Comparison of PDX, PDC, and PDOrg models from the National Cancer Institute’s Patient-Derived Models Repository (PDMR)

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Leidos Biomedical Research, Inc. In Support of the Division of Cancer Treatment and Diagnosis, NCI
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American Association for Cancer Research Annual Meeting

https://pdmr.cancer.gov
Overview

• Status of PDXs, organoids (PDOrgs), tumor cell lines (PDCs), and cancer associated fibroblasts (CAF) available today and in the upcoming 6-months

• Comparative analysis of WES and RNASeq data from matched PDXs, organoids (PDOrgs), and tumor cell lines (PDCs)

• Preliminary comparison of a set of PDX, PDOrg, and PDC models derived from a rapid autopsy case from one patient with Pancreatic Adenocarcinoma
NCI’s Patient-Derived Models Repository

- A national repository of Patient-Derived Models (PDMs) to serve as a resource for academic discovery efforts and public-private partnerships for drug discovery

- Clinically-annotated & early-passage models with comprehensive molecular-characterization and quality control metrics

- Complement existing PDM collections and focus on under-represented model types such as rare cancers and models representing racial and ethnic minorities

- Provide models to the research community at a modest cost compared to other distributors

- Provide all related metadata including: deidentified patient clinical history and outcomes, model histology, WES and RNASeq of models, and SOPs through a public website: [https://pdmr.cancer.gov](https://pdmr.cancer.gov)

Ship overnight from Clinic to FNLCR
- Attempt to generate multiple patient-derived model types

PDX

PDOrg Culture

PDC/CAF Culture
Model Development and Characterization
# PDX Take-Rate from Tumor Tissue Implantations

<table>
<thead>
<tr>
<th>Body Location</th>
<th>Total Specimens Received</th>
<th>Total Assessable Specimens</th>
<th>%Take-Rate of Assessable Specimens</th>
<th>Histology-Confirmed Tumor</th>
<th>Discontinued</th>
<th>Not Yet Assessable: P0 tumors</th>
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</thead>
<tbody>
<tr>
<td>Breast</td>
<td>307</td>
<td>238</td>
<td>38%</td>
<td>91</td>
<td>147</td>
<td>69</td>
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<tr>
<td>Digestive/ Gastrointestinal</td>
<td>692</td>
<td>614</td>
<td>52%</td>
<td>321</td>
<td>293</td>
<td>78</td>
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<tr>
<td>Endocrine/ Neuroendocrine</td>
<td>194</td>
<td>166</td>
<td>24%</td>
<td>40</td>
<td>126</td>
<td>28</td>
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<tr>
<td>Genitourinary</td>
<td>528</td>
<td>453</td>
<td>37%</td>
<td>168</td>
<td>285</td>
<td>75</td>
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<tr>
<td>Germ Cell</td>
<td>4</td>
<td>4</td>
<td>0%</td>
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<td>0</td>
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<tr>
<td>Gynecologic</td>
<td>327</td>
<td>265</td>
<td>52%</td>
<td>137</td>
<td>128</td>
<td>62</td>
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<tr>
<td>Head and Neck</td>
<td>168</td>
<td>162</td>
<td>54%</td>
<td>88</td>
<td>74</td>
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<tr>
<td>Hematologic/Blood</td>
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<td>13</td>
<td>54%</td>
<td>7</td>
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<td>7</td>
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<tr>
<td>Musculoskeletal</td>
<td>385</td>
<td>351</td>
<td>34%</td>
<td>118</td>
<td>233</td>
<td>34</td>
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<tr>
<td>Neurologic</td>
<td>30</td>
<td>27</td>
<td>63%</td>
<td>17</td>
<td>10</td>
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<tr>
<td>Respiratory/Thoracic</td>
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<td>198</td>
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<td>95</td>
<td>103</td>
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<tr>
<td>Skin</td>
<td>80</td>
<td>75</td>
<td>64%</td>
<td>48</td>
<td>27</td>
<td>5</td>
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<tr>
<td>Unknown Primary</td>
<td>20</td>
<td>19</td>
<td>26%</td>
<td>5</td>
<td>14</td>
<td>1</td>
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<tr>
<td><strong>Totals</strong></td>
<td><strong>2980</strong></td>
<td><strong>2585</strong></td>
<td><strong>44%</strong></td>
<td><strong>1135</strong></td>
<td><strong>1450</strong></td>
<td><strong>395</strong></td>
</tr>
</tbody>
</table>

All tumor material collected and shipped priority overnight in CO2-independent media for next-day implantation into NSG host mice
Rare Cancer Histology PDX Models Available

- Merkel Cell Carcinoma
- Mesothelioma
- Hurthle Cell Neoplasm of the Thyroid
- Malig. Periph. Nerve Sheath Tumor
- Salivary Gland SCC
- Pharyngeal SCC
- Nasopharyngeal SCC
- Laryngeal SCC
- Vaginal Cancer
- Cervical SCC
- Carcinosarcoma of the Uterus
- Synovial Sarcoma
- Liposarcoma
- Leiomyosarcoma – uterine and non-uterine
- Rhabdomyosarcoma
- Osteosarcoma
- Chondrosarcoma
- Malignant fibrous histiocytoma
- Fibrosarcoma – not infantile
- Ewing sarcoma/Peripheral PNET
Patient-Derived Xenografts (PDXs)

- **222 PDX models publicly available** (pdmr.cancer.gov).
  - 210 models going through final QC (final pathology, NGS, STR, regrowth from freeze,…)
  - ~200-300 models in Passage 1-4 expansion
  - ~600 models in Passage 0

- Clinically-annotated, early-passage, molecularly-characterized patient-derived models

- **Distribution Material**
  - Median Passage = 2
    - Range for NCI-generated models: 1-6
    - Range for Contributor models: 1-24

- Current distribution within the US (pdmr.cancer.gov).
  - Model information also available through PDX Finder at www.pdxfinder.org

- Specimens are from patients with both primary and metastatic disease from treatment naïve to heavily pretreated.
Patient/PDX-Derived Cancer Cell Lines (PDCs) and Cancer Associated Fibroblast Cultures (CAFs)

- **52 PDCs**
  - Colorectal Cancer
  - Upper GI Cancers
  - Head & Neck Cancers
  - Urothelial/Bladder Cancer
  - Melanoma & Skin Cancers
  - Pancreatic Adenocarcinoma
  - Non-Small Cell Lung Cancer
  - Small Cell Lung Cancer
  - Adult Soft Tissue Sarcoma
  - Renal Cancer
  - Gynecologic Cancers
  - Endo/Neuroendocrine Cancers
  - Other Cancers

- **Adherent & Suspension Cultures**
- **Requires use of defined media**
- **Distribution Material**
  - Median Passage = 20
  - Range: 12-51

- **125 CAFs**
  - Colorectal Cancer
  - Upper GI Cancers
  - Head & Neck Cancers
  - Urothelial/Bladder Cancer
  - Melanoma & Skin Cancers
  - Pancreatic Adenocarcinoma
  - Non-Small Cell Lung Cancer
  - Small Cell Lung Cancer
  - Adult Soft Tissue Sarcoma
  - Renal Cancer
  - Gynecologic Cancers
  - Endo/Neuroendocrine Cancers
  - Other Cancers

- **Not Transformed - Limited Lifespan**
- **Requires use of defined media**
- **Distribution Material**
  - Median Passage = 14
  - Range: 9-26
Patient/PDX-Derived Organoids (PDOrg)

- First 46 models are publicly available with another 30 going through QC (NGS, tumorigenicity verification, STR, etc)

- **Goal**: Wherever possible develop a PDX, 2D *in vitro* PDC, and PDOrg culture for comparative preclinical studies

- Provide all related metadata and SOPs through the PDMR website and public database: [pdmr.cancer.gov](http://pdmr.cancer.gov)

- Requires use of defined media
- Distribution Material
  - Median Passage = 10
    - Range: 6-30
Matched PDX, PDOrg, PDC, and CAF Models

Includes models that are either

(1) Publicly Available or

(2) Going through final QC for Public release (pathology confirmation of all contributing material, NGS, STR, regrowth from cryopreservation, etc)

<table>
<thead>
<tr>
<th></th>
<th>PDX</th>
<th>PDC</th>
<th>PDOrg</th>
<th>CAF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>421</td>
<td>95</td>
<td>54</td>
<td>131</td>
</tr>
</tbody>
</table>
Molecular Characterization
Comparison of PDX, PDC, and PDOrg Model Types
Comparing PDX, PDC, and PDOrg Model Types

• Data for Comparison
  o 413 PDX samples from 75 PDX models
  o 43 PDOrg models
  o 73 PDC models

• Whole Exome Sequence Comparisons
  o Driver Allele Frequency (VAF)
  o Loss of Heterozygosity (LOH)
  o Microsatellite Instability

• RNASEq
  o RNASEq Correlation Matrix
  o tSNE Plots

✓ Driver mutations are present in all model types (PDX, PDOrg, PDC)

✓ Irrespective of model type (PDX, PDOrg, PDC), gene expression profiles cluster by histology and by patient

✓ The majority of models are internally consistent for LOH; however, models can be found with differences at the LOH level between model types (PDX, PDOrg, PDC)
Variant Allele Frequency and Loss of Heterozygosity is Consistent Across Model Types

Driver Allele Frequency Consistent Across Majority of Models

Percent LOH Consistent

Germline not subtracted
Majority of Models have Consistent LOH Across Model Types

Originating Patient: 84yo, Treatment Naïve, Urothelial Transitional Cell Carcinoma

<table>
<thead>
<tr>
<th>Model</th>
<th>Sample</th>
<th>Passage</th>
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<td>159449-244-R</td>
<td>J1-PDC</td>
<td>Passage 19</td>
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<tr>
<td>159449-244-R</td>
<td>PDX</td>
<td>Passage 2</td>
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<td>PDX</td>
<td>Passage 1</td>
</tr>
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<td>Passage 0</td>
</tr>
<tr>
<td>159449-244-R</td>
<td>V1-organoid</td>
<td>Passage 14</td>
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</tbody>
</table>

- LOH on 3p, 4q, 9, 11, 13, and 14 are preserved
- Imbalance on 1q, 8, and 10p are preserved
## MSI-High Status is Consistent Across Model Types

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Originator</th>
<th>PDX</th>
<th>PDOrg</th>
<th>PDC</th>
<th>Originator</th>
<th>PDX</th>
<th>PDOrg</th>
<th>PDC</th>
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<tr>
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<tr>
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<td>Uterine Ca</td>
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<td></td>
</tr>
</tbody>
</table>
RNASeq Correlation Matrix

[Image: Heatmap of gene expression correlation with annotations highlighting colorectal/GI cancers and small cell carcinoma of colonic origin]

- **Diagnosis Key**: Bladder/Urothelial, Head & Neck, Other Squamous Cell, Melanoma, Sarcoma, Lung, Gynecologic, Pancreatic, Colon/GI, Neuroendocrine, Other

- **Sample Types**: PDX, PDC, Organoid

- **Color Key**: -1 to 1, indicating correlation values
tSNE for all models w PDXs, PDCs, and/or PDOrgs

- Every point represents an individual PDX, PDC, or PDOrg. Within one model, 4-6 PDXs can be sequenced.
- Data represent:
  - 413 PDX samples for 75 PDX models
  - 73 PDC models
  - 43 PDOrg models
tSNE for all models w PDXs, PDCs, and/or PDOrgs

- Every point represents an individual PDX, PDC, or PDOrg. Within one model, 4-6 PDXs can be sequenced.
- Data represent:
  - 413 PDX samples for 75 PDX models
  - 73 PDC models
  - 43 PDOrg models
Expression Profiles Correlate Across the Majority of Models for Different Model Types: PDX, PDC, PDOrg

Colon/Digestive and Pancreatic Cancer Expression Clusters

- PDOrg
- PDC
- Gynecologic (Pink)
- Pancreatic (Blue)
- Colon/GI (Brown)

176247-063-R, Colon Adeno
514821-333-R, Colon Adeno
521955-158-R6, Pancreatic Adeno
292921-168-R, Pancreatic Adeno
Expression Profiles Correlate Across the Majority of Models for Different Model Types: PDX, PDC, PDOrg

Melanoma and Sarcoma Expression Clusters

- PDOrg
- PDC
- Melanoma
- Sarcoma
- Other

425362-245-T, Melanoma
128128-338-R, Melanoma
698357-238-R, Osteosarcoma
287954-098-R, Ewings Sarcoma

NCI Patient-Derived Models Repository
An NCI Precision Oncology Initiative Resource
Preliminary data from a set of Pancreatic Adenocarcinoma Models Derived from a Rapid Autopsy Case
<table>
<thead>
<tr>
<th>Model ID</th>
<th>Resection Site</th>
<th>Tissue Origin</th>
<th>PDX</th>
<th>PDOrg</th>
<th>PDC</th>
<th>Pt Tumor</th>
<th>NGS Status</th>
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</thead>
<tbody>
<tr>
<td>521955-158-R2</td>
<td>Liver (A)</td>
<td>Metastatic Site</td>
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<td>✔</td>
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<td>✔ NGS Complete</td>
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<tr>
<td>521955-158-R3</td>
<td>Liver (B)</td>
<td>Metastatic Site</td>
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<td>✔ NGS Complete</td>
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<td>521955-158-R4</td>
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<td>Metastatic Site</td>
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<td>Colon</td>
<td>Metastatic Site</td>
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<td></td>
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<td></td>
<td>NGS In Progress</td>
</tr>
</tbody>
</table>

A CAF has been generated and will be sequenced as a surrogate germline reference.
**Rapid Autopsy Pancreatic Adenocarcinoma Models**

**521995-158-Rn**

**All driver mutations present**
- KRAS-G12D (~60% AF)
- TP53-R158Sfs*8 (~100% AF)
- Homozygous Deletion of CDKN2A, CDKN2B, MAP2K4

2D/3D Growth Conditions likely drive difference at expression level
- PDC grown on Matrigel-coated flasks in DMEM/F12 Complete Media + Y compound
- PDOrg grown in Panc media in BME2 domes

### WES

<table>
<thead>
<tr>
<th>Model ID</th>
<th>Resection Site</th>
<th>Tissue Origin</th>
<th>PDX</th>
<th>PDOrg</th>
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<th>Pt Tumor</th>
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</thead>
<tbody>
<tr>
<td>521955-158-R2</td>
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<td>✓</td>
<td>✓</td>
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<td>521955-158-R3</td>
<td>Liver (B)</td>
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</table>

### RNASeq

- **521955-158-R2**: 158-R2-J5-PDC
- **521955-158-R3**: 158-R3-J6-PDC
- **521955-158-R4**: R2-IV-organoid
- **521955-158-R5**: R2-W9
- **521955-158-R6**: R2-W9
- **521955-158-R7**: R2-V5-organoid

**Δ PDOrg**
- PDOrg

**PDC**
- PDC
521955-158-R2, Adenocarcinoma - pancreas

Glandular architecture is present: nests of glands with areas of back-to-back gland formation.
Differences in LOH observed on Chromosomes 7, 8, and 11
Rapid Autopsy Pancreatic Adenocarcinoma Models

521955-158-Rn

<table>
<thead>
<tr>
<th>Model ID</th>
<th>Resection Site</th>
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<th>PDOrg</th>
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<td>Liver (A)</td>
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<td>✔</td>
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<td>〉</td>
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<tr>
<td>521955-158-R3</td>
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</tr>
<tr>
<td>521955-158-R4</td>
<td>Colonic Fat</td>
<td>Metastatic Site</td>
<td>✔</td>
<td>〉</td>
<td>〉</td>
<td>〉</td>
</tr>
</tbody>
</table>

Differences in LOH observed on Multiple Chromosomes

<table>
<thead>
<tr>
<th>Model ID</th>
<th>Sample</th>
<th>Passage</th>
</tr>
</thead>
<tbody>
<tr>
<td>521955-158-R2</td>
<td>PDX</td>
<td>Passage 0</td>
</tr>
<tr>
<td>521955-158-R2</td>
<td>PDC</td>
<td>Passage 18</td>
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<td>521955-158-R2</td>
<td>PDOrg</td>
<td>Passage 14</td>
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<td>521955-158-R3</td>
<td>PDX</td>
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<td>521955-158-R3</td>
<td>PDC</td>
<td>Passage 25</td>
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<tr>
<td>521955-158-R4</td>
<td>PDX</td>
<td>Passage 0</td>
</tr>
</tbody>
</table>
Conclusions

• Driver mutations are maintained within a model across all model types: PDX, PDOrg, PDC

• Irrespective of model type (PDX, PDOrg, PDC), gene expression profiles cluster by histology and with other models from the same patient

• The majority of models are internally consistent for LOH; however, models can be found with differences at the LOH level between model types (PDX, PDOrg, PDC)
The NCI expresses its deepest thanks to the patients, families, and clinical teams that make this effort possible.