



# CBI

# Capabilities

with a focus on Oncology Studies

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COMPARATIVE BIOSCIENCES, INC.  
A TRANSLATIONAL APPROACH TO PRECLINICAL RESEARCH

# COMPARATIVE BIOSCIENCES, INC.

## Premier Preclinical Contract Research Organization

- **20 years** of experience
- Conveniently **located in the heart of Silicon Valley**, amidst many biotech companies
- **State of the art, purpose-built facility**
- Approximately **30 employees**
- **Highly experienced staff**
- **GLP, OECD, FDA, USDA, OLAW**
- **AAALAC Accreditation**



# Scientific Overview

***We specialize in developing a custom study plan in order to best meet your pre-clinical research needs and prepare for regulatory submission.***

- GLP and Non-GLP
- Toxicology
- Efficacy
- Pharmacokinetics
- Pharmacology
- Oncology Studies
- In-house histopathology, immunohistochemistry & TCR



# Our Staff

- **Study Directors**

- PhD level scientists
- Appointed by management for each job
- Serves as single point of control and is responsible and accountable for study conduct and scientific interpretation
- Experienced, attentive, and communicative
- Rapid study initiation and report preparation

- **Research Associates**

- Bachelor level scientists
- Extensive technical training

- **Quality Assurance**

- Rigorous Training Program



# CBI Animal Facility

- **Dedicated Rooms:**
  - Six small animal and four large animal rooms
  - Two procedure rooms, two surgery rooms
  - Two rooms with ventilation for immuno-compromised animals
- **Air Quality Systems:**
  - HVAC, light control in each room
  - 10-15 air changes per hour with positive air flow and filtered air
- **Cleanliness:**
  - Dual corridor system with pass-thru Basil cage washer
  - Daily environmental monitoring
  - Regular disease, bacterial and water surveillance
- **24/7 Staff**



# Species

- **Mice and Rats:** transgenic, knockout, immuno-compromised, and wild-type strains
- **Guinea Pigs, Hamsters, Gerbils**
- **Rabbits:** New Zealand White, Dutch Belted
- **Ferrets**
- **Chinchillas**
- **Dogs:** Lab Beagles
- **Mini-Pigs:** Gottingen, Yucatan



# Test Articles

- **Small molecules**
- **Biologics**
  - Peptides
  - Antibodies
  - Vaccines
  - siRNA & Nucleic Acids
- **Stem cells & cell therapies**
- **Devices**
- **Device and drug combinations**
- **Regenerative medicine**



# Toxicology Studies

- **GLP and Non-GLP studies**
- **Single-dose and multiple-dose studies**
- **All routes of administration**
- **Acute & chronic studies**
- **Discovery and Investigative toxicology**
- **Non-standard routes of administration**
- **Complete, prompt reports**





# Pharmacokinetic Studies

- **GLP and Non-GLP studies**
- **Single-dose or multiple-dose studies**
- **Sample analysis from blood, urine, CSF, feces**
- **Measure C<sub>max</sub>, T<sub>max</sub>, AUC**
- **Metabolic and pharmacodynamics studies**
- **Non-standard routes of administration**



# Pharmacology and Efficacy Studies

- **Pharmacology and efficacy modeling in multiple areas**
- **Custom model development**
- **Surgical modeling**
- **Investigative studies**
- **Combination GLP efficacy and toxicology**



# Pharmacology and Efficacy Studies

- **Animal Disease Models for Multiple Indications**
  - Ocular
  - Otic
  - Cardiovascular
  - Inflammation
  - Dermatology
  - Arthritis
  - Allergic and Immune Mediated studies
  - Wound healing and scarring
  - Fibrotic: skin and lung
  - Anti-infective studies
  - Oncology
  - Botulinum toxin
  - Central nervous system
  - Regenerative medicine
  - Osteogenesis imperfecta/  
Osteoporosis



# Histopathology Services

- **Complete In House Laboratories**
  - Paraffin, cryotomy, plastic
  - Immunohistochemistry
  - Special Stains
- **Toxicology Pathology:** Necropsy, Histopathology, Histomorphometry
- **Molecular Pathology:** Procurement, embedding and sectioning of tissue studies
- **Highly skilled staff**
  - Trained technicians
- **Validated pathology data acquisition system**
- **Evaluation and report by Board Certified Pathologist**



# Investigative Pathology

- **Histomorphometry:** quantitative analysis of IHC stained slides; Capillary angiogenesis in whole mount Retina , and Fibrosis
- **Tissue cross reactivity studies (TCR)**
- **Immunofluorescence** and Confocal laser scanning microscopy, Transmission electron microscopy (TEM)
- **Synthesis of DNA/RNA Probes**
- **In situ Hybridization** (CISH and FISH)
  
- Histopathology evaluation by board certified pathologist
- Report with representative photomicrographs
- Archiving: Report, Specimens, Slides, FFPE, FROZEN and Plastic blocks



# COMPARATIVE BIOSCIENCES, INC.

## Tour of Facility



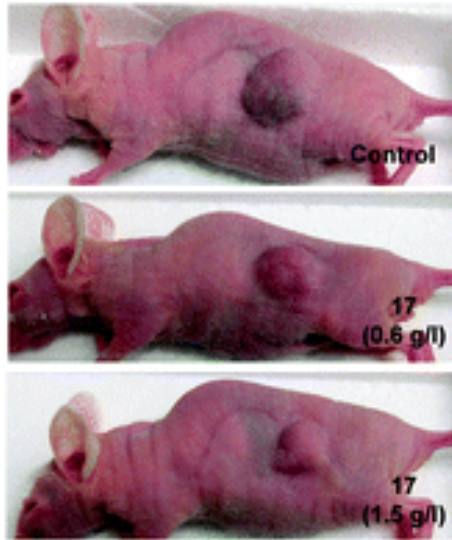
# Oncology Study Capabilities

## **CBI's experience includes**

- **Establish in-house xenograft with numerous cell lines**
- **Human xenograft implants (PDX)**
- **Rodent syngeneic implants**
- **Orthotopic Implants**
- **Metastatic models**
- **Angiogenesis models**
- **Biomarker Discovery and Validation**
- **In vitro assays**

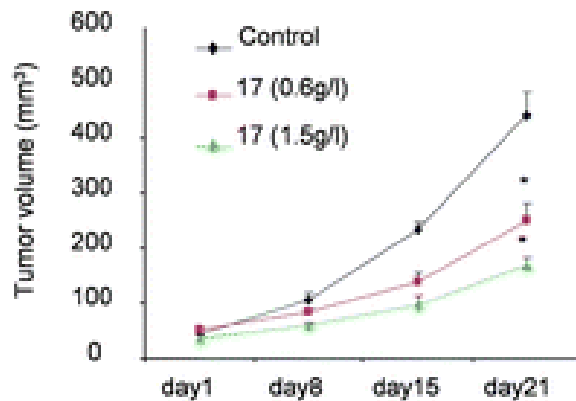


# Xenograft Model: Study Design



## Available Cell Lines Include:

- **Brain:** U87, CMLV
- **Breast:** MCF7, MDA MB-231, MDA-MB-435, MDA-MB-468, T-47D
- **Cervical:** C33A, Swiss HeLa
- **Colon:** KM12, COLO 205, HCT-15, HT-29, HCT-116, LS174T, DLD-1, LS1034
- **Epidermoid Carcinoma:** A431
- **Fibrosarcoma:** HT-1080
- **Gastric Carcinoma:** MKN-45, SNU-638, SNU-620, SNU-5, Hs746T
- **Glioma:** GOGUVM, U87MG
- **Head and Neck:** Hep-2 (contaminated w/ HeLa)
- **Lung:** A549, NCI-H69, NCI-H226, NCI-H441, NCI-H460
- **Melanoma:** LOX-IMVI, SKMEL-28
- **Myeloid:** Daudi Cell, Kasumi-1, Jeko, Rabbit VX2, HL60
- **Ovarian:** SK-OV-3, OVCAR-3, OVCAR-5, ES-2
- **Pancreas:** CFPAC-1, PANC-1, BxPC-3, MIA PaCa-2
- **Prostate:** CRW22, LNCaP, PC-3, DU-145, MDA PCa 2b
- **Renal:** CAKI-1, A498, SN12C, ACHN, 769-P





# Xenograft Model: Study Design

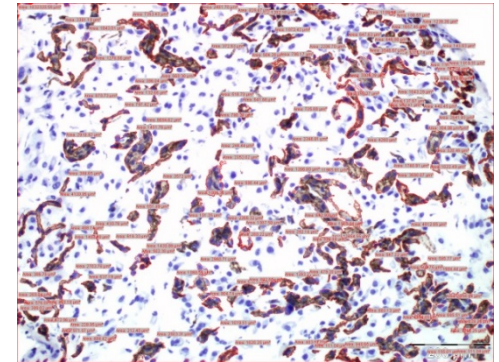
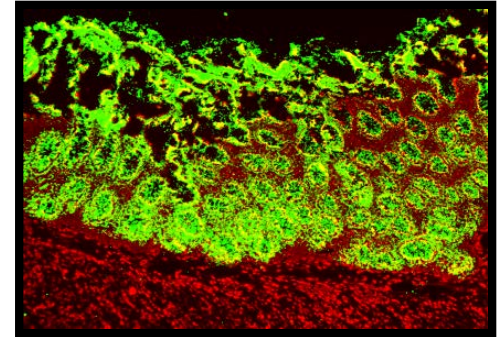
## Supporting Capabilities for Assessment of Anticancer Candidates include:

- Pharmacokinetics including radiolabeling
- Maximum tolerated dose, acute and subacute toxicity
- Hematology and clinical chemistry
- Histopathology including GLP toxicological pathology
- Immunohistochemistry, immunofluorescence, in situ hybridization
- Antibody Cross Reactivity Studies (research and GLP)



# Xenograft Model: Histological Results

- **Histopathology is a powerful tool in the assessment of anti-cancer agents**
  - Complete necropsy, organ weights, clinical chemistry
  - Routine paraffin sectioning and special stains
  - Cryotomy and Immunohistochemistry including:
    - TUNEL , PCNA, Transferrin, Angiogenesis, Markers
    - Necrosis
  - In Situ Hybridization, Fluorescent Angiography, Plastics
  - Antibody Cross Reactivity
  - Digital Image Analysis
  - ACVP Board Certified Veterinary Pathologist



# PDX Mouse Models

- **Patient derived tumor xenografts (PDX)**
  - Cancerous tissue from a patient's primary tumor is implanted directly into an immunodeficient mouse.
  - Test article efficacy will be tested on tumor bearing mice.
  - Partnership is local collaborators that can provide custom PDX lines for studies



# Syngenic Tumor Model



- Syngenic tumor models utilize immunocompetent animals as the host, enabling the efficacy evaluation of cancer therapeutic.

- Breast Cancer: *4T1 (BALB/c), DB7*
- Colon Cancer: *C51, CT-26*
- Lung Cancer: *Lewis lung carcinoma*
- Melanoma and Ocular melanoma: *B16F10, B16F1 (C57B6)*
- Renal Cell Carcinoma: *RENCA*
- Teratoma: *F9*



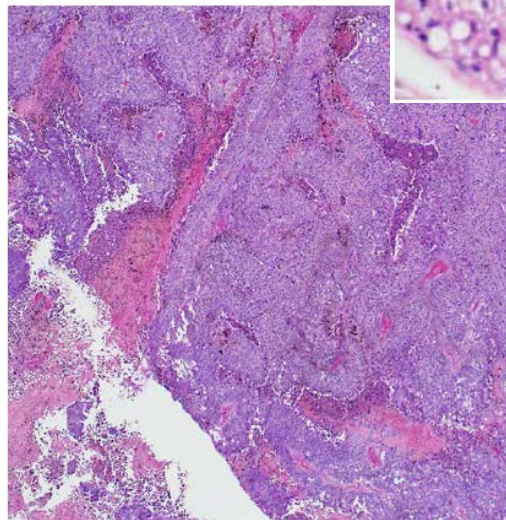
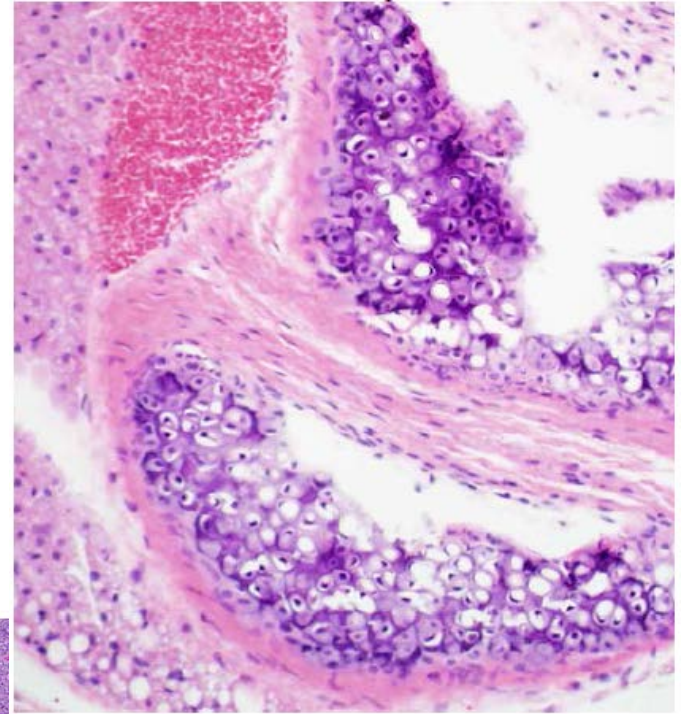
# Orthotopic Tumor Model

- Implantation of tumor cells into the organ of origin allows organotypical interaction between tumor cells and surrounding stroma.
- Tumor cells are implanted surgically at target sites
  - Pancreas
  - Prostate
  - Spleen (LS174T)
  - Brain



# Metastatic Tumor Model

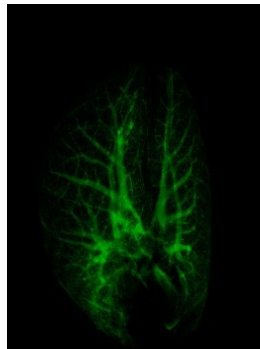
- Certain tumor lines readily metastasize
- Metastases may be measured or counted
- Metastatic lines LS174T, MCF-7, HT29 well established
- B16-F10 metastatic ocular melanoma



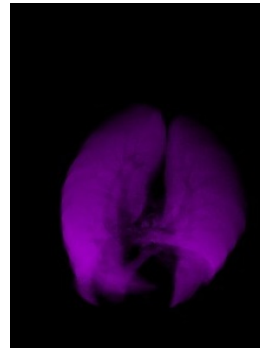


# Metastatic Lung Imaged by microCT

Normal Mouse Lung

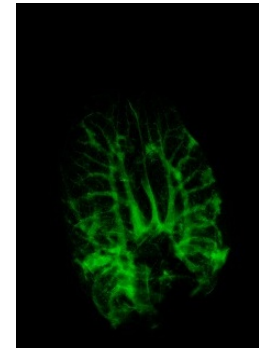


Tissue Volume

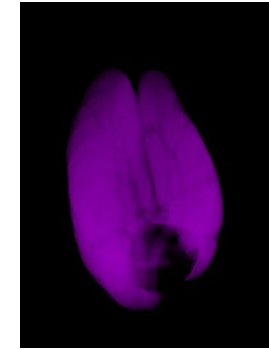


Airway Volume

EBC-1 Bearing Mouse Lung

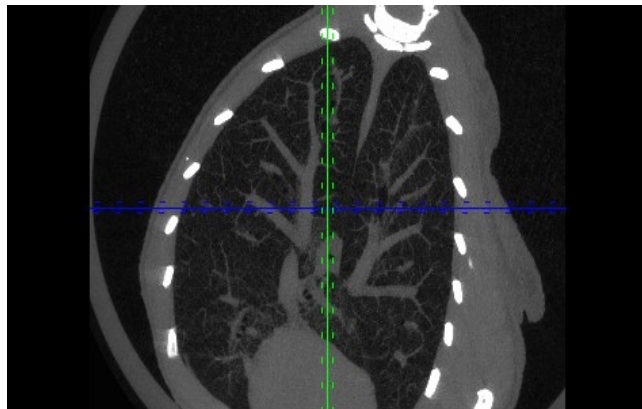


Tissue Volume

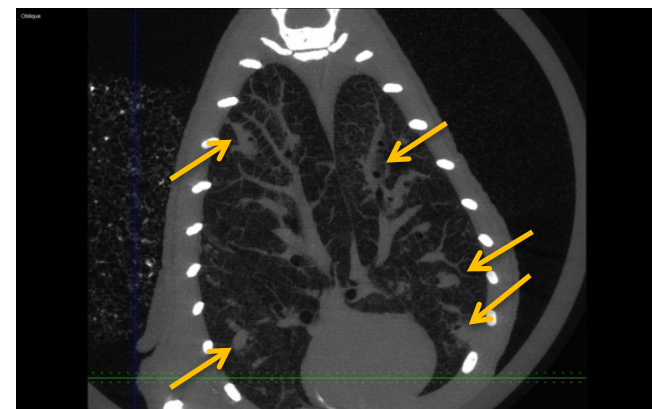


Airway Volume

3D Volumetric  
images



CT Slice



# Angiogenesis

- Mechanism of Angiogenesis critical area of cancer research
- Neovascularization of tumor, metastases, implants, corneal implants may be assessed
  - Fluorescent angiography
  - Histopathology
  - Immunohistochemistry
  - Immunofluorescence
- Knockout and transgenic mice with vascular defects available
- Corneal Micropocket in Mice and Rats
- Analytical peptide
  - apoptosis tracers *in vivo or ex vivo* stain apoptotic cells prior to explanation and analysis of the tissue





# Angiogenesis

- Chick Chorioallantoic Membrane (CAM) assay; Stimulation and Inhibition of angiogenesis
- *Ex-Vivo* angiogenesis; Tube formation



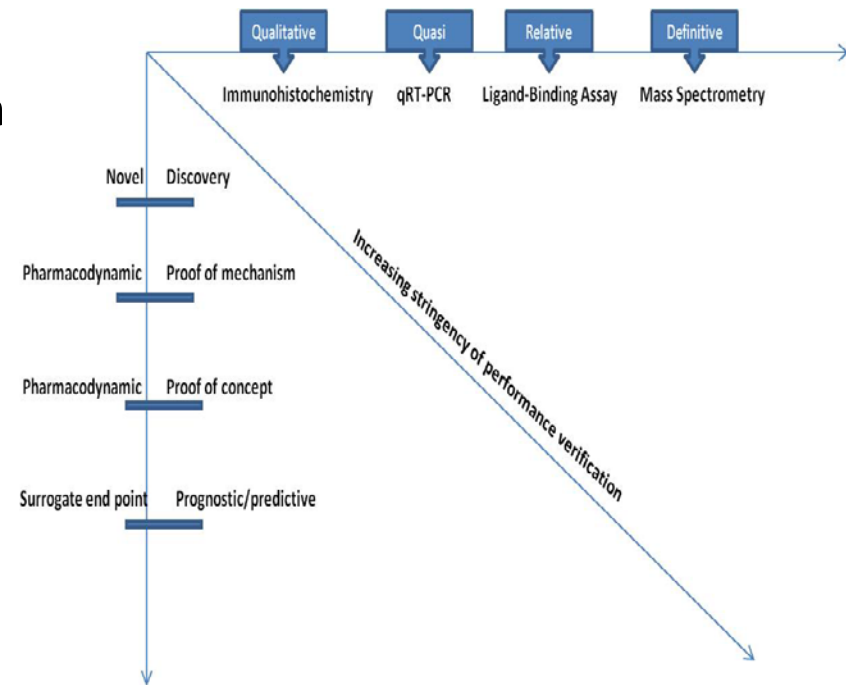
# Angiogenesis – Corneal Micropocket Assay

- The study and development of anti-angiogenic therapies depends on reliable and reproducible stimulation models of neovascular response. The corneal micropocket provides a robust and rapid assay
- Hydron pellet preparation with varying basic fibroblast growth factor (bFGF) concentrations, which is adjusted for specific study requirements
- Pellet is surgically implanted into the base of the corneal micropocket
- Histologic examination records neovascularization in the cornea using a slit lamp microscope connected to a camera
- Intravital fluorescent angiography is conducted
- Pharmacokinetics of aqueous or vitreous
- Histopathology and immunohistochemistry



# Biomarker Discovery and Validation

- Target discovery
- Mechanism of action using knockdown and over expression of target molecule
- Human primary cell assays (using HUVEC or human primary cells from patients)
- Enzyme immunoassay (EIA) for Collagen, Cytokines
- TUNEL, MTT, ADCC, CDC assay
- Bioluminescence and MicroPET
- Protein analytic ELISA, WB, IP
- patients)
- Enzyme immunoassay (EIA) for Collagen,
- Protein analytic ELISA, WB, IP



Overview of Biomarker method validation

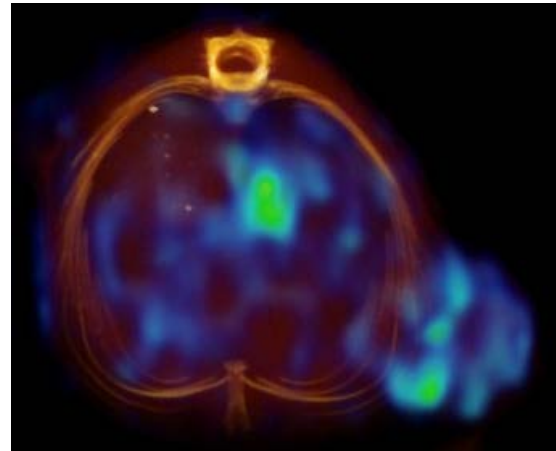
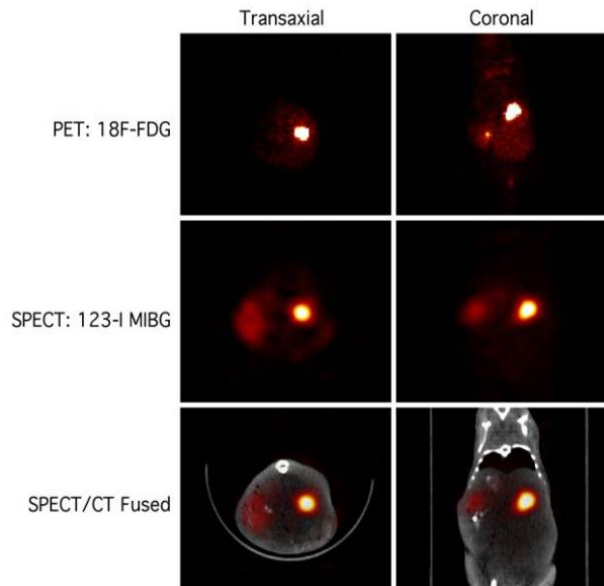
# Quantitative Molecular Imaging

- Compelling Advantages of this New Additional Capability
  - Disease staging
  - Decreased animal usage
  - Decreased drug needed
  - Imaging complements histology
  - Data mining – 4D image replicas can be re-interrogated
  - FDA endorsed, IND accepted – becoming expected, Critical Path Initiative

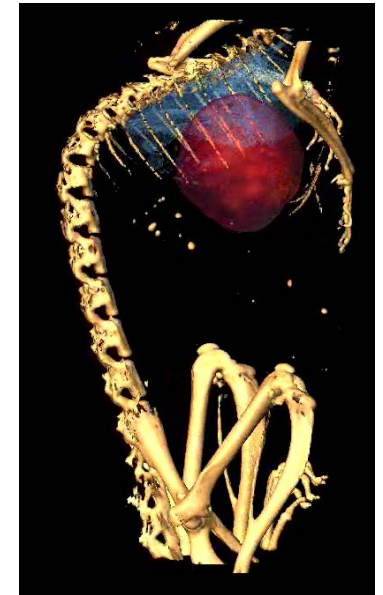


# SPECT/CT Tumor Imaging

A wide range of biomarker and therapeutic biologics have been imaged and quantitated by SPECT. The longer half-life of SPECT radionuclides compared to PET allow for imaging over several weeks.



$^{123}\text{I}$  - MAB



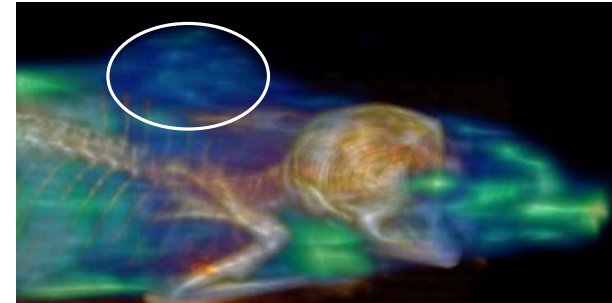
$^{99\text{m}}\text{Tc}$  - VEGF

$^{123}\text{I}$ -metaiodobenzylguanidine (MIBG) microSPECT/CT and FDG PET comparison in a mouse model of neuroblastoma

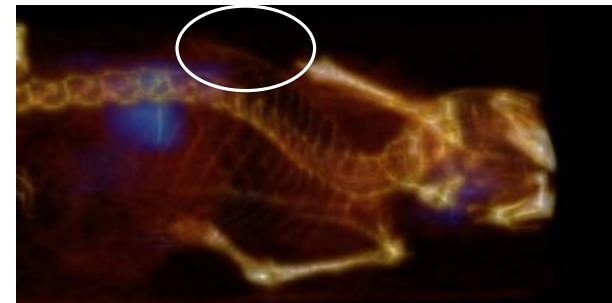


# Imaging Tox (apoptosis) by SPECT

- Many cancer drugs have off target toxicity at lower concentrations that are undetected in standard preclinical GLP tox studies.
- Functional organ toxicity at early stages can only be determined in vivo by imaging.
- Apoptosis is an early indicator of cell toxicity long before even functional damage can be detected.
- In this example the cancer drug doxorubicin induced apoptosis not only in the tumor but in a variety of normal organs.



Dox



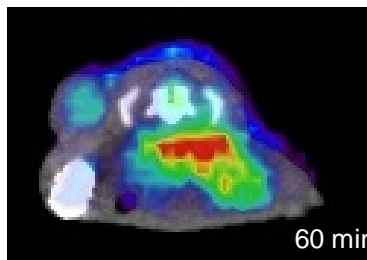
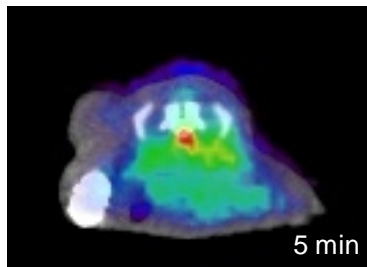
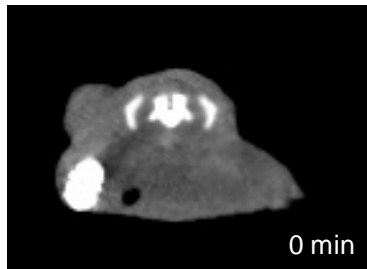
CONTROL

Drug induced apoptosis imaged with  $^{99m}\text{Tc}$  – annexin V – tumor model

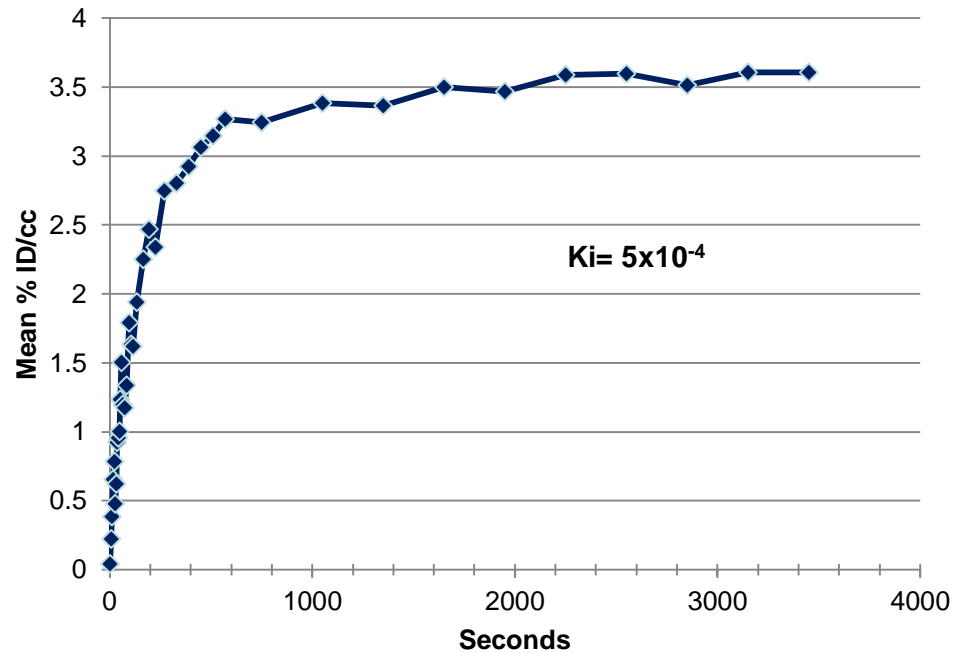


# PET PK (Dynamic Imaging) in Cancer Tissue

- An alternative when not possible by conventional PK (Blood)

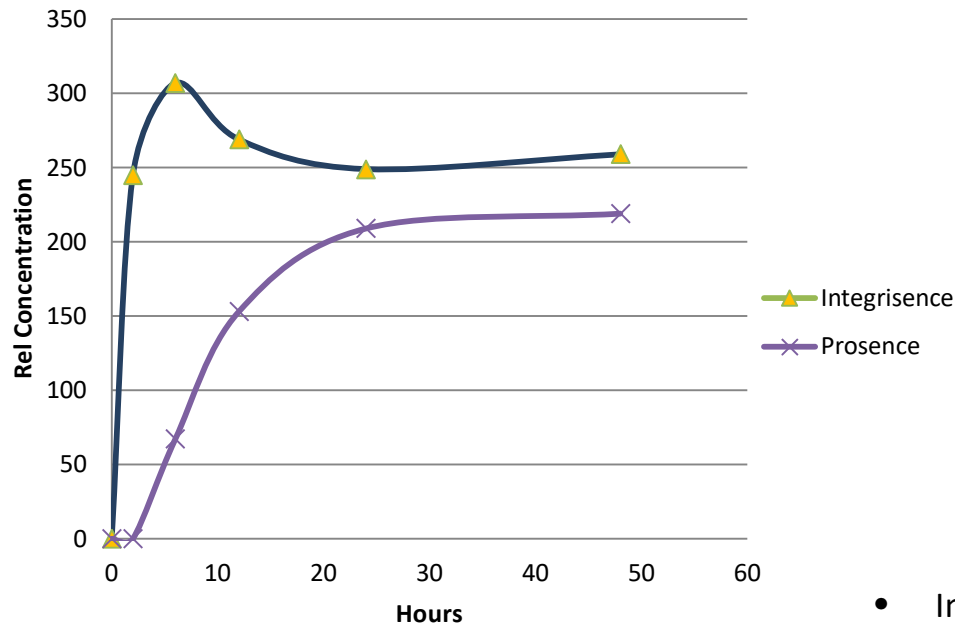


Time Activity Curve of FDG Uptake  
in Colo 205 Tumor Xenograft

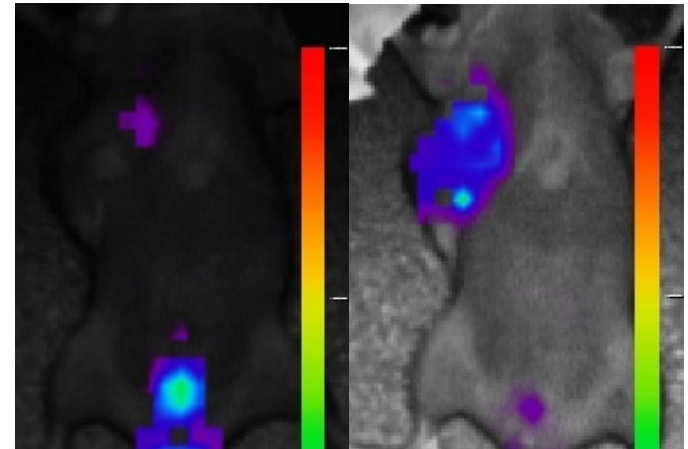


# FLI Imaging of Tumor Markers

## Longitudinal Concentration Analysis



HCT-116 xenograft tumor.



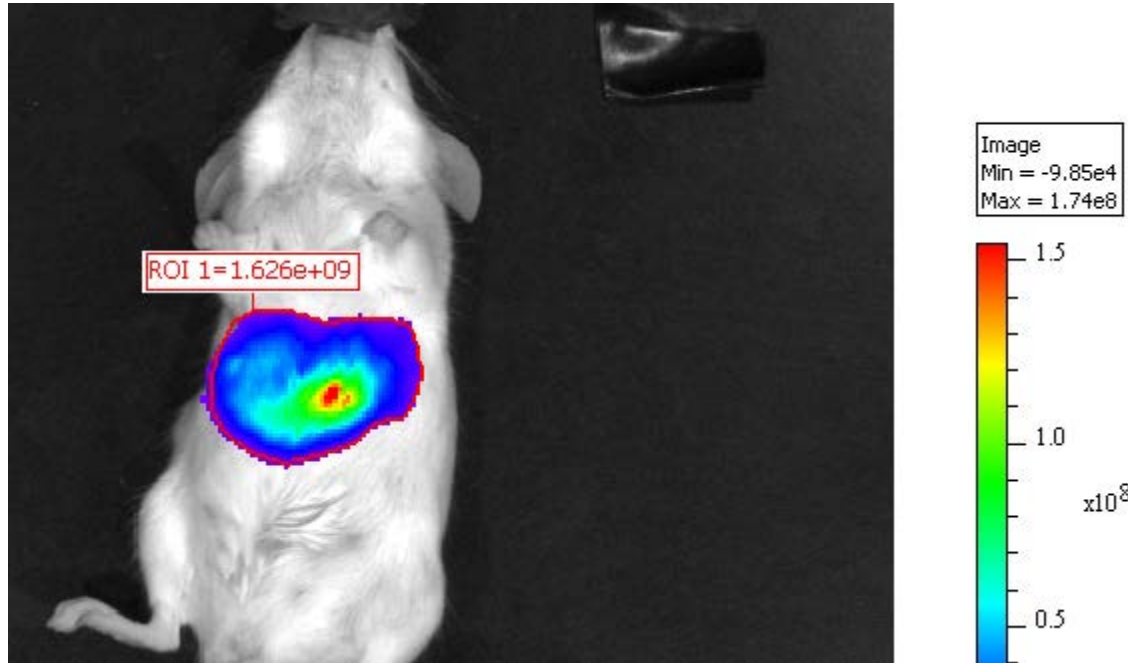
- Integrisence is an  $\alpha v$  integrin receptor marker on tumor blood vessels and tumor cells.
- Prosenice is a cathepsin D activated fluorophore. Cathepsin D is overexpressed in many tumors.





# BLI Imaging

## In Vivo Transfected Liver



### Luciferase labeled cell lines

- HT-29.CMV.Luc.59
- HT-29.CMV.Luc.101
- HT-29.CMV.Luc.118
- HT-1080.CMV.Luc

Photon Intensity Map: In Vivo transfection of liver cells by HDTV procedure.

Mouse was transfected 7 days earlier with pCBA-Luc.

Image was acquired following ip injection of Luciferin.

Data expressed as p/s/cm<sup>2</sup>/sr

Cells can be labeled in vivo as above or labeled in vitro and injected in vivo as in cancer or stem cell studies



# Service and Quality

- ***Thoroughness in planning and execution is key to a successful study.*** All protocols are vetted and approved by multiple personnel. Our QAU has a rigorous training program. All non-GLP studies are conducted in the spirit of GLP.
- ***We believe in sound science.*** Our ratio of scientists to non-scientists is one of the highest in the industry. Every study director is a PhD-level scientist.
- ***We believe in communication.*** Timely responses to your inquiries and frequent updates on your study are mandatory.
- ***We welcome visitors.*** You are always welcome at CBI to meet the staff, tour the laboratory and discuss the progress and results of your study.



# Summary

- **CBI provides state of the art:**
  - Toxicology
  - Pharmacokinetics
  - Efficacy
  - Pharmacology
  - In house histopathology
  - Imaging
- **Experienced attentive and communicative study directors**
- **Rapid study initiation and report preparation**
- **Established, stable business**
- **Regulatory compliance**
- **Favorable pricing structure**
- **Conveniently located facility**

