Role of Companion Animals in the Development of new PET Agents

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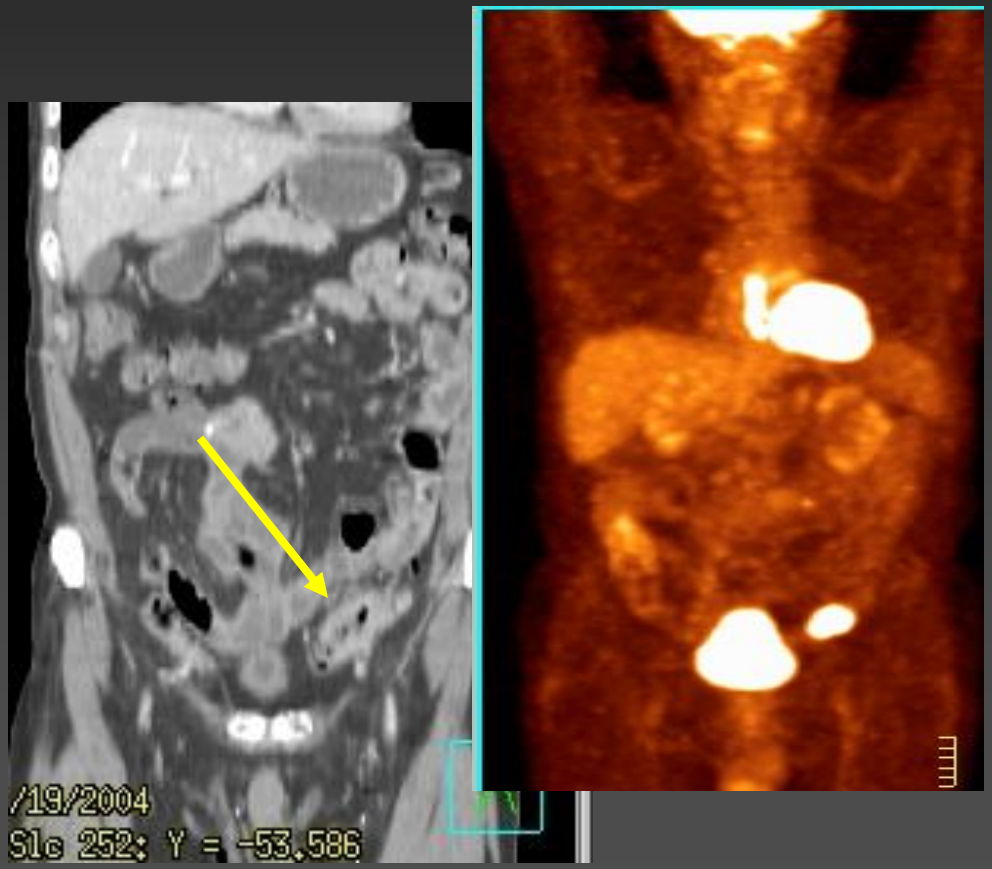
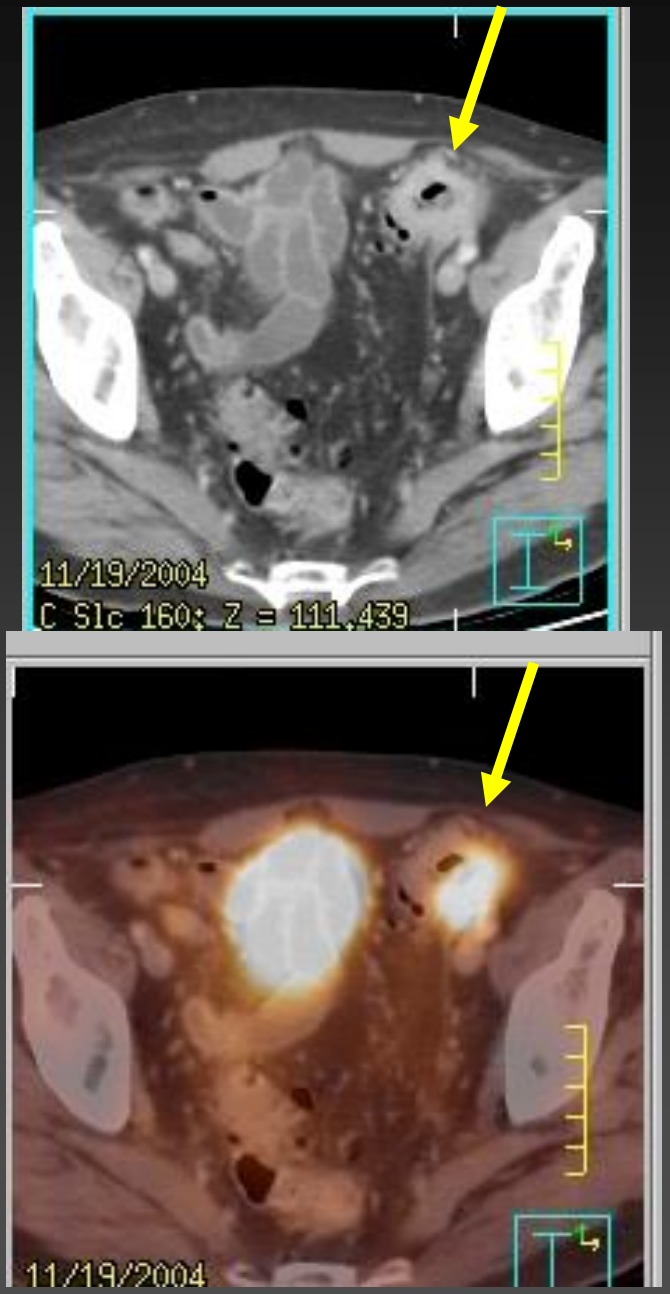
F18 FDG PET/CT
Colon cancer





Rationale for use of companion animals in PET imaging

- Accelerate development of drugs/imaging agents
- Go-No-go decisions regarding drugs/imaging
- Conduct realistic experiments without IND
- Toxicity easy to ascertain
- Size, weight, PK, PD, efficacy response
- Feasibility to obtain validation tissue (IACUC easier than IRB)
- Can be used in context of clinical trials

Companion animal "models" are more realistic than mouse models

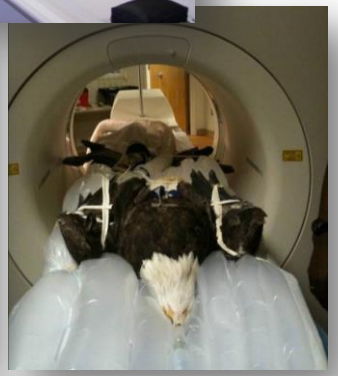
- Companion animal
  - Realistic size, human scanner
  - Spontaneous tumors with stroma
  - Immune system
  - Heterogeneous
  - Realistic growth rates
  - Realistic physiology (e.g. heart rate 100bpm)
- Mouse Model
  - Small size, large tumor, micro PET/CT
  - Cell lines with mixed stroma
  - Immune compromised
  - Homogeneous
  - Accelerated growth rates
  - Rodent physiology (e.g. heart rate 300bpm)

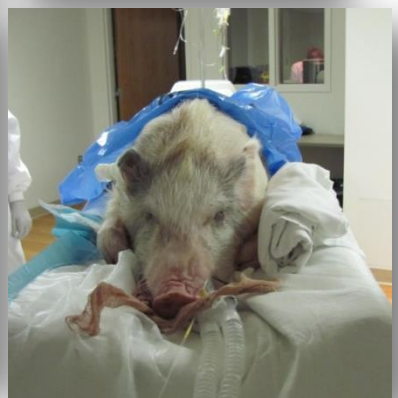
Challenges of testing PET Agents

- PET agents depend on high target to background ratio(TBR)
- Mouse models over-estimate target affinity (monoclonal-high expressing) and underestimate background.
  - Companion animal tumors are polyclonal+stromal
- Background is related to pharmacokinetics
  - PK of rodents is much different than higher order mammals.
  - Larger mammals provide a more realistic view of TBR

Patient preparation for PET

- Sedation with cage confinement vs. general anesthesia during uptake time
- General anesthesia to facilitate positioning
- Consideration of radioactive waste(s) during and after scan





Courtesy Amy LeBlanc DVM

Technical Considerations

- Dual Use Equipment
  - Human PET/CT scanners are generally not dedicated to animal research therefore companion animals need to be separated from human schedule
- Staffing
  - PET/CT Technologist (Radiation), Veterinary Staff, Imaging Specialists
- Radiation Safety

Technical Issues

- Radiation Safety
  - Number one issue: Peeing and Pooping
  - Concerns:
    - Public Relations
    - Heightened awareness vis a vis terrorist threats
  - Temporary Housing
    - C11-20 minute half life (clear in 2 hours)
    - Ga68- 70 min half life (clear in 7 hours)
    - F18 – 110 min. half life (clear in 12-24 hours)
    - Zr-89-3 day half life (clear in 20 days!)

P5H/P39 scanner : 2005-2006

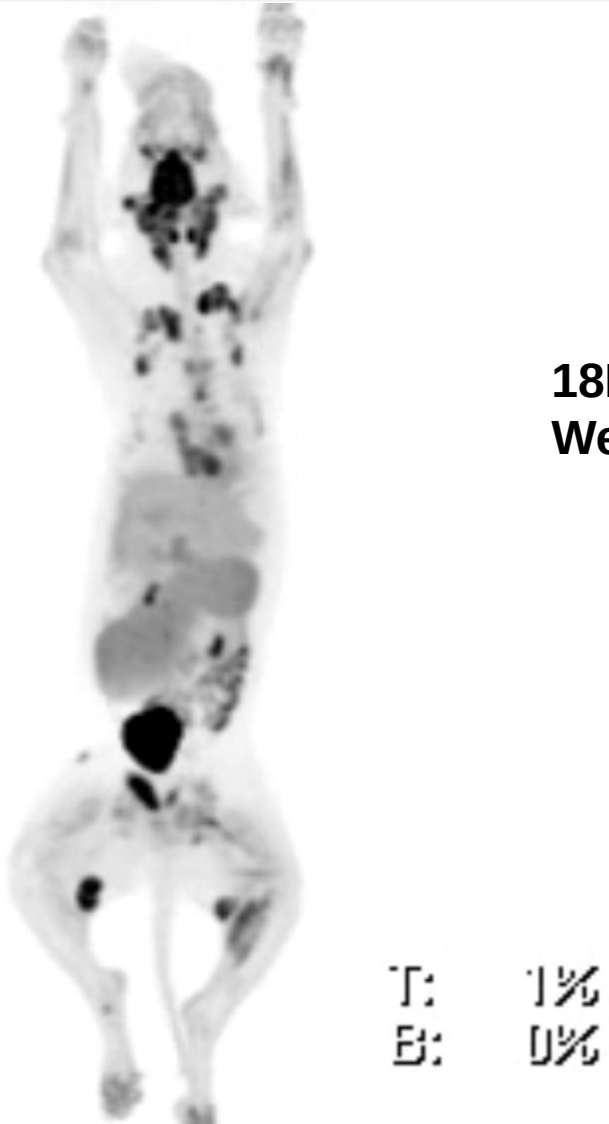
Courtesy Amy LeBlanc DVM

PET imaging agents

- Receptor Specific imaging
  - Ga68-DOTATE: Somatostatin Receptor
  - Zr89 Panutumumab (EGFR)
- Cancer "Hallmark" Tracers
  - F18 FDG: Warburg phenomenon
  - F18 FAZA: Hypoxia
  - F18 CP18: Apoptosis
  - F18 FLT: Proliferation

"Winston" 6 yr mixed-breed dog
B cell non-Hodgkin's lymphoma

18F FDG
Baseline



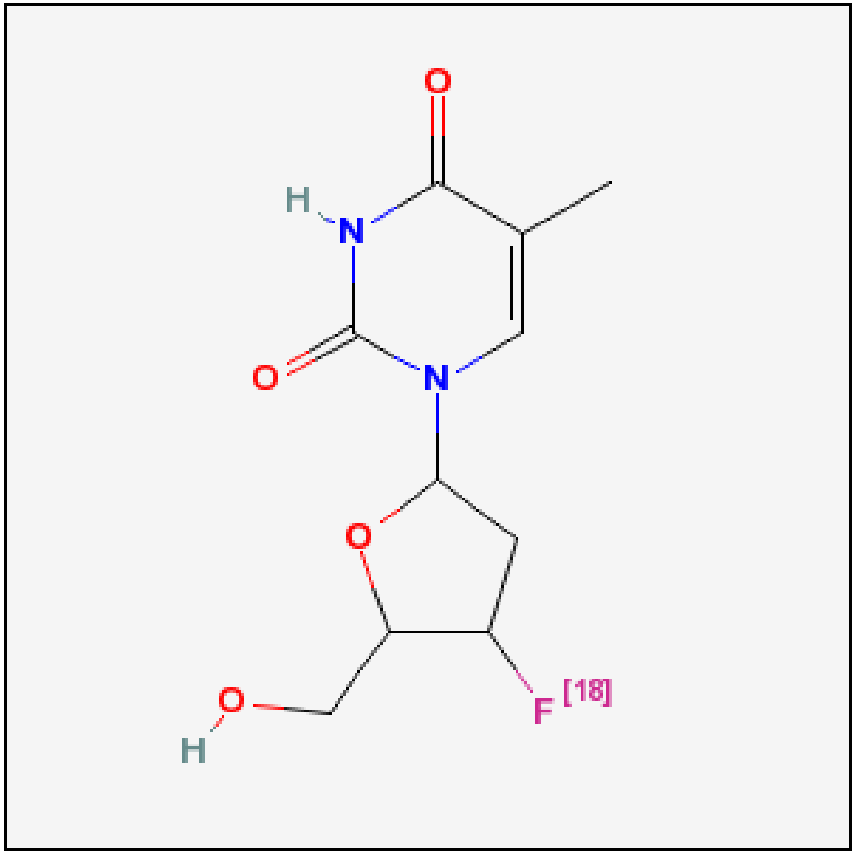
18F FDG
Week 9 CHOP

Images courtesy of Misty Long RT (R)(N)

^{18}F-fluoro-3′-deoxy-3-L-fluorothymidine (^{18}FLT)

- Marker of cellular proliferative activity
- Analog of AZT

- Accumulation in cells dependent on:
  - Thymidine kinase 1 (TK1) activity
  - Activation of salvage pathways for nucleoside transport

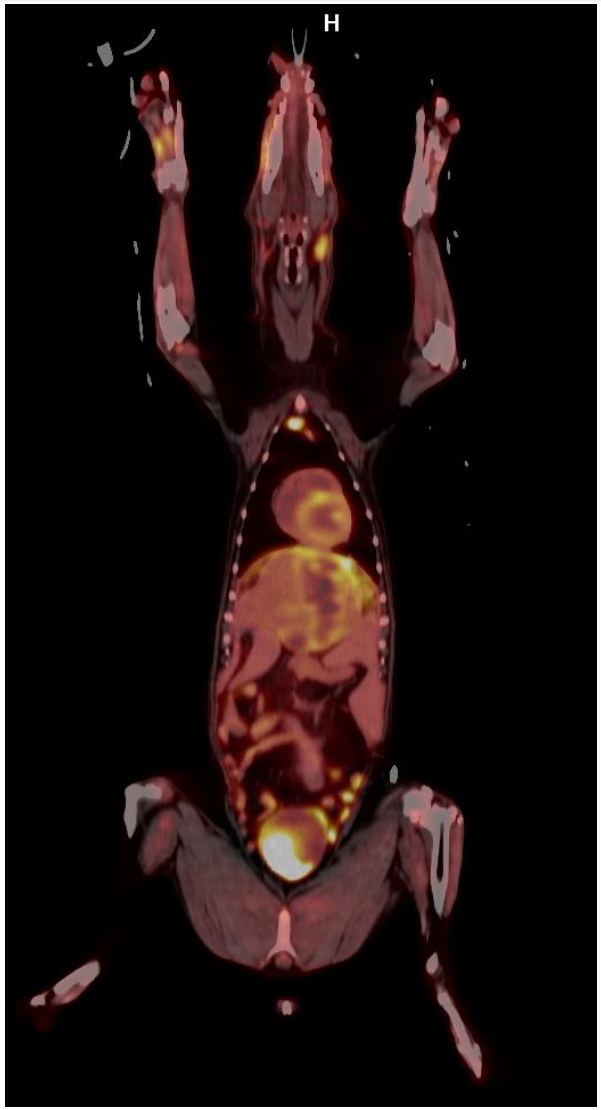


Dual-tracer studies

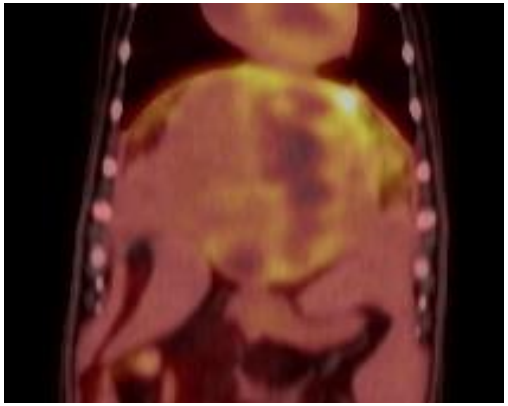
- ^{18}FDG = cellular glucose metabolism
- ^{18}FLT = cellular proliferation

- "Ike" 9 yr MC mixed breed dog
  - Large hepatic mass
  - US guided FNA = mixed inflammation and necrosis
  - Staging = no signs of metastasis

"Ike" – ^{18}FDG PET/CT imaging

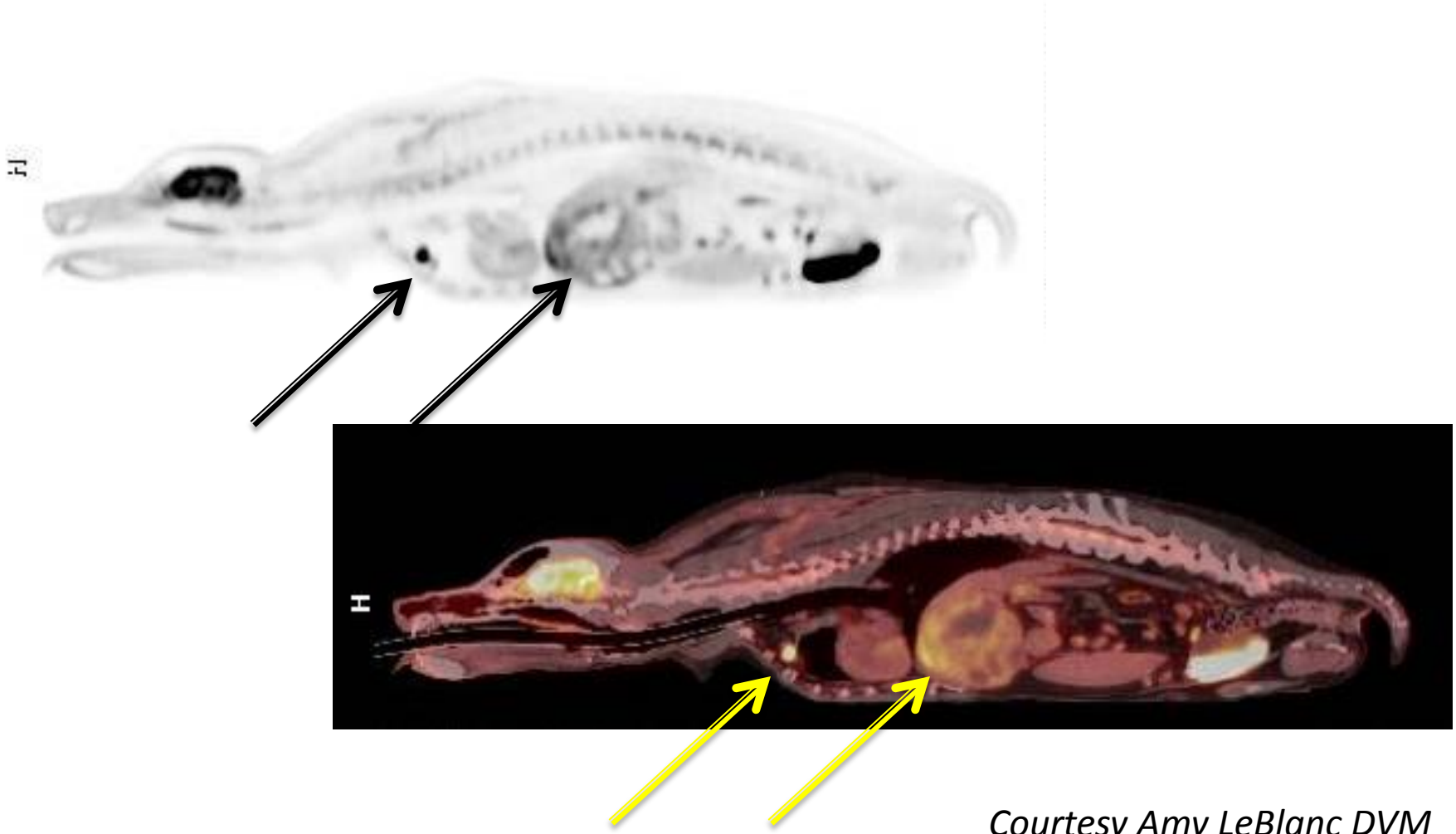


5 mCi ^{18}FDG
60 min uptake
5 min/bed



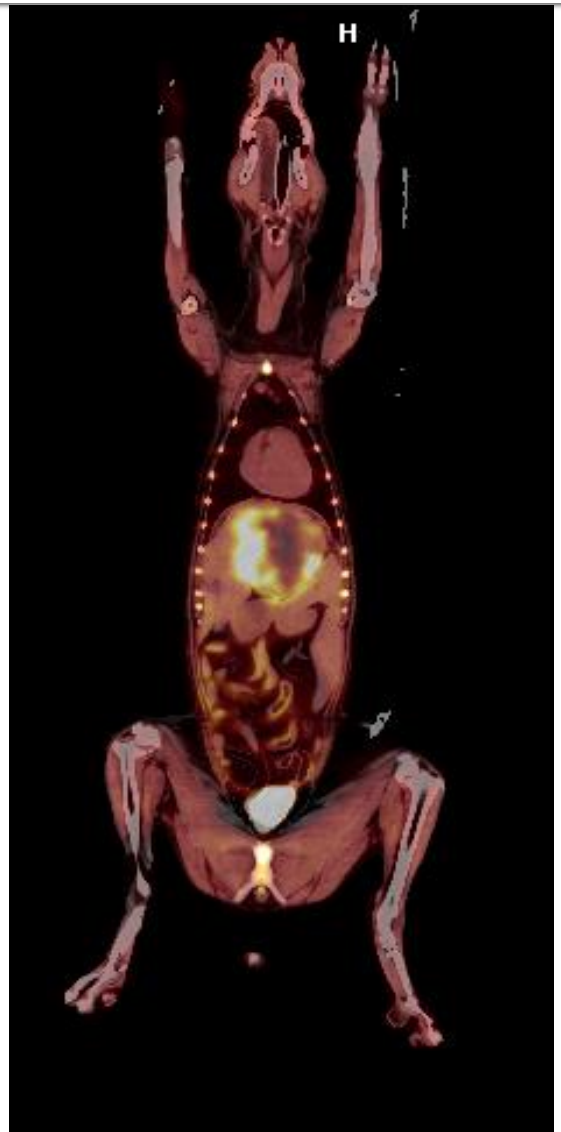
Courtesy Amy LeBlanc DVM

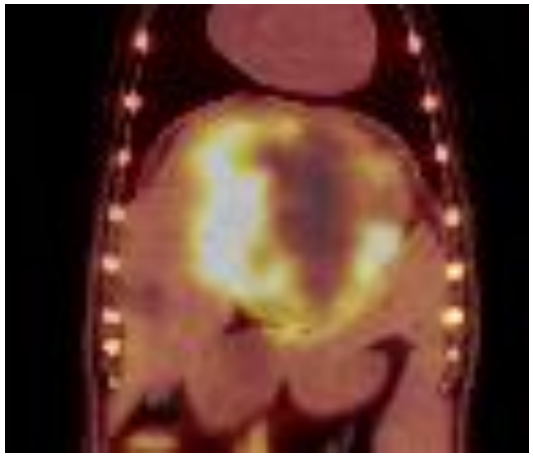
"Ike" – ^{18}FDG PET/CT imaging

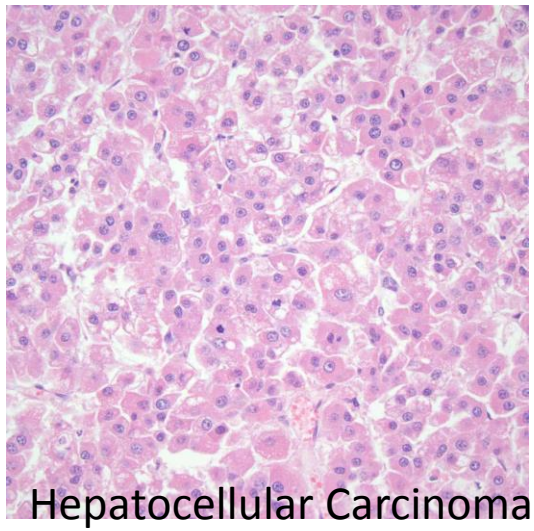


Courtesy Amy LeBlanc DVM

"Ike" – ^{18}FLT imaging



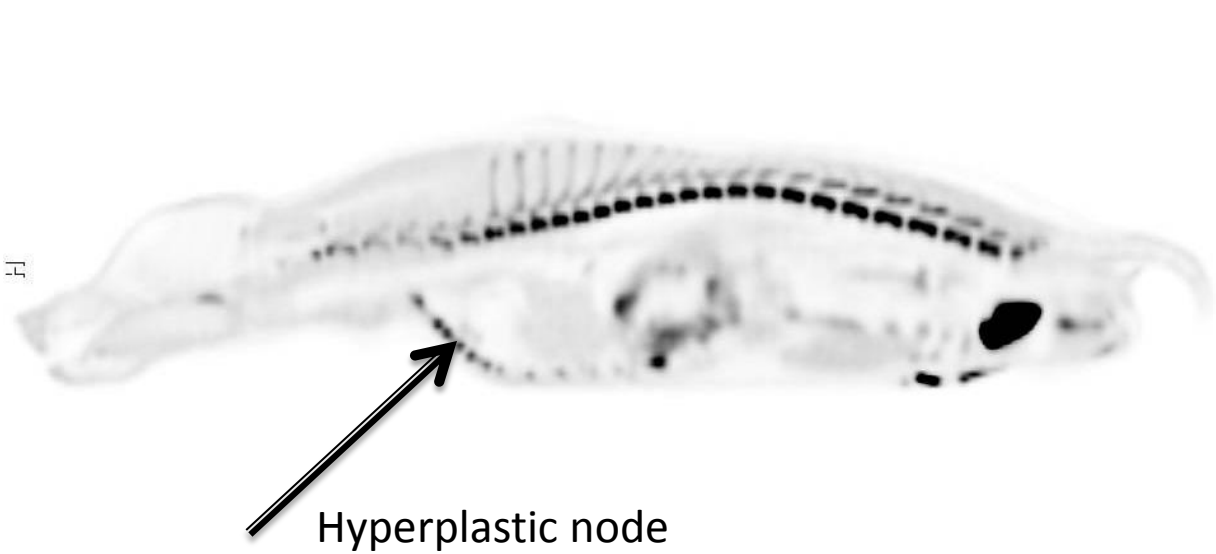




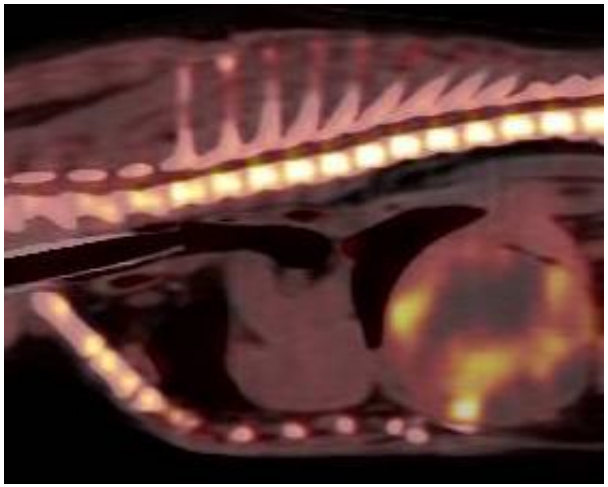
Hepatocellular Carcinoma

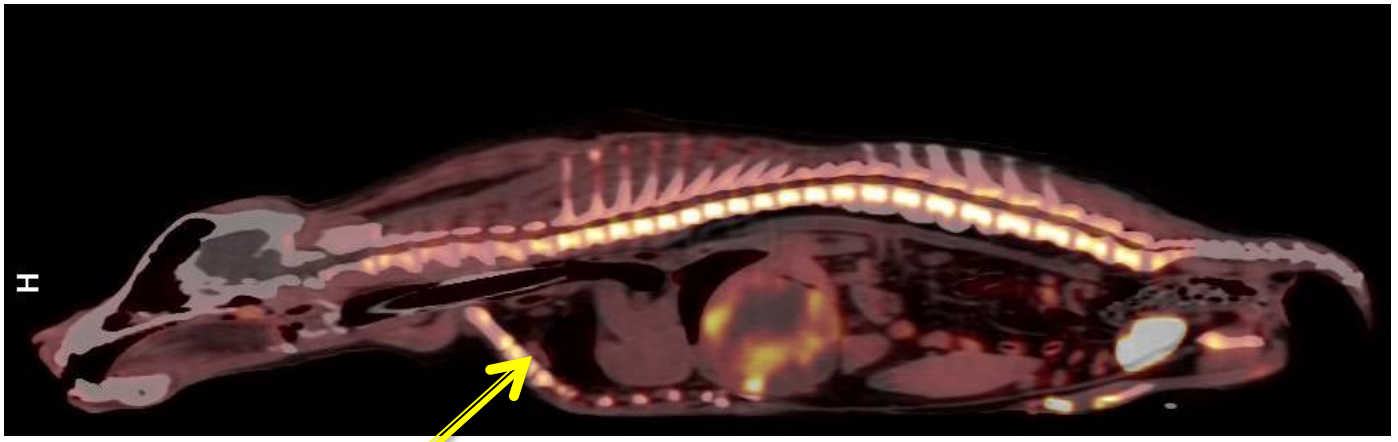
- *Courtesy Amy LeBlanc DVM*

"Ike" – ^{18}FLT imaging



Hyperplastic node

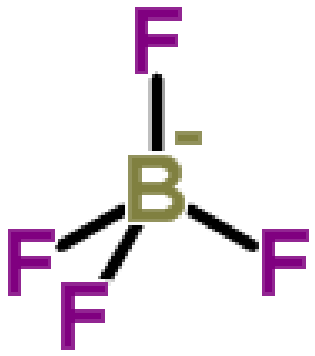




Hyperplastic node

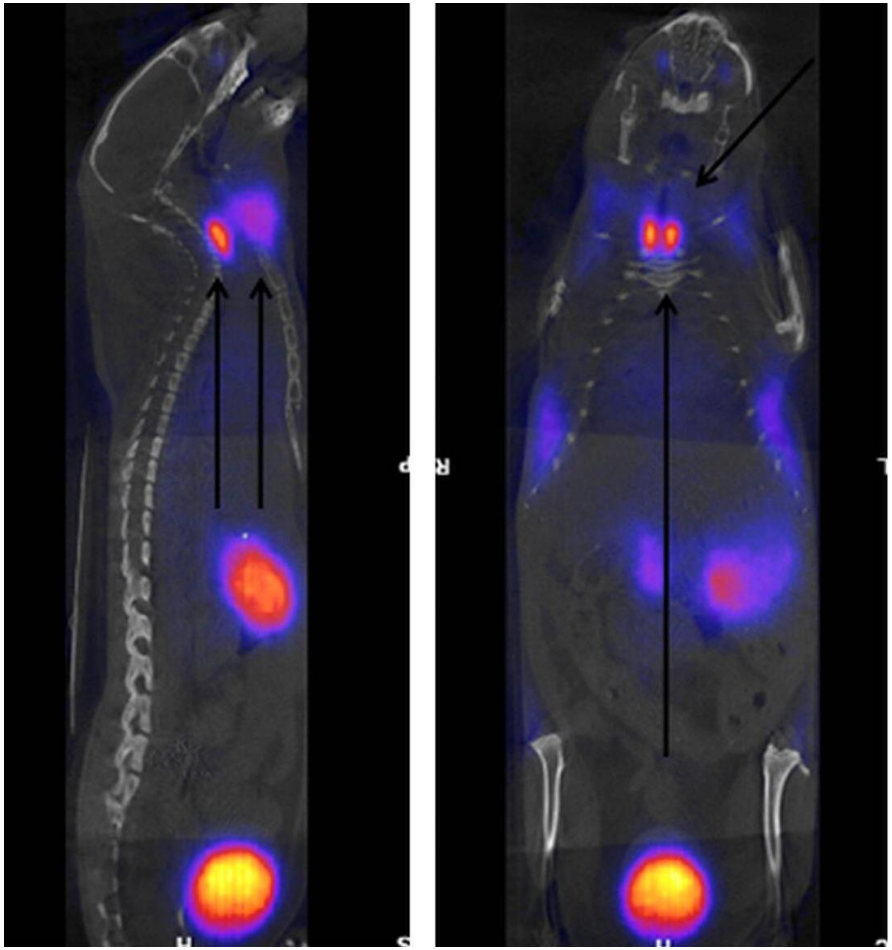
Courtesy Amy LeBlanc DVM

^{18}F-tetrafluoroborate (^{18}F-TFB)



Imaging of sodium/iodide symporter (NIS) protein

Traditionally accomplished with ^{123}I, ^{131}I, and ^{99m}Tc pertechnetate



Courtesy Amy LeBlanc DVM

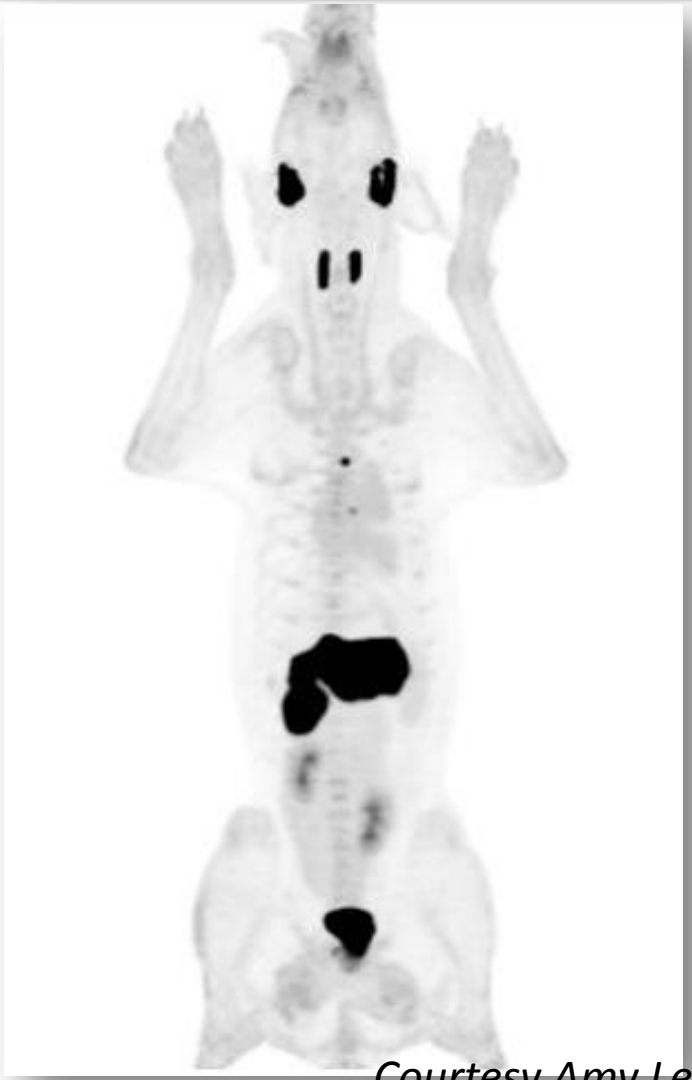
^{18}F-TFB, 5 mCi, 60 min uptake 9 yr MC 80 lb hound dog

Salivary gland

Thyroid gland
Ectopic thyroid tissue - myocardium/LV outflow tract

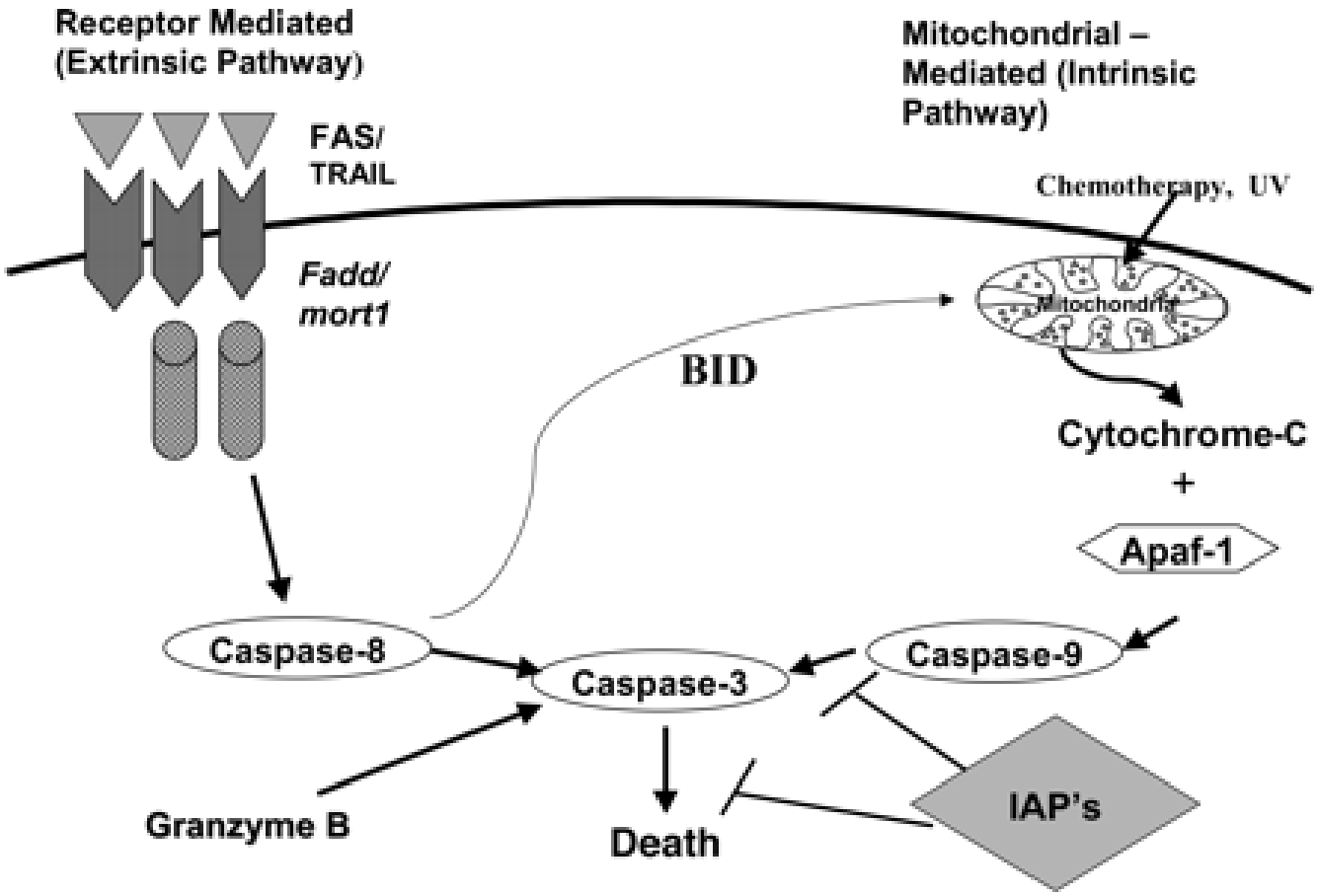
Stomach

Free ^{18}F- uptake in bone, excretion via urinary system



Courtesy Amy LeBlanc DVM

Schematic of Apoptosis



CP-18 is an enzyme substrate of caspase 3

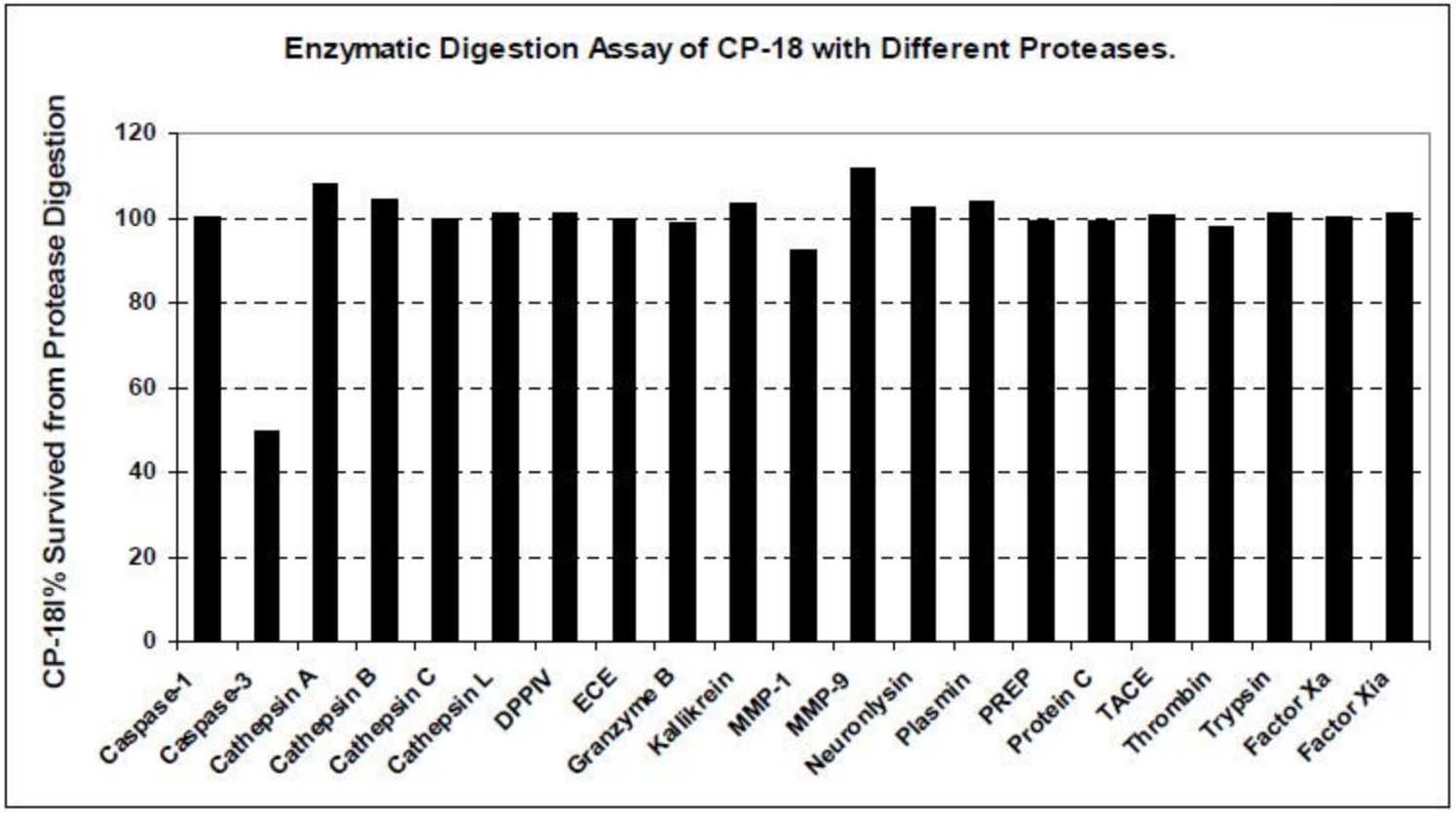
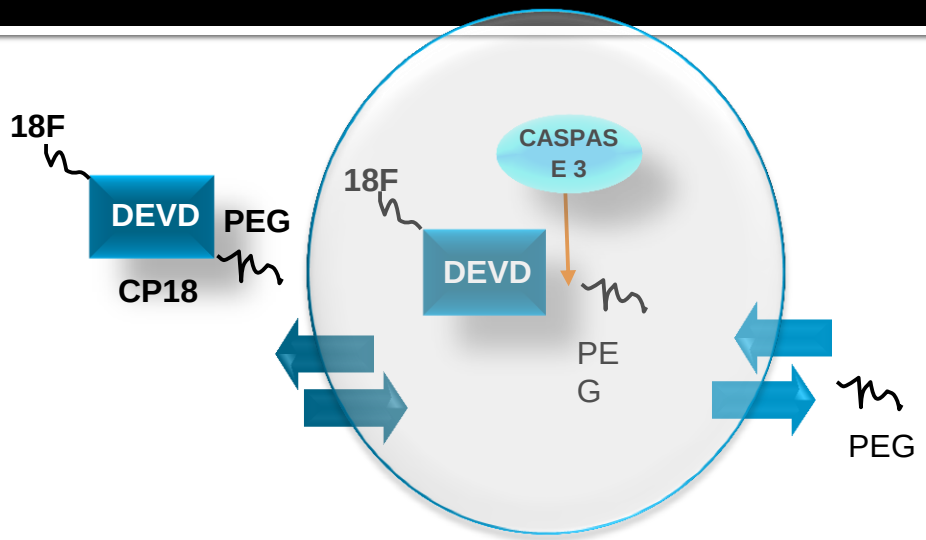


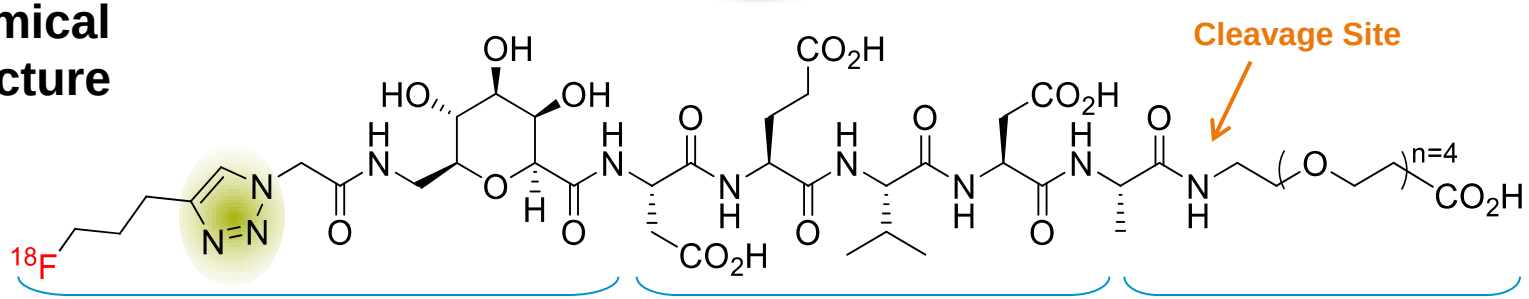
Figure 5-12 Digestions of CP-18 with various proteases. Results normalized for CP-18 in controls.

Design Concept for Apoptosis Imaging Agent
Click Chemistry Caspase-3 Substrate ^{18}F-CP18

Apoptotic Cell



Chemical Structure



Easy to Label: Click chemistry enabled [F-18] labeling

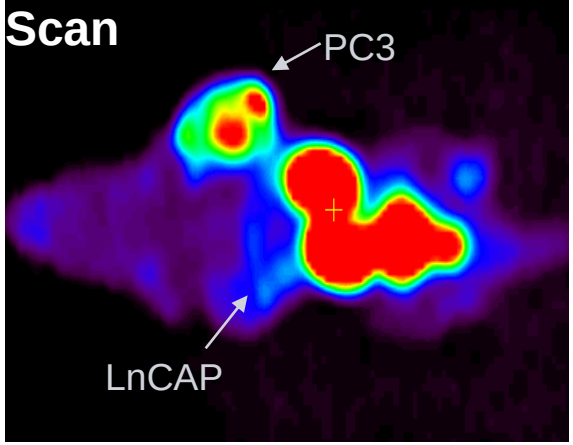
Superior PK Properties: Triazole and galactose groups urge renal clearance

DEVD Sequence: Based on well-established fluorescent apoptosis reporters

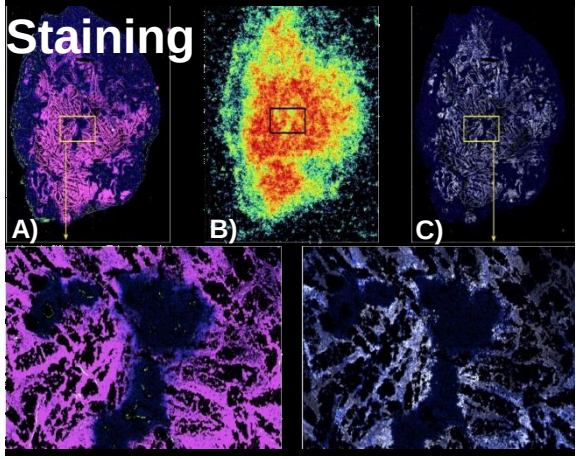
Localizes in Cells: PEG facilitates transport into cells

Apoptosis Imaging Agent
Caspase-3 substrate ^{18}F-CP18 pre-clinical evaluation

^{18}F-CP18 Mouse Scan



Autoradiography & Staining



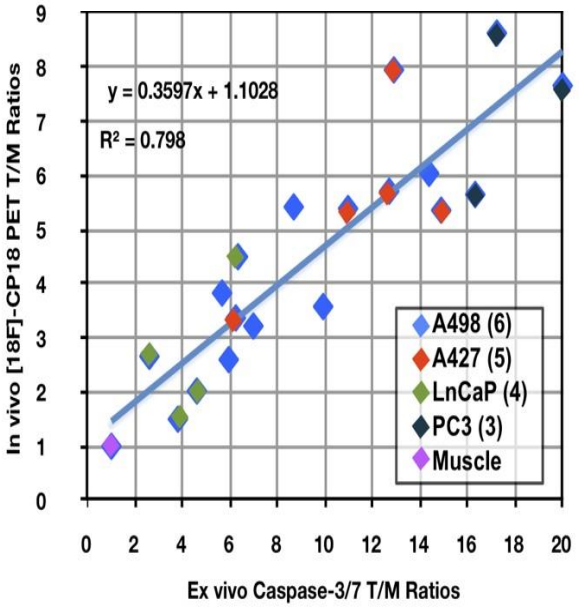
Tumor Type	PET T/M	C-3 T/M
LnCaP	4.5	6.3
PC3	8.6	17.2

A) CD31, tunel, and hematoxylin of PC3 tumor

B) ^{18}F-CP18 *in vivo* uptake, ex vivo autoradiography of PC3 tumor

C) Caspase-3 staining and hematoxylin

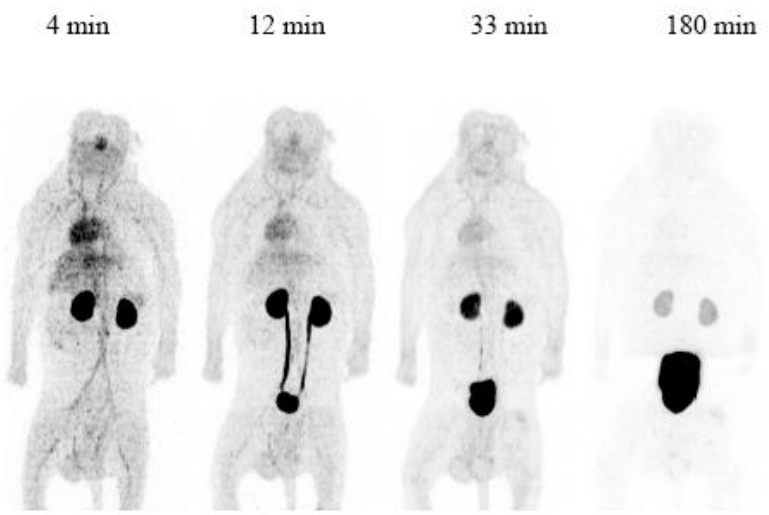
Correlation of Caspase-3 & ^{18}F-CP18 PET



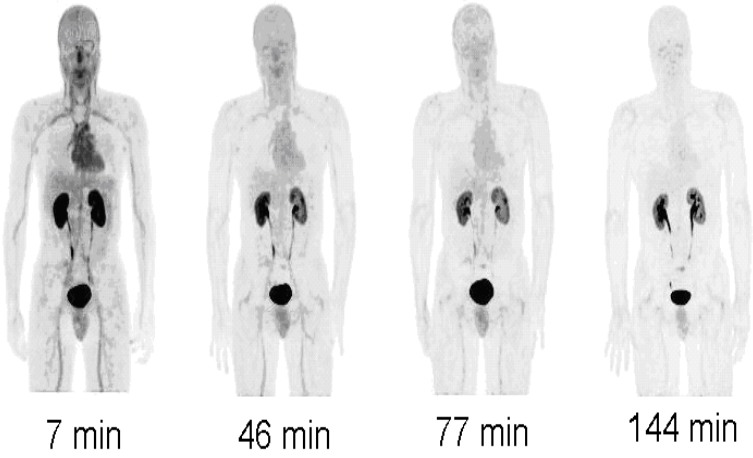
Caspase-3 actvity assay: Ac-DEVD-AFC (fluorogenic substrate), tissue homogenate or Caspase-3. Measure the cleavage rate of substrate. Fluorescent units of samples were converted to Caspase-3 activity units (from standard curve) and were normalized by protein concentration.

NHP studies

Non human primate



Human volunteer



No safety signals
Rapid renal excretion
Low background
Granted IND
No evidence that it shows Apoptosis in man

Imaging Endpoints in Clinical Trials

- Positives to companion animals
  - Validation with tissue confirmation
  - Survival data
- Open questions:
  - Are animal patients recognized by FDA?
  - Will metabolism differences render data useless?
- Strategy:
  - Start small with commonly available PET agents and proceed with more exotic tracers

Conclusions

- Companion animals offer many potential advantages in developing new PET agents
  - Larger, heterogeneous, with stroma
- Companion animals present some unique challenges for the clinical environment
  - Radiation safety, Dual use issues
- "Hallmark" Tracers are likely more relevant than receptor-transporter tracers
  - Species differences
- PET agent testing in companion animals could be very useful in accelerating tracer/drug development

Acknowledgement

- Thanks to Amy LeBlanc for supplying several of the slides in this talk and for helpful discussions