Molecule-Targeted Immune Modulation: Current Summary

**Antibody-based modulation of tumor immunity**

**Ab antagonists of suppression elicit durable responses in patient subsets**
- $\alpha$CTLA4 (ipilimumab)
  - response rates 11-13\
  - FDA approved for late-stage melanoma
  - active clinical trials for several cancers
  - success similar at multiple independent clinical sites
  - significant autoimmune toxicity
- $\alpha$PD-1 (nivolumab, lambrolizumab, pidilizumab, AMP-224), and
- $\alpha$PD-L1 (MPDL3280A, MEDI-4736)
  - response rates 13-38\
  - activity demonstrated in non-traditional immune-responsive cancers (lung, colon H&N, gastric)
  - toxicity can be significantly lower than $\alpha$CTLA4

**Ab agonists of stimulators show early promise after initial setback**
- $\alpha$CD40
- $\alpha$OX40
- $\alpha$GITR
Therapies Targeting the Cancer-Immunity Cycle

1. Release of cancer cell antigens
   - Chemotherapy
   - Radiation therapy
   - Targeted therapy

2. Cancer antigen presentation
   - Vaccines
   - IFN-α
   - GM-CSF
   - Anti-CD40 (agonist)
   - TLR agonists

3. Priming and activation
   - Anti-CTLA4
   - Anti-CD137 (agonist)
   - Anti-OX40 (agonist)
   - Anti-CD27 (agonist)
   - IL-2
   - IL-12

4. Trafficking of T cells to tumors

5. Infiltration of T cells into tumors
   - Anti-VEGF

6. Recognition of cancer cells by T cells
   - CARs

7. Killing of cancer cells
   - Anti-PD-L1
   - Anti-PD-1
   - IDO inhibitors

Chen and Mellman, Immunity 2013
National Laboratory “Hub and Spoke” Execution to Solve Major Biomedical Challenges

“With their centralization of resources (both monetary and intellectual), the national labs serve as an exemplar for Big Science...”

Timely Advancement of Immunomodulation for Cancer Patient Management Requires Major Effort in Integrated Team Science

- Community-based exploration
- Leidos technology development, execution, facilitation
New Paradigms for Potential Therapeutic Development Workflows

- HTP ex vivo models
  - organoids
  - live tissue arrays

- mammalian models
  - GEMs
  - GDAs
  - (PDX-hulms)
  - PDXs

- clinical trials
- canine trials

Molecular architecture, genomics, systems of disease and therapeutic response

signaling targeted therapies
immunomodulatory, signaling-targeted therapies
Mission: *to facilitate development of guiding preclinical workflows for clinical research and effective cancer management*

- Novel hybrid culture: integrated research rigor and project/goal management
- Business development (Leidos Biomedical)
- Dedicated staff drawn from public/private sectors along with NCI/NIH/LB technologies & research
- Integrated expertise in cancer mechanisms, pathways, murine models, genetics, drug development
- Dedicated pathology, histopathology, molecular pathology, quantitative morphometrics
- Cost effective through economy of scale and GEM model management
CAPR Interface with the National Laboratory

Leidos NCI

NCI Center for Cancer Research

Community-based exploration
Leidos technology development, execution, facilitation
Objectives for Preclinical Development

- Generation/adaptation of biologically and genetically engineered mouse models
  - *PDXs, GEMs, syngeneic GEM-derived allograft transplants (GDAs)*
  - *best possible representation of human cancer tractable for preclinical scale and timetables*
- Assessment of relative predictive power among engineered models
  - *treatment /organ-specific PK/PD evaluation compared to clinical outcomes*
- Hypothesis generation for clinical and basic research
- Biomarker discovery via dynamic systems assessment
- Development of imaging technologies to monitor disease and treatment
- Development of preclinical/clinical interactive data management systems
Leidos Biomedical Scientific Technologies Integration

Laboratory of Molecular Technology
*Microarray Analysis*

Laboratory of Molecular Technology
*Genotyping*

Laboratory Animal Sciences Program
*Animal Resources*

Laboratory Animal Sciences Program
*Pathology/Histotechnology*

Advanced Biocomputing Center
*Bioinformatics Analysis*

Laboratory Animal Sciences Program
*Small Animal Imaging*
### Major Models

<table>
<thead>
<tr>
<th>Model Type</th>
<th>Genetic Events</th>
<th>Induction Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Small Cell Lung Cancer:</td>
<td>EGFR-L858R</td>
<td>Adeno-Cre/Lenti-Cre</td>
</tr>
<tr>
<td>Lung Adenocarcinoma</td>
<td>EGFR-L858R/T790M</td>
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<tr>
<td>Squamous Cell Carcinoma</td>
<td>Lkb1/Kras</td>
<td>Doxycycline</td>
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<tr>
<td>Anaplastic Astrocytoma III</td>
<td>pRb/Kras/PTEN</td>
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</tr>
<tr>
<td>Glioblastoma</td>
<td>pRb/p53/Brca1</td>
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</tr>
<tr>
<td>Serous Ovarian Carcinoma</td>
<td>pRb/p53</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>BRAF-V600E</td>
<td></td>
</tr>
<tr>
<td>Pancreatic Adenocarcinoma</td>
<td>p53/Kras</td>
<td></td>
</tr>
<tr>
<td>In vivo</td>
<td>Ink4a/Kras</td>
<td></td>
</tr>
<tr>
<td>PDX-Cre</td>
<td>de novo, orthotopic</td>
<td></td>
</tr>
<tr>
<td>Prostate Carcinoma</td>
<td>pRb/PTEN</td>
<td></td>
</tr>
<tr>
<td>de novo, orthotopic</td>
<td>Tamoxifen</td>
<td></td>
</tr>
<tr>
<td>Small Cell Lung Cancer</td>
<td>pRb/p53</td>
<td></td>
</tr>
<tr>
<td>Lenti-Cre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous Ovarian Carcinoma</td>
<td>BRAF-V600E</td>
<td></td>
</tr>
<tr>
<td>HGFR/MET</td>
<td></td>
<td></td>
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<tr>
<td>N-Ras</td>
<td></td>
<td></td>
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<tr>
<td>UV, Tamoxifen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>de novo, orthotopic</td>
<td></td>
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</tr>
</tbody>
</table>

*in therapeutic/biomarker evaluation*

*in characterization or available*
CAPR Preclinical Evaluation Workflow

Tumor/model-derived primary cells
- Tumor-initiating cells
- Reprogrammed Models
- Efficacy Assays
- PD Assays
- Combinatorial Therapies
- Network Analysis
- Molecular Profiling
- Resistance Mechanisms

Integrated Analysis:
- efficacy assessment data
- disease endpoints/biomarkers
- collections of reagents
- preclinical SOP development

Mice with disease progression or tumor burden

Model Evaluation

Model Engineering/Retooling

Clinical Results

PK/PD
- Disease Biomarkers
- Drug Efficacy: Tumor Architecture
- Drug Efficacy: Biomarkers
- Biobanking

Drug Efficacy: Imaging
- Resistance Mechanisms

Clinical Research Hypotheses
CAPR Projects and Partnerships

- Internal
- Extramural
- Private Sector
- Foundations
- Intramural
- International
- NIH
Representative Internal Developments for Export

- Matched GEM/GDA/primary culture sets established for GBM and serous ovarian cancers
- Inducible human EML4-ALK lung cancer models (3 primary, 2 resistant)
- Inducible human EGFR erlotinib-resistant focal lung cancer model established
- p53 missense-mutant alleles retooled
- Therapeutic and biomarker evaluation SOPs developed for all models
- ESC-derived non-germline cohorts developed for complex genetic models
- Colony management database developed
- Preclinical workflow database developed
Long-Term Treatments in GDA-SEOCs Mirror Lesion-Specific Human Responses

- Olaparib treatment in mice - suppressed tumor growth but did not translate into sustained increase in survival
- Olaparib treatment in humans: progression-free survival benefit but no overall survival benefit
Extramural Collaboration Examples

Molecular Responses to Treatment of Erlotinib-Resistant Lung Cancer
Kwok-Kin Wong Dana Farber
Julien Carretero Spanish Nat’l Cancer Centre
Fatima Al-Shahrour Broad Institute


Biological Systems in the Progression to GBM
Lee Hood Institute for Systems Biology
Terry Van Dyke Center for Cancer Research ,NCI

Integrated Basic/Translational/Clinical Team Exploration in Cutaneous Melanoma

**Driver**
Merlino Lab
CCR, NCI

**Pharma**
- BRAF^{V600E} Inhibition
- MEK/ERK Inhibition
- PI3K/mTOR Inhibition
- MET inhibition
- Anti-CTLA-4
- Anti-PD-1

**Clinical Consultants**
- Keith Flaherty (targeted therapy)
- Nick Restifo
- Jedd Wolchok (immunotherapy)

**Outreach**
- Society for Melanoma Research
- Melanoma Research Alliance
- Melanoma Research Foundation Breakthrough Consortium
- Preclinical Subcommittee
- Meenhard Herlyn

**Basic Collaborators**
- Martin McMahon
- Marcus Bosenberg
- Thomas Tueting (mouse melanomas)

**Facilitator Collaborator**
Center for Advanced Preclinical Research (CAPR)
Driver-Specific Models of Primary and Metastatic Melanoma in Preclinical Evaluation

- Collect melanoma tissue
- Label with Luciferase/GFP
- Transplant to syngeneic mice
- Establish metastatic model

**Gene Therapy**
- Mutant BRAF Model
  - \( \text{BRAF}^{V600E} \)
  - 50%

- Mutant NRAS Model
  - \( \text{NRAS}^{Q61K} \)
  - 20%

- HGF/SF Model
  - HGF/SF
  - 30%

**GDA: GEM-derived allograft model**

**Targeted Therapy**
- \( \text{BRAFi} \)
- \( \text{MEKi/ERKi} \)
- \( \text{PI3Ki} \)
- \( \text{METi} \)

**Immunotherapy**
- Anti-CTLA-4
- Anti-PD-1

**Chemotherapy**
- Dacarbazine
- Paclitaxel
- Cisplatin

Glenn Merlino and CAPR, NCI
Responses to Anti-CTLA4 Mono and Combination MEK Inhibition Therapy in HGF/MET Melanoma GDAs

- Vehicle
- GSK1120212: 3mg/kg daily, PO
- Anti-CTLA-4: ~150μg Q3D x 4, IP
- GSK1120212 + Anti-CTLA-4

HGF;CDK4R24C allograft model

Merlino collaboration
CAPR Projects and Partnerships

- Internal
- Extramural
- Private Sector
- Foundations
- International
- NIH

CAPR Center for Advanced Preclinical Research
NIH Initiative Partnerships

Division of Cancer Treatment and Diagnosis

Cross-utilization and Development of PDX and GEM-related Preclinical Models in Clinical Guidance

Integration of Preclinical Systems (Ex Vivo and In Vivo) into Target Identification and Treatment Development
CAPR Projects and Partnerships

- Internal
- Extramural
- Private Sector
- Foundations
- Intramural
- International
- NIH
• PREDECT/CAPR MOU in progress to collaborate in development of live tissue slice arrays for therapeutic screening
• Invited partnership possibility under investigation

A consortium of 19 members (Pharma, Academic, CROs) aligned to develop

**New models for preclinical evaluation of drug efficacy in common solid tumours**

*funded by the IMI, EFPIA and other entities*

Specific areas:
- in vitro 2D/3D organotypic (co-)cultures, stirred bioreactor aggregates and tissue slice systems
- novel (orthotopic) grafts of human and mouse tumour samples
- genetically-engineered and mosaic mouse models.
CAPR Projects and Partnerships

- Internal
- Extramural
- Private Sector
- Foundations
- International
- NIH
- Intramural
• RFAs for Partnership Opportunities

Eight thematic RFA’s enlisting CAPR support (Released April 1st, 2013):

- Early Detection and Risk Assessment in Pancreatic Cancer
- Testing New Drug Delivery Approaches in Pancreatic Cancer
- Novel Imaging Technologies in Pancreatic Cancer
- Therapies for Pancreatic Cancer
- Novel Agents for Pancreatic Cancer
- Identification of Clinical and Molecular Markers for Metastatic Burden
- Early Target Validation for Pancreatic Cancer
- New Models for PDAC

• Active Partnership: Lustgarten/Evans (Salk)/CAPR (NCI)
**Kras-G12D/p53-R172H Driven Pancreatic Ductal Adenocarcinoma (KPC Model)**

**Cre-dependent de novo induction:**
- Pdx-Cre
- Kras-G12D (Cre-dependent)
- P53-R172H (Cre-dependent)

**De novo model: Cre-dependent activation of Kras (G12D) and p53 (R172H) mutations**

**KPC Model Workflow**

<table>
<thead>
<tr>
<th>Age (weeks):</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
<th>24</th>
</tr>
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<tbody>
<tr>
<td>Start of weekly palpations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultrasound imaging (twice weekly)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Enrollment Drug studies Monitoring outcomes</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

- Dosing
- Imaging
- End-Point

**Ultrasound analysis: advanced PDAC tumor**

*Consultants: Dr. David Tuveson, CSHL
Dr. Kenneth Olive, Columbia U.*
CAPR Projects and Partnerships

- Internal
- Extramural
- Private Sector
- Foundations
- International
- NIH
- Intramural
Discovery of a Mutant-Selective Covalent Inhibitor of EGFR that Overcomes T790M-Mediated Resistance in NSCLC

Annette O. Walter¹, Robert Tjin Tham Sjin², Henry J. Haringsma¹, Kadoaki Ohashi³, Jing Sun³, Kwangho Lee², Aleksandr Dubrovskiy², Matthew Labenski², Zhendong Zhu², Zhigang Wang², Michael Sheets², Thia St Martin², Russell Karp², Dan van Kalken², Prasoon Chaturvedi², Deqiang Niu², Mariana Nacht², Russell C. Petter², William Westlin², Kevin Lin¹, Sarah Jaw-Tsai¹, Mitch Raponi¹, Terry Van Dyke⁴,⁵, Jeff Etter¹, Zoe Weaver⁵, William Pao³, Juswinder Singh², Andrew D. Simmons¹, Thomas C. Harding¹, and Andrew Allen¹
New Paradigms for Potential Therapeutic Development Workflows

- HTP ex vivo models
  - organoids
  - live tissue arrays

- mammalian models
  - GEMs
  - GDAs
  - (PDX-hulms)
  - PDXs

- clinical trials

Molecular architecture, genomics, systems of disease and therapeutic response

- signaling targeted therapies
- immunomodulatory, signaling-targeted therapies

- canine trials
Project Focus

Mechanisms and optimization of therapeutic responses by targeted immunomodulation

- Selective patient response mechanisms
- Optimization of efficacy and breadth of individual responses

- Why are positive responses patient-specific?
- Can combination signaling-targeted and immune-targeted therapies improve efficacy?
- What dosing schedules are most effective?
- What are the potential resistance rates? Mechanisms?
- To what extent can immunocompetent mouse model studies predict human mechanisms? (success in αCD40)
- Can biomarkers of responsiveness be developed to identify susceptible individuals? To monitor PD? Successful outcomes?
- Can PDX models harboring humanized immune systems be incorporated into effective preclinical workflows?
Value Added

• Robust preclinical technologies to provide reproducible bridge to basic and clinical investigators
• Integration of ex vivo technologies already in development in the research community into the FNLCR and collaborators
• Community access to tractable vetted GEM and GDA models
• Development of PDX-hu-Imm models
• Development of robust preclinical SOPs and export to community
• Facilitation of collaborations/partnerships, including the private sector
• Resource optimization through economy of scale, team science, and shared technologies
• Training
Center for Advanced Preclinical Research

Scientific Director: Terry Van Dyke, PhD
Administrative Director: Lionel Feigenbaum, PhD

Zoë Weaver Ohler, PhD
Serguei Kozlov, PhD, MBA

Preclinical Evaluation
- Efficacy studies on therapeutic candidates
- Molecular and in-vivo imaging endpoints
- Biodistribution (PK/PD)
- Biomarkers/molecular signatures of treatment response.

Research and Development
- Derivation, modification & validation of hypothesis-predictive GEMMs
- Biomarkers/molecular signatures of tumorigenesis
- Breeding strategies for scale-up

Technology and Optimization
- Throughput/scale up facilitation
- ES and iPSC technologies for non-germline cohorts and preservation
- Optimization/retooling of GEMs
- Preclinical evaluation of/in PDAC models

Animal Research Support

Philip Martin, DVM, DACVP
- Histopathology, Molecular Pathology, Quantitative Morphometrics,
- BioBank: TMAs, tissues, blocks, slides (glass/digital), fluids, nucleic acids
Conversion of Complex GEMs to Non-Germline Cancer-Bearing Cohorts

Tg/KI-G(Z)T_{122};KRas^{G12D/+};PTEN^{fl/+};GFAPCre_{ERT2}

Develop GBM upon 4OHT induction
penultimate cross → blastocysts → ES cells

4OHT
Mouse Models of Pathway-Specific Serous Epithelial Ovarian Cancer (SEOC)

**Induced events:**
- Rb\textsubscript{tumor} suppression inactivation
- p53 mutation/loss
- Brca1 or Brca2 loss

**De novo model:** Intra-bursal injection of adeno-Cre

<table>
<thead>
<tr>
<th>Months p.i.</th>
<th>3</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 wk old females</td>
<td>super-ovulation</td>
<td>adeno-Cre injection</td>
<td>MR imaging</td>
<td>papillary hyperplasia</td>
<td>ovarian papillary carcinoma</td>
</tr>
</tbody>
</table>

**GDA model:** Intra-bursal passage of tumor fragments

Recapitulates histopathology and molecular profiles of GEM models
Tumors develop in 1-2 months
Preclinical Models of Serous Ovarian Cancer: Similarity to Human Disease
Optimal Preclinical Guidance via Cross-model Cross-species Unbiased Evaluation

- Consentng, therapy-eligible patient with core biopsy
- Patient (therapy-naïve)
- Patient therapy-refractory
- Patient – therapy resistant (prior response, now PD)
- Patient-derived xenografts (PDXs)
- Disease appropriate, pathway-specific GEMs/GDAs

Combined Systems Biology Analyses

- Standard of care (SOC)
- SOC + drug A
- SOC + drug B
- Drug A + drug B

distinct target and therapy

therapeutic

Consenting, therapy-eligible patient with core biopsy

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distinct target and therapy

therapeutic
A Path to Improved Cancer Patient Care: Development and Implementation of Preclinical Systems Predictive of Human Hypotheses

- Basic discovery
- Clinical discovery, patient care
- Education

Academia/Basic Institutes

Frederick National Labs
- Only HHS Nat’l Lab
- “Big science”
- Major health challenges solved (e.g. facilitate preclinical → cancer patient care)

Pharma
- Applied discovery/development
- Marketing
- Improved patient care

With their centralization of resources (both monetary and intellectual), the national labs serve as an exemplar for Big Science