The Center for Advanced Preclinical Research: Evaluation of Cancer Therapeutics in Translational Mouse Cancer Models

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Mouse 101
6/5/18
• Introduction to CAPR Mission and Workflow

• Brief model overview:
  – Lung cancer
  – Ovarian cancer
  – Glioblastoma
  – Melanoma
  – PDX
Develop strategies for effective preclinical evaluation in murine cancer models

AND facilitate routine application in clinical research for optimal outcomes in cancer patient management.

- combination of internal research and intra/extramural collaborations
- Genetically engineered mouse (GEM) models, GEM-derived allograft models (subcutaneous and orthotopic), investigator-requested models, and “custom” PDX
Improving outcomes for GEM/orthotopic model studies

• Well-characterized model
  – tumor histopathology
  – latency and time to endpoint - metastases?
  – positive control for efficacy or standard of care
  – expressing biomarkers related to oncogene(s) of interest?
  – for IMT: T cell markers
  – can a large enough cohort for statistical significance be produced?

• How will we monitor growth of internal (autochthonous/orthotopic) tumors?
  – palpation, live animal imaging: MRI, ultrasound, BLI

• Decide at the start of the study what the endpoints will be, and the necropsy plan
  – ex: fresh or viably frozen tissue for transplant and/or cell line
  – flash-frozen
  – fixed tissue for pathology
  – blood
Improving outcomes for GEM/orthotopic model studies (cont.)

- Establish (preclinical friendly) timeline for the study
  - Does recruitment need to be staggered to get well-randomized tumor sizes?
  - In vivo imaging intervals?

- What is the treatment regimen/route/dose?
  - Drug tolerance may be different in wildtype mice vs. nudes
  - Availability of drug in tumor tissue - short-term PK/PD needed?
Available preclinical cancer models

<table>
<thead>
<tr>
<th>Model Type</th>
<th>Genetic Events</th>
<th>Induction</th>
<th>Allograft</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung AdCa</td>
<td>EGFR^{L858R/T790M}</td>
<td>Doxycycline</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Kras^{G12D/Lkb1}; Kras^{G12D/p53}</td>
<td>AdCre/Lenti-Cre</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>EML4-ALK</td>
<td>Doxycycline</td>
<td>-</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>pRb/Kras^{G12D}/PTEN</td>
<td>GFAP-CreER/tam Lenti-Cre</td>
<td>✓</td>
</tr>
<tr>
<td>Serous Ovarian Carcinoma</td>
<td>pRb/p53^{c/c or m/-}</td>
<td>AdCre</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>pRb/p53^{c/c or m/-}/Brca1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pRb/p53^{c/c or m/-}/Brca2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>HGF/MET</td>
<td>UV or DMBA</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>BRAF^{V600E}</td>
<td>UV, Tyr-Cre</td>
<td>✓</td>
</tr>
<tr>
<td>Pancreatic Ductal AdCa</td>
<td>Kras^{G12D}/p53</td>
<td>PDX1-Cre</td>
<td>✓</td>
</tr>
</tbody>
</table>

- In development: Brca1/pRB/p53 mutant mammary cancer models, both GEM and orthotopic
CAPR translational research tools/workflow

- Potency assays
- PD assays
- Molecular profiling

GEM and allograft

- Tolerability assays
- PK/PD assays
- Efficacy
- Prevention studies

In vivo monitoring

- Enrollment based on tumor volume
- Model-specific live imaging to track efficacy
- Defined endpoints
- Pathology/histology
- Qualitative and quantitative molecular profiling of blood/tissue

- MRI
- BLI
- H&E
- IHC
- Transcriptome
- Metabolome
### CAPR models for lung adenocarcinoma

<table>
<thead>
<tr>
<th>Lung cancer subtype</th>
<th>Non-small cell</th>
<th>Small cell</th>
</tr>
</thead>
</table>
| **MOUSE model available at CAPR (genetic alterations)** | • Kras;Lkb1  
• Kras;p53  
• EGFR-L858R (TKI sensitive)  
• EGFR-L858R-T790M (TKI insensitive)*  
• EML4-ALK | • Rb;p53 |

**PK/PD**

- Tumor-bearing lung
- Vehicle vs. MK-2206

**PET-CT**

- Image of PET-CT scan

**MRI**

- Image of MRI scan

**Histology**

- Image of histology

*Example: Worked with a pharmaceutical company using an EGFR mutant-driven lung cancer model to predict effect in target patient population*
Evaluation of a 3rd generation covalent inhibitor of EGFR (with Clovis Oncology)

- Study design used for both erlotinib-sensitive and insensitive (human)EGFR-driven GEM models

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Treatment</th>
<th>Dose/Schedule</th>
<th>Harvest Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>Vehicle</td>
<td>PO, QD</td>
<td>21 days post treatment</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>Afatinib (BIBW2992)</td>
<td>20mg/kg PO, QD</td>
<td>21 days post treatment</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>CO-1686</td>
<td>50mg/kg PO, BID</td>
<td>21 days post treatment</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>CO-1686</td>
<td>100mg/kg PO, QD</td>
<td>21 days post treatment</td>
</tr>
</tbody>
</table>

- Readouts in the EGFR-L858R-T790M model
  - Tumor volume (MRI)
  - Lung tissue – Histopathology (H&E), Proliferation (Ki67), IHC (pEGFR)
  - Continue dosing n=6 mice to generate resistant tumors
CO-1686 is efficacious as a single agent

CO-1686 is very potent in EGFR^{L858R/T790M} transgenic model (similar response in EGFR^{L858R})
Activity is associated with reduced proliferation and pEGFR
_Afatinib dosed at MTD due to wild type EGFR blockade; efficacy limited_

Efficacy:
- CO-1686: Baseline
- CO-1686: 3W

Change from Baseline (%)

Walter A.O. et al., Cancer Discovery 2013;3:1404-1415
CO-1686-treated lungs have almost no evidence of tumor burden

Vehicle
Score 4.5

Afatinib (20 mg/kg QD)
Score 3.5

CO-1686 (50 mg/kg BID)
Score 0.5

CO-1686 (100 mg/kg QD)
Score 1.0

Small atypical adenoma
(n=1)
CAPR OVARIAN CANCER MODELS
Serous epithelial ovarian cancer GEM recapitulates features of human disease

Transcriptome analysis

Mouse model for SEOC
- RB-TS inactivation
- p53 mutation/loss
- BRCA1 or BRCA2 loss

Histology
- Papillary structures

Gross pathology/MRI

Analysis of blood metabolites

<table>
<thead>
<tr>
<th>Human ovarian cancer TISSUE</th>
<th>Mouse ovarian cancer SERUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-hydroxybutyrate</td>
<td>↑</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>↑</td>
</tr>
<tr>
<td>β-hydroxybutyrate</td>
<td>↑</td>
</tr>
<tr>
<td>citrate</td>
<td>↑</td>
</tr>
<tr>
<td>short chain acyl carnitines</td>
<td>↑</td>
</tr>
<tr>
<td>taurine</td>
<td>↓</td>
</tr>
</tbody>
</table>

Szabova et al., Cancer Res, 2012
Orthotopic model maintains features of GEM with a preclinical–friendly timeline

- Multifocal peritoneal carcinomatosis with tumor spread to all abdominal organs + lung mets as in GEM
CAPR GLIOBLASTOMA MULTIFORME MODELS
Orthotopic GDA model recapitulates features of GEM and human GBM

- Pseudopalisading tumor cells
- Necrosis
- Invasive

- Potency assays
- Target pathway analyses

Kras;Rb;Pten GFAP-CreER tamoxifen induction

Intracranial implant into wildtype recipients
Neural stem cell and astrocytic markers are expressed in invasive regions of the orthotopic tumors

- Invasion into non-neoplastic parenchyma
- Heterogenous cell population as in human GBM
GBM model evaluated in preclinical study evaluating PI3K and MAPK inhibitor combination

- Short term tumor suppression led to a small survival benefit

El Meskini et al., Disease Models & Mech, 2015
Melanoma models represent pathways perturbed in human melanoma

**HGF/SF-Tg;CDK4^{R24C}**

- HGF expression in BRAF-resistant melanoma
- Epitheloid histology
- (in vivo pagetoid spread)

**BRAF^{V600E};PTEN+/-;CDKN2A+/-**

- BRAF mutated in >50% of human melanoma
- Sarcomatous histology

**GDA: GEM-derived allograft model**

- Collect melanoma tissue
- Label with Luciferase/GFP
- Transplant to syngeneic mice
- Establish metastatic model by resection (HGF/SF-Tg;CDK4^{R24C})

- Lung and liver metastasis
Evaluation of therapeutic responses in metastatic melanoma GDA

- Resection of primary tumor
- Adjuvant treatment
- Time to metastasis/regrowth
- Targeted chemotherapy
- Immunotherapy

Primary melanoma

First-line treatment

Transplantation to syngeneic recipient mice (GDA)

HGF;CdK4\textsuperscript{R24C} GEM primary melanoma
Melanoma models with diverse drivers respond differently to anti-CTLA-4 antibody.

- BRAF-driven model is sensitive to vemurafenib, no additional effect from anti-CTLA-4.
- HGF/MET-driven model responds to anti-CTLA-4.
CAPR PDX studies
Evaluation of therapeutic responses in Patient-Derived Xenografts (PDX)

• Using fresh patient tumor tissue to implant either subcutaneously or orthotopically in immunocompromised mice

• NSG (NOD-SCID-gamma) mice developed at the Jackson Laboratory commonly used as recipients (B and T cell deficient; also deficient in functional NK cells)

• Advantages:
  – ability to treat actual patient tumor material and evaluate drug efficacy
  – Can do longitudinal studies if multiple biopsies are part of clinical trial

• Disadvantages:
  – Tumors may change in mutation spectrum, gene expression after serial passaging in mice
  – Take rate may not be ideal (depends on tissue origin)
  – Immunocompromised mice are not useful for evaluating most immunotherapies
PDX example longitudinal study:

Acquired resistance to targeted drugs in EGFR-driven lung cancer (collaboration with Dr. Udayan Guha, TGOB NCI)

- Clinical trial NCI-16-C-0092: A Pilot Study of Local Ablative Therapy for Treatment of Oligoprogressive, EGFR-Mutated, Non-Small Cell Lung Cancer (NSCLC)

- Take tumor samples (from biopsy, lung surgery, or surgery on metastasis) prior to treatment, at progression, and at progression post-treatment

- Characterize implanted tumors for lung adenocarcinoma markers; sequencing and gene expression to confirm mechanisms of resistance

- Therapeutic efficacy study with combination treatment to inhibit pathways contributing to resistance

Patient with EGFR-activating mutation

Biopsy/PDX

EGFR inhibitor

Patient with progression

Surgery/PDX

EGFR inhibitor re-challenge

Patient with progression/metastasis to other sites

Surgery/PDX
Efficacy study results for PDX (sample taken at surgery) with EGFR mutation/cMET amplification

Treatment with EGFR inhibitor + cMET inhibitor

Vehicle (untreated)

MET inhibitor

EGFR inhibitor

Combination treatment
• Development and retooling of relevant mouse models for preclinical research
• Genetically engineered mice or allografts for small molecule and immunotherapy evaluation
• Development of new PDX models
• Facilitation of preclinical translation to the clinic
• Collaboration mechanisms available for CCR through RFA
  - LOI due in October, Application due in November
• You’re invited! CAPR minisymposium: 9/6/2018, Lipsett and webcast

![CAPR logo]

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