Molecular Imaging for Cancer Visualization

American College of Surgeons
Surgical Simulation Summit
Chicago, IL
March 16-17, 2018

Michael Bouvet, MD
Professor of Surgery
University of California San Diego
San Diego VA Medical Center
Molecular Imaging for Cancer Visualization - Outline

• Preclinical mouse models
• Options for molecular imaging of tumors
• Current technology for fluorescence guided surgery that is available in the clinic
• Future directions
Orthotopic injection of pancreatic cells
Whole body Imaging

46 days

50 days

57 days

64 days
Fluorescence-guided surgery of human colon cancer increases complete resection resulting in cures in an orthotopic nude mouse model

Cristina A. Metildi, MD, a Sharmeela Kaushal, PhD, a Cynthia S. Snyder, MD, a Robert M. Hoffman, PhD, a,b and Michael Bouvet, MD a,*

a Department of Surgery, University of California San Diego, San Diego, California
b AntiCancer, Inc., San Diego, California
Imaging of Primary and Metastatic Pancreatic Cancer Using a Fluorophore-Conjugated Anti-CA19-9 Antibody for Surgical Navigation

Michele McElroy · Sharmeela Kaushal · George A. Luiken · Mark A. Talamini · A. R. Moossa · Robert M. Hoffman · Michael Bouvet

Peritoneal carcinomatosis imaged after I.V. delivery of conjugated CA19-9

CA19-9 treated animals

Brightfield

Fluorescence

metastases

2 mm

metastases

2 mm
Fluorophore-conjugated anti-CEA antibodies for rapid staged pancreatic cancer using fluorescence laparoscopy

Cristina A. Metildi, Sharmeeha Khanh, Huy Tran Cao, Chasee Hartzman, Cynthia S. Snyder, George A. Lusch
Department of Surgery, University of California San Diego, San Diego, CA, United States

Goal: To use fluorophore-conjugated anti-CEA antibody to improve detection of primary and metastatic deposits in an orthotopic mouse model

Background
- Aggressive surgical resection provides an additional survival benefit in the presence of metastatic pancreatic cancer
- Standard staging laparoscopy has a false-negative rate as high as 20%
- The use of fluorescent protein conjugated with anti-CEA antibody to label tumor has improved identification of primary and metastatic lesions

Materials & Methods
- Stryker Laparoscopic Setup
- Fluorescence laparoscopy was performed with Stryker equipment that permitted simultaneous detection of different fluorescent proteins while maintaining background visualization
- A Stryker Lumen-View light source was used with adjustments to the red, blue and green wavelengths
- Images were acquired with a digital camera

Results
- Images were acquired with a digital camera
- Images were acquired with a digital camera

Conclusion: Fluorescence laparoscopy with and LED light source and fluorophore-labeled anti-CEA permits rapid detection and staging of lesions of CEAs-expressing PC

Cristina Metildi, MD
New Technique Could Improve Pancreatic Cancer Staging, Treatment

Laparoscopy Using Fluorescent Markers and LED Light

BY CHRISTINA FRANGOU

A new laparoscopic technique that uses fluorescent antibody markers and an LED light source has the potential to improve pancreatic staging and treatment, according to a report presented at the 2011 Clinical Congress of the American College of Surgeons.

Researchers took two antibodies commonly expressed by pancreatic cancer and tagged them with a fluorescent marker, making the cancer cells light up in bright green or red. The researchers then administered the fluorescent antibodies into mice and studied the mice under LED light and during traditional laparoscopy.

Analysis showed that the LED light vividly identified the primary and metastatic tumors, with a sensitivity rate of 96% compared with 40% for traditional laparoscopy. Fluorescent laparoscopy rendered fewer false-positives than traditional laparoscopy and was sensitive enough to illuminate metastatic lesions smaller than 1 mm, which are not visible with a standard laparoscope.

The combination of fluorescent markers and LED light potentially could sharpen how surgeons detect and treat pancreatic cancer in human patients, said investigators Michael Bouvet, MD, and Robert M. Hoffman, PhD, both professors of surgery at the University of California, San Diego (UCSD).

The LED light improves visualization of both the tumor and the surrounding anatomy in the abdominal cavity of the mice. “You can see both the normal background of the anatomy plus the fluorescent tumor signal at the same time,” said Dr. Bouvet, in a press release.

The technology also could be used to target tumor cells for treatment with drugs, but the work is still in early stages. The surgeons plan to join forces with industry to secure FDA approval for clinical trials of the fluorescent antibodies in humans.

The research was funded by a five-year grant to UCSD and AntiCancer, Inc. from the National Cancer Institute. The UCSD team worked with AntiCancer Inc. on the mouse model and with laparoscopic technicians at Stryker Corporation.

The LED light improves visualization of both the tumor and surrounding anatomy in the abdominal cavity.
Comparison of a chimeric anti-carcinoembryonic antigen antibody conjugated with visible or near-infrared fluorescent dyes for imaging pancreatic cancer in orthotopic nude mouse models

Ali A. Maawy
Yukihiro Hiroshima
Sharmeele Kaushal
George A. Luiken
Robert M. Hoffman
Michael Bouvet
## Depth of penetration

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MUC1 Selectively Targets Human Pancreatic Cancer in Orthotopic Nude Mouse Models

Jeong Youp Park¹²,³, Yukihiko Hiroshima¹²,³, Jin Young Lee³, Ali A. Maawy¹, Robert M. Hoffman¹², Michael Bouvet¹⁵

¹ Department of Surgery, University of California San Diego, San Diego, California, United States of America, ² AntiCancer, Inc., San Diego, California, United States of America, ³ Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea, ⁴ Department of Surgery, Yokohama City University Graduate School of Medicine, Yokohama City, Japan, ⁵ Surgical Service, VA San Diego Healthcare System, San Diego, California, United States of America

Fig 1. Characterization of pancreatic cancer cell lines. (A) Western blot analysis shows MUC1 expression in pancreatic cancer cell lines (BxPC-3 and Panc-1). (B) Flow cytometric analysis shows the expression of MUC1 on the surface of BxPC-3 and Panc-1 cell lines. (C) Immunoperoxidase and fluorescence on live cells shows multiple fluorescent dots on the surface of Panc-1 cells. Representative fluorescence images merged with corresponding DIC (differential interference contrast) images (40x water immersion objective on Fluorplex, using the 559 nm laser).

Fig 4. Imaging of MUC1 targeting of orthotopically-transplanted Panc-1 and BxPC-3 pancreatic tumor in vivo. Fluorescence signals from pancreatic tumors orthotopically transplanted at the tail of the pancreas were detected. Other than the tumor, fluorescence signal was detected from the skin and, bladder and intestinal contents but at lower intensity than the tumor. White arrows indicate pancreatic tumor.
Humanization of Anti-CEA Antibody (M5A)

CDR grafting onto Herceptin framework

Dr Paul Yazaki
City of Hope,
Beckman Research Institute

Yazaki et al. PEDS 2004
55 yo M T3N1 rectal cancer with small lung mets and circulating CEA levels of 142. FDG-PET uptake in large primary tumor and peri-rectal nodes.

FOLFOX x 8 with reduction of primary, no change in lung mets (felt benign), and CEA normal. Neoadjuvant chemo-radiotherapy: 54 Gy + capecitabine.

$^{64}$Cu-M5A scan at 7 weeks post RT – primary and nodal uptake at 24 and 48 hours
Surgical resection of primary planned
Tumor specific antibody labeling of pancreatic cancer using a fluorescent humanized anti-CEA antibody

Thinzar M. Lwin, MD, MS. 2; Takashi Murakami, MD1, 2, 3; Paul J. Yazaki, PhD4; Bryan Clary, MD1, Robert M. Hoffman, Ph.D. 2, Michael Bouvet, MD1, 2

Department of Surgery, University of California San Diego, San Diego, CA; AntiCancer, Inc., San Diego, CA; Department of GI Surgery, Yokohama City University, Graduate School of Medicine, Yokohama, Japan; City of Hope National Medical Center, Duarte, CA

Background
Achieving negative tumor margins is critical in oncologic surgeries, especially during surgery for GI malignancies. Currently, surgeons rely on visual and tactile cues to distinguish tumors from surrounding tissue, but this is inadequate. Positive resection margins (R1-2) are found in up to 86% of pancreatic cancer patients after curative intent surgery. Positive margins lead to early recurrence and decreased overall survival. Techniques to visualize resection margins are necessary to increase the rate of complete (R0) surgical resections and improve survival. Navigation with fluorescence guidance using tumor specific probes has emerged as a promising strategy to improve the efficacy of oncologic surgery. In the present study, we show that the use of a humanized anti-CEA antibody conjugated to an 800nm NIR fluorescent dye can selectively label pancreatic cancer in both pancreatic cancer cell line and patient derived xenograft mouse models (PDOX).

Materials and Methods

Results

Conclusions

Humanized anti-CEA-800 tumor-specific dye specifically labeled orthotopically implanted pancreatic cancer xenografts from cell line and patient derived tissue. Tumor specific fluorescence imaging clearly identifies the primary tumor and is especially useful in detection of satellite lesions. The longer wavelength allowed for deeper tissue penetration, particularly in areas of the tumor covered by normal pancreatic parenchyma. Humanized anti-CEA antibody conjugated to a radio-labeling agent is already in Phase I/II trials. Humanized anti-CEA conjugated with the IR-800 dye is a promising agent for future clinical FGS applications.

References

Spectral Overlap: ICG & IRDye800

Da Vinci Si HD Surgical System
Stryker AIM
Fluorescence Imaging in the OR

PerkinElmer Solaris
Novadaq SPY-Elite
Quest Spectrum
Curadel ResVet LAB-Flare
VisionSense Iridium
SurgVision prototype

Intuitive Firefly
Novadaq Pinpoint

Stryker Aim
Integration with Surgical Technology

Imaging with the Firefly Camera, Intuitive Da Vinci Robot System


Author information

Abstract

Navigation with fluorescence guidance has emerged in the last decade as a promising strategy to improve the efficacy of oncologic surgery. To achieve routine clinical use, the onus is on the surgical community to objectively assess the value of this technique. This assessment may facilitate both Food and Drug Administration approval of new optical imaging agents and reimbursement for the imaging procedures. It is critical to characterize fluorescence-guided procedural benefits over existing practices and to elucidate both the costs and the safety risks. This report is the result of a meeting of the International Society of Image Guided Surgery (www.isigs.org) on February 6, 2015, in Miami, Florida, and reflects a consensus of the participants' opinions. Our objective was to critically evaluate the imaging platform technology and optical imaging agents and to make recommendations for successful clinical trial development of this highly promising approach in oncologic surgery.

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Cetuximab-IRDye800 for multimodality optical imaging: Potential for guiding surgical therapy and pathological analysis

Eben Rosenthal, MD
SGM-101: An innovative near-infrared dye-antibody conjugate that targets CEA for fluorescence-guided surgery

Marian Gutowski a,1, Bérénice Framery b,1, Martin C. Boonstra c, Véronique Garambois a,d,e,f,François Quenet a, Karen Dumas b, François Scherninski g, Françoise Cailler b, Alexander L. Vahrmeijer c, André Pèlegrin a,d,e,f,*

a Institut régional du Cancer de Montpellier, ICM, Montpellier, F-34298, France
b SurgiMAb, 10 Parc Club du Millénaire, 1025 Avenue Henri Becquerel, 34000, Montpellier, France
c Department of Surgery, Leiden University Medical Center, Leiden, The Netherlands
d IRCM, Institut de Recherche en Cancérologie de Montpellier, Montpellier, F-34000
*e INSERM, U1194, Montpellier, F-34298, France
f Université de Montpellier, Montpellier, F-34298, France
g Laboratoires Synth-Innove, 2bis rue Dupont de l'Eure, 75020, Paris, France
**CLINICAL DATA**

SGM-101 allows a clear visualization of tumors within a healthy environment, with both a high specificity and sensitivity.

**PANCREAS CANCER**

**PRIMARY TUMOR**

- Proposed resection line based on visual inspection/palpation
- Proposed resection line based on fluorescence
- Tumor Bulk

**RECTUM CANCER**

**METASTATIC COLORECTAL CANCER**

Following very good results in both pancreas and rectal cancer, clinical investigators requested authorization to use SGM-101 to detect metastatic colorectal cancer. Metastasis were visible even at the lowest tested dose of 5mg/patient.
**Table 1. Techniques for in vivo labeling of cancer cells with fluorescence**

<table>
<thead>
<tr>
<th>Technique</th>
<th>Tumor type</th>
<th>Fluorescence type</th>
<th>References</th>
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<tbody>
<tr>
<td>Fluorophore-conjugated antibodies</td>
<td>pancreas, colon</td>
<td>Alexa 488 or 550</td>
<td>[4, 5, 19, 20]</td>
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<td>Activatable cell penetrating peptides</td>
<td>melanoma, sarcoma</td>
<td>Cy5</td>
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<td>Telomerase-dependent adenovirus</td>
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<td>GFP</td>
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<td>GFP</td>
<td>[9, 10, 21]</td>
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<td>gGlu-HMRG</td>
<td>ovarian</td>
<td>rhodamine green</td>
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<td>malignant glioma</td>
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gGlu-HMRG = γ-Glutamyl hydroxymethyl rhodamine green; ICG = indoyanine green; MB = methylene blue; GFP = green fluorescent protein. ¹ These techniques are currently being used in the clinical setting.
Ratiometric Activatable Cell-Penetrating Peptides Label Pancreatic Cancer, Enabling Fluorescence-Guided Surgery, Which Reduces Metastases and Recurrence in Orthotopic Mouse Models

Cristina A. Metildi, MD¹, Csilla N. Felsen, BS², Elamprakash N. Savariar, PhD², Quyen T. Nguyen, MD, PhD¹, Sharmeela Kaushal, PhD³, Robert M. Hoffman, PhD¹,²,³,⁴, Roger Y. Tsien, PhD²,³,⁵, and Michael Bouvet, MD¹,³
Folate Receptor Targeting

Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor-α targeting: first in-human results

Gooitzen M van Dam¹, George Themelis², Lucia M A Crane¹, Niels J Harlaar¹,², Rick G Pleijhuis¹, Wendy Kelder¹, Athanasios Sarantopoulos², Johannes S de Jong¹, Henriette J G Arts³, Ate G J van der Zee³, Joost Bart⁴, Philip S Low⁵ & Vasilis Ntziachristos²
OTL38 and lung cancer

Preoperative CT scan (Right Upper Lobe Nodule)

Sunil Singhal, MD
Indocyanine Green
ICG
Parathyroidectomy with ICG

- 17 yo female
- Calcium 11.9
- PTH 113
- Kidney stones
- Bone pain
- Sestamibi scan and ultrasound were negative
Parathyroidectomy with ICG

- 17 yo female
- Calcium 11.9
- PTH 113
- Kidney stones
- Bone pain
- Sestamibi scan and ultrasound were negative
Parathyroidectomy with ICG
Endocrine
Presented at the Academic Surgical Congress 2017

Indocyanine green fluorescence-guided parathyroidectomy for primary hyperparathyroidism

Jonathan C. DeLong, MD, Erin P. Ward, MD, Thinzar M. Lwin, MD, Kevin T. Brumund, MD, Kaitlyn J. Kelly, MD, Santiago Horgan, MD, and Michael Bouvet, MD *

Department of Surgery, University of California San Diego, San Diego, CA

ABSTRACT

Background. Our aim was to evaluate the ease and utility of using indocyanine green fluorescence angiography for intraoperative localization of the parathyroid glands.

Methods. Indocyanine green fluorescence angiography was performed during 60 parathyroidectomies for primary hyperparathyroidism during a 22-month period. Indocyanine green was administered intravenously to guide operative navigation using a commercially available fluorescence imaging system. Video files were graded by 3 independent surgeons for strength of enhancement using an adapted numeric scoring system.

Results. There were 46 (77%) female patients and 14 (23%) male patients whose ages ranged from 17 to 87 (average 60) years old. Of the 60 patients, 43 (71.6%) showed strong enhancement, 13 (21.7%) demonstrated mild to moderate vascular enhancement, and 4 (6.7%) exhibited little or no vascular enhancement. Of the 54 patients who had a preoperative sestamibi scan, a parathyroid adenoma was identified in 36, while 18 failed to localize. Of the 18 patients who failed to localize, all 18 patients (100%) had an adenoma that fluoresced on indocyanine green imaging. The operations were performed safely with minimal blood loss and short operative times.

Conclusion. Indocyanine green angiography has the potential to assist surgeons in identifying parathyroid glands rapidly with minimal risk.

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Laparoscopic Adrenalectomy with ICG

- 27 year old female with Cushing's syndrome
- 5 cm mass in left adrenal gland
Laparoscopic Left Adrenalectomy with ICG
Photoimmunotherapy lowers recurrence after pancreatic cancer surgery in orthotopic nude mouse models


Ali A. Maawy, MD, Yukihiro Hiroshima, MD, PhD, Yong Zhang, MD, Miguel Garcia-Guzman, PhD, George A. Luiken, MD, Hisataka Kobayashi, MD, Robert M. Hoffman, PhD, Michael Bouvet, MD
Wave length: 690 nm  
Intensity: 150 mW/cm²  
Duration: 30 min
Figure 3

BLS-only group

After surgery Brightfield
After surgery GFP
1 wk
2 wks
3 wks
4 wks
6 wks

BLS + PIT group
Targeted Photoimmunotherapy Approach for Cancer Moves Forward

April 25, 2016, by NCI Staff
Conclusions

• Curative surgery is dependent on removing all primary and metastatic cancer cells

• Techniques in fluorescence guided surgery (FGS) are emerging that selectively illuminate cancer cells

• FGS enhances tumor detection, surgical navigation, margin confirmation, and in some cases can be combined with therapeutic techniques to eliminate microscopic disease
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