LIMITATIONS OF TRADITIONAL PRE-CLINICAL TUMOR MODELS

Beverly A. Teicher, PhD
DCTD/NCI

The content reflects my professional opinions, not an NCI policy statement.
Outline

1. Transplantable Syngeneic Tumors
2. Human Tumor Xenografts
3. Disseminated Disease Models
4. ‘Labeled’ Tumor Models
5. PDX: Personal Cancer Avatars
6. PDX: New Xenograft Models
7. GEMM Tumor Models
8. The Mouse is a Hardy Host
9. Tumor Microenvironments
Transplantable Syngeneic Tumor Models

Advantages

- Low cost
- Reproducible
- Immuno-competent host
- Some variety
- Non-immunogenic
- Long history/strong baseline data
- Hosts readily available
- Statistically valid numbers

Disadvantages

- Rodent tumor cells
- Tumor lines are old
- Often implanted sc
- Rodent targets
- Rodent hosts
- Rodent immune system
- Grow very fast
Human Tumor Xenografts (Subcutaneous implant)

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>Human Tumor Cells</td>
<td>More costly</td>
</tr>
<tr>
<td>Reproducible</td>
<td>Rodent stroma</td>
</tr>
<tr>
<td>Wide variety</td>
<td>Immuno-deficient hosts</td>
</tr>
<tr>
<td>Long history</td>
<td>Non-natural site (sc)</td>
</tr>
<tr>
<td>Strong baseline data</td>
<td>Most tumor lines old</td>
</tr>
<tr>
<td>Hosts readily available</td>
<td>Genetic diversity limited</td>
</tr>
<tr>
<td>Tumor growth easily followed</td>
<td></td>
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<tr>
<td>Statistically valid numbers</td>
<td></td>
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Disseminated Disease Models (syngeneic or xenograft)

**Advantages**
- More like clinical disease
- Tumor cells home to tissues
- Good Variety
- Hosts readily available
- Syngeneic immuno-competent

**Disadvantages**
- Intracardiac/iv injection
- Low animal numbers
- Rodent stroma
- Rodent immune system
- Tumor difficult to follow
- Most tumor lines old
- Statistics difficult
- Survival endpoint
Disseminated Disease Models: Tumor Excision
Quantify tumor cell killing in vivo

- Grow tumor
- Treat the host
- Excise the tumor

- Prepare single cell suspension
- Count cells
- Plate known number of cells

- Count colonies
- Calculate survival
Disseminated disease Models:
Metastatic Spread & Kinetics of Tumor Growth

Mouse EMT6 Mammary Ca
sc implant
Day 9
Disseminated Disease Models: 4T1 Intra-cardiac injection model
Rat 13672 Mammary Carcinoma Bone Metastasis & TGF-β Antagonists
‘Labeled’ Tumor Models (synegenic or xenograft): Orthotropic or subcutaneous

<table>
<thead>
<tr>
<th>Advantages</th>
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<tbody>
<tr>
<td>Tumor can be followed by fluorescence or luminescence</td>
</tr>
<tr>
<td>Metastasis visualized</td>
</tr>
<tr>
<td>Tumor measurement</td>
</tr>
<tr>
<td>Variety limited</td>
</tr>
<tr>
<td>Hosts readily available</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetically altered sub-lines</td>
</tr>
<tr>
<td>Many clonal lines</td>
</tr>
<tr>
<td>Poor representation of disease</td>
</tr>
<tr>
<td>Low animal numbers</td>
</tr>
<tr>
<td>Rodent stroma</td>
</tr>
<tr>
<td>Immuno-deficient host</td>
</tr>
<tr>
<td>Costly equipment</td>
</tr>
<tr>
<td>Statistics difficult</td>
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</table>
Development of an Orthotopic Human Pancreatic Cancer Xenograft Model Using Ultrasound Guided Injection of Cells

Amanda Shanks Huynh¹, Dominique F. Abrahams², Monica S. Torres², Margaret K. Baldwin², Robert J. Gillies¹, David L. Morse¹*
PDX (Patient-Derived Xenograft) Models

1) Personal Cancer Avatars

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor cells recently from patient (P1)</td>
<td>Require large tumor specimen to start</td>
</tr>
<tr>
<td>Genetically similar to patient</td>
<td>Slow growing</td>
</tr>
<tr>
<td>Tumor measurement (sc)</td>
<td>Immuno-deficient host</td>
</tr>
<tr>
<td>Hosts readily available</td>
<td>Mouse stroma</td>
</tr>
<tr>
<td></td>
<td>Low animal numbers</td>
</tr>
<tr>
<td></td>
<td>Very costly</td>
</tr>
<tr>
<td></td>
<td>Statistics difficult</td>
</tr>
<tr>
<td></td>
<td>Not validated predictors</td>
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</table>
As understanding of human malignant disease complexities emerge & preclinical investigators attempt to match clinical disease to available human tumor xenograft models, it is strikingly apparent that there are not enough xenograft models & that those used as drug discovery drivers fall short of representing the diversity of the clinical disease.

Advantages
- Recently from patient (P4-?)
- Many genetically characterized
- Tumor measurement (sc)
- Hosts readily available

Disadvantages
- Immuno-deficient host
- Rodent stroma
- No baseline data
- Very costly
- Low animal numbers
- Often slow growing
- Statistics difficult
- Not validated
GEMM Tumor Models

GEMM models have come a long way; however, genetically they are too ‘clean’ compared with human malignant disease. GEMM models can be useful for understanding the biology & kinetics of changes caused by specific mutations.

Advantages

- Tumor arises in desired tissue
- Well-defined lesion; defined mutations
- Can follow time course to cancer
- Immunocompetent host

Disadvantages

- Limited mutations not reflective of human disease
- Rodent tumors
- Slow tumor development
- Very costly breeding
- Variable tumor stage
- Tumor difficult to follow
- Statistics difficult
- Survival endpoint
The Mouse is a Hardy Host

The mouse remains the most useful & most maligned host for cancer models. In-bred strains of conventional & immuno-deficient mice are, generally, hardy upon exposure to anticancer agents compared with humans.

The most commonly used measure of mouse normal tissue toxicity is body weight change. Treatments resulting in 20% net body weight loss associated with a moribund condition &/or >20% lethality are designated ‘toxic’.

Bone marrow is often a dose-limiting toxicity in patients.
Response of Human & Mouse Bone Marrow: Toposioemerase 1 Inhibitors in Culture

**SN-38**
- **Human**
  - $IC_{50}$: 10 nmol/L; $IC_{90}$: 26 nmol/L
- **Mouse**
  - $IC_{50}$: 108 nmol/L; $IC_{90}$: 331 nmol/L

**Topotecan**
- **Human**
  - $IC_{50}$: 6.5 nmol/L; $IC_{90}$: 18.8 nmol/L
- **Mouse**
  - $IC_{50}$: 166 nmol/L; $IC_{90}$: 518.8 nmol/L

**Surviving Fraction** vs. **Concentration, nM**
Tumor Microenvironment

The tumor microenvironment plays a very important role in tumor progression. In fact, the microenvironment is involved during carcinogenesis initiation, progression to malignancy, & treatment response & resistance.

The mouse subcutaneous space is a poor microenvironment for syngeneic or xenograft tumors. Most orthotopic implantations are confounded with the cytokine & growth factor ‘storm’ produced by wound healing.

The ‘communication’ between human and mouse signaling pathways is imperfect & in some cases such as interferons, some interleukins & some growth factors (HGF), non-functional.

The mouse immune system is quite different from the human & has led to a great effort to produce humanized mice.

The mouse pharmacokinetic & metabolic handling of drugs can be very different from human.
Microenvironment effect on tumor response

EMT6 parent tumor

EMT6/CTX tumor
Conclusions

- Transplantable tumors in mice are poor mimics of human cancer whether syngeneic or xenograft
- GEMM models have advantages and disadvantages. Are they ‘better’ models of human disease?
- The mouse is a hardy host & thus has led to inaccurate predictions of activity in human cancer
- The mouse microenvironment does not accurately reflect the microenvironment of human cancer