Breast Cancer Avatars: Patient-derived Xenografts As A Platform For Drug Development

Michael T. Lewis
Baylor College of Medicine

IMPAKT 2014 Breast Cancer Conference
May 8-10, 2014

Founder and Limited Partner – StemMed Ltd
Member – StemMed Holdings LLC
Cancer Avatars: Can An Alternate "You" Provide Unique Insight Into New Cancer Treatments?
Human Clinical Trial Sequence

• Phase I: Safety, MTD
• Phase I/II: Drug combinations; Dose-response
• Phase I/II: Drug sequencing
• Phase II/III: Drug superiority
Basic Clinical Trial Design In Humans

Recruit Patients

Dozens to Thousands

Randomize

Standard/No treatment

Experimental arm 1

Experimental arm 2

Measure Outcome:

Percentage Of Patients That Respond.

Survival.
Human Clinical Trial Limitations

• Cannot have untreated patients as controls
• Cannot predict which patients will respond a priori
• Cannot treat same patient with multiple therapies
• Cannot evaluate scheduling efficiently
• Number of treatment arms limited by:
  – patients available
  – ethical considerations
  – financial considerations
Can Xenograft-bearing Mice Serve As Useful Avatars?
And If So ....

- Can we discover resistance mechanisms more efficiently?

- Can we develop predictors of differential treatment response?

- Can we change the way in which drug evaluation is done?

- Can we reduce the cost of drug development?
Animal Clinical Trial

Each PDX "Patient" Receives Each Treatment

- Vehicle
- Therapy 1
- Therapy 2
- Therapy 3
- Combo Therapy 1
- Combo Therapy 2
- Combo Therapy 3
- Drug Sequence 1
- Drug Sequence 2
- Drug Sequence 3
Animal Clinical Trial Advantage

- Transplant Tumors
- Randomize Each Tumor To Multiple Treatment Arms
- Compare Outcomes Across Treatment Arms
- Correlate Outcome With Patient of Origin and Xenograft-based Molecular data:
  1. Single agent response prediction
  2. Drug combinations and sequencing
  3. Resistance mechanism discovery
  4. Superiority. “GO/NO GO” decision making in drug development
  5. Translation to clinical decision making?

Dozens To Hundreds
As many as you can manage
Breast Cancer PDX Resources

- Curie Institute (France)
- Baylor College of Medicine (USA)
- University of Utah (USA)
- Washington University (USA)
- University of Colorado (USA)
- Walter and Eliza Hall Medical Research Institute (Australia)
Baylor College of Medicine Xenograft Renewable Tissue Resource

Overall:

43 Lines
33 patients

“Triple negative”: 27

ER+ PR- HER2-:  1
ER+ PR+ HER2-:  1
ER+ PR+ HER2+:  1
ER- PR- HER2+:  3
• Represent all clinically-defined subtypes
• Histologically nearly identical to tumor of origin
• Cluster molecularly with human tumors
• Phenotypically stable at the genomic, transcriptomic, and proteomic levels
• Treatment responses match tumor of origin
Chemotherapy In Clinical Practice - “Triple Negative” Breast Disease

• Neoadjuvant or Adjuvant Setting:
  – Taxanes (T)
  – Adriamycin/Cytoxan (AC)
  – Platinum-based agents (less common)

• How is the decision made?
  – T vs. AC - “Essentially identical” from a clinical perspective
  – Based largely on the experience of the clinician
  – No predictive tools available to determine best agent, or to determine who will likely not respond at all
Might There Be A Better Way?
Animal Clinical Trials

• How Many Xenograft Models Are Required?

• How Many Animals Per Treatment Arm?

• Are All Chemotherapy Agents Really Identical?

• Can Differential Response Predictors Be Identified?

• What Is The Cost To Conduct An Animal Clinical Trial?
How Many Mice Per Treatment Arm? A Continuum of Purpose

Determine efficacy in an **individual** xenograft model

Determine rate of efficacy in a **population** of xenograft models

**Middle ground:**
Determine rate of efficacy in a **population** of xenograft models; **AND** which **individual** models are most responsive

**Number Of Animals/Tumor/Tx**

MANY       SOME       1
Animal Clinical Trial Design I: The Human Paradigm (Each Patient Represented Only Once)

Recruit “Patients”

Randomize

Vehicle
Doxorubicin
Carboplatin

Outcome:
Percentage Of “Patients” That Respond To Each Treatment

20/arm
60 “Patients” Total
Animal Clinical Trial Design II: The Mouse Advantage

Randomize Each “Patient” To Each Arm
N= 1/arm

Outcome: Percentage Of “Patients” That Respond To Each Treatment
Differential Responses?

Recruit “Patients”

20/arm
20 “Patients” Total

Vehicle
Doxorubicin
Carboplatin
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Tumor Size

Vehicle
Doxo
Carbo
Animal Clinical Trial Design III: The Mouse Advantage

Assign Each “Patient” To Each Arm with Replicates.
N= 3/arm with control oversample

Recruit “Patients”

Vehicle
Doxorubicin
Carboplatin

Outcomes:
Percentage Of “Patients” That Respond To Each Treatment
Differential Responses?

20/arm
20 “Patients” Total
Putting Your Money Where Your Mouth Is: Proof-of-concept Trial

Assign Each “Patient” To Each Arm
N= 9 Vehicle control
3 per treatment arm

Recruit “Patients”

Vehicle
Docetaxel
Doxorubicin
Carboplatin

Outcomes:
Percentage Of “Patients” That Respond To Each Treatment
Differential Responses

20/arm
20 “Patients” Total
...But Do Treatment Responses In Mouse Avatars Match Responses In Women?
Xenografts Show Treatment Responses Similar To Tumor of Origin

Supplemental Table 11. Patient And Xenograft Treatment Response

<table>
<thead>
<tr>
<th>Patient</th>
<th>Xenograft Line(s)</th>
<th>Tumor Source</th>
<th>Clinical Treatment(s)</th>
<th>Clinical Response</th>
<th>Docetaxel (20 mg/kg)</th>
<th>Doxorubicin (3 mg/kg)</th>
<th>Trastuz + Lap</th>
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<td>Ascites</td>
<td>Xeloda (5FU), Pac. Others</td>
<td>Xeloda Res; Pac Res</td>
<td>Sen</td>
<td>nd</td>
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Pre, pre-treatment; Post, post-treatment; P.Br, primary breast; AC, doxorubicin (Adriamycin) and cyclophosphamide (Cytoxan); Pac, Paclitaxel; Doc, Docetaxel; Lap, Lapatinib; Trastuz, Trastuzumab; GSI, gamma secretase inhibitor; Das, Dasatinib; Sen, ≥30% regression; Res, <30% regression, stable disease or continued growth; nd, not determined.

Expected concordance = 66%  
Observed concordance = 92%  
Kappa = 0.75, p=0.003
### Table 1. Patient And Xenograft Treatment Response

<table>
<thead>
<tr>
<th>Patient</th>
<th>Xenograft Line</th>
<th>Tumor Source</th>
<th>Patient Ethnicity</th>
<th>Clinical Treatment(s)</th>
<th>Clinical Response</th>
<th>Docetaxel (20 mg/kg)</th>
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<td>Doc</td>
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<td>Sen</td>
<td>Res</td>
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Pre, pre-treatment; Post, post-treatment; P.Br, primary breast; CWR, chest wall recurrence; IDC, invasive ductal carcinoma; Mpap, micropapillary; Met, metastatic disease; AC, doxorubicin (Adriamycin) and cyclophosphamide (Cytoxan); Pac, Paclitaxel; Doc, Docetaxel; Lap, Lapatinib; Trastuz, Trastuzumab; GSI, gamma secretase inhibitor; Das, Dasatinib; Sen, .30% regression; Res, stable disease or continued growth; AA, African American; nd, not determined; nr, not reported.
Ongoing and Upcoming Animal Trials

- Sunitinib/Crizotinib alone and in combination (Westbrook)
- MEK/AKT alone and in combination (Wicha)
- SMO/GSI (Wicha)
- CHK1/PARP (Wicha)
- CXCR1 (Wicha)

- +/- Chemotherapy (all unique targets)
  - C188-9 (STAT3 inhibitor - StemMed)
  - MCB-1 (O’Malley)
  - MCB-2 (O’Malley)
  - MCB-3 (O’Malley)
  - MCB-4 (O’Malley)
  - MCB-5 (O’Malley)
  - DP-1 (Pati)
“Omics” – Linking Sensitivity To Molecular Phenotype

- Patient-matched whenever possible
- RNAseq
- RPPA proteome
- Metabololome
- Copy number analysis
- Whole genome/Exome sequencing
- Methylolome sequencing
RNAseq Principle Component Analysis (N=33)
RNAseq: PDX Models Allow Assay of Epithelial – Stromal Interactions
Chemotherapy Increases the Proportion of CD44+/CD24\textsuperscript{low/-} and Mammosphere-Initiating Cells

Li et al (2008) JNCI 100:672-9
Identification of a “TIC Signature”

A

RNA transcripts elevated in cancer MS vs bulk tumor

RNA transcripts elevated in CD44+/CD24− flow-sorted cells

2542
154
2067

significance of overlap p=1E-5

B

RNA transcripts diminished in cancer MS

RNA transcripts diminished in CD44+/CD24− flow-sorted cells

4489
339
1498

significance of overlap p=1E-15

Creighton et al., PNAS (2009) 106: 13820-13825
Targeting STAT3 Activity

Transactivates anti-apoptotic Bcl-2 proteins, cell survival factors, c-myc, enhances transactivation of androgen receptor, favors cell proliferation.

Inactivation of Forkhead & AFX TX Factors, which induce FasL, and other Apoptosis inducers, i.e., Bad, Caspase-9, and MDM-2. Activated AKT upregulates NFKB and iNOS activity; inactivating pro-apoptotic Bcl-2 proteins mediators, inactivates p53, etc., via phosphorylation events.

Piperlongumine C188
PDX Models For Use In Drug Repositioning

ORIGINAL ARTICLE
Drug-repositioning screening identified piperlongumine as a direct STAT3 inhibitor with potent activity against breast cancer

U Bharadwaj, TK Eckols, M Kolosov, MM Kassembeli, A Adam, D Torres, X Zhang, LE Dobrolecki, W Wei, MT Lewis, B Dave, JC Chang, MD Landis, CJ Creighton, MA Mancini, and DJ Tewardy
Flow Cytometric Assay For pSTAT3
Model Selection For Validation Of Drug Repositioning Screens

pSTAT3 and Total STAT3 Levels In PDX
Piperlongumine Inhibits Mammosphere Formation By PDX-derived Breast Cancer Cells
Model Selection For Validation Of Novel Compounds Targeting STAT3

pSTAT3 and Total STAT3 Levels In PDX In Two Chemoresistant Lines
A Third Generation STAT3 Inhibitor Overcomes Docetaxel Resistance
Pathway-Specific Reporters

- **3XTCF sequence**
- **7XGli sequence**
- **4XM67 sequence**
- **TATA**

**Basal**
- **Constitutive**
- **Catenin**

**TOP**
- **TCF**
- **Stat3**

**M67**
- **Gli**

**7Gli**
- **M67**

**TATA GFP**
Reporter Specificity confirmed by STAT3 Agonist Treatment (HepG2 Cells)

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<tr>
<td>STAT3</td>
<td>M67</td>
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**Bar Graph**

- IL6: 
  - non: 0
  - EFS: 0
  - TATA: 0
  - STAT3: 0
  - TATA + IL6: 100
  - STAT3 + IL6: 100

**Images**

- non
- TATA
- TATA + IL6
- EFS
- STAT3
- STAT3 + IL6
Reporter Activity In Cell Line Xenografts

IHC:pSTAT3

IHC:GFP
Mammosphere Assay Shows Enrichment Of TIC Cells In The GFP+ Subpopulation (MDA231)
Limiting Dilution Transplantation Assay Shows Enrichment Of TIC Cells In GFP\(^+\) Subpopulation (MDA231)

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Mammosphere Assay Shows Enrichment Of TIC Cells In The GFP+ Subpopulation (SUM159)
Limiting Dilution Transplantation Assay Shows Enrichment Of TIC Cells In GFP\(^+\) Subpopulation (SUM159)

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Drug Sequencing: PDX-Based Sequencing Informs Clinical Trial Design

Preclinical and Clinical Studies of Gamma Secretase Inhibitors with Docetaxel on Human Breast Tumors

Anne F. Schott¹, Melissa D. Landis², Gabriela Dontu⁴, Kent A. Griffith¹, Rachel M. Layman⁵, Ian Krop⁶, Lacey A. Paskett², Helen Wong², Lacey E. Dobrolecki², Michael T. Lewis³, Amber M. Froehlich², Jaya Paranilam², Daniel F. Hayes¹, Max S. Wicha¹, and Jenny C. Chang²,⁷
Cost Comparison

• Phase I - Human:
  – Two arms
  – 30 Patients/arm (60 total)

  $1.5-2.5M

  • Little or no efficacy data
  • No correlative biomarkers discoverable
  • No predictive analysis

• Phase I - Animal:
  – Two arms
  – 30 PDX/arm (30 total)

  $50K-60K

  • Quantitative efficacy data
  • Correlative biomarkers discoverable
  • Predictive analysis possible
PDX-Based Studies

• Representative models exist, particularly as a community
• Accurately reflect patient biology
• Accurately reflect patient treatment response
• Differential treatment response prediction should be possible to inform phase II studies
• Cost effective relative to human phase I
My Avatar Now That All This Is Working
Acknowledgements

**Lewis Lab**
Xiaomei Zhang
Ivana Petrovic
Lacey Dobrolecki
Wei Wei
John Landua

**Rosen Lab**
Jeffrey Rosen
Mei Zhang

**Westbrook Lab**
Trey Westbrook
Sid

**Tweardy Lab**
David Tweardy
Uddalak Bharadwaj

**O’Malley Lab**
Bert O’Malley
David Lonard

**Breast Center**
Susan G. Hilsenbeck
Mothaffar F. Rimawi
Meng-Fen Wu
Anne C. Pavlick
Alejandro Contreras
Carolina Gutierrez
Rachel Schiff
Mario Giuliano

**BCM**
Chad Shaw
Lisa White

**University of Michigan/Karmanos Cancer Institute**
Max Wicha
Pat Lorusso

**TMHRI**
Jenny C. Chang

**MDACC**
Sofie Claerhout
Gordon B. Mills

**UNC**
Charles M. Perou
Aleix Pratt

**Hollywood**
James Cameron