A Versatile Tool to Study Immune Checkpoint Therapeutics: Syngeneic Tumor Mouse Models in vivo

Holger Weber
Drug discovery platform for oncology at ProQinase

- Recombinant Protein Production
- Biochemical Kinase Assay Services
- Cellular Assay Services
- In Vivo Testing Services

ProQinase
Targeting Cancer

Syngeneic Tumor Mouse Models, July 23rd, 2015
In vivo drug testing

Today/past

Drug A
Drug B
Drug C
Drug D

Human xenografts

Drug B

Role of immune system neglected
In vivo drug testing

future/now

Syngeneic models

Human xenografts

drug A

drug B

drug C

drug D

drug B

drug C

testing of immunomodulatory activity obligatory
Syngeneic models – revival of “old” tumor models

- matched cytokine network
- labeled cell lines
- functional immune system
- orthotopic
- metastasis
- tumor tissue = host cells
- reduced cost
- rapid
- reliable
ProQinase’s syngeneic tumor model platform

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Entity</th>
<th>Validated syngeneic models</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>subcutaneous</td>
</tr>
<tr>
<td>4T1</td>
<td>Breast</td>
<td>✓</td>
</tr>
<tr>
<td>B16.F10</td>
<td>Skin</td>
<td>✓</td>
</tr>
<tr>
<td>Clone M-3</td>
<td>Skin</td>
<td>✓</td>
</tr>
<tr>
<td>Ct26wt</td>
<td>Colon</td>
<td>✓</td>
</tr>
<tr>
<td>LL/2</td>
<td>Lung</td>
<td>✓</td>
</tr>
<tr>
<td>MC38-CEA</td>
<td>Colon</td>
<td>✓</td>
</tr>
<tr>
<td>RENCA</td>
<td>Kidney</td>
<td>✓</td>
</tr>
</tbody>
</table>
Tumor infiltrating leukocytes

**Tumor regression**

- M1 Macrophage
- CD8+ CTL
- tumor cell

**Tumor promotion**

- M2 Macrophage
- CD4+ Treg
- CD4+ tumor cell
- MDSC

Syngeneic Tumor Mouse Models, July 23rd, 2015
Frequency of immune cell populations in syngeneic tumors

- 4T1
- Ct26wt
- B16.F10
- RENCA
Frequency of immune cell populations in syngeneic tumors

- **4T1**
  - M1 Macrophages
  - PMN-MDSC
  - CD8+ Tcells

- **Ct26wt**
  - M2 Macrophages
  - CD8+ Tcells

- **B16. F10**
  - M-MDSC
  - CD8+ Tcells

- **RENCA**
  - M2 Macrophages
  - M1 Macrophages
  - MDSC
  - CD8+ Tcells
Testing the immune checkpoint inhibitor α–PD-L1

**Implantation:**
4T1, B16.F10, Ct26wt, RENCA

**Treatment:**
α-PD-L1, biw

**Analysis:**
tumor growth, flow cytometry

Nguyen & Ohashi, Nature Reviews Immunology, 15, 2015
Effect of $\alpha$–PD-L1 treatment on tumor growth

- 4T1
- B16.F10
- Ct26wt
- RENCA

$\Rightarrow$ Ct26wt is inhibited by treating with $\alpha$-PD-L1

Group 1: vehicle  Group 2: $\alpha$ PD-L1 antibody
Long lasting treatment effect

**Implantation:** Ct26wt tumor cells, s.c.

**Treatment:** α-PD-L1 antibody

![Graph showing tumor volume over time after implantation and treatment with α-PD-L1 antibody. The graph includes a re-challenge with Ct26wt tumor cells.]
**T cell analysis in Ct26wt tumor tissue**

- **Leukocytes**
- **T cells**
- **CD4 T cells**
- **CD8 T cells**
- **FoxP3**

![Flow cytometry plots for leukocytes, T cells, CD4 T cells, CD8 T cells, and FoxP3](image)

**Graph showing the number of cells per 1 million leukocytes**

- **Vehicle**
- **α-PD-L1**

<table>
<thead>
<tr>
<th>CD4 T cells</th>
<th>CD8 T cells</th>
<th>T_reg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Legend**

- **CD45**
- **SS**
- **CD4**
- **CD8**
- **CD25**
- **FoxP3**
MDSC analysis in Ct26wt tumor tissue

- **Leukocytes**
- **Myeloid cells**

**CD11b**
**Ly6G**
**Ly6C**

**PMN-MDSCs**
**M-MDSCs**

**CD45**

**Graph**
- **Vehicle**
- **α-PD-L1**

**X-axis**: Number of cells per 1 mio. leukocytes
**Y-axis**: 0 - 200,000

**Bars**
- **PMN-MDSCs**
- **Neutrophils**
- **M-MDSCs**
Macrophage analysis in Ct26wt tumor tissue

leukocytes

macrophages

CD11b

F4/80

CD45

CD206 (MMR)

MHC II

SS

N° of cells/1 mio. leukocytes

ratio M2/M1

M2/M1 macrophage ratio

r²=0.69

tumor volume [mm³]

vehicle

α-PD-L1

Ct26wt vehicle tumor

0 1 2 3 4

0

1000

2000

3000

4000

5000

6000

7000

8000

9000

10000

N° of cells/mg tumor

M2

M1

ratio M2/M1

0 1 2 3 4

0

1

2

3

4
Summary

4T1 tumor
• not inhibited by α-PD-L1 treatment
• high number of PMN-MDSCs

B16.F10 tumor
• not inhibited by α-PD-L1 treatment
• high number of M-MDSCs

Ct26wt tumor
• inhibited by α-PD-L1 treatment
• high number of Macrophages
• high number of T cells

RENCA tumor
• marginally affected by α-PD-L1 treatment
• low number of T cells

→ Assistance in selecting the best model for your target
In Vivo Tumor Models – ProQinase

Thank you

*In Vivo Veritas*