Limitations of Preclinical Models: Immune Modulators

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• Proof of principle of pathway importance *can* be modeled in mice.

• The extremes of *toxicity profile* can be gathered from knock-out models.

• Predicting a *specific disease application* *not* generally realistic with mouse models.
• Background
• Pros and cons of implantable versus spontaneous models
• Principle advantages to preclinical models:
  – Inform targets and combinations
  – Investigate related questions, e.g. microbiome
• Major limitations:
  – Variable resemblance to parallel human disease: specific malignancies and autoimmunity
  – Lack human diversity
  – Tumor time course
• Conclusions
Topics for Discussion

• **Background**
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Background

Research in Tumor Immunology

Birth of Tumor Immunology

1880s
- 1891 William Coley injected live bacteria into human tumors

1915-1920
- Strong & Little: Generation of inbred (congenic) strains of mice
- Burnet & Thomas: Concept of immune surveillance of cancer

1970-1982
- First human Tumor Associated Antigen cloned (MAGE-1)

1986
- Interferon-α approved by FDA

1982
- Interleukin-2 approved by FDA

1986
- Anti-CTLA4 (Ipilimumab) approved by FDA

Future
- Other checkpoint blockade
- T cell co-stimulation
- Vaccines/Tumor cell death
- Combination with other therapies

Immunotherapy in the clinic

Present
- First cellular immunotherapy (Sipuleucel-T) approved by FDA

Budhu, Wolchok, Merghoub, Curr Opin Gen Dev 2014
Background
Topics for Discussion

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Implantable vs. Spontaneous Models: Advantages
Implantable vs. Spontaneous Models: Advantages

**Implantable**
- ✓ Rapid/reliable tumor growth
- ✓ Multiple cancer types
- ✓ Intervene upon cell lines before implantation
- ✓ Orthotopic (into organ of tumor origin) or subcutaneous (easily monitored)
- ✓ Homogeneity of system

**Spontaneous**
- ✓ Carcinogen-induced or genetically engineered
- ✓ Heterogeneous, slower tumor development may be more similar to human tumors
- ✓ Tumor immune response/escape may also be more similar to human tumors (carcinogen-induced)
- ✓ Organ-specific tumor development (some)
## Implantable vs. Genomic Models: Disadvantages

<table>
<thead>
<tr>
<th>Implantable</th>
<th>Spontaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ Does not recapitulate human tumor growth rate/context</td>
<td>✓ Longer time to develop/higher cost</td>
</tr>
<tr>
<td>✓ Rapid time course may be associated with immune interaction different from humans</td>
<td>✓ Inter-mouse heterogeneity can make results difficult to interpret</td>
</tr>
<tr>
<td></td>
<td>✓ Mutations continuously present from birth in some genetically modified models → tolerance, immunoRx less effective</td>
</tr>
</tbody>
</table>
Implantable vs. Genomic Models: Example

KRAS G12D; p53 deficient
2 mutations

Carcinogen-induced sarcoma
2,830 mutations

Days Post Transplant

DuPage...Jacks Nature 2012
Matsushita...Schreiber Nature 2012
Implantable vs. Genomic Models: Example

Grm1
Benign disease

del-TG3
Aggressive metastatic disease
• Background
• Pros and cons of implantable versus spontaneous models

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Preclinical Models: Inform Targets, e.g. CTLA-4

Heart

Wild Type

CTLA4 -/-

Pancreas

Average tumor size (mm²)

Days after tumor injection

Tivol...Bluestone, Sharpe Immunity 1995
Leach, Krummel, Allison Science 1996
Preclinical Models: Inform Targets, e.g. PD-(L)1

4-1BB, OX40, GITR, LAG3 & other molecules targeted in clinical trials all developed based on mouse models.

Iwai...Honjo T, Minato N PNAS 2002
Preclinical Models: Inform Combinations

C4=anti-CTLA-4
P1=anti-PD-1

Twyman-Saint victor...Vonderheide, Minn Nature 2015
Winograd...Wherry, Vonderheide Cancer Immunol Research 2015
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Investigate Related Questions: Microbiome
• Background
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Variable Resemblance to Human Disease: Mouse vs. Human Malignancies

• Melanoma, B16, *quite similar*
  – CDKN2A homozygous deletion
  – 962 nonsynonymous SNVs
  – 16/50 expressed neoantigens elicited a response (ELISPOT)

• Ovarian, ID8, *somewhat similar*
  – Derived from spontaneous *in vitro* malignant transformation of C57BL6 mouse ovarian surface epithelial cells
  – Resemble serous ovarian cancer histologically and in tumor behavior
  – Sequence not published
• Myeloma, J588L, dissimilar
  – Used as a model for anti-PD-L1 efficacy
  – One of the least checkpoint blockade-sensitive diseases in human studies

• Colon, MC38 (BL6) dissimilar, CT26 (BALB/c) somewhat similar
  – MC38: 4,285 nonsynonymous SNV; 1,290 expressed
  – CT26: KRAS is homozygously mutated at p.G12D, APC and TP53 are not mutated, and CDKN2A is homozygously deleted; MHC I but not II is expressed
Variable Resemblance to Human Disease: Autoimmunity

- Seminal papers on CTLA-4/PD-1 showed cardiac toxicity of receptor KO not observed in humans
- Autoimmunity infrequently observed in mice and difficult to measure
- Certain strains more prone to autoimmune side effects

Tivol...Bluestone, Sharpe Immunity 1995
Nishimura...Honjo Science 2001
Iwama...Wolchok, Caturegli. Sci Transl Med 2014
Mouse Models Lack Human Diversity

Areas of Human Diversity Lacking in Mouse Models

- HLA alleles
- Germline genetics
- Exposures-environmental and therapeutic
- Microbiome
- Age
- ...

HUMAN

H-2 Class I

<table>
<thead>
<tr>
<th>Strain</th>
<th>Appearance</th>
<th>Appearance</th>
<th>Haplotype</th>
<th>K</th>
<th>D</th>
<th>L</th>
<th>IA</th>
<th>IE</th>
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<tbody>
<tr>
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<td>albino</td>
<td>d</td>
<td>Kd</td>
<td>Dd</td>
<td>Ld</td>
<td></td>
<td>IAd</td>
<td>IEd</td>
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<tr>
<td>C3H/He</td>
<td>agouti</td>
<td>k</td>
<td>Kk</td>
<td>Dk</td>
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<td></td>
<td>IAk</td>
<td>IEk</td>
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<tr>
<td>C57BL/6</td>
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<td>Kb</td>
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<tr>
<td>CBA</td>
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<td>Dk</td>
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</table>

H-2 Class II

<table>
<thead>
<tr>
<th>Locus (IMGT/HLA)</th>
<th>Alleles</th>
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<tr>
<td>HLA-A</td>
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<tr>
<td>HLA-B</td>
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<tr>
<td>HLA-C</td>
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<tr>
<td>HLA-DMA</td>
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<tr>
<td>HLA-DMB</td>
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<tr>
<td>HLA-DOA</td>
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</tr>
<tr>
<td>HLA-DOB</td>
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</tr>
<tr>
<td>HLA-DPA1</td>
<td>39</td>
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<tr>
<td>HLA-DPB1</td>
<td>520</td>
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<tr>
<td>HLA-DQA1</td>
<td>54</td>
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<tr>
<td>HLA-DQB1</td>
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<td>HLA-DRA</td>
<td>7</td>
</tr>
<tr>
<td>HLA-DRB1</td>
<td>1,719</td>
</tr>
</tbody>
</table>
Tumor Time Course

Days

Versus

Years

2yo mouse ≈ late middle aged human

Gubin...Schreiber Nature 2014
Yachida...Vogelstein, Iacobuzio-Donahue Nature 2010
Proof of principle of pathway importance can be modeled in mice.

– Novel combination therapies/modalities are under development based on these models.

– The extremes of toxicity profile can be gathered from knock-out models.

– Predicting a specific disease application not generally realistic with mouse models.