The Mouse Hospital for Co-Clinical Trials and Precision Medicine

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- ILAR Roundtable -
Advancing Disease Modeling in Animal Based Research in Support of Precision Medicine

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Improving human health through preclinical trials in mouse models of human disease
To develop mouse trials in parallel with human clinical trials to enable rapid, real-time transfer of information from mouse experiments to human trials and allows optimization and improved outcomes of clinical efforts.

Approach:

1. Patient tumors can be engrafted in immunodeficient mice to generate PDX models.

1. Appropriate GEMMs can be identified that act as a surrogates, based on the tumor genetics.

Clinical and Co-Clinical PDX and GEMM model Trials can be enrolled in parallel.

Sensitivity of tumors to the same drugs, novel drugs or combinations thereof can be rapidly evaluated in these co-clinical trials, and real time integration of data between clinical and co-clinical trials can inform patient treatment protocols.
Drug Development Challenges

Traditional Drug Development:

- Disease classification (e.g., cancer types) based upon antiquated nomenclature and histopathology

- Statistics-based clinical development (low response rates in oncology trials of > 20% response rates often deemed adequate for approval):
  - re-imbursement despite poor response rates

- Proof of Concept determined at end of Phase II based upon statistical trend (some PR activity)
Drug Development Challenges

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Optimizing Cancer Drug Development: Challenges and Opportunities

The number of new agents for cancer treatment approved by the FDA between 1996–2007

Hambley T W Cancer Res 2009

Attrition rates during new drug development


Phase II is the focal point for PoC de-risking prior to major $ investments of Phase III
Cancer Cell Survival Strategies

Addiction

Sensitivity Cancer Cells

Tolerant Cancer Cells

Resistant Cancer Cells

Adaptive Compensatory Modification

Genetic Modification

Sensitive Cancer Cells

Addiction

Single Target Agent

Tolerant Cancer Cells

EGFR T790M

Resistant Cancer Cells

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Sensitive Cancer Cells

Adaptation

Tolerant Cancer Cells

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Acetylation

Methylation

Phosphorylation

HSP90

E-Cad

Amplification

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Drug Development Needs

- **Improved Disease Models (Surrogates for Patients)**
  - Account for complexity of biologic systems
  - Account for inherent inter- and intra-patient heterogeneity of human cancers

- **Early and Improved linkage of targeted agents to their molecular targets and predictive modelling**
  - Early biomarker development decrease risk of failure
  - Accelerate rate of new drug development and decrease patient resources required

- **Improved clinical trial designs**
  - Predictive biomarker validation designs
  - Test and validate strategies with a single trial
MISSION: Provide expertise in the design and implementation of preclinical trials to test new drugs in mouse models of human disease

- Resource specializing in Pre- and Co-Clinical testing in GEMMs (genetically engineered mouse models) and PDX models.

- This enables efficient stratification of patients that are most likely to respond to novel therapeutic agents on the basis of their own specific genetic makeup.

- Streamline the progression from bench to bedside for promising agents, or new combinations of approved drugs.
Mouse Hospital for Co-Clinical Trials

Preclinical and Co-clinical Testing of Novel Therapeutics and SOC in Mouse Models of Human Diseases

State-of-the-Art Animal Facility

SOPs e.g. “auditable” notebooks, husbandry etc..

Training to create professional personnel in “diseased” mouse care, husbandry, and therapeutic treatments

Dedicated in vivo imaging, behavioral test and surgical procedure areas within the “vivarium”
Tiny Patients, Major Goals
In a hospital for mice, researchers pursue better treatments for men with prostate cancer.

By GINA KOYATA

BOSTON — Here at Beth Israel Deaconess Medical Center, a black mouse lies on a miniature exam table, its tail dangling off the end. A plastic tube carries anesthetic to his nose and mouth. He is asleep.

The mouse is about 3 feet long, yet he is injected with two mutated genes often found in human prostate cancer. As he lies on the table, a technician is measuring his two-millimeter prostate tumor with a petite ultrasound machine — the very exam a man would undergo, only on a dollhouse scale.

"There's the tumor," says the technician, Bhawik Padmanab, sliding a probe over the mouse as a bright white amorphous shape comes into view.

This is what is called a "mouse hospital," a new way of using mice to study cancer. Although mice have been studied in regular labs for years, the results often have been disappointing. Usually, the cancer isn't the same, not in the organs where they originated. And drugs that seemed to work in mice often proved useless in humans.

The mouse hospital at Beth Israel Deaconess and a few similar ones elsewhere are at the forefront of a new approach to studying human cancers. The mice are given genes that make their tumors develop in a way that mimics human cancers more closely than conventional mice. This means the researchers need scanners to track the tumors' growth inside the animal's body. So the mouse hospitals have tiny ultrasound machines, CT and PET scanners, and magnetic resonance imaging machines with little stretchers to slide mice onto the machines.

"It's mouse medicine," said Paul Weisberg, a scientist at Beth Israel Deaconess. "It's mouse pharmacies to formulate medicines in mouse-size doses and mouse clinical laboratories specially designed to do analysis on minute drops of mouse blood and scanning small quantities of mouse urine. That lets them follow cancer growth.

What's more, with genetic advances in studies of human tumors, the researchers do not have to implant human cancer cells into mice. Instead, they can give the mice just a few mutated genes that seem to drive a tumor.

They genetically alter the mice before they are born and then, with scanners, watch what happens as a cancer develops in the affected organ, the prostate, in this case. Then they can try out drugs designed to attack the gene mutations and CONTINUES ON PAGE 31
Integration of Human and Mouse Hospitals

Clohessy and Pandolfi, (2015) NAT REV CLIN ONCOL
Clinical/Co-Clinical Trials for Cancer

Clohessy and Pandolfi, (2015) NAT REV CLIN ONCOL
Co-Clinical modeling: Strategies and Approaches

• Re-Evaluation and Repurposing of Existing Models
  • New ways to analyze and utilize existing models for co-clinical utilization
    (Lung cancer treatment – response to therapy at a single cell level)
    Elena Levantini & Dan Tenen - BIDMC

• New approaches for model development
  • Off the shelf approaches for GEMMs in co-clinical approaches
  • More ‘faithful’ modeling of human disease
    (Economical modeling of heterogeneity in prostate cancer)
    Pier Paolo Pandolfi & Marco Bezzi - BIDMC

• Co-Clinical implementation of models towards therapy
  • GEMMs in action – co-clinical development
    (Defining and overcoming resistance in leukemia)
    Pier Paolo Pandolfi, Vera Mugoni, Lourdes Mendez - BIDMC
Re-evaluation and Repurposing of existing models for co-clinical approaches

- **Lung Cancer Modeling – Elena Levantini (BIDMC)**
  - Variety of GEMMs already exist to study lung cancer
  - A number have been utilized in the context of pre-clinical studies.
  - Frequently, studies evaluate response at the level of histopathology and bulk tumor analysis (DNA RNA, protein)

- **Need for more in-depth approach to analysis of tumors at single cell level**
  - Evaluate sub-populations of cells that exist within tumors
  - Study the dynamics of tumor evolution – looking to stromal, immune, tumor populations
  - Establish response of sub-populations to treatment (acute/chronic) – and the emergence of resistance
Re-evaluation and Repurposing of existing models for co-clinical approaches

- inDrop single cell sequencing to study $\text{Kras}^{G12D}$ and $\text{Kras}^{G12D;\text{Trp53}^{-/-}}$ GEMMs
  - In collaboration with Dr. Allon Klein (HMS) applying inDrop to evaluate cellular populations at early and late stage tumor development
  - Examining population dynamics in response to treatment
  - Computational analysis for deconvoluting cellular populations
  - Oncology Model Fidelity Scores – Ted Goldstein (UCSF)

Comparison with single cell data from primary human lung tumors
(in collaboration with Dr. Raphael Bueno – Brigham and Women’s Hospital)

Ultimately – Can these models be repurposed for co-clinical application

Data courtesy of Drs. E. Levantini, A. Klein & V. Savova
New approaches for model development – The OGGO Platform for Prostate Cancer

• Challenges confronting GEMMs in Co-Clinical Application
  • Two predominant challenges:
    – Time
    – Money
  • Frequently models require multiple genetic lesions to mimic human disease
    – Complex breeding/genotyping
  • Time to disease development and cohort establishment
  • New strategy to facilitate rapid and faithful models of disease: Orthotopic Graft of GEMM-derived Organoids (OGGO)

Establishment and characterization of a GEMM-derived organoid biobank for prostate cancer

(Pier Paolo Pandolfi & Marco Bezzi)
Application of GEMM Derived Organoids (GDO’s) for modeling human cancer

Harvest prostate cells for growth in 3D organoid culture systems

Co-culture systems

Drug screening

Gene knockdown/overexpression
CRISPR library
shRNA library

Data courtesy of Drs. Pandolfi & Bezzi
Building a GEMM-derived organoid (GDO) Biobank

- Utilizing established GEMMs for prostate cancer in a homogenous BL6 genetic background

- Enables easy manipulation and modification

Ubi-CreERT2; PTEN$^{\text{lox/lox}}$

Ubi-CreERT2; TRP53$^{\text{lox/lox}}$

Ubi-CreERT2; Myc-\text{IRES-hCD2}^{\text{*lox/STOP/lox}}$

Ubi-CreERT2; ERG-\text{IRES-GFP}^{\text{lox/STOP/lox}}$

pLVX-BFP

pLVX-mAR-IRES-RFP

Basal vs Luminal

Ex vivo CRE activation

Homogeneous organoid population

Traceable
Application of GDO’s for modeling human cancer

- Ubi-CreERT2; PTEN\textsuperscript{lox/lox}
- TRP53\textsuperscript{lox/lox}
- Wild type syngeneic mice
- Special diet-fed syngeneic mice (e.g. High Fat)
- Knockout syngeneic mice (e.g. CXCR2 KO)
- Any of the above syngeneic mice

Anterior prostate injection - 30 days post-injection

Data courtesy of Drs. Pandolfi & Bezzi
Co-Clinical application of the OGGO platform
Current application for Co-Clinical studies

- Validation of Organoid Modeling for tumor heterogeneity
- Immune Landscaping
- Clonal Tracing upon therapy (ADT – Immunotherapy)
- Target Discovery
- Testing drug combination
The 3 year relapse-free survival for patients with AML is 29%. Novel agents and combination therapies are needed to induce durable responses and prolong survival.

IDH1/2 mutations in 20% of AML

IDH2 mutant has neomorphic activity catalyzing αKG → 2-HG, oncometabolite.

2-HG competes with αKG. For binding to histone and DNA demethylases, resulting in epigenetic dysregulation and a block in differentiation.

Extensive efforts to develop clinical agents targeting mIDH2 for therapy

Agios/Celgene (Enasidenib; AGI-221)
Developing GEMMs to model mutant IDH2 AML

Hematopoietic Inducible IDH2<sup>R140Q</sup>

+ HoxA9-Meis1a

Model for AML

Kats et al., Cell Stem Cell, 2014
Serial Transplantation results in mIDH2 independence

Tg (M2-rtTA / IDH2<sup>R140Q</sup>)

DONOR

HoxA9 Meis1A

- Doxy

BMT

1<sup>st</sup> RECIPIENT

SENSITIVE to IDH2<sup>R140Q</sup> genetic de-induction

+ Doxy

2<sup>nd</sup> RECIPIENT

BMT

- Doxy

3<sup>rd</sup> RECIPIENT

RESISTANT to IDH2<sup>R140Q</sup> genetic de-induction

IDH2<sup>R140Q</sup> OFF

IDH2<sup>R140Q</sup> ON

% of GFP+/YFP+ Leukemic cells in BM

0 20 40 60 80 100

SENSITIVE RESISTANT

Data courtesy of Drs. Pandolfi & Mugoni
“Omics” approaches identify resistance changes that

- Combination of high-throughput analysis:
  - Metabolomics
  - Transcriptomics (RNAseq)
  - Genomics (Whole Exome)

- Enabled identification of novel resistance mechanisms and vulnerabilities for therapeutic intervention.

Data courtesy of Drs. Pandolfi & Mugoni
Developing Co-Clinical Trials

- **In vitro and in vivo validation of findings**
  - Mechanisms and therapeutic targets have been validated both:
    - *In vitro* - Serial replating, differentiation, apoptosis assays
    - *In vivo* - GEMM and PDX pre-clinical trials evaluating response to therapy

- **Consultation with clinical and pharma partners**
  - Consultation and engagement with pharmaceutical partners
  - Trial design, set-up and implementation

- **Iterative analysis and real-time co-clinical execution**
  - Utilization of patient derived data and integration with GEMMs for optimal trial execution
Phase Ib/II trial of AG-221 + Combinations

• Currently planned trial in conjunction with Celgene Pharmaceutical:

  - Relapsed/refractory AML
  - Untreated AML unfit for IC
  - Untreated High risk MDS unfit for IC

  Phase Ib
  Dose escalation

  Phase II
  Randomized

Pandolfi & Mendez
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