016
May 25, 1961

And 8 years later...

July 20, 1969

“I believe we should go to the moon… It is a most important decision that we make as a nation.”
Key facts, concerns, and hope in the field of pancreatic cancer research

1. Genetic mutations: We know a lot about the genes involved or mutated in pancreatic.
2. Treat every aspect of the tumor (the complex TME): We know that this is a complex tumor compared to other tumor systems.
3. Update on early detection, monitoring disease
4. Optimize agents with existing and proven activity (even gemcitabine)
5. Understand and identify new targets beyond genetic lesions
1. Personalized approach

The progression model has not changed....

August, 2008

Normal ductal epithelium

PanIN-1

PanIN-2

PanIN-3

KRAS mutations
Telomere shortening

CDKN2A loss

TP53 loss
SMAD4 loss
BRCA2 loss
The RAS Initiative

More than 30 percent of all human cancers — including 95 percent of pancreatic cancers and 45 percent of colorectal cancers — are driven by mutations of the RAS family of genes. NCI established the RAS initiative in 2013 to explore innovative approaches for attacking the proteins encoded by mutant forms of RAS.
Developing a targeted therapy against these mutated pathways takes time and money....
In vivo Therapeutic Responses Contingent on Fanconi Anemia/BRCA2 Status of the Tumor


Clin Cancer Res 2005;11:7508-7515
Case study: BRCA2

- Mrs. Smith: Prior tongue cancer; uterine cancer and breast cancer
- Surgery—pancreatic ductal adenocarcinoma (PDA) by Dr. Yeo early January 2007

Team: Charles Yeo, MD, Sarah Charles, Ph.D.

BRCA2 (mutation, inactivation)

1. The cause of her tumor: via a defect in genome maintenance
2. An Achilles Heal of the tumor: giving Mrs. Smith a great response

Platinum-based adjuvant therapy
Patient alive and well today (~8 years)

An example (rare)
Gemcitabine

Radiation

FOLFORINOX
World with no biomarkers....
Early detection

Surgery or 
Biopsy

Laboratory work: analysis to predict patient therapy
Predictive biomarkers

Optimized adjuvant therapy

Clinical benefit

Days

Surgery or Biopsy

Laboratory work: analysis to predict patient therapy
Predictive biomarkers

Optimized adjuvant therapy
What are Biomarkers?

Sequence:
- BRCA2 mutation
- Immunohistochemistry

Expression Heatmap:
- Long survival
- Short survival

Diagram:
- Pie chart with percentages labeled as DPC4, IDO2, BRCA2/FA, SPARC, and a question mark for unknowns.
- Arrows indicating HuR status.

Blood sequencing chart and tissue sections are also present.
Future of medicine: Personalized Medicine

WE HAVE BANKED >400 specimens to date!
ERCC1 and RRM1 might be Useful
Chemotherapy Predictive Markers

- Pilot Trial in Pancreatic Cancer
- Patient “Tailored” Therapy (*2000 version)

Predictive Markers

- Gemcitabine
  - RRM1
- Platinum
  - ERCC1
- 5-FU
- TS

Tumor Biopsy

Low RRM1
- Gem-Based
  - Low ERCC1
    - GemOX
    - Low TS
      - Gem 5FU
  - No Platinum
    - High ERCC1
      - No Platinum

High RRM1
- No Gem
  - High ERCC1
    - No Platinum
    - Platinum-Based
      - Low ERCC1
        - Ox-Tax
        - Low TS
          - 5-FU Tax
    - Tax - Iri

Michael Pishvaian, MD, Ph.D., LCC, Georgetown University
Clinical trial over 4 institutions: TJU the lead institution
Is the pancreatic cancer field ready for “personalized” therapy?
(Overview of the grant) **Patient chronology:** Metastatic pancreatic cancer patients

**Metastatic pancreatic cancer patients**

1. **1st Disease progression**
   - Biopsy
   - Multi-Omic analysis
     - NGS (DNA)
     - Expression (RNA)
     - Immunohistochemistry
     - Phosphoprotein
   - Physician’s discretion (SOC)
   - Molecular Tailored Therapy

2. **2nd line therapy**
   - Biopsy
   - Multi-Omic analysis
     - Focused genomic sequencing
     - RNA seq
     - Phosphoprotein

3. **2nd Disease progression**
   - Biopsy

**Aim 1: Clinical trial**

*2016*

**Biopsy while on Front line therapy (prior to progression)**

**Aim 2:** Molecular Tailored Therapy Version 2.0 for Phase III trial

**Aim 3:** Resistance Pathways

**Additional Omic analysis**

- Resistant CRCs
- New targets and Resistance pathways

---

**Front line therapy**

**1st Disease progression**

**2nd line therapy**

**2nd Disease progression**
5 core Biopsies

- Drug sensitivity
- MOP
  - Protein
  - RNA
  - NGS

- Zebratars

- Orthotopic mouse
- Xenograft

- Validate sequencing
- SNPs

Develop CRCs 2D

Correlative science
Sue Chand, a graduate student, actively plating cancer cells for organoid growth.

On top of the 1 million dollar grant we raised >500K for the organoid lab.
PERTHERA & Organoids: Trial Design (stage I & 2)

SURGICAL BIOPSY

PERThERA TESTING

PROFILE
ANALYSIS
REVIEW
REPORT

G
Genome

P
Protein

PP
Phosphoprotein

PH
Patient History

Molecular Profiling & Patient History

Perthera ExpO
Clinical Knowledge

Web Service

Expert Oncology

Expert Medical Review

Physician’s Report

GENERATE ORGANOIDs

WHOLE EXOME SEQUENCE

FOCUSED DRUG SCREENING

Jordan Winter
Talar Tatarian
Organoids and Pharmacologic Tailored Therapy (PTT)
KYT-Perthera Workflow

1. Patient or caregiver calls PanCAN
2. Patient meets eligibility requirements
   - Patient consent
   - Talks to treating physician
   - Biopsy by SOP
   - Sends sample to labs
3. Multi-omic Molecular Testing:
   - Genomic
   - Protein
   - Phosphoprotein
   - Data analysis and integration
   - Current literature and knowledge of field
   - Patient history
4. Treatment physician
5. Report with treatment options
   - PanCAN clinical trials database
   - Solid tumor Phase 1's
6. Expert Medical Review Board-certified medical review panel
7. PanCAN follows up with patient
   - Outcomes data collected and organized
8. PanCAN follows up with physician
Patient 576: IDH1 Mutation

An Inhibitor of Mutant IDH1 Delays Growth and Promotes Differentiation of Glioma Cells

- 48-year-old female diagnosed with metastatic pancreatic cancer
- Started FOLFIRINOX – progression after nine months
- Started on a clinical trial of gemcitabine plus nab-paclitaxel plus pembrolizumab
- Biopsy of a liver metastasis was performed while on study
- FM identified an established IDH1 R132H mutation in GBMs (frequency of 12% in GBM-Cosmic)
- Mutation was confirmed via targeted next-generation sequencing pan cancer panel
- Mutant IDH1 specific Ab H & E IHC confirmation with an Ab against mutant IDH1
Part II: Treat every aspect of the tumor (the complex TME): We know that this is a complex tumor compared to other tumor systems.
The many facets of targeting pancreatic cancer...

2. Know your entire tumor

Weinberg BA, Yabar CS, Brody JR, Pishvaian MJ. 
Witkiewicz AK1, Costantino CL, Metz R, Muller AJ, Prendergast GC, Yeo CJ, Brody JR.
Table 1. Reduced PDAC formation in IDO2 null genotypes

<table>
<thead>
<tr>
<th>K-rasG12D (♂ and ♀)</th>
<th>WT</th>
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</thead>
<tbody>
<tr>
<td>Invasive cancer (N)</td>
<td>16/54</td>
</tr>
<tr>
<td>Invasive cancer (%)</td>
<td>30%</td>
</tr>
<tr>
<td>Endpoint age (weeks ± SE)</td>
<td>47.5 ± 2.6</td>
</tr>
</tbody>
</table>

Two tailed P values:

Figure 6. Representative pancreas histologies from PDX/K-Ras mice at 11-13 months of age.

Cox Adjusted Survival Function

IDO2-null genotype aids therapy

IDO2 -/- genotype

Wild type genotype

Wild type

Heterozygous

Homozygous

A)

R235W

Y359STOP
Part III: early detection and monitoring

T1: tumor initiation to parental clone
T2: parental clone to metastatic clone
T3: dissemination to death

Interval: yrs
-20 -15 -10 -5

Clinical relevance

Histologic progression

 Detectable By CT
Surgical intervention
Death

Duct carcinoma
Disseminated pancreatic cancer

T1: tumor initiation to parental clone
T2: parental clone to metastastic clone
T3: dissemination to death

Nature 2010; 467: 1114
Clin Im. 2010; 34:277.
Figure 1. Clinical applications of CTCs and ctDNA as liquid biopsy for personalized medicine. Blood samples can be sampled repeatedly to predict relapse in M0 patients or metastatic progression in M1 patients, monitor the efficacy of therapies and understand potential resistance mechanisms. Before therapy, patients can be stratified to the most effective drugs, whereas after initiation of treatment persistence in increases of CTCs/ctDNA indicates resistance to therapy, and this information may allow an early switch to a more effective regimen before the tumor burden is excessive and incurable. mt, mutation; BC, breast cancer; PC, prostate cancer; CRC, colorectal cancer.
A. RESPONDERS: Most tumor cells from the primary tumor and/or metastatic sites are killed by therapy.

- Therapy-sensitive tumor cells: undergo apoptosis and release DNA
  - ctDNA DNA of sensitive clones

B. NONRESPONDERS: Most tumor cells from the primary tumor and/or metastatic sites survive therapy.

- Therapy-resistant tumor cells: do not undergo apoptosis and can disseminate through the blood
  - no ctDNA
  - CTCs available for DNA analyses
Part IV: Optimize agents with existing and proven activity (even gemcitabine)

Genetic events → Pancreatic tumorigenesis → Infiltrating/metastasis → Lethality

- KRAS → TP53

- microRNAs/Chromatin changes

- Disseminated pancreatic cancer

- Duct carcinoma

Darwinian selection

2 years 12 years
Genomic analyses identify molecular subtypes of pancreatic cancer

Pencil sketch of the DNA double helix by Francis Crick in 1953
dCK expression correlates with 5-fluorouracil efficacy and HuR cytoplasmic expression in pancreatic cancer
A dual-institutional follow-up with the RTOG 9704 trial

Florenic MctAllister1, Danielle M Pineda3, Masaya Jimbo2, Shrutl Lal3, Richard A Burkhat3, Jennifer Moughan4, Kathryn A Winter4, Kotb Abdelsmosri5, Myriam Gorospe5, Ana de Jesus Acosta6, Rachana H Lankapalli7, Jordan M Winter3, Charles J Yeo3, Agnieska K Witekiewicz6, Christine A Iacobuzio-Donahue1, Daniel Lahu6, and Jonathan R Brody3,*

HuR Posttranscriptionally Regulates WEE1: Implications for the DNA Damage Response in Pancreatic Cancer Cells

Shrutl Lal1, Richard A. Burkhat1, Neil Beeharry4, Vikram Bhattacharjee4, Eric R. Londin2, Josea A. Cozzitorte1, Carmella Romeo1, Masaya Jimbo1, Zoë A. Norris1, Charles J. Yeo1, Janet A. Sawicki3,5, Jordan M. Winter1, Isidore Ric paysos2, Timothy J. Yen4, and Jonathan R. Brody1

Oncogene (2015), 1–13
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www.nature.com/onc

ORIGINAL ARTICLE
The mRNA-binding protein HuR promotes hypoxia-induced chemoresistance through posttranscriptional regulation of the proto-oncogene PIM1 in pancreatic cancer cells
FF Blanco1,2, M Jimbo2, J Wulfkuhle3, I Gallagher3, J Deng3, L Enyenih7, N Meisner-Kober4, E Londin3, I Ricoutsos3, J A Sawicki6, MV Risbud7, AK Witekiewicz6, PA McCue6, W Jiang9, H Rui10, C J Yeo1, E Petricoin5, JM Winter1 and JR Brody2
# Risks Associated with WEE1 INDEL vs. WEE1 WT (N=99)

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<tr>
<th>Risk Factor</th>
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<td>Any Fam. Hx of Cancer</td>
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<tr>
<td>Fam. Hx of Colon Cancer*</td>
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<td>(Non-Smoker)§</td>
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<tr>
<td>Fam. Hx of Pancreas Cancer</td>
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<td>Group 1*</td>
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<td>Group 2§</td>
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<td>(Non-Smoker)§</td>
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<td>(Smoker)</td>
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<td>5.294118</td>
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<td>2.384868</td>
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<tr>
<td>3.340659</td>
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</table>

Smokers (N=37), Non-Smokers (N=51).
Risk of Cancer in 1st degree relatives, Odds Ratio (95% Confidence Interval).
* P < 0.05; § P = 0.06

Group 1: Colon, Rectal, Gastric, Endometrial, Ovarian
Group 2: Colon, Rectal, Gastric, Breast, Hepatobiliary, Pancreas, Endometrial, Ovary, Melanoma, Brain, Urinary

Avi Nevler, MD
Struan Grant, Ph.D. (CHOP)
James Eshleman, MD/PhD (Johns Hopkins)
ORIGINAL ARTICLE
The mRNA-binding protein HuR promotes hypoxia-induced chemoresistance through posttranscriptional regulation of the proto-oncogene PIM1 in pancreatic cancer cells

1. Lower microvessel density: drug penetration
2. Hypoxic niches: Arrested or quiescent cells become refractory to agents targeting rapidly-dividing cells

Drug Resistance
Poor Prognosis
Nature, 379 (1996),
Cancer Res, 60 (2000)
The Hypoxia-Inducible Proto-Oncogene PIM1 Promotes Chemoresistance

PIM1 is a serine-threonine kinase that regulates DNA repair and therapeutic response.

Hypoxia

→

PIM1

Inhibition of apoptosis e.g., BAD

Cell Cycle Arrest
DNA Repair

Chemoresistance

The role of PIM1 expression in hypoxia-induced chemoresistance to Oxaliplatin

Blanco et al Oncogene 2015
# Sensitivity to Oxaliplatin in Normoxia and Hypoxia

**Oxaliplatin IC$_{50}$ values (± SEM), μM**

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Hypoxia</th>
<th>Fold Difference</th>
<th>p-value (two-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MiaPaCa2</td>
<td>1.96 (0.18)</td>
<td>11.43 (3.08)</td>
<td>5.83</td>
<td>0.0009</td>
</tr>
<tr>
<td>Capan-1</td>
<td>1.34 (0.49)</td>
<td>12.91 (0.60)</td>
<td>9.63</td>
<td>&gt;0.0001</td>
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<tr>
<td>Su.86.86</td>
<td>1.81 (0.22)</td>
<td>4.76 (0.60)</td>
<td>2.63</td>
<td>&gt;0.0001</td>
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<tr>
<td>Hs766t</td>
<td>1.41 (0.29)</td>
<td>&gt;100</td>
<td>NA*</td>
<td>NA*</td>
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<td>PL5</td>
<td>0.75 (0.15)</td>
<td>1.78 (0.40)</td>
<td>2.37</td>
<td>0.0029</td>
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<tr>
<td>BxPc3</td>
<td>1.50 (0.88)</td>
<td>14.65 (5.92)</td>
<td>9.77</td>
<td>0.0046</td>
</tr>
<tr>
<td>Panc-1</td>
<td>10.78 (3.14)</td>
<td>&gt;100</td>
<td>NA*</td>
<td>NA*</td>
</tr>
</tbody>
</table>

NA = Not available
* Value extrapolated, cannot be determined
In patient samples cyto HuR correlates with PIM1 levels

<table>
<thead>
<tr>
<th>HuR</th>
<th>Low n (%)</th>
<th>Moderate / High n (%)</th>
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<tbody>
<tr>
<td>Low PIM1</td>
<td>5 (11)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Med / High PIM1</td>
<td>7 (16)</td>
<td>30 (68)</td>
</tr>
</tbody>
</table>

*Fisher's Exact Test, p-value = 0.011*

Blanco et al Oncogene 2015
# Phase I/II Trial of vABT-888 in Pancreatic Cancer

- **Phase I** – ABT-888 dose escalation
  - Mixture of untreated and previously treated patients
- **Phase II** - two strata
  - Untreated vs. previously treated

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Day</th>
<th>ABT-888 (Orally BID)</th>
<th>Oxaliplatin (85mg/m²)</th>
<th>Leucovorin (400mg/m²)</th>
<th>5-FU CI (2400mg/m²)</th>
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</table>


Pishvaian MJ, Brody JR.... PARP inhibitor + mFOLFOX for the treatment of pancreatic cancer, GI ASCO and ASCO presentations
PARP inhibitors translocate HuR

Cytoplasmic

Untreated  Veliparib  Olaparib  Rucaparib  Niraparib

Nuclear

Untreated  Veliparib  Olaparib  Rucaparib  Niraparib

Total

Untreated  Veliparib  Olaparib  Rucaparib  Niraparib

Lamin A/C  α-Tubulin

HuR

DAPI

HuR

HuR

HuR

HuR

HuR

HuR
Xenograft studies with Mia.sh290 and Olaparib treatment

- Vehicle only
- shHuR
- + Olap
- + Olap, shHuR

**Graph:**
- Tumor Volume (mm³)
- Days

**Legend:**
- Vehicle
- shHuR
- + Olap
- + Olap, shHuR

**Images:**
- Tissue samples for different treatment groups.
HuR regulates Poly ADPribose Glycohydrolase (PARG) to modulate PARPi resistance

Chand et al., unpublished
A novel, alternative non-genetic target for pancreatic cancer.

Part V: Understand and identify new targets beyond genetic lesions

Chemotherapeutic Efficacy: PIM1, PARG, WEE1

TME: PIM1, IDH1
Targeting the mRNA-binding protein HuR impairs malignant characteristics of pancreatic ductal adenocarcinoma cells

Masaya Jimbo¹, Fernando F. Blanco¹,², Yu-Hung Huang³,⁎, Aristeidis G. Telonis⁴,⁎, Brad A. Scrceni¹, Gabriela L. Cosma⁵, Vitali Alexeev⁶, Gregory E. Gonye⁷, Charles J. Yeo¹, Janet A. Sawicki⁸, Jordan M. Winter¹, Jonathan R. Brody¹

Would targeting RNA regulation be more effective than targeting DNA?
A Global Synthetic Lethal approach: Multiple targets at once

**Chemotherapeutic Efficacy:**
PIM1, PARG, WEE1

<table>
<thead>
<tr>
<th>Table 1: H</th>
<th>Upregulated HuR knock</th>
<th>1 change</th>
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<td>ERBB2</td>
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<td>DAP3</td>
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<td>L1CAM</td>
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<td>MTA1</td>
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<td>MYC</td>
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**TME:**
PIM1, IDH1

\[ EGF eIF4E \]

<table>
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<th>Enhancement of cell survival</th>
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<tbody>
<tr>
<td>ProTox Bcl-2 Mcl-1 SIRT1 p21 Mdm2 c-Myc*</td>
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<thead>
<tr>
<th>Evasion of immune recognition</th>
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<tr>
<td>TGF-β</td>
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<tr>
<th>Promotion of cell proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclin D1 Cyclin E1 Cyclin A2 Cyclin B1 p27*</td>
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<th>Elevation of local angiogenesis</th>
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<tbody>
<tr>
<td>HIF-1α VEGF Cox-2 TSP1*</td>
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<th>Invasion and metastasis</th>
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<tbody>
<tr>
<td>Snail MMP-9 uPA uPAR</td>
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\[ \text{Cytoplasm} \]

\[ \text{Nucleus} \]
Wave of the future: Latest in DNA/RNA tech

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system

- Discovered in bacteria as a key part of a precise immune reaction
- Short DNA sequences are recognized and put back together
CRISPR plasmid was transfected into MiaPaCa2 cells in 6-well plate.

48 h later, GFP positive cells were single sorted by FACS in 96-well plate.

2-3 weeks later, single cell colonies were expanded into 24-well plate.

Cells were then expanded into T-75 flask.

Expanded colonies were assessed for mutations with sequencing, qPCR and Western.

Cells were then expanded into 6-well plate.
RNP-IP with HuR CRIPSRred in MiaPaCa2 cells

Lamin A/C
α-Tubulin
HuR

Lamin A/C
α-Tubulin
HuR

Pre-Clear Trial
Cyttoplasmic Fraction
G10  G1C  G3E  G3F

α-Tubulin
HuR

NT  IgG  NT  HuR  Oxall IgG  Oxall HuR

IP

Relative Binding (Normalized to NT IgG)

IDH1  WEE1  PARP

IDH1  WEE1  PARP

G2C (+/+)
G3E (+/+)

Stanger Sequencing

Whole cell lysate

HuR

α-Tub
Delivery of Therapeutics Targeting the mRNA-Binding Protein HuR Using 3DNA Nanocarriers Suppresses Ovarian Tumor Growth

Yu-Hung Huang¹,², Weidan Peng¹, Narumi Furuuchi¹, Jacquelyn Gerhart³, Kelly Rhodes³, Neelanjana Mukherjee⁴, Masaya Jimbo⁵, Gregory E. Gonye⁶, Jonathan R. Brody⁵, Robert C. Getts³, and Janet A. Sawicki²,⁷
Take home points

• High throughput DNA sequencing of pancreatic cancer genomes has provided us with some validating pearls, BRCA1ness: Best example for personalized therapy

• Alternative approach: Post-transcriptional gene regulation can provide us with information about the biology of the tumor now (not yesterday but when the tumor is treated): Personalized therapy=Present focus Therapy: “Pancreatic cancer Transcriptionally addicted”

• Understanding these acute, stress response mechanisms by tumor cells will allow us to NOW:
  • 1: Optimize current therapies
  • 2: Attack and find new targets
How to make a difference against this foe?

• Multi-disciplinary conferences (surgeons, PhDs, medical oncologists, Radiation Oncology, nutrition, geneticists, basic scientists, medicinal chemists, bioinformatics): patient centric

• Programmatic initiatives with authentic questions: think out of the box type strategies

• Development/philanthropic strategies to support pilot awards and bridge funding

• LiveBiobank: How many important questions have been answered from a frozen BioBank (except for genetics)
Summary of key concepts

1. Genetic mutations: We know a lot about the genes involved or mutated in pancreatic.
2. Treat every aspect of the tumor (the complex TME): We know that this is a complex tumor compared to other tumor systems.
3. Update on early detection, monitoring disease
4. Optimize agents with existing and proven activity (even gemcitabine)
5. Understand and identify new targets beyond genetic lesions
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Struan Grant (CHOP)
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Tim Yen (FC)
Igor Astsaturov (FC)

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