WIN 2015 Symposium
Radiation and immunology: a new therapeutic partnership
Dr. Ralph Weichselbaum
Conflict of Interest

• Nothing to Disclose
Limitations of Radiotherapy

- Radiotherapy cannot be given in high doses to large normal tissue volumes for very large primary tumors or widespread metastasis.

- Normal tissue complications are common following high curative doses in some sites.

- Need newer therapies for effective control of radioresistant histology e.g. Glioblastoma, large (T4), and micro and gross metastasis. Even with local control metastasis will defeat the purpose of treatment.

- Immunotherapy?
Immunodeficiency Abrogates the Anti-tumor Effect of RT. 

A. **Wt**

B. **Nude**

C. **C**

D. **Percent survival**
How Can RT Induce Antigen-specific T cells Priming?

Day 0
5x10^5 B16-SIY

Day 16
20Gy
CFSE 2C transfer

5 d
Harvest Draining Lymph Nodes, Spleen

No RT

RT

Increase antigen availability

Reduced suppression

Increase positive cytokines or other molecules

Tolerance may be reversed?
Can RT increase the Cross-priming Capacity of Intratumoral DCs?

**Functional capacity of tumor infiltrating DCs directly \textit{ex vivo}**

- **B16-SIY**
  - 14 days
  - 20 Gy
  - 3 days
  - CD11c$^+$ from tumor
  - 2C T cells
  - Proliferation Assay

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**Graph:**
- Y-axis: T cell Proliferation (cpm)
- X-axis: Treatment groups
  - No RT-DC+T
  - RT-DC+T
  - No RT-DC only
  - RT-DC only

- *** indicates statistical significance

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Proliferation Assay
Local RT Increases CD8+ T cell Infiltration

Day 7 Post-RT Analysis of Tumor Infiltrating Lymphocytes (TIL)

CD90+ Lymphocytes

CD8+ T cell

CD4+ (Non-Treg)

Local Chemokine Expression

No RT
20 Gy

Ccl5
Cxcl11
Cxcl12
Cxcl9
Cxcl11
Cxcl16
Cxcl10

24hrs
Type I Interferon is Essential for RT

Interpretation: Type I interferon signaling on hematopoietic cells is required for tumor reduction following RT
Radiation & Interferon Induction

In situ vaccination against mycosis fungoides by intratumoral injection of a TLR9 agonist combined with radiation: a phase 1/2 study

What are we actually modeling?

- No T cell response
- Existing T cell response
- Generate Systemic cell response
- Quantitatively Enhance Existing T cell response
- Qualitatively Enhance Existing T cell response

Vaccines:
- Anti-PD-1
- Anti-PD-L1
- Anti-CTLA4
- Anti-OX40

Vaccines:
- Anti-CTLA4
How to Improve the Radiation Response?

• Strategies that induce host Interferon production

• Immune Modulating Antibodies
  Anti-CTLA-4 (Ipilimumab)
  Anti-PD-1 / Anti-PD-L1
  OX40
Blockade of PD-1 or CTLA-4 Signaling in Tumor Immunotherapy

Immunologic Correlates of the Abscopal Effect in a Patient with Melanoma

Michael A. Postow, M.D., Margaret K. Callahan, M.D., Ph.D., Christopher A. Barker, M.D., Yoshiya Yamada, M.D., Jianda Yuan, M.D., Ph.D., Shigehisa Kitano, M.D., Ph.D., Zhenyu Mu, M.D., Teresa Rasalan, B.S., Matthew Adamow, B.S., Erika Ritter, B.S., Christine Sedrak, B.S., Achim A. Jungbluth, M.D., Ramon Chua, B.S., Arvin S. Yang, M.D., Ph.D., Ruth-Ann Roman, R.N., Samuel Rosner, Brenna Benson, James P. Allison, Ph.D., Alexander M. Lesokhin, M.D., Sacha Gnjatic, Ph.D., and Jedd D. Wolchok, M.D., Ph.D.

Case Report #1: Postow, NEJM, 2012

9.5 Gy x 3
Clinical Observations Radiation & anti-CTLA-4

Immunologic Correlates of the Abscopal Effect in a Patient with Melanoma

Michael A. Postow, M.D., Margaret K. Callahan, M.D., Ph.D., Christopher A. Barker, M.D., Yoshiya Yamada, M.D., Jianda Yuan, M.D., Ph.D., Shigehisa Kitano, M.D., Ph.D., Zhenyu Mu, M.D., Teresa Rasalan, B.S., Matthew Adamow, B.S., Erika Ritter, B.S., Christine Sedrak, B.S., Achim A. Jungbluth, M.D., Ramon Chua, B.S., Arvin S. Yang, M.D., Ph.D., Ruth-Ann Roman, R.N., Samuel Rosner, Brenna Benson, James P. Allison, Ph.D., Alexander M. Lesokhin, M.D., Sacha Gnjatic, Ph.D., and Jedd D. Wolchok, M.D., Ph.D.
Radiation-Induced Equilibrium Is a Balance between Tumor Cell Proliferation and T Cell–Mediated Killing
Three major outcomes after SBRT as monitored by MRI

A: Early responses but relapse

- 2 weeks before SBRT
- 4 months after SBRT
- 9 months after SBRT
- 10 months after SBRT

B: Cure or not

- 0 day before SBRT
- 2 months after SBRT
- 22 months after SBRT

Hurthle cell thyroid

Melanoma

(20Gy x 1)
RT-induced dominancy can be relapse for years

(1) before SBRT

(2) Post SBRT 1 month

(3) Post SBRT 11 months

(4) Post SBRT 25 months

(5) Post SBRT 49 months

(6) relapse Post SBRT 57 months
T cell – Tumor Cell Interactions

The specificity and magnitude of the T cell response against tumor antigens determines the outcome between tumor suppression/elimination and tumor outgrowth.

Immunotherapy, or modification of existing or induced (vaccination) T cells responses, can push this interaction in the direction of tumor elimination.
Ablative RT Can Suppress Local Tumor Regrowth and Induce Stable Disease

Radio-sensitivity of Cancer Cells was not Correlated with the Efficacy of RT

A

B

C

7 days post RT

21 days post RT

7-8 days later

Remove tumor

collagenase

Clonogenic assay

0, 2, 5 or 10 Gy

7 days

Dormant (D)

Partial Response (PR)

Non-RT control

Non-responsive (NR)

responsive (R)

Days post RT

Tumor Volume (mm$^3$)

Percentage of survival cells

Fraction of survival cells

p=0.057

A                                            B                                             C

Non-RT control

Non-responsive (NR) responsive (R)

Percentage of survival cells

Fraction of survival cells

p=0.057

no-IR 2Gy 5Gy 10Gy

0.0
0.2
0.4
0.6
0.8
1.0
1.2

Non-RT PR D

0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2

Non-RT

NR

R
Host immune responses, not the radiosensitivity of cancer cells, correlate with efficacy of RT

Apoptosis co-localized with CD8+ cells
PD-L1 Blockade Breaks the Equilibrium of Stable Disease to Favor Tumor Regression

A

B

C

Days post RT

Days after RT

Tumor Volume (mm$^3$)

Tumor-bearing (%)

IFN$\gamma$+cells / 2x10$^5$ DLNs

3T3KB (Control)

3T3NKB

Ctrl

anti-PD-L1

*: P < 0.05

**: P < 0.01

***: P < 0.001

Ctrl

D

D+αPDL-1

RT
Infiltration of T cells: A better predictor for colorectal cancer patients survival

PD-1/PD-L1 Checkpoint
Clinical Activity of Anti–PD-1 Antibody in the Efficacy Population

<table>
<thead>
<tr>
<th>Dose of Anti–PD-1 Antibody</th>
<th>Objective Response</th>
<th>Objective-Response Rate</th>
<th>Duration of Response</th>
<th>Stable Disease ≥24 wk</th>
<th>Progression-free Survival Rate at 24 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no. of patients/total no. of patients</td>
<td>% (95% CI)</td>
<td>mo</td>
<td>no. of patients/total no. of patients</td>
<td>% (95% CI)</td>
</tr>
<tr>
<td><strong>Melanoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>4/14</td>
<td>29 (8–58)</td>
<td>7.5+, 5.6+, 5.6, 5.6</td>
<td>1/14</td>
<td>7 (2-24)</td>
</tr>
<tr>
<td>0.3 mg/kg</td>
<td>3/16</td>
<td>19 (4–46)</td>
<td>3.8+, 2.1+, 1.9+</td>
<td>1/16</td>
<td>6 (2–10)</td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td>8/27</td>
<td>30 (14–50)</td>
<td>24.9+, 22.9, 20.3+, 19.3+, 18.4+, 7.6+, 5.6+, 5.3+</td>
<td>3/27</td>
<td>11 (2-29)</td>
</tr>
<tr>
<td>3.0 mg/kg</td>
<td>7/17</td>
<td>41 (18–67)</td>
<td>22.4+, 18.3+, 15.2+, 12.9, 11.3, 9.3, 9.2+</td>
<td>1/17</td>
<td>6 (0.1–29)</td>
</tr>
<tr>
<td>10.0 mg/kg</td>
<td>4/20</td>
<td>20 (6–44)</td>
<td>24.6+, 23.9+, 18.0+, 17.0</td>
<td>0/20</td>
<td>0</td>
</tr>
<tr>
<td>All doses</td>
<td>26/94</td>
<td>28 (19–38)</td>
<td></td>
<td>6/94</td>
<td>6 (2–13)</td>
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<tr>
<td><strong>Non–small-cell lung cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Squamous</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td>0/5</td>
<td>0</td>
<td>0/5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3.0 mg/kg</td>
<td>3/6</td>
<td>50 (12–88)</td>
<td>ND</td>
<td>0/6</td>
<td>0</td>
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<tr>
<td>10.0 mg/kg</td>
<td>3/7</td>
<td>43 (10–82)</td>
<td>ND</td>
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<tr>
<td>All doses</td>
<td>6/18</td>
<td>33 (13–59)</td>
<td>ND</td>
<td>0/18</td>
<td>0</td>
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<tr>
<td>Nonsquamous</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1.0 mg/kg</td>
<td>0/12</td>
<td>0</td>
<td>1/12</td>
<td>8 (0.2–39)</td>
<td>14 (0–17)</td>
</tr>
<tr>
<td>3.0 mg/kg</td>
<td>3/13</td>
<td>23 (5–54)</td>
<td>ND</td>
<td>2/13</td>
<td>15 (2–45)</td>
</tr>
<tr>
<td>10.0 mg/kg</td>
<td>4/31</td>
<td>13 (4–30)</td>
<td>ND</td>
<td>2/31</td>
<td>6 (0.8–21)</td>
</tr>
<tr>
<td>All doses</td>
<td>7/56</td>
<td>12 (5–24)</td>
<td>ND</td>
<td>5/56</td>
<td>9 (3–20)</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td>1/1</td>
<td>NA</td>
<td>ND</td>
<td>0/1</td>
<td>0</td>
</tr>
<tr>
<td>10.0 mg/kg</td>
<td>0/1</td>
<td>0</td>
<td>0/1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>All types</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td>1/18</td>
<td>6 (0.1–27)</td>
<td>9.2+</td>
<td>1/18</td>
<td>6 (0.1–27)</td>
</tr>
<tr>
<td>3.0 mg/kg</td>
<td>6/19</td>
<td>32 (13–57)</td>
<td>30.8+, 7.6+, 5.5+, 3.7+, 1.9+, NA**</td>
<td>2/19</td>
<td>11 (1–33)</td>
</tr>
<tr>
<td>10.0 mg/kg</td>
<td>7/39</td>
<td>18 (8–34)</td>
<td>14.8+, 7.6+, 7.3+, 6.7, 4.2, 3.7, 3.7</td>
<td>2/39</td>
<td>5 (0.6–17)</td>
</tr>
<tr>
<td>All doses</td>
<td>14/70</td>
<td>18 (11–29)</td>
<td></td>
<td>5/76</td>
<td>7 (2–15)</td>
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<td><strong>Renal-cell cancer</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td>4/17</td>
<td>24 (7–50)</td>
<td>17.5+, 9.2+, 9.2, 5.6+</td>
<td>4/17</td>
<td>24 (7–50)</td>
</tr>
<tr>
<td>10.0 mg/kg</td>
<td>5/16</td>
<td>31 (11–59)</td>
<td>22.3+, 21.7+, 12.9, 12.0, 8.4</td>
<td>5/16</td>
<td>31 (11–59)</td>
</tr>
<tr>
<td>All doses</td>
<td>9/33</td>
<td>27 (11–46)</td>
<td></td>
<td>9/33</td>
<td>27 (13–46)</td>
</tr>
</tbody>
</table>

Association between Pretreatment Tumor PD-L1 Expression and Clinical Response

<table>
<thead>
<tr>
<th>Response Status</th>
<th>PD-L1–Positive</th>
<th>PD-L1–Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number (percent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Objective response</td>
<td>9 (36)</td>
<td>0</td>
<td>9 (21)</td>
</tr>
<tr>
<td>No objective response</td>
<td>16 (64)</td>
<td>17 (100)</td>
<td>33 (79)</td>
</tr>
<tr>
<td>All</td>
<td>25</td>
<td>17</td>
<td>42</td>
</tr>
</tbody>
</table>

P = 0.006 for association by Fisher’s exact test
A All Patients

B Previous Treatment

C No Previous Treatment

No. at Risk

<table>
<thead>
<tr>
<th>PS ≥50%</th>
<th>119</th>
<th>86</th>
<th>66</th>
<th>60</th>
<th>38</th>
<th>20</th>
<th>13</th>
<th>8</th>
<th>4</th>
<th>3</th>
<th>3</th>
<th>3</th>
<th>3</th>
<th>1</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS 1–49%</td>
<td>161</td>
<td>122</td>
<td>70</td>
<td>45</td>
<td>21</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PS &lt;1%</td>
<td>76</td>
<td>52</td>
<td>29</td>
<td>17</td>
<td>11</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

No. at Risk

| PS ≥50% | 99 | 67 | 53 | 47 | 30 | 19 | 12 | 8 | 4 | 3 | 3 | 3 | 1 | 0 |
|---------|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|
| PS 1–49% | 127 | 93 | 48 | 31 | 15 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| PS <1%   | 68  | 44 | 26 | 16 | 11 | 6 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

No. at Risk

<table>
<thead>
<tr>
<th>PS ≥50%</th>
<th>20</th>
<th>19</th>
<th>13</th>
<th>13</th>
<th>8</th>
<th>1</th>
<th>1</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS 1–49%</td>
<td>34</td>
<td>29</td>
<td>22</td>
<td>14</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PS &lt;1%</td>
<td>8</td>
<td>8</td>
<td>3</td>
<td>1</td>
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<td>0</td>
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<td>0</td>
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</tr>
</tbody>
</table>
Irradiation and anti–PD-L1 treatment synergistically promote antitumor immunity in mice
Expression of PD-L1 and PD-1 in the tumor microenvironment

A

CD11c+  CD11b+Gr1+  CD11+F4/80+  CD45-

B

CD8+T  CD4+T

PD-L1

PD-1
Anti-PD-L1 blockade synergizes with Radiation

Experiments on different tumor models:

- **TUBO**
  - Days after tumor challenge
  - Controls (Ctrl), αPD-L1, RT, RT+αPD-L1
  - Tumor Volume (mm$^3$)

- **MC38**
  - Days post RT
  - Controls (Ctrl), αPDL-1, RT, RT+αPDL-1
  - Tumor Volume (mm$^3$)

- **Days after tumor rechallenge**
  - Naive (n=4), RT+αPD-L1 (n=4)
  - Tumor Volume (mm$^3$)

- **Days after tumor challenge**
  - MC38
  - Control, αPD-L1, RT, RT+αPD-L1
  - Tumor Volume (mm$^3$)

Significance levels:
- *: p < 0.05
- **: p < 0.01
- ***: p < 0.001
CD8+ T Cells are Required for Combination Therapy with IR and PD-L1 Blockade

(A) Tumor Volume (mm^3)

(B) IFNγ+cells / 2x10^5 DLNs

9 days post RT
IR and PD-L1 blockade induce the reduction of MDSCs

Day 10 after IR

Day 3 after IR

<table>
<thead>
<tr>
<th>Isotype ctrl</th>
<th>αPD-L1</th>
<th>IR</th>
<th>IR+αPD-L1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD11b+Gr1+</td>
<td>15.1%</td>
<td>5.71%</td>
<td>0.27%</td>
</tr>
<tr>
<td>CD11b+F4/80+</td>
<td>15.1%</td>
<td>5.71%</td>
<td>0.27%</td>
</tr>
<tr>
<td>CD8+ T</td>
<td>15.1%</td>
<td>5.71%</td>
<td>0.27%</td>
</tr>
<tr>
<td>CD4+ T</td>
<td>15.1%</td>
<td>5.71%</td>
<td>0.27%</td>
</tr>
</tbody>
</table>

IR and PD-L1 blockade induce the reduction of MDSCs
**Figure 5**

**A**

- **Gr1**
- **CD8**
- **Merge**

**Isotype Ctrl**

**IR+ αPD-L1**

Gr1

CD8α

Caspase3

**B**

- **Intercellular distance between Gr1+ and CD8+ T (µm)**

- **Isotype Ctrl**
- **IR+αPD-L1**

**C**

- **Isotype Ctrl**
- **IR+αPD-L1**
- **IR+αPD-L1+αCD8**

- **% (MDSCs in CD45+ TILs)**

**Gr-1**

**CD11b**

**CD11b**

**CD8α**

**Caspase3**

---

Intercellular distance between Gr1+ and CD8+ T (µm)
**A**

<table>
<thead>
<tr>
<th></th>
<th>Gr1(^+) alone</th>
<th>Gr1(^+) + Naïve CD8(^+)T</th>
<th>isotype</th>
<th>αTNF-α</th>
<th>αIFN-γ</th>
<th>αTNF-α + αIFN-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ly-6C</td>
<td>5.96%</td>
<td>12.6%</td>
<td>42.5%</td>
<td>22.8%</td>
<td>38.7%</td>
<td>20.5%</td>
</tr>
<tr>
<td>Annexin V+ MDSC(%)</td>
<td>5.96%</td>
<td>12.6%</td>
<td>42.5%</td>
<td>22.8%</td>
<td>38.7%</td>
<td>20.5%</td>
</tr>
</tbody>
</table>

**B**

- **Annexin-V**
- **Ly-6C**
  - Gr1\(^+\) alone: 5.96%
  - Gr1\(^+\) + Naïve CD8\(^+\)T: 12.6%
  - isotype: 42.5%
  - αTNF-α: 22.8%
  - αIFN-γ: 38.7%
  - αTNF-α + αIFN-γ: 20.5%

**C**

- **Figure 6**
- **Days after tumor challenge**
- **Tumor Volume (mm\(^3\))**
  - non-IR
  - IR
  - IR + 1A8

**D**

- **Days after tumor challenge**
  - Isotype Ctrl
  - IR + αPD-L1
  - IR + αPD-L1 + TNFRhIgG

**E**

- **Tumor Volume (mm\(^3\))**
  - non-IR
  - IR
  - IR + 1A8

**F**

- **Myeloid cells**
- **B7-H1**
- **PD-1**
- **CD8\(^+\) T cells**
- **Tumor cells**
- **MDSCs**

---

**Figure 6**
Schematic of proposed mechanism for tumor destruction induced by IR and PD-L1 blockade
Questions to be answered:

• What is mechanism behind the synergistic combination of PD-L1/PD-1 blockade?
• Does radiation provide a window for anti-PD-L1/PD-1 axis inhibitors?
• What is the best sequence/timing for RT and Ab?
• Does RT induce PD-L1 directly? If so, what is the mechanism?
• Study the effect of RT and anti-PD-L1 in several different tumor models? (e.g. B16.SIY, MC38, Autochthonous Tumor Models)
• What changes in tumor microenvironment after the combination of radiation and anti-PD-L1/PD-1? A) Immune infiltration B) cytokine/chemokine profile
• Is anti-PD-1 superior to anti-PD-L1 when combined with radiation?
The IRDS is expressed by primary breast cancer and a wide variety of other human tumors in a manner resembling Nu61/SCC61.

Weichselbaum R R et al. PNAS 2008;105:18490-18495
IRDS-positive clones can be selected by tumor microenvironment due to their resistance to the host defense systems.
Acknowledgements

Weichselbaum Lab
Radiation and Cellular Oncology

Liufu Deng
Hua “Laura” Liang
Mike Beckett
Helena Mauceri
Byron Burnette
Nikolai Khodarev
Tom Darga

Fu Lab. – Department of Pathology/Immunology
Yang-Xing Fu, M.D., Ph.D