^{89}Zr-ImmunoPET: Harnessing Antibodies for Diagnostic and Theranostic Nuclear Imaging

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Disclosure

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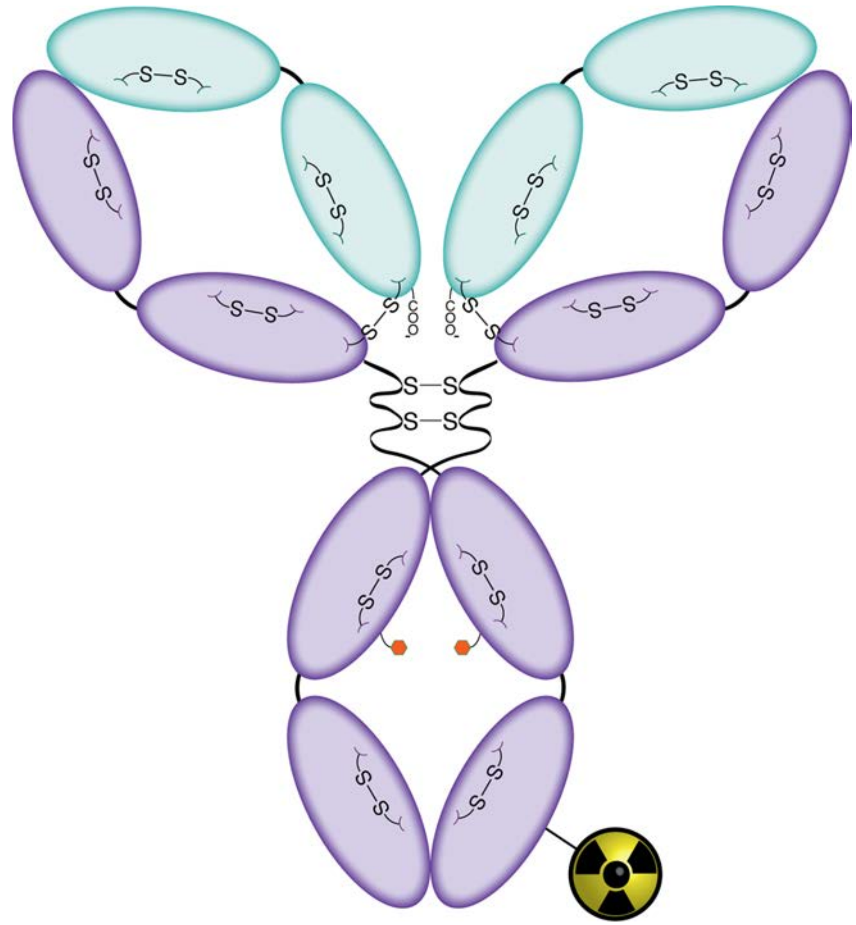
ImmunoPET: The Hallmarks

- **Advantages:**
  - Specificity
  - Affinity
  - Stability
  - Biochemical information
- **Obstacles:**
  - Immunogenicity
  - Extracellular targets
  - Slow pharmacokinetics

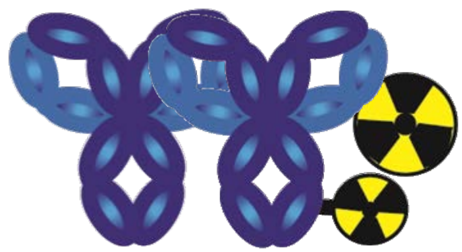


- **Key:** *Matching radionuclidic $t_{1/2}$ to pharmacokinetic $t_{1/2}$*

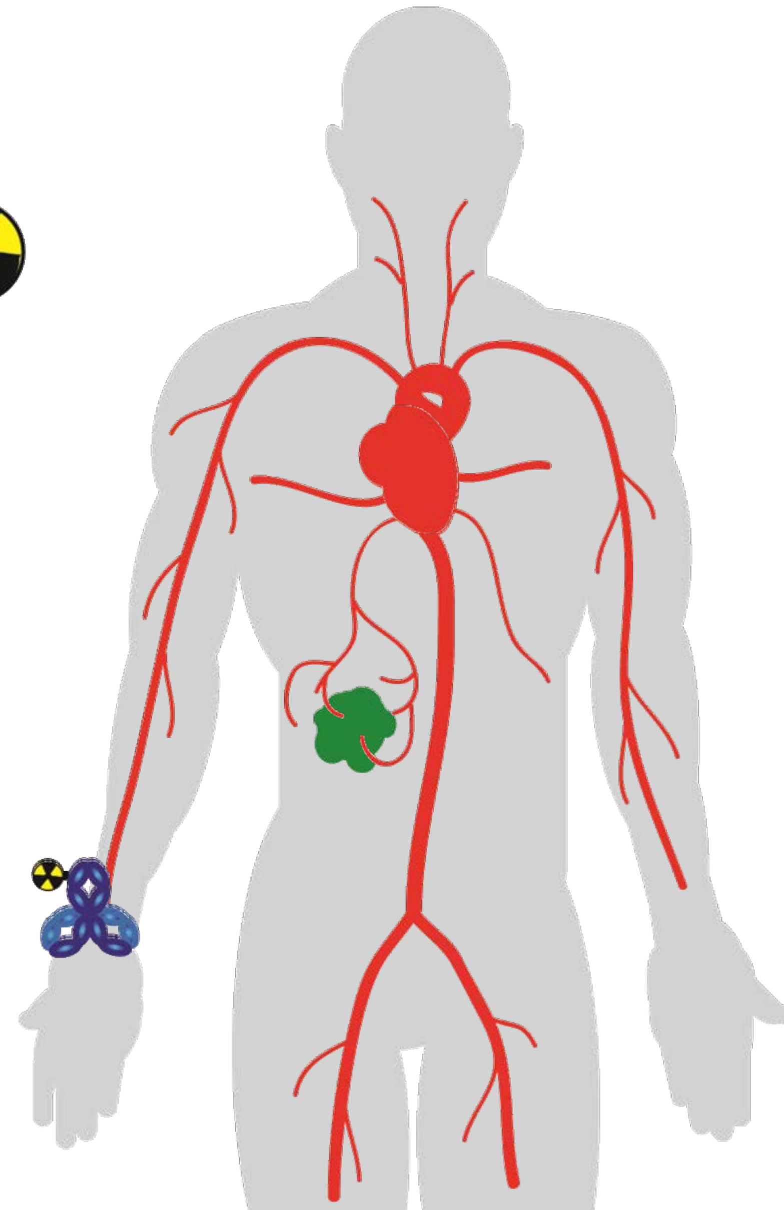
Radioimmunoconjugates

Advantages and Disadvantages

- Advantages:
  - Specificity
  - Affinity
  - Stability



- Disadvantages:
  - Immunogenicity
  - Extracellular targets
  - Imprecise Construction
  - Slow pharmacokinetics



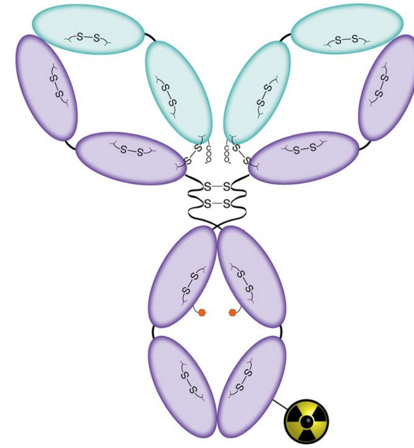
Wu, *et al.* J Nucl Med, 2009
Van Dongen, *et al.* Oncologist, 2007

ImmunoPET: Choosing an Isotope

Half-Life Too Short (< 6 h)

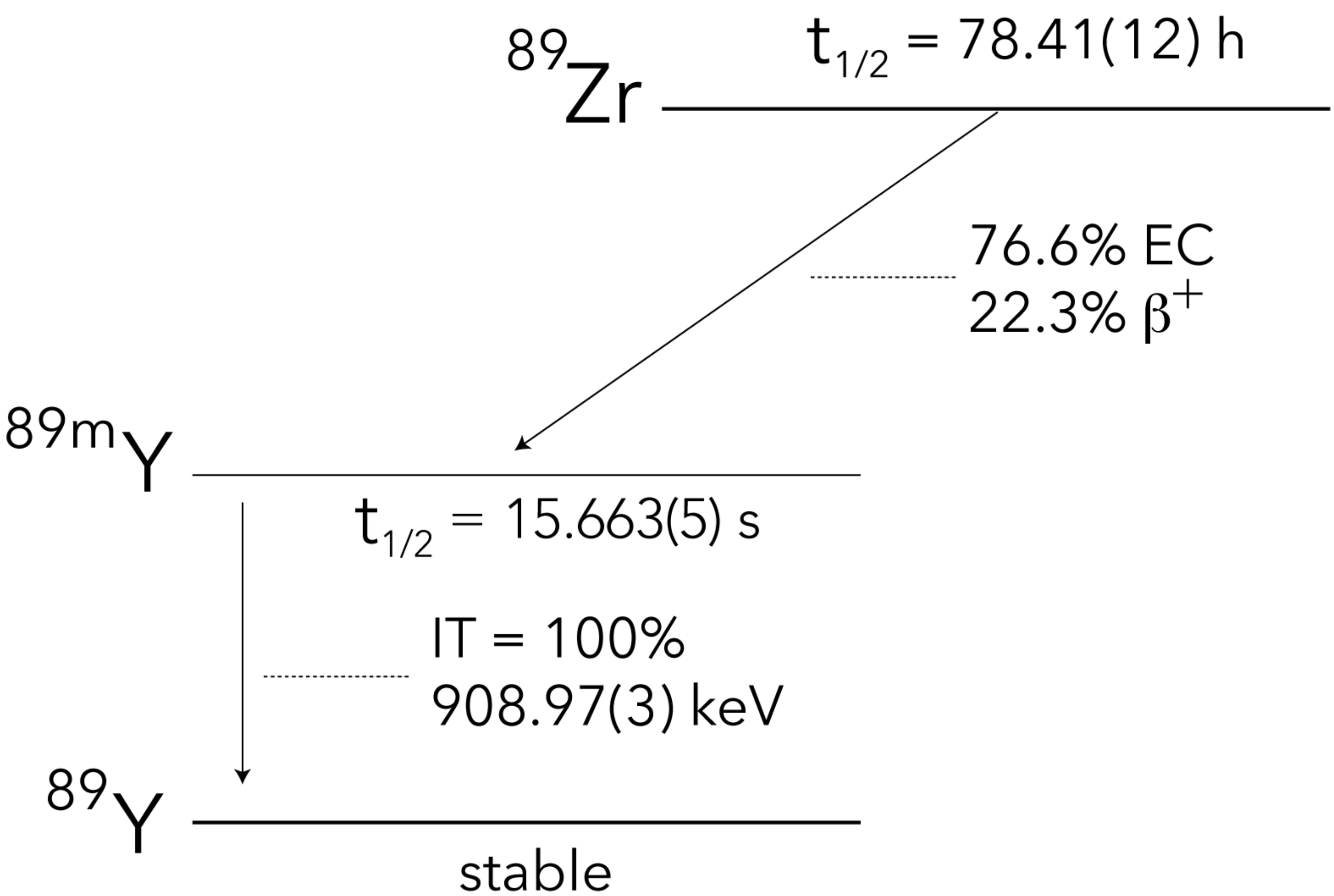
Half-Life So-So (6-24 h)

Half-Life Just Right (24-96 h)



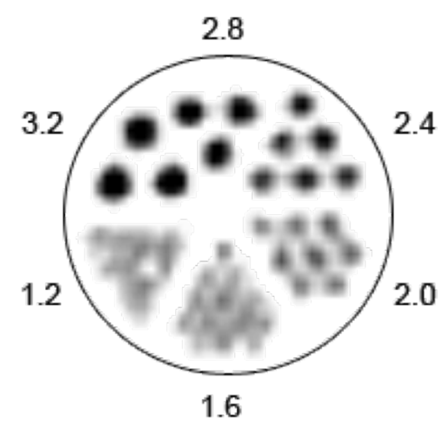
1 H Hydrogen																		2 He Helium
3 Li Lithium	4 Be Beryllium												5 B Boron	6 C Carbon	7 N Nitrogen	8 O Oxygen	9 F Fluorine	10 Ne Neon
11 Na Sodium	12 Mg Magnesium												13 Al Aluminum	14 Si Silicon	15 P Phosphorus	16 S Sulfur	17 Cl Chlorine	1 Ar Argon
19 K Potassium	20 Ca Calcium		21 Sc Scandium	22 Ti Titanium	23 V Vanadium	24 Cr Chromium	25 Mn Manganese	26 Fe Iron	27 Co Cobalt	28 Ni Nickel	29 Cu Copper	30 Zn Zinc	31 Ga Gallium	32 Ge Germanium	33 As Arsenic	34 Se Selenium	35 Br Bromine	36 Kr Krypton
37 Rb Rubidium	38 Sr Strontium		39 Y Yttrium	40 Zr Zirconium	41 Nb Niobium	42 Mo Molybdenum	43 Tc Technetium	44 Ru Ruthenium	45 Rh Rhodium	46 Pd Palladium	47 Ag Silver	48 Cd Cadmium	49 In Indium	50 Sn Tin	51 Sb Antimony	52 Te Tellurium	53 I Iodine	54 Xe Xenon
55 Cs Cesium	56 Ba Barium	57-70 Actinides	71 Lu Lutetium	72 Hf Hafnium	73 Ta Tantalum	74 W Tungsten	75 Re Rhenium	76 Os Osmium	77 Ir Iridium	78 Pt Platinum	79 Au Gold	80 Hg Mercury	81 Tl Thallium	82 Pb Lead	83 Bi Bismuth	84 Po Polonium	85 At Astatine	86 Rn Radon
87 Fr Francium	88 Ra Radium	89-103 Lanthanides	103 Lr Lawrencium	104 Rf Rutherfordium	105 Db Dubnium	106 Sg Seaborgium	107 Bh Bohrium	108 Hs Hassium	109 Mt Meitnerium	110 Ds Darmstadtium	111 Rg Roentgenium	112 Cn Copernicium	113 Uut Ununtrium	114 Fl Flerovium	115 Uup Ununpentium	116 Lv Livermorium	117 Uus Ununseptium	118 Uuo Ununoctium

Decay Scheme of ^{89}Zr



Physical Properties of ^{89}Zr

- ^{89}Zr decay properties
  - $t_{1/2}$ = 78.41 (12) h
  - β^+ = 22.3%, $E_{max}(\beta^+)$ = 897 keV, $E_{ave.}(\beta^+)$ = 396.9 keV, $R_{ave.}(\beta^+)$ = 1.18 mm
- ^{89}Y target
  - 100% abundant & commercially available
- High $^{89}Y(p,n)^{89}Zr$ production yields on <15 MeV cyclotrons
  - 1.52 ± 0.11 mCi/μA·h
  - Typical 2 - 3 h bombardments yield 45 – 65 mCi
- High purity
  - >99.99% radionuclidic and radiochemical purity
  - [^{89}Zr]Zr-oxalate and [^{89}Zr]Zr-chloride readily accessible
- High specific-activity
  - 5.3 – 13.4 mCi/μg (470 – 1195 Ci/mmol)

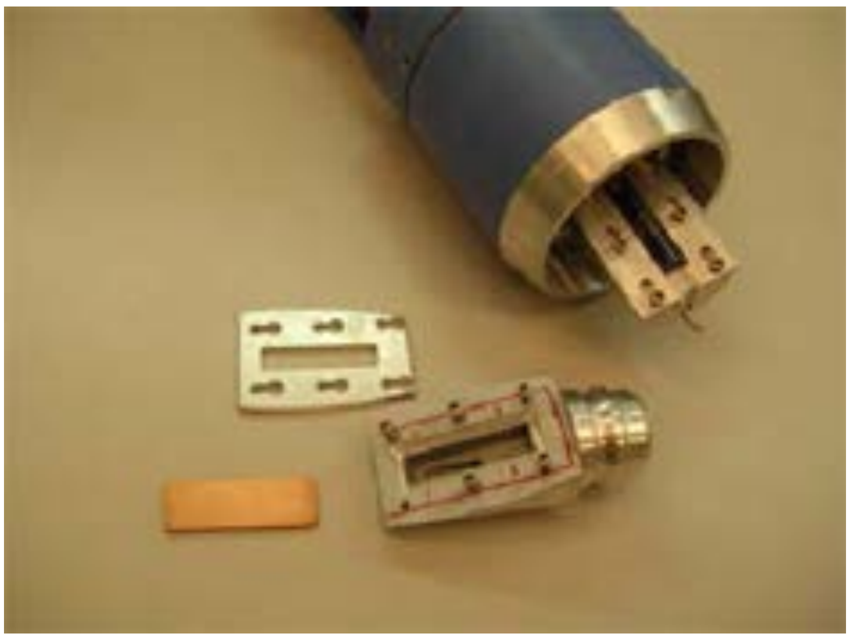


Phantom
(d = 1.2 – 3.2 mm)

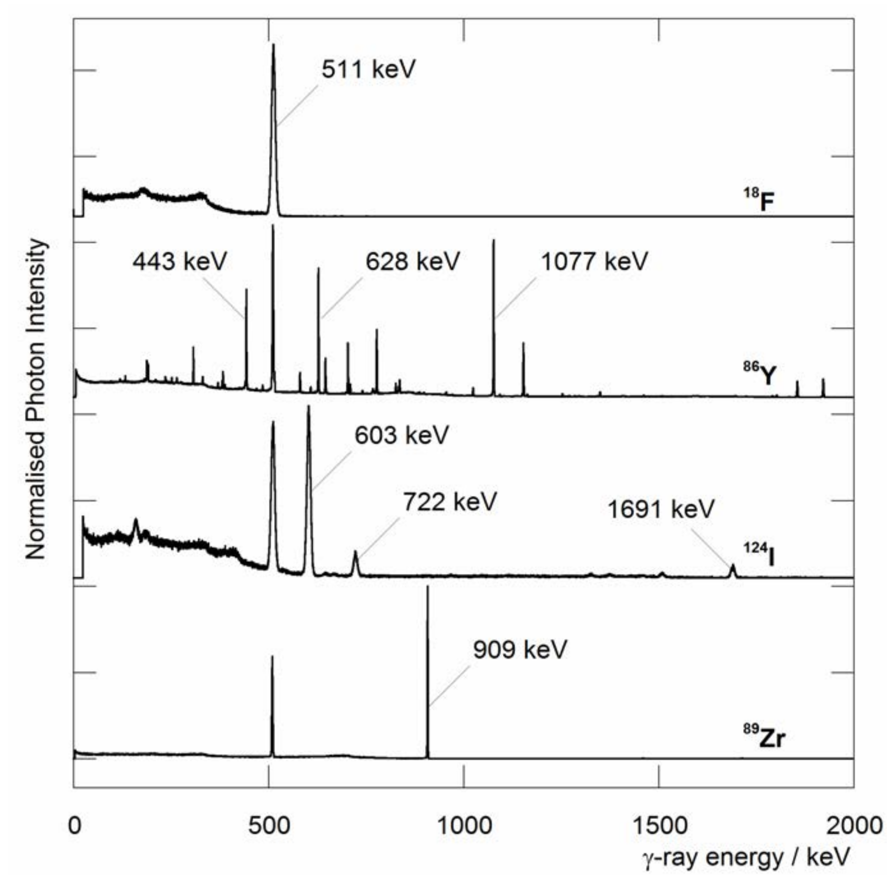
Deri, Zeglis, *et al.*, Nucl. Med. Biol. **2013**, *40*, 3-14.

The Production of ^{89}Zr

- Solid-phase purification
  - Hydroxamate resin (0.25 ± 0.08 mmol/g) gives >98% recovery of ^{89}Zr activity
  - >99.99% radionuclidic purity
  - [^{89}Zr]Zr-oxalate or [^{89}Zr]Zr-chloride



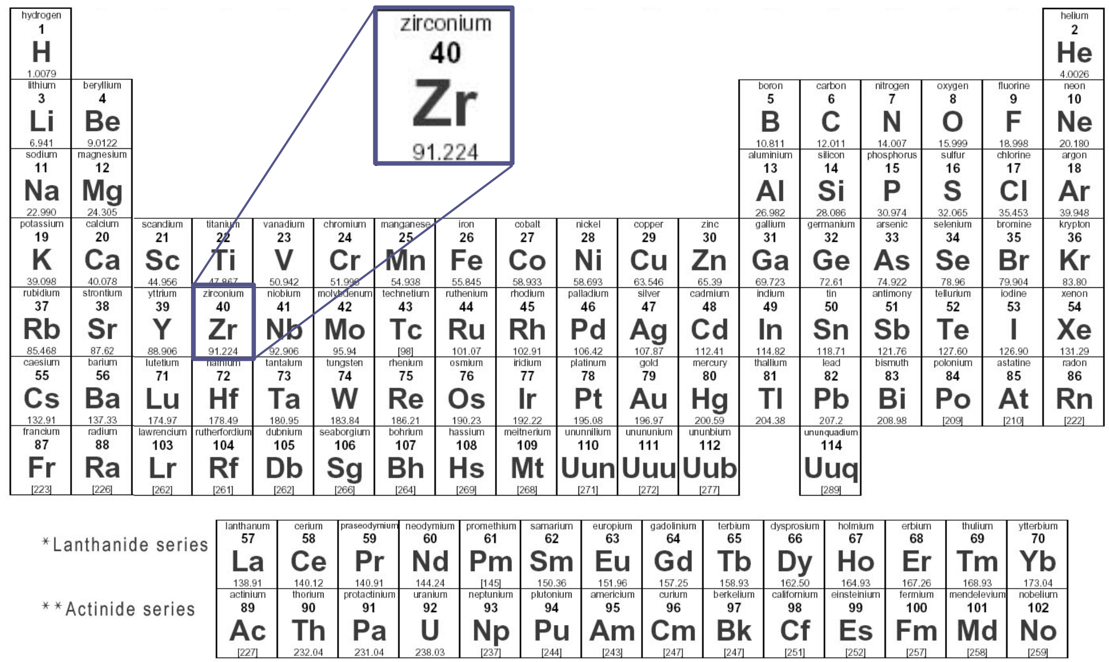
Solid ^{89}Y-foil
0.1 mm thick, 10° angle,
15 MeV, 15 µA



Verel *et al.* J. Nucl. Med. **2003**, *44*, 1271.

The Chemistry of ^{89}Zr

- Hard Metal
- Oxophilic
- Primarily a +4 cation in aqueous solution
- Effective ionic radius of Zr^{4+} cation: 0.84 Å

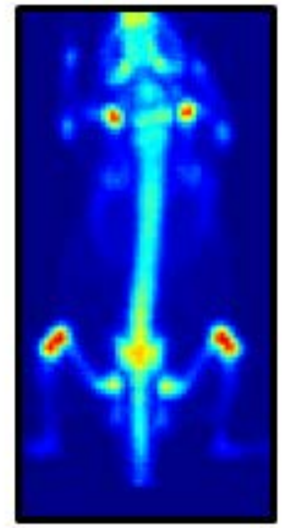


- Forms complexes with high coordination numbers
  - Typically eight-coordinate species

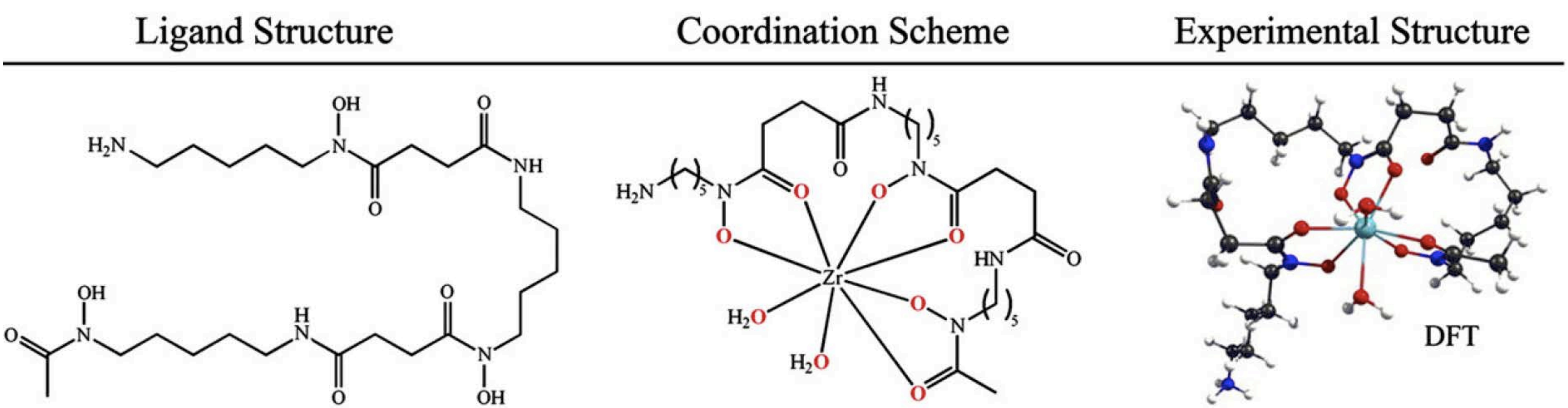
Coordination Chemistry of ^{89}Zr

- Uncomplexed $^{89}Zr^{4+}$ accumulates in the bone (osteophilic)
- $^{89}Zr^{4+}$ is a very 'hard', oxophilic cation
- Common chelators such as DOTA, NOTA, and DTPA do not work well
- Works well with the siderophore-derived chelator desferrioxamine (DFO)
  - O_6 coordination environment
  - Three hydroxamate groups = 3 anionic oxygens + 3 neutral oxygens
  - Two exogenous waters may also be involved in coordination

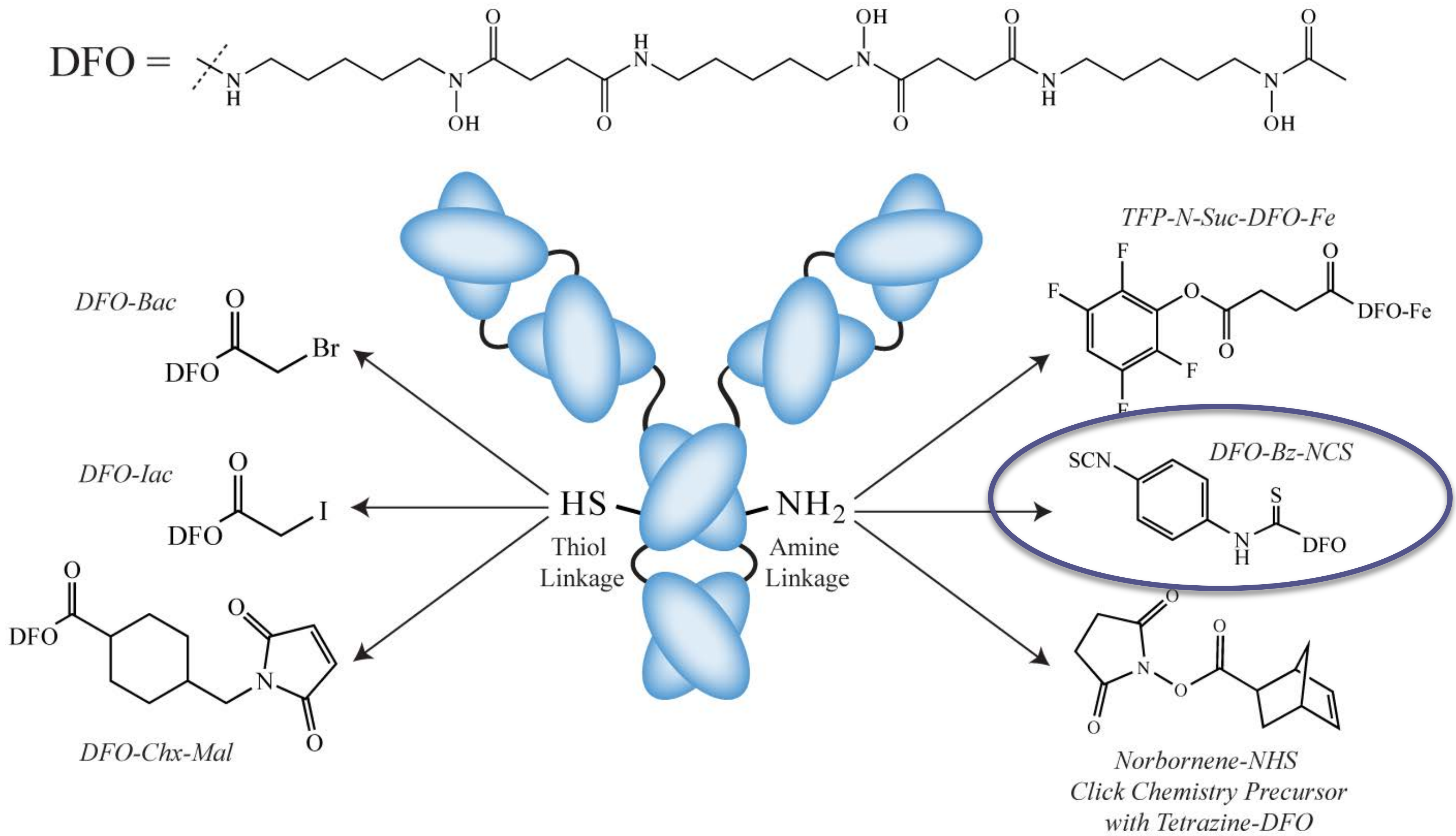
[^{89}Zr]Zr-Oxalate



MIP image (24 h)



Bioconjugation of DFO



An Example From Our Lab:
^{89}Zr-ImmunoPET of Ovarian Cancer

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Ph.D.

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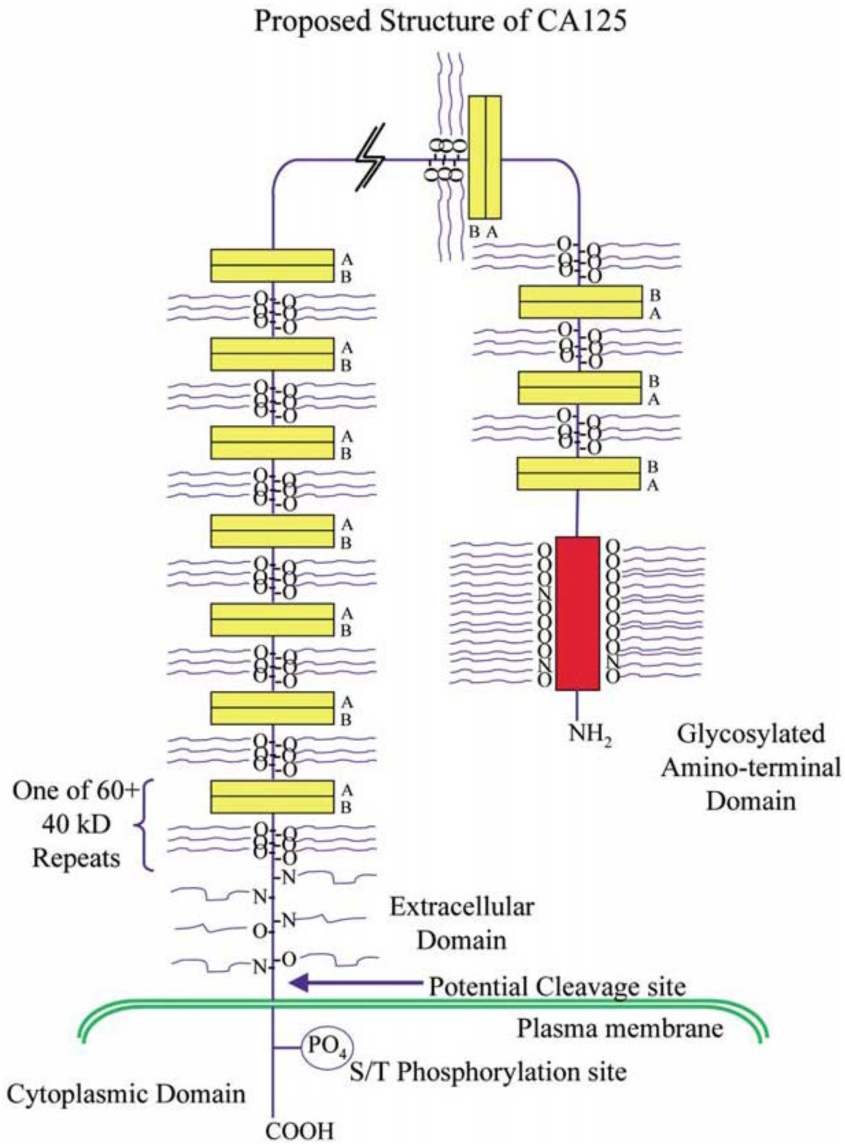
HC '14
GC '19

Ovarian Cancer

- 5th leading cause of cancer-related deaths in women
- 'Most **lethal** gynecologic malignancy'
- 21,290 new cases will be diagnosed and **14,180** women will die from **cancer of the ovary** in 2015'
- > 70% women diagnosed with OC are at 'advanced stage'
- Spread to lymph nodes is particularly hard to detect

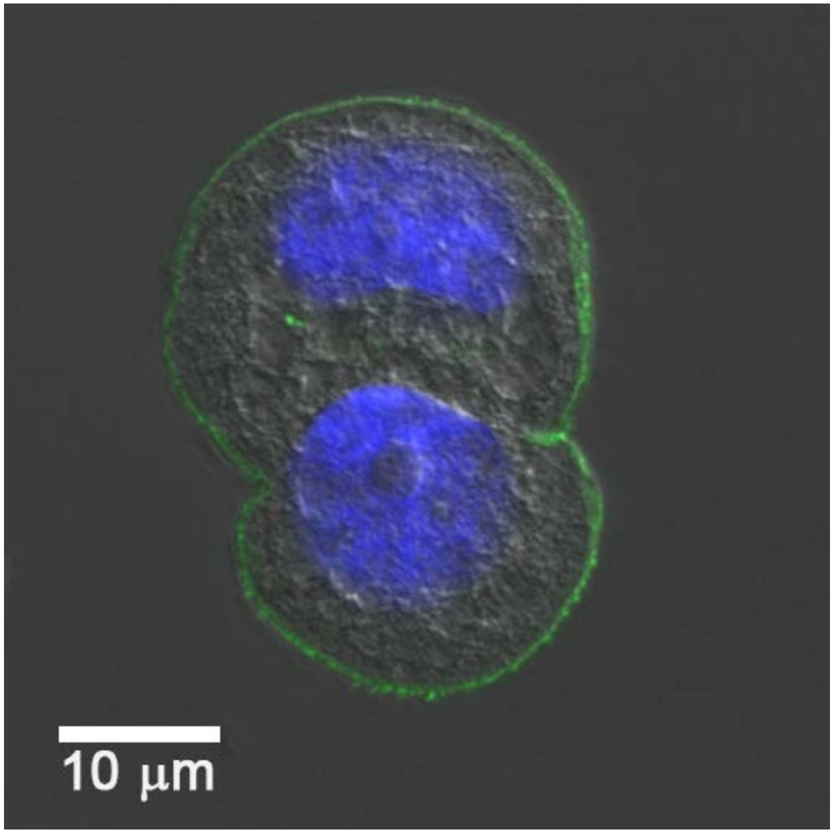
Our Target: CA125



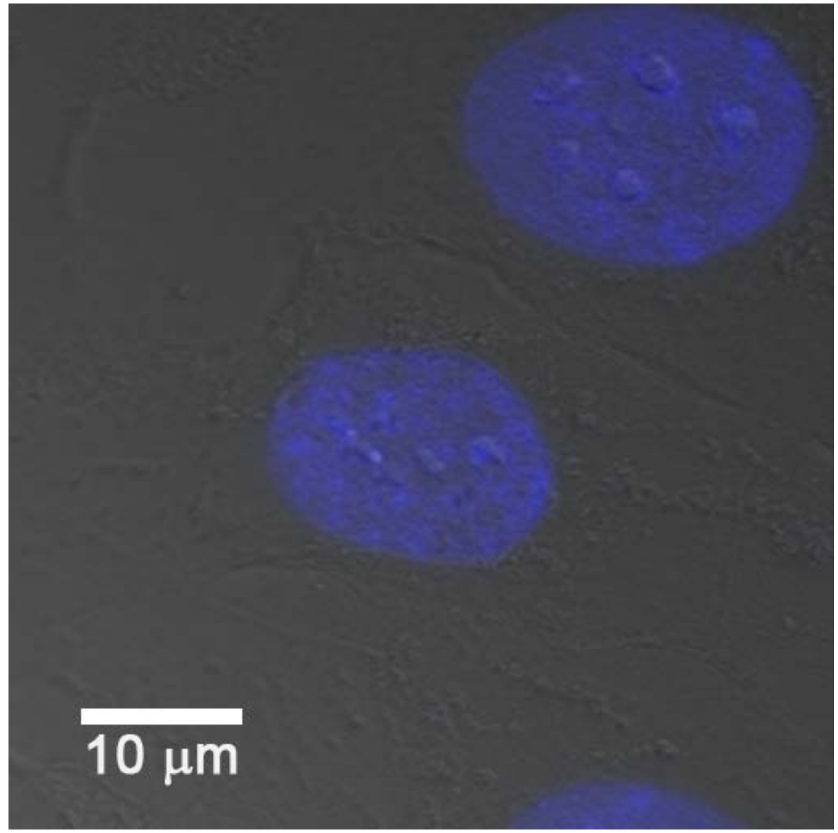
- Ovarian Tumor Associated Antigen
- High MW glycoprotein (~5 MDa)
- High diagnostic / prognostic value in recurrent EOC

O'Brien et al. Tumour Biol 2001;22(6): 348-66.

Our Target: CA125



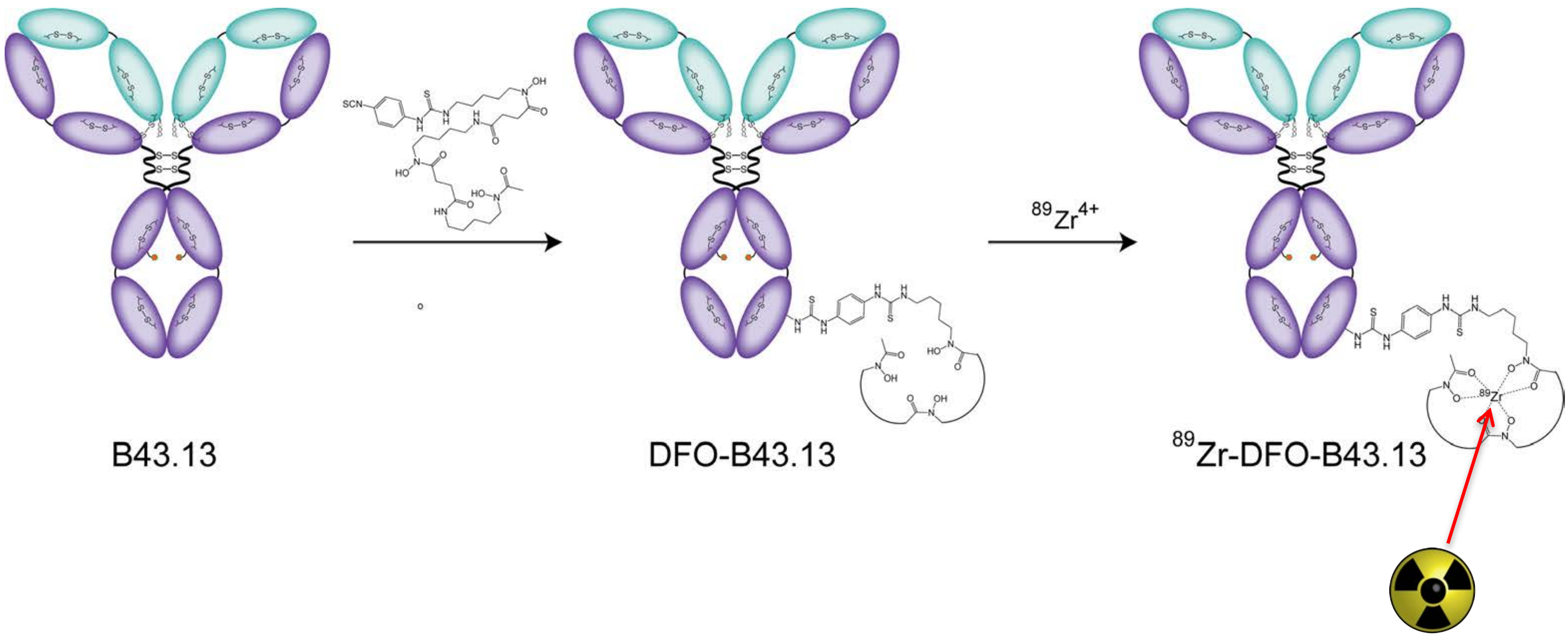
OVCAR3
CA125 - positive



SKOV3
CA125 - negative

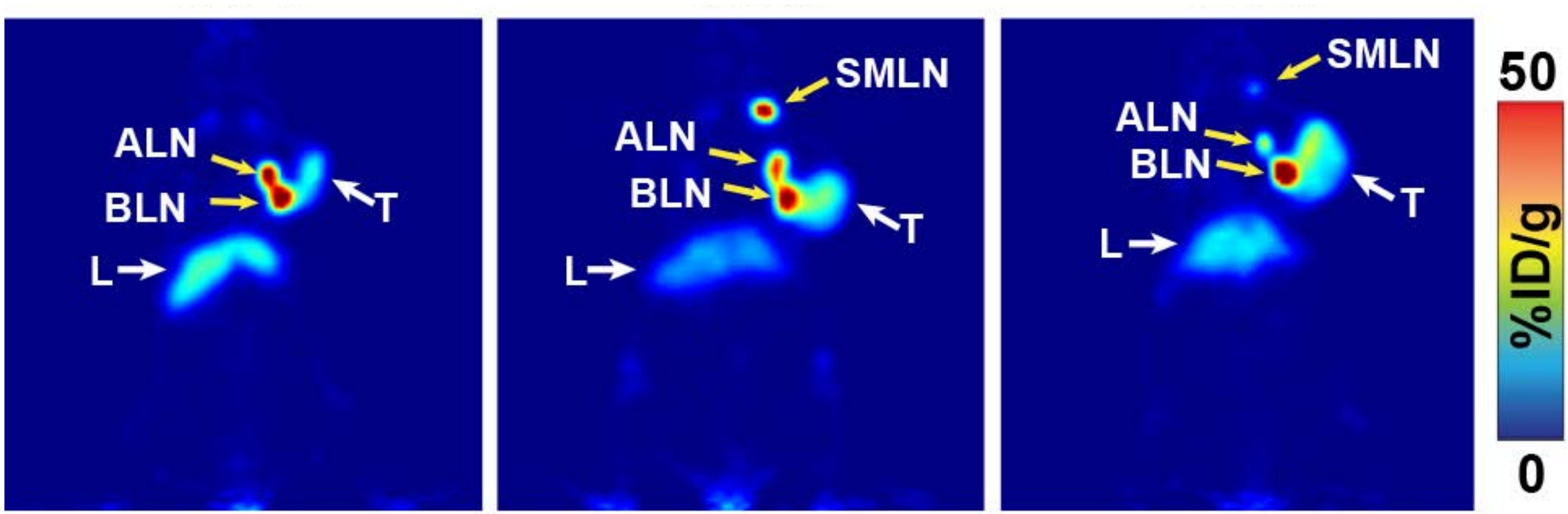
Sharma et al. EJNMMI Research 2014, 4:60.

PET Imaging of CA125

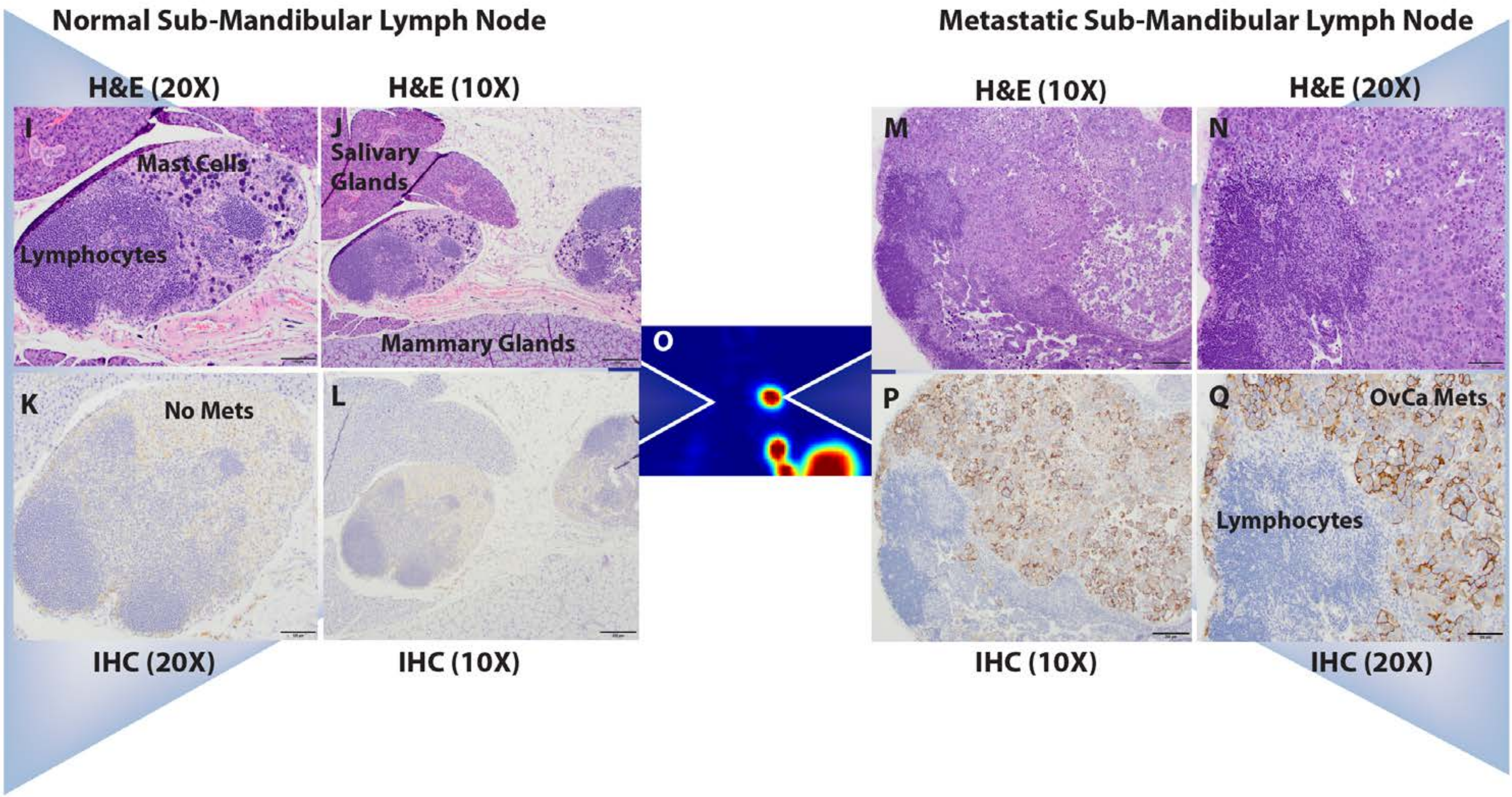


PET Imaging of Ovarian Cancer

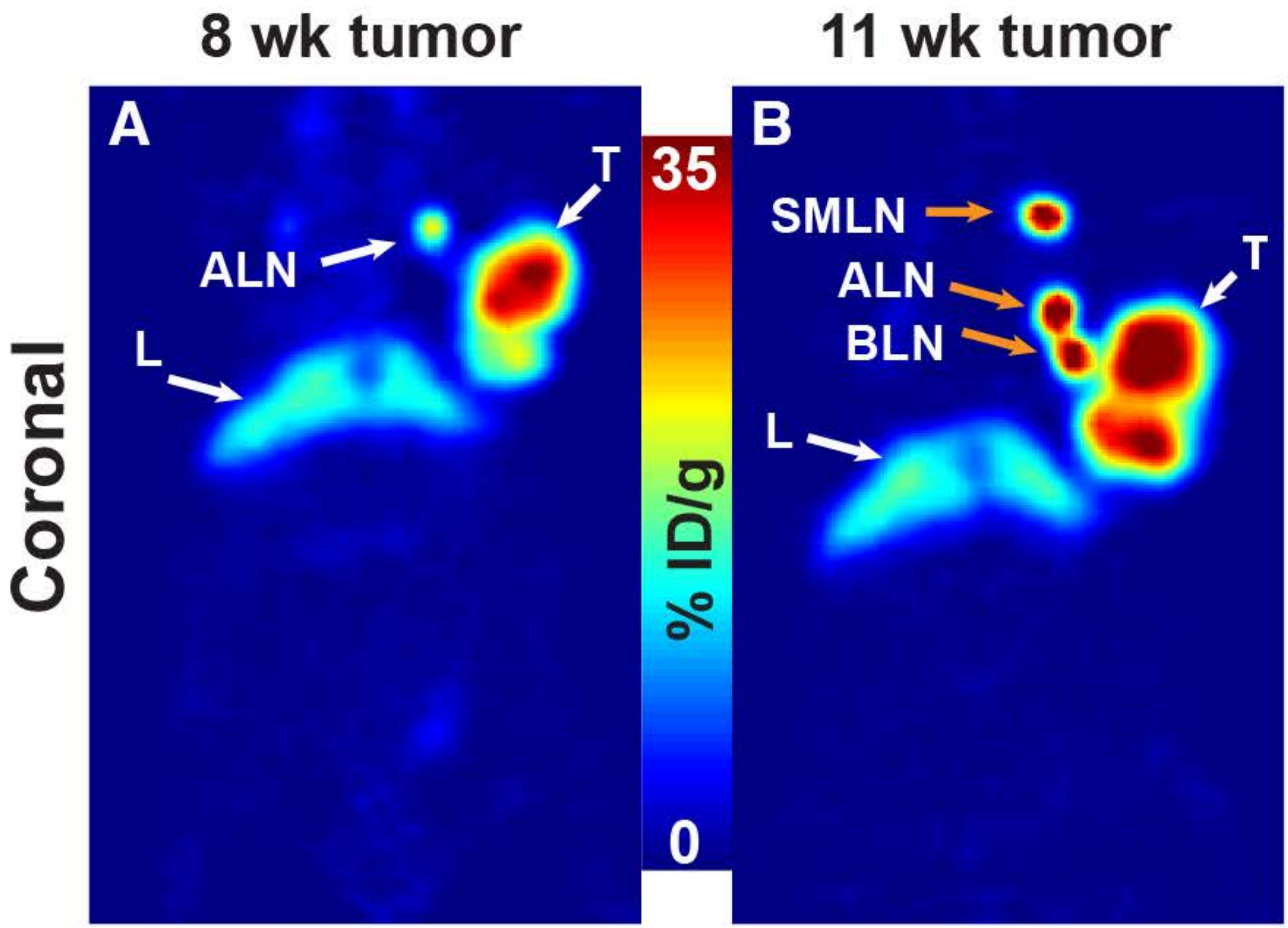
- Inject human ovarian cancer cells on the shoulder of mice
- Let the tumors grow for 3-4 weeks
- Inject the mice with ^{89}Zr-DFO-B43.13



PET Imaging of CA125



PET Imaging of CA125



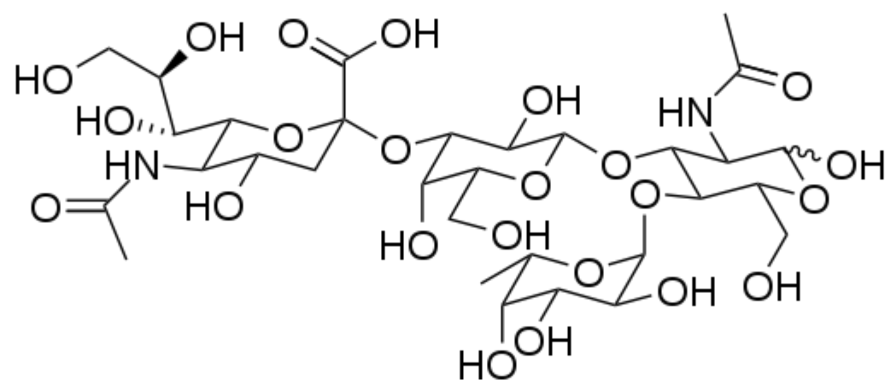
Sharma et al. J Nucl. Med. 2016 (in press).

PET Imaging of CA125

An Example from the Clinic: ImmunoPET of Pancreatic Cancer

Pancreatic Cancer, CA19.9, and 5B1

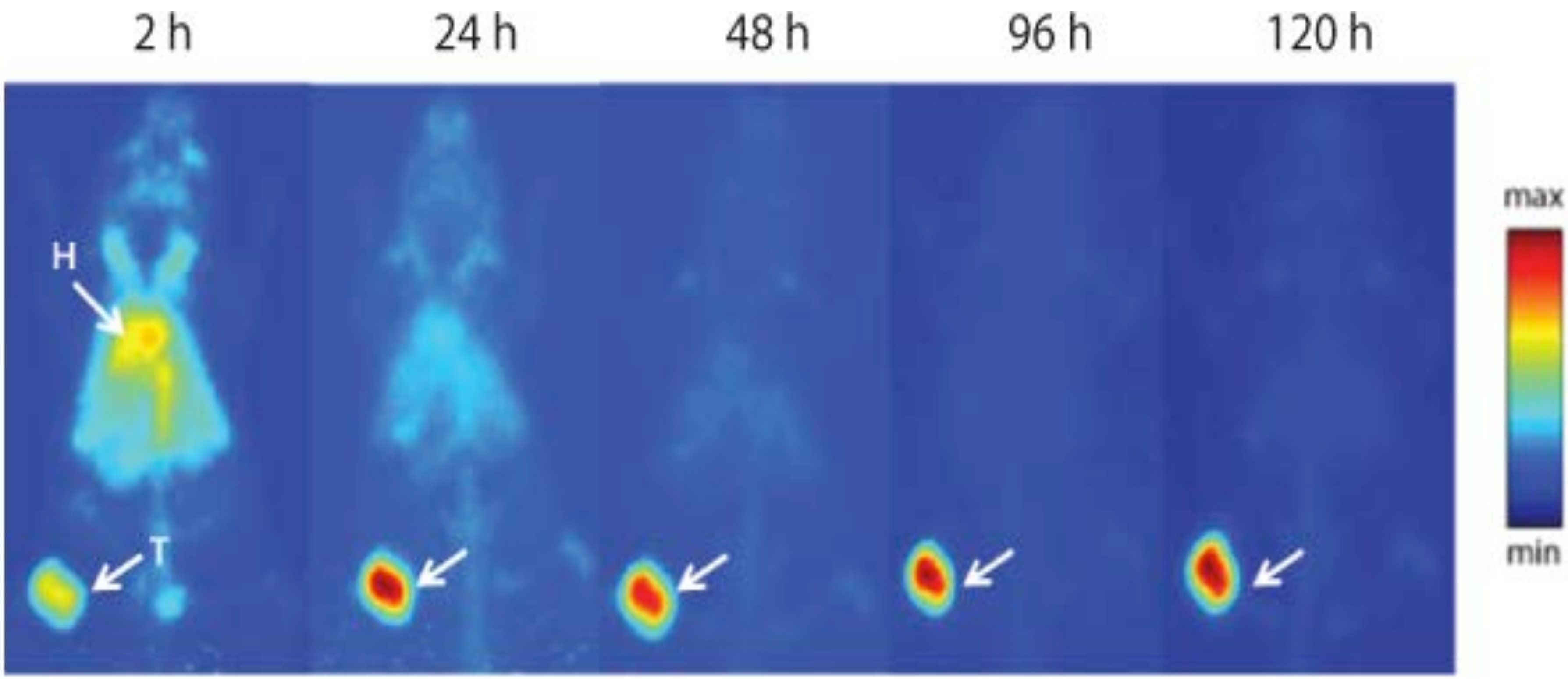
- Pancreatic cancer is the 4th leading cause of cancer-related deaths.
- Pancreatic cancer lacks effective treatment and imaging modalities.
- Carbohydrate antigen 19.9 (CA19.9) is a promising biomarker for pancreatic cancer
  - Supports selectin-dependent adhesion
  - Up to 200 copies/protein
  - Attached to as many as 50 proteins
  - Elevated in several types of cancer, including PDAC (~90%)



- 5B1 is a fully human antibody that specifically binds CA19.9

Imaging Pancreatic Cancer

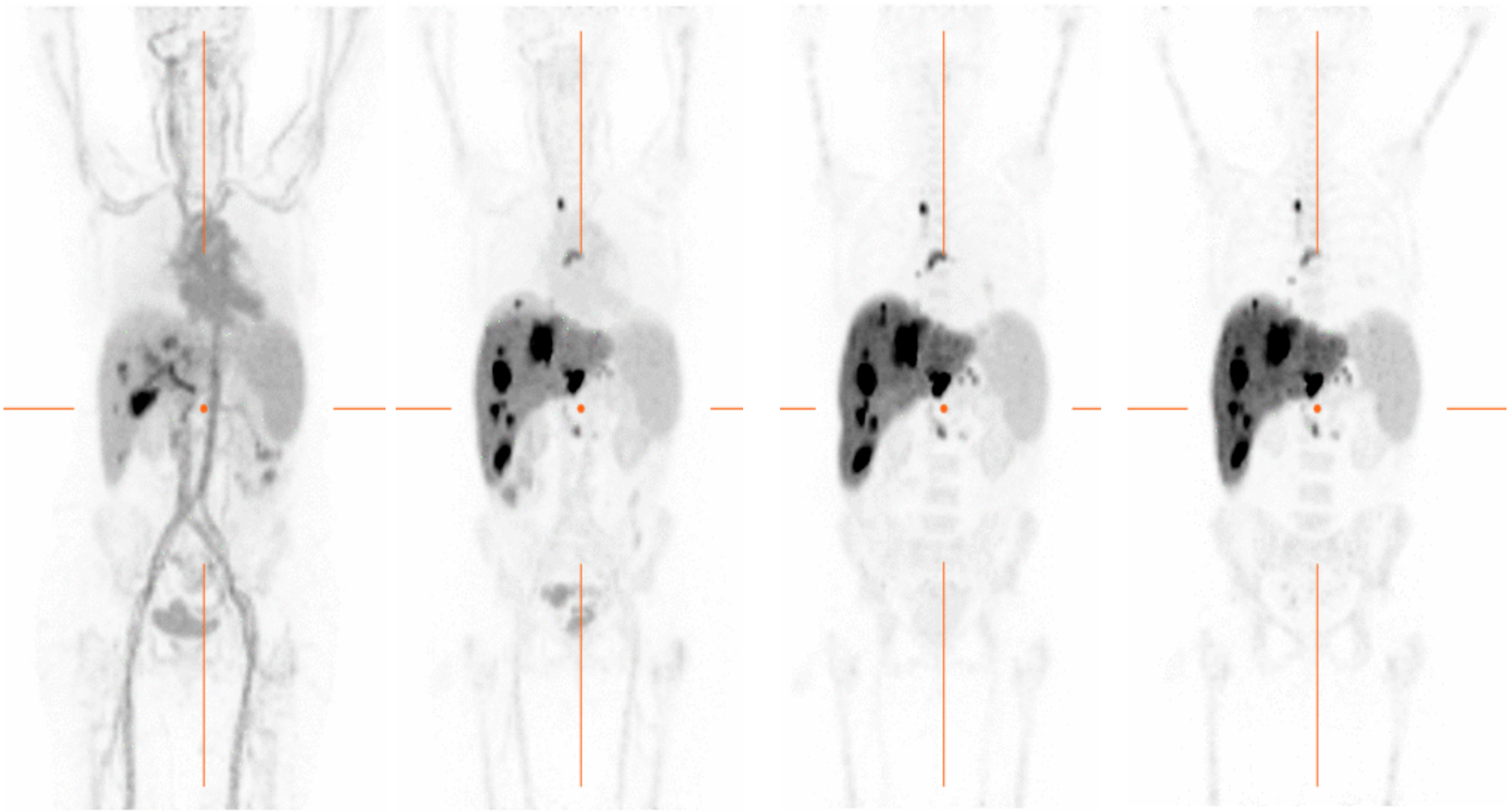
Targeting CA19.9 with hu5B1



Coronal PET images acquired with ^{89}Zr-DFO-5B1 in athymic nude mice bearing CA19.9-expressing BxPC3 PDAC xenografts.

Villegas, *et al.* J Nucl Med 2013.

PET Imaging with ^{89}Zr-DFO-5B1

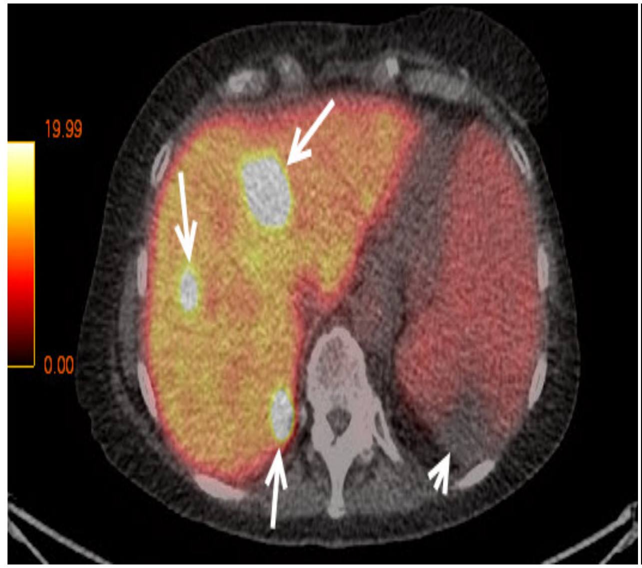


PET/CT imaging study with MVT-2163 (^{89}Zr-DFO-HuMab-5B1)

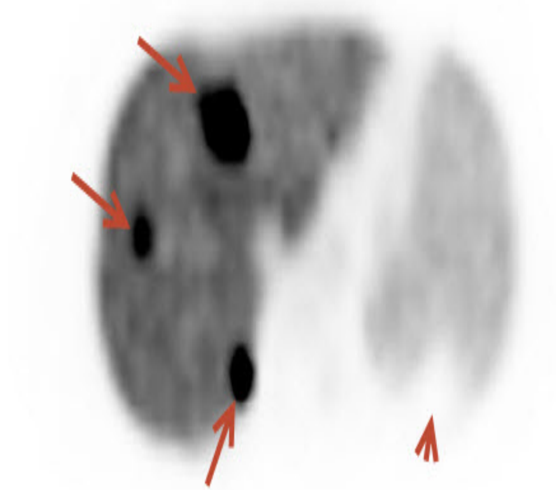
PET Imaging with ^{89}Zr-DFO-5B1

- **Liver Metastases**

67-year-old female with metastatic pancreatic adenocarcinoma and rising CA 19-9 antigen levels of 2119 U/mL



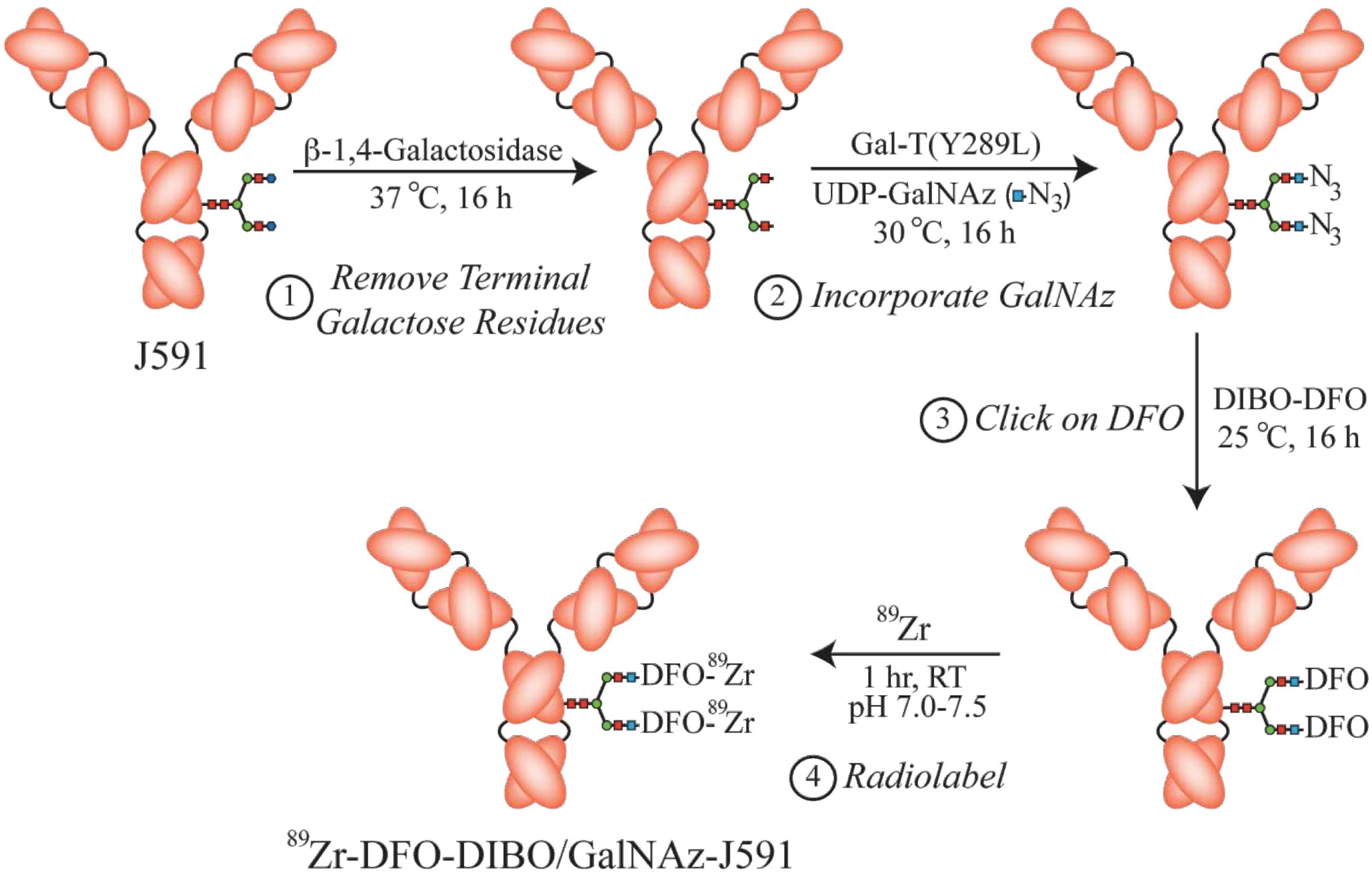
Diagnostic CT



PET/CT image after injection of 47 mg MVT-5873 and 3 mg MVT-2163

What's Next?

Site-Specific Bioconjugation

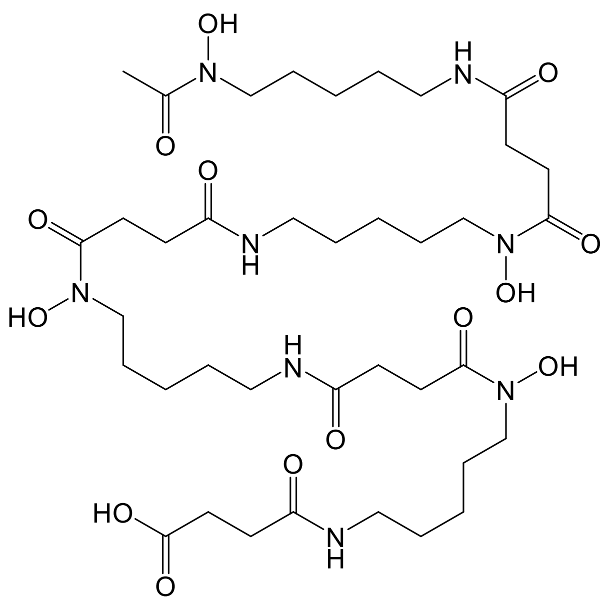


Zeglis BM, et al., Bioconjugate Chemistry, 24, 1057-1067, 2013.

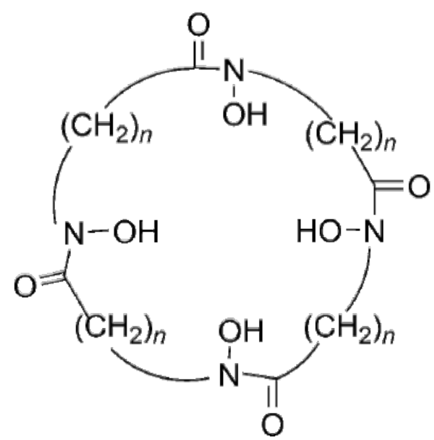
What's Next?

New Chelators for $^{89}Zr^{4+}$

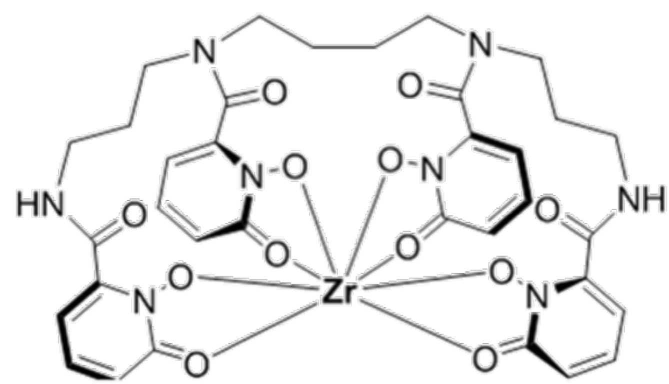
- A more robust ligand designed specifically for Zr^{4+} would demonstrate greater stability and, therefore, less *in vivo* release and bone uptake.
  - HOPO: Deri, et al. *J. Med. Chem.* **2014**, 57, 4849
  - C5-C7: Guérard, F. et al. *Chem. Eur. J.* **2014,** *20,* 5584.
  - DFO*: Patra, M. et al. *Chem. Commun.* **2014,** *50,* 11523.
  - BFC1: Pandya, D. N. et. al *Chem. Commun.* **2015,** *51,* 2301.
  - FSC: Zhai, C. et al. *Mol. Pharm.* **2015,** *12,* 2142.



DFO*



C5-C7



HOPO

Conclusions

- Antibodies are an effective vector for the delivery of positron-emitting radionuclides to tumor tissue.

- The long serum half-life of immunoglobulins means that they must be radiolabeled with long-lived radionuclides such as ^{89}Zr.

- $^{89}Zr^{4+}$ is an osteophilic radiometal so it must be covalently attached to the antibody and stably coordinated with a chelator such as desferrioxamine (DFO)

- ^{89}Zr-labeled antibodies have shown immense potential for the nuclear imaging of ovarian and pancreatic cancer.

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