“Can we use patient-derived xenografts to assess new lymphoma therapies?”

Lymphoma and Myeloma: An International Congress on Hematologic Malignancies
An international Congress on Hematologic Malignancies

22-24 Ottobre, 2015

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Cost to Develop New Pharmaceutical Drug Now Exceeds $2.5B

A benchmark report estimates that the cost of bringing a drug to market has more than doubled in the past 10 years.

By Rick Mullin and Chemical & Engineering News | November 24, 2014

A new report published by the Tufts Center for the Study of Drug Development (CSDD) pegs the cost of developing a prescription drug that gains market approval at $2.6 billion, a 145% increase, correcting for inflation, over the estimate the center made in 2003.

CSDD’s finding, a bellwether figure in the drug industry, is based on an average out-of-pocket cost of $1.4 billion and an estimate of $1.2 billion in returns that pharmaceutical companies have earned on their drugs since 2003.
Mouse xenograft models of cancer, understandably, have a terrible reputation. Although researchers and companies routinely use these human tumors in mice for preclinical drug testing, individual models poorly predict how drugs will act in the clinic. Retrospective reviews published by the National Cancer Institute in 2001 and the National Cancer Institute of Canada in 2003 came to the same conclusion: Drugs that work against cancer in xenograft mice rarely work in people with the same tumor.

New drug testing in mouse models by the NCI-supported Pediatric Preclinical Testing Program. The 3-year-old program, which has already sent several drugs into clinical trials...
**Mouse models: old and new strategies**

**Cornell’s Stem Cell and Transgenic Core Facility**

- **Genetically engineered mice (GEM):**
  - Potential analysis of many genetic backgrounds by using a variety of mouse strains.
  - Tumor exists in the presence of a realistic immune system.
  - Defined mutations mimic those identified in human tumors.
  - Can follow tumor growth from early stages.

- **Orthotopic xenograft of human tumors:**
  - Cancers are placed in their normal anatomical location.
  - Allows for rapid analysis of human tumor response to a therapeutic regime.

- **Xenograft of human tumor in humanized mice:**
  - Appropriately mimics human tumor microenvironment.
  - Can predict the drug response of a tumor in a human patient.
  - Provides realistic heterogeneity of tumor cells.
  - Expensive.
  - Technically complicated.

- **Limited % of engraftment**
**Humanized mouse models**

**Timeline** | Important events in the development of humanized mice

- **1966**: Description of the nude mutation
- **1983**: CB17-scid mice engrafted with human fetal tissues, adult blood cells and HSCs
- **1988**: Rag1- and Rag2-targeted mutations described
- **1992**: Description of NOD-scid mice and increased engraftment of human PBMCs and HSCs
- **1997**: Description of NOD-Rag1−/− mice
- **2002**: Pre-clinical bridge between mouse and human
- **2007**: The NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ mice

**Description**:

- CB17-scid mice: Engrafted with human fetal tissues, adult blood cells, and HSCs.
- NOD-scid mice: Increased engraftment of human PBMCs and HSCs.
- NOD-Rag1−/− mice: Description of the model.

**Key Cytokines**:

- IL3
- CSF1
- GM-CSF

**Notes**:

- B2m, β2-microglobulin; HSC, haematopoietic stem cell; Il2rg, interleukin-2 receptor γ-chain; NOD, non-obese diabetic; PBMC, peripheral-blood mononuclear cell; Praf1, perforin 1; Rag, recombination-activating gene; scid, severe combined immunodeficiency.

PDT Research Challenges

**Pro**
- Patient derive tumors
- Pre and post therapies
- Clinical annotation
- Molecular annotation
- Slow/intermediate growth rate
- Cell heterogeneity?
- Maintain Treatment History
- Predict drug response and resistance
- Experimental replicates
- Pre-clinical drug efficacy

**Con**
- High cost
- Tissue procurements
- Limited rate of implant
- Long latency period
- End stage diseases
- Lack immunobiology
- Mouse/human chimera
- Implant attrition
- Changes overtime/passages
PDT are very popular
PDX Models: Initial Progress

• Tissue/Model Acquisition:
  – 16 NCI-Designated Cancer Centers just reviewed and funded to *each* supply 20 tumor and 20 matched blood samples with clinical annotation in FY14; focus on less common malignancies: small cell lung cancer; head and neck cancer; sarcoma; thymic carcinoma; bladder cancer; melanoma; prostate cancer:
    **Total ≈ 300 tumors & 300 blood samples for CTCs**
  – Offered existing Cancer Center collections (>25 unique PDX models *each* ovary and breast)
    **Total 50 (so far)**
  – >300 unique PDXs from Pharma/Biotech: in negotiation
  – ≈ 1000 tumor biopsies from NCI MPACT Trial: starts Q1 2014

• PDX tumors in hand:
  – 27 GBMs
  – 31 Lung (adeno>squamous; 3 sclc’s)
  – 7 Bladder
  – 5 Colon
  – 5 Sarcoma
  – 3 Head & Neck
  – 1 NHL
  – Take rate:
    • 70% for 18 gauge needle biopsies
    • 7/21 implants directly from CTCs growing as PDXs
PDT biobanking and Academia/Health Sector
Precision Therapeutic Medicine: new ideas at work

Molecular morphology

Biorepository

Preclinical "tailored" trials

HTP molecular stratification

Tumor expansion

Molecular stratifiers

Results

Tumor growth inhibition results delivered to oncologist
Experimental Therapeutics Program (ETP)

Present:
Heme Malignancy

Ongoing:
Multiple Platforms

ETP phase I

ETP phase II
High-Performance MRI Systems
**Why we need reproducible PTCL models?**

- Although several cell lines derived from T-ALL exist. Rare in vitro models for neoplastic post-thymic lymphocytes are available, with HTLV-I+, CTCL and ALK+ ALCL lines representing the exception.

- Few spontaneous (Roquinsan) or engineered (i.e. ITK-SYK, NPM-ALK) mouse models, faithfully reproducing their corresponding human counterparts, have been successfully used to define the pathogenetic mechanisms leading to T-cell transformation and/or design and validate therapeutic protocols.

- ‘Xenografts do not predict for human effects, with 90% of novel antineoplastic drugs failing in the clinic despite antitumor efficacy in classical preclinical models.'
Translational Discovery in Peripheral T-Cell Lymphomas

In vitro drug responses

2D

Targeted agents

Lymphomagenesis

T-cell differentiation

Patient-Derived-Tumor Xenografts (PDTX)

Primary lymphoma & PDTX cell lines

Transgenic Mice

Pathology Core
Characterization, Banking, NGS, Phospho-mapping Spectral Imaging

Surgery

Model Generation Core
PDTX development
Cell line generation

WCMC

Nebraska

MSKCC

Dana-Farber/BWH

Milano

PDTX clinical trials

Primary lymphoma & PDTX cell lines

Primary lymphoma & PDTX cell lines
Can different implantation routes improve PDTX grafting?

Diagnostic sample

Sub cutaneous

Intra venous

Intra peritoneal

Intra bone

Intra liver

Intra Spleen
Representative ALCL tumorgraft expansion

ALCL-1
ALCL tumorgraft on disseminate to secondary lymphoid and parenchymal organs
Do PDTX fully recapitulate their corresponding primary lesions
ALCL PDTX faithfully recapitulate the immunophenotype of their corresponding primary lesions

A  Primary ALCL-1

B  ALCL-1-T3

C  ALCL-1

ALCL-2

ALCL-3
ALCL PDTX overtime disseminate to all mouse organs
Gene expression signatures of ALCL-PDT identified prognostic classifiers

A

 Preferential expression in Primaries

Low Expression in PDT

High Expression in PTD

B

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C

D

Funzioni di sopravvivenza
Does clonal evolution take place in NSG models?
Clonal dynamics of PDTX
Treatment of ALK+ ALCL PDXT predicts clinical responses

[Diagram showing TRAF1 and ALK with different domains such as TRAF Domain, Coiled Coil, NPXY sequences, and Tyrosin Domain.]

[Graph showing MRI spleen volume over time with variation from baseline.]

[Graph showing percent survival over days with different treatment groups indicated.]
ALCL GEP signature allows the discovery of novel pathogenetics events
NSG tumorgrafts respond to conventional treatments and matched the clinical responses of their donor patients.

1st line CHOP

2nd line CHOP

Anti-ALK(CEP28122)
The efficacy of SGN-35 is related to the total tumor burden.
Deregulated activation of JAK1/STAT3 is pathogenetic in a subset of ALK- ALCL
Jak1/2 inhibitors control the lymphoma growth of Jak1/Stat3 mut ALK- ALCL PDTX
cALCL Patient Derived Tumor Xenograft

<table>
<thead>
<tr>
<th>Primary</th>
<th>CD3</th>
<th>CD7</th>
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<tr>
<td>PDTX</td>
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20 µm
Genomic and biological characterization of cALCL PDTX
MSKCC-WCMC-6
Angioimmunoblastic T-cell Lymphoma

Liver
Kidney
Lung
Heart

CD4
PD1
Therapeutic strategic with AITL PDTX models
Leukemia fitness improves along serial passages

- ALL 1° round (red)
- ALL 2° round (green, blue, orange)
- ALL 3° round (fuchsia)
- ALL 4° round (black)
- ALL 5° round (gray)

Day of injection
-60
-66
-53
-40
-33
-32

%CD19+CD34+CD10
Combination of corticosteroid and high dose of dasatinib leads to a sustainable near mCR
Bifunctional antibodies and CIK can eradicate Bcr-Abl resistant ALL cells
CLL/RICHTER MODEL

Diagnosis: Richter

Cryopreserved as single cell suspension

s.c. nodule

s.c. nodule

K-R-CHOP

Fludarabine-based Protocol+Camptos

Autologous BM transplant

CD3

CD5

CD20

Ki67

CD45

KNUCA 24/7 1056-5/19/45

KNUCA 24/7 1056-DM/45

KNUCA 24/7 1056-38/5/45

CD45

CD20

CD3

CD5

Ki67

CG primary tumor
Monoallelic deletion:
(los D13S19) 83% of nuclei

CG primary tumor
Monoallelic deletion:
27.2% of nuclei

CG primary tumor
Amplification:
38% of nuclei

CG primary tumor
Gain: 57.1% of nuclei

CG primary tumor
Gain: 73% of nuclei

CG primary tumor
Gain: 71.4% of nuclei

1. RS2123
2. RS1042
BACK TUMOR
NAPE TUMOR
DEL: 89%
DEL: 95%

1. RS2123
2. RS1042
BACK TUMOR
NAPE TUMOR
AMPL: 27.2%
AMPL: 28.9%

1. RS2123
2. RS1042
BACK TUMOR
NAPE TUMOR
GAIN: 55%
GAIN: 56%

1. RS2123
2. RS1042
BACK TUMOR
NAPE TUMOR
GAIN: 38%
GAIN: 20%

1. RS2123
2. RS1042
BACK TUMOR
NAPE TUMOR
GAIN: 54.5%
GAIN: 56%
Bifunctional antibodies and CIK can eradicate DLBCL cells
A diffuse large B cell lymphoma (DLBCL) derived xenograft to evaluate the effect of the XPO1 inhibitor Selinexor (KPT-330)

Patient (R1) presenting a DLBCL with XPO1 amplification and triple-hit disease (BCL6, MYC, BCL2)

In vivo testing

Ex vivo testing

Selinexor IC50: 1.2 μM

Tumor size vs Treatment day

Placebo vs Selinexor

15 generations of PDXs in NSG mice from patient R1
Columbia University
Raul Rabadán
Francesco Abate
Sakellarios Zairis

IOSI
Francesco Bertoni
Michela Boi
Ivo Kwee

Hugef, Torino
Sivia Deaglio
Tiziana Vaisitti

Icahn School of Medicine at Mount Sinai
Joshua Brody

Jackson Laboratory
Lenny Shultz

IRCC-Candiolo
Andrea Bertotti
Livio Trusolino
Enzo Medico
Barbara Martinoglio

The European T-cell Lymphoma Study Group

Genetics-driven targeted management of lymphoid malignancies
AIRC 5x1000