Drug Discovery in the Immuno-Oncology Era: Applications for Humanized Mouse Models

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Immunotherapy: Game Changer for Metastatic Melanoma

• 3 year overall survival (OS) before immune checkpoint inhibitors (ICI) ~ 15%
• 3 year OS after ICIs = 58% (anti-CTLA-4/anti-PD-1 combination)


Types of Immunotherapy

- Immuno-oncology (I/O) agents activate a patient’s immune system to fight the disease
  - ICIs – target immune regulators
  - Adoptive T cell therapies (CAR-T) – modify patient T cells to boost anticancer patient immune response
  - Oncolytic virotherapy selectively kills tumor cells while stimulating the patient’s anti-tumor immune response
  - Cancer vaccines – use cancer neoantigen(s) to activate or boost the patient’s immune system against the cancer
Challenges

- Limited availability of *in vitro* validation assays
  - Hampered by donor-dependent variability
  - Difficult to model interactions between multiple cell types
- *In vivo* testing requires models with a functional immune system
  - Traditional xenograft models use immunocompromised mice
  - Mouse and human immune systems are not completely analogous
- Immune response can be variable even under identical conditions
CrownBio’s Integrated I/O Platform

I/O Platform Technologies

- Syngeneic MuPrime™ GEMM
- Mouse
- CAR-T Therapy
  - HuPrime®
- Human-specific biologics
  - HuGEMM™/HuCELL™
  - MiXeno™
  - HSC-PDX

Human

Mouse
CrownBio’s Integrated I/O Platform

I/O Platform Technologies

Human-specific agents
- HuGEMM™/HuCELL™
- MiXeno™
- HSC-PDX

CAR-T Therapy
- HuPrime®
Introduction to CAR-T Therapy

- Highly personalized approach
- Neoantigen expression is key to a successful CAR-T therapy
- Two CAR-Ts currently approved with many more on the way
CrownBio CAR-T Therapy Platform

- **HuPrime**: well characterized collection of 2,500 patient-derived xenografts (PDXs)
  - Highly predictive models, preserving original patient tumor pathological features
  - Reflective of the genetic diversity from the patient population
- **HuBase™**: online searchable database to access
  - PDX phenotypic and genotypic data
  - Patient clinical information
  - Growth curves
  - Standard of care treatment data
- TMAs for additional biomarker analysis by IHC
GPC3-Targeted CAR-T Development

- PDX model selection via HuBase
• Validation of GPC3 expression in the NSCLC LU1542 PDX model via IHC
• Evaluation of model LU1542 response to CAR-GPC3 T cell therapy
CrownBio Chimeric Models

- Syngeneic mouse tumor models widely used for testing I/O agents
  - Not suitable for testing human-specific therapeutics
- CrownBio has developed HuGEMM and HuCELL models to evaluate human specific agents
  - Chimeric knock-in mice that render syngeneic models effective in evaluating targeted human immunotherapies in vivo
• A chimeric mouse tumor model with fully functional murine immune system but a humanized drug target
• Mouse tumor cells that have been engineered to express humanized ligands
Validated PD-1 HuGEMM Models

- Validated models:
  - huPD-1 HuGEMM host with MC38 murine colon adenocarcinoma model expressing mouse PD-L1
  - huPD-1 HuGEMM host with HuCELL MC38 model expressing human PD-L1 (exon 3 human PD-L1 knock-in)

- Model response to anti-hPD-1 mAb treatment (Keytruda® and Opdivo®) evaluated
• Anti-PD-1 (Opdivo) treatment effectively reduced tumor burden with 4/8 mice cured
Anti-PD-1 (Keytruda) treatment effectively reduced tumor burden with 3/8 mice cured.

92% TGI (Day 17)

3/8 mice cured
TIL analysis 48hrs following 2 doses of Opdivo

Correlation of tumor volume and CD8+ T cells following Opdivo treatment

\[ y = -0.0041x + 11.153 \]

\[ R^2 = 0.5047 \]
Donor containing CTLA-4 Exon 2 and Exon 3 plus CRISPR/Cas9 cassette for recombination

Mouse genome

Targeted mouse genome

Cas9 and sgRNA
CTLA-4 HuGEMM Efficacy Study: Yervoy® Treatment

- Anti-CTLA-4 (Yervoy) treatment effectively reduced tumor burden with 2/8 mice cured

TGI: 94% (Day 19)

2/8 mice cured
CTLA-4 HuGEMM TIL Analysis

CD4+ T Cells

CD8+ T Cells

- Isotype control, 10mg/kg, BIW
- Yervoy analog, 10mg/kg, BIW
<table>
<thead>
<tr>
<th>Single KI</th>
<th>Status</th>
<th>Double KI</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-1</td>
<td>Available</td>
<td>PD-1/PD-L1</td>
<td>Available</td>
</tr>
<tr>
<td>CTLA-4</td>
<td>Available</td>
<td>PD-1/TIM-3</td>
<td>Homozygous breeding</td>
</tr>
<tr>
<td>CD137</td>
<td>Available</td>
<td>PD-1/LAG3</td>
<td>Heterozygous breeding</td>
</tr>
<tr>
<td>TIM-3</td>
<td>Available</td>
<td>PD-L1/CTLA-4</td>
<td>Heterozygous breeding</td>
</tr>
<tr>
<td>OX40</td>
<td>Available</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD-L1</td>
<td>Available</td>
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<tr>
<td>LAG3</td>
<td>Validating</td>
<td></td>
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</tr>
<tr>
<td>GITR</td>
<td>Homozygous breeding, validating in 2 months</td>
<td></td>
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</tr>
<tr>
<td>CD40</td>
<td>Homozygous breeding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICOS</td>
<td>Homozygous breeding, validating in 2 months</td>
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<tr>
<td>TIGIT</td>
<td>Validating</td>
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</table>

**Immune Regulators**

- Activating receptors: CD27, CD28, CD137, GITR, HVEM, OX40
- Inhibitory receptors: BTLA, CTLA-4, LAG-3, PD-1, TIM-3
- Blocking antibodies: VISTA
- T-cell stimulation
CrownBio Humanized Model

MiXeno

Donor PBMC

Human tumor cells or tissue

Intravenous or admix with tumor cells

Tumor xenografts

Human T cells

MiXeno Mouse

Anti-tumor immunotherapeutic approaches
### MiXeno Immuno-Oncology Applications

<table>
<thead>
<tr>
<th>Immune Function Involved</th>
<th>Test Substance</th>
<th>CrownBio Experience</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cell function</td>
<td>BiTE®-like Ab</td>
<td>CD19, HER2, EGFR BiTEs</td>
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<tr>
<td></td>
<td>Immune checkpoint inhibitors/agonists</td>
<td>PD-1, PD-L1, CTLA-4 inhibitors</td>
</tr>
<tr>
<td>NK cell function</td>
<td>ADCC</td>
<td>Cetuximab</td>
</tr>
<tr>
<td></td>
<td>NK modulating agents</td>
<td>N/A</td>
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</table>
Keytruda demonstrates significant antitumor activity
Donor Variability Affects Efficacy in the HCC827 MiXeno Model

**NOG mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumor Volume (mm³)</th>
<th>T/C Value (%) on Day 13</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human IgG4 (Donor A)</td>
<td>120 ± 19</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>MPDL3280A (Donor A)</td>
<td>145 ± 38</td>
<td>131</td>
<td>0.567</td>
</tr>
<tr>
<td>Human IgG4 (Donor B)</td>
<td>166 ± 41</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>MPDL3280A (Donor B)</td>
<td>54 ± 10</td>
<td>32</td>
<td>0.067</td>
</tr>
</tbody>
</table>
• MPDL3820A demonstrates significant antitumor activity
• The number of lymphocytes increases in treated animals
A CD3/CD19 BiTE molecule effectively redirects T cells to the tumor in the Jeko-1 MiXeno model.

**Relevance To Human-Specific Antibodies**
Essential when no mouse surrogate exists or there is no cross-reactivity

**Combination: IO+Targeted**
Access full diversity of cancer genetics from PDX collection (e.g. PD-1/EGFR, CTLA-4/BRAF)

**Relevance To Known And Novel IO Targets**
Ability to evaluate multiple IO targets
Model captures diversity of human lymphocyte targets (e.g. model is not humanized for just PD-1)

**Combination: IO/IO**
Access full diversity of human lymphocyte targets (e.g. PD-1/CTLA-4)

**HuTrial™: Assess Diversity Across Population**
Both immune system diversity (multiple stem cell donors) & tumor diversity (multiple PDX models)
CD34+ Humanized Mice

Myeloablation via irradiation

Mature lymphoid cells (PBMC) → Immunodeficient mouse → Human hematopoietic stem cells (HSC, CD34+)

2-3 weeks 12 weeks

Engraftment validation

~20 to 30% hCD45+ cells in peripheral blood

>25% hCD45+ cells in peripheral blood

hPBMC mouse hCD34+ mouse
Humanization of NSG Mouse Does Not Effect MDA-MB-231 Growth
Humanization of NSG Mouse Required for Keytruda Effect: MDA-MB-231

- Humanized mice respond to an anti-PD-1 mAb
Humanized Mice Display a Memory Response

- Previously treated humanized NSG mice are rechallenged with tumor cells to evaluate memory response
  - Re-implanted tumors fail to grow in pretreated animals, indicating a memory response
• Checkerboard study design
  – Reduces the number of humanized animals required per each arm by increasing the number of models tested
  – Allows assessment of donor-to-donor variability
Case Study: Humanized Tumor Bearing Models Help Predict Response

- **Objective:** evaluate response to Keytruda in SCLC PDX models
- **Study design:** 7 SCLC PDX models, 5 immune donors, N=1
Case Study: Humanized Tumor Bearing Models Help Predict Response

- 36% of SCLC PDX in the study respond to treatment (TGI≥48%)
- Translatable study design with outcomes comparable to the clinic
Case Study: Humanized Tumor Bearing Models Help Predict Response

- Increased % of TILs in tumors from responder PDXs
- M1 (CD38) macrophage polarization in responders vs M2 (PTGS1) in non-responders
# Summary of CrownBio Humanized Models

<table>
<thead>
<tr>
<th>CrownBio Platform</th>
<th>Model Type</th>
<th>Immune System</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>HuPrime</td>
<td>PDX</td>
<td>Immunocompromised animals</td>
<td>CAR-T or other cell therapies with immune cells supplied by infusion</td>
</tr>
<tr>
<td>HuGEMM</td>
<td>Syngeneic mouse tumor</td>
<td>Competent mouse immunity</td>
<td>Known ICI targeting agents, combinations of ICIIs with novel mouse cross reactive test articles</td>
</tr>
<tr>
<td>MiXeno</td>
<td>Cell line derived xenografts or fast growing PDX</td>
<td>PBMC reconstituted human immunity. GvHD limits experimental window. Only compatible tumor models</td>
<td>BiTEs, ICIIs, other immunomodulatory reagents with a fast response (21 days or less)</td>
</tr>
<tr>
<td>HSC-PDX</td>
<td>PDX</td>
<td>CD34+ reconstituted human immunity</td>
<td>All immunomodulatory reagents</td>
</tr>
</tbody>
</table>
Get in Touch

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