PDX mouse models integrated into a precision medicine initiative for ovarian cancer

Tim Starr, University of Minnesota
Micro Biobanks?

AllofUs Million People Biobank
Mayo Clinic receives $142 million over 5 years

Starr Lab Biobank
Starr Lab receives $50,000 over 1 year
Disease Course of an Ovarian Cancer Patient

- **Years**
  - 1
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7
  - 8
  - 9

- **CA125 (Units/mL)**
  - 0
  - 35
  - 60
  - 90
  - 120
  - 150
  - 180
  - 210
  - 240
  - 270
  - 300

- **Diagnosis and Treatment Timeline**
  - **Primary tumor resection**
  - **Recurrence detection**
  - **Stop treatment**
  - **Resection**

- **Diagnosis Sites**
  - Vagina
  - Diaphragm
  - Liver
  - Spleen

- **Treatments**
  - PET
  - IP cisplatin
  - Paclitaxel
  - IV carboplatin
  - Pegylated liposomal doxorubicin
  - CT scan
  - Gemcitabine
  - Bevacizumab
  - Cyclophosphamide
  - Pemetrexed
  - Topotecan

Jimenez-Sanchez, Miller, et al., Cell 2017
Minor improvement in survival rate for ovarian cancer patients over the last 40 years

Ovarian Cancer 5-Year Survival Rates 1975 - 2012

Survival and Stage at Diagnosis

Institute of Medicine, 2016, SEER Statistics 2017
Based on a molecular analysis of a patient’s tumor, can we predict their response to treatment and their long-term prognosis?
Ovarian Cancer Precision Medicine Initiative at the University of Minnesota

Prospective study of 100 patients

- scRNA-seq
- scDNA-seq

**PDX models** *

Clinical data
- CLIA Lab targeted exome
  - somatic
  - germline

Goal: Identify biomarkers that predict chemotherapy resistance and identify targetable genes and pathways
Why do it ourselves?

Patient-Derived Xenograft - PDX Models

Over 2,500 clinically-relevant PDX models that truly mimic human disease.

Why do it ourselves?

Tumor Model Collection

- 459 Oncotest™ Patient-Derived Xenografts (PDX)

Welcome

Welcome to the Champions TumorGraft® database. The database includes molecular characterization data obtained through Whole Exome Sequencing and RNA-Seq technology, and represents the most annotated compendium of patient-derived xenograft (PDX) models available for searching through a web-based interface.

Please select a query below to browse models and characterization data of frequently-requested model sets. You can also go directly to the model filter page to start building your own queries.

If you have any questions, suggestions or need for assistance, please email database@championsoncology.com.

PDX Models Syngeneic Models Human Cell Lines Knowledgebase
Why do it ourselves?

Cost

Experimental control

Integration of local patients

Access to follow-up clinical data and possible recurrent specimens

Patient Derived Xenograft/PDx Models Market Worth 167.6 Million USD by 2022

Costs involved

Project costs
Develop protocols, train personnel, get approvals (IACUC, IRB) = ? Salaries
Recruit patients = ? Salaries and support of clinical collaborators and staff
Maintain records = ? Salaries and administrative support

Per patient costs
Obtain sample (UMN Biospecimen charge) = $60
Purchase mice (5-10 mice/patient, NSG or Nude) = $350 - $1,000
Surgery costs (salaries, isofluorane, etc) = $200+
Husbandry costs (5-10 mice, 2 cages, 1 year, $1/day/cage) = $700
Weekly measurement costs = ? Salaries
Our Funding

OCRA ovarian cancer research alliance

Chorzempa Ovarian Cancer Research Endowed Fund

University of Minnesota Driven to Discover

MASONIC CANCER CENTER

MOCA Minnesota Ovarian Cancer Alliance 20 Years of Progress

Department of Obstetrics, Gynecology and Women's Health

Masonic Cancer Center

Comprehensive Cancer Center designated by the National Cancer Institute
# Methods – Mouse Model Choice

<table>
<thead>
<tr>
<th>Model Choice</th>
<th>Nude</th>
<th>SCID</th>
<th>NSG</th>
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<tbody>
<tr>
<td>Cost</td>
<td>$72</td>
<td>$123</td>
<td>$171</td>
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<tr>
<td>Ease of surgery</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ease of measurement</td>
<td>+++</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Engraftment rate</td>
<td>?</td>
<td>?</td>
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</table>
### Methods – Implantation Site

**Transplant Site Choice:**
- Subcutaneous surgery
- Intraperitoneal injection
- Ovarian bursa surgery

<table>
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<tr>
<th>Orthotopic</th>
<th>Procedure</th>
<th>Measurement</th>
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<tr>
<td>No</td>
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<td>Yes</td>
<td>Easy</td>
<td>Difficult</td>
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<tr>
<td>Yes</td>
<td>Very difficult</td>
<td>Difficult</td>
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Methods – Obtain Sample

Considerations

Coordination between providers, consenting staff, surgical team, Biospecimen procurement staff and lab staff

Surgery is rarely done when planned

Protocols in place for selecting samples and temporary storage

Minimize time from cutting blood flow to implantation into mice
Methods – Surgery

Procedure
Anesthetization: Isoflurane.
Incision: Dorsal/Ventral (Problems with tumors growing together). Small incision (large enough for tweezers). Detach from inner layer (make "pouch"). Place specimen (~2-4 mm chunk).
Wound closure: Staples (removed 7+ days later)
Pain: Analgesics (ketoprofen)
Methods – Monitoring & Chemotherapy Administration

Considerations
Weekly tumor measurements (longest diameter vs two measurements, minimum measurement, variability)
Published chemotherapy doses are not always applicable. Perform mini-MTD testing before treating experimental animals. Be flexible with administration by monitoring “patient”. Our current dosage: Carboplatin (15 mg/kg) and Paclitaxel (6 mg/kg) on days 1, 4 and 7.
OCPMI Enrollment Statistics

First Patient Sample: 12/11/14

As of 10/30/19 (4.9 years):
Consented: 154 (~30/yr)
Enrolled: 72 (~15/yr)

Attempted PDX models: 45
Engraftment Rate: 56% (25/45)

Mice receiving chemo: 79

Mouse Statistics

<table>
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<tr>
<th>Description</th>
<th># of mice</th>
<th>% engrafted</th>
<th>Avg Days to &gt; 0.7 cm</th>
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<tbody>
<tr>
<td>All Enrolled mice</td>
<td>593</td>
<td>38%</td>
<td>128</td>
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<tr>
<td>N1 enrolled mice</td>
<td>324</td>
<td>25%</td>
<td>178</td>
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<tr>
<td>N2+ enrolled mice</td>
<td>269</td>
<td>55%</td>
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Use of PDX Models – Mesenchymal Stem Cells as Drug Delivery

Two-step drug delivery via *in vivo* click chemistry

Human mesenchymal stem cells (MSCs)

IL-15 expressing glycoengineered MSCs

Tumor bearing mice imaged by IVIS

Paclitaxel

AM6 antibody

DBC0-conjugated nanoparticles
Experimental Design – PDX models for testing MSC drug delivery

Inject 50 mice
Separate into cohorts
Tumor size > 0.7 cm
Inject MSCs & NPs
Age & monitor

Cohort 1
Saline control

N = 6

Cohort 2
Paclitaxel

N = 9

Cohort 3
Paclitaxel in NPs

N = 9

Cohort 4
MSCs + NPs

N = 9

Cohort 5
Paclitaxel in NPs + MSCs

N = 9
Two-step drug targeting using MSCs and NPs improves anti-tumor efficacy
Example 2 – Analysis of single cell gene expression

Control

Chemo

10X Genomics

Illumina NGS
scRNA-Seq Objectives

1) Identify and quantify cell types based on gene expression
   - Cancer cells, Stromal cells, Immune cells, others

2) Identify rare cell types, including cancer stem cells

3) Correlate presence/quantity of cell types with clinical findings
   - Platinum response (sensitive, resistant, refractory)
   - Histotypes (serous, endometrial, clear cell, mucinous)
   - Molecular subtypes based on bulk sequencing

4) Identify cell types associated with chemotherapy resistance
   - Validate with PDX models treated with chemotherapy
10X Technology: Drop-Seq, 3’ polyA, Unique Molecular Identifier

Zheng, et al., Nature Communications 2017
Data output is a gene expression matrix of genes x cells

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<th>D</th>
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Based on gene expression can identify human vs mouse cells

Quantification of mouse cells vs human cells in two mouse PDX grafts from a single patient
Clustering based on gene expression identifies cell groups

Original Patient Sample

Mouse PDX untreated

Recurrent Patient Sample

Mouse PDX treated with chemo
Use knowledge-based lists to assign cell types to groups

Overlap of up-regulated genes in a cluster with knowledge gene lists
Single cell analysis of chemotherapy response

Original Patient Sample
Mouse PDX untreated
Recurrent Patient Sample
Mouse PDX treated with chemo

Comparison of cell types present before and after chemotherapy resistance used to understand mechanisms of resistance
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