Engineered PSCA antibody for Targeted Radiotherapy

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City of Hope

Oct 2022

Executive Summary

- **Target:**

  Prostate Stem Cell Antigen (PSCA)

- **Therapeutic hypothesis / MoA:**

  Engineered anti-PSCA antibody delivering therapeutic radionuclides to prostate cancer

- **Proposed disease indications:**

  Prostate Cancer

- **Current status**
  - Key assays: Antibody characterization, immunoreactivity, PET imaging ✓
  - Screening: Well-characterized murine monoclonal antibody ✓
  - Hits / leads and attributes: **^{177}Lu-scFv-Fc;** high affinity, high specificity to PSCA ✓
  - Lead optimization / plans: Humanized, affinity matured engineered Ab fragments ✓
  - Preclinical disease models and PoC / working plan: Xenograft, KI models available ✓
- **Major Issue / Challenge / Risk:**

  Dose regimen and dosimetry; therapeutic benefits vs safety; competition

- **COH IP status:** Provisional patent application filed

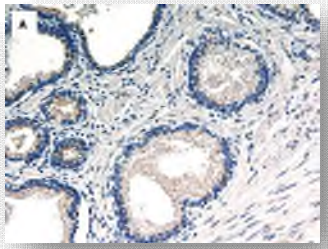
TPP: Target Product Profile

Goal: *Targeted delivery of therapeutic radionuclides to PSCA-prostate cancer*

Attribute	Desired criteria
Indication and patient population	Patients with metastatic prostate cancer
Selectivity	Specific targeting and delivery of radionuclide to PSCA-expressing prostate cancer. Low uptake in normal tissues including kidney, bladder, stomach. Timely hepatobiliary clearance, minimizing radiation dose to sensitive tissues such as bone marrow and kidney
Mechanism-based activity	Radiolabeled with Lu-177 (beta-emitter) to deliver high radiation doses to kill tumor cells while sparing normal tissues.
Safety	Comparable safety profile to approved targeted radioligand agents (Zevalin, Bexxar, Lutathera)
Desired dose & schedule	10-30 mCi single administration or potentially q 4-8 wks
Pharmaceutical properties	Suitable for intravenous administration
PK / ADME Properties	Rapid uptake in tumors (6-12 hrs); blood half-life 24-48 hrs; hepatic clearance

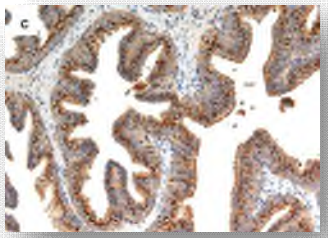
Biological Rationale: PSCA

- Target background – Prostate Stem Cell Antigen (PSCA)
  - 123 aa cell-surface glycoprotein
  - Low background expression in normal organs
  - Highly expressed in prostate cancer (~95%), also bladder and pancreatic cancers

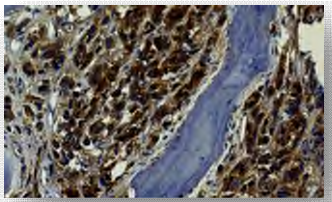


Normal Prostate

- Evidence of target validation
  - huPSCA knock-in mice show physiologic expression in stomach, pancreas, prostate, bladder
  - Anti-PSCA antibodies have been evaluated clinically in pancreatic cancer (Astellas)
  - PSCA-CAR-T cells currently under clinical investigation prostate cancer (CoH)



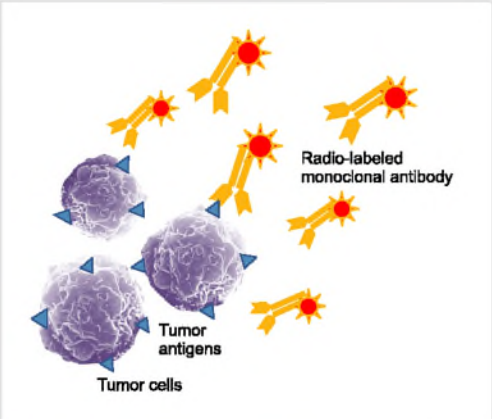
Primary Prostate Ca



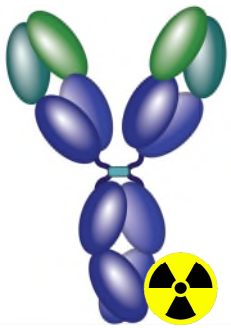
Bone metastasis

Biological Rationale: Radioimmunotherapy

- Therapeutic hypothesis / Mechanism of action



- Radioimmunotherapy uses radiolabeled antibodies recognizing tumor-specific targets for direct delivery of toxic radionuclides to cancer cells
- FDA approval of two radiolabeled anti-CD20 antibodies for lymphoma (Bexxar and Zevalin)



Challenge and opportunity:

- Radiolabeled antibodies offer highly specific delivery to tumors
- However, efficacy is hampered by their long circulating half-life
- Blood pool radioactivity leads to dose-limiting bone marrow toxicity.

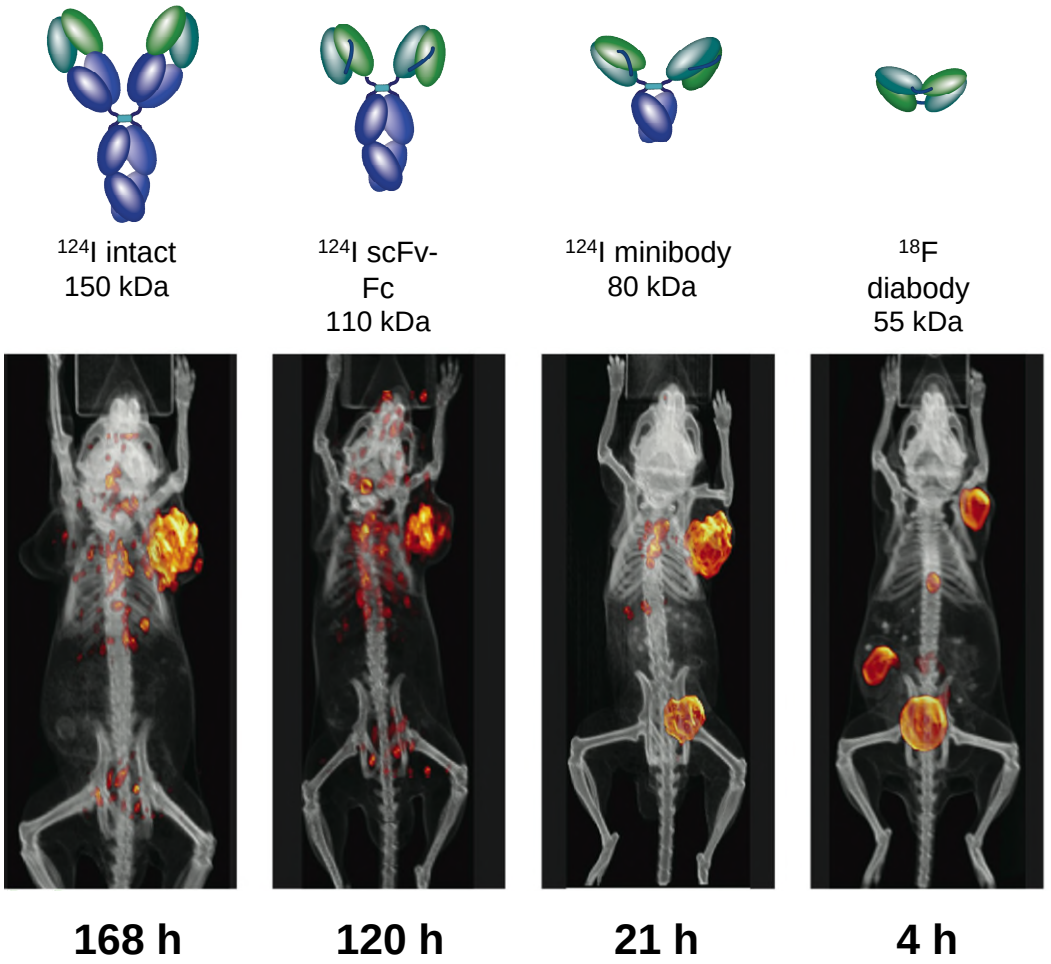
Biological Rationale: Technology (1)

Intact Antibodies and Engineered Fragments

- Protein engineering offers extensive control over biological properties such as PK and organ of clearance
- Critical for efficacious delivery of therapeutic radionuclides

PET imaging to evaluate tumor targeting and clearance

- MicroPET imaging in xenograft-bearing mice using ^{124}I-labeled antibodies and fragments
- Requires up to a week using intact antibodies
- 20-120 h for scFv-Fc
- 20 h (minibody) or 4 h (diabody) for smaller fragments



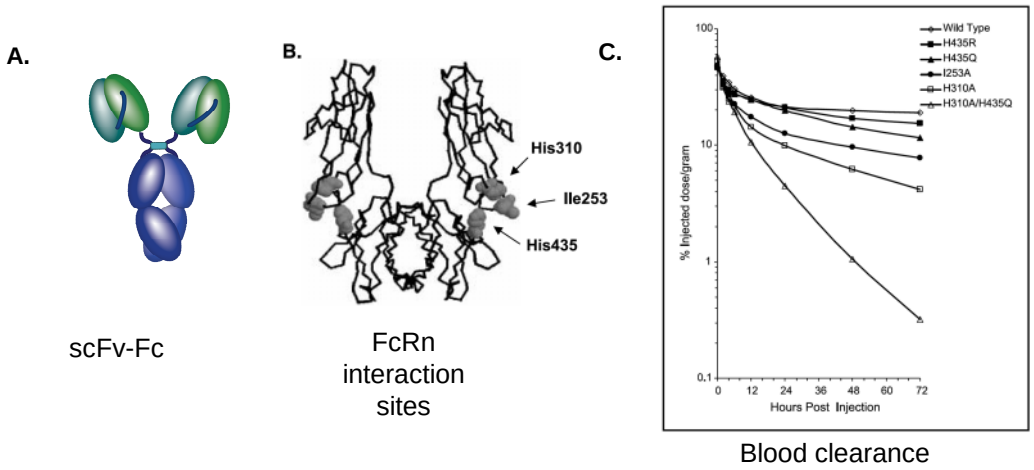
Wu AM, Protein Engineering, in: Molecular Imaging Principles and Practice (ed. Weissleder, Gambhir) 2010

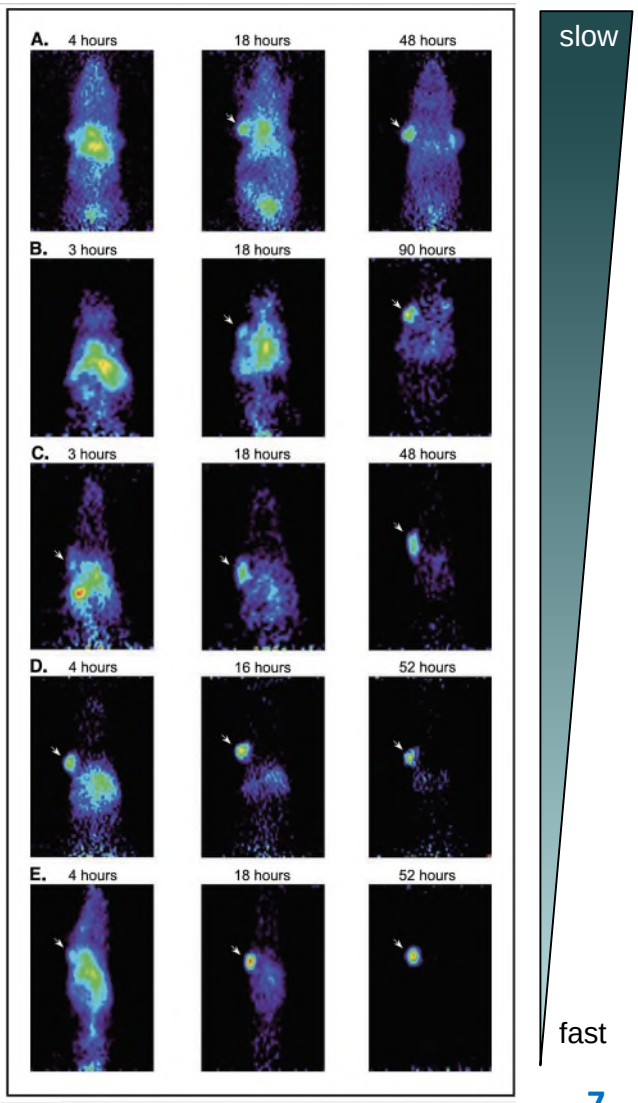
Biological Rationale: Technology (2)

ScFv-Fc fragments with tailored PK

- Serum persistence of intact antibodies is governed by interaction between Fc and FcRn receptor
- Point mutations can weaken or eliminate FcRn interactions and accelerate clearance
- **CEA**-specific scFv-Fc fragments:
  - Wild-type
  - Single mutant: H435Q, I253A, H310A
  - Double mutant (DM) H435Q/H310A

- **Serum half-lives from 8 – 80 h**





Kenanova V, ...Wu AM Ca. Res. 2005

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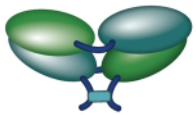
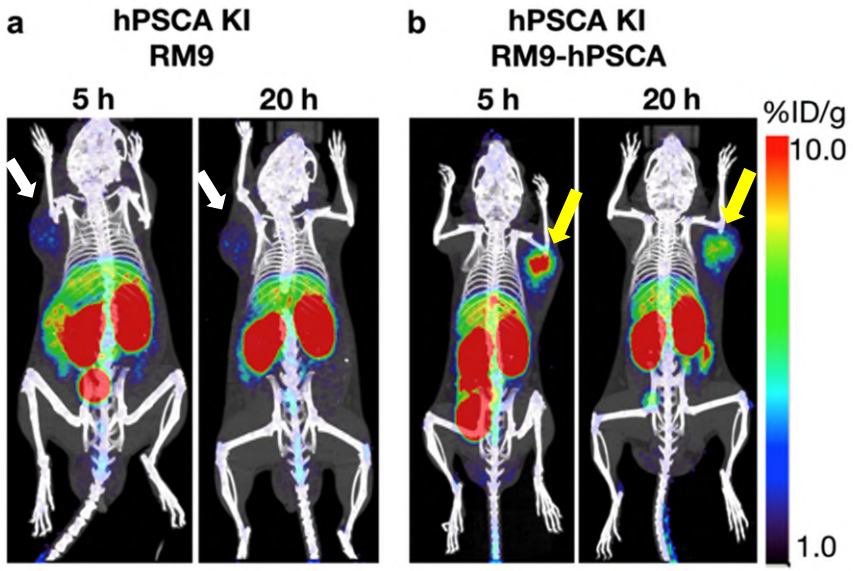
Biological Rationale: Published Data (1)

Prototype anti-PSCA cys-diabody
Targeting and imaging syngeneic tumor in hPSCA KI model

- Prototype anti-PSCA cys-diabody radiolabeled with ^{89}Zr for PET
- HuPSCA knockin (KI) mice
- Murine RM9 and RM9-hPSCA prostate cancer s.c. tumors
- Serial PET imaging shows high uptake of ^{89}Zr-DFO-anti-PSCA cys-diabodies in tumors on right shoulders
- Clearance and retention of activity in kidneys



Prototype
A2 anti-PSCA cys-diabody

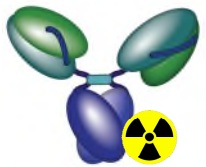


- Serial PET images of hPSCA KI mice bearing control (white arrows) or PSCA-expressing (yellow arrows) s.c. tumors using ^{89}Zr-A2 cys-diabody.
- High activity is observed in tumors, while normal organ activity remains low

Zettlitz KA, ...Wu AM Mol Imag Biol. 2020

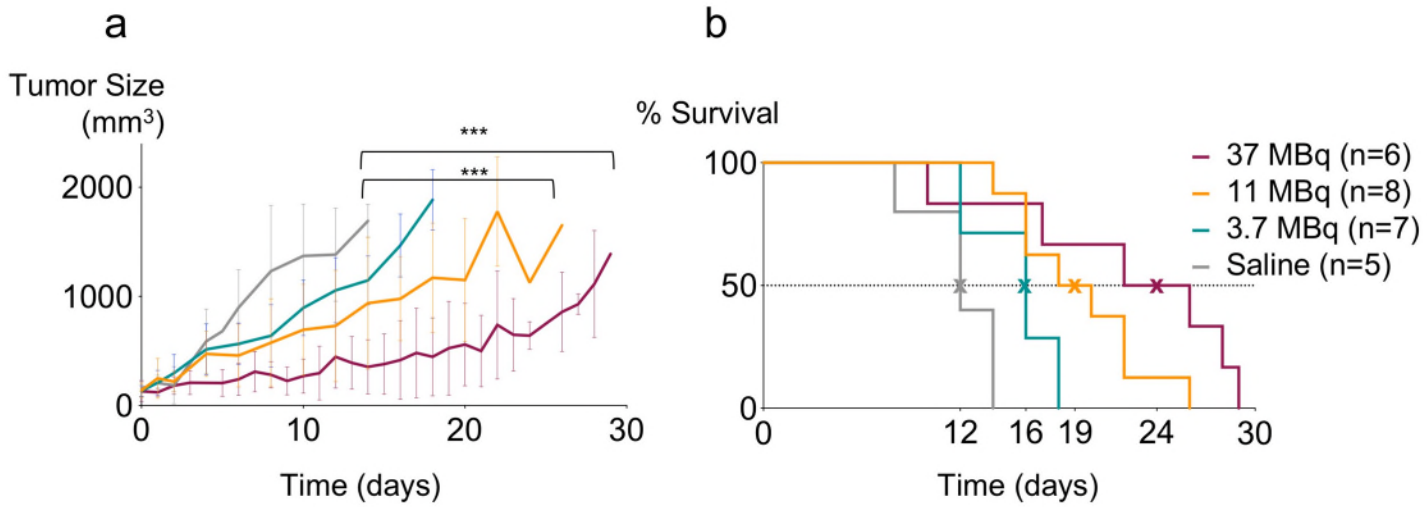
Biological Rationale: Published Data (2)

Prototype anti-PSCA minibody
Targeted radionuclide delivery in xenograft mouse model



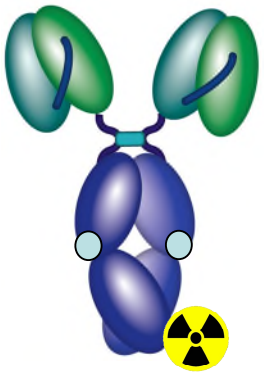
Prototype minibody

- ^{131}I-A11 anti-PSCA minibody in 22rv1-PSCA xenograft-bearing mice
- Dosimetry predicts ^{131}I version has higher therapeutic index
- Radioimmunotherapy showed dose-dependent growth inhibition and improved survival using ^{131}I-anti-PSCA minibody
- However, high kidney uptake limits the utility of the prototype for radioimmunotherapy, especially with radiometals



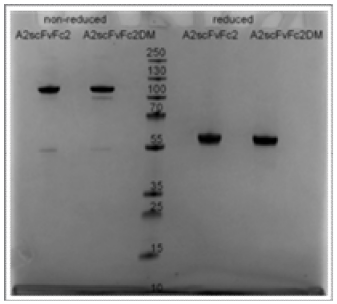
Tsai W., ...Wu AM Mol. Imag. Biol. 2020

Lead candidate: Engineered anti-PSCA scFv-Fc

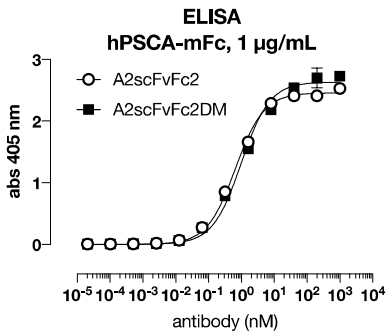


Optimized scFv-Fc antibody fragment will maintain high delivery to tumor while minimizing normal tissue toxicity

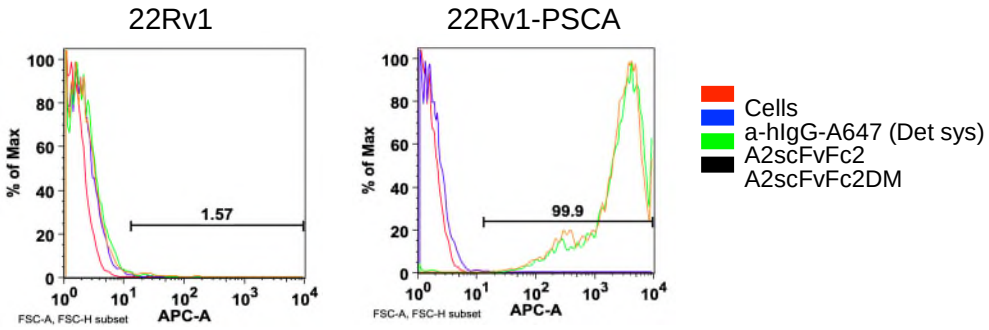
- Fully humanized, affinity-matured anti-PSCA antibody
- Two versions: parental A2scFvFc2 (slow clearance kinetics) and variant A2scFvFc2DM (accelerated clearance)
- Retention of high affinity for PSCA (0.4 nM)
- Rapid tumor targeting combined with hepatic clearance will minimize dose to bone marrow and kidney
- Ideal for delivery of Lu-177 for radioimmunotherapy



SDS-PAGE



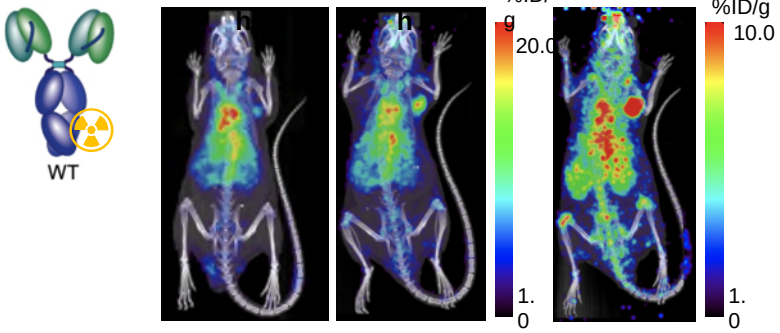
ELISA



Cell binding by flow cytometry

Lead candidate: Engineered anti-PSCA scFv-Fc

A.



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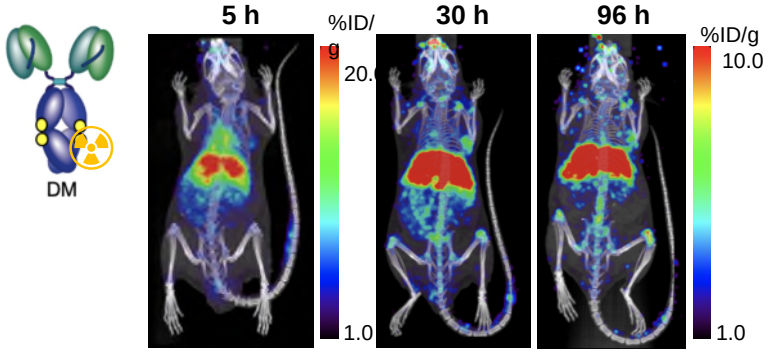

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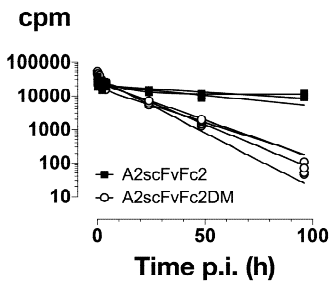


Serial immunoPET imaging confirms expected properties:

- ^{89}Zr-labeled scFv-Fc wild type (WT, A) vs candidate scFv-Fc DM (B)
- DM Candidate demonstrates accelerated clearance via the liver
- This is reflected in the blood half-lives and tumor:blood ratios

Half lives: **12.2 h (DM)** vs 74.7 h (WT)

T:B ratio at 96 h: **32.8 (DM)** vs 1.9 (WT)

Proposed Disease Indication

- Overall utility: Targeted therapy of PSCA-expressing cancers such as prostate, pancreatic, bladder cancer
- Lead indication: Metastatic, castrate-resistant prostate cancer
- Current unmet need: patients who progress through hormonal therapy need alternative therapies
- Radiolabeled ligand therapies (e.g., PSMA-617) are promising but patients recur. PSMA-targeted agents are limited by side effects such as severe xerostomia
- Specificity of PSCA-targeted agents is high and complementary to PSMA-targeted agents
- 20-40% of prostate cancer patients recur, and once prostate cancer has spread, ~30% 5-year survival

Current Project Status and Hit / Lead Profile

- **Biology:** Well characterized and established target (Human Protein Atlas; IHC; under active investigations by industry and academic groups). Low normal tissue expression; overexpression in key cancers including prostate cancer, pancreatic cancer, bladder cancer
- **Key Assays:** Biochemical (SDS-PAGE, SEC-HPLC), functional (PSCA binding by ELISA or flow cytometry), in vivo (image and therapy in xenograft and syngeneic knock-in preclinical models). Assays are all established
- **Screening:** Mouse monoclonal antibody, humanization, affinity maturation and reformatting are all completed
- **Hits / leads /prototype products and key attributes:** Lead candidate has the required biochemical and functional properties. *In vivo* characterization is in progress

Competitive Landscape for CoH's PSCA[1] modified Antibody [scFv-Fc][2] Radiotherapy Program [in mCRPC][3]

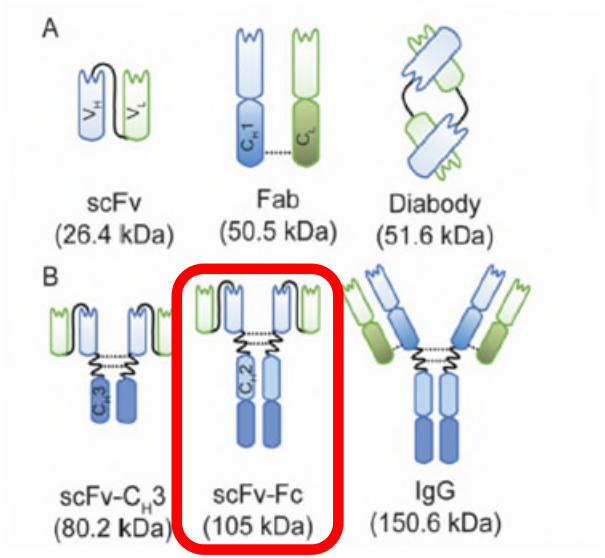
Key Takeaways: (1) Currently no marketed therapies targeting PSCA [target currently not validated with/without radiolabeling]; late stage industry pipeline mainly exploring PSMA[4] and next gen AR[4] in mCRPC (2) CoH program potentially can be first in class; no publications found covering indication/modality/target (3) Big Pharma activity in mCRPC very high; several blockbuster brands on market [may present high scientific, regulatory and commercial hurdles to future agents]; GRx presence will increase [Zytiga copycats on market; 1st Gen anti-AR expected to follow; will impact cost/benefit of future agents] (4) SM[5] is the dominant modality in mCRPC but I/O presence growing [e.g. BiTEs; CAR-Ts; checkpoints] (5) Various combination options being explored; assumed to be approved; will address unmet need impacting later (last) line therapies (7) Xofigo latest radiolabeled agent approved in space [bone mets]; industry/academia is exploring other assets targeting later (last) line therapies; including novel radiopharma agents.

PSCA in mCRPC

- **Market:** Currently none on market
- **Pipeline:** Two Inactive: (a) CV-9104 [Curevac AG; last development stage phase II; inactive since 2017; failed to improve OS); an mRNA therapeutic comprising of 6 mRNA-encoded prostate cancer-associated antigens including PSCA] (b) CV-9103 [by Curevac AG; last development stage phase II; inactive since 2015] a chemically unmodified mRNA immunotherapy targeting four antigens including PSCA]

Other mCRPC Agents

- **Marketed:** ~20 drugs [Astellas (Xtandi, anti-AR SM, ~$3B[8]); J&J (Zytiga, CYP17[9] inhibitor SM, ~$3B[8], 12 generic versions {US}); Sanofi (Jevtana, Tubulin inhibitor SM, ~600M[8]; Taxotere, Tubulin inhibitor SM, ~200M[8], 5 generic versions {US}); Dendreon Pharmaceuticals (sipuleucel-T, anti-PAP[10] dendritic cell therapy vaccine); others]
- **Pipeline: (a)** ~10 assets in Ph3 [BMS (nivolumab, anti-PD1[11] mAb); Merck & Co (pembrolizumab, anti-PD1 mAb); J&J (niraparib, PARP-1/2 inhibitor SM; apalutamide, anti-AR SM); others] **(b)** ~70 in Ph2 [Pfizer (palbociclib, CDK4/6[12] inhibitor SM); AbbVie (venetoclax. Bcl 2[13] inhibitor SM); Eli Lilly (abemaciclib, CDK4/6 inhibitor SM); Novartis (ribociclib succinate, CDK4/6 inhibitor SM); others] **(c)** ~50 in Ph1 [Amgen; Bayer AG; Novartis; others] across modalities including SMs [23], mAb [12], Vaccines [3], and others **(d)** ~40 in Preclinical [J&J; Bayer AG; others] across modalities including SM [25], cell therapies [8] and others



1: **PSCA**= Prostate **S**tem **C**ell **A**ntigen and not imaging
2: **scFv-Fc** = modified **s**ingle-**c**hain Variable **F**ragment- **C**rystallizable **F**ragment (PSCA antibody fragment)
3: **mCRPC** = metastatic Castrate-Resistant Prostate **C**ancer [lead indication]
4: **PSMA=** Prostate-Specific Membrane Antigen
AR= Androgen **R**eceptor
5: **SM**= **S**mall Molecule
6: **mAb** = monoclonal Antibodies
7: Only considered therapeutic application
8: 2019 Global Sales
9: **CYP17** = Steroid 17 Alpha Hydroxylase
10: **PAP** = **P**rostatic Acid Phosphatase
11: **PD-1** = **P**rogrammed Cell **D**eath Protein **1**
12: **CDK4/6** = **C**yclin Dependent Kinase **4/6**
13: **Bcl-2** = **B C**ell **L**ymphoma 2

Preclinical development in progress

Current steps in progress:

- ImmunoPET, biodistribution and pharmacokinetics in syngeneic hPSCA knock-in model to confirm targeting efficiency and pharmacokinetics

- Optimization of radiolabeling to produce high specific activity radioimmunoconjugates for therapy

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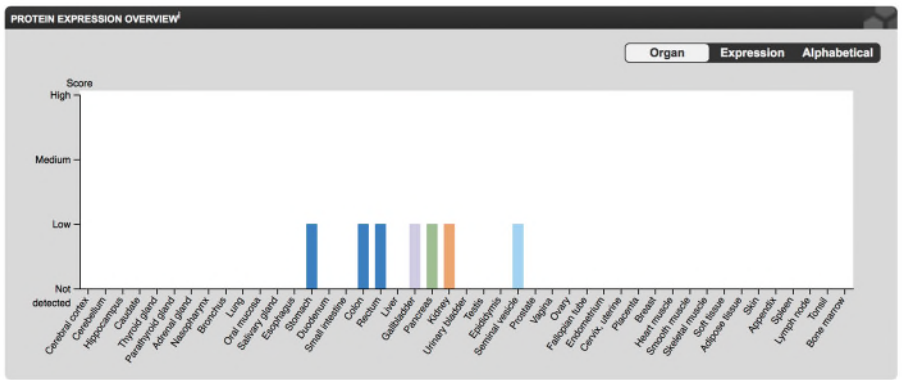
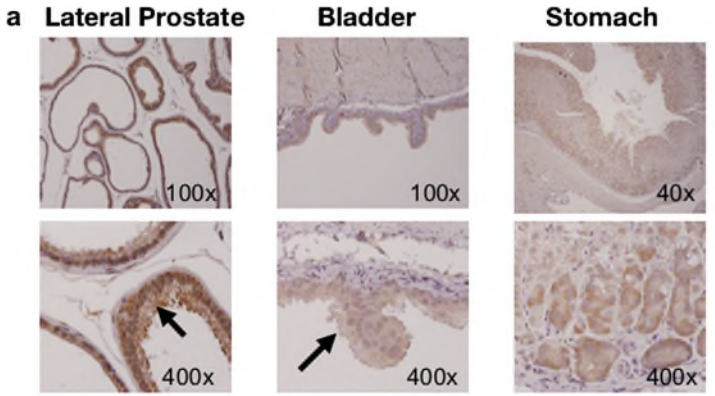
Appendix

Human PSCA expression and knock-in (KI) mouse model

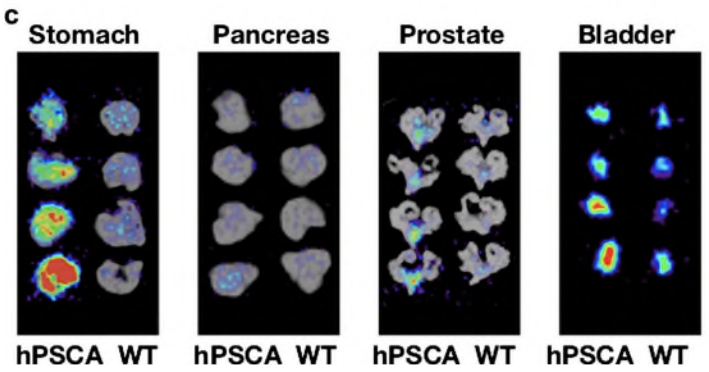
- In humans: no or low normal organ expression of PSCA (stomach/GI tract, pancreas, kidney, prostate)
- HuPSCA knock-in (KI) mice reproduce physiologic expression in stomach, pancreas, prostate, bladder
- Radiolabeled anti-PSCA antibodies localize *in vivo* to normal PSCA+ tissues



Source: The Human Protein Atlas



IHC of normal tissues in huPSCA KI mice

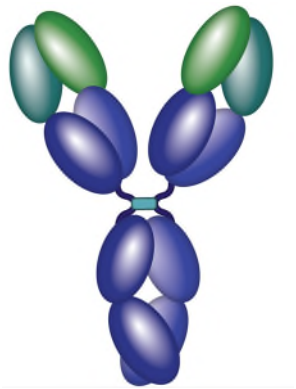


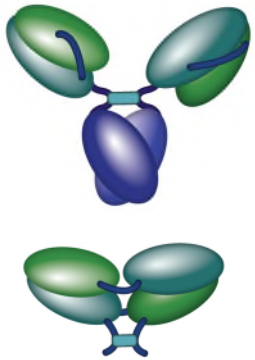
Localization of radiolabeled PSCA antibody fragments in huPSCA KI mice

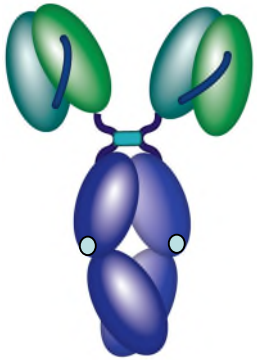
Zettlitz KA, ...Wu AM Mol Imag Biol. 2020

Biological Rationale

- Therapeutic hypothesis / Mechanism of action







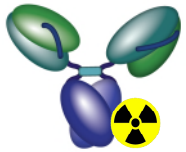
Intact antibody
- Bivalent, high affinity
- Prolonged blood persistence
- Bone marrow background and toxicity

Prototype small antibody fragments
minibody, cys-diabody
- Bivalent, high affinity
- Rapid clearance via kidney
- Ideal for imaging

Lead antibody fragment
scFv-Fc with tailored PK
- Bivalent, high affinity
- Moderately rapid clearance via liver
- Lower exposure to radiosensitive bone marrow and kidney
- **Ideal for radioimmunotherapy**

Biological Rationale: Published Data

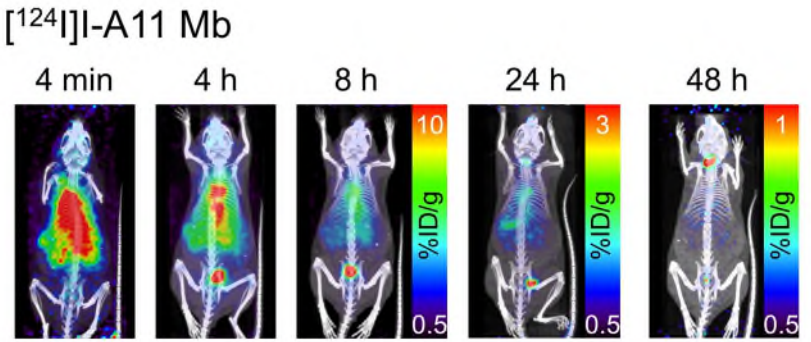
Prototype anti-PSCA minibody
Serial PET imaging, biodistribution, and dose estimation

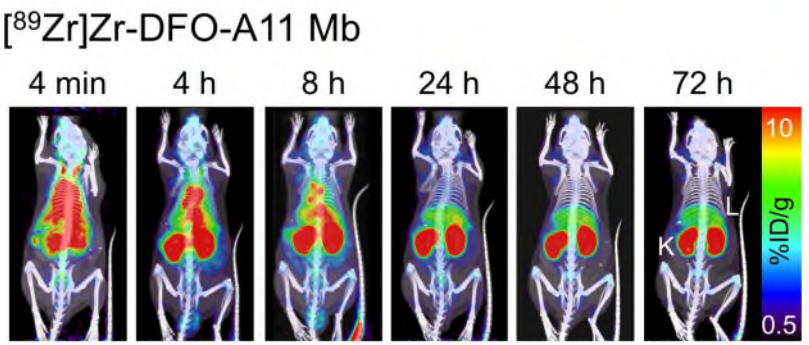


Prototype
A11 anti-PSCA minibody

1. A11 anti-PSCA minibody labeled with ^{124}I or ^{89}Zr for comparison of radioiodine vs radiometal
2. Serial PET imaging in mice with 22RV1-PSCA xenografts
3. A11 anti-PSCA minibody labeled with ^{131}I or ^{177}Lu for full biodistribution
4. Calculated radiation dose estimates to tumor and normal tissues

(note unexpected kidney accumulation by prototype minibody)

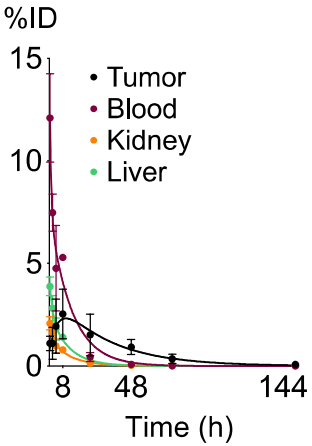




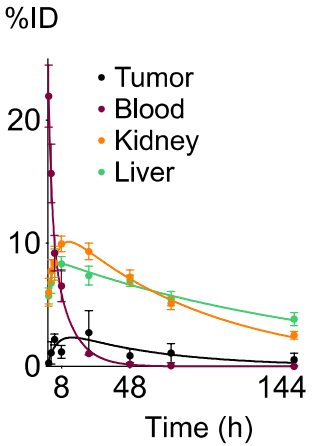
Tsai W., ...Wu AM Mol. Imag. Biol. 2020 in press

Biodistribution and dose estimation

a [131I]I-A11 Mb



b [177Lu]Lu-DTPA-A11 Mb

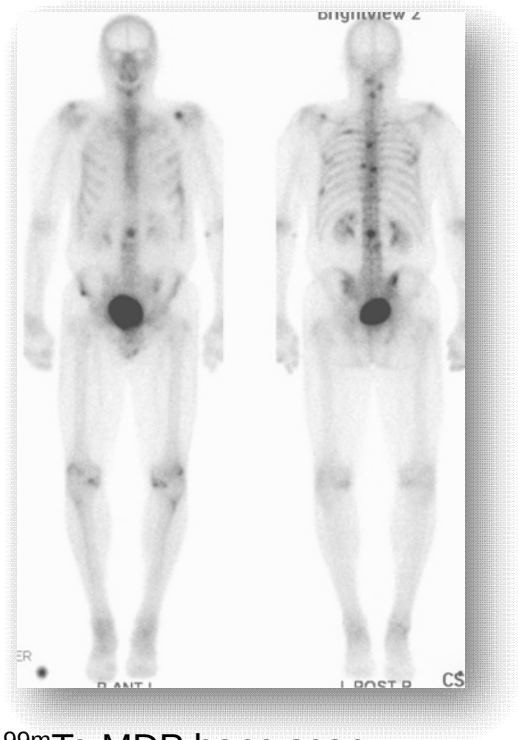


RIT agent	Maximum allowed activity	Dose to tumor	Dose-limiting tissues
^{131}I minibody	37 MBq	35 Gy	Bone marrow
^{177}Lu minibody	7.4 MBq	11 Gy	Kidney

- Prototype minibody radiolabeled with either ^{131}I or ^{177}Lu-DTPA for biodistribution studies
- Time-activity curves generated for formal dose estimation
- Results predicted ^{131}I version would have higher therapeutic index (due to kidney metabolism and clearance)
- Prototype minibody still falls short since at least 60 Gy needed for effective radiotherapy

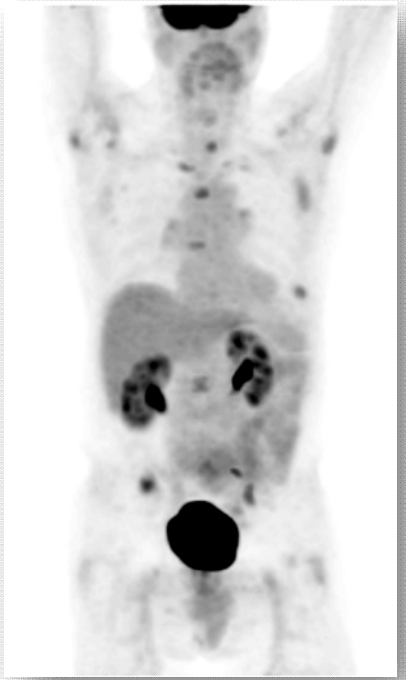
Tsai W., ...Wu AM 2020 in press

Clinical POC: Anti-PSMA minibody (ImaginAb)



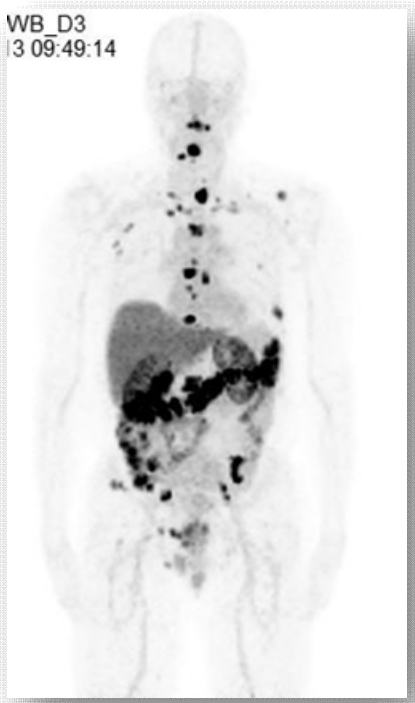
^{99m}Tc-MDP bone scan

Anterior and posterior



FDG PET scan

MIP



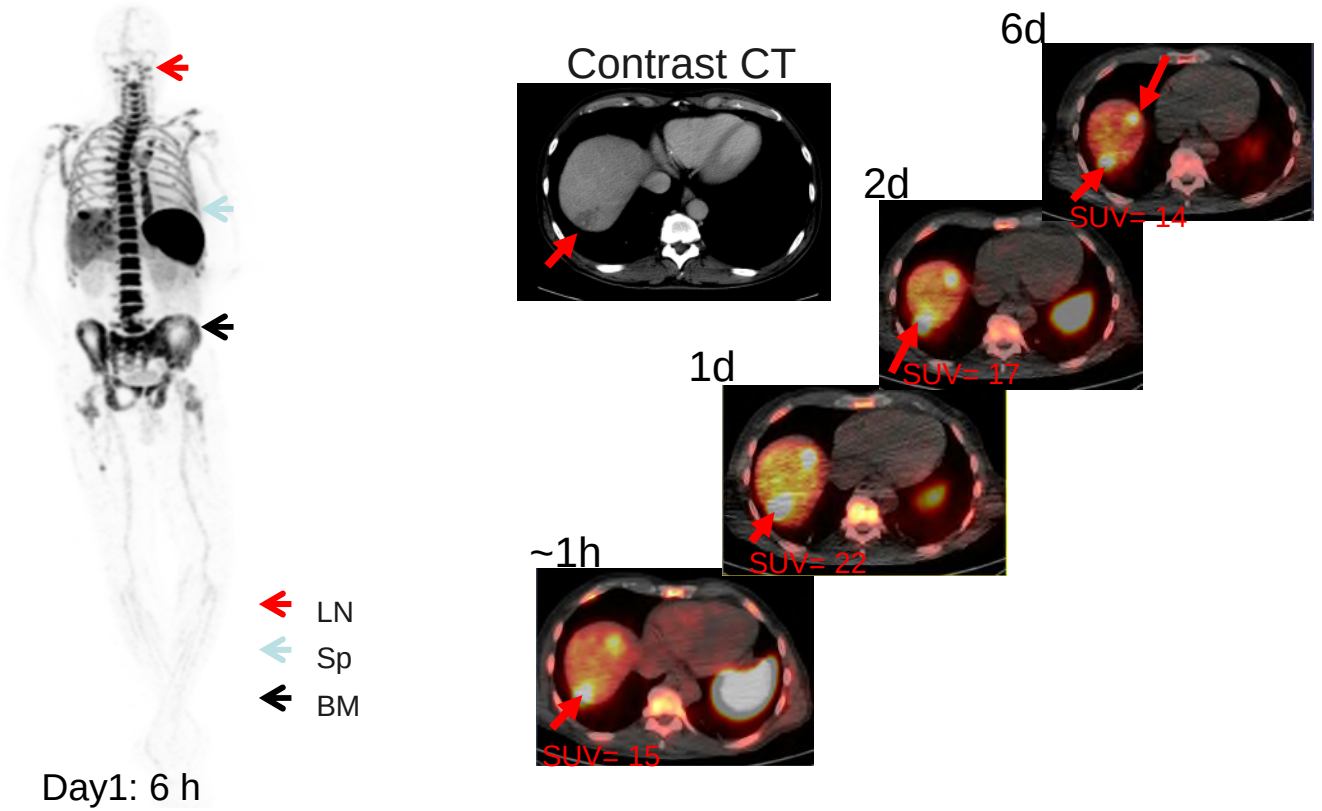
^{89}Zr-Df-IAB2M scan

MIP

Patient with metastatic prostate cancer; conventional bone scan and FDG-PET, and PSMA-specific ^{89}Zr-Df-IAB2M minibody

Pandit-Taskar et al., J. Nucl. Med. 2016

Clinical POC: Anti-CD8 minibody (ImaginAb)



Patient with newly-diagnosed hepatocellular carcinoma; initiated treatment with checkpoint inhibitor 2 weeks prior to scanning with CD8 cytotoxic T cell-specific ^{89}Zr-Df-IAB22M2C. Intratumoral CD8 signal is clearly visible in liver.

Pandit-Taskar et al., J. Nucl. Med. 2020

CONTACT INFORMATION

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