Patient-Derived Tumor Xenograft Models

‘An emerging way to personalized medicine in translational cancer research’

Webinar Taconic
Leuven, 10/03/2016

Department of Oncology, UZ Leuven – KU Leuven
Els Hermans, DVM
Introduction
Establishment
Quality control & characterization
Applications
Drawbacks
Problem in Cancer R&D

Pre-Clinical Phase

Clinical Phase

Economical and ethical impact

How can we improve the efficiency of drug development in oncology?

- Use preclinical models which more closely recapitulate the heterogeneity of human cancers

- Perform studies for genotype /response correlation on a population scale, to achieve scientific solidity.
An emerging platform in translational cancer research

The genomic landscape of response to EGFR blockade in colorectal cancer

Antisense oligonucleotide-mediated MDM4 exon 6 skipping impairs tumor growth

Characterization of patient-derived tumor xenograft models of endometrial cancer for preclinical evaluation of targeted therapies

Stromal contribution to the colorectal cancer transcriptome
Patient-Derived Tumor Xenograft

‘To improve accuracy and accelerate R&D in oncology’

Based on National Cancer Plan Grant – End 2012

Platform in UZ Leuven – KU Leuven
in collaboration with other hospitals and research institutes
Introduction

Establishment

Quality control & characterization

Applications

Drawbacks
One mouse, one patient paradigm: new avatars of personalised cancer therapy

Malaney P.; Cancer Lett, 2013
PATIENT RECRUITMENT @

ESTABLISHMENT OF PDXS
- Transplantation of tumor biopsy in immunocompromised mice over different passages
- Tumor take rate: 60%
- Tumor biopsies – FNB

STORAGE OF PDXS
- PDX Tissue: FFPE, snap frozen, cryopreserved
- Frozen & Viable Biobank

PDX RECRUITMENT @

PDX ANNOTATION
- Clinical data
- Preclinical data
- Histopathology
- Genomics and/or transcriptomics
- Pharmacologic analysis

QUALITY CONTROL
- Histology
- SNP-Based Fingerprinting

APPLICATION OF PDXs
- Pre-clinical drug screening
- Biomarker analysis
- Identify sensitivity/resistance mechanisms

www.europdx.eu/
Develop a new systematic approach to evaluate response to targeted therapy
Delineate new avenues for intervention in managing a variety of cancers
Input

Future: Centre Hospitalier Universitaire de Liège, Universitair ziekenhuis Brussel, Universiteit Gent, Universiteit Antwerpen ... ?
Number of Tumors  (tumor types=11)
Implanted (244) - Engrafted (123)
# COMPARISON OF ONCOLOGY MOUSE AND RAT MODELS

<table>
<thead>
<tr>
<th>MODEL NUMBER</th>
<th>MODEL NAME</th>
<th>COAT COLOR</th>
<th>T,B &amp; NK CELL DEFICIENCES</th>
<th>OTHER IMMUNODEFICIENCIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALBNU</td>
<td>BALB/c nude mouse</td>
<td>NU</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>B6NU</td>
<td>B6 nude mouse</td>
<td>NU</td>
<td>T</td>
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<tr>
<td>NCRNU</td>
<td>NCr nude mouse</td>
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<td>T</td>
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<tr>
<td>NMRINU</td>
<td>NMRI nude mouse</td>
<td>NU</td>
<td>T</td>
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<tr>
<td>NIHNRU</td>
<td>NIH nude rat</td>
<td>NU</td>
<td>T</td>
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<tr>
<td>9066*</td>
<td>HRN™ nude mouse</td>
<td>NU</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>CB17SC</td>
<td>C.B-17 scid mouse</td>
<td></td>
<td>T</td>
<td>B</td>
</tr>
<tr>
<td>ICRSC</td>
<td>ICR scid mouse</td>
<td></td>
<td>T</td>
<td>B</td>
</tr>
<tr>
<td>NODSC</td>
<td>NOD scid mouse</td>
<td></td>
<td>T</td>
<td>B</td>
</tr>
<tr>
<td>RAG2</td>
<td>Rag2 (129S6) mouse</td>
<td></td>
<td>T</td>
<td>B</td>
</tr>
<tr>
<td>461</td>
<td>Rag2 (B6.5JL) mouse</td>
<td></td>
<td>T</td>
<td>B</td>
</tr>
<tr>
<td>601</td>
<td>Rag2 (BALB/c) mouse</td>
<td></td>
<td>T</td>
<td>B</td>
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<tr>
<td>RAGN12</td>
<td>Rag2 (C57BL/6) mouse</td>
<td></td>
<td>T</td>
<td>B</td>
</tr>
<tr>
<td>CBSCBG</td>
<td>Sdd-beige mouse</td>
<td></td>
<td>T</td>
<td>B</td>
</tr>
<tr>
<td>4111</td>
<td>Rag2/129rg Double Knockout Mouse</td>
<td></td>
<td>T</td>
<td>B</td>
</tr>
<tr>
<td>11503</td>
<td>CIEA BRG mouse</td>
<td></td>
<td>T</td>
<td>B</td>
</tr>
<tr>
<td>NOG</td>
<td>CIEA NOG mouse®</td>
<td></td>
<td>T</td>
<td>B</td>
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</tbody>
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**KEY:**

- **COAT COLOR**
  - Black and White Nude
  - Black and White
  - White-bellied Agouti
  - Albino

- **CELL DEFICIENCES**
  - T Cell Deficient
  - B Cell Deficient
  - NK Cell Deficient

- **Notes:**
  - Reduced complement activity, dysfunctional macrophages and dendritic cells, deficiencies in immune signaling, including cytokine production. The most immune deficient mouse available.
Individual Ventilated Cages (IVC)
Introduction
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Quality control & characterization

Validation of PDX Model

Tumor tissue in different conditions & therapy response

Patient (F0)

Engraftment Phase (F1)

Expansion Phase (F2)

Validation Phase (F3)
Endometrial Carcinoma

Mesonephric  Serous  Endometrioid

F0 - Humane

F1 - PDTX

F3 - PDTX

Depreeuw J. et al, Gynaecologic Oncology 2015
Specific markers: tumor type

**PDX PDAC003** *Pancreatic Ductal Adenocarcinoma*

- Cytokeratin CK7 – 20x
- F0 - Humane F3 - PDTX

**PDX EMC016** *Endometrioid endometrial carcinoma, grade 2*

- Human progesterone receptor – 20x
- F0 - Humane F3 - PDTX

**PDX OVC005** *Serous ovarian carcinoma, grade 3*

- Human WT-1 – 20x
- F0 - Humane F3 - PDTX

**PDX BRC008** *Breast Invasive Ductal Adenocarcinoma*

- HER2 – 20x
- F0 - Humane F3 - PDTX
Specific markers: Micro-environment

Pancreas cancer
Genetic profile: Shallow Sequencing

Similarity between human tumor & xenograft: 80 – 100%

Depreeuw J. et al, Gynaecologic Oncology 2015

Endometrial cancer
Genetic profile: Whole Exome Sequencing

(PDXT-EMC003: 9 (0) 52 (7) 30 (0)
PDXT-EMC007: 10 (0) 25 (4) 36 (0)
PDXT-EMC015: 11 (0) 75 (6) 36 (2)
PDXT-EMC053: 7 (0) 41 (1) 15 (0)

Primary tumor (F0) unique
Primary tumor (F0) and Xenograft (F3)
Xenograft (F3) unique

(): Cancer Consensus Genes
Endometrial cancer

Depreeuw J. et al, Gynaecologic Oncology 2015
Clonal dynamics over multiple passages in time

Clonal dynamics are reproduced in replicate transplants

Eirew P, Nature 2014
Transcription profile: RNA Sequencing

PRO gene list (764) (Verfaillie et al. 2015)

INV gene list (591) (Verfaillie et al. 2015)

Unpublished data by Rambow F, Lab. Of Molecular Cancer Biology
Transcription profile: RNA Sequencing

Isella et al, Nature genetics, 2015
Pharmacologic validation

<table>
<thead>
<tr>
<th>Tumor ID</th>
<th>Stage</th>
<th>Treatment</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVC-020</td>
<td>FIGO IIIb</td>
<td>TC 3w (6 cycles) Last 29/11/2013</td>
<td>Primary tumor Surgery 16/07/2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>On AMG386/placebo</td>
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<tr>
<td></td>
<td></td>
<td>Till Dec 2014—Relapse(!)</td>
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<tr>
<td></td>
<td></td>
<td>SENSITIVE</td>
<td></td>
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</tbody>
</table>

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<th>Tumor ID</th>
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<th>Treatment</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVC-002</td>
<td>FIGO Ic</td>
<td>Several TC 3w (6 cycles)</td>
<td>Recurrence Surgery 6/11/2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>From 2004 till Nov 2012 – partial remission</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Acquired resistance</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olaparib (2013) -- Progr</td>
<td></td>
</tr>
</tbody>
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Unpublished data by Annibali D, Translat. Research GNC
Establishment of PDXs

Pre-clinical drug screening and biomarker analysis

Co-clinical trial approach

Personalized medicine strategy

Develop humanized xenograft mice
ESTABLISHMENT OF PDXs

PRE-CLINICAL DRUG SCREENING AND BIOMARKER ANALYSIS

CO-CLINICAL TRIAL APPROACH

PERSONALIZED MEDICINE STRATEGY

DEVELOP HUMANIZED XENOGRAFT MICE
Proposed preclinical screening and biomarker study in PDX models.

Proposed preclinical screening and biomarker study in PDX models.

Proposed preclinical screening and biomarker study in PDX models.

Population-based preclinical drug screening and biomarker analysis

The genomic landscape of response to EGFR blockade in colorectal cancer

The Novartis approach: A proof of concept

The Novartis approach: A proof of concept

The genomic landscape of response to EGFR blockade in colorectal cancer

The Novartis approach: A proof of concept

The genomic landscape of response to EGFR blockade in colorectal cancer

The Novartis approach: A proof of concept
Searching for therapeutic targets in uterine sarcoma

- Comprehensive NGS project
- IHC for known targets
- In vivo treatment experiments using patient-derived models

*Unpublished data by Cuppens T., Translat. Research GNC*
MOUSE CLINICAL TRIAL
- A dual PI3K/mTOR inhibitor
- A chemotherapy
- Combined treatment (PI3K/mTORi and chemo)

CONCLUSION
- Combination of chemo and PI3K/mTOR: no synergistic effects
- Do primary tumors respond better to PI3K/mTORi and chemo than recurrent tumors?

Unpublished data by Cuppens T., Translat. Research GNC
ESTABLISHMENT OF PDXs

PRE-CLINICAL DRUG SCREENING AND BIOMARKER ANALYSIS

CO-CLINICAL TRIAL APPROACH

PERSONALIZED MEDICINE STRATEGY

DEVELOP HUMANIZED XENOGRAFT MICE
Co-clinical trial approach with PDX models.

Co-clinical trial approach with PDX models.

Co-clinical trial approach with PDX models.

Co-clinical trial approach with PDX models.

OBJECTIVES

• Investigation of resistance mechanism of action

• Development of new strategies to overcome resistance

• Design of novel and effective personalized targeted therapies
ESTABLISHMENT OF PDXs

PRE-CLINICAL DRUG SCREENING AND BIOMARKER ANALYSIS

CO-CLINICAL TRIAL APPROACH

PERSONALIZED MEDICINE STRATEGY

DEVELOP HUMANIZED XENOGRAFT MICE
Personalized medicine strategy.

Exome sequencing

Normal DNA (blood)

Tumor

Genomic profiling

Detecting biomarkers

Transcriptional profiling

Copy number and sequencing (somatic mutations)

Identify drug sensitivity based on tumor genomics alterations

Personalized medicine strategy.

Exome sequencing

Genomic profiling
- Detecting biomarkers
  - Transcriptional profiling
  - Copy number and sequencing (somatic mutations)

Identify drug sensitivity based on tumor genomics alterations
- Drug 1

Potential drugs based on genomic profiling

Personalized medicine strategy.

Personalized medicine strategy.
Personalized medicine strategy.

Exome sequencing

- Normal DNA (blood)
- Tumor

Avatar

- Drug response in Avatar mouse

Potential drugs based on genomic profiling

- Genomic profiling
  - Detecting biomarkers
  - Transcriptional profiling
  - Copy number and sequencing (somatic mutations)

Identify drug sensitivity based on tumor genomics alterations

Drug 1

Sensitive Resistant

Custom database with response biomarkers

Genomic profiling
- Predictive and prognostic methodology
- Personalized drug therapy

Personalized medicine strategy.

ESTABLISHMENT OF PDXs

Pre-clinical drug screening and biomarker analysis

Co-clinical trial approach

Personalized medicine strategy

Develop humanized xenograft mice
GOAL:
Reconstitution of human immune system and co-transplantation of a PDX to allow preclinical testing of drugs targeting immune-checkpoints and humane T cell function

NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup-tm1Sug/JicTac</sup> • Polymorphic SIRP-α • Complement C5 Deficient • Impaired Dendritic cell Function • Impaired Macrophage Function • B and T cell Deficient • NK cell Deficient • Impaired cytokine signaling
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Drawbacks

• Lack of immune system of host

• Substitution of human stroma by murine tissue

• Engraftment failure: 30 – 40 %

• Cost & resources
Take home message
Innovative strategy in R&D Oncology

Experimental data

Pre-Clinical Phase  PDX

Clinical data

Clinical Phase

TCGA/CGP: Descriptive data

Cost saving & increase patient OS/DFS

www.uzleuven-kuleuven.be/lki/trace
Trace
Frédéric Amant
Debby Thomas
Ellen Gommé
Marie-Astrid Van Hoorick

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