APPLYING HUMANIZED MOUSE MODELS TO IMMUNE THERAPY RESEARCH

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Outline

• Humanization
• Managing Heterogeneity
  – Hu-NSG IO studies
• Hu-SGM3 – new applications
  – Immuno-oncology
    • Anti-PD-1
    • Anti-DLL4 (Preliminary Data)
  – Immuno-toxicity (CRS)
  – Infectious disease
Creating Humanized Mice: Timeline

- Whole body irradiation
- Tail vein injection
- Human B cells appear
- Human T cells appear

3 weeks
12 weeks
15 weeks
Human Immune Cell Reconstitution in the Blood of Hu-NSG vs. Hu-NSG-SGM3:
Window for drug treatment can depend on cellular target
Human Immune Cell Reconstitution in the Blood of Hu-NSG vs. Hu-NSG-SGM3

**HuCD3+ Cell Number in Peripheral Blood** (Donor 0787)

- **NSG (n=10)**
- **NSG-SGM3 (n=10)**

**HuCD3+CD8+ Cell Number in Peripheral Blood** (Donor 0787)

- **NSG (n=10)**
- **NSG-SGM3 (n=10)**

**HuCD3+CD4+ Cell Number in Peripheral Blood** (Donor 0787)

- **NSG (n=10)**
- **NSG-SGM3 (n=10)**
BR1126 in Hu-NSG mice: Tumor Growth Curve

- Fresh tumor tissue engraftment
- HuCD45+ in Hu-NSG mice: >25%
- BR1126 PD-L1 surface expression: 56.9%

<table>
<thead>
<tr>
<th>HLA match</th>
<th>CD34+HPC donor</th>
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<tbody>
<tr>
<td>Tumor</td>
<td>1</td>
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<tr>
<td>BR1126</td>
<td>HLA-C, DPA1</td>
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*P = 0.0127; Compared to Vehicle group. Two-tailed unpaired t test.
Preliminary Efficacy Results of BR1126 PDX in HuCD34 NSG-SGM3 Mice

- Fresh tumor tissue engraftment
- HuCD45+ in whole blood: 50-88%
- HuCD3+/HuCD45: average 34%
- BR1126 PD-L1 surface expression: 56.9%
BR1126 in Hu-SGM3 mice: Flow Data (PD-1)

**PD-1 Level (%) of Treg or CD8+ Cells in BR1126 Tumor (Hu-SGM3)**

*Vehicle vs Pembrolizumab; Two tailed unpaired t test.*

- **P=0.0137**
- **P=0.0102**
Immune cell infiltration of humanized PDX NSG™ mice in response to pembrolizumab

Vehicle

Pembrolizumab

CD45 CD8 Cytokeratin

- Pembrolizumab-treated mice displayed increased total immune cell and CD8+ T lymphocyte tumor infiltration compared to vehicle-treated mice.
hHSC Donor Delated Variability

- ~15% of hHSC donors exhibit PDX tumor rejection
- ~85% PDX or cell line tumor growth
  - 100% statistical significant decrease in immune cell PD-1 detection with keytruda treatment
  - ~70% of these we have statistically significant tumor growth reduction with keytruda treatment
Efficacy Results of Pembrolizumab on LG1208 PDX tumors in Hu-NSG Mice

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<th>LG1208</th>
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<tr>
<td>Tumor</td>
<td>CD34+ HPC Donor</td>
<td>HLA-DRB4, DPA1</td>
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PD-1 and PD-L1 Signal in The Tumor Tissue:
No correlation of PD-1 loss of signal or blockage with tumor growth rate reduction

- PD-1 Expression: Activated T cells, Tregs, B cells, NK cells and monocytes; occasionally on tumor cells.
- PD-L1 Expression: Mainly on tumor cells and normal tissues; also on T cell, B cells, macrophages, DCs.
Heterogeneity

- Early passage (P4 – P6) PDX tumors
  - Not dissociated
  - Passaged as fragments

- hHSC
  - Every study is a unique patient
  - Engraftment levels within individual mice will vary

- HLA match
  - Is a Class I match not really a mismatch
Significant variability in response to Pembrolizumab within a treatment arm: Each PDX engrafted mouse has its own unique tumor.
LG1306 in Hu-NSG mice: Individual PDX Tumor Volume Changes
Variability of tumor growth in humanized mice

Individual Tumor Volume of TM00302 (LG1306P5) PDX in Hu-NSG Mice

- Vehicle (saline) ip Q5Dx6
- Pembrolizumab (5mg/kg) ip Q5Dx6 *

(Tumor Volume (mm³))
(Days 0 = treatment initiation)
Mouse as a patient

- Variability of immune cell levels between individual mice (even in a cohort)
- Variability in growth kinetics of PDX tumors
- Variability in drug response

To my preclinical colleagues - congratulations, your life is now as complicated as a clinician’s

(Isn’t this what we wanted?)
Significant variability in response to Pembrolizumab within a treatment arm: Each PDX engrafted mouse has its own unique tumor.
What’s new – What’s coming

- Additional PDX models – hu-Onc studies
- Non-checkpoint IO targets in hu-SGM3
- Infectious Disease – Myeloid specific
Additional PDX Models for IO

Mean Tumor Volume of TM00212 (LG0978P5) PDX in Hu-NSG Mice (donors 7206 and 7096)
* P<0.05; Compared to Vehicle group. Two-tailed unpaired t test.

Kras mutant
Additional PDX Models for IO

**Mean Tumor Volume of TM00199 (LG0703P4) PDX in Hu-NSG Mice (two donors)**

- **Vehicle (Saline) ip Q5Dx9**
- **Pembrolizumab (10->5mg/kg) ip Q5Dx9***

*** P<0.0001; Compared to Vehicle group. Two-tailed unpaired t test.

(Estimates and errors are shown for each treatment group.)

EGFR mutant
Novel applications - hu-SGM3

**Strain:** NSG-Tg(CMV-IL3,CSF2,KITLG)1Eav/MloySzJ (#013062)

**Common name:** NSG-SGM3

- Immuno-oncology
- Infectious disease
  - Ebola
    - Poster
    - Submitted manuscript
Anti-DLL4 Treatment of NSCLC PDX Tumors in Humanized SGM3 Mice Shows Immune Cell Engagement

- Collaboration with Chris Murriel and Tim Hoey, OncoMed Pharmaceuticals
- DLL4, Delta-like ligand 4, activates the Notch pathway and is important in cancer stem cell survival, angiogenesis, and recently shown to play a role in anti-tumor immune response
- Demcizumab, anti-DLL4, is in phase 2 for NSCLC
Anti-DLL4 Significantly Inhibits OMP-LU121 NSCLC AdC Growth in Hu-SGM3 Mice

*\( p \leq 0.009 \) 21MR ± PD1 vs Control, D11

**\( p \leq 0.0006 \) 21MR ± PD1 vs Control, D19
Anti-DLL4 Significantly Inhibits OMP-LU121 NSCLC AdC Growth in Hu-SGM3 Mice

Control IgG2

21MR

Anti-PD1 (Keytruda)

21MR + Keytruda

Tumor Volume (mm³)

Tumor Volume (mm³)

Tumor Volume (mm³)

Tumor Volume (mm³)

Treatment (Days)

Treatment (Days)

Treatment (Days)

Treatment (Days)
Anti-DLL4 Increases Intra-splenic and Tumor-associated Human CD45+ Immune Cells

IHC: Hu CD45+

Splenic Hu CD45+ (Flow cytometry)

Tumor Hu CD45+ (IHC)

p = 0.0046

p = 0.0438
Anti-DLL4 Decreases Intra-splenic Human Myeloid Cells
Immuno-Toxicity: Cytokine Release Syndrome

• T cell, macrophages, DCs and NK cells are principally responsible for the secretion of the cytokines associated with an immuno tox response.

• Hu-NSG mice may not have enough T cells and macrophages response for testing some drugs like anti-CD28.

• To enhance the sensitivity of the testing, T cells and macrophages response we are humanizing a variety of NSG tg models include the hu=SGM3, hu-NSG-CSF-1 and others

• Dual utility may be possible – efficacy and immuno tox. (Blue Sky)
Ebola Replication in Humanized SGM3:

29th International Conference on Antiviral Research April 17-21, 2016

• Humanized Mouse Models of Filovirus Disease: Screening Model for Vaccines and Therapeutics

• Jessica Spengler, D.V.M., Ph.D., Stuart Nichol, Ph.D., Christina Spiropoulou, Ph.D., James Keck, Ph.D., Dana Scott, D.V.M., Heinz Feldmann, M.D., Ph.D., Joseph Prescott, Ph.D.

• 1 Viral Special Pathogens Branch, CDC, Atlanta, Georgia, USA;
• 2 In Vivo Services, The Jackson Laboratory, Sacramento, California, USA;
• 3 Rocky Mountain Veterinary Branch, NIAID, NIH, RML, Hamilton, Montana, USA;
• 4 Laboratory of Virology, NIAID, NIH, RML, Hamilton, Montana, USA

Ebola infection is mediated through myeloid cells – hu-SGM3 is a new model to study this disease.
Preliminary Findings and Conclusions

• PDX growth is not affected by HLA-type matching
• PDX engraftment into hu-CD34 NSG™ or hu-CD34 SGM3 mice does not significantly impact growth kinetics
• Hu-CD34 NSG™ and hu-CD34 NSG-SGM3 mice demonstrate immune-cell infiltration of tumors
• Hu-CD34 NSG™ and hu-CD34 NSG-SGM3 PDX respond to anti-tumor agent Pembrolizumab
• Treatment of NSCLC tumors in hu-SGM3 with anti-DLL4 shows up-regulation of splenic and tumor huCD45+ cells and a down-regulation of splenic huCD33+ cells suggesting an increase in anti-tumor immune response
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