

Engineered Antibody Fragments for ImmunoPET Detection of Cancer



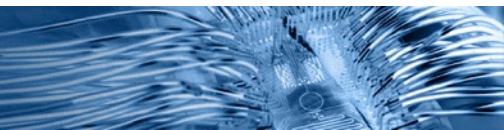
Anna M. Wu, Ph.D.

Crump Institute for Molecular Imaging
Department of Molecular and Medical Pharmacology
David Geffen School of Medicine at UCLA

Founder and Chief Scientific Advisor, ImaginAb, Inc.

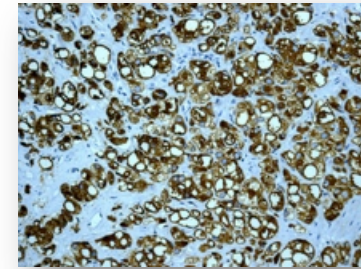


Crump Institute
for
Molecular Imaging



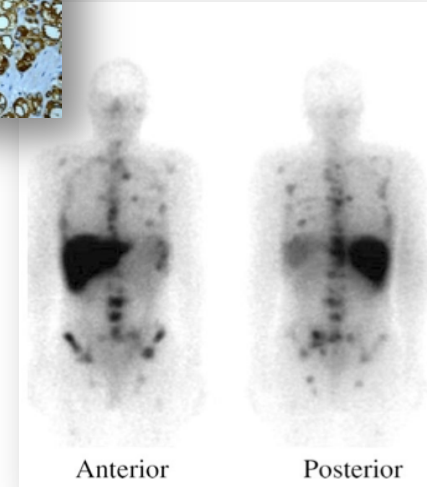
Why Image? Why ImmunoPET?

- Molecular therapeutics need molecular diagnostics
- Limitations of *in vitro* diagnostics
- Molecular imaging of *in vivo* biomarkers
 - Localization – staging
 - Biologically characterize disease
 - Quantify disease
 - Patient selection/stratification – target expression
 - Delivery and disposition of therapeutic
 - Response to therapy
- Broad availability of clinical PET
 - [¹⁸F]-2-fluorodeoxyglucose (FDG-PET) for imaging elevated metabolism in cancer and other diseases



α -PSMA IHC

α -PSMA imaging for metastatic prostate cancer



N. Bander et. al



A Long, and Short, History of (US FDA-approved) Antibody Imaging

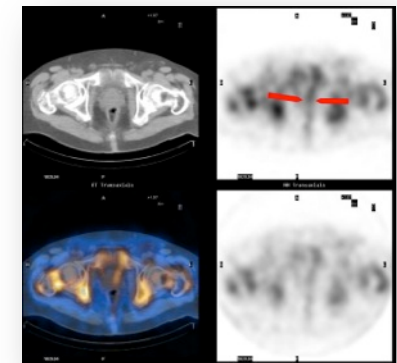
- ^{99m}Tc -arcitumomab (anti-CEA murine Fab)
- ^{111}In -satumomab (anti-TAG72 murine Mab)
- ^{99m}Tc -novetumomab (Nr-Lu-10 murine Fab)
 - Approved 1990's; Never marketed or no longer marketed in US



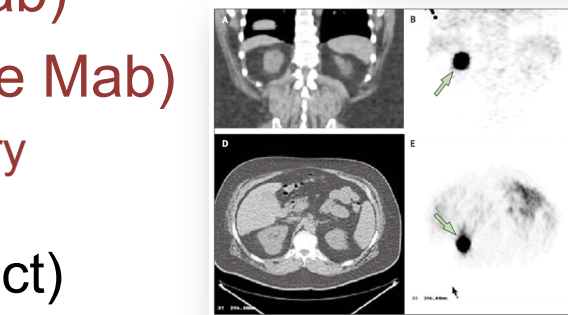
^{99m}Tc -arcitumomab

- ^{111}In -capromab (anti-PSMA murine Mab)
 - Fusion (SPECT/CT) imaging as revived interest

^{111}In -capromab
Nagda et al., Int J Rad Onc Biophys 2007
Mouraviev et al., J Urol 2009



- ^{131}I -tositumomab (anti-CD20 murine Mab)
- $^{111}\text{In}/^{90}\text{Y}$ ibritumomab (anti-CD20 murine Mab)
 - Radioimmunotherapy with imaging/dosimetry



- ^{124}I -girentuximab (anti-CA IX chimeric intact)
immunoPET detection of renal carcinoma
Completed Phase III

^{124}I -cG250

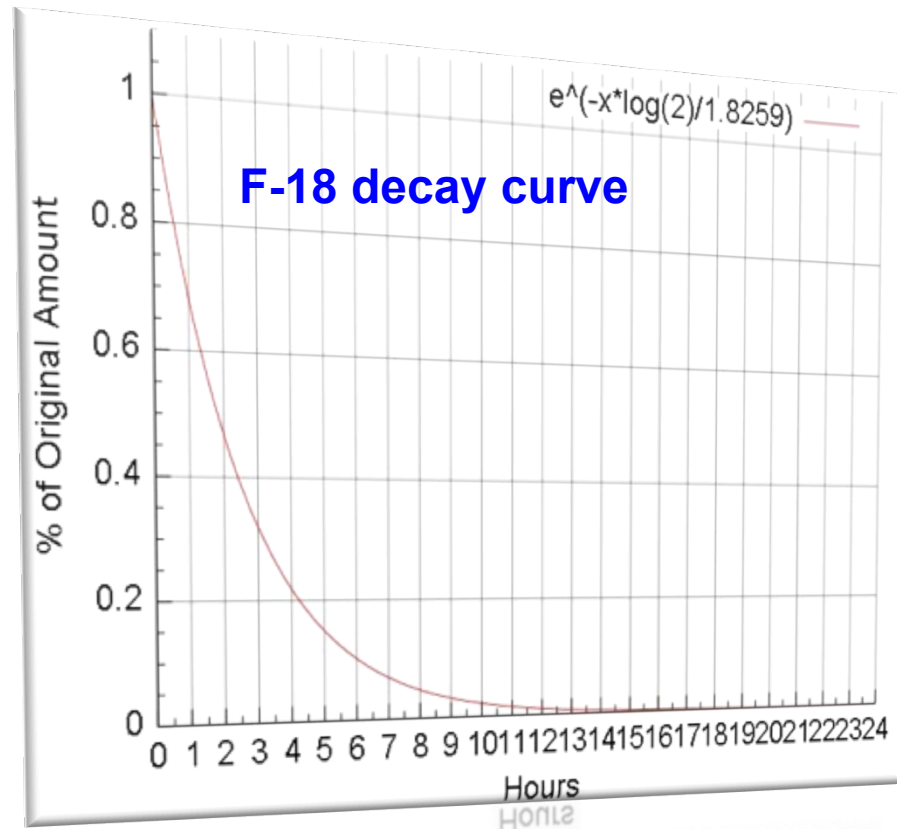
Divgi et al. Lancet Oncol. 2007
Uzzo et al. AUA 2010

The Challenge

Irresistible force

vs.

Immovable object



$$t_{1/2} = 109 \text{ min}$$



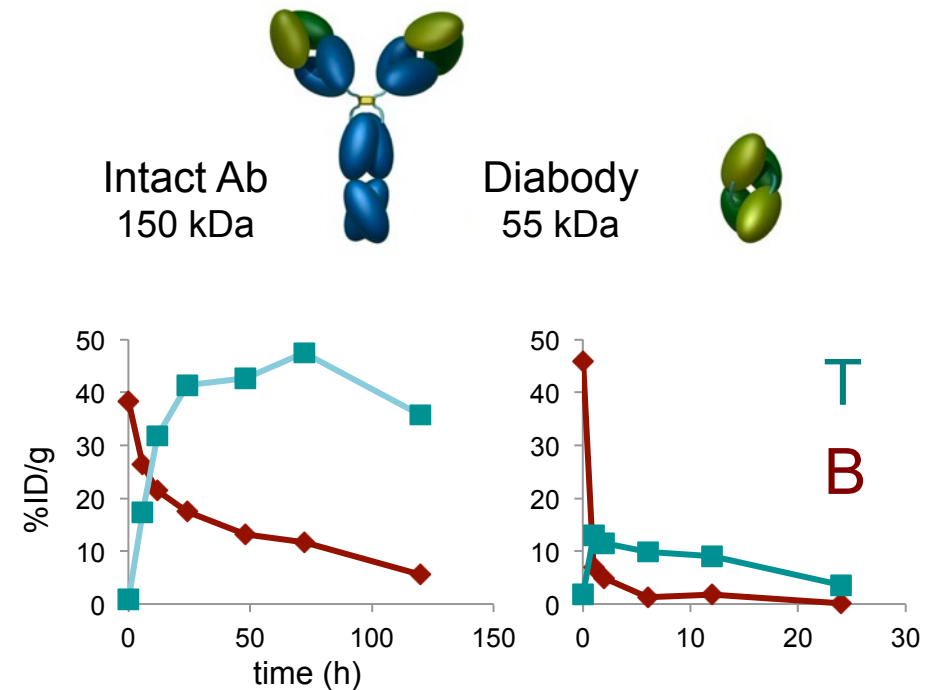
$$t_{1/2} = 2 \text{ wks}$$

The Solutions

Positron-emitting radionuclides with *longer* half-lives

Isotope	$T_{1/2}$
^{68}Ga	1.1 h
^{18}F	1.8 h
^{64}Cu	12.7 h
^{86}Y	14.7 h
^{76}Br	16.2
^{89}Zr	78.5
^{124}I	100.3

Engineered antibody fragments with *shorter* half-lives

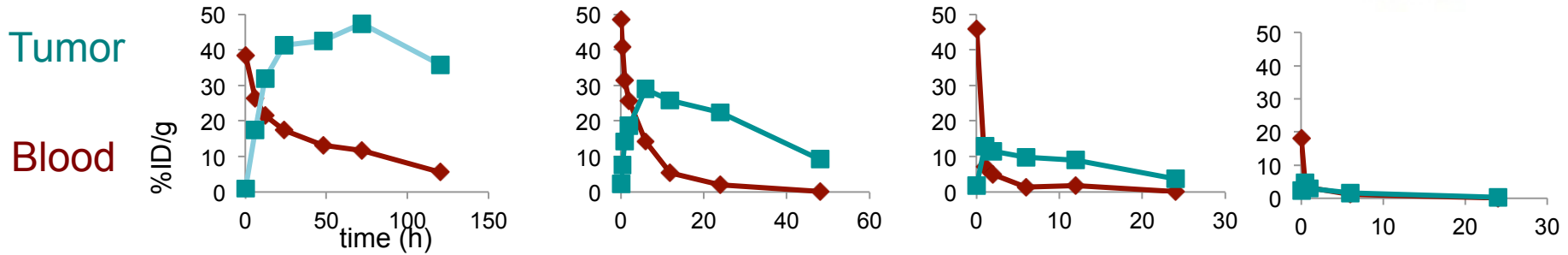
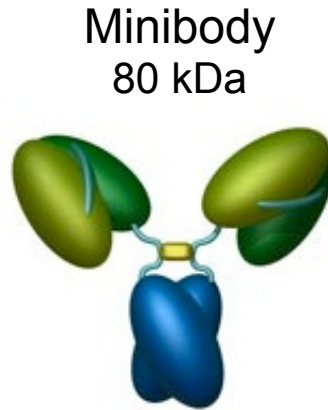
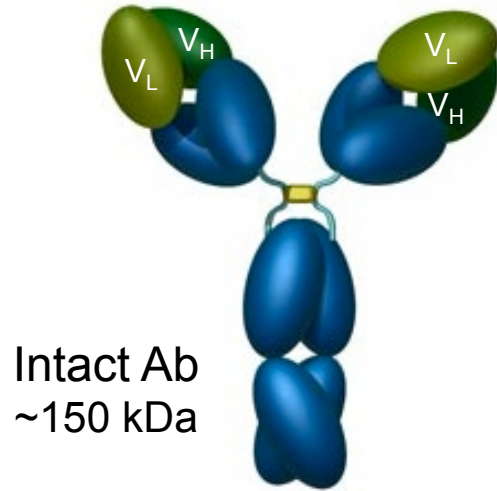


Diabody: Peak uptake at 1-2 h post injection
3-4 h half-life

Engineering Antibodies for Therapy vs. Imaging

- Engineering antibodies for *therapy*
 - Reduction of immunogenicity
 - Half-life extension (1-3 wks and longer)
 - Enhanced effector function
- Engineering antibodies and fragments for *imaging* (and *RIT*)
 - Reduction of immunogenicity
 - Half-life reduction / accelerated clearance
(clinical imaging w/intact typically requires 5-7 d delay)
 - Removal of effector functions
 - Drive clearance to kidneys or liver based on size
 - Improved diffusion / transport in target tissues
 - Sites for conjugation of imaging moiety

Engineering Antibodies for ImmunoPET: Control of PK and Targeting

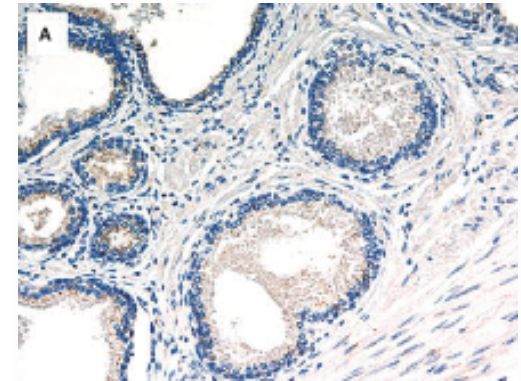


Exposure vs. Clearance

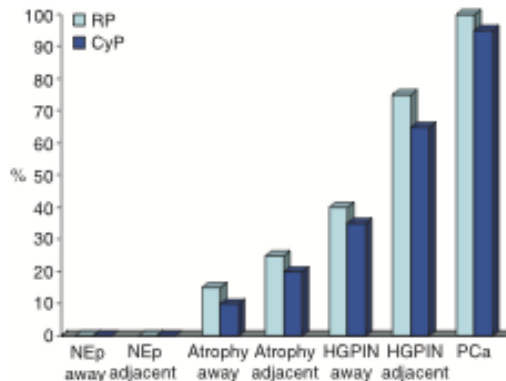
PSCA expression in normal tissue and prostate cancer

- GPI-anchored 123 aa glycoprotein
- Low expression in prostate basal and secretory cells
- Also expressed in transitional epithelium of bladder and stomach
- Overexpressed in 94% of primary prostate cancer
- Expression correlates with the Gleason score
- Highly overexpressed in 87-100% of prostate cancer bone metastases

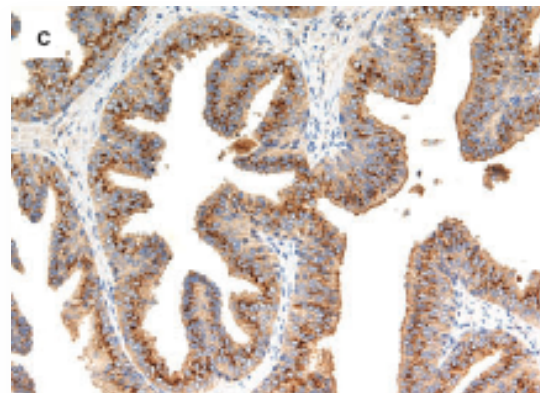
Normal Prostate



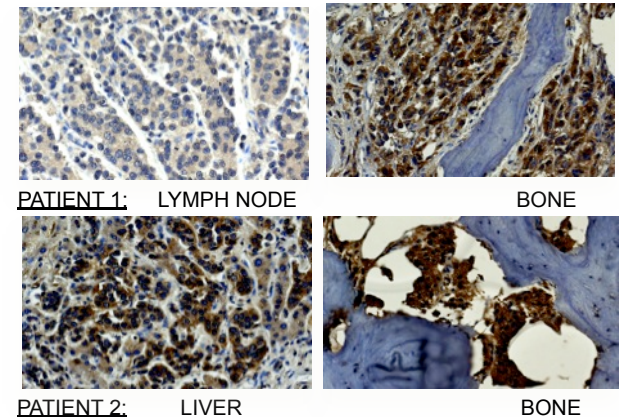
% of patients with overexpression



Primary prostate cancer



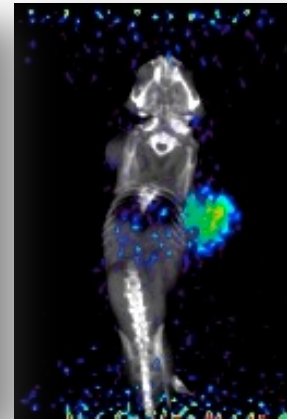
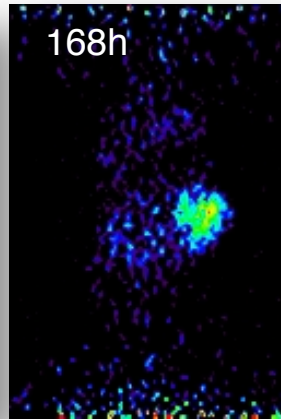
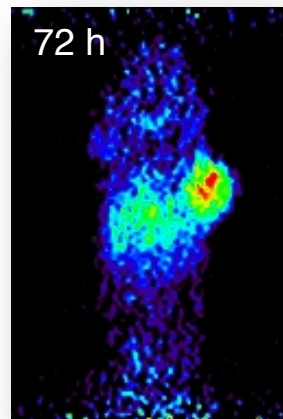
Metastatic prostate cancer



MicroPET Imaging using Intact ^{124}I m1G8 and hu2B3 anti-PSCA Antibodies



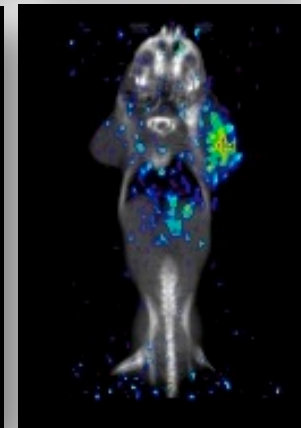
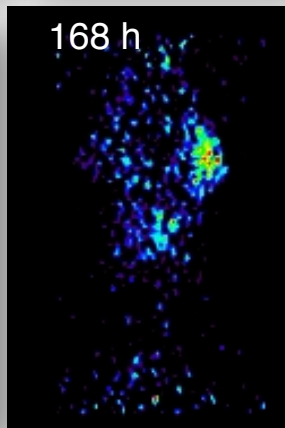
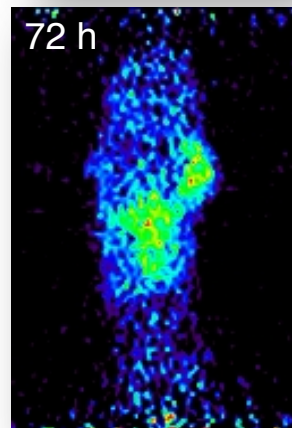
Murine 1G8
2.6 nM



LAPC-9 = 5.8 (0.8)% ID/g
PC-3 = 2.6 (0.5)% ID/g
Tumor:negative tumor = 2.23



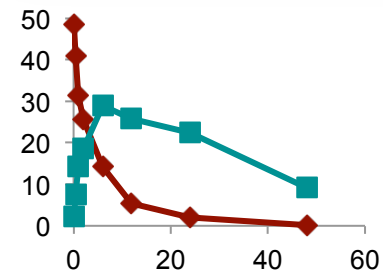
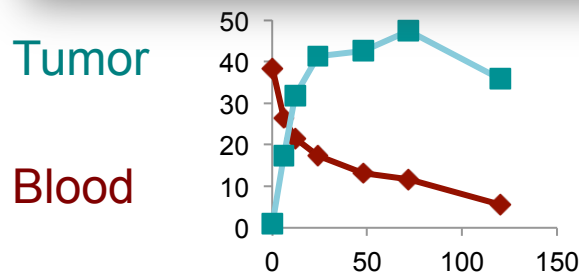
Humanized 2B3
16.7 nM



LAPC-9 = 6.6 (0.9)% ID/g
PC-3 = 3.3 (1.1)% ID/g
Tumor:negative tumor = 2.0

LAPC-9 (PSCA-positive, right shoulder) vs. PC-3 (PSCA-negative, left shoulder)
Clear microPET imaging obtained at **168 h** for both intact antibodies.

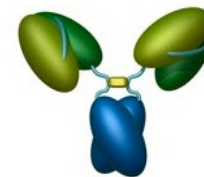
Engineering Antibodies for ImmunoPET: Minibody for Next-Day Imaging



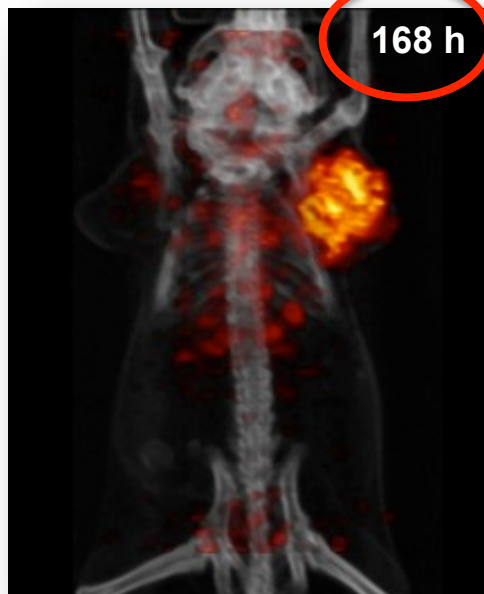
Minibody: Peak uptake at 4-6 h post injection; 5-11 h half-life

Choosing the right vehicle

Imaging PSCA: Intact Antibody vs. Minibody



2B3 Intact



2B3 minibody



- ^{124}I -labeled humanized minibody displays comparable biodistribution and imaging results
- However, at a **much earlier time** (20 h)

Images are adjusted to same scale

LAPC-9 5.3 (1.6)% ID/g

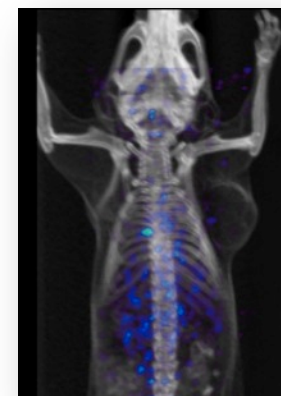
PC-3 (NT) 2.6 (0.2)% ID/g

T:NT = 2.0

LAPC-9 6.6 (0.9)% ID/g

PC-3 3.3 (1.1)% ID/g

T:NT = 2.0

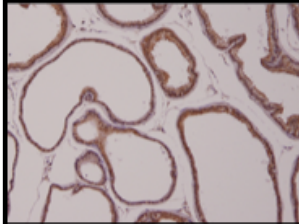
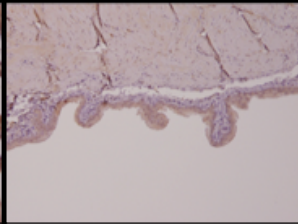
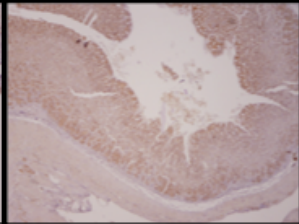
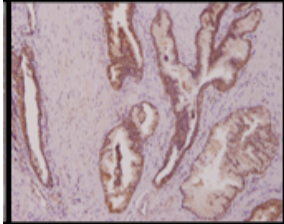
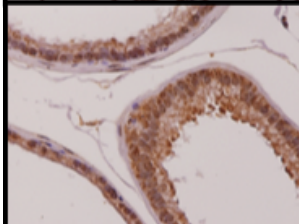
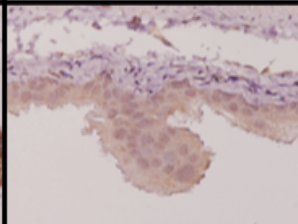
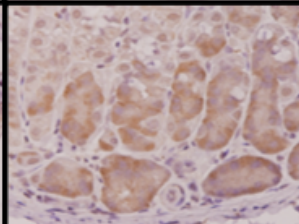
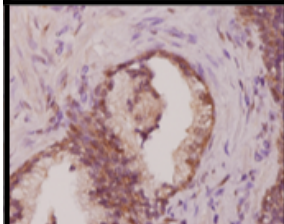


Control anti-CD20
minibody

Transgenic mouse model: hPSCA expression similar to mPSCA in WT

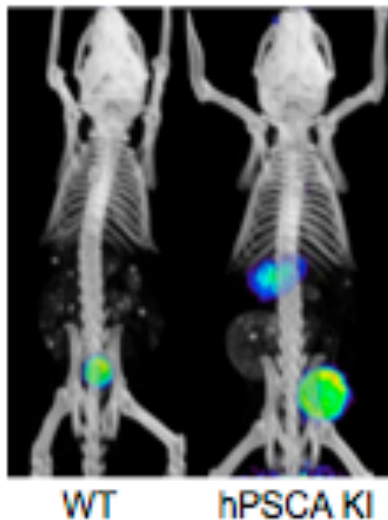


- 4 lines of hPSCA knockin mice were generated
- hPSCA expression in hPSCA^{+/-} KI mice was examined with immunohistochemistry and RT-PCR.

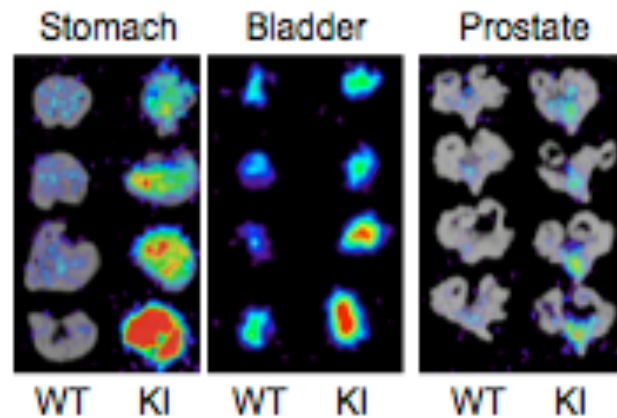
Prostate	Bladder	Stomach	Human Prostate
			
			
+++	+	+++	

A11 immunoPET of hPSCA^{+/-} KI vs WT

20 hr
A11 ImmunoPET/CT



20 hr
A11 ex vivo PET/CT



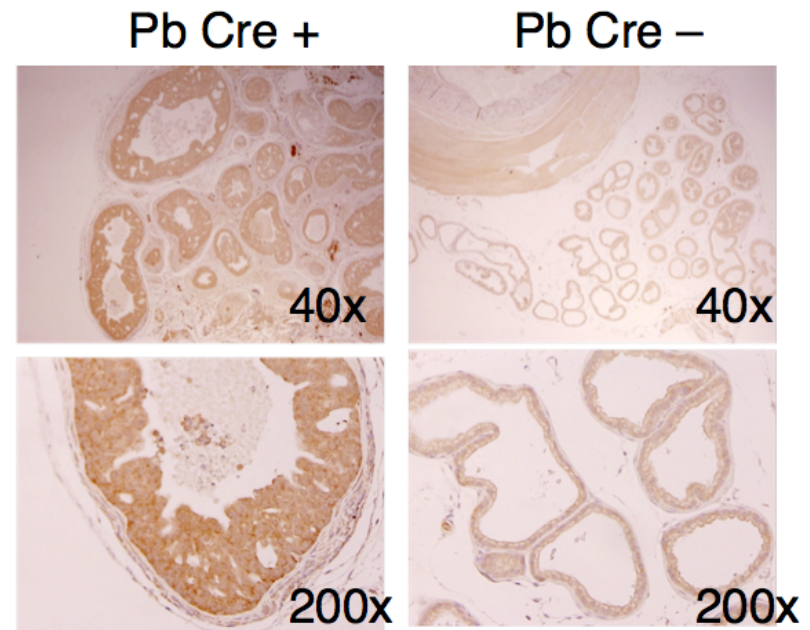
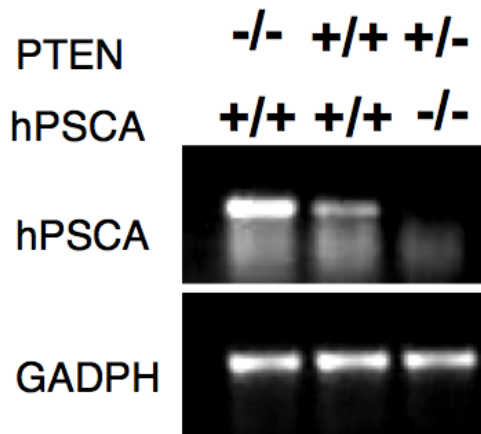
20 hr
A11 biodistribution

	WT %ID/g	KI %ID/g	p
Prostate	0.49±.04	0.83±.13	0.04
Bladder	1.65±.26	3.21±.42	0.02
Stomach	0.79±.05	2.01±.22	0.002
Pancreas	0.51±.02	0.63±.09	0.26
Muscle	0.38±.04	0.43±.02	0.30
Liver	1.09±.03	1.23±.12	0.32
Kidney	1.86±.10	2.05±.24	0.51
Lung	2.8±.25	2.92±.33	0.78
Spleen	1.31±.03	1.43±.19	0.54
Blood	4.67±.21	5.27±.48	0.29

- hPSCA^{+/-} KI imaging shows increased stomach uptake compared to WT at 20hrs
- hPSCA^{+/-} ex vivo imaging and biodistribution show increased uptake in the prostate, bladder, and stomach of hPSCA mice
- Biodistribution correlates well with hPSCA expression by IHC and RT-PCR

hPSCA KI mice crossed into a PTEN^{Loxp/Loxp} transgenic prostate cancer model

- hPSCA PTEN^{Loxp/Loxp} Pb-Cre⁺ mice progress from PIN to invasive adenocarcinoma
- Tumor over-expresses hPSCA as it progresses



A11 minibody can image local prostate cancer in hPSCA $PTEN^{Loxp/Loxp}$ Pb Cre+ mice

7 month old hPSCA $PTEN^{Loxp/Loxp}$ Pb Cre+ mice show significant increase in prostate uptake at 44 hrs *in vivo*

CT windows:

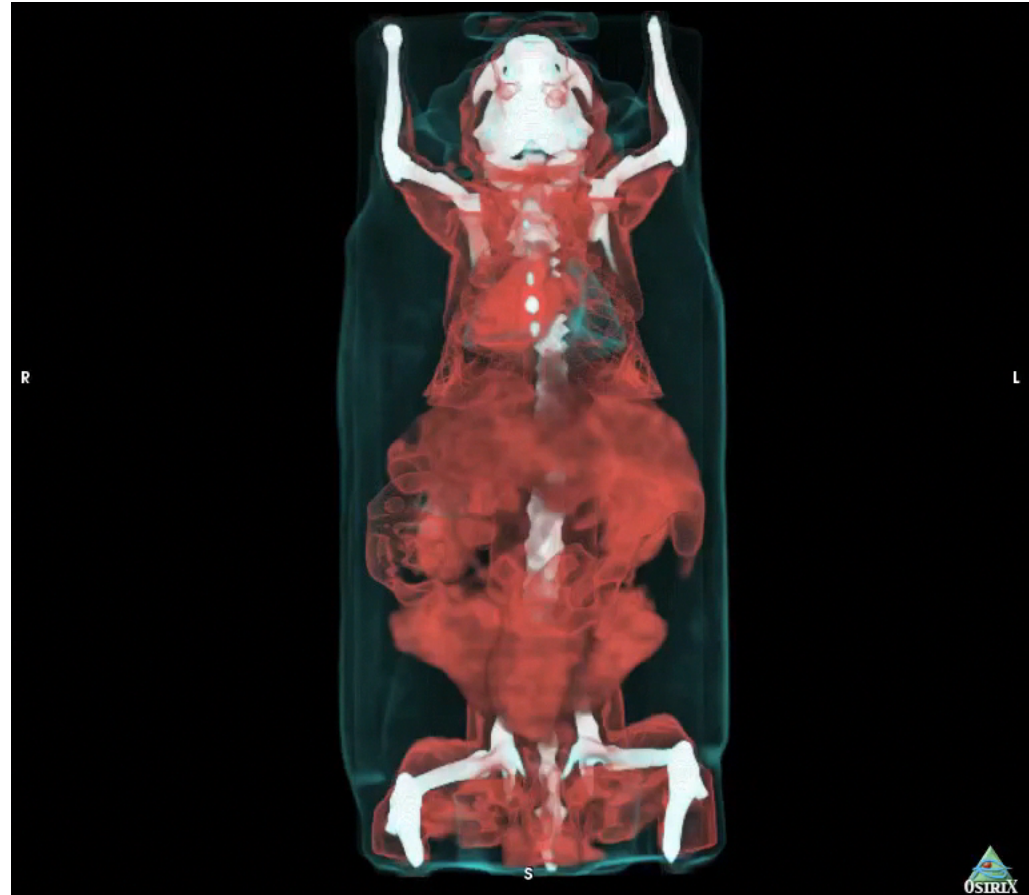
White: bone

Green: skin

Red: soft tissue

PET:

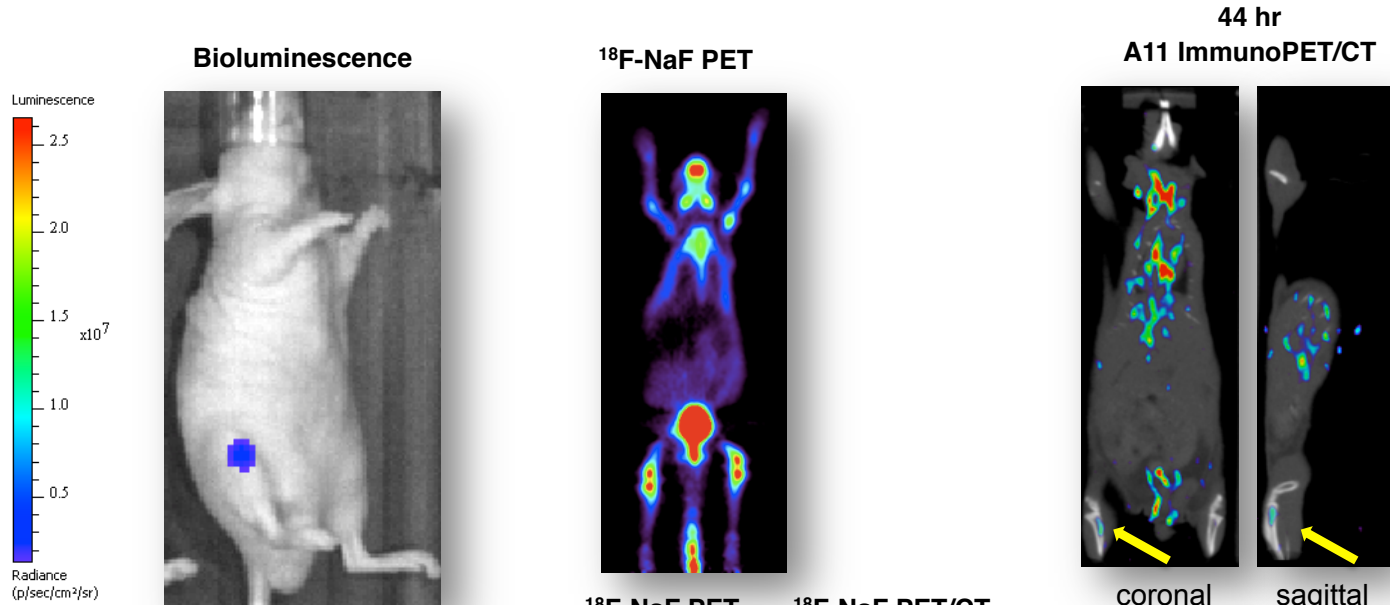
I-124 A11 anti-PSCA minibody



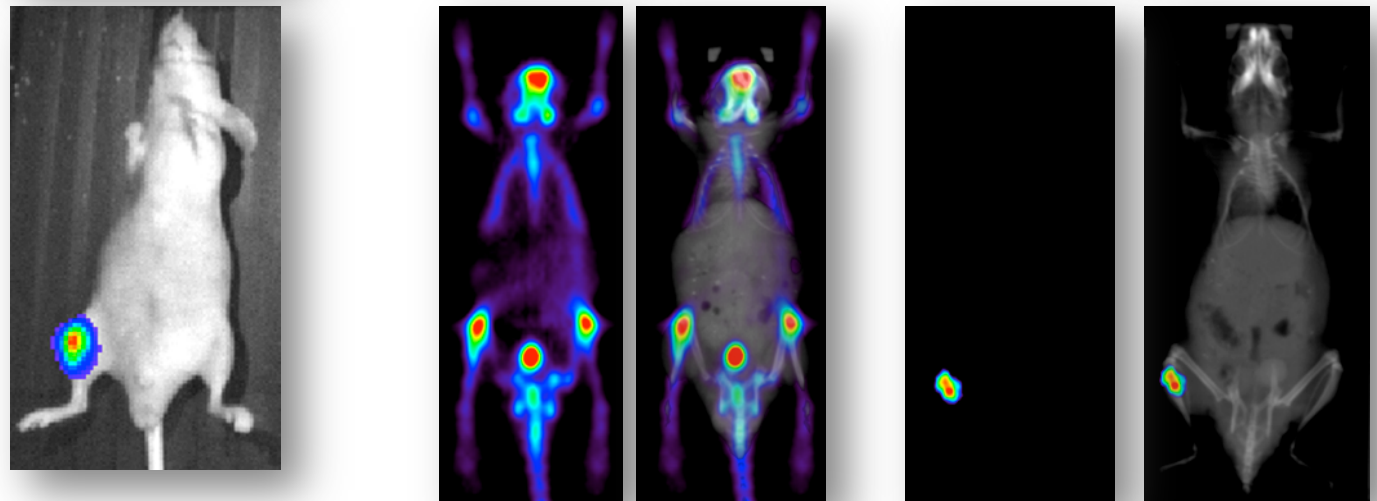
Intratibial tumors: 22rv1-PSCA Fluc

Optical vs. NaF vs. A11 minibody

2 weeks

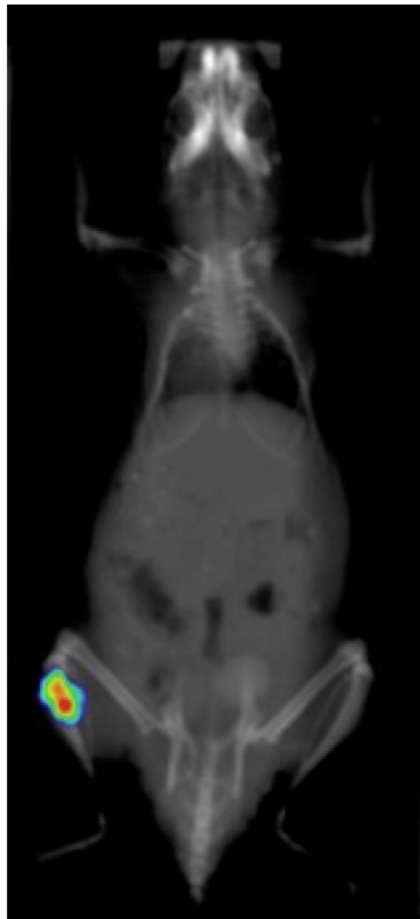


6 weeks

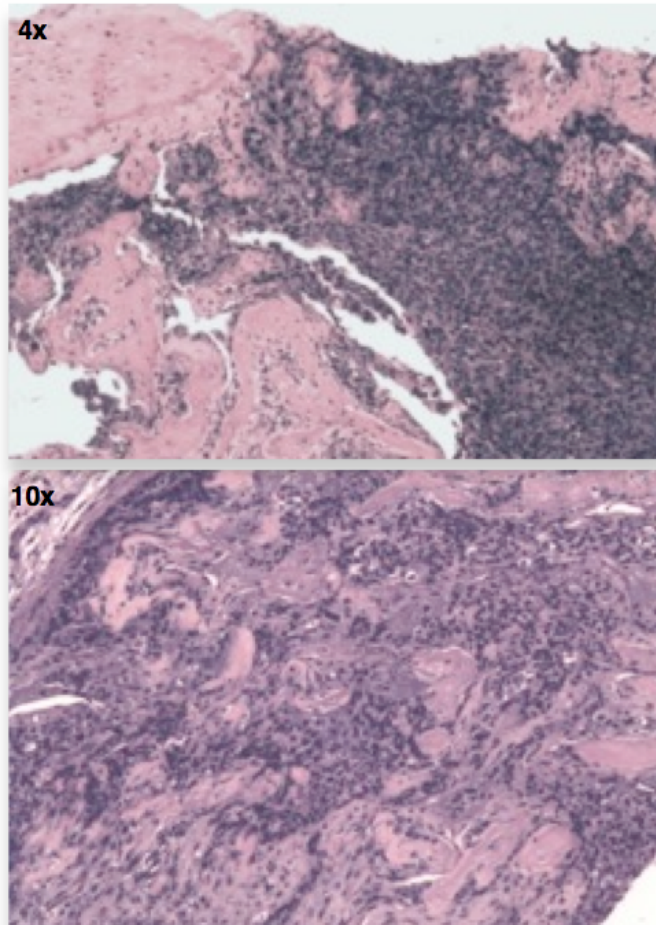


A11 minibody imaging is more specific than ^{18}F -NaF PET in detecting intratibial tumors

- Six weeks post-injection A11 minibody detects intratibial tumors with higher specificity than ^{18}F -NaF PET bone scans



44 hr A11 ImmunoPET/CT

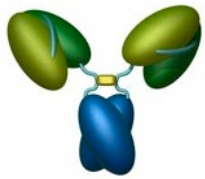


Intratibial Xenograft H&E

44 hr A11 Biodistribution

	Mean %ID/g
22rv1xPSCA Tibia	3.31
22rv1 Tibia	0.45±0.11
Negative Tibia	0.07±0.004
Blood	0.31

22rv1xPSCA Tibia: 22rv1 Tibia	7.28
22rv1xPSCA Tibia: Blood	10.71
22rv1xPSCA Tibia: Negative Tibia	46.86

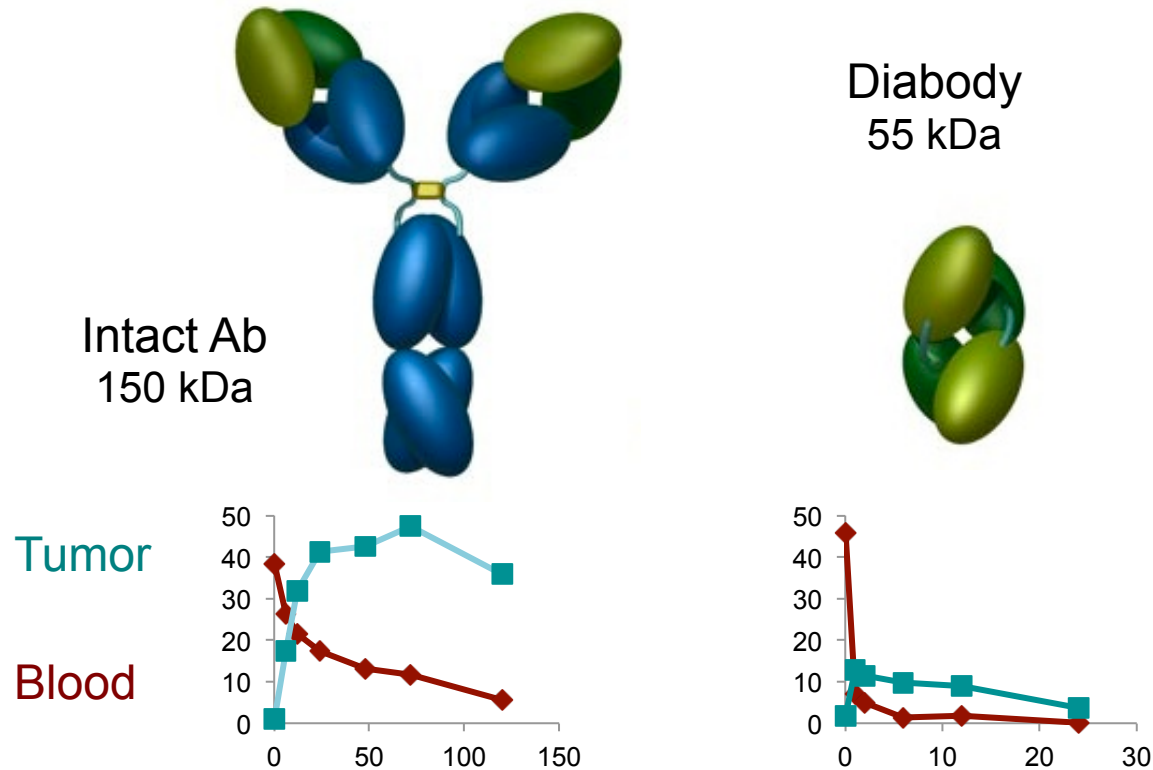


Clinical development of A11 anti-PSCA minibody



- Affinity-matured, humanized anti-PSCA minibody
- Collaboration with City of Hope - cGMP production and pilot clinical imaging studies
- cGMP production, purification, vialing, safety studies, test radiolabeling complete. IND in preparation
- Goal: compare **I-124 PSCA minibody** imaging to conventional imaging (bone scan and CT) in men with metastatic prostate cancer

Engineering Antibodies for ImmunoPET: Diabody for Same Day Imaging

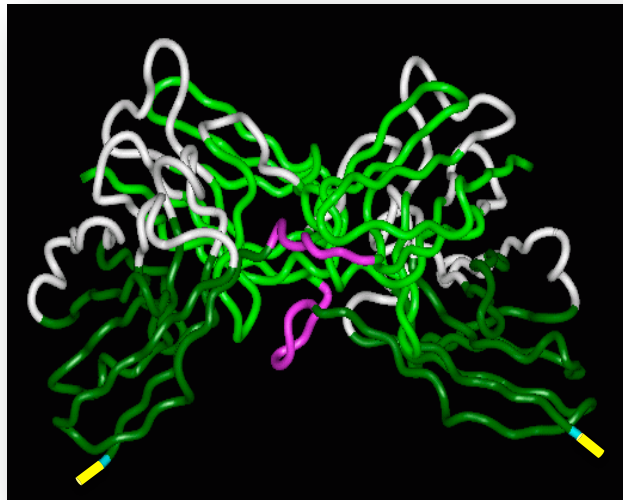


Diabody: Peak uptake at 1-2 h post injection; 3-4 h half-life

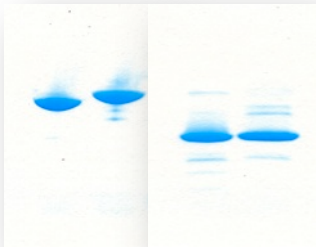
Engineering for site-specific conjugation



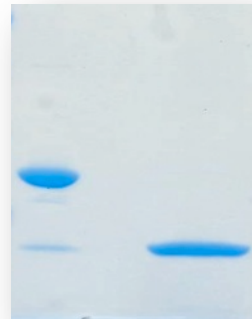
T84.66 anti-CEA diabody



CEA



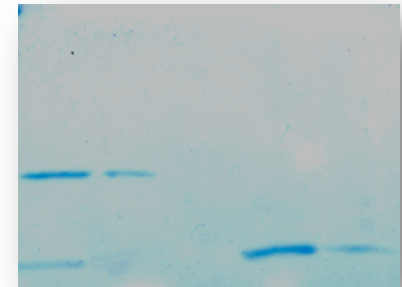
Her2/neu



CD20

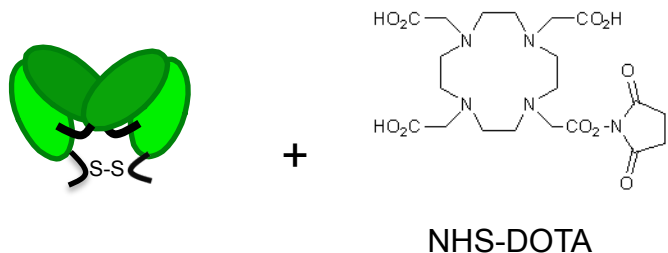


PSCA

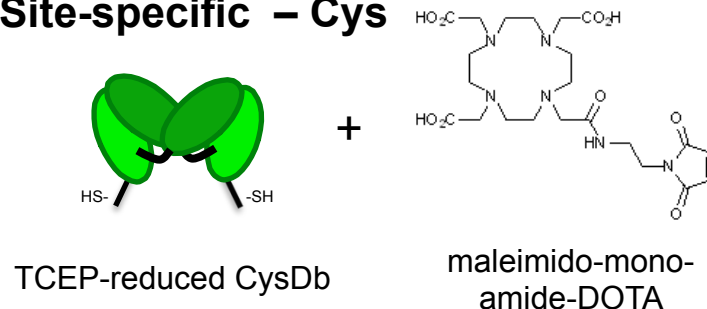


Conjugation of DOTA to anti-ALCAM CysDb

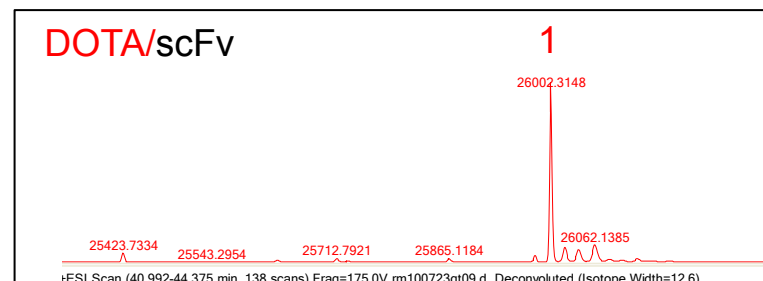
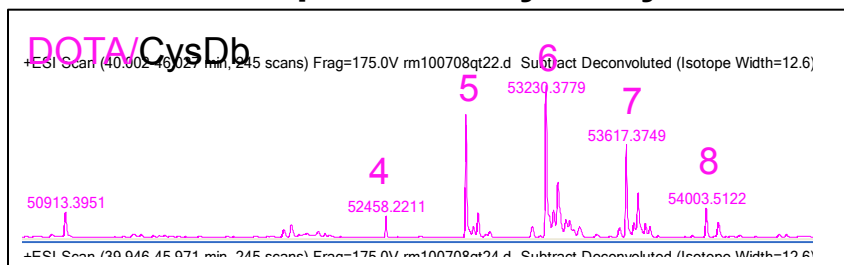
Random – Lys



Site-specific – Cys



Q-TOF mass spectrometry analysis



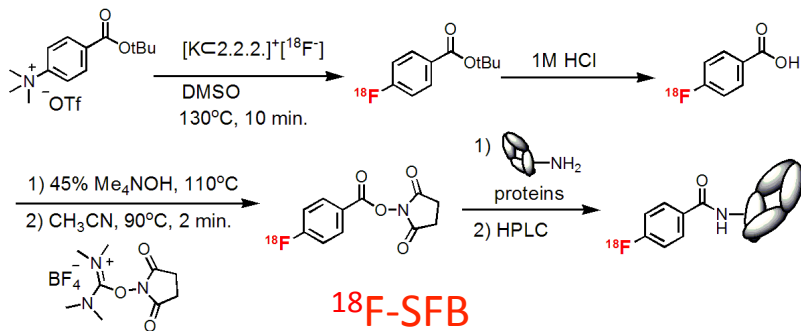
LC-MS/MS analysis

QVQLVESGGG	LVQPGGSLRL	SCAASGFTFS	SYAMSWVRQA	PGKGLEWVSA
ISGSGGSTYY	ADSVKGRFTI	SRDNSKDTLY	LQMNSLRAED	TAVYYCASRS
LLDYWGQGTLL	VTVSSGGGGS	GGGNFMLTQD	PAVSVALGQT	VRITCQGDSL
RSYYASWYQQ	KPGQAPLLVI	YGKNNRPSGI	PDRFSGSSSG	NTASLTITGA
QAEDPADYYC	NSRDSSGNPV	FGGGTKVTVL	GAAAGCGGHH	HHHH
QVQLVESGGG	LVQPGGSLRL	SCAASGFTFS	SYAMSWVRQA	PGKGLEWVSA
ISGSGGSTYY	ADSVKGRFTI	SRDNSKDTLY	LQMNSLRAED	TAVYYCASRS
LLDYWGQGTLL	VTVSSGGGGS	GGGNFMLTQD	PAVSVALGQT	VRITCQGDSL
RSYYASWYQQ	KPGQAPLLVI	YGKNNRPSGI	PDRFSGSSSG	NTASLTITGA
QAEDPADYYC	NSRDSSGNPV	FGGGTKVTVL	GAAAGCGGHH	HHHH

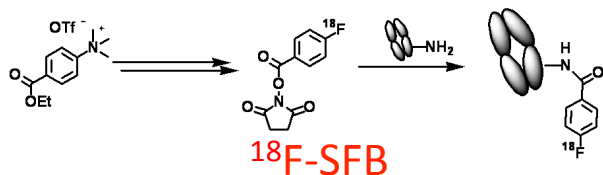
Site-specific oriented conjugation of a variety of cargoes

^{18}F -FB-diabodies for same-day imaging

Conventional bench-scale synthesis



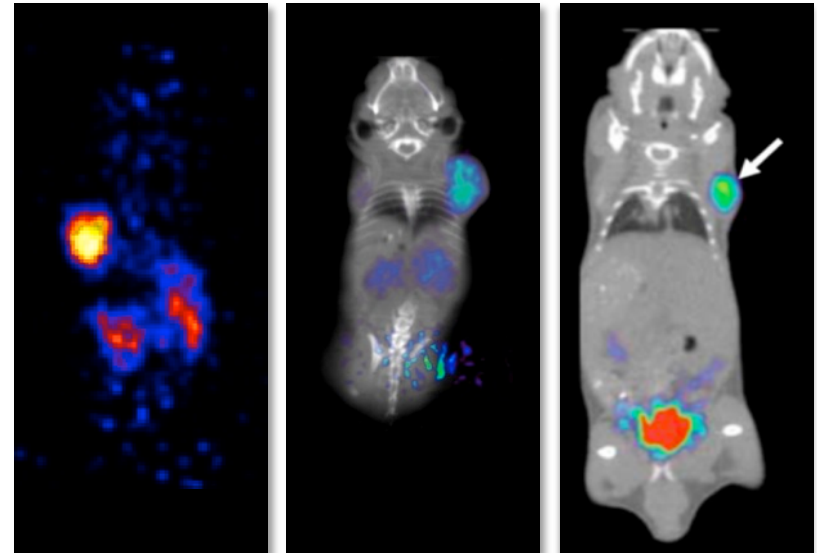
One-pot, microwave-assisted synthesis



CEA

HER2

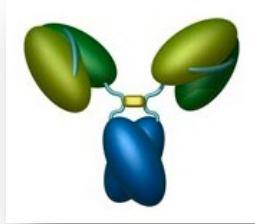
PSCA



4 h

Diabodies with any specificity can be routinely labeled with F-18 and used to tumors at 1-4 h post injection by microPET

Minibodies and Diabodies as a Platform for Cell-surface Imaging



CEA

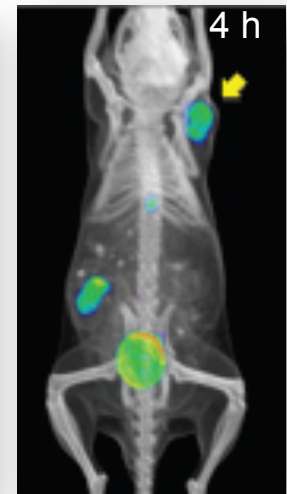
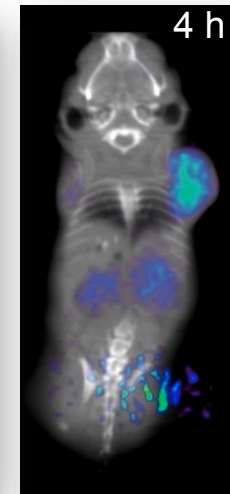
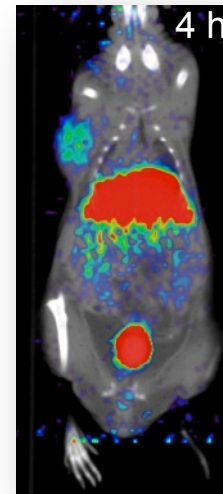
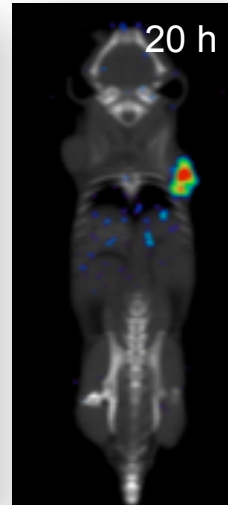
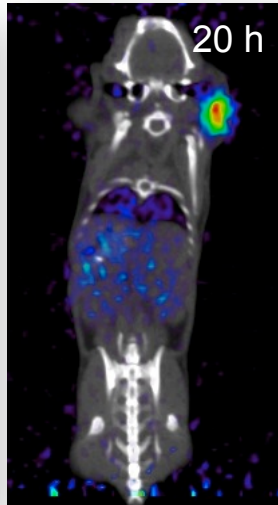
CD20

PSMA

ALCAM

HER2

PSCA

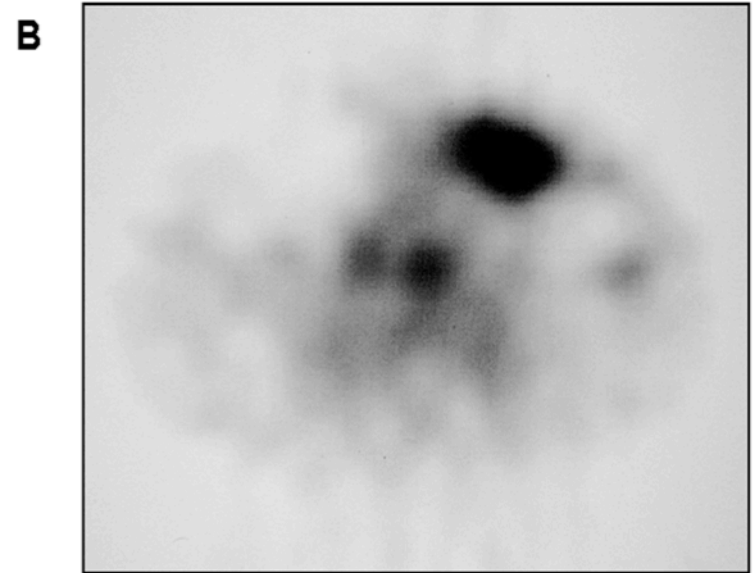
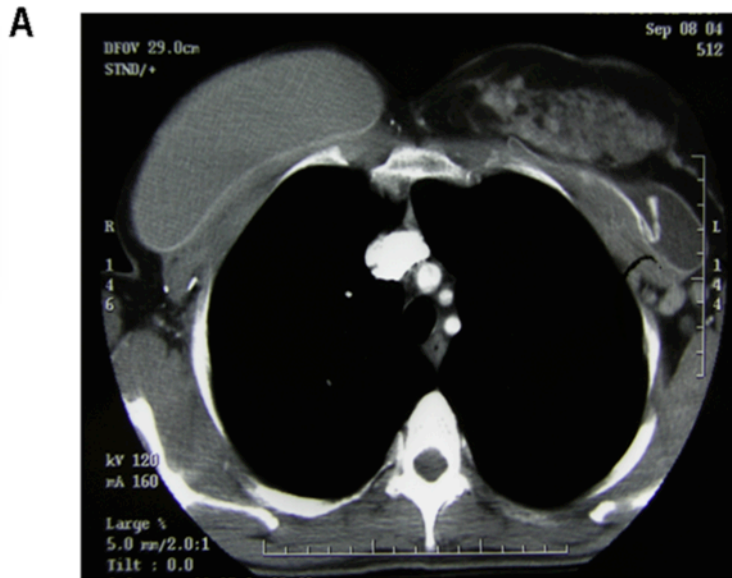
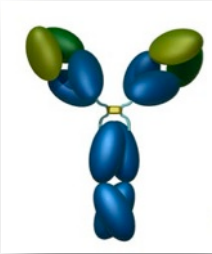


ImmunoPET detection at 18-20 h for minibodies; 1-4 h for diabodies

Immunohistochemistry in vivo

Applications

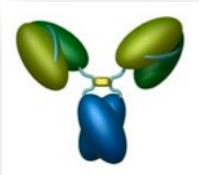
Clinical Imaging study using In-111-Mx-DTPA-trastuzumab (anti-Her2)



36 year old woman with diffuse involvement of the left breast seen on axial CT (A) and demonstrating diffuse uptake of ^{111}In -MxDTPA-trastuzumab to the left breast (B) on 48 hour axial images.

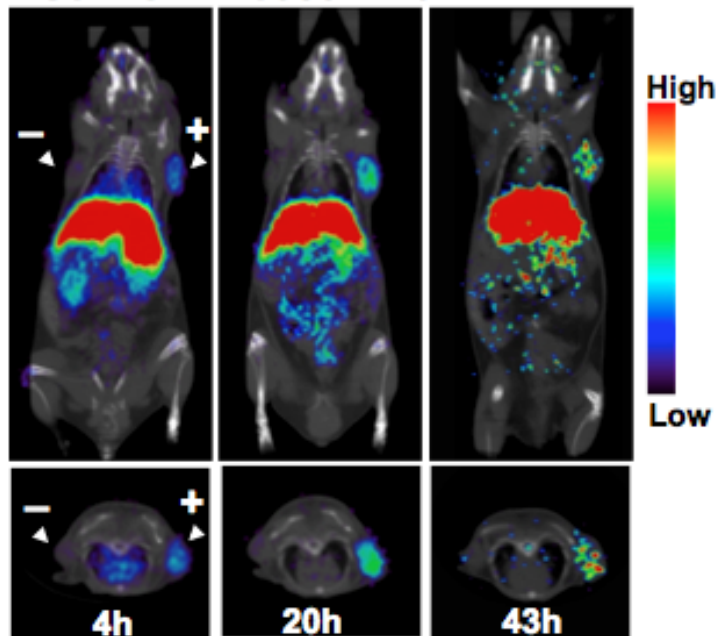
Targeting in 3/7 patients
Increased uptake in cardiac muscle
Dosimetry for radioimmunotherapy using ^{90}Y

Target expression
Delivery of therapeutic
Therapy selection

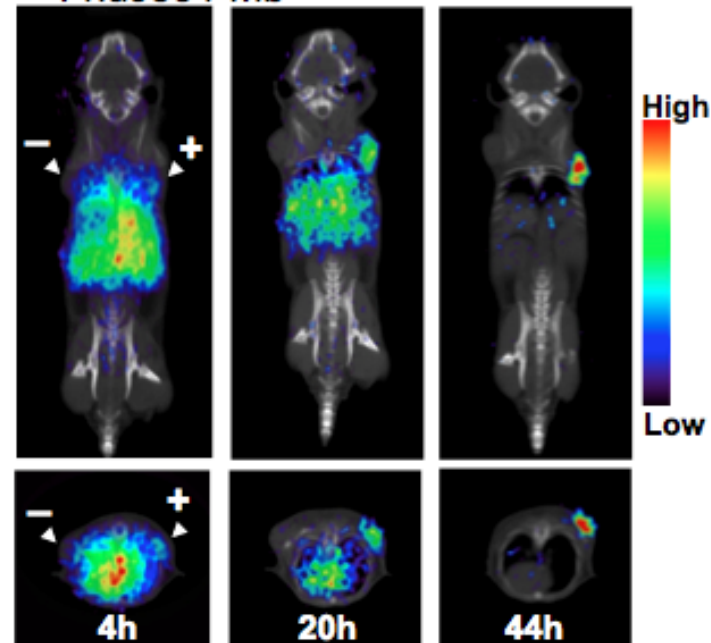


ImmunoPET detection of PSMA⁺ prostate cancer xenografts

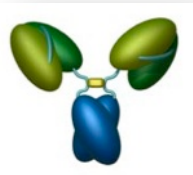
⁶⁴Cu-DOTA huJ591 Mb



¹²⁴I-huJ591 Mb

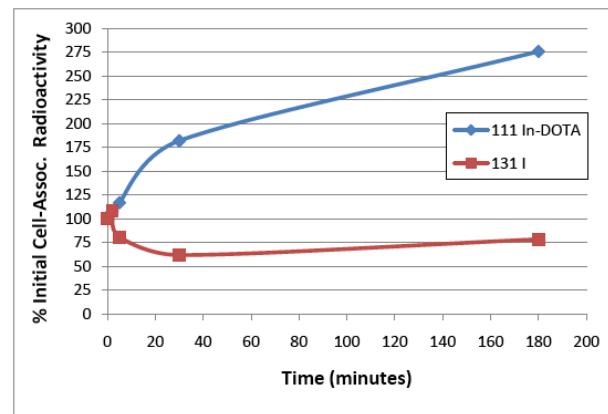


Both Cu-64 and I-124 radiolabeled huJ591 minibody demonstrate excellent localization to PSMA⁺ xenografts, reaching 13.2 vs 8.3 %ID/g, respectively, at 43-44 h.

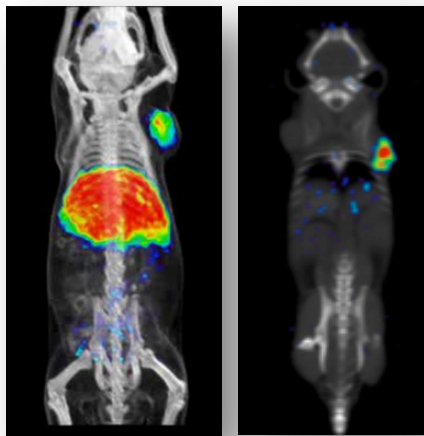


Internalization of anti PSMA minibodies *in vitro* and *in vivo*

- HuJ591 anti-PSMA (prostate membrane-specific antigen)
- Minibodies labeled with radiometal (stable) or iodine (labile)
- Internalization studies on CWR22rv1 cells

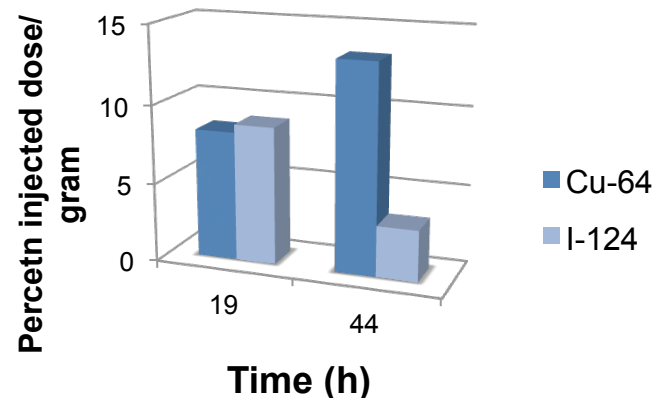


- MicroPET/CT imaging of CWR22rv1 tumor-bearing mice at 4, 19, and 44 h



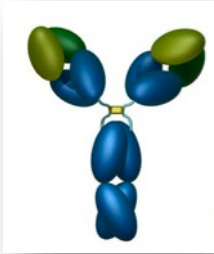
Cu-64

I-124



*Internalization/
catabolism in vivo*

Imaging Cell Surface PSMA: Downstream Readout of Anti-Androgen Tx

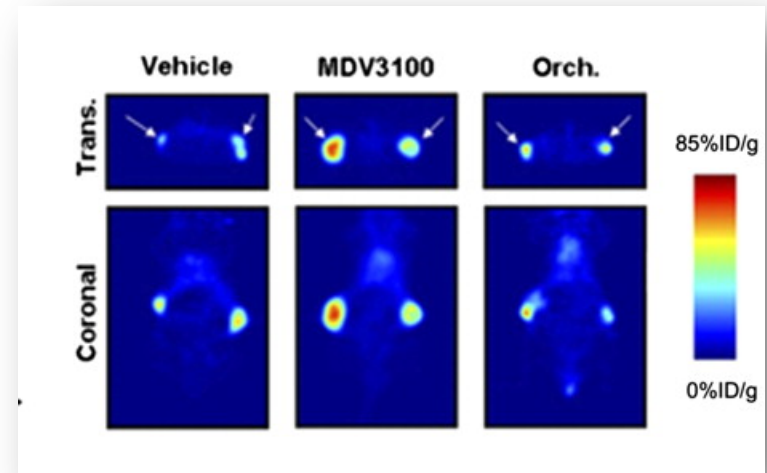


Noninvasive measurement of androgen receptor signaling with a positron-emitting radiopharmaceutical that targets prostate-specific membrane antigen

Michael J. Evans^a, Peter M. Smith-Jones^b, John Wongvipat^a, Vincent Navarro^c, Sae Kim^c, Neil H. Bander^{c,d}, Steven M. Larson^{b,e,f}, and Charles L. Sawyers^{a,g,1}

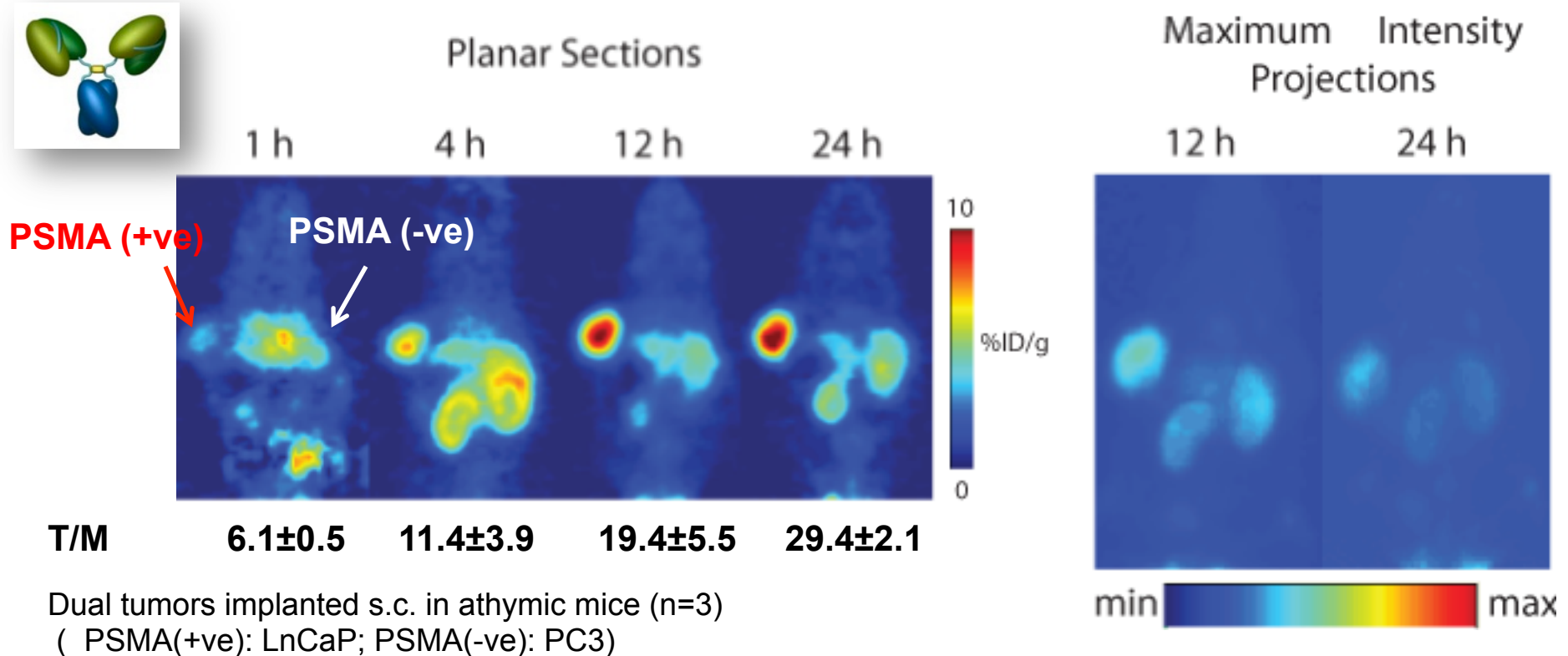
- LNCaP-AR s.c. xenografts
- Treated for 6 days with MDV3100
- ⁶⁴Cu-J591 anti PSMA PET imaging

Detects upregulation of PSMA in response to androgen deprivation therapy



Response to therapy

^{89}Zr -DFO-huJ591 anti-PSMA Mb ImmunoPET



- Fully humanized
- Extracellular epitope of PSMA
- Minibody
- PET

Acknowledgments

Wu Lab

Tove Olafsen
Richard Tavaré
Kirstin Zettlitz
Scott Knowles
Keyu Li
Felix Bergara
Wei Wu
James Tomlinson
Mark Girgis
Matthew Rochefort



Past:

Zoo Hwang
Vania Kenanova
Eric Lepin
Shannon Sirk
Bhaswati Barat
Karl Bauer
Katy Venisnik
Jeff Leyton
Katelyn McCabe

UCLA

Mike Phelps
Johannes Czernin
Arion Chatziioannou
David Stout
Rob Reiter
John Timmerman
Jerry Zack
Clifton Shen
Mike van Dam

UCSF

Jim Marks
Eunice Zhou



City of Hope

Andrew Raubitschek
Paul Yazaki
David Colcher
Jeff Wong
Jack Shively



Memorial Sloan-Kettering Cancer Center

Nerissa Viola-Villegas
Jason Lewis



David Ho Jason Romero
Arye Lipmann Giti Agahi
Lola Rahib Lizzie Work
Derek Bartlett Celia Adelson
Katy Sorenson Jenny Keppler

Funding support:

UCLA Prostate SPORE, CoH Lymphoma SPORE, UCLA ICMIC, UCLA SAIRP, CoH RIT PPG, Stanford CCNE, CIRM Tools and Technologies, SNM Molecular Imaging Scholar Award, NCI SBIR

