METABOTROPIC GLUTAMATE RECEPTOR 1 (GRM1), IS A MOLECULAR TARGET FOR MELANOMA
-Lifetime melanoma incidence in the U.S.
  -men  1:53
  -women  1:78
- common type of cancer and cancer death between the ages of 20-35 (Houghton, 2002)
- 80% of skin cancer deaths are from melanoma
- surgical removal at early stages cures the disease in most cases

But…
-5 year survival rate if melanoma has spread to lymph nodes is 60%
- 5 year survival rate if melanoma has spread to distant organs (liver, bones, brain, etc.) is 16%

(Cancer Facts & Figures 2007, American Cancer Society)
An estimated 60,000 new cases of melanoma will be diagnosed in 2007. Close to 8000 of which will result in death.

(Cancer Facts & Figures 2007, American Cancer Society)
Background

- Our laboratory has a transgenic mouse model (TG-3) with predisposition to melanoma.

- At transgene integration site, about 70kb of host DNA was deleted. The deleted DNA was part of intron 3 of Grm1.

- This disruption of host DNA resulted in ectopic expression of Grm1 in melanocytes.

- We showed that the aberrant expression of Grm1 in melanocytes is sufficient to induce melanoma development in vivo as demonstrated by a new transgenic line constructed with Grm1 cDNA regulated by Dct promoter.
Conclusion: *In vivo*, ectopic expression of Grm1 in melanocytes is sufficient to induce melanocytic neoplasia.
What is Grm1?

- Grm1 is Metabotropic Glutamate Receptor 1. When activated, it modulates the production of second messenger(s) through G-proteins.

- Grm1 (150kD) is a member of the G-protein-coupled-receptor family (GPCR). Grm1 is normally expressed in neuronal cells and involved in neuronal signaling.

- The ligand for Grm1 is glutamate, which is the predominant neurotransmitter in the CNS.

- Grm1 expression has not been detected in normal melanocytes from either murine or human.
GRM1 Expression in Human Melanoma Biopsy Samples

No cDNA control
Benign nevus 1
Benign nevus 2
Marker
Nodal Metastasis 1
Primary tumor
In-Transit Metastasis
Nodal Metastasis 2

Size (Kb)
1353
1078
872

GRM1 (1.1kb)
DCT (0.75kb)
Expression of GRM1 in Human Melanoma Biopsies

![Expression of GRM1 in Human Melanoma Biopsies](image)

**HEM**

**Dysplastic Nevi**

1. 2. 3. 4. 5.

**GRM 1**

**TYRP1**

**Dysplastic Nevi**

6. 7. 8. 9. 10. 11. 12.

**GRM 1**

**TYRP1**
IHC of GRM1 in Human Melanoma Tissue Samples

- Isotype Control
- Negative GRM1 Stain
- Positive GRM1 Stain
- Positive GRM1 Stain
IHC of GRM1 in Normal Human Skin

<table>
<thead>
<tr>
<th>Pathology</th>
<th>IHC Result</th>
<th>Total</th>
<th>Positive Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>23</td>
<td>15</td>
<td>38</td>
</tr>
<tr>
<td>Normal skin</td>
<td>15</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>15</td>
<td>53</td>
</tr>
</tbody>
</table>
GRM1 Expression in Human Melanoma Cell Lines
GRM1 Expression in Human Melanoma Cells

Positive control

Normal human melanocytes

C8161

UACC903

WM115
(Marín and Chen, 2004)
GRM1 Antagonists

- Competitive antagonist binds to the same site as the ligand, glutamate

- Non-competitive antagonist modifies the coupling between the extracellular and the 7TM domain or it may stabilize the inactive state of the 7TM domain
Accumulation of IP3 is induced by GRM1-agonist and suppressed by GRM1-competitive or GRM1-non-competitive antagonist in human melanoma cells

Q = Quisqualate, Group I metabotropic glutamate receptor agonist

Ly = Ly367385, metabotropic glutamate receptor 1 specific competitive antagonist

Bay= Bay36-7620, metabotropic glutamate receptor 1 specific non-competitive antagonist
Activation of ERKs in Human Melanoma Cell Lines by GRM1-Agonist

<table>
<thead>
<tr>
<th>Q</th>
<th>0</th>
<th>2</th>
<th>3</th>
<th>5</th>
<th>10</th>
</tr>
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<tbody>
<tr>
<td>C8161</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
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LY367385 (30min)

<table>
<thead>
<tr>
<th>Q</th>
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<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
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</table>

LY367385 (30min)
Suppression of C8161 human melanoma cell growth by GRM1-competitive antagonist

<table>
<thead>
<tr>
<th></th>
<th>Veh 100µM</th>
<th>500µM</th>
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</thead>
<tbody>
<tr>
<td>NT</td>
<td>100</td>
<td>53</td>
</tr>
<tr>
<td>Veh</td>
<td>80</td>
<td>28</td>
</tr>
<tr>
<td>LY367385</td>
<td>100</td>
<td>80</td>
</tr>
</tbody>
</table>

Day 4

% of no treatment (NT)

![Graph showing suppression of C8161 cell growth](graph.png)
Excess glutamate is released by human melanoma cells

**Glutamate Release**

- C8161
- WM239A
- WM115
- UACC903+
- UACC930-
- HEK293A
- Media Only

**Cell Proliferation**

- C8161
- WM239A
- WM115
- UACC903+
- UACC930-
- HEK293A
- Media Only

* p<0.001  ** p<0.008  *** p<0.139

**[Glu] (uM)**

- Day1: 22.49 ± 3.08
- Day2: 31.23 ± 0.02
- Day3: 35.73 ± 0.27
- Day4: 38.13 ± 6.62
- Day5: 50.34 ± 3.55
- Day6: 119.03 ± 15.54
Suppression of C8161 human melanoma cell growth by GRM1-non-competitive antagonist
C8161 melanoma cells accumulated in sub-G1 phase in the presence of GRM1 non-competitive antagonist, BAY 36-7620

<table>
<thead>
<tr>
<th>Conditions - 24hrs</th>
<th>Sub-G1</th>
<th>G0/G1</th>
<th>S</th>
<th>G2/M</th>
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</thead>
<tbody>
<tr>
<td>No Treatment</td>
<td>2.60</td>
<td>50.36</td>
<td>25.44</td>
<td>21.60</td>
</tr>
<tr>
<td>Vehicle</td>
<td>3.30</td>
<td>48.22</td>
<td>50.42</td>
<td>23.06</td>
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<tr>
<td>BAY 36-7620</td>
<td>5.90</td>
<td>35.32</td>
<td>23.28</td>
<td>20.68</td>
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</table>

<table>
<thead>
<tr>
<th>Conditions - 48hrs</th>
<th>Sub-G1</th>
<th>G0/G1</th>
<th>S</th>
<th>G2/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Treatment</td>
<td>9.74</td>
<td>55.44</td>
<td>17.60</td>
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<tr>
<td>Vehicle</td>
<td>7.62</td>
<td>57.86</td>
<td>17.18</td>
<td>17.34</td>
</tr>
<tr>
<td>BAY 36-7620</td>
<td>18.62</td>
<td>32.64</td>
<td>28.28</td>
<td>20.46</td>
</tr>
</tbody>
</table>

NT   Veh   BAY 36-7620

PARP  Cleaved PARP  Tubulin
Riluzole (Rilutek®)

- Riluzole is an inhibitor of glutamate release and inactivates voltage-dependent sodium channels.
- Riluzole is currently being used to treat patients with amyotrophic lateral sclerosis (ALS), glutamate is believed to be involved in pathogenesis of this disease.
- Group I metabotropic glutamate receptors (Grm1 and Grm5) have been demonstrated to be involved in glutamate release, however data for Grm1 is not so clear.
- Glutamate is the natural ligand of Grm1.
Suppression of C8161 human melanoma cell growth by Riluzole
Apoptotic Responses of Human Melanoma Cells in the Presence of Riluzole

<table>
<thead>
<tr>
<th>Conditions</th>
<th>SubG1</th>
<th>G0/G1</th>
<th>S</th>
<th>G2/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Treatment</td>
<td>2.60</td>
<td>50.36</td>
<td>25.44</td>
<td>21.60</td>
</tr>
<tr>
<td>Vehicle</td>
<td>3.30</td>
<td>48.22</td>
<td>25.42</td>
<td>23.06</td>
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<tr>
<td>Riluzole</td>
<td>2.94</td>
<td>5.58</td>
<td>16.38</td>
<td>75.10</td>
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<table>
<thead>
<tr>
<th>Conditions</th>
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<th>G0/G1</th>
<th>S</th>
<th>G2/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Treatment</td>
<td>9.74</td>
<td>55.44</td>
<td>17.60</td>
<td>17.22</td>
</tr>
<tr>
<td>Vehicle</td>
<td>7.62</td>
<td>57.86</td>
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<td>17.34</td>
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<tr>
<td>Riluzole</td>
<td>22.72</td>
<td>10.32</td>
<td>30.44</td>
<td>36.52</td>
</tr>
</tbody>
</table>

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**C8161**

**WM239A**

Riluzole

24 Hrs

48 Hrs

NT

Veh

PARP

Cleaved PARP

Tubulin
Suppression of human melanoma cell xenografts growth by Riluzole

Oral Treatment

IV Treatment
### Apoptotic responses of Riluzole treated human melanoma cell xenografts

<table>
<thead>
<tr>
<th></th>
<th>M</th>
<th>NT</th>
<th>NT</th>
<th>Veh</th>
<th>Veh</th>
<th>4mM</th>
<th>IV</th>
<th>IV</th>
<th>8mM</th>
<th>IV</th>
<th>IV</th>
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</thead>
</table>

- PARP
- Cleaved PARP
- Tubulin
Induction of apoptotic cells by dominant negative mutants of GRM1 in human melanoma cells
Stable siGRM1-human melanoma clones showed reduced cell growth
The Glutamatergic System in Cancer

- Glutamate release promotes growth of malignant gliomas

- Antagonists of ionotropic glutamate receptors exert a concentration dependent anti-proliferative effect in human thyroid carcinoma, lung carcinoma, astrocytoma, colon adenocarcinoma and breast carcinoma

Summary

- Expression of GRM1 is detected in about 40% of human melanoma cell lines and biopsies.

- ERK is activated by GRM1-agonist (L-Quisqualate) treated human melanoma cell lines. Pretreatment of these cells with a GRM1-antagonist (LY367385) abolished ERK activation.

- Proliferation of human melanoma cells is suppressed by dN mutants of GRM1, GRM1-antagonists, LY367385 or BAY 36-7620, as well as an inhibitor of glutamate release, Riluzole.
Summary

• Treatment of human melanoma cells with Bay36-7620 or Riluzole resulted in the accumulation of treated cells in subG1 phase of the cell cycle

• Xenografts of GRM1 positive human melanoma cells show apoptotic responses to treatment with Riluzole

• GRM1 negative human melanoma cells are unresponsive to stimuli/inhibitors of GRM1
<table>
<thead>
<tr>
<th>Rutgers University</th>
<th>Cancer Institute of New Jersey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lili Chan</td>
<td>James Goydos</td>
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<tr>
<td>Karine Cohen-Solal</td>
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<td>Ryan Gleason</td>
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<tr>
<td>Hwa Jin Lee</td>
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<td>Yari E. Marin</td>
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