Seeing Beyond the Visible: Fluorescent Imaging of Brain Tumors

Jarrod D Predina MD MS, Ryan Zeh BS, Sunil Singhal MD, John YK Lee MD MSCE

Center for Precision Surgery
Department of Surgery, Department of Neurosurgery
University of Pennsylvania School of Medicine
Cancer Surgery in the US

Surgery improves survival 10-fold

Survival

Month

Surgical Oncology 22 (2013) 156–161
Cancer Surgery in the US

Surgery improves survival 10-fold

Surgery

No Surgery

“THE GOOD”

Survival

Month

Surgical Oncology 22 (2013) 156–161

Why is surgery good?

Tumor Removal + Accurate staging
Cancer Surgery in the US

Surgery improves survival 10-fold

Surgical Oncology 22 (2013) 156–161

Why do surgeons have these problems?

Limited tools
Mission Statement:
To improve the cancer care of surgery patients through intraoperative molecular imaging techniques.

13 Surgeons (Thoracic, Neurosurgery, Surg Onc, Breast, ENT, Gyn Onc)
2 Staff PhDs
11 Active Clinical Trials
Over 500 patients enrolled in IMI trials
IMI Example:

72F, smoker, 2.0cm LUL (SUV 8.6)
FR-Targeted NIR contrast agent

NIR Fluorophore

Folate

NIR Fluorophore
Preoperative holding area
Folate
NIR Fluorophore
Lung adenocarcinoma
FR
IMI Example:

White Light
Molecular Imaging for the Neuro-Oncologist
Optical contrast agents

5-ALA (1990s)

Second Window ICG (2011)

EC17 (2014)

OTL0038 (2015-current)
FGS Improves Gross Total Resection and Progression Free Survival
Intraoperative Biopsy in GBM

LIMITATIONS:
- autofluorescence
- poor depth of penetration
How to Improve??

NIR > Visual Range

Better depth of detection
Less autofluorescence
NIR contrast agents

5-ALA (1990s)

Second Window ICG (2011)

OTL0038 (2015-current)

EC17 (2014)
Approach #1: Non-Targeted NIR contrast agent
NIR Agent 1:
Second Window ICG
Indocyanine green
Tumor microenvironment

- Extensive production of vascular permeability enhancing substances
- Differences in capillary fluid transport
Infusion Center--
1 day prior to surgery

5mg/kg!!
Intraoperative Imaging

5 mg/kg ICG one day prior to surgery
In Vivo Support:
ICG in Preclinical Model of GBM:
Methods

- U251-\textit{Luc}-GFP cell line
- Stereotactic intracranial implantation
- NIR Imaging with IVIS Spectrum
Orthotopic Model of GBM: U251-\textit{Luc}-GFP cell line
From Mice to Men
Trial Design:

18 Subjects
(1st analysis)

Preop MRI

ICG (5.0mg/kg)
Day before surgery

Review by Pathology

Intraoperative imaging

Anterior Margin

Tumor
Intraoperative Near-Infrared Optical Imaging Can Localize Gadolinium-Enhancing Gliomas During Surgery

BACKGROUND: Although real-time localization of gliomas has improved with intraoperative image guidance systems, these tools are limited by brain shift, surgical cavity deformation, and expense.

OBJECTIVE: To propose a novel method to perform near-infrared (NIR) imaging during glioma resections based on preclinical and clinical investigations, in order to localize tumors and to potentially identify residual disease.
Patient Example 1:
Example of what you see in OR:

SBR = 6.3
Dura Opened

SBR = 7.3
Removing Tumor

SBR = 9.5
Higher SBR than with previous visual agents
SBR proportional Gadolinium-enhancement
Margin Detection

- **Visible Light Only**
  - Sensitivity = 84%
  - Specificity = 80%
  - PPV = 91%
  - NPV = 66%

- **With NIR Imaging**
  - Sensitivity = 98%
  - Specificity = 45%
  - PPV = 82%
  - NPV = 90%

<table>
<thead>
<tr>
<th>A. Enhancing TUMORS</th>
<th>Bright Light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor pathology</td>
<td>Yes  No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Yes</td>
<td>43</td>
</tr>
<tr>
<td>No</td>
<td>4</td>
</tr>
<tr>
<td>Total N</td>
<td>71</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Enhancing TUMORS</th>
<th>Near Infrared Camera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor pathology</td>
<td>Yes  No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Yes</td>
<td>50</td>
</tr>
<tr>
<td>No</td>
<td>11</td>
</tr>
<tr>
<td>Total N</td>
<td>71</td>
</tr>
</tbody>
</table>
Summary

• Strong SBR in 100% of patients with high grade glioma

• Strong correlation between MRI-gadolinium enhancement and SBR

• Helpful for detection of residual disease at margins

• Phase II trial in the works
Approach #2: 
Targeted NIR contrast agent
2. Targeted NIR contrast agent

Folate

NIR Fluorophore

NIR Fluorophore

Folate
High FR expression in Pituitary Neoplasms
Folate receptor overexpression can be visualized in real time during pituitary adenoma endoscopic transsphenoidal surgery with near-infrared imaging

John Y. K. Lee, MD, MSCE, Steve S. Cho, BS, Ryan Zeh, BS, John T. Pierce, MS, Maria Martinez-Lage, MD, Nithin D. Adappa, MD, James N. Palmer, MD, Jason Newman, MD, Kim O. Learned, MD, Caitlin White, MD, Julia Kharlip, MD, Peter Snyder, MD, Philip Low, PhD, Sunil Singhal, MD, and M. Sean Grady, MD
Trial Design:

15 Subjects (First analysis)

PreOp MRI

OTL (0.025mg/kg) 4 hours prior to surgery

Review by Pathology

Outcomes Analysis

IMI with OTL0038
Dura Opened
Human Experience with OTL38 for pituitaries
FRα Immunohistochemistry

- Kidney – positive control
- Pituitary Adenoma
- Normal Pituitary Gland
Study Results
Folate Receptor Expression vs SBR

Folate Receptor H-score vs Signal to Background Ratio

High FRα = 3.0 ± 0.29 (n=3)

Low FRα = 1.6 ± 0.43 (n=11)
Test Characteristics for Margin Specimen

- For 6 patients with NF adenoma
  - 100% Sensitivity
  - 100% Specificity
Endoscope Distance to Target

Signal to Background Ratio versus Endoscope Distance

- Low FRalpha
- High FRalpha

Graphs by fra
Summary

- Folate receptor overexpression can be visualized with fluorescence in the operating room
- Low N, but perfect sensitivity and specificity for NF adenomas
Conclusion

• NIR Fluorescence Guided Surgery is Feasible

• Has advantages over traditional Visual Contrast Agents (5-ALA, Fluorescein)

• Evaluating in Phase II Trials
Acknowledgements:

Fluorescence Guide Surgery Research Lab:
Sunil Singhal and John Lee (Director)

Singhal Imaging Lab
Sunil Singhal, MD
Jarrod Predina, MD
Andrew Newton, MD
Leilei Xia, MBBS
Ashley Dunbar, BA
Courtney Connolly, BA
Mike Baldassari, BA
Jack Mizelle, BS

John YK Lee Tumor Visualization Lab
John YK Lee, MD
Ryan Zeh, BS
John Pierce, MS
Ryan Salinas, MD
Steve Cho, BS
Shayoni Nag, BS

Center for Precision Surgery:
Singhal Singhal (Director)

Special Acknowledgments:
Jay Dorsey Lab, Penn
Philip Low, PhD, Purdue University
ICG: For Vascular Imaging
Pituitary Neoplasms

Folate

NIR Fluorophore

FR
# Histopathology

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>FRα over-expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonfunctioning</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Cushing’s</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Somatotroph</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Cortico-somatotroph</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>