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Cancer-Targeted Diapeutics

Radioiodinated Phospholipid Ether Analogs for Broad-Spectrum Imaging and Therapy

EMIT: Targeted Radiotherapy Conference

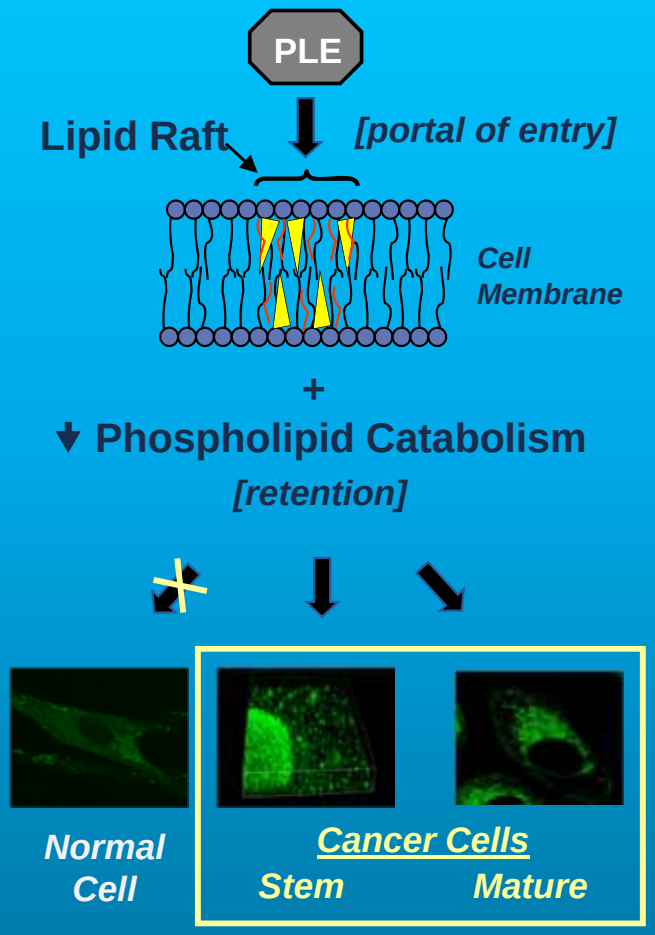
Washington, DC *January 31, 2013*

Cancer-Targeted Approaches: Desirable Features

- **Selective for cancer vs. normal cells/tissues**
- **Broad-spectrum targeting across/within cancer types**
- **Targets cancer stem cells and mature cancer cells**
- **Targeting vehicle can deliver a range of effectors**
  (radioisotopes, chemo agents, imaging agents)
- **Broad-spectrum efficacy across/within cancer types**
- **Cancer-targeting examples**
  - **Active targeting**
    - **mAbs/fragments; peptides, derivitized nanoparticles**
  - **Passive targeting**
    - **enhanced vascular permeability ("EPR-effect")**
    - **nanoparticles, liposomes**

Cancer-Targeted, Broad-Spectrum, Multi-Product Technology Platform

Cancer-Targeting Mechanism



Cancer-targeted Radiopharmaceuticals

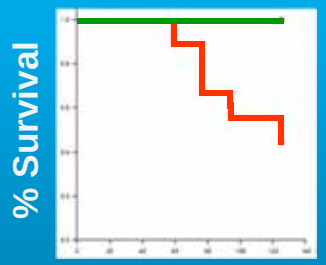
PET Imaging

^{124}I PLE

Phase 1-2

Therapy

^{131}I PLE

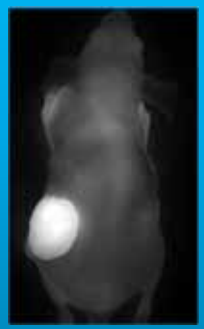


Phase 1b

Cancer-targeted Optical Imaging

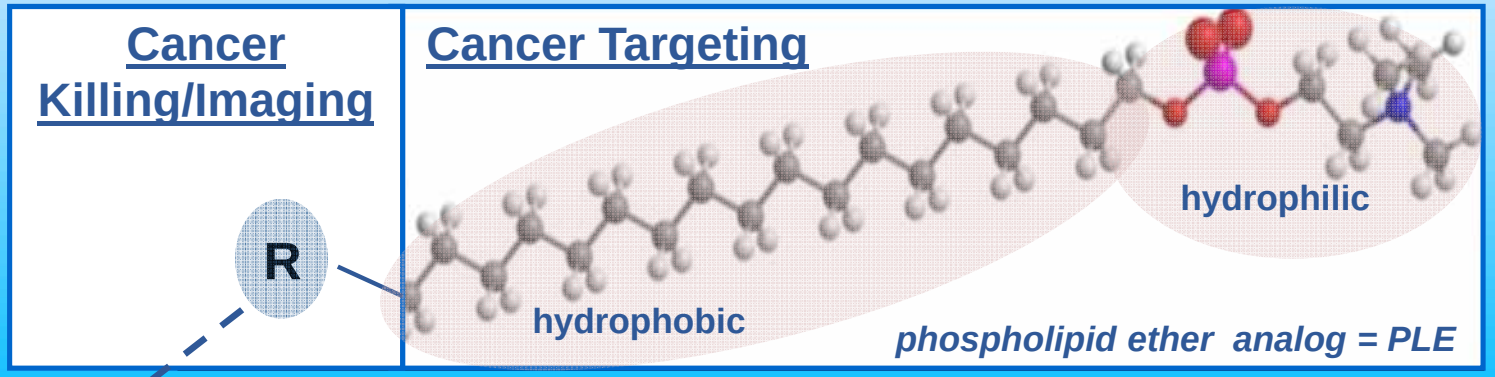
Intraoperative Margin Illumination & Non-Invasive Imaging

nIR PLE



Pre-IND

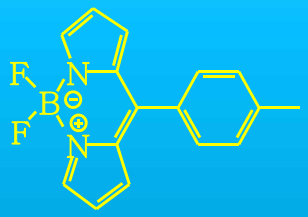
Cancer-Targeting Technology Platform



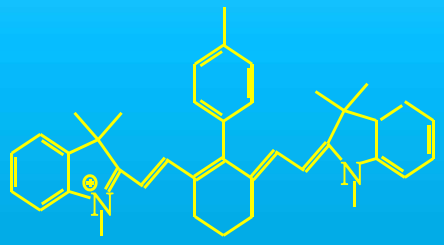
^{124}I-CLR1404 - *PET*

^{131}I-CLR1404 - *Radiation therapy*

^{127}I-CLR1404 - *Chemotherapy*



CLR1501
Optical, visible



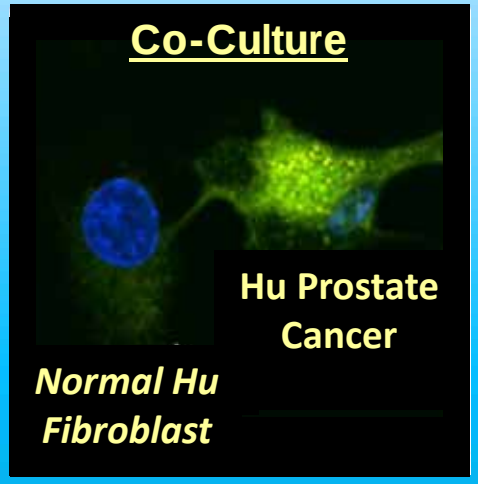
CLR1502
Optical, near-infrared

- Proprietary PLE chemical scaffold derived from substantial exploration of cancer-targeting SAR *(Pinchuk, et al. 2006, J Med Chem, 49:2155)*
- Aryl iodine bond very stable (free iodine not released)
- Selective uptake and prolonged retention in cancer cells
  - Bulk tolerance in R position

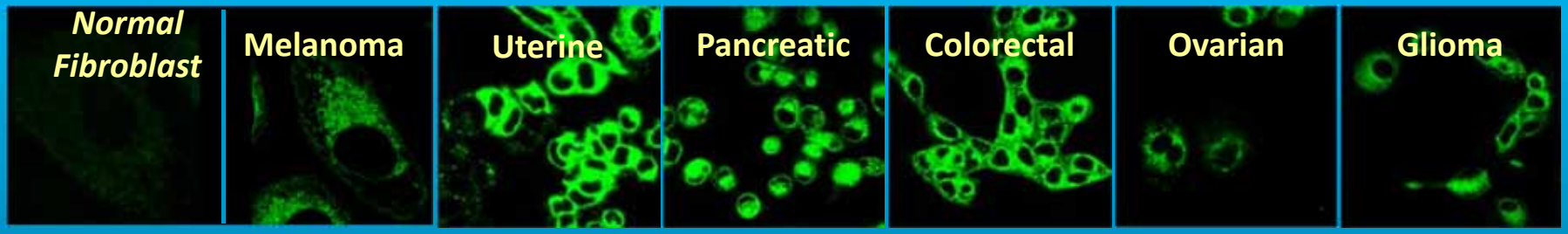
PLEs Selectively Target Cancer Cells

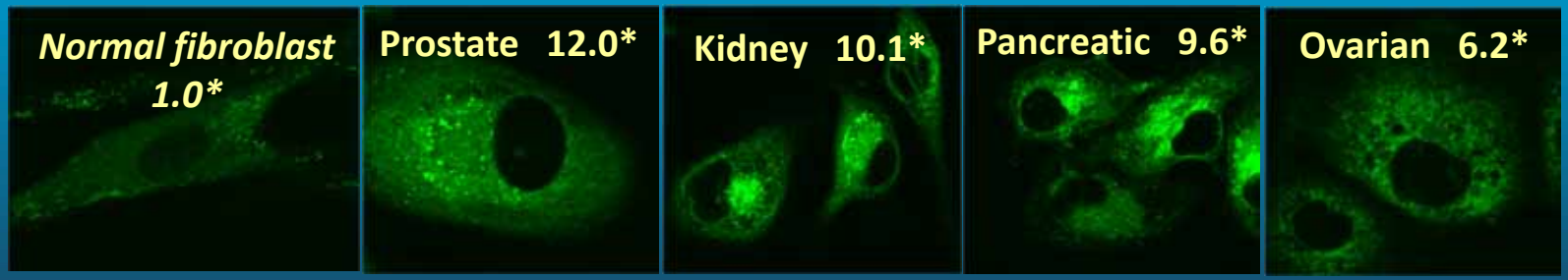
- **PLEs accumulate in cancer vs. normal cells**

  (24 hr incubation)



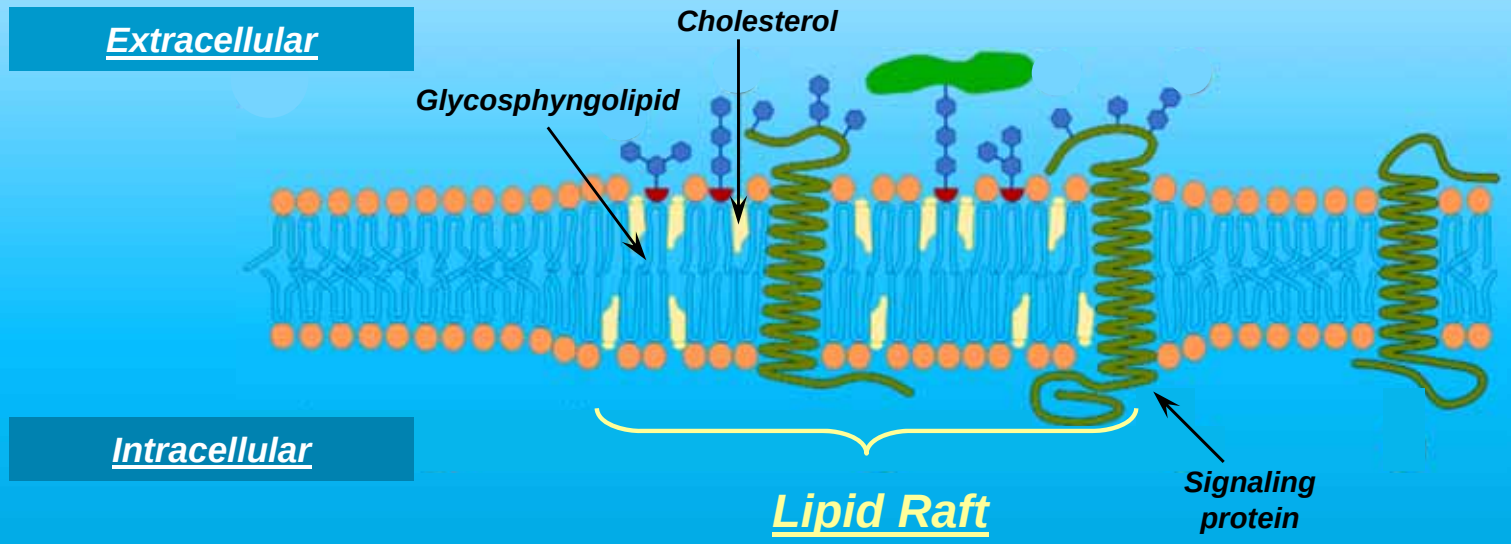
Green = CLR1501
Blue = cell nucleus





* *Fluorescent signal normalized to normal fibroblast (=1.0)*

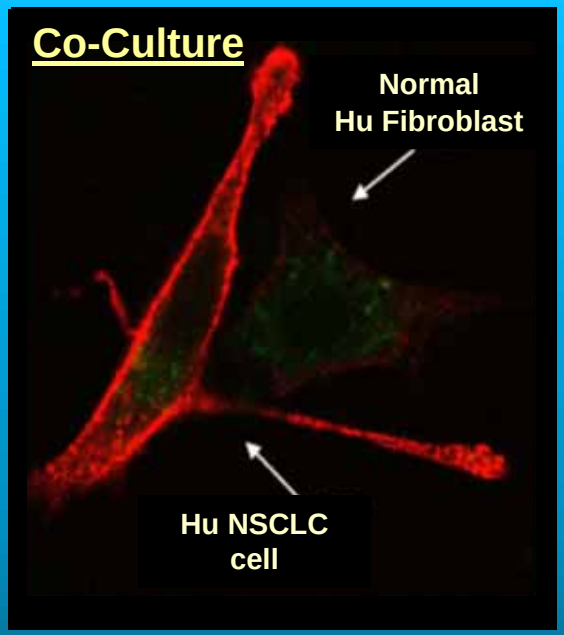
Lipid Rafts

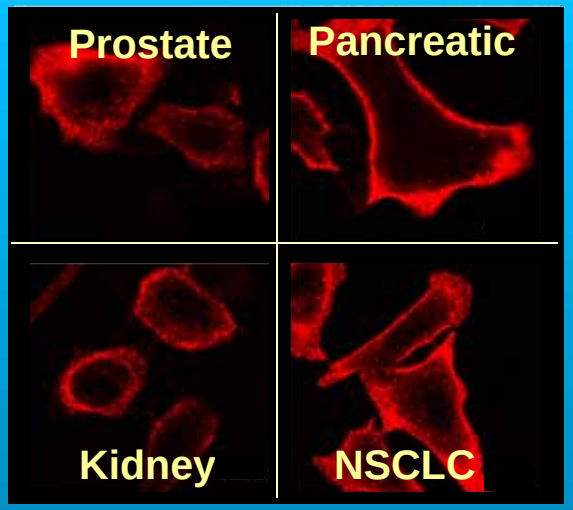


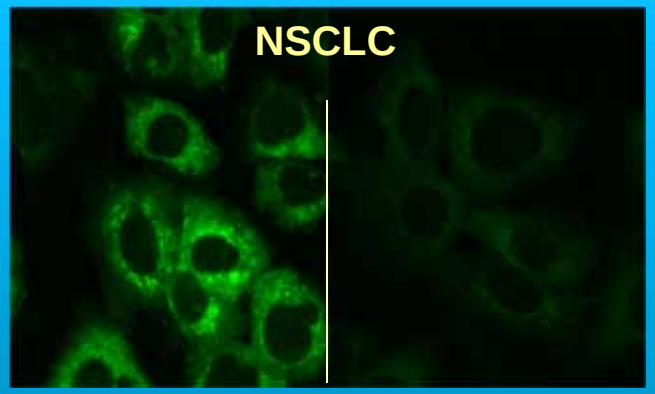
- Lipid rafts are specialized microdomains of plasma membrane that are enriched in cholesterol and glycosphingolipids
- Lipid rafts serve as molecular platforms that spatially organize molecules for specific signaling pathways including those involved in regulation of apoptosis and cell proliferation (e.g. growth factor receptors, Akt, TNF receptors)

Lipid Rafts are Over-Expressed in Cancer Cells

- PLE uptake into cancer cells is, at least in part, dependent upon intact plasma membrane lipid rafts







Intact Rafts

Disrupted Rafts
(Me-β-cyclodextrin pretreatment)

(red = fluorescent-labeled cholera toxin subunit B)

Green = CLR1501

Reduced PLE Catabolism in Neoplastic Cells/Tissues May Contribute to Retention

Tissue	PLE Catabolic Activity [a]
Rat Liver (normal)	*7.3*
RatMorris Hepatoma 7794A	5.8
RatMorris Hepatoma 7777	1.4
Mouse Sarcoma 180	0.42
Mouse Melanoma B-16	0.31
Mouse Ehrlich Ascites Carcinoma	0.14
Mouse KHZ Mam Tumor	0.11
RatWalker-256	0.10

[a] *Expressed as μmol of PLE cleaved/20 min/mg protein*

Soodsma, Piantadosi, Snyder, Cancer Research, 30:309 (1970)

Cell/Tissue	PLD [b] Protein [c]	PLD mRNA [d]
Rat Liver (normal)	14.1	12.2
Mouse CT26 colorectal	7.8	2.4
Mouse hepa-1 hepatoma	3.3	6.2
Mouse TS/A breast	2.8	4.0

[b] *PLD = phospholipase D*

[c] *mU fluorescence/μg protein/ml*

[d] *μg x 10^{-5}/0.01 μg total cDNA*

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PLEs Selectively Target a Wide Range of Malignant Tumors *In Vivo*

Yes *(52)*	Selective Uptake & Retention of ^{124}I-CLR1404	No *(2)*

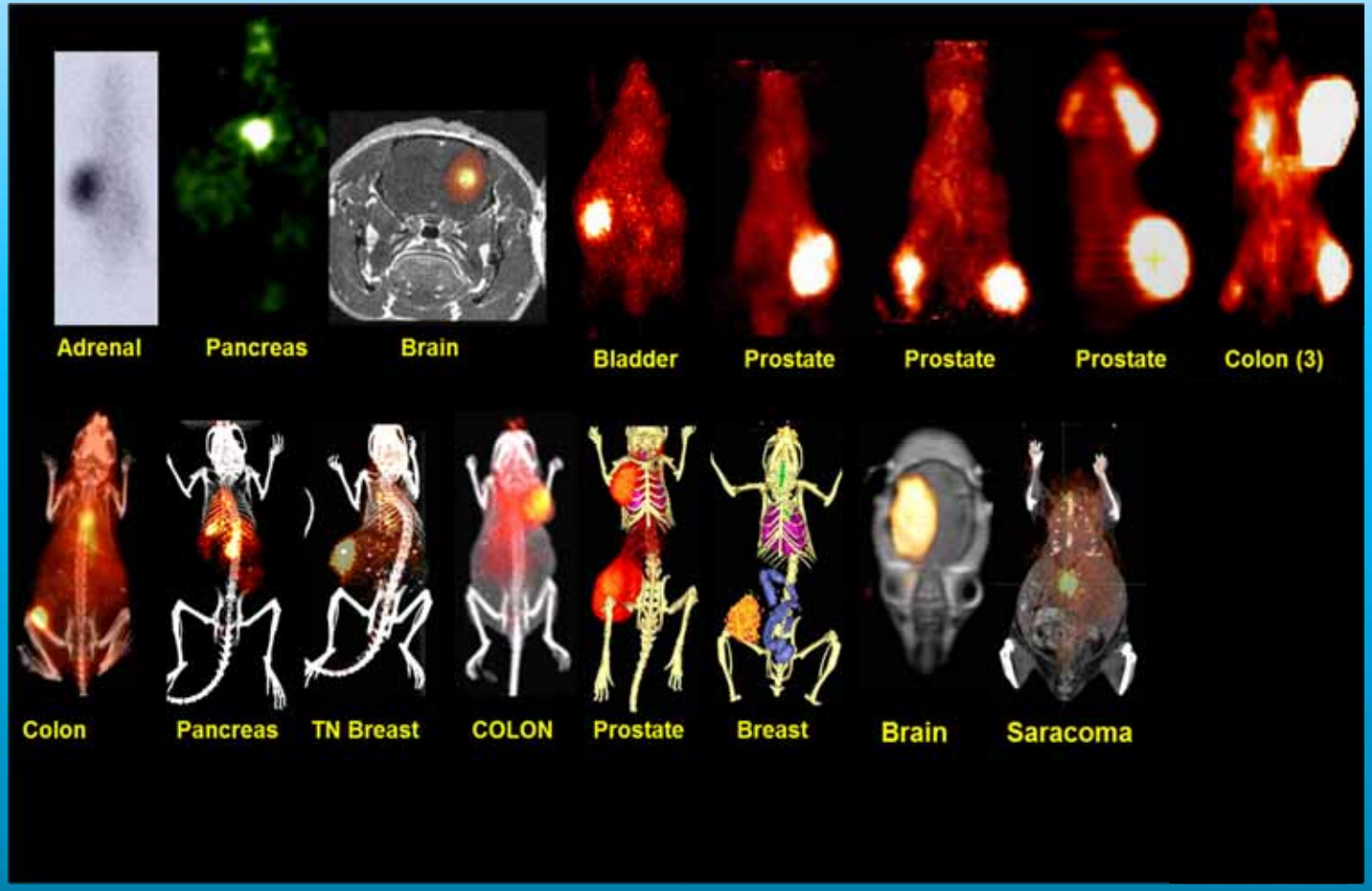
Human cancer xenografts
Prostate
Non-small cell lung
Adrenal
Colon
Melanoma
Ovarian
Pancreatic
Renal Cell
Prostate
Breast *(triple-negative)*

Rodent malignant tumors	
Breast	Glioma
Prostate *	Retinoblastoma
Colon	Pancreatic *
Intestinal *	Cervical *
Melanoma	Sarcoma
Mammary *	Esophageal *
Hepatocellular Carcinoma *	Hepatic

Mouse benign tumors
Intestinal polyp
Mammary alveolar hyperplasia

** Includes transgenic tumor models*

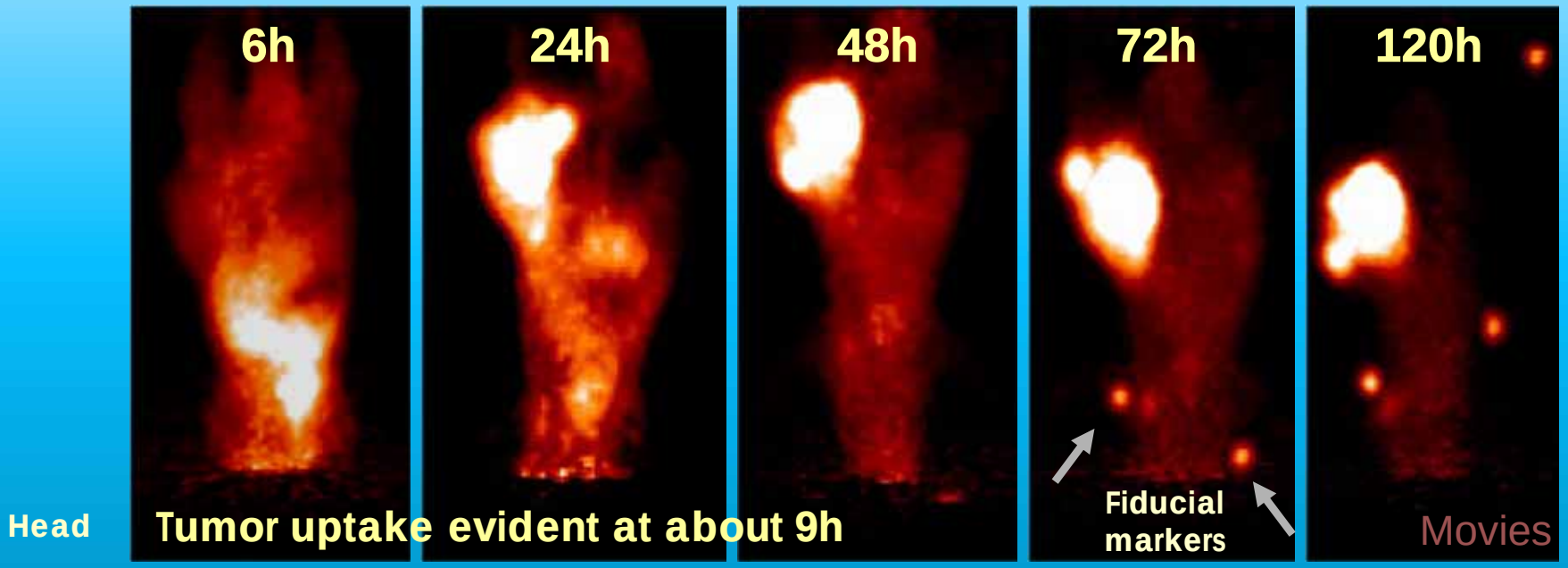
PLEs Selectively Target a Wide Range of Malignant Tumors *In Vivo*



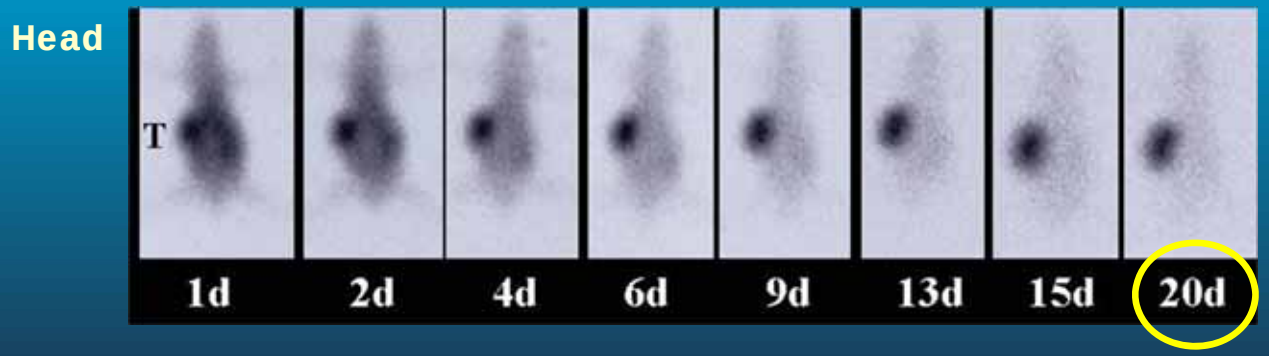
Representative nuclear and/or microPET/CT or MRI hybrid images demonstrating excellent primary and metastatic tumor conspicuity. Images were acquired from 24-96h post-i.v. injection (80-140 µCi of ^{124}I-CLR1404) in a variety of human subcutaneous or orthotopic xenograft, spontaneously induced, or transgenic in vivo tumor models.

CLR1404 Tumor Uptake/Retention *In Vivo*

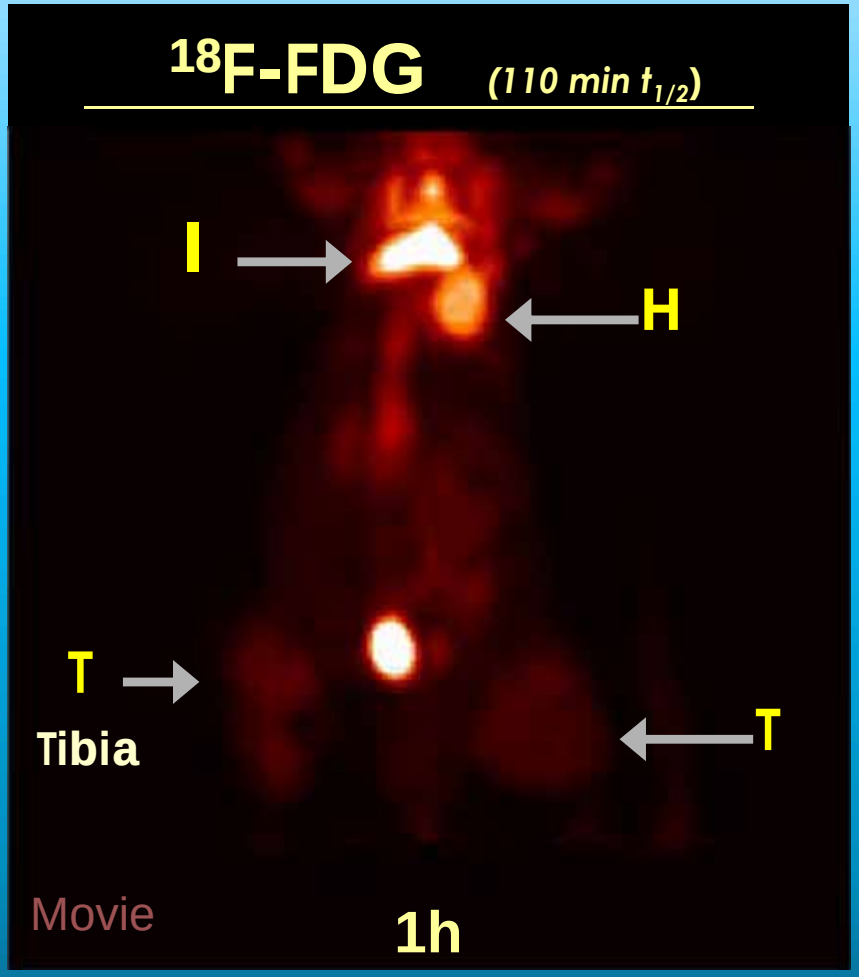
^{124}I-CLR1404 - PC3 hu prostate xenograft *(μPET scans)*

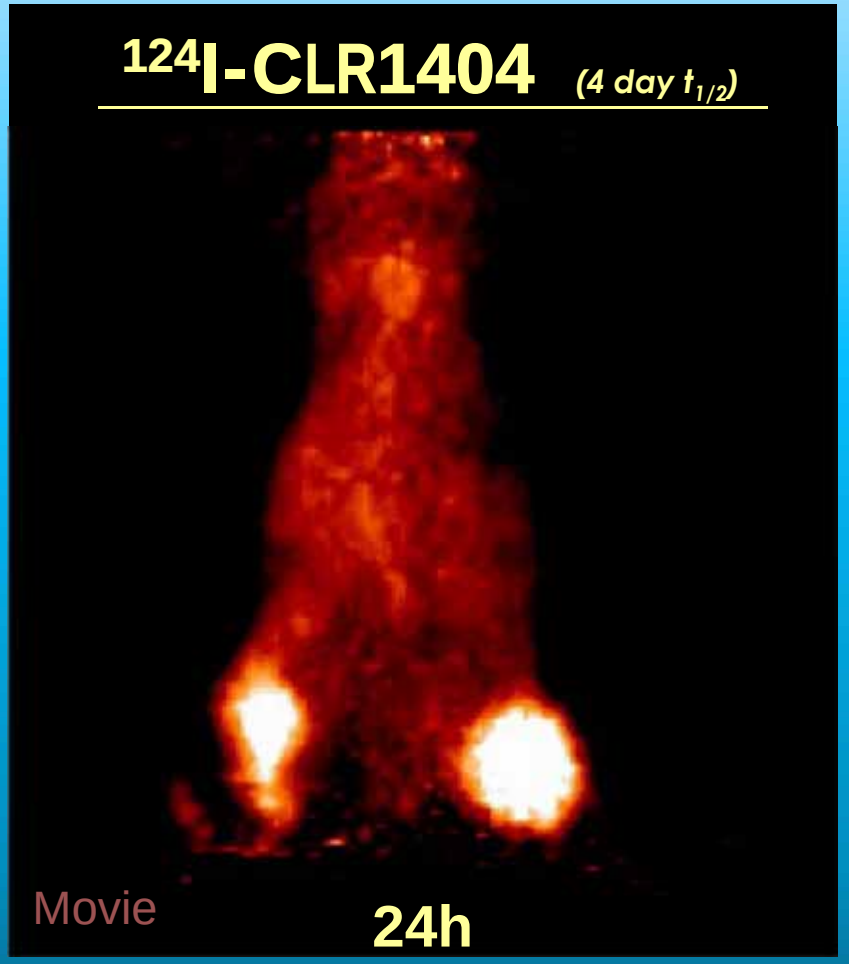


^{125}I-CLR1404 - 251 hu adrenal xenograft



Unlike FDG, CLR1404 Does Not Accumulate at Sites of Inflammation



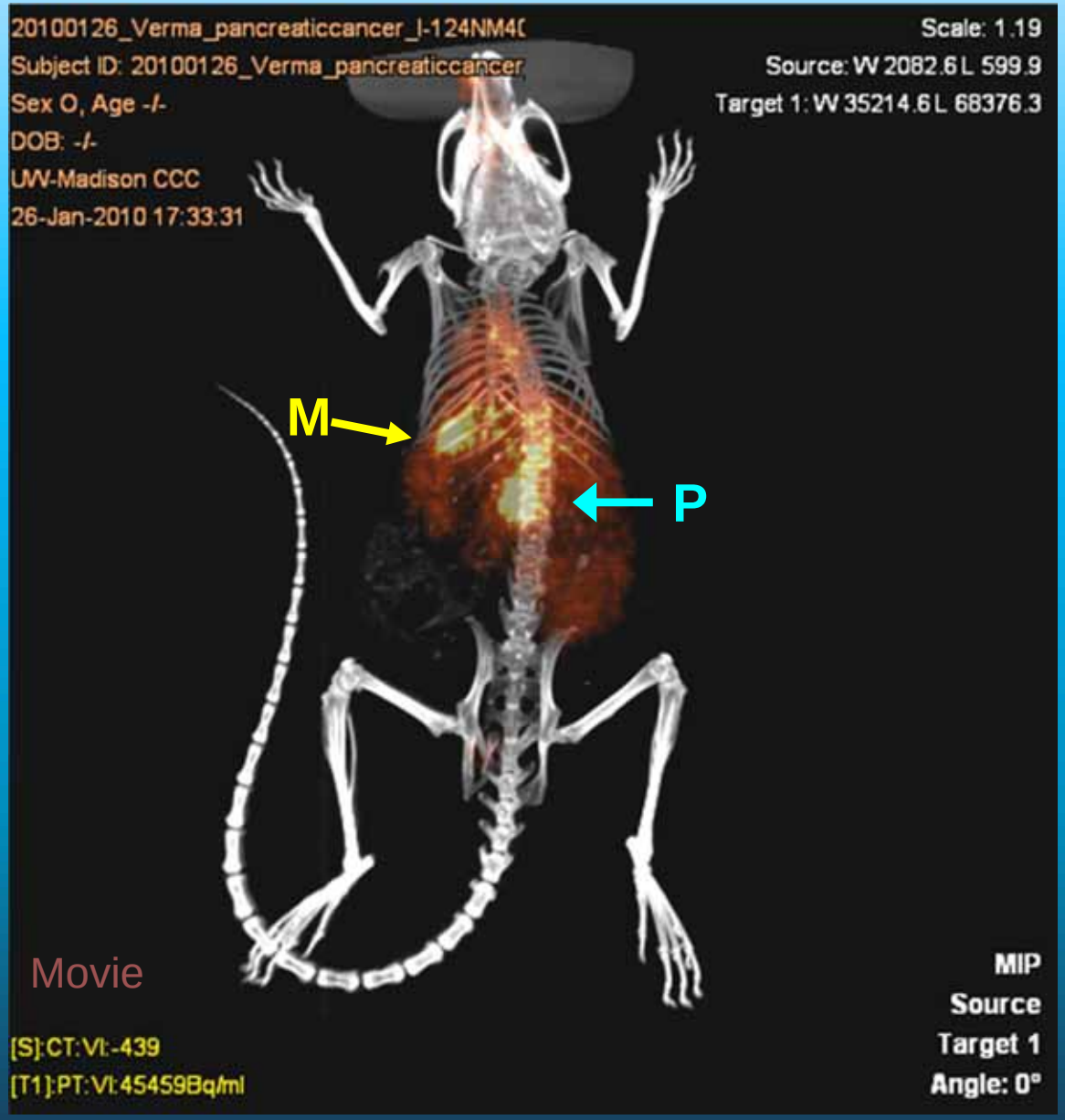


PC3 human prostate xenograft

***I** = carrageenan induced inflammatory lesion, **H** = heart, **T** = tumors*

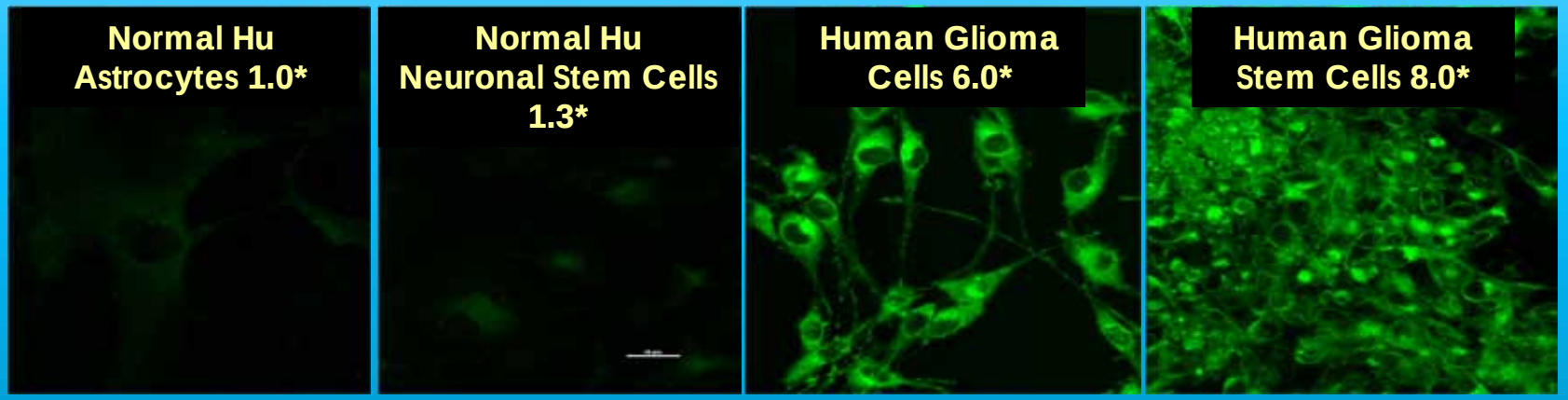
CLR1404 Targets Primary and Metastatic Tumors

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.

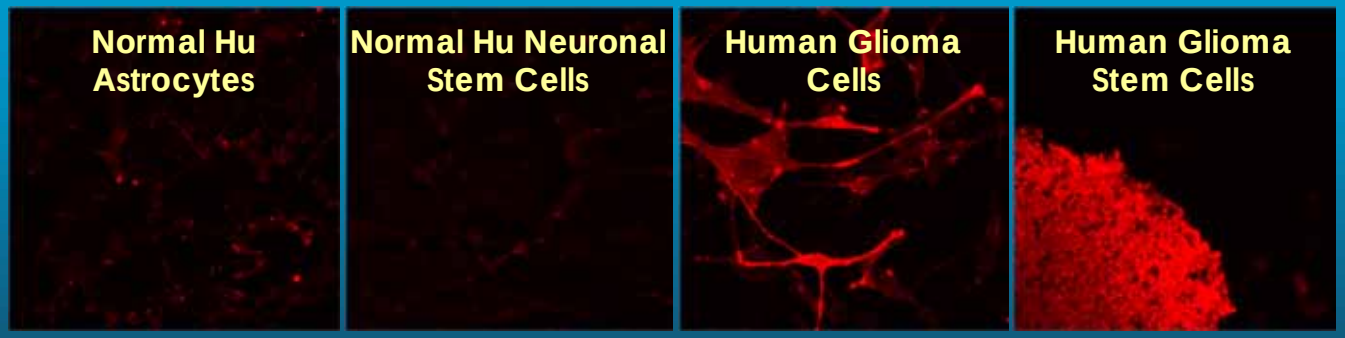


PLEs Target Cancer Stem Cells

- Growing database implicates cancer stem cells in
  - Tumor growth, metastasis
  - Resistance to chemotherapy, radiotherapy
  - Cancer relapse

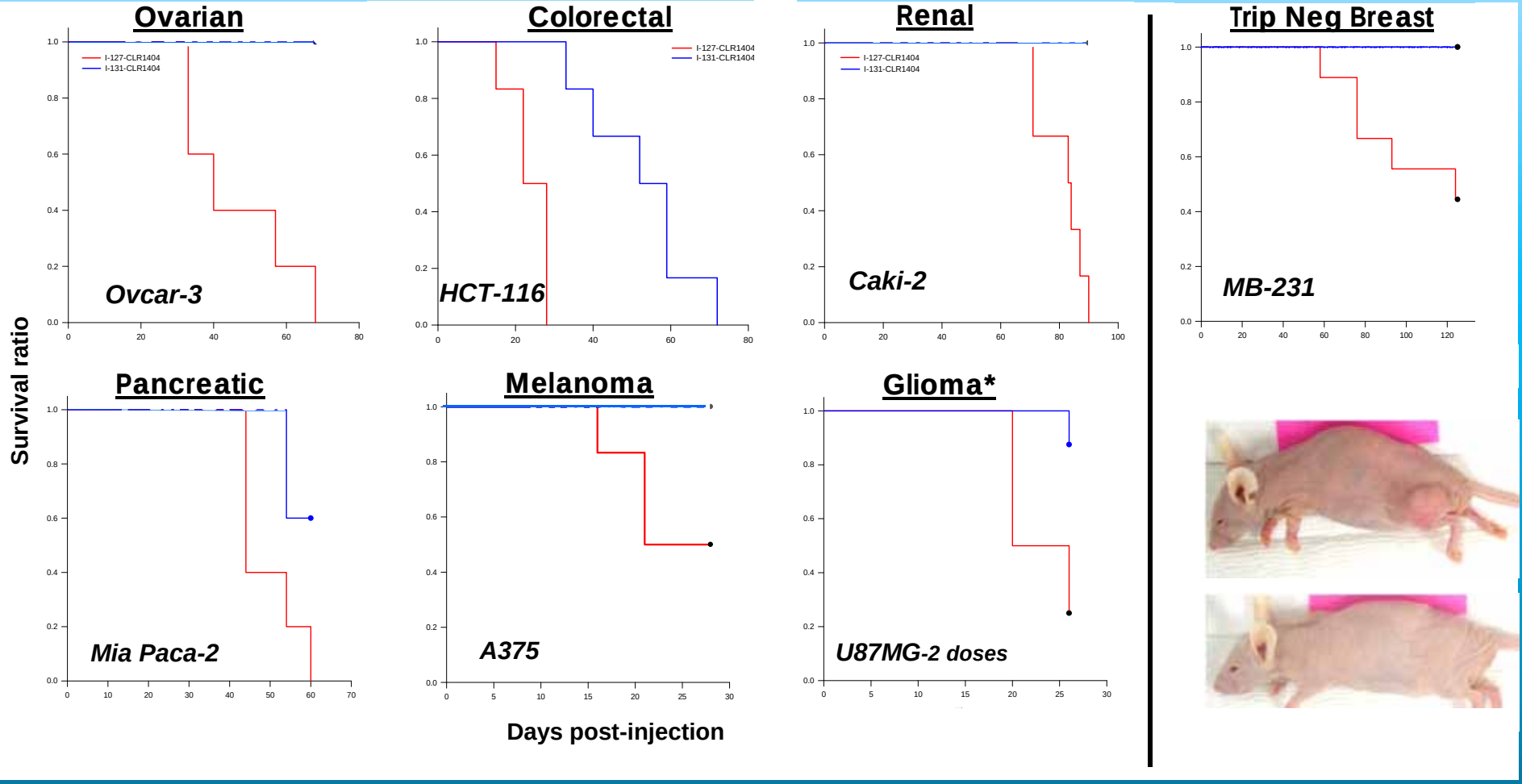


** fluorescent signal normalized to normal human astrocyte (=1.0); Green = CLR1501*



(red = lipid rafts; fluorescent-labeled cholera toxin subunit B)

^{131}I-CLR1404 is Highly Efficacious in Mouse Xenograft Models



A single dose of ^{131}I-CLR1404 (100 uCi, i.v., n=6 **BLUE**) was administered after tumors became established (~200 mm^3 = Day 0). Control = ^{127}I-CLR140(0.19 mg/kg; n=6 **RED**)*

(Two doses, one week apart for glioma model)*

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 of imperfect surrogates

- **The PLE-based diapeutic treatment paradigm offers advantages over existing approaches**
  - Chemically identical biomarker (^{124}I-)and therapeutic (^{131}I-) molecules (CLR1404) which are administered in ~ equal mass doses
  - PET/CT allows full-body, quantitative, 4-D mapping of biodistribution, and localization of primary tumors/metastases for diagnosis and disease staging
  - PET/CT based dosimetry may predict personalized therapy dose
    - Or no treatment if imaging shows suboptimal tumor or normal organ uptake

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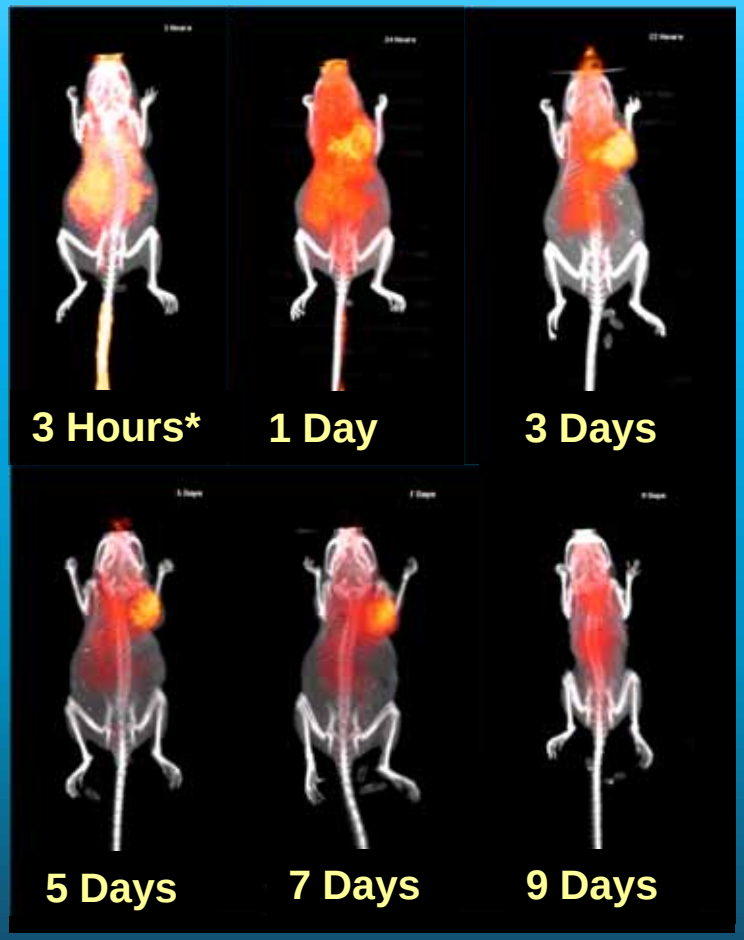
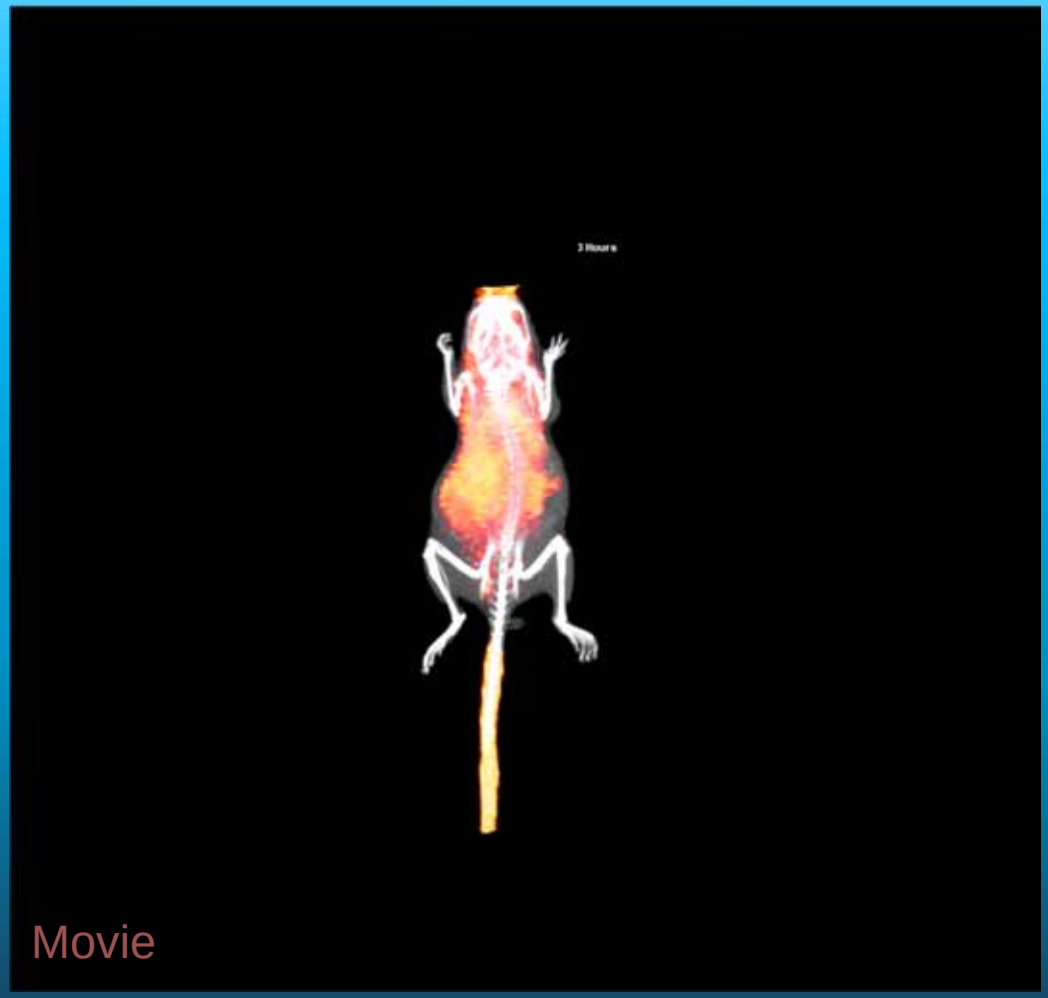
$^{124/131}$I-CLR1404 Diapeutic Paradigm

- PET/CT time course of an LS180 colon CA xenograft-bearing mouse injected i.v. with a single injection of a mixture of ^{124,131}I-CLR1404 (200 µCi each). Tumor shrinkage confirmed by CT. Weight loss seen near the end of the study.



* time post-injection

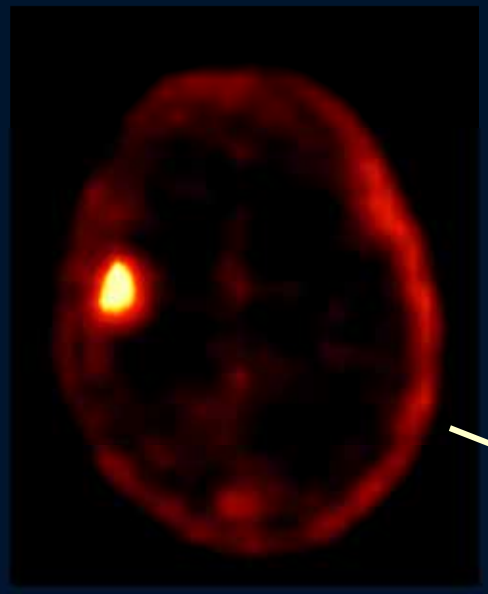


CLR1404 Platform – Clinical Studies

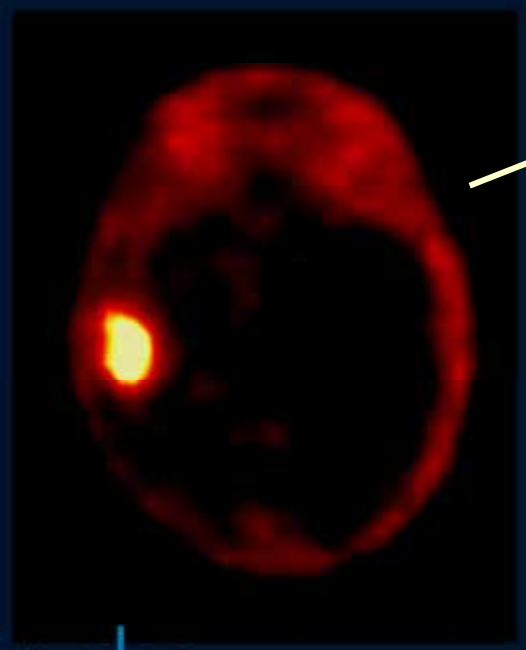
- **^{124}I-CLR1404 PET imaging agent**
  - **Ongoing Phase 1-2 trials in multiple tumor types**
    - **NSCLC, brain (primary and metastases), triple negative breast, soft tissue sarcoma, colorectal, gastric, esophageal, prostate, ovarian, pancreatic, and head & neck cancers**

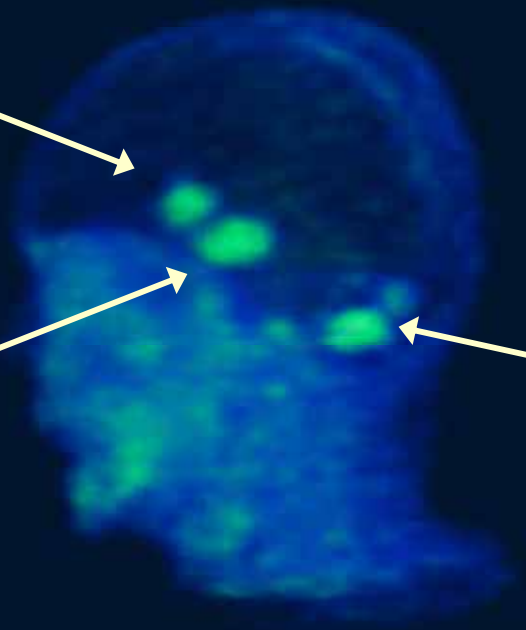
- **^{131}I-CLR1404 molecular radiotherapeutic agent**
  - **Phase 1a dosimetry trial successfully completed**
  - **Phase 1b escalating dose, MTD-seeking, multi-site trial is ongoing**
    - **NSCLC, triple negative breast, soft tissue sarcoma, colorectal, gastric, esophageal, prostate and ovarian cancers**

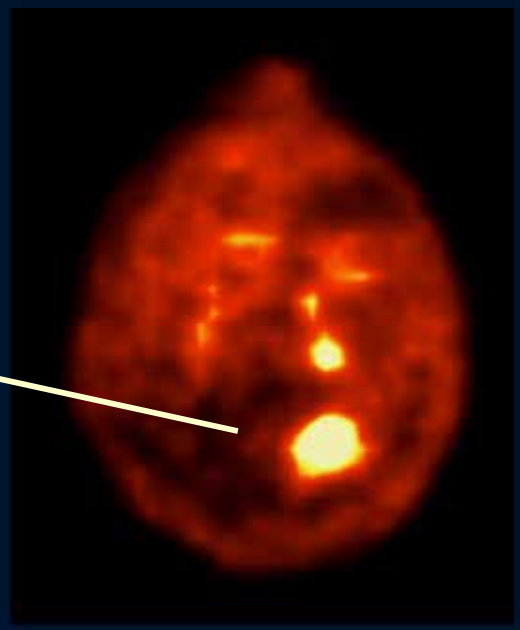
^{124}I-CLR1404 PET - NSCLC Brain Tumor Metastases



- Three previously unknown brain mets were discovered, altering treatment plan
- Selective tumor uptake against very low background in normal brain tissue







Movie

Imaged 6 days following a 5 mCi dose; confirmed with MRI

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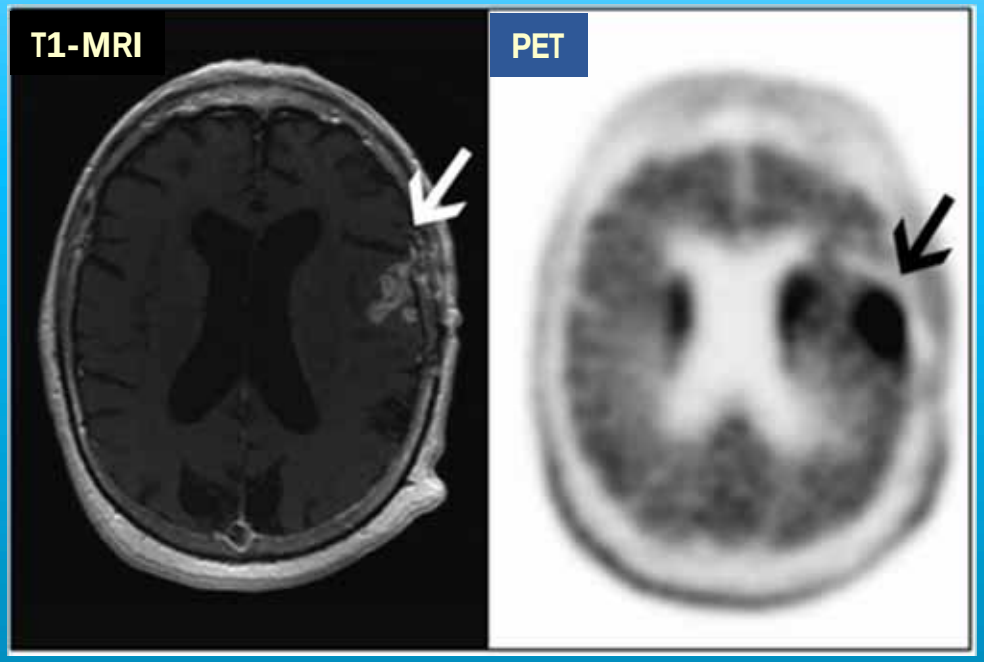
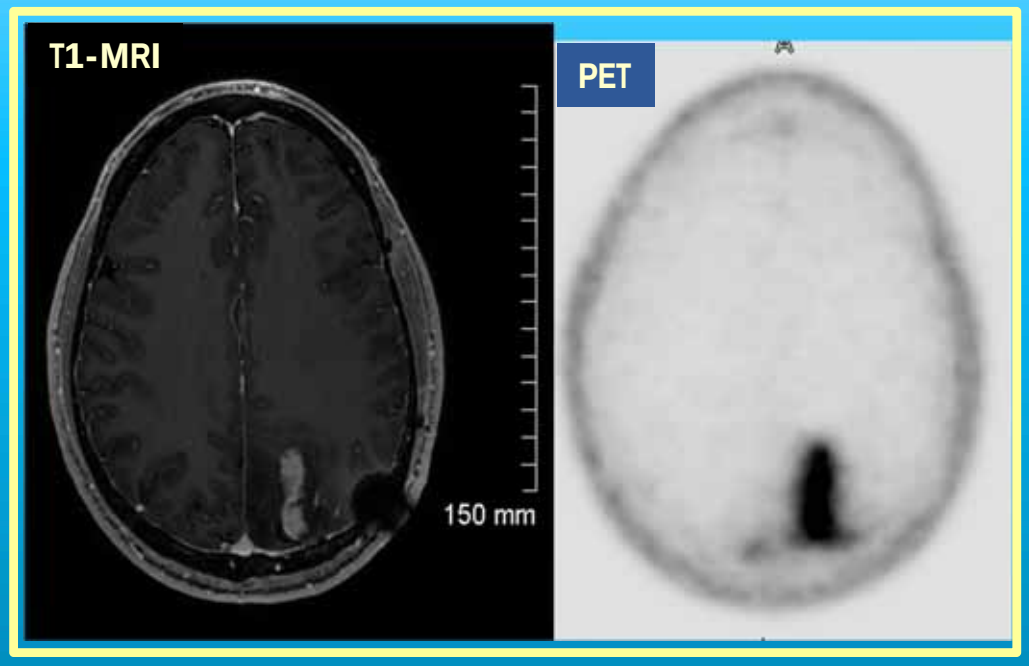
^{124}I-CLR1404 PET – Recurrent Glioblastoma

- ^{124}I-CLR1404 PET image shows **tumor to brain ratio of 30:1**
  (3-5 typically considered adequate in PET imaging)



^{18}F-DOPA PET

JNM Cover Image
Walter F, et al, J Nuc Med,
March, 2012, 53:393



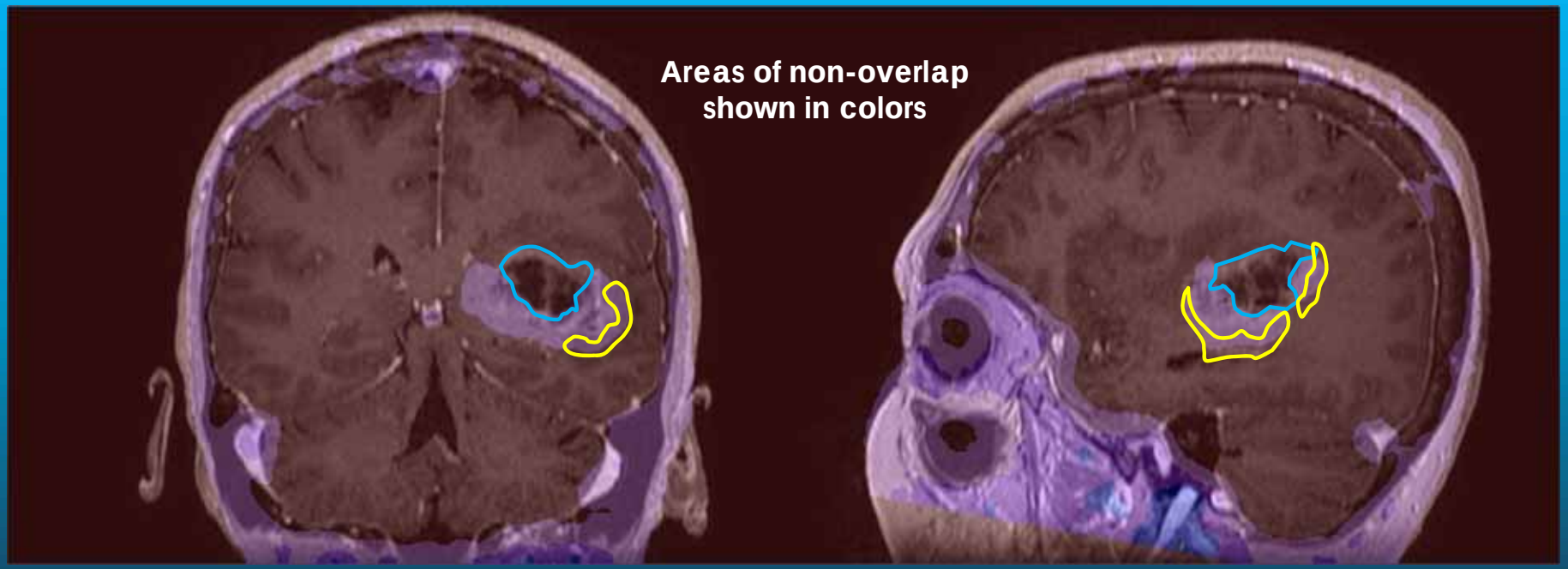
^{124}I-CLR1404 PET

48h post-5mCi dose

^{124}I-CLR1404 PET - Glioblastoma

- ^{124}I-CLR1404 PET and MRI tumor images are only partially overlapping
- This could reflect more accurate imaging of living, malignant tissue by ^{124}I-CLR1404 PET compared to MRI *(note: histopathology not performed on resected tumor)*

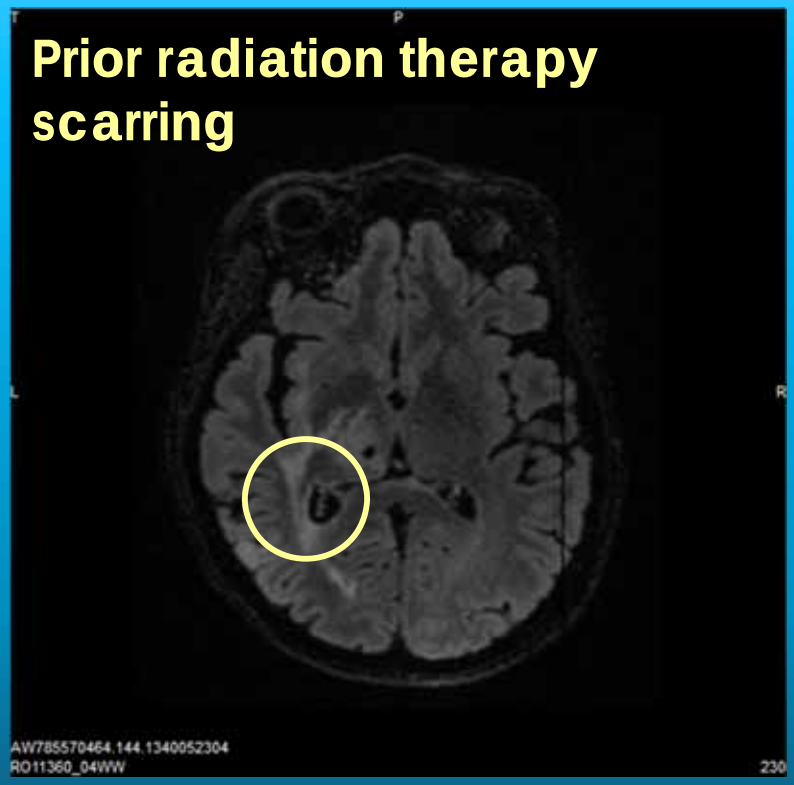
^{124}I-CLR1404 PET (*48h post-5mCi dose*)	MRI	Possible Interpretation
–	+	Necrotic tissue?
+	–	Malignant tissue?



Fused PET/MRI Images

^{124}I-CLR1404 PET – No Uptake in Glioma Scar Tissue

- ^{124}I-CLR1404 PET has the potential to differentiate growing tumor from pseudoprogression, enabling more timely and certain diagnosis



MRI

^{124}I-CLR1404 PET
(day 2 post-dose, 5 mCi)

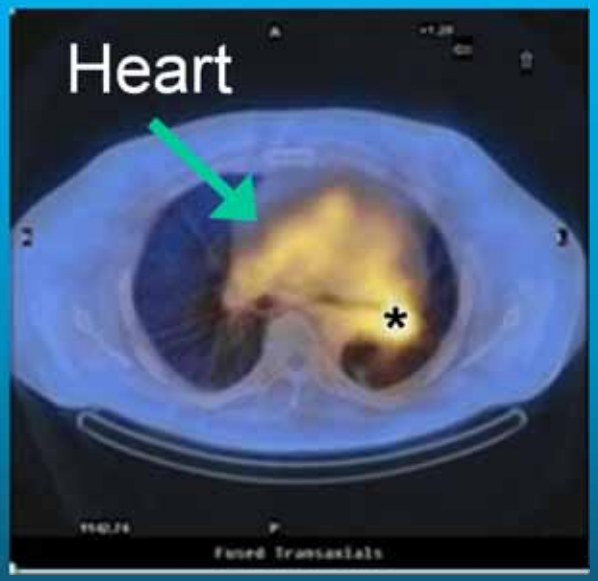
^{131}I-CLR1404 PET – Targets Tumors in Man

- SPECT/CT images from Phase 1a dosimetry study (10 mCi, Day 6)
- Demonstrated uptake and prolonged retention of ^{131}I-CLR1404 in cancerous tumors but not normal tissues

Prostate Cancer Metastases in Lumbar, Spine, Pelvis, Sacrum

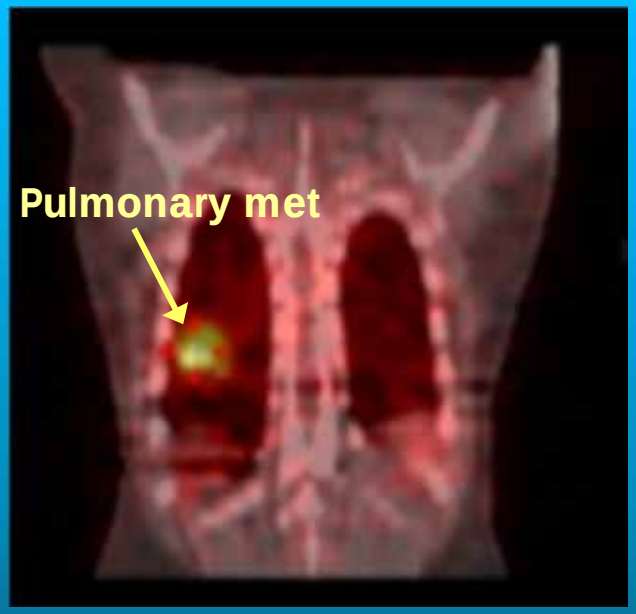
* = Tumor

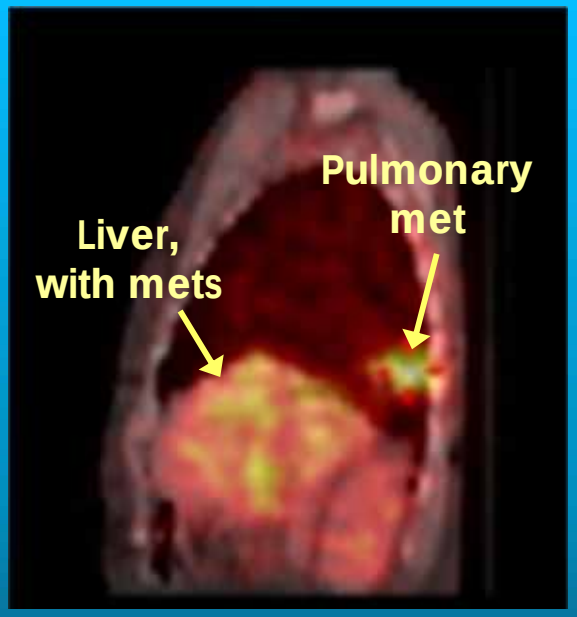
Colorectal Cancer Pulmonary Metastasis

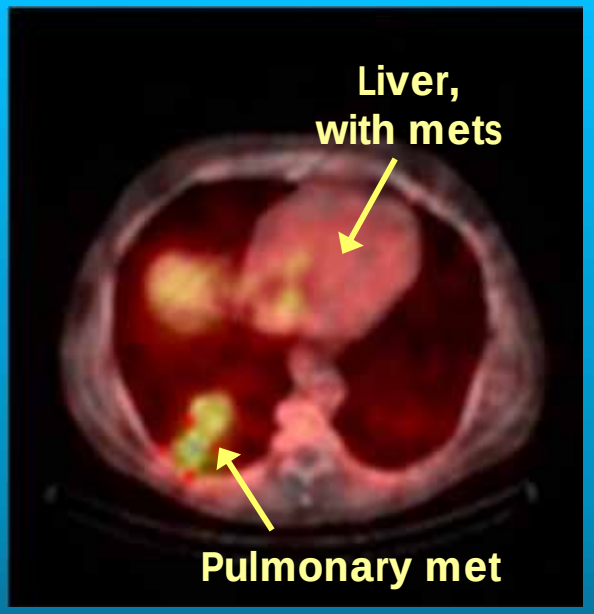


^{131}I-CLR1404 PET – Targets Tumors in Man

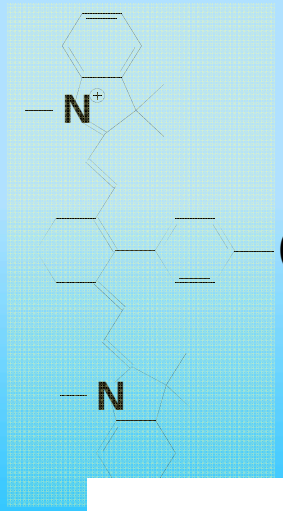
- SPECT/CT images from Phase 1b MTD study (27 mCi, Day 21)
- Demonstrated uptake and prolonged retention of ^{131}I-CLR1404 in cancerous tumors but not normal tissues







Colorectal cancer patient

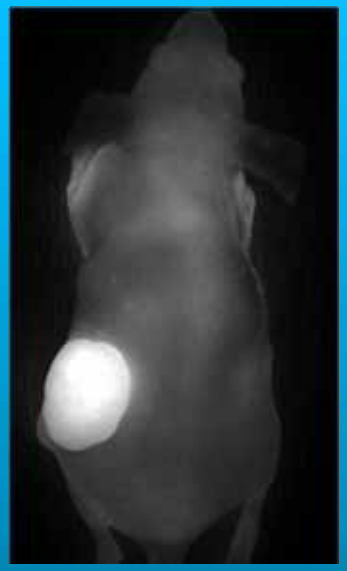


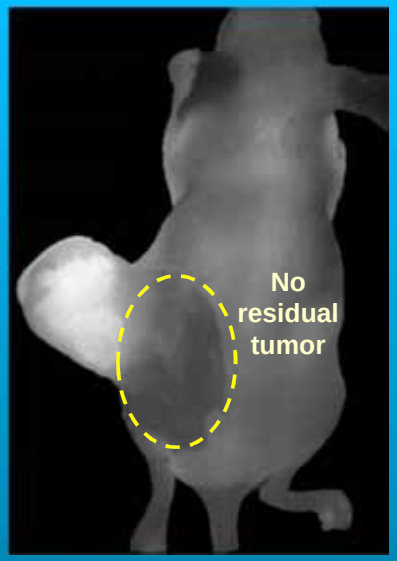
$(CH_2)_{18}OP(O)(O^-)OCH_2CH_2N^+(CH_3)_3$

CLR1502 - Intraoperative Tumor Margin Illumination and Non-Invasive Tumor Imaging

ve Tumor Imaging

Fluobeam™ (near-IR)





Intraoperative Tumor Margin Illumination in Real Time

Fluobeam ™ Fluoptics

HCT116 human colon tumor xenograft; 4 days post-CLR1502 injection

Summary

- Overabundant lipid rafts and deficits in PLE catabolism are believed to be involved in selective targeting of both differentiated cancer cells and cancer stem cells by PLEs
- As a consequence, PLE targeting of primary and metastatic tumors is broad-spectrum across a wide range tumor types
- ^{124}I-CLR1404 may have distinct advantages over ^{18}F-FDG as a PET agent
- Selective and prolonged accumulation in human cancer has been routinely observed in imaging with ^{131}I-CLR1404 and ^{124}I-CLR1404 in initial clinical studies
- Significant therapeutic efficacy has been seen with ^{131}I-CLR1404 in a wide range of xenograft models (tumor growth suppression and increased survival)
- Diapeutic pairing of ^{124}I-CLR1404 and ^{131}I-CLR1404 may offer a truly individualized approach to cancer diagnosis, staging, therapy and efficacy assessment
- Optical imaging PLEs show early promise for intraoperative tumor margin illumination and diagnosis

Thank you!

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Clinical Trial Sites

COH-Mortimer
Duke
Johns Hopkins
Georgetown
Univ Wisconsin

Univ Wisconsin

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

National Cancer Institute

UWCCC Grant
2 R21Grant (breast and lung)
1 RO1 Grant (glioma, brain mets)

Patient Volunteers