Molecular Diapeutics: Phospholipid Ether Analogs for Broad Spectrum Cancer and Cancer Stem Cell Detection and Treatment

Jamey Weichert, PhD
U of Wisconsin Radiology, Medical Physics, and Pharmaceutics
Novelos, Inc, Madison, WI

Imaging in 2020
Jackson Hole, WY
Oct 3, 2012

Novelos
find. treat. follow.

University of Wisconsin
Paul P. Carbone
Comprehensive Cancer Center

Disclosure Statement

Coinventor of CLR1404 technology and technical founder/CSO/Director of Novelos, Inc (Madison, WI) which owns all rights to CLR1404 and related technologies.

NM404=CLR1404

Novelos
find. treat. follow.

Improving Outcomes in Cancer Therapy

- **Diagnosis**
  - Sensitive, early detection and localization of primary tumors and metastases for disease staging and treatment planning
- **Therapy**
  - Tumor kill and metastasis blockade while addressing phenotypic heterogeneity and cancer stem cells, all with low toxicity/side effects

Ether Cleavage Enzyme Activity in Normal Liver and Neoplastic Tissues

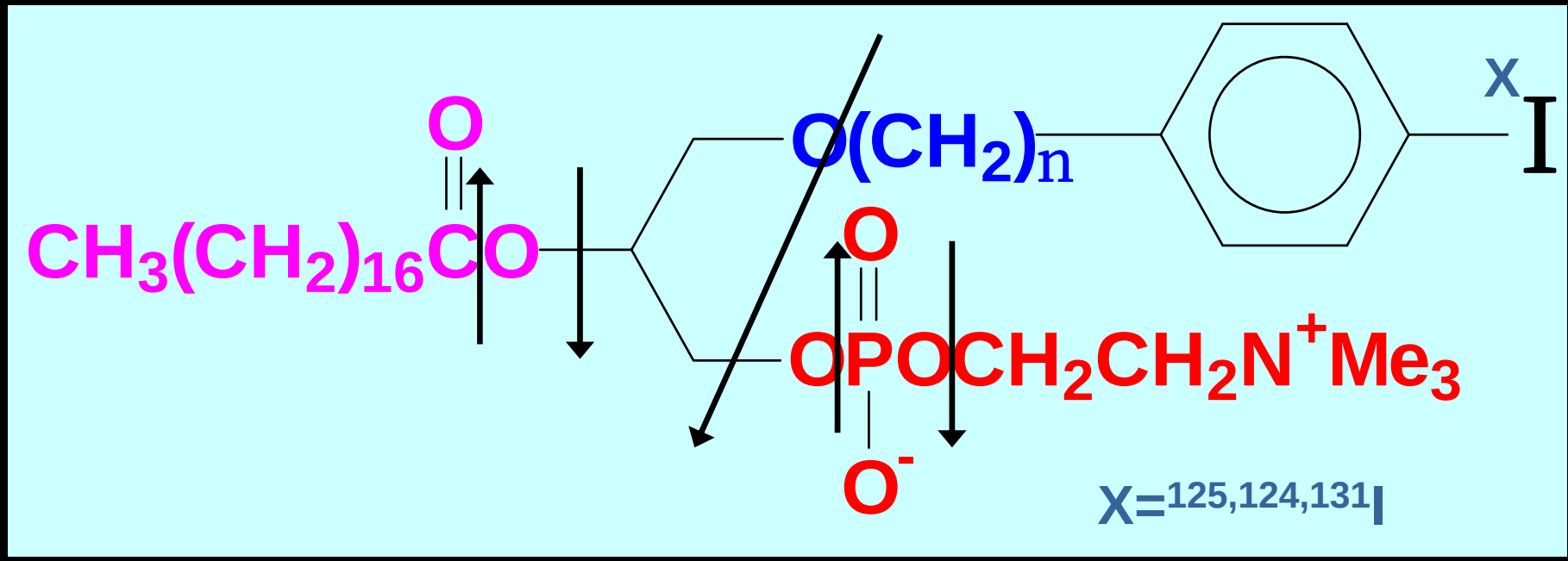
Tissues	Host Animals	Activity[a]
Rat Liver	Buffalo[b]	7.3
Morris Hepatoma 7794A	Buffalo[b]	5.8
Morris Hepatoma 7777	Buffalo[b]	1.4
Sarcoma 180	HA/ICR[c]	0.42
Melanoma B-16	C57BL/6[c]	0.31
Ehrlich Ascites Carcinoma	HA/ICR[c]	0.14
KHZ Mammary Tumor	C3H[c]	0.11
Walker-256	Carsworth Farms Nelson[b]	0.10

[a] Expressed as mμmol of ether cleaved/20 min/mg protein
[b] Rat strains
[c] Mouse strains

Ref. Soodsma, Piantadosi, and Snyder
Cancer Research, 30:309-311 (1970)

↓Ether cleavage enzyme→↑ cellular PLE's

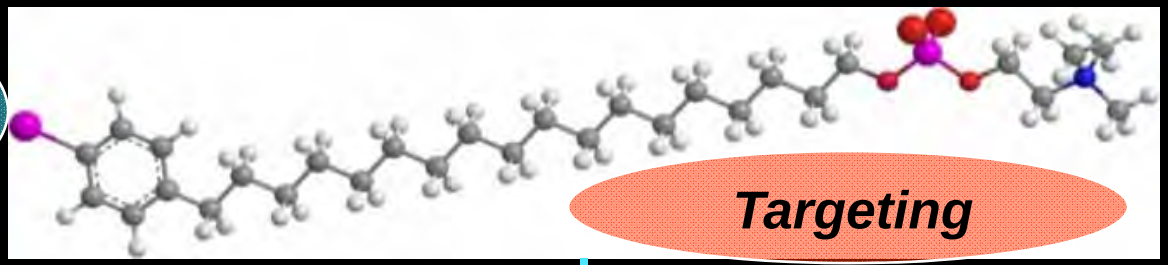
PLE Structure Activity Relationships



>30 analogs synthesized, radiolabeled, and evaluated

Synthesis and Structure Activity Relationship Effects on the Tumor Avidity of Radioiodinated Phospholipid Ether Analogues. **J Med Chem (2006) 49:2155-2165**

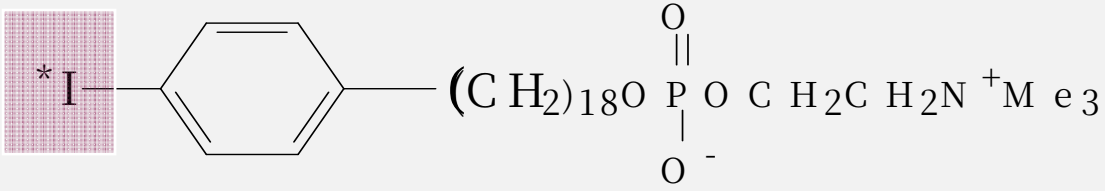
Diapeutic Phospholipid Ether Analogs



^{131}I-CLR1404 Molecular Radiotherapy	^{124}I-CLR1404 PET Imaging	CLR1401 Cytotoxic Chemotherapy	CLR1501 Optical 500 nm	CLR1502 Optical 800 nm (Near IR)
Phase 1b MTD	Phase 1-2	Preclinical stage	Preclinical stage	Preclinical stage

Complete safety pharmacology & toxicology package

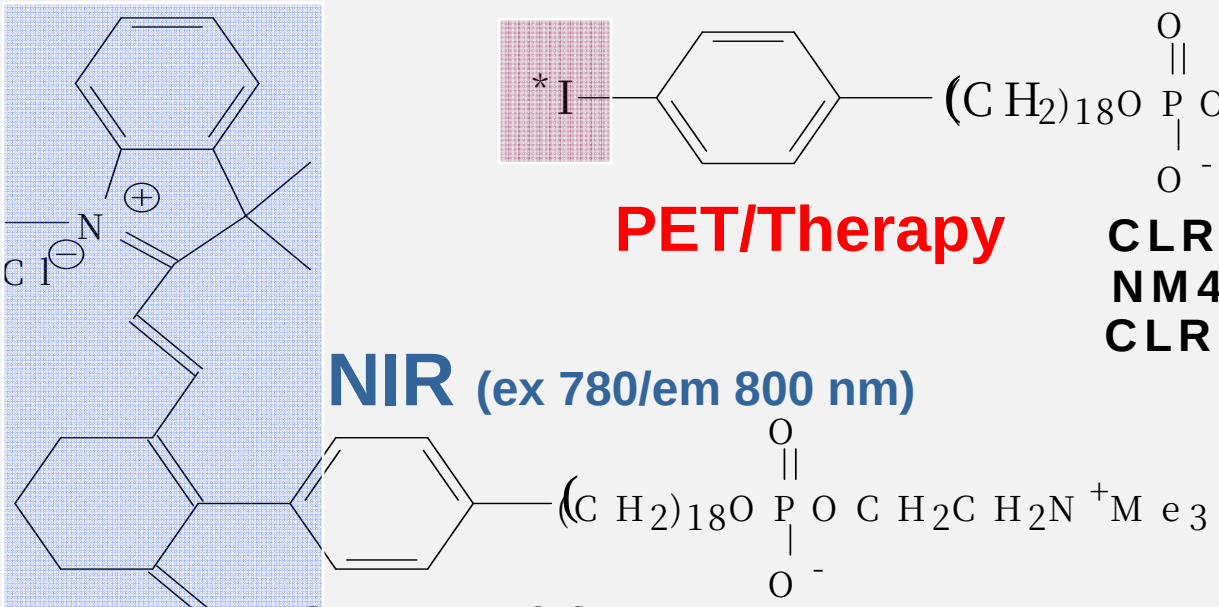
PLE Tumor Imaging Agents



PET/Therapy

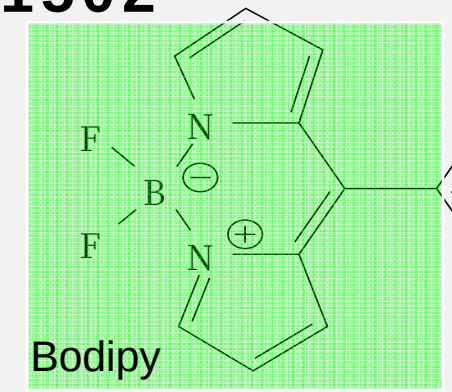
CLR1401 *= I-127 Chemo
NM404 *= I-124 PET
CLR1404 *=I-131 Rad Therapy

NIR (ex 780/em 800 nm)



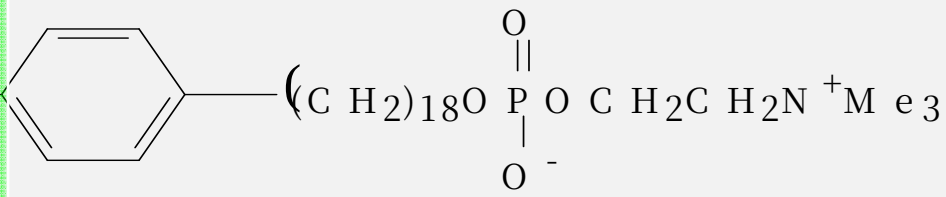
CLR1502

Indocyanine



Bodipy

Fluorescent



CLR1501 (ex 495/em 515 nm)

Alkylphosphocholine Class

Diapeutic CLR1404 Radiopharmaceutical

- Small molecule simplicity and advantages
  - Stabile aromatic iodine resists *in vivo* deiodination
  - Can be radiolabeled with any iodine isotope
    - Iodine-124 (PET isotope with 4.2 day half-life)
    - Iodine-131 (SPECT and therapy isotope with 8 day half-life)
    - Iodine-125 (low E gamma/therapy isotope with 60 day half-life)
- CLR1404 is taken up and **selectively retained** by **52/54** xenograft, orthotopic, and transgenic solid tumor models examined to date.

  adenoma vs hyperplasia vs malignancy

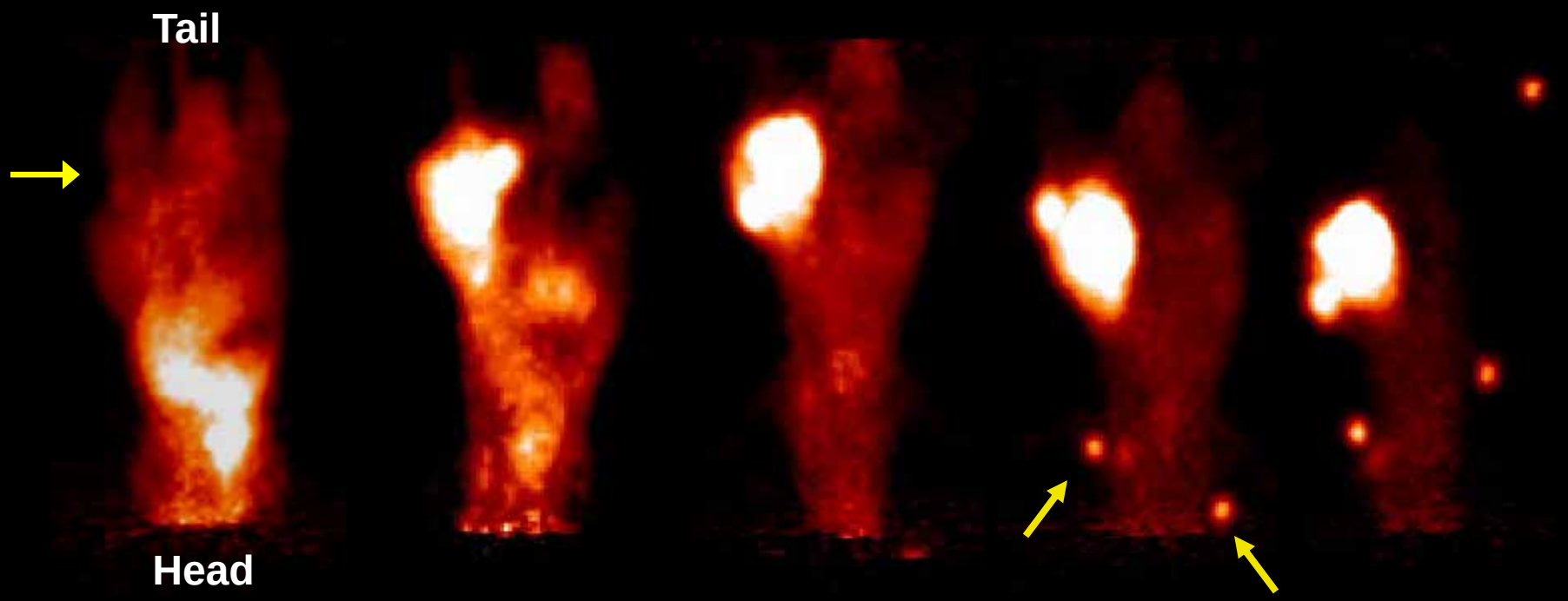
  No No Yes
- Tumor uptake is independent of anatomic location with little or no tumor clearance
- Avoids inflammatory lesions
- GLP safety/pharm/ tox study (>20 total studies) results in rodents and non-human primates indicate an exceedingly high safety index even at >800 times the anticipated human mass dose.

Tumor Imaging with ^{124}I-CLR1404 PET

University of Wisconsin
Paul P. Carbone
Comprehensive Cancer Center

CLR1404 Tumor Time Course

^{124}I-CLR1404 in PC3 SCID Mouse



µPET scans: Head down/tail up with flank tumor
Fiducial markers (arrows)

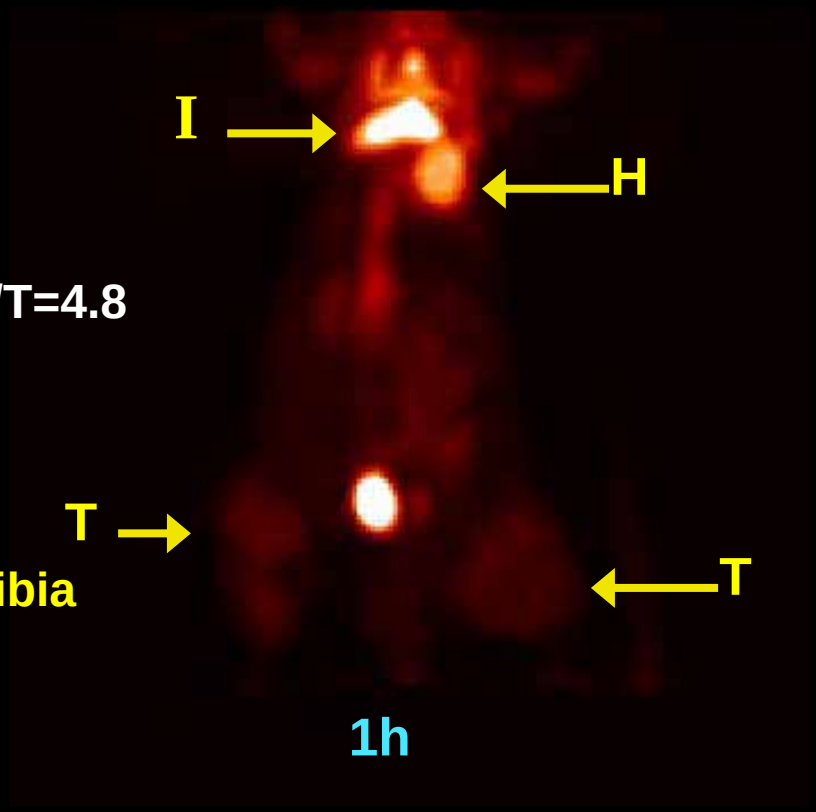
Tumor uptake evident in about 9h

Inflammation: CLR1404 vs FDG PET

PC3 Prostate CA

^{18}F-FDG

^{124}I-CLR1404



1h

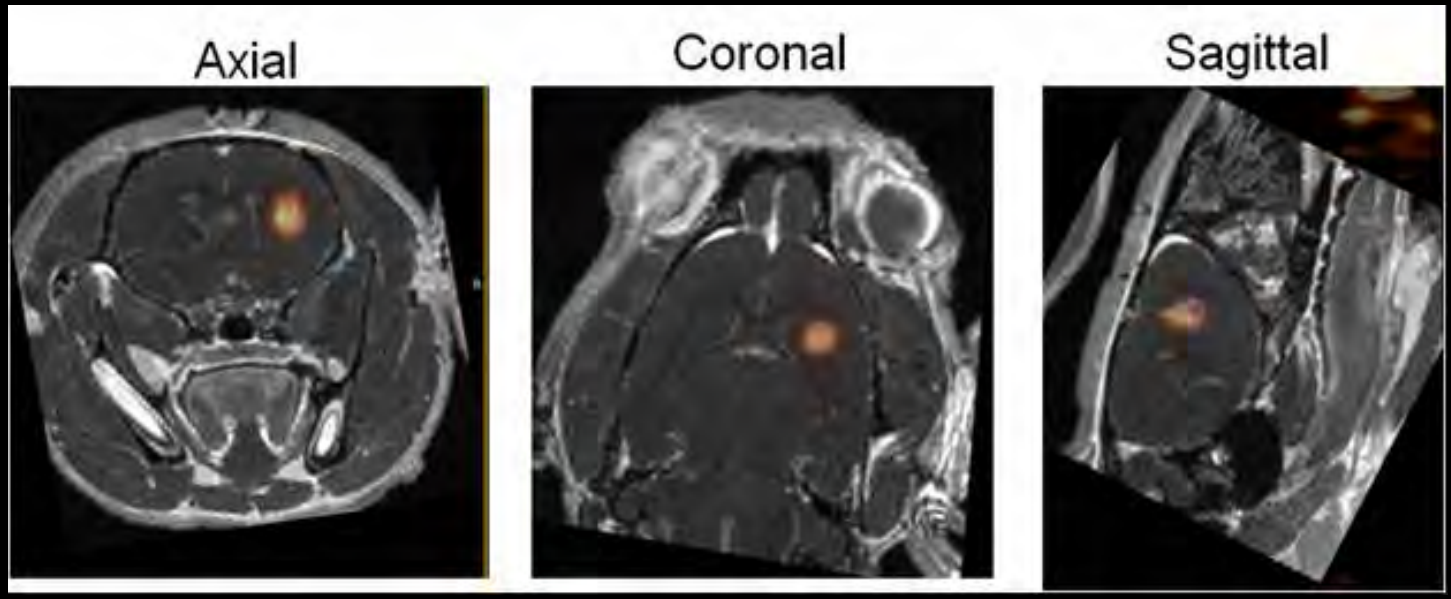
24h

3D cine projection (I=carrageenan induced inflammatory lesion, H=heart, T=human PC3-prostate tumors, SCID mouse)

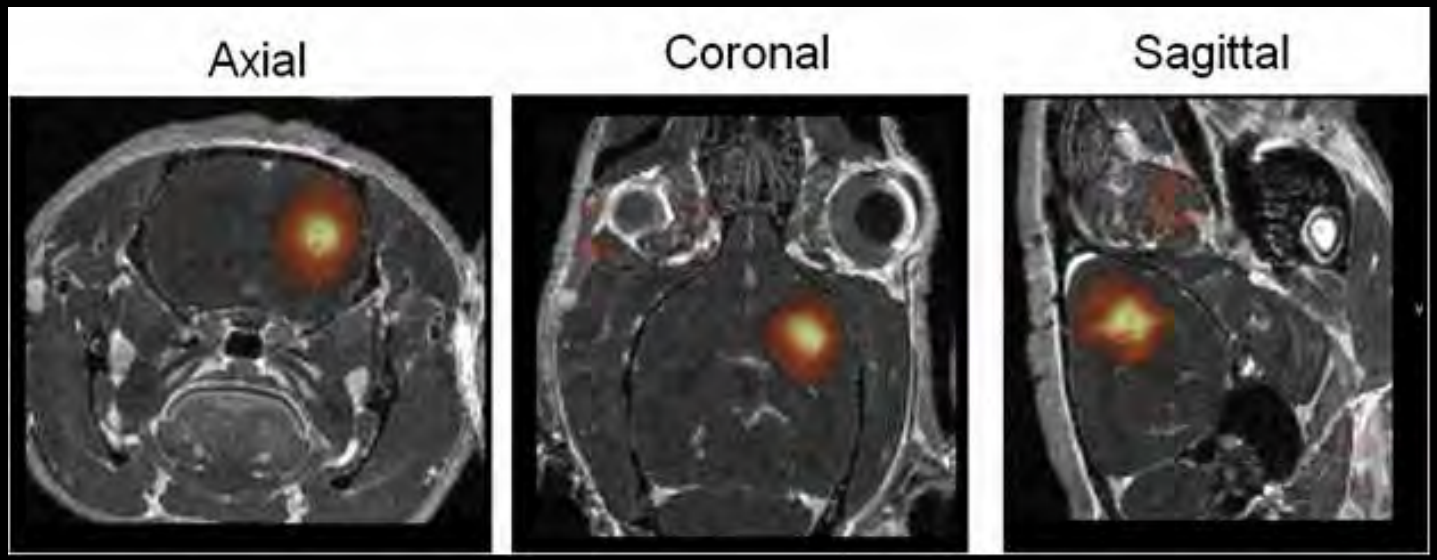
CLR1404 PET/MRI U87 MG-nuRat

48h

2 mm tumor



72h



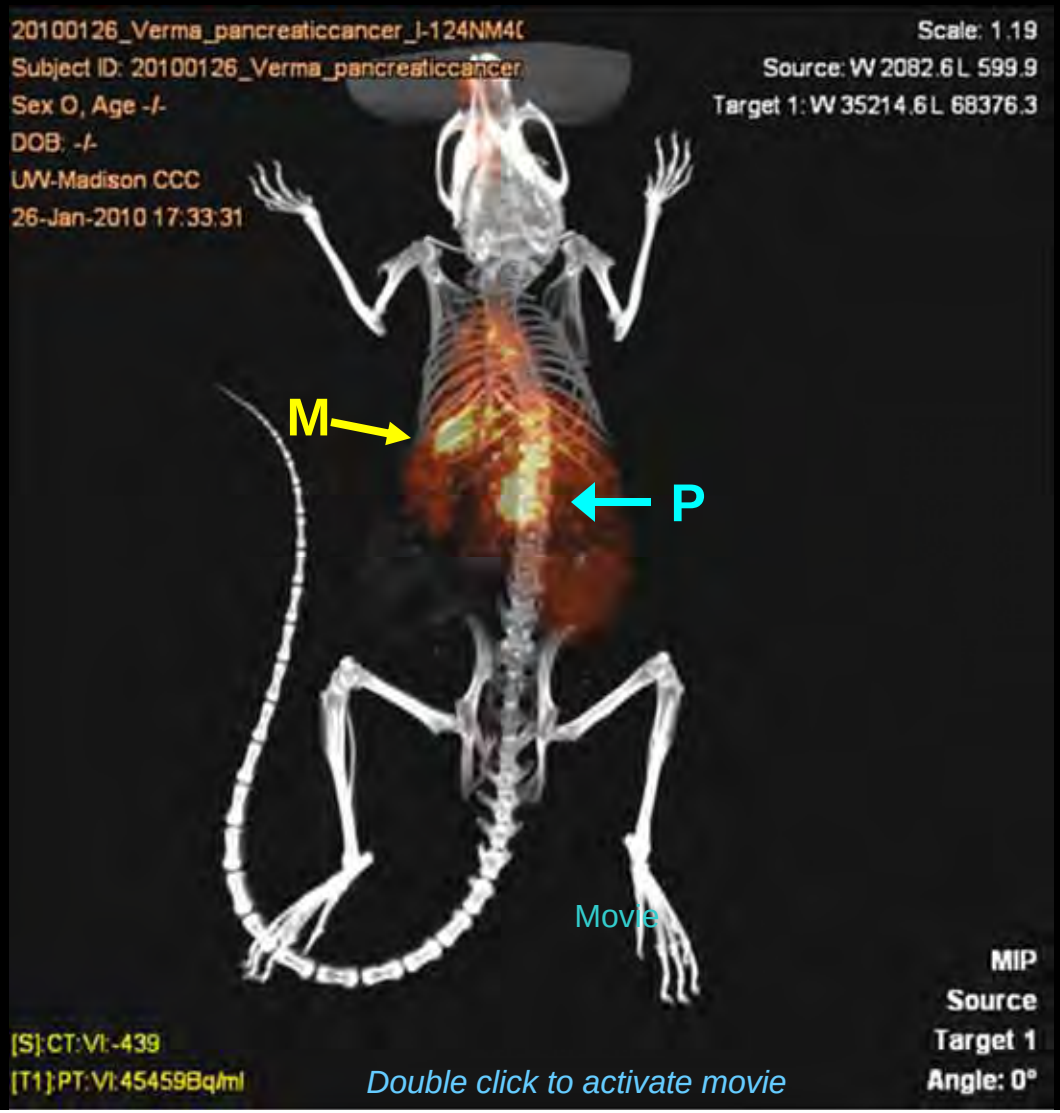
4.7T MRI

Primary and Metastatic Pancreatic Cancer Images

First Demonstration of Spontaneous Liver Metastasis Imaging

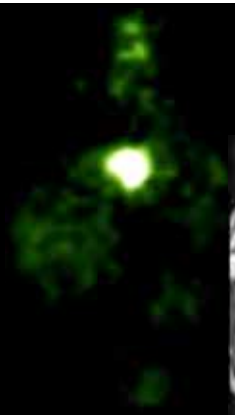
3D Hybrid microPET/CT image of an anesthetized orthotopic BxPC3 pancreatic tumor-bearing Nude mouse 48h post iv administration of ^{124}I-CLR1404, The presence of the primary pancreatic tumor (P) as well as a spontaneous liver metastasis (M) is evident on the 3D scan. The presence of both tumors was verified at necropsy.

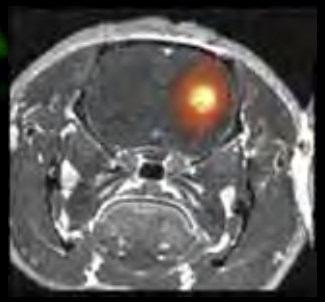
With Verma/Hafeez

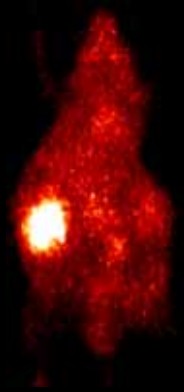


Pan Cancer Imaging with ^{124}I-CLR1404

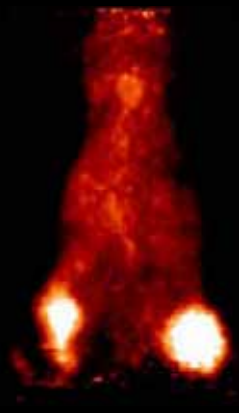

Adrenal


Pancreas

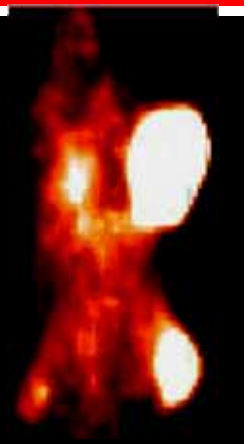

Brain


Bladder


Prostate


Prostate


Prostate


Colon (3)


Colon


Pancreas

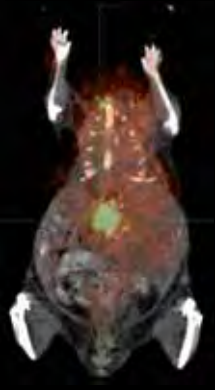

TN Breast


COLON


Prostate


Breast


Brain

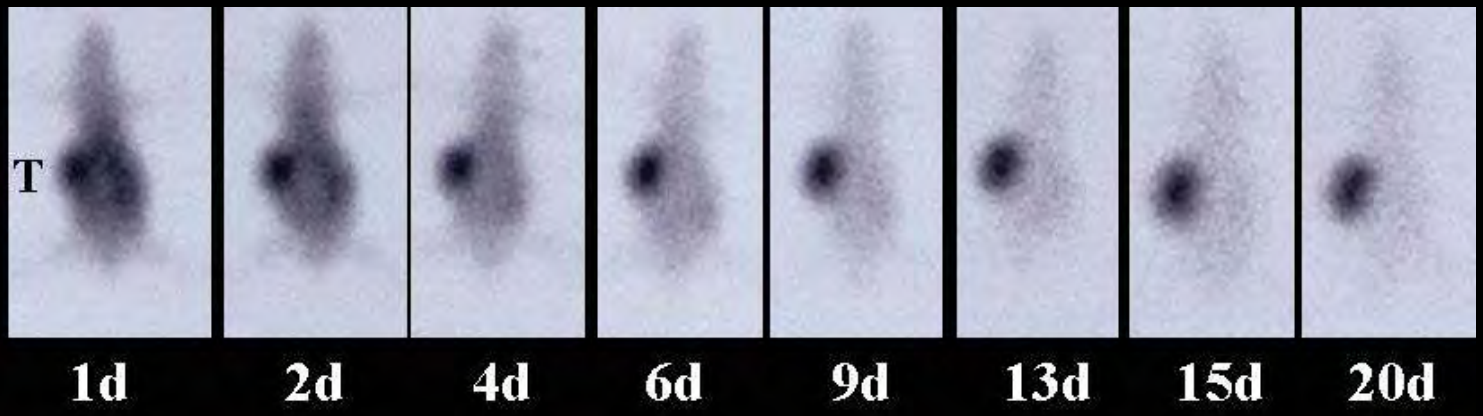

Sarcoma


Human Colorectal Lung Met

Primary+Mets in 52/54 xenograft and spontaneous models

Radiotherapy or Diapeutic Potential of ^{131}I-CLR1404

Prolonged Tumor Retention

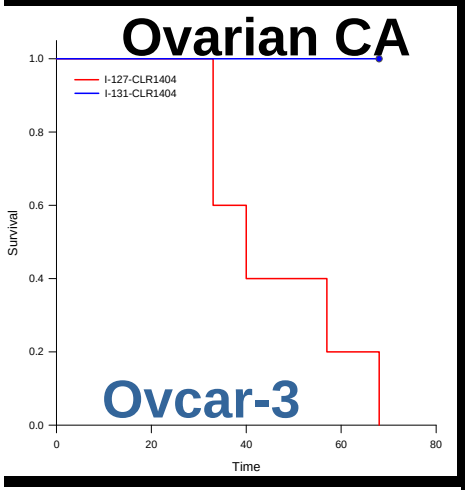


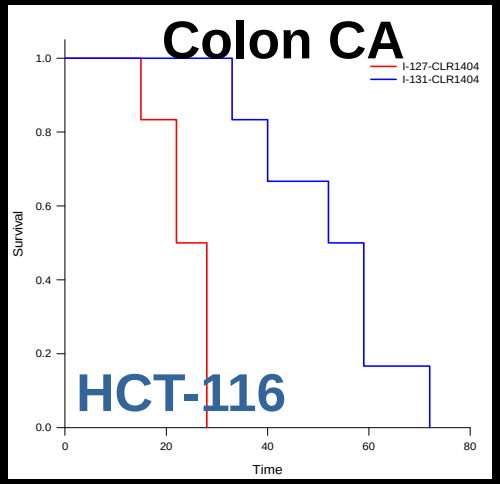
RL251 Adrenal CA

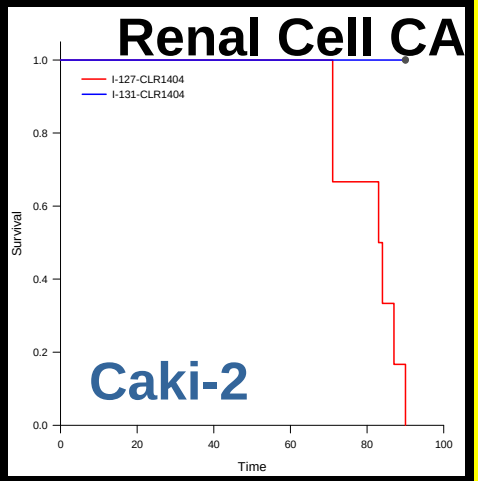
University of Wisconsin
Paul P. Carbone
Comprehensive Cancer Center

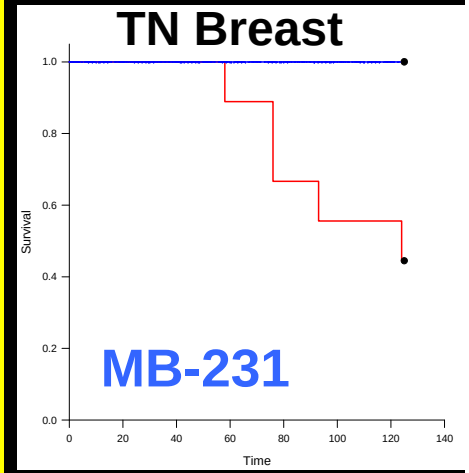
^{131}I-CLR1404 Kaplan-Meier Survival Results

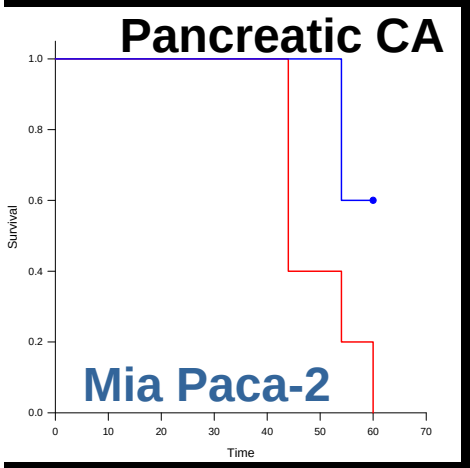
Mouse tumor models-Single injection*

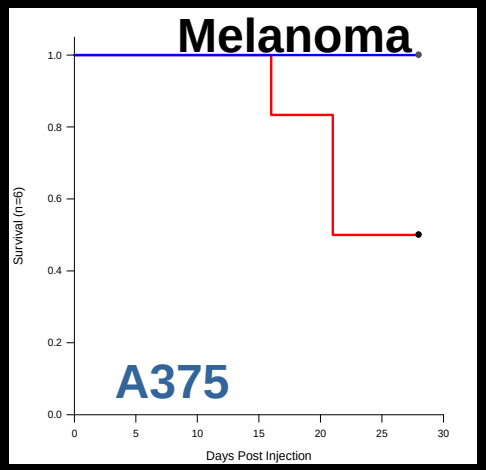


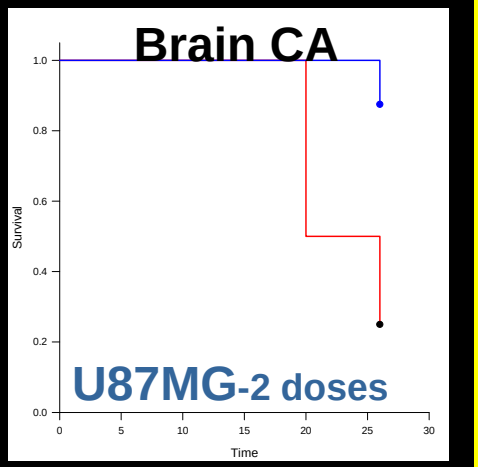










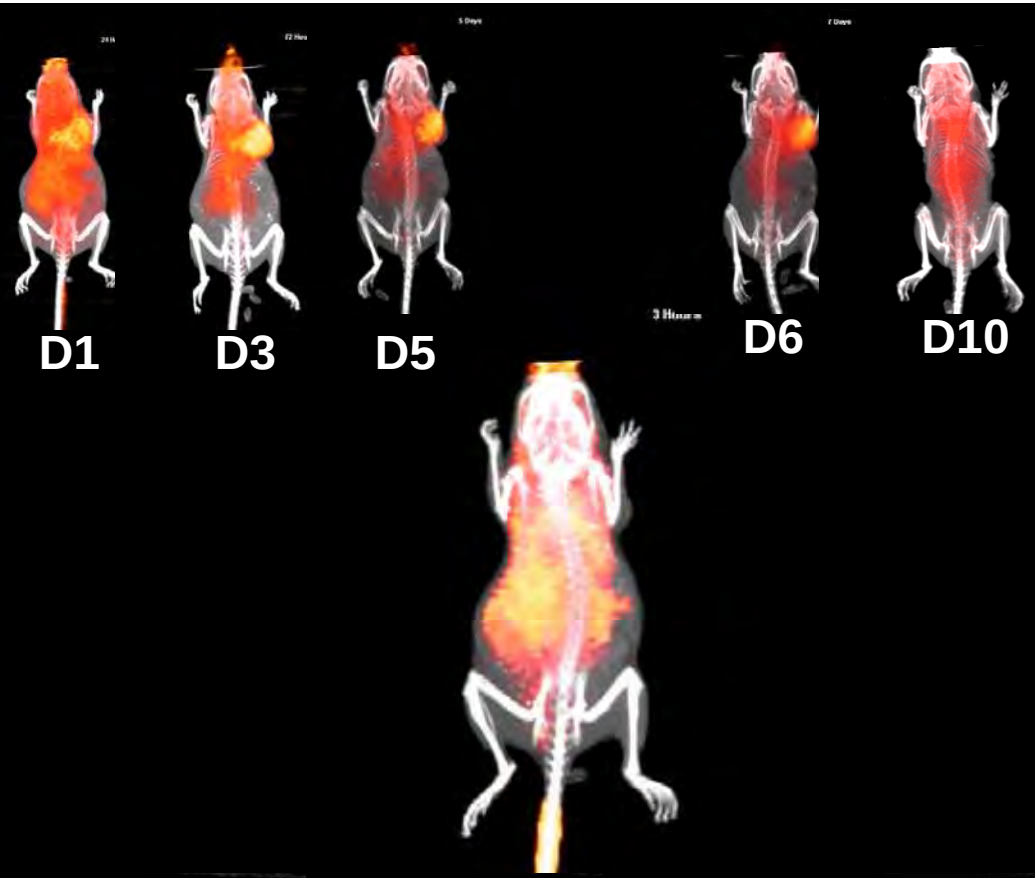


Starting tumor size was 200 mm^3 for both treated and control groups

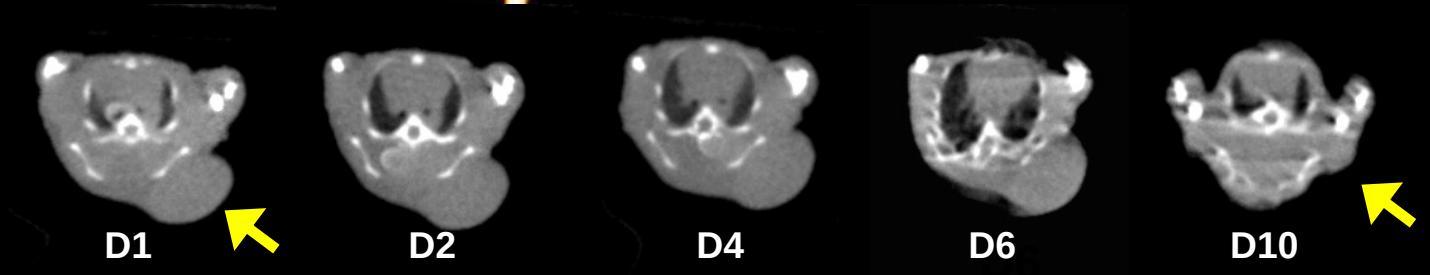
Control Groups (n=6, RED) received equal mass dose of CLR1401 when tumor size reached 200 mm^3

Treated groups (n=6, BLUE) received single 100 µCi dose of ^{131}I-CLR1404 except where noted (U87MG)

$^{124/131}$I-CLR1404 Diapeutic Response

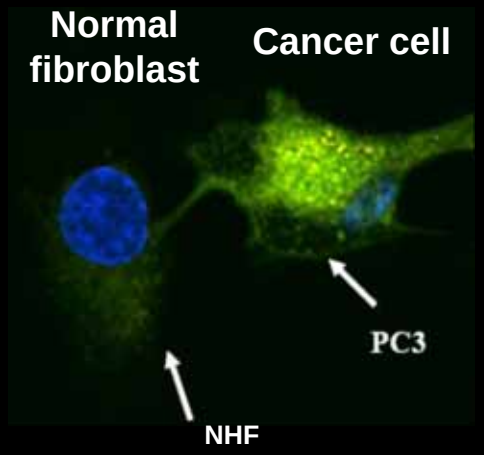


PET/CT time course of an LS180 colon CA xenograft bearing mouse injected simultaneously with a mixture of ^{124,131}I-NM404 (200 µCi each). Time course is from 3h to 10 days. Animal lost weight near the end of the study. Images are not normalized for exposure levels.

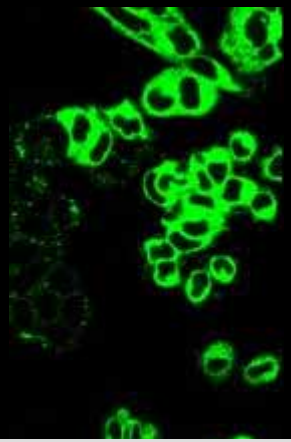


1 cm tumor regression over 10 days following single CLR1404 injection

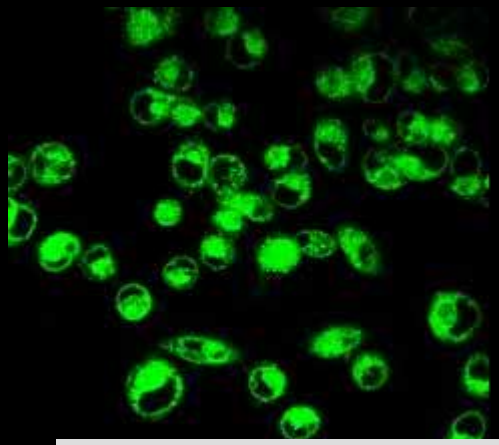
CLR1501 Selectively Targets Cancer Cells



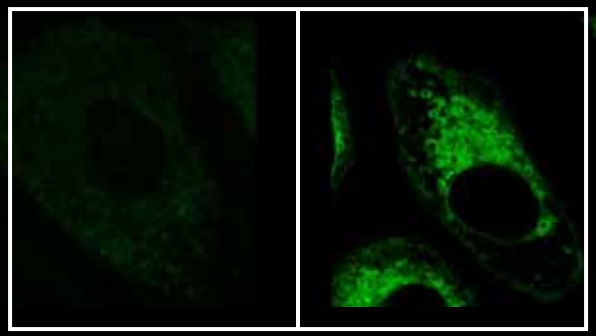
Co-cultured



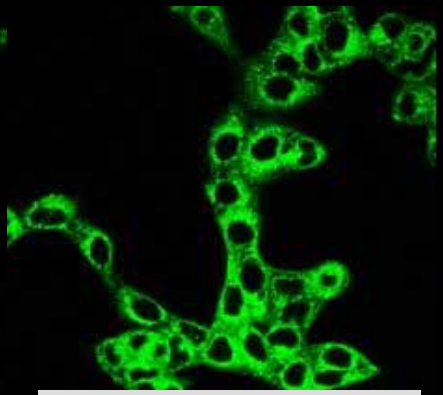
MES SA/Dx5 Uterine



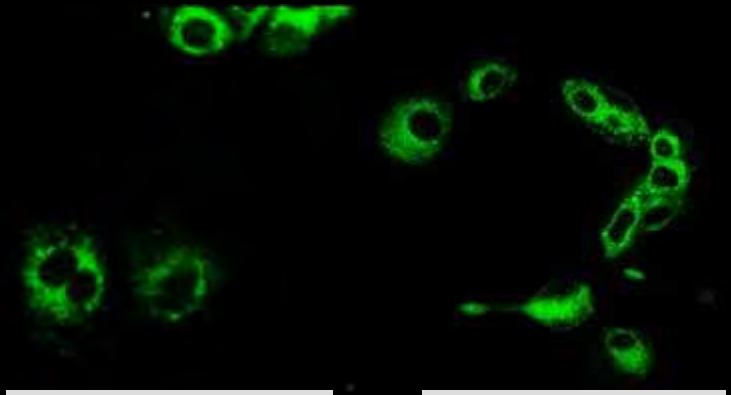
Mia Paca-2 Pancreatic



SK704-skin A375 Melanoma



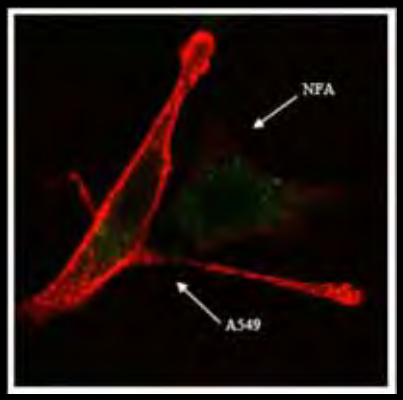
HCT-116 Colorectal



Ovcar-3 Ovarian U87MG Glioma

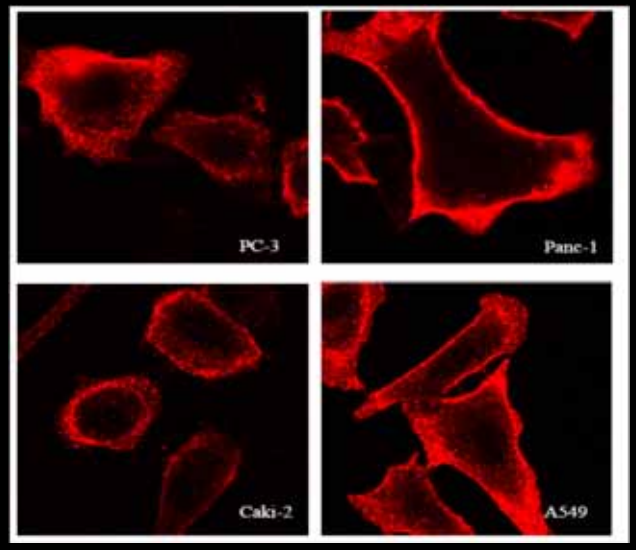
CLR1501 incubated 24h with cells then washed 2X with PBS prior to z-stack confocal microscopy. All exposure settings are the same. Blue is nuclear stain.

Lipid Rafts are Over-Expressed in Cancer Cells



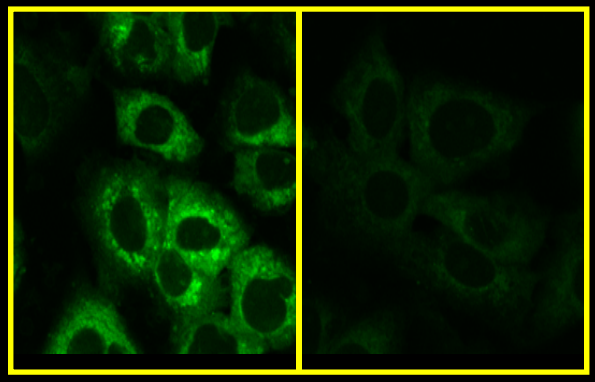
Co-cultured
Fibroblast vs A549

Prostate Pancreatic



Kidney Lung

A549 NSCLC

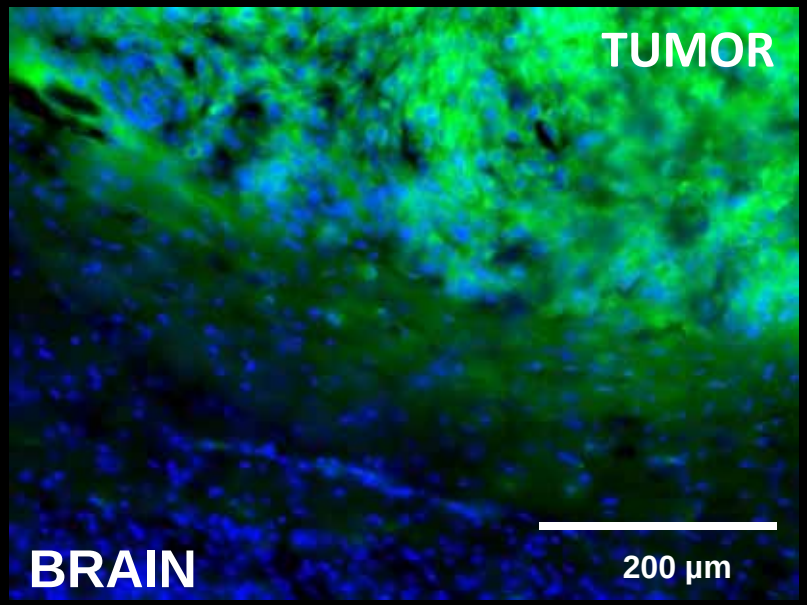


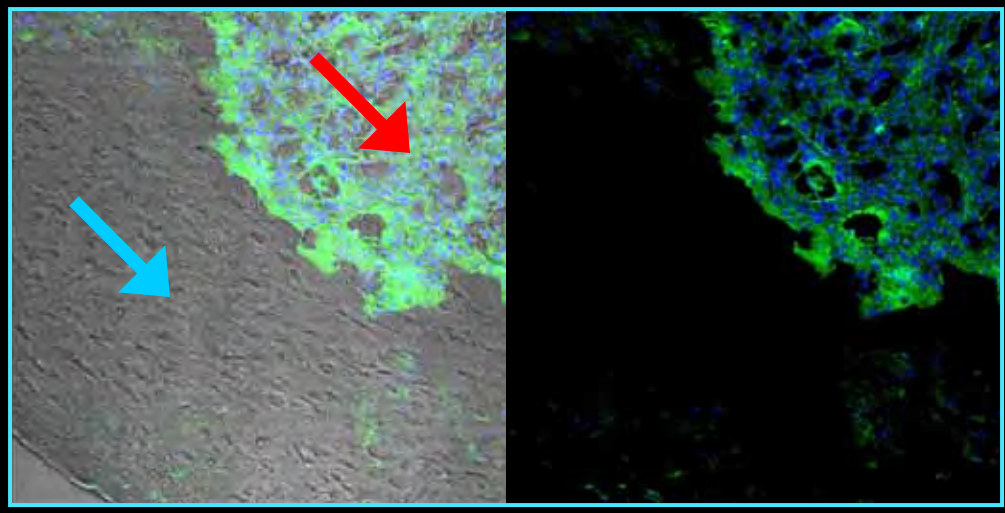
Intact Rafts

MBCD
Pretreatment
Decreased 60%

CLR1501 is taken up by cells via "lipid rafts", specialized regions of cell plasma membranes *(= red; fluorescent-labeled cholera toxin subunit B). Methyl-β-cyclodextrin selectively disrupts rafts.*

Glioma Tumor Margin with CLR1501





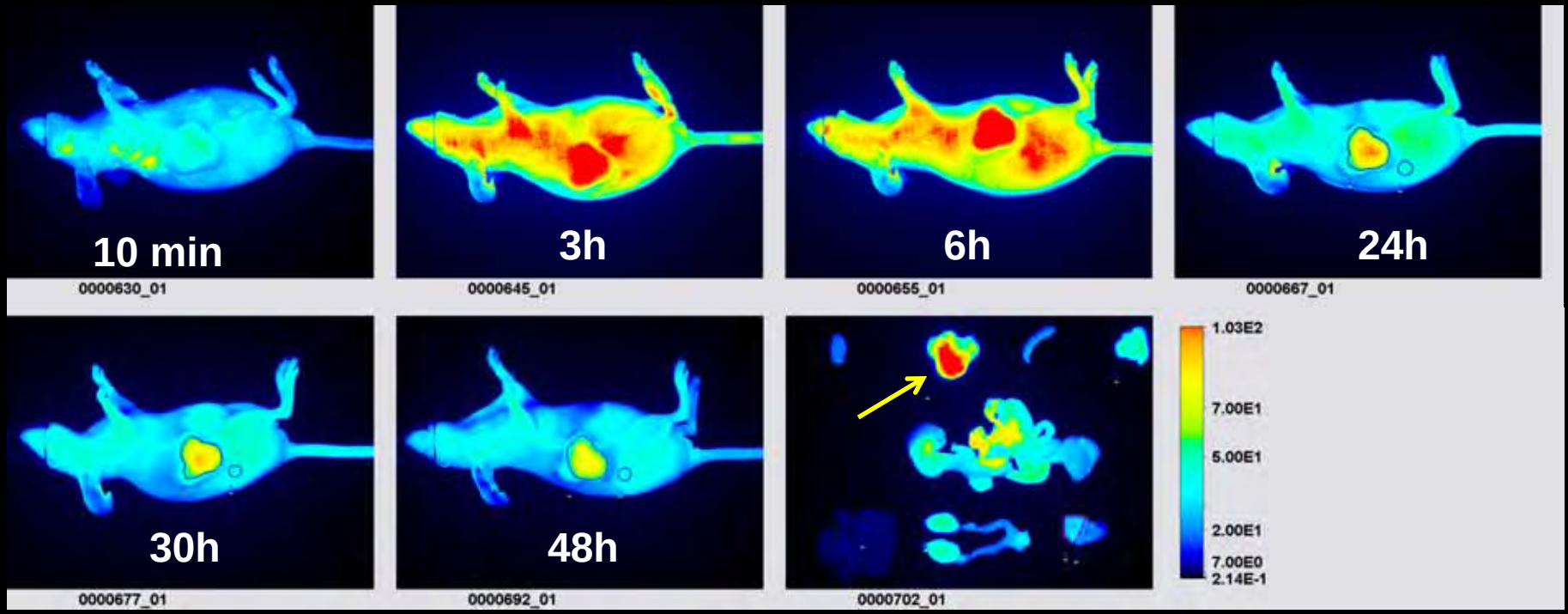
Tumor border with bright field and without bright field

Fluorescence micrograph of a brain section (20 µm) 24h post CLR1501 (1 mg, iv) injection. 22T cell line **blue** is a nuclear stain (**To-Pro-3**) and **1501** is **green**.

22T Glioma Margins: Confocal
Green stain: **CLR1501** and **Blue** stain: Hoechst 33452 (**nucleus**)
Red arrow=Tumor
Blue arrow=Normal brain parenchyma

In Vivo NIR Optical Scanning with CLR1502

Colorectal Carcinoma (HCT-116)



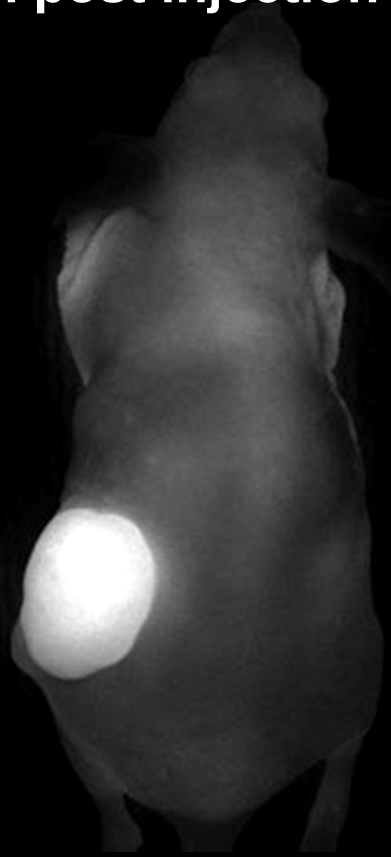
Injected with 1 mg of CLR1502. Monitored the intensity in vivo over time. The color reflects the intensity. At 48 hours, animals euthanized and organs excised and scanned ex vivo. The organs clockwise starting from top left corner: heart, tumor, spleen, lung; middle: GI tract (not flushed); skin, kidneys and liver. The signal intensity in tumor is 200 times higher than signal from liver.

Intraoperative Tumor Margin Illumination

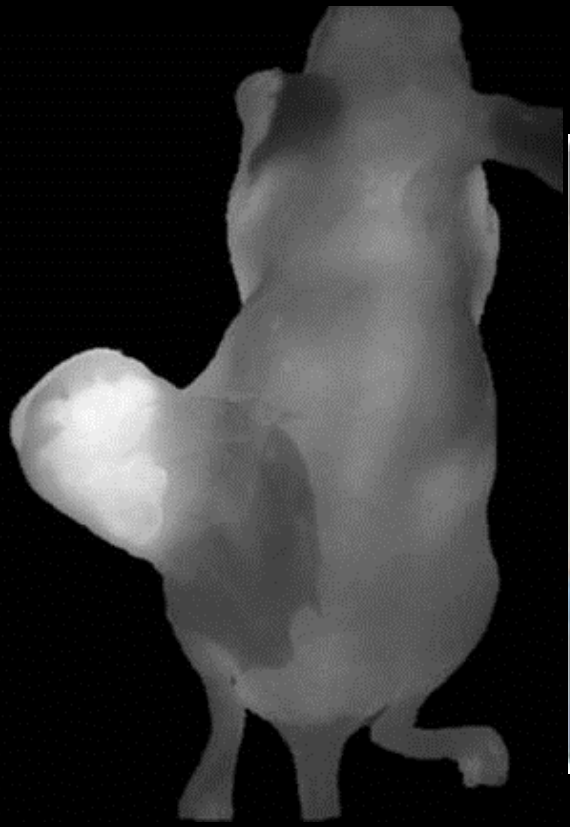
CLR1502

96h post injection HCT116 Xenograft

In vivo (IVIS Spectrum)



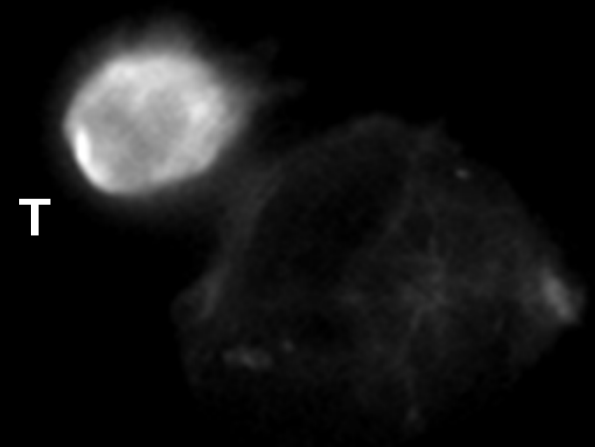
In vivo (Fluobeam)



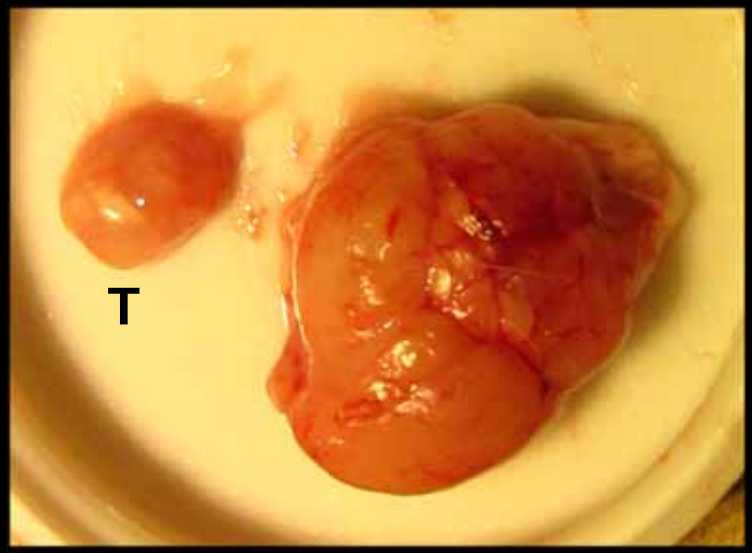
In vivo Post Partial Dissection (Fluobeam)

Fluobeam™ Fluoptics

Mouse Brain Tumor Illumination-Fluobeam



Fluobeam Image of excised mouse brain and tumor 96h post CLR1502 injection



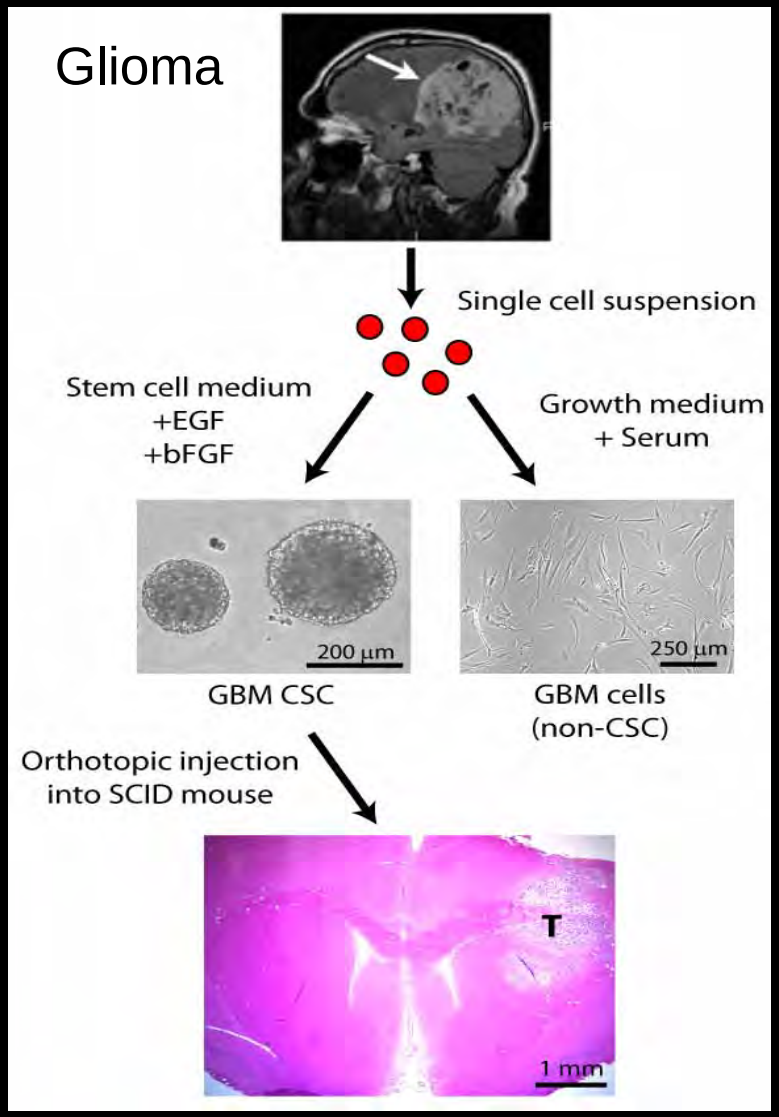
Photograph of excised brain and GSC-derived tumor

Cancer Stem Cell Paradigm

- Cancer stem cells have now been firmly associated with **most if not all** major cancer types.
- Numerous recent reports confirm that cancer stem cells do exist and are **chemotherapy resistant.**
- Glioma stem cells are also known to be up to **30% more radiation resistant** relative to normal cancer cells.
- These cells **affiliated with tumor regrowth and metastasis** following chemo and radiation therapy.
- Tumor **hypoxia** stimulates **CSC propagation** leading to increased resistance and metastatic potential.
- ***"Any new cancer treatment paradigm must address tumor heterogeneity including cancer stem cells"*** **-Jeremy Rich and others**

CSCs extremely tumorigenic: (1 cell→tumor in melanoma)

Cancer Stem Cell Isolation and Properties



Kuo, Clark

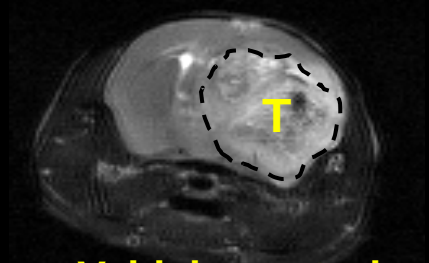
Isolation of Cancer Stem Cells

	Marker(s)	Reference
Leukemia	CD34	Lapidot et al. 1994 Bonnet and Dick 1997
Brain	CD133 Neurospheres	Singh et al., 2004 Hemmati et al., 2003 Clark et al., 2007
Breast	CD24, CD44	Al Hajj et al., 2003
Colorectal	CD133	O'Brien et al., 2007 Ricci-Vitiani et al., 2007
Prostate	CD44, CD133	Collins et al., 2005

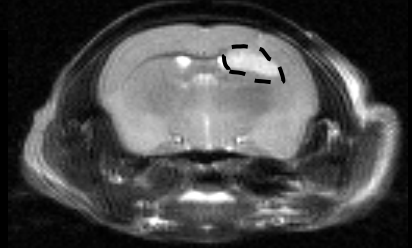
Cancer Stem Cell Properties

	Reference
Genetically/ Phenotypically similar to parental tumor	Lee et al., 2006
Enhanced chemoresistance	Eramo et al., 2006 Liu et al., 2006
Enhanced radioresistance	Bao et al., 2006a Diehn et al., 2009
Release angiogenic factors	Bao et al., 2006b Bruno et al., 2006 Calabrese et al., 2007

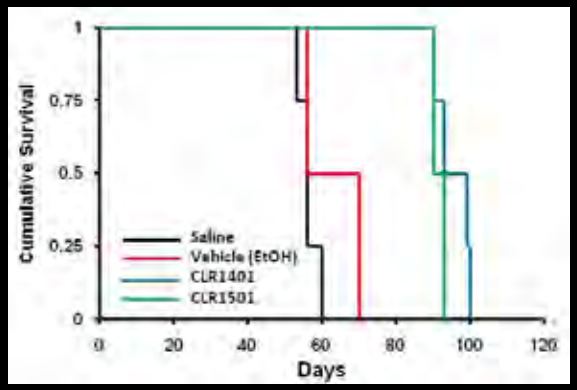
Glioma Stem Cell Results-Kuo Group



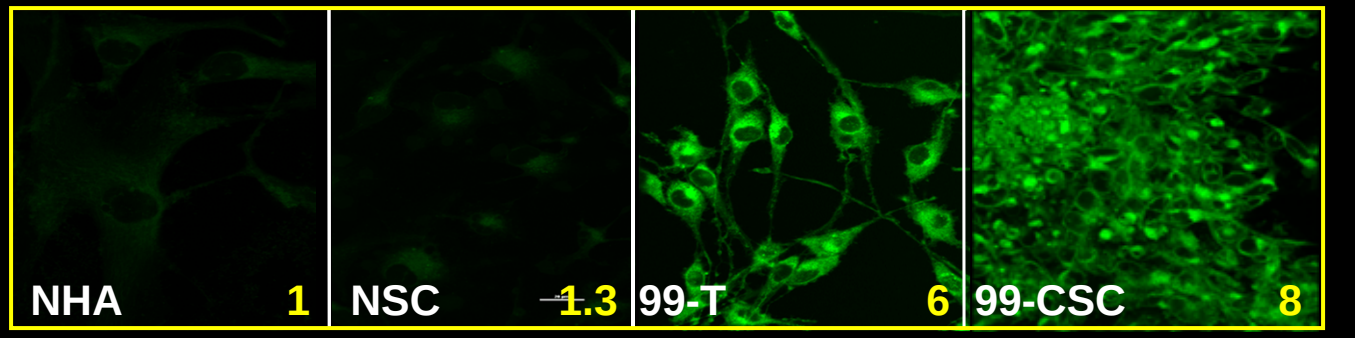
Vehicle control



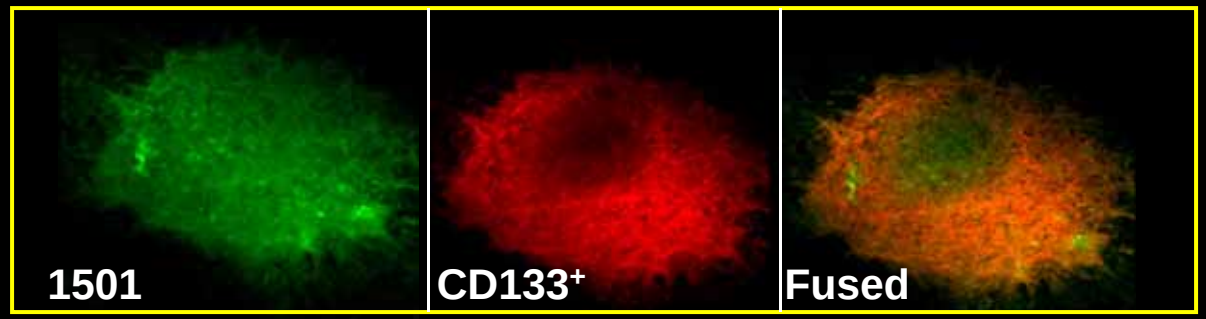
CLR1401



Cells pretreated with 1401 or 1501-24h followed by ortho-inoculation with subsequent MRI monitoring. Survival: Control 59±6.1 days; CLR1404 94±4.4 days.

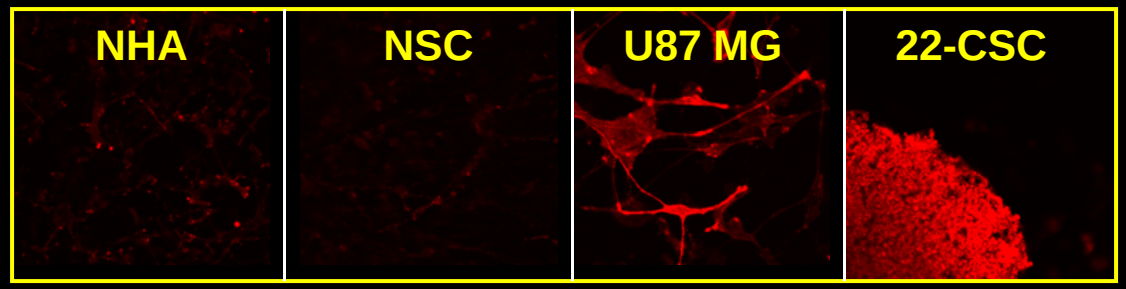


Comparative uptake of 1501 in normal vs malignant cells



In vivo uptake and serial GSC regrowth (3wk) with 1501

Prolonged retention in GSCs



Glioma Stem Cell Lipid Raft Status (Alexa Fluor-594)

CLR1404 Clinical Development-2012

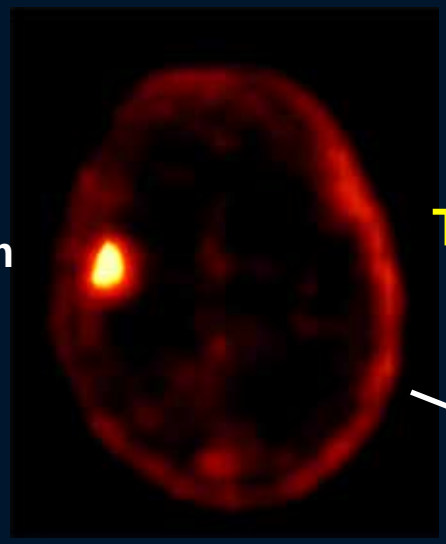
- **124I-PET Imaging Phase1-2 Clinical Trials (UW IND)**
  - Lung Cancer (Traynor/Perlman) *(Image and dose optimization)*
  - Glioma/ Brain tumors or Mets *(Image and dose optimization)*
  - Multiple Tumor Protocol (Liu) *(Image and dose optimization)*
    - Pancreas
    - *Breast*
    - *Prostate (Wilding, Liu, Med Onc)*
    - *Head and neck (Speer/Harari. Rad Onc)*
    - *Others-9 total*
- **131I-Therapy Phase 1b MTD Trial (Novelos IND)**

  (UW, Georgetown, City of Hope)
  - 20-40-60 etc (12.5 mCi/m^2)
  - 2nd dose cohort completed (3rd cohort ongoing)

Phase 1a dosimetry completed in 2010: 8 patients, 10 mCi ^{131}I-CLR1404, 4 tumor types

- No adverse events
- Low coefficient of variance among subjects
- Strong visual evidence of tumor uptake

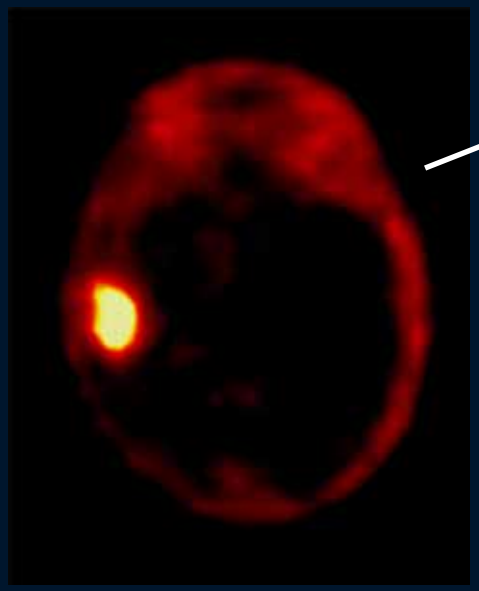
NSCLC Brain Tumor Metastasis with ^{124}I-CLR1404

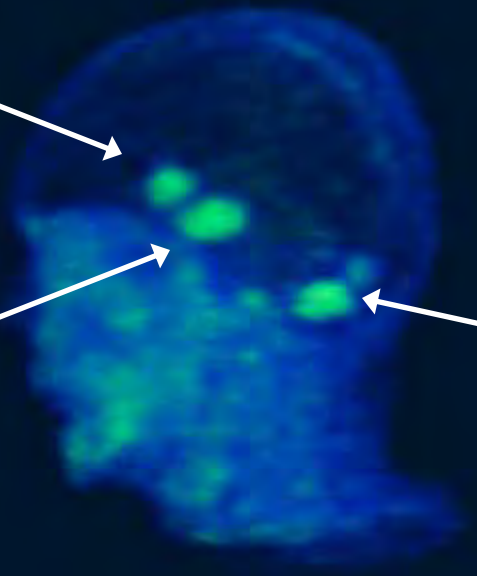


8 mm

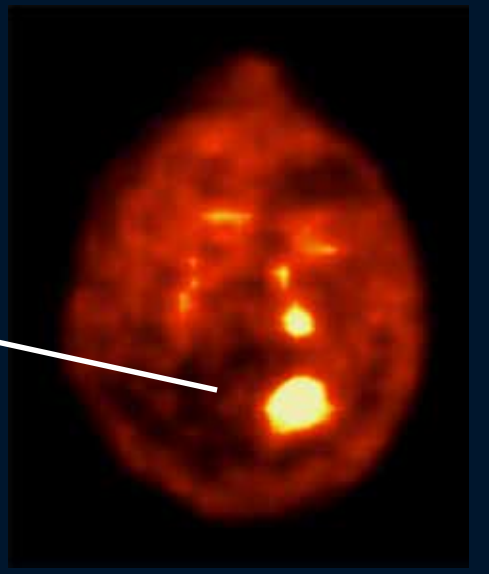
3 Previously unknown brain mets were discovered with CLR1404 (5 mCi) in this lung cancer patient.

Treatment plan was altered because of this finding



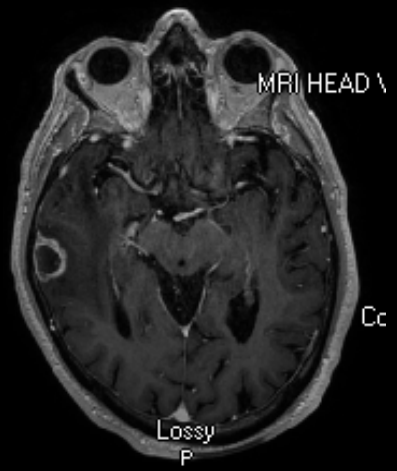


Movie

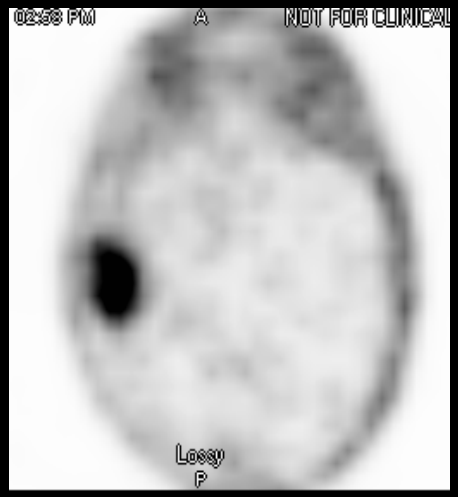


- **Extremely low background activity afforded tumor to brain ratio of >28 (3-5 typically considered adequate)**
- ***Tumor uptake at 24h***

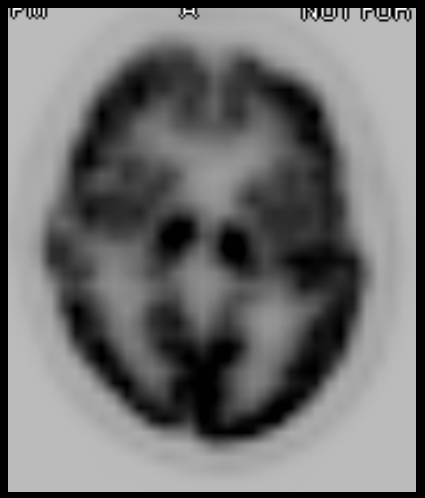
Human CLR1404 Brain Tumor Imaging



CE-MRI

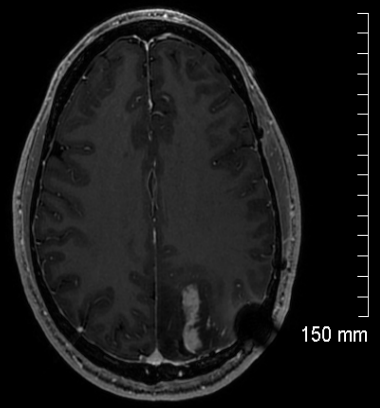


^{124}I-CLR1404

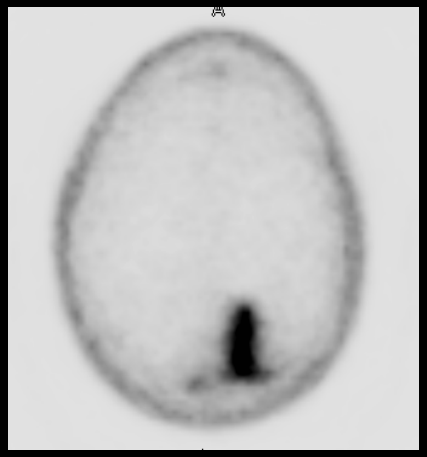


FDG

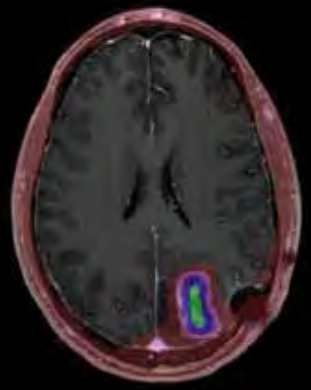
Lung Mets



CE-MRI



^{124}I-CLR1404

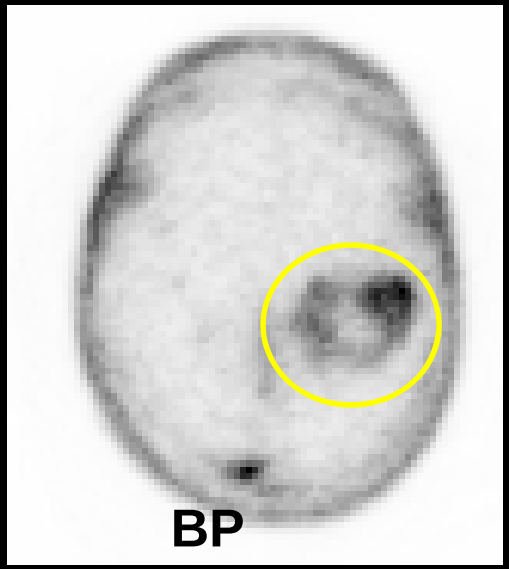


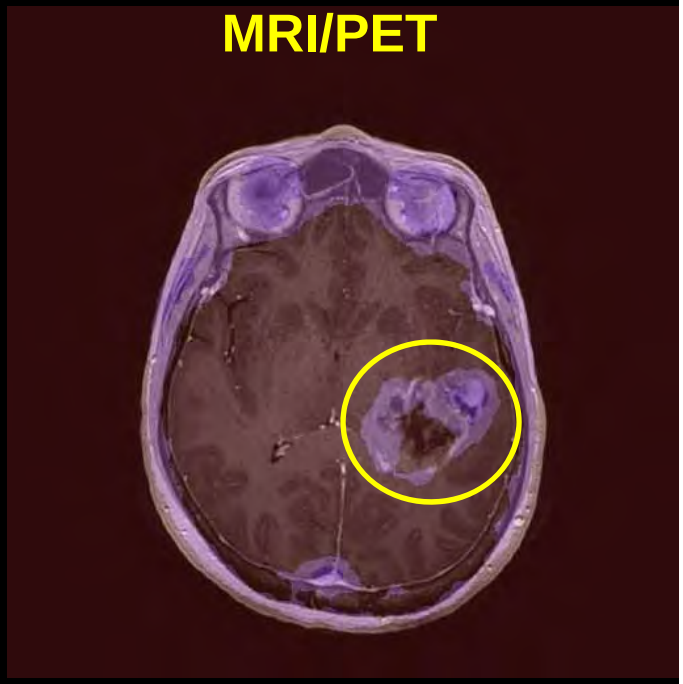
^{124}I-CLR1404/MRI

Recurrent Glioma
Grade 3

Primary Human Glioma (Grade 4)

CE-MRI **CLR1404 PET** **MRI/PET**

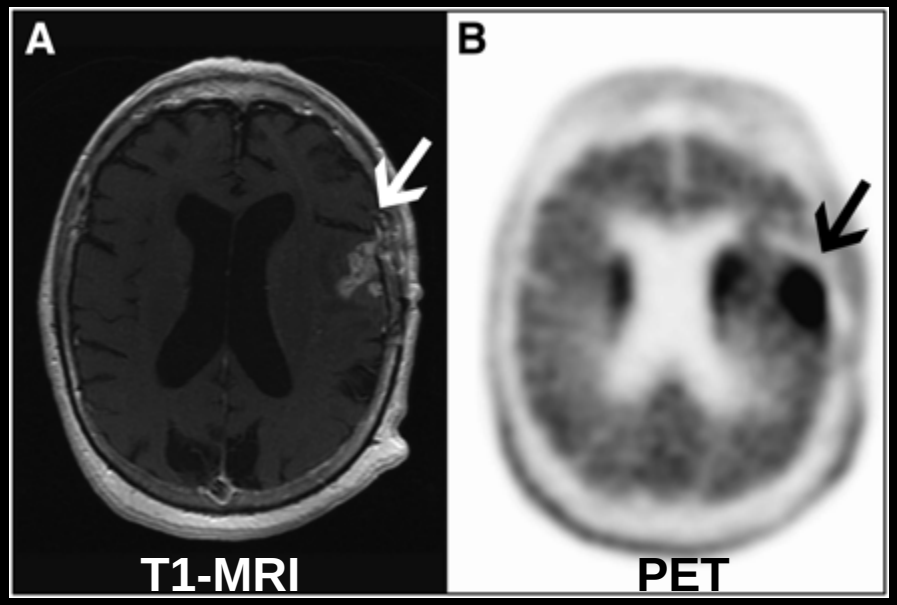




Newly diagnosed GBM. CE-MRI (left), 48h ^{124}I-CLR1404 PET (center), and fused PET/MRI image (right). Blood pool activity from venous sinus (BP).

"NM404 PET shows heterogeneous avidity throughout the tumor, likely showing more uptake in viable parts of tumor and lack of uptake in areas of necrosis. If NM404 PET can better identify viable tumor and tumor infiltration compared to MRI, this could have a positive impact on treatment strategies and patient survival." Lance Hall

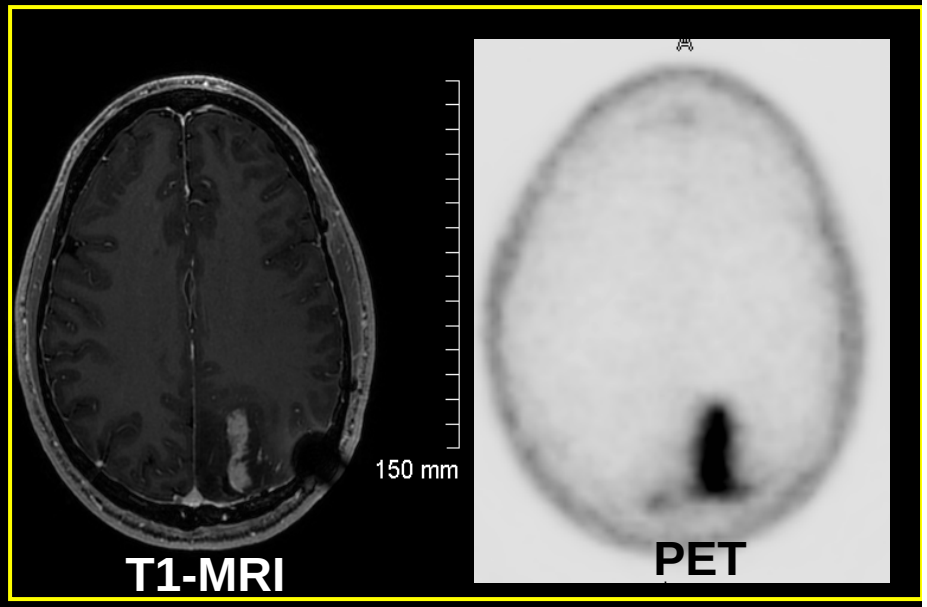
CLR1404 PET vs F-DOPA PET



^{18}F-DOPA Image of Recurrent Glioma

JNM Cover Image-March, 2012

Walter F, et al, J Nuc Med **2012** 53:393-98.



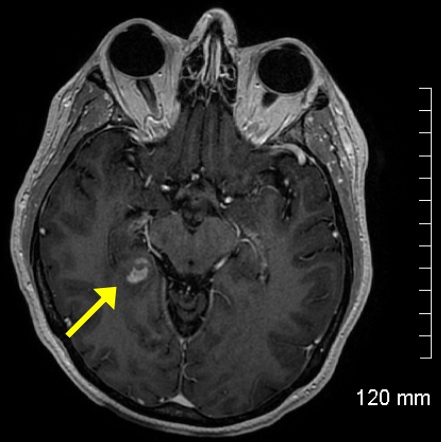
^{124}I-CLR1404 Image of Recurrent Glioma

(Tumor uptake at 6h)

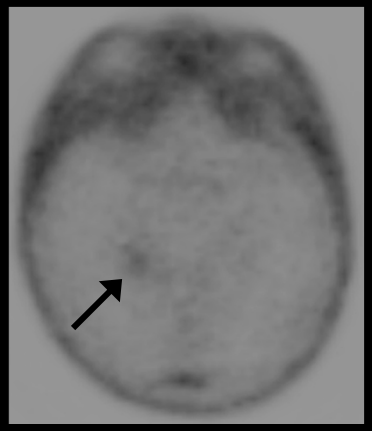
Surgical efficacy due to lack of tumor clearance?

High Grade Astrocytoma Previously Resected and Radiated

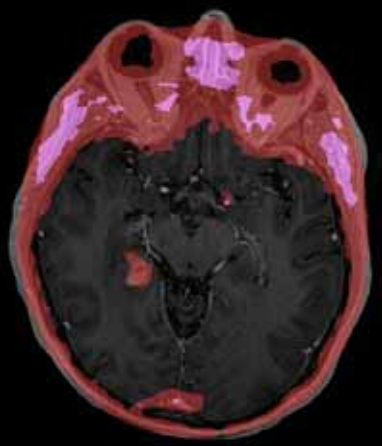
MRI demonstrated new small enhancing lesion (<1 cm) in posterior right temporal lobe/hippocampus



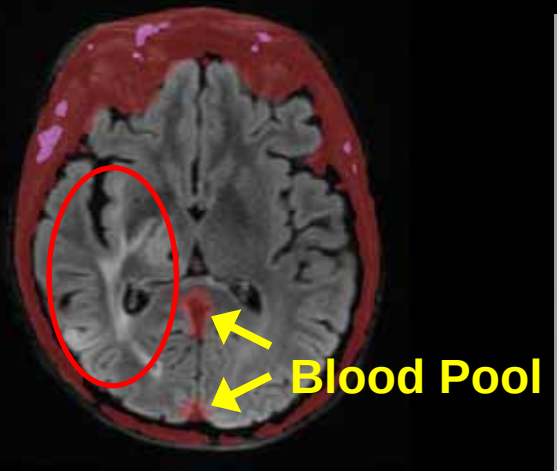
T2-MRI



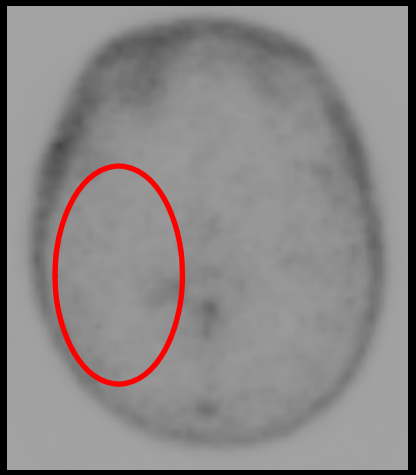
PET



PET/MRI



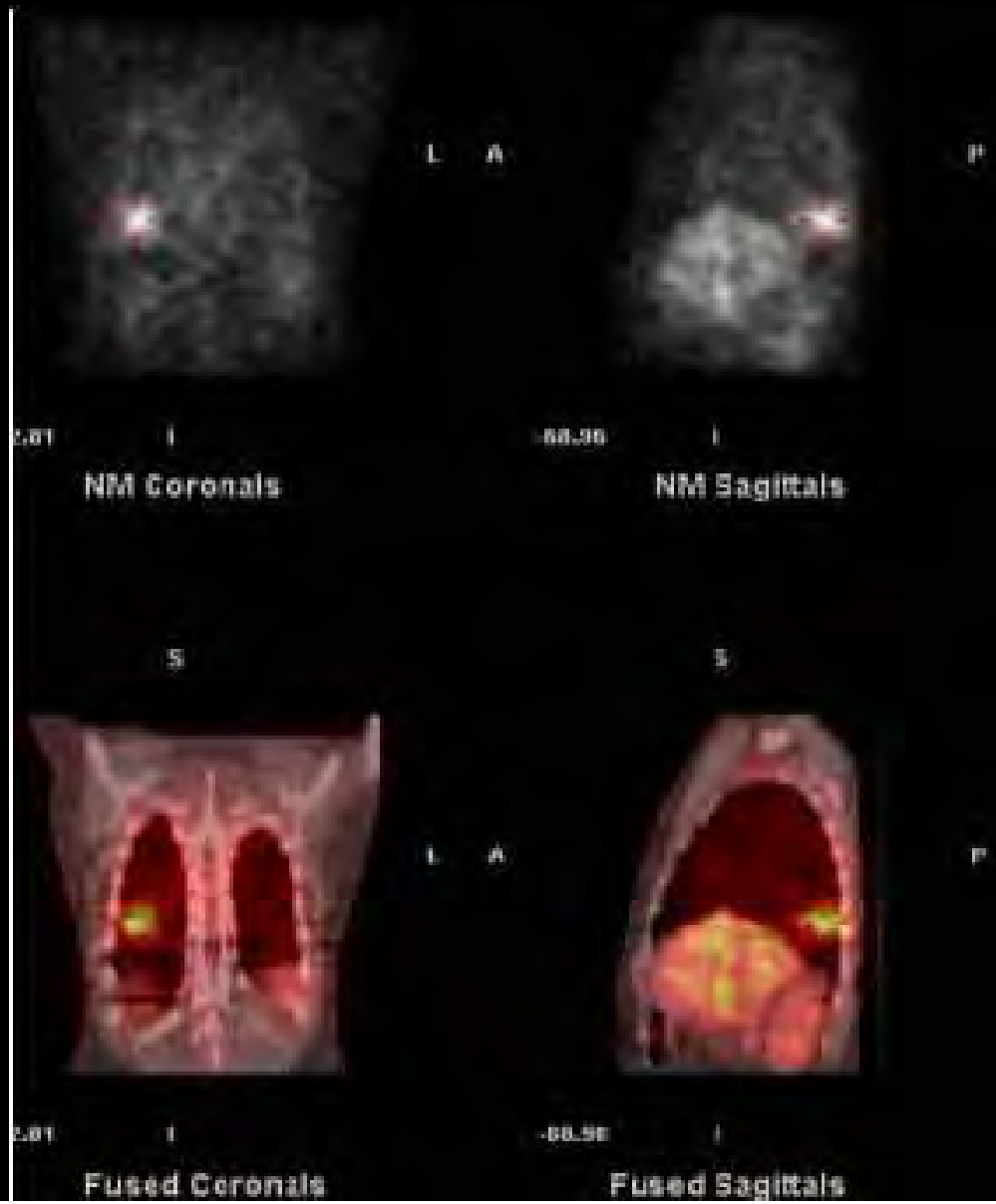
PET/MRI



PET

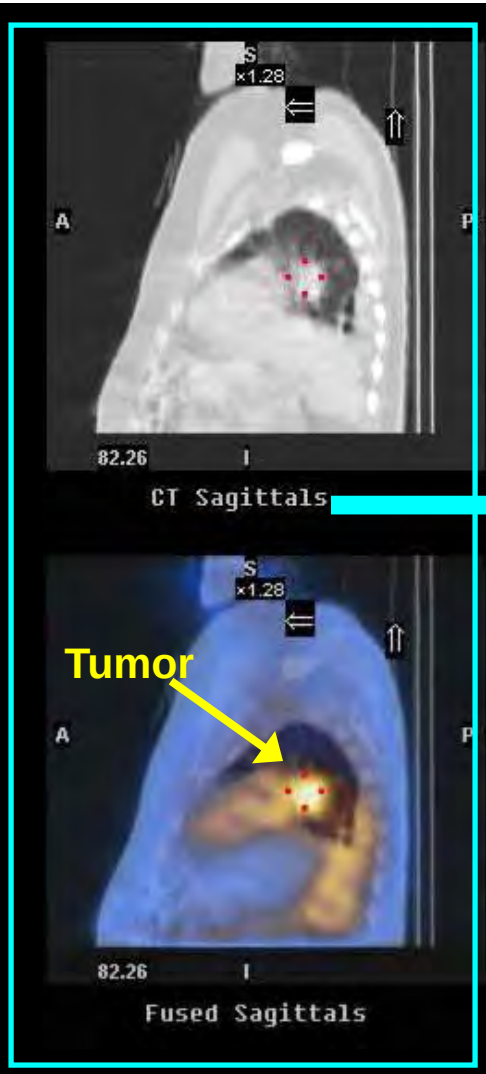
Radiation changes also evident throughout right hemisphere on T2 FLAIR MRI. No 1404 uptake.

Phase 1b MTD Patient SPECT



SPECT/CT image of Phase 1b patient with colorectal cancer metastases to the liver (multiple) and lung 21 days post injection of 25 mCi of ^{131}I-CLR1404 confirming prolonged tumor retention.

Diapeutic Treatment Paradigm



^{124}I-NM404 PET Image

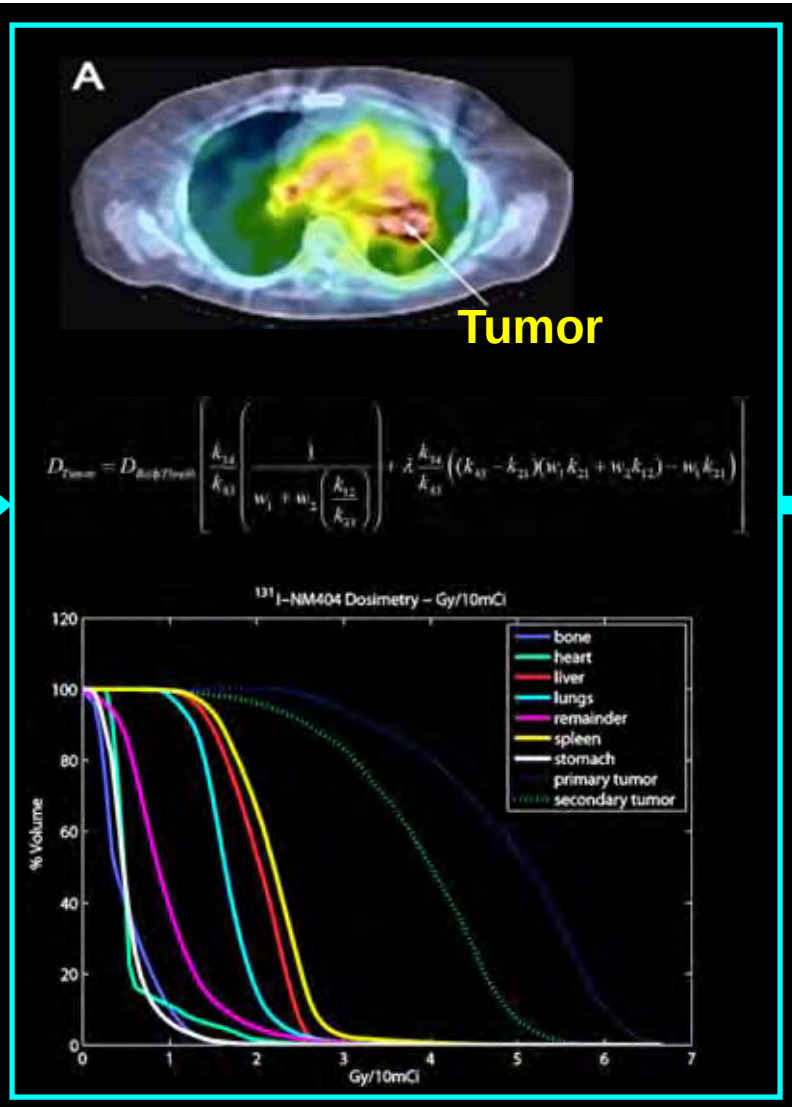


Image Quantification/Dose Calculation

Find-Treat-Follow

^{131}I-CLR1404 Therapy Dose Injection

Monitor Response Imaging

The Diapeutic Cancer Treatment Paradigm

A major goal of oncology today is to predict which patients will respond to a molecularly targeted drug. This is done by using biomarkers or imaging surrogates which are selective for the pathway or target of interest. Limitations still exist.

The PLE based diapeutic treatment paradigm has the following advantages over existing approaches:

- **Identical biomarker and therapy molecule (CLR1404) which are administered in nearly the same mass dose.**
- **PET/CT allows full body quantitative 4-D mapping of *in vivo* biodistribution**
- **PET/CT based dosimetry may predict personalized therapy dose or no treatment if imaging shows suboptimal tumor or normal organ uptake.**

PLE Delivery Platform Summary

- **Unique preclinical tumor targeting and retention properties of CLR1404 appear to translate to primary and metastatic human cancers (lung and others?)**
- **Optical and radioactive CLR1404 analogs target and undergo prolonged retention in glioma stem cells.**
- ***The longer half-life of I-124 coupled with the prolonged tumor cell retention of CLR1404 may enable tumor resection efficacy quantification by utilizing pre- and post surgical image comparisons. (see residual tumor)***
- **The unique *diapeutic treatment paradigm* we are attempting to define continues to progress and show promise.**
- **The optical PLE platform shows early promise for intraoperative tumor margin illumination and staging.**

Thank you!

Novelos Colleagues

Maria Dawson
Jill Erwin
Chris Blakley
Dennis Tate
Jason Larrabee
Brad Wallom
Angki Kandella
Anatoly Pinchuk
Marc Longino
Abe Vicaro
Harry Palmin
Chris Pazoles
Kim Hawkins
Joanne Protano
Patrick Genn

UW Students and Faculty

Lance Hall
Fred Lee
Perry Pickhart
Sharon Weber
Anne Traynor
Glen Liu
Rock Mackie
John Kuo
Paul Clark
Joe Grudzinski
John Floberg
Mohammed Farhoud
Ben Durkee
Rich Halberg
Bill Dove

Patient Volunteers

UW

Carbone Cancer Center
Radiology
Medical Physics
Human Oncology
WARF
Clinical Trial Group

National Cancer Institute

UWCCC Grant
2 R21 (Breast and Lung)
RO1-CA158800
(Glioma and Brain mets)

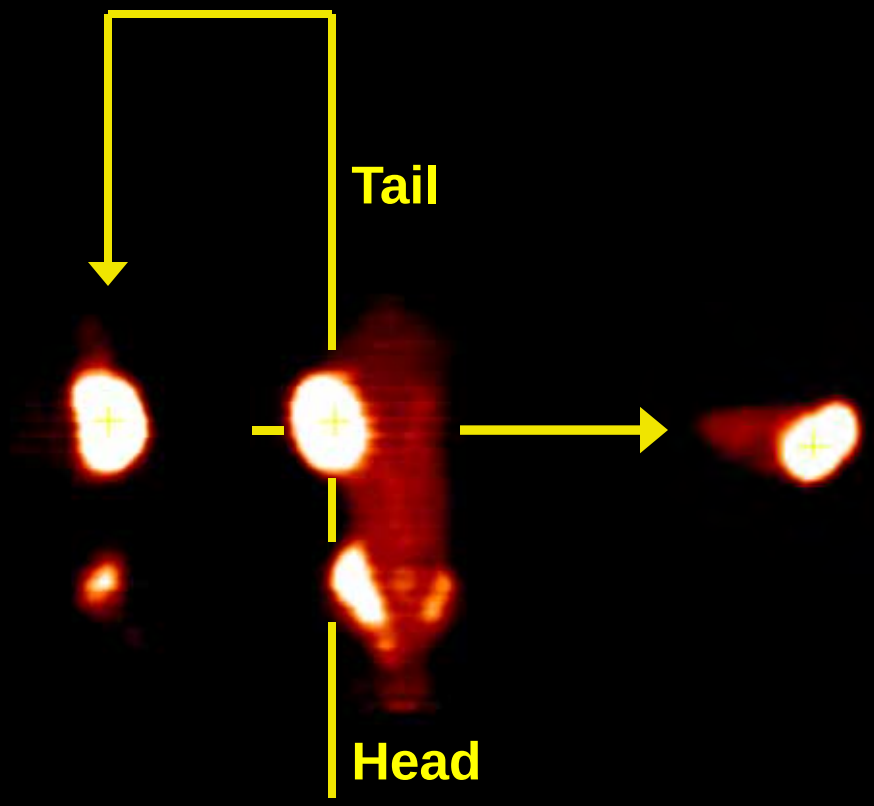
Clinical Trial Sites

City of Hope
Duke
Johns Hopkins
Georgetown
UW

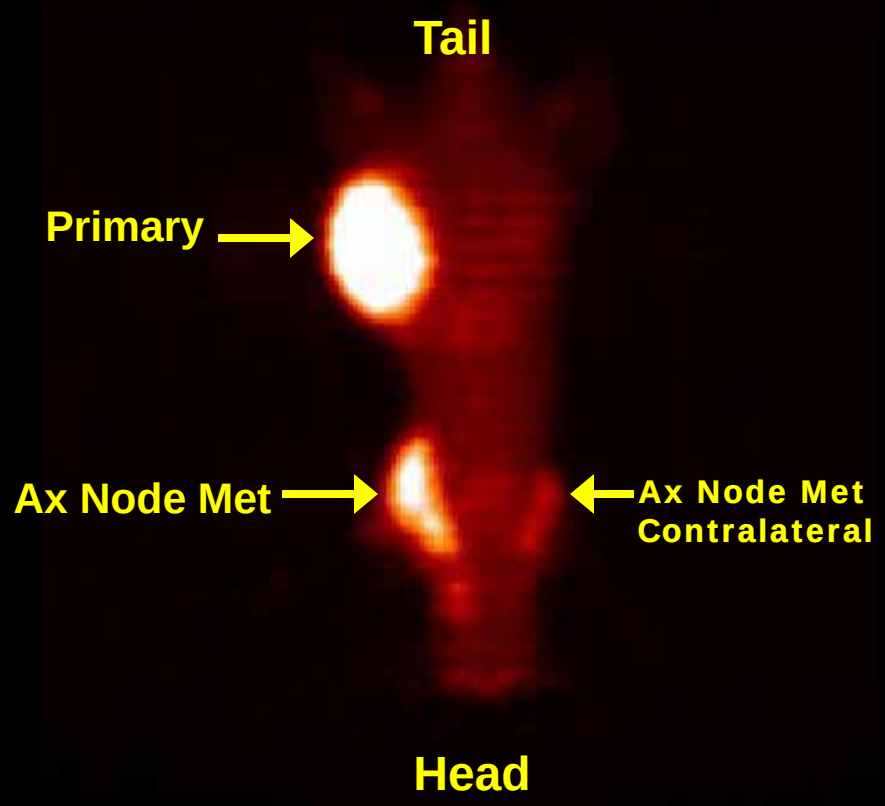
Supplemental Slides

PC-3 Prostate Cancer MicroPET with ^{124}I-CLR1404

Lymph Node Metastasis

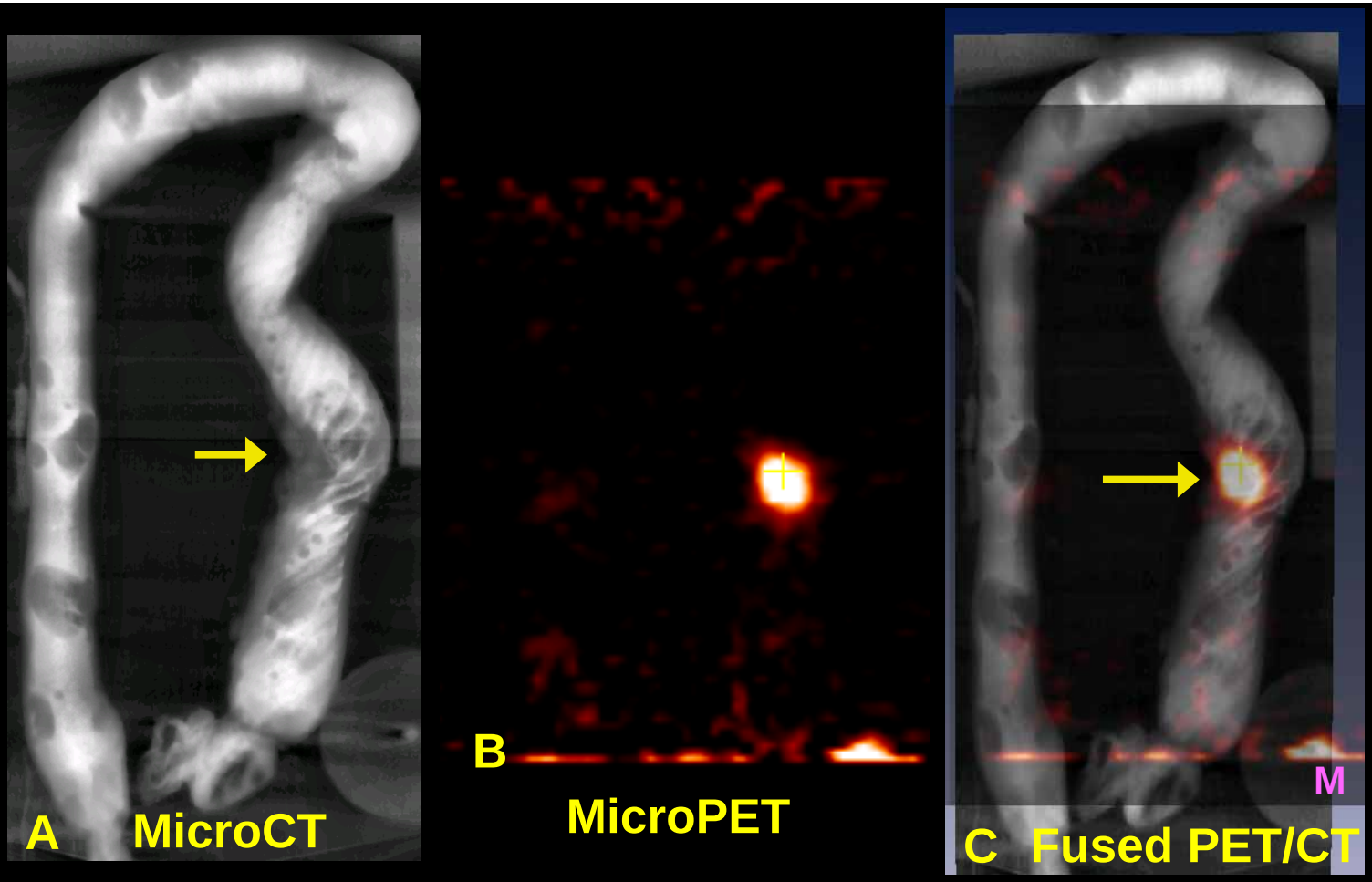


PET Scanning



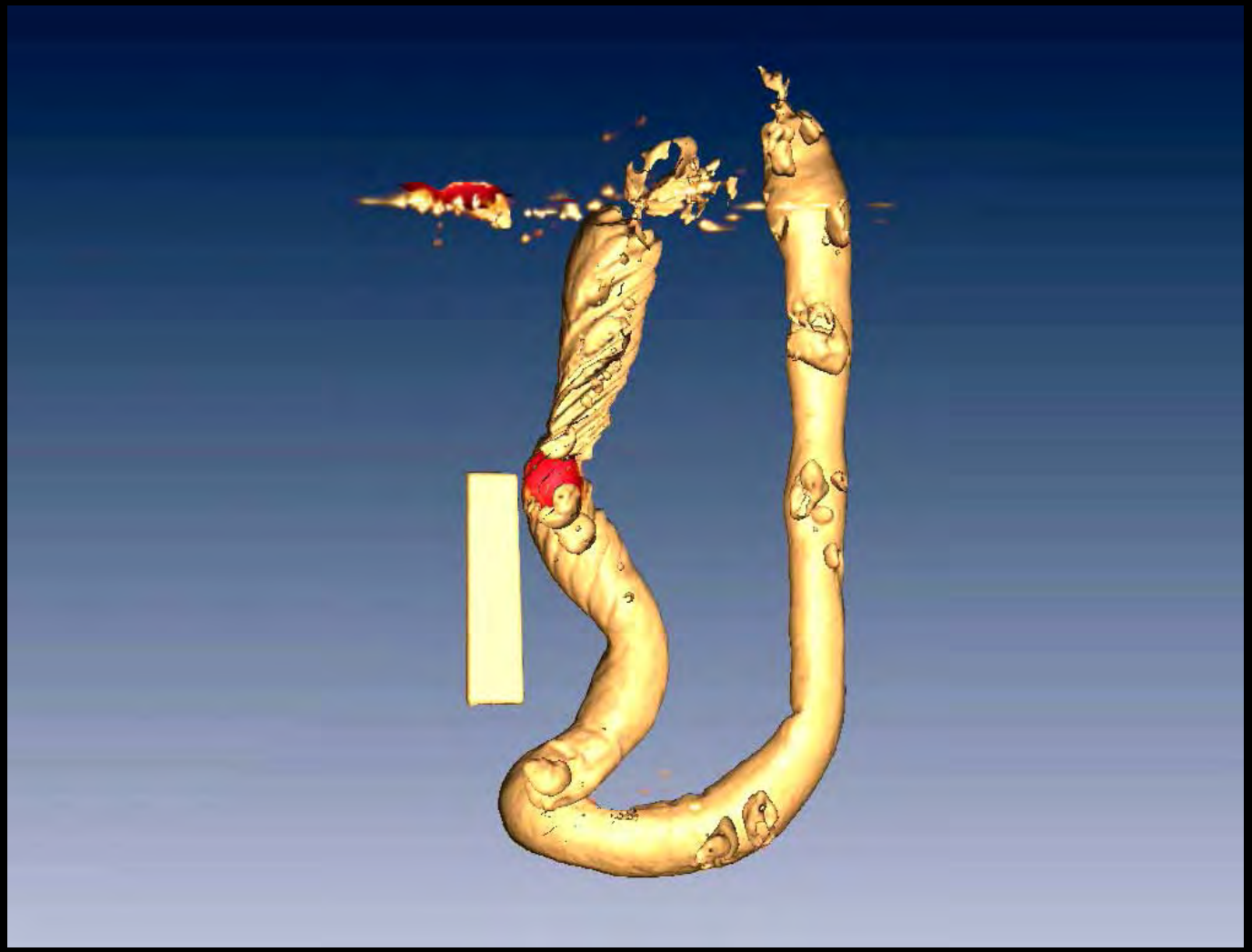
48h post CLR1404 injection

^{124}I-CLR1404 DMVC in PIRC Rat



Fused 2D microCT projection (A) and ^{124}I-CLR1404 microPET image (B) and fused microPET/microCT image (C) of excised PIRC rat colon filled with 2% barium. Fiducial marker (M), Tumor (arrow)

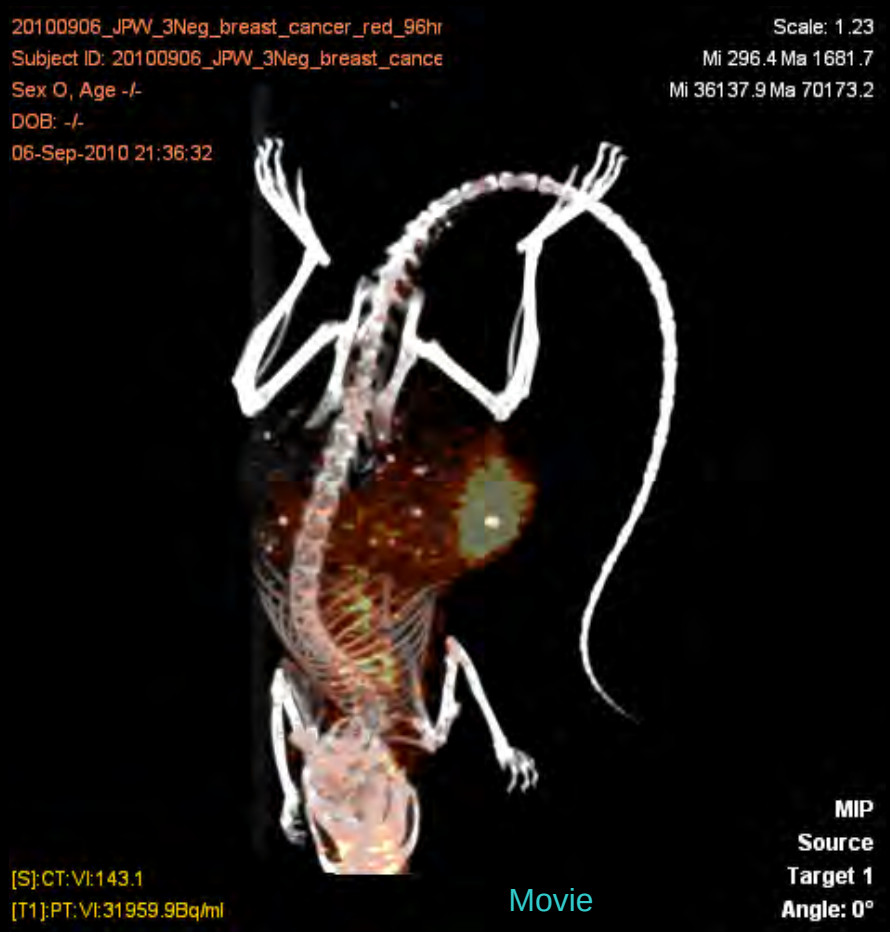
^{124}I-CLR1404 DMVC Luminal Flythrough



Virtual Biopsy?

with Dove/Amos-Landgraph

Triple Negative Breast Cancer Images



^{124}I-CLR1404 microPET/CT image of a mouse with triple negative breast cancer (MB-231).

Triple negative Breast Cancer

- Estrogen receptor negative
- Progesterone receptor negative
- Her2 receptor negative
- 15-20% of breast cancer patients have this form

Doesn't respond to hormonal or epidermal growth factor targeted therapies

First Demonstration of Triple Neg Breast Cancer Imaging

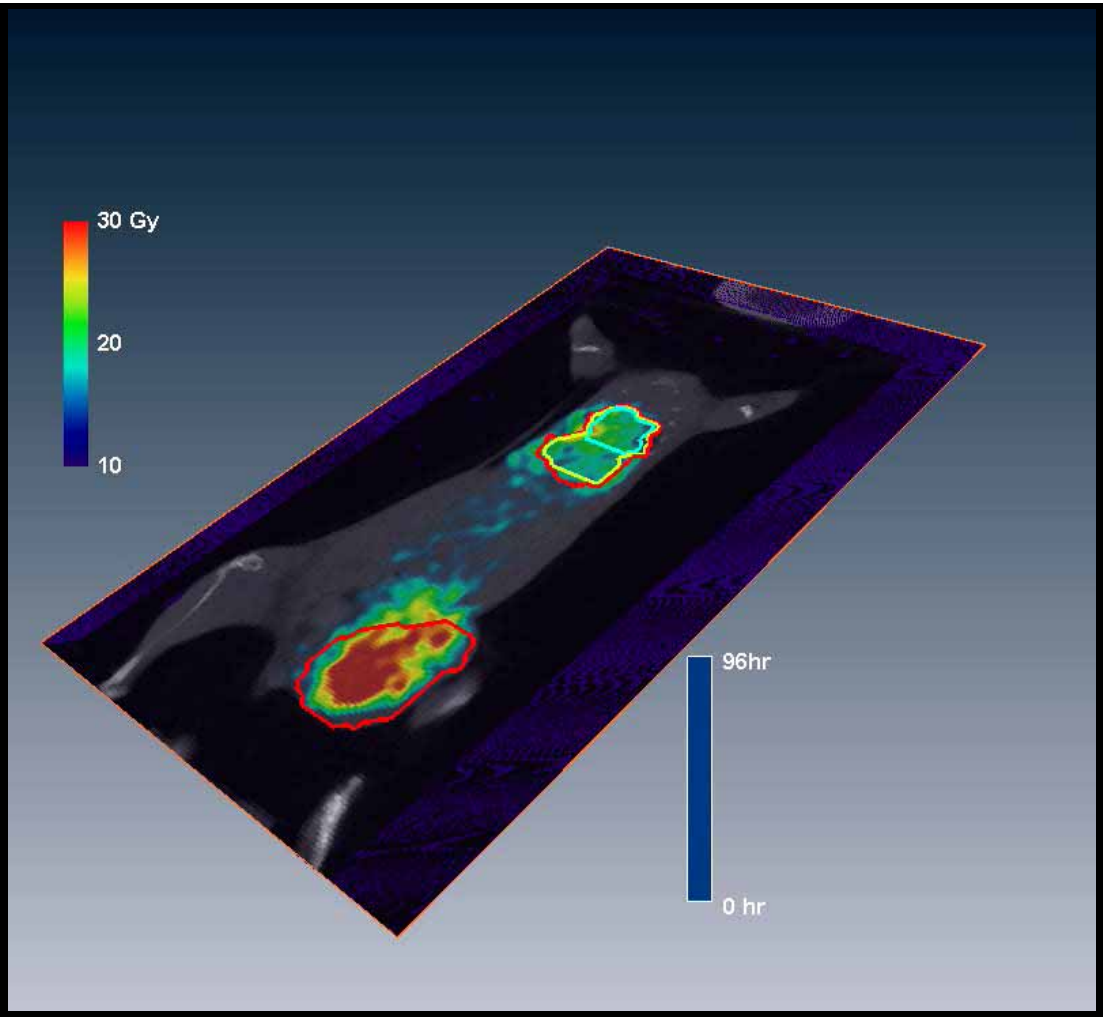
^{131}I-NM404 Max Tolerable Dose in Rats

Rat Dose (mCi)	Human (70kg) Equiv dose (mCi)	Rad tox findings
0.5	150*	None
2.5	750	None
4.0	1200	Slight platelet drop and recovery
5.0	1500	Grade 3 platelet drop and recovery
7.0	2100	Grade 4 platelet drop and death

Normal rats, N=6 for each cohort

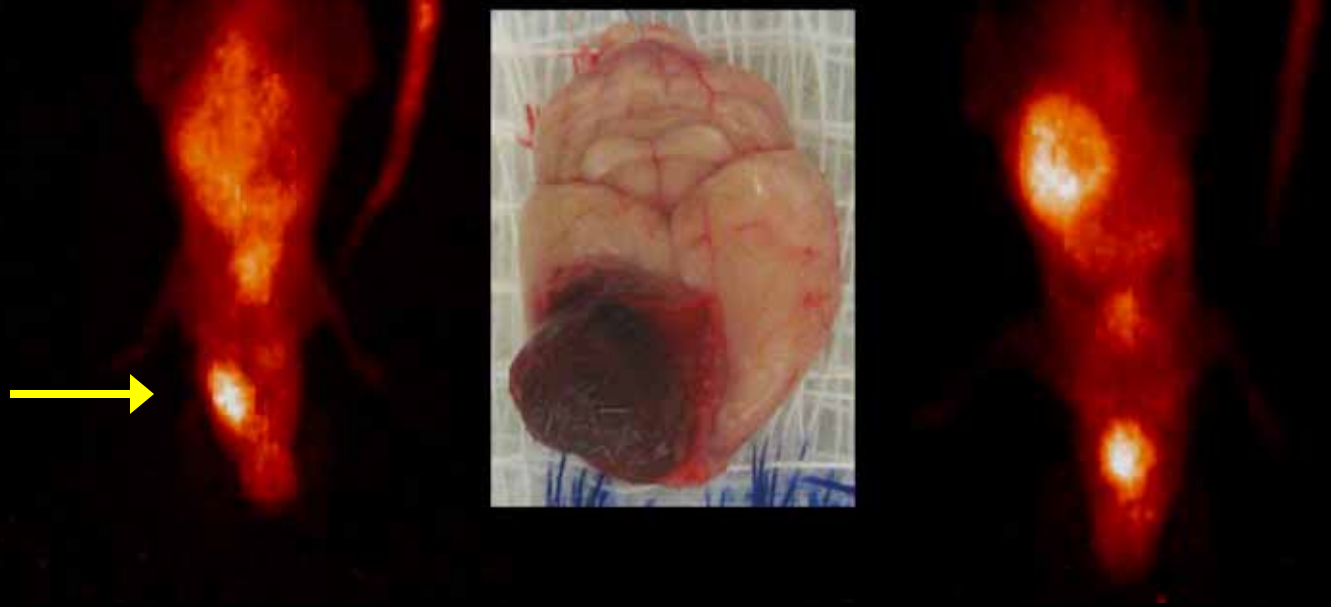
* Anticipated max human dose

MicroPET/CT Based 4D-Treatment Planning for IV Radiotherapeutics: ^{124}I-CLR1404



Siemens 2008 Inveon Image of the Year-
World Molecular Imaging Congress

^{124}I-CLR1404 MicroPET of Human Glioma Stem Cell Derived Orthotopic Brain Tumor



24h after injection
[Tumor/Brain]=5.4

48h after injection
[Tumor/Brain]=7.0

With Kuo and Clark

Clinical Phase 1a Dosimetry Results-Therapy

- ✓ Eight patients enrolled; four cancer types; 10 mCi 131I-CLR1404
- ✓ Zero drug attributable serious adverse events
- ✓ Consistent distribution from patient to patient – increases safety profile
- ✓ Distribution and elimination *exactly* as predicted from animal studies; minimal renal elimination increases safety profile
- ✓ Strong visual evidence of tumor uptake

Clinical Trial Partner Sites	1st Completed Patient Cancer Type	2nd Completed Patient Cancer Type	3rd Completed Patient Cancer Type
Duke	Colorectal	Prostate	
Georgetown	Colorectal	Colorectal	Colorectal
City of Hope	Breast	Prostate	
Johns Hopkins	Esophageal		

Results provided starting dose (12.5 mCi/m^2) for MTD therapy trial

Potential Uses for CLR1404 Analogs

- **Diagnosis, characterization, and staging of tumor masses regardless of location**
- **Guiding or in conjunction with Tomotherapy**
  - **Endo/Exo Radiotherapy Synergy**
  - **PET Guided Tomotherapy**
- **Monitoring tumor response to therapies**
- **Radiotherapy *(Diapeutic)* [^{131}I, ^{125}I, Both]**
- **Dual Modality Virtual Colonoscopy *(Virtual Biopsy)***
- **Optical Versions for detecting surface-oriented cancers**

  -Colorectal /esophageal/ cervical/melanoma/nodes

  -Intraoperative tumor margin illumination

CLR1404 Summary

- Preclinical imaging has shown selective tumor uptake and prolonged retention in **52/54** tumor types in mice.
- Preliminary radiotherapy results with ^{131}I-CLR1404 in mice (>12 models) are very promising and show significant life extension.
- Rat MTD studies suggest acceptable dosimetry tolerance profile.
- Phase 1a dosimetry/safety study (8 cancer patients) with 10 mCi ^{131}I-NM404 - safe and low variance pkinetics). Phase 1b MTD study and imaging trials now FDA approved and ongoing at **UW (main site), COH and Georgetown.**
- Initial cancer stem cell results appear very promising.