

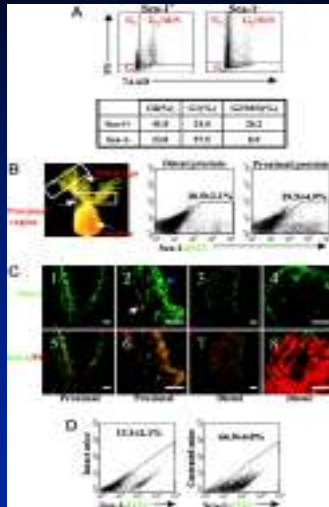
Stem Cell Antigens as Therapeutic Targets in Prostate Cancer

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Co-Director, Prostate Cancer Program
Jonsson Cancer Center
Geffen School of Medicine at UCLA

Cancer Stem Cell Hypothesis

- ◆ Cancers arise from transformation of stem/progenitor cells
- ◆ Cancer stem cells can self renew and give rise to differentiated progeny
- ◆ Cancer stem cells are tumorigenic whereas progeny are not
- ◆ Cancer stem cells should be targeted therapeutically to improve results of anticancer therapy

SCA-1+ Cell Fraction Has Stem Cell Features

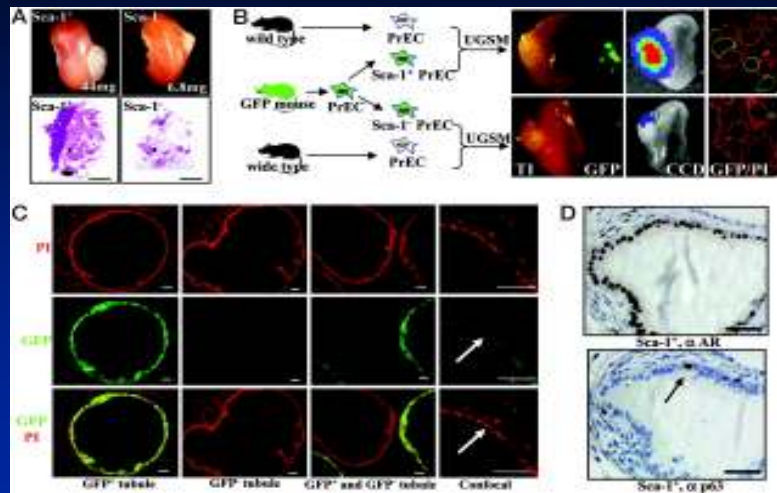


Sca-1+ prostate cell fractions contain increased numbers of replication-quiescent, androgen-independent cells that cluster in the proximal region of murine prostatic tubules

Xin, Li et al. (2005) Proc. Natl. Acad. Sci. USA 102, 6942-6947

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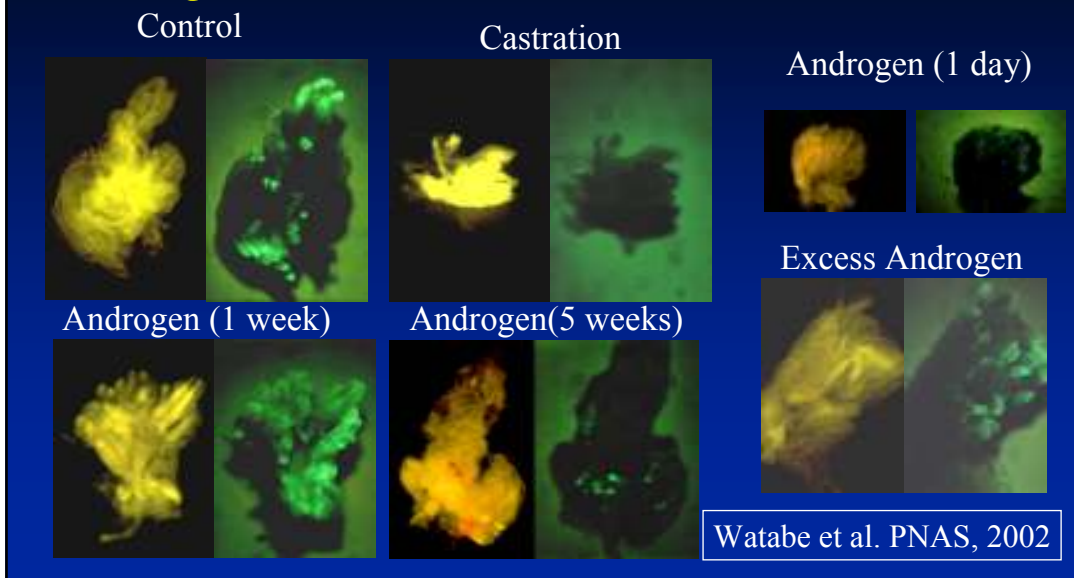
Sca-1 Enriches for Cells with Prostate-Regenerating Activity



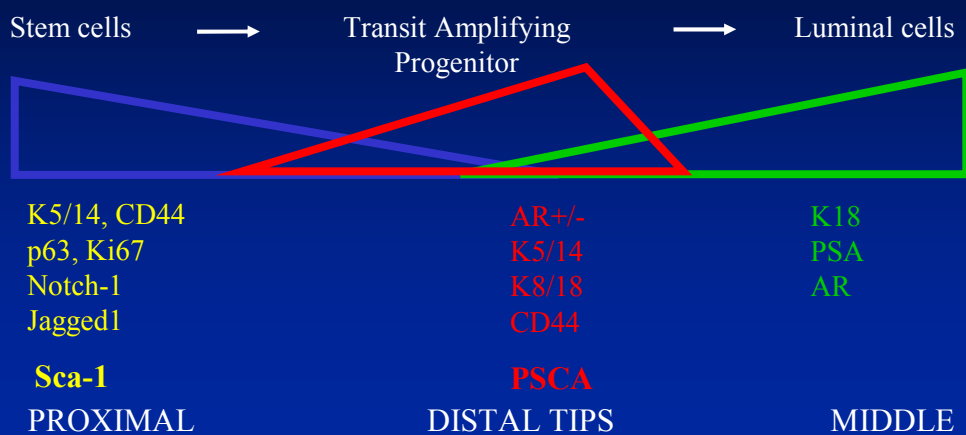
Xin, Li et al. (2005) Proc. Natl. Acad. Sci. USA 102, 6942-6947

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PSCA Expression is Associated with Growth and Regeneration of the Prostate

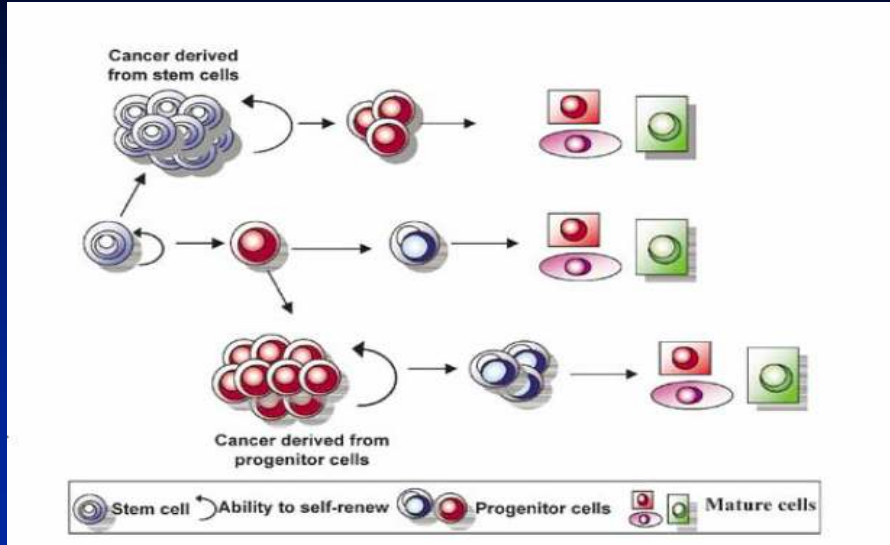


Model of Normal Human and Mouse Prostate Epithelium: SCA-1 vs. PSCA Positive Progenitors

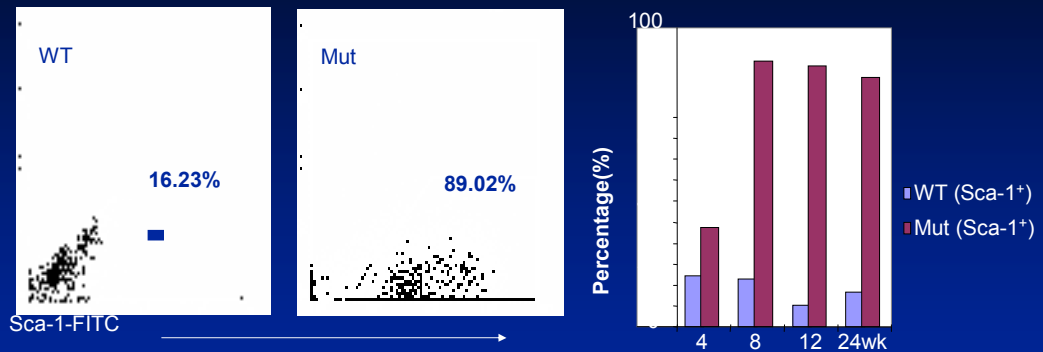


Adopted from Tran et al. Molecular Cancer Res, 2002

Cancer Stem Cell Model



Increased Sca-1+ cells in Pten null prostate cancer



Hong Wu and Owen Witte

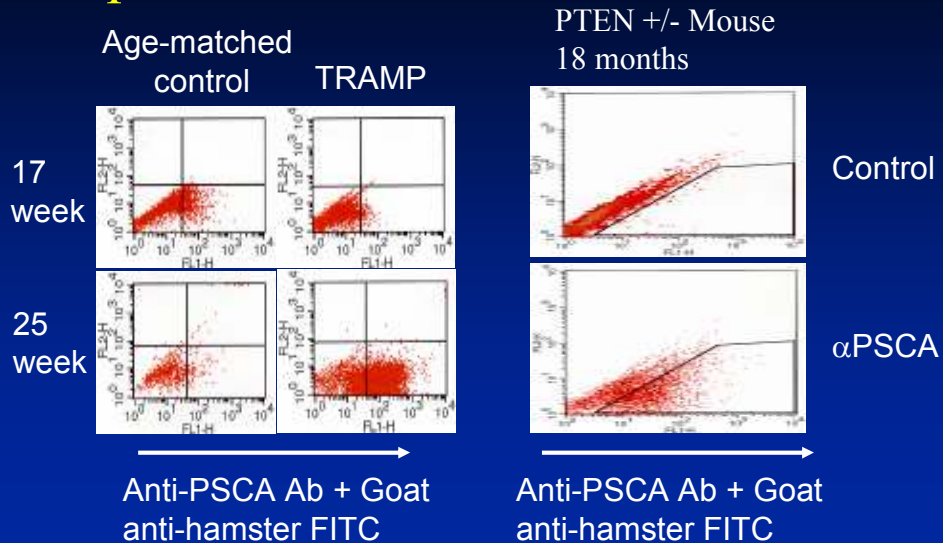
Expansion of PSCA/GFP Positive Cells in TRAMP/PSCA-GFP Tumors (23 weeks)



Gross Pathology
TRAMP-/GFP+ vs
TRAMP+/GFP+

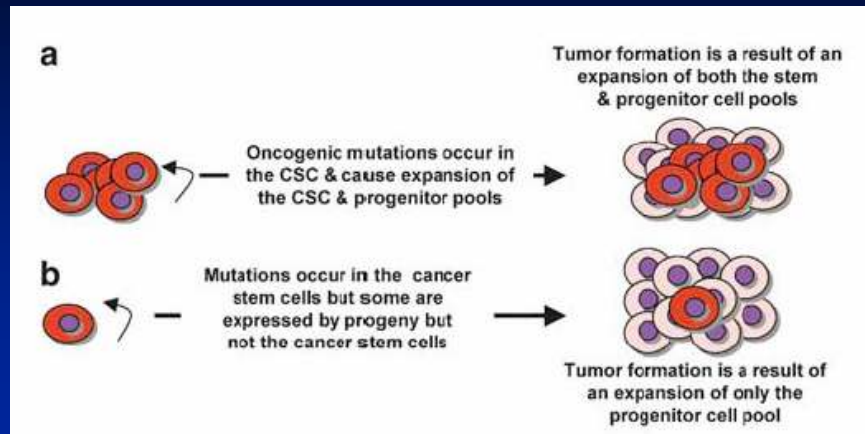
Ventral prostate
TRAMP-/GFP+ vs.
TRAMP+/GFP+

Altered mPSCA Expression in Multiple Murine Prostate Cancers



Dubey et al. Cancer Res 2001

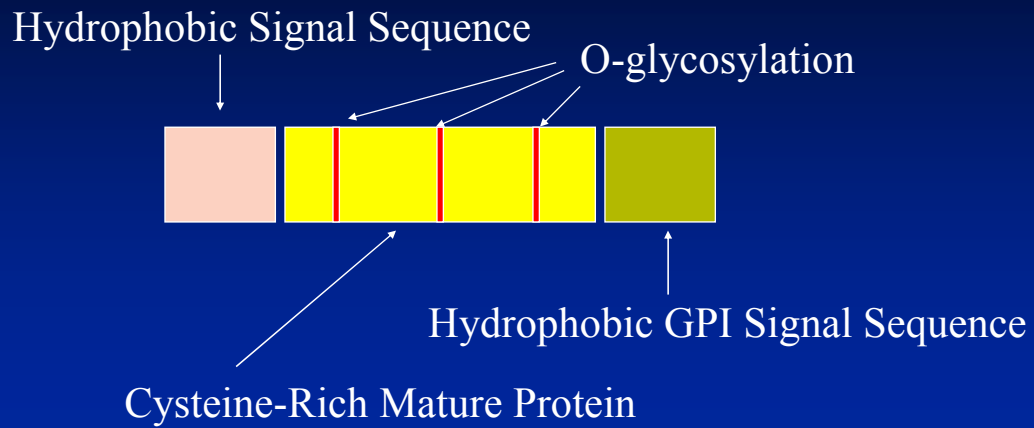
Expansion of Stem and/or Progenitor Cells in a Cancer Stem Cell Model



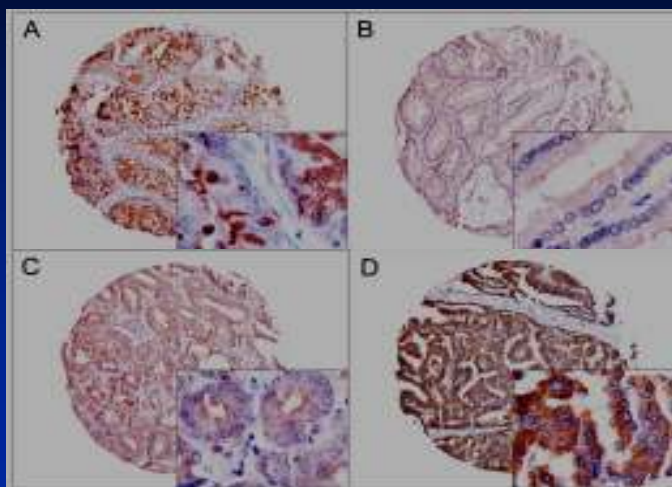
Targeting PSCA in Prostate Cancer: Preclinical Validation to the Clinic

- ◆ Expression of Target
- ◆ Target Function
- ◆ Drug that "Hits" Target
- ◆ Preclinical Efficacy
- ◆ Mechanism of Action
- ◆ GMP Grade Product
- ◆ Toxicity Studies
- ◆ IND
- ◆ Phase I Clinical Trial

PSCA: Structure



PSCA Expression



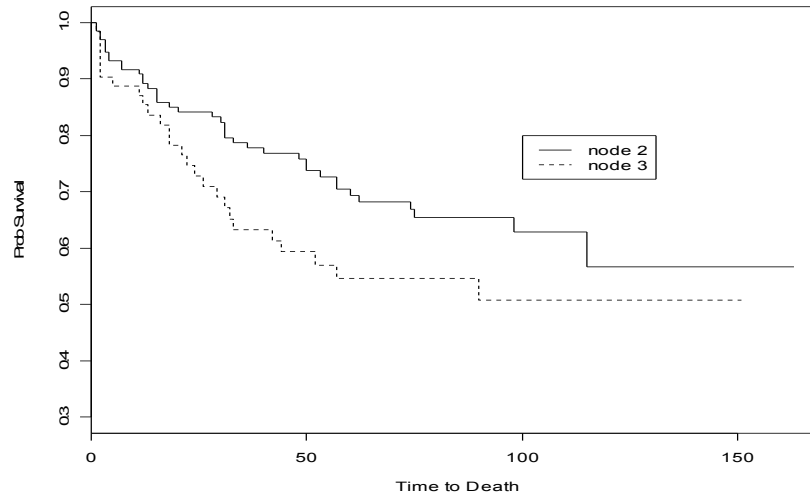
A. Intensity = 3
(Gleason 4)

B. Intensity = 1
(Gleason 3)

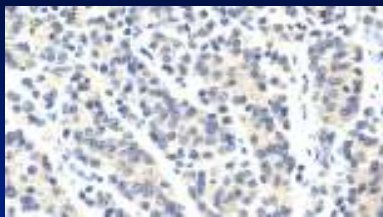
C. Intensity = 2
(Gleason 3)

D. Intensity = 3
(Gleason 4)

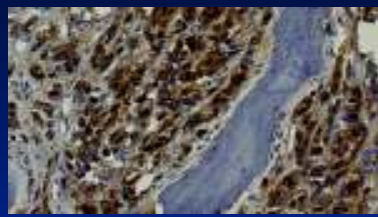
PSCA Expression and Survival



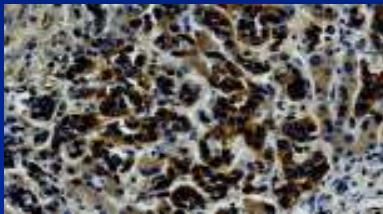
PSCA Expression in Matched Soft Tissue and Bone Metastases



PATIENT 1: LYMPH NODE



BONE

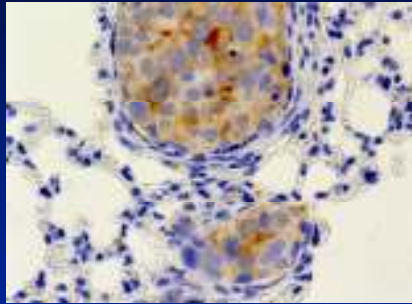


PATIENT 2: LIVER

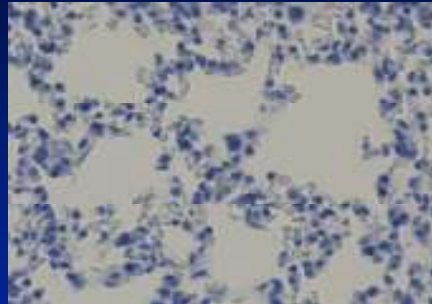


BONE

PSCA mAB 1G8 Blocks LAPC 9 Metastasis

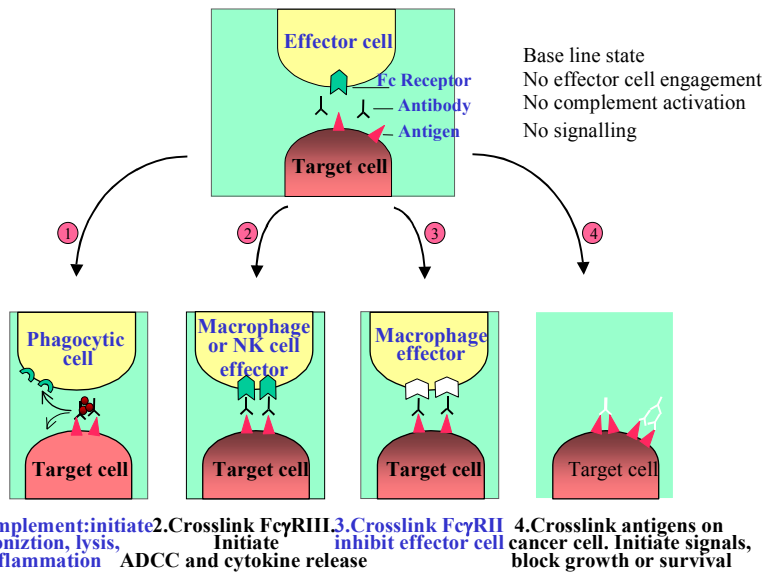


Control Lung
0.3 mets/high power field
100% mice

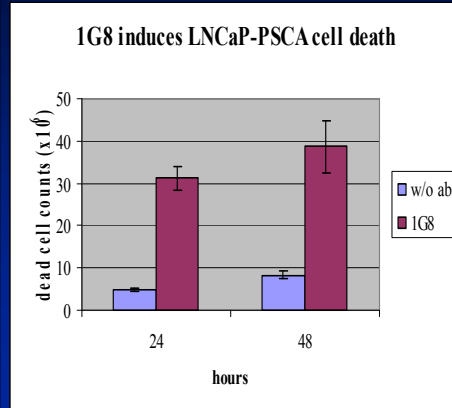
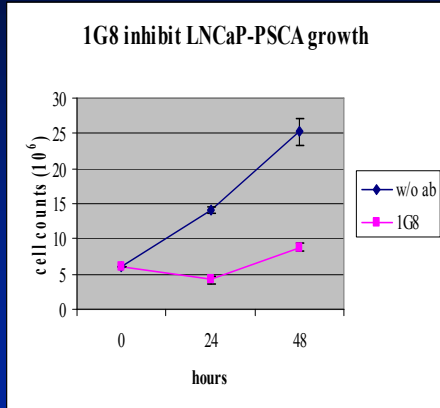


1G8 Treated Lung
0.029 mets/high power field
12.5% mice

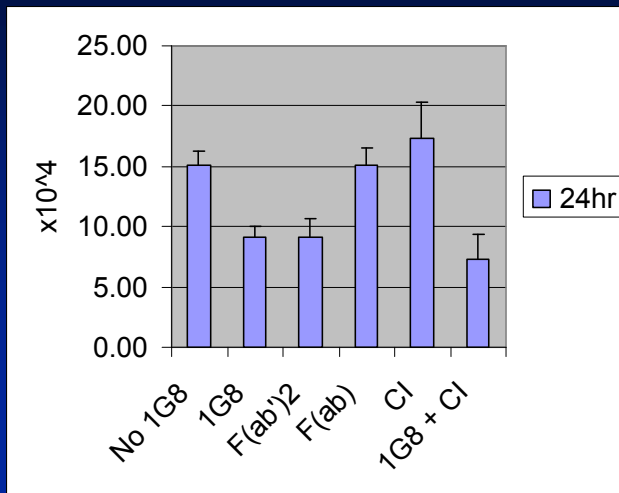
Mechanisms of Antibody Mediated Tumor Rejection



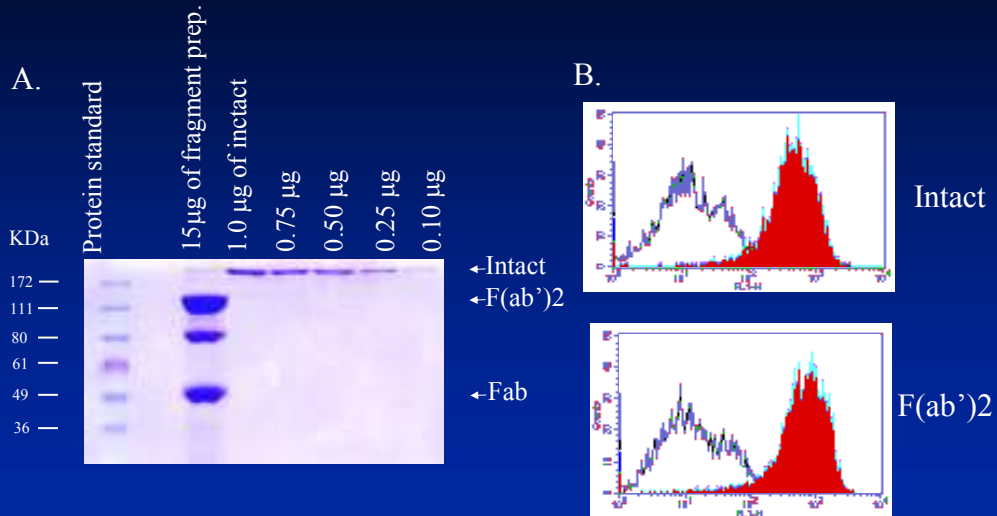
1G8 inhibits LNCaP-PSCA growth and induces cell death



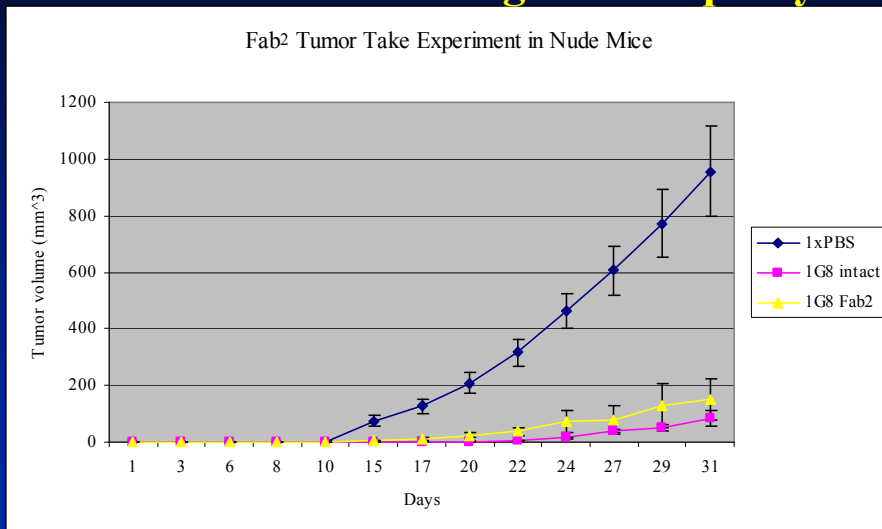
PSCA Antibody-mediated Cell Death is Caspase Independent and Requires Antigen Crosslinking



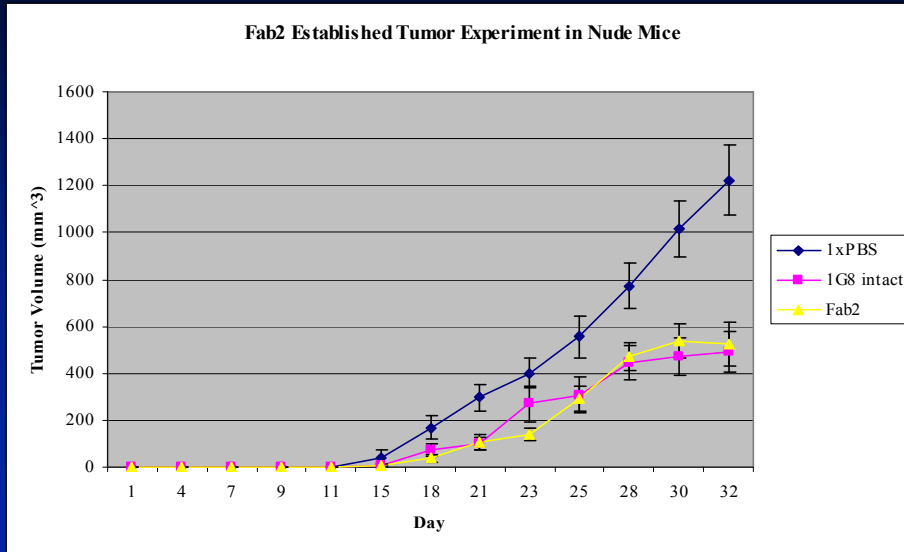
F(ab')₂ Retains the Same Binding Affinity and Specificity as Intact 1G8



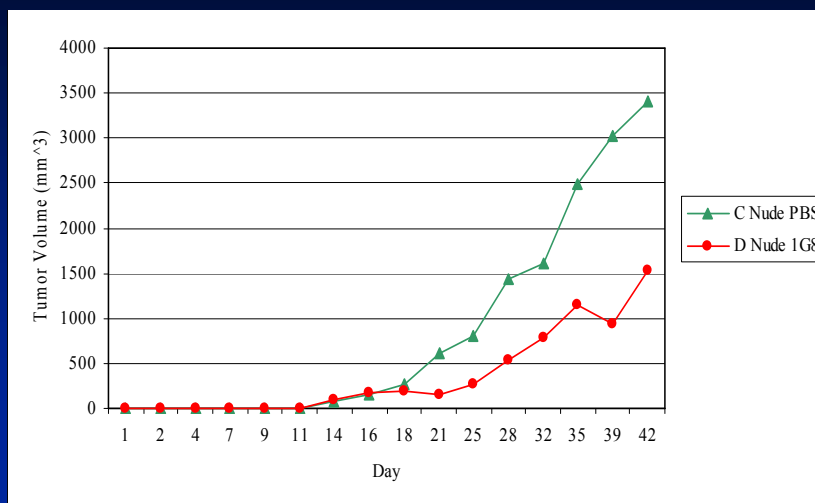
Intact 1G8 and It's F(ab')₂ Fragment Inhibit LAPC9 Tumorigenesis Equally





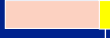

PSCA mAb and It's F(ab')₂ Fragment Slow LAPC9 Tumor Progression Equally



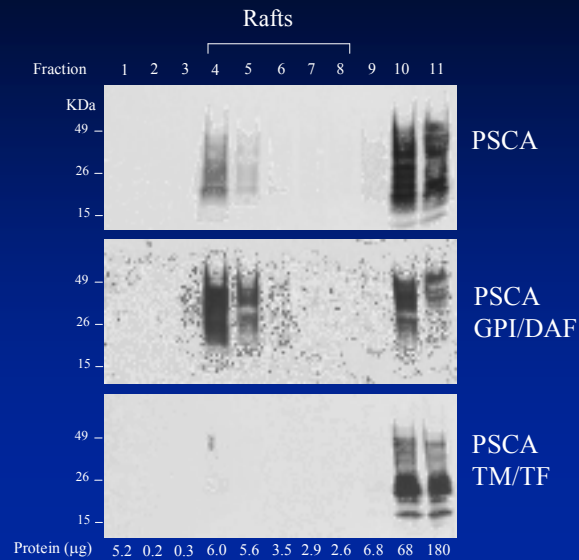
PSCA mAb 1G8 Inhibits LAPC 9 Growth in FCR γ RIII^{-/-} Mice



Chimeric PSCA Constructs

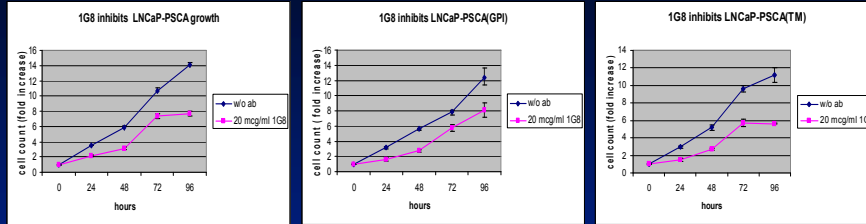
			Internalization	Rafts
PSCA		GPI	+	+
PSCA/DAF-GPI		GPI	+	+
PSCA/TF-TM		TM	+	??
PSCA/IL2-TM		TM	-	

PSCA Cellular Distribution

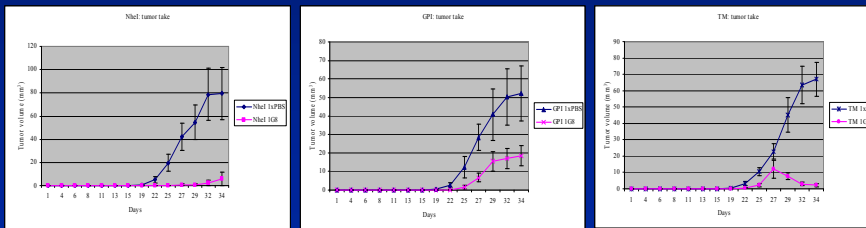


1G8 inhibits LNCaP-chimeric PSCA growth in vitro and in vivo

In vitro



In vivo



PSCA

PSCA-GPI (DAF)

PSCA-TM

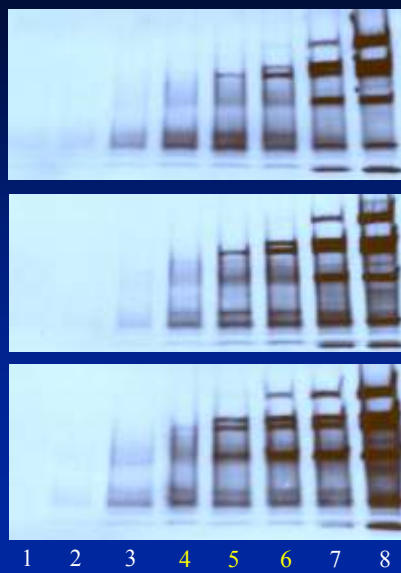
Translocation of TM-PSCA to Rafts Following Treatment with 1G8

No Ab

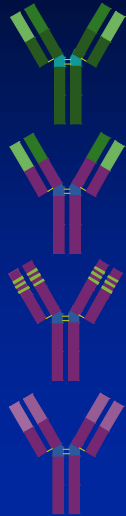
1G8 30'

1G8 60'

Fraction

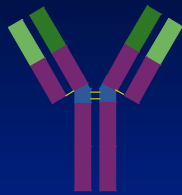


Engineering Anti-PSCA Antibodies for Clinical Use: Humanization



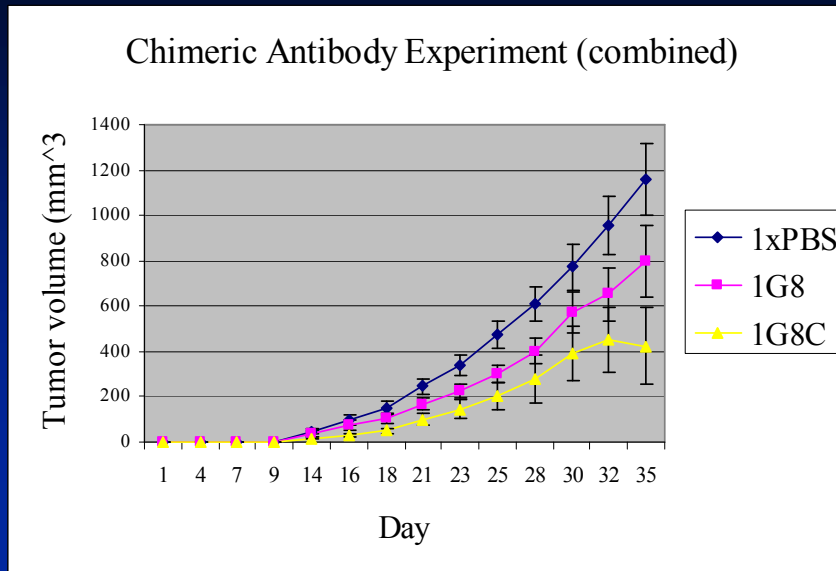
- Murine (mouse)
- Chimeric (mouse variable, human constant regions) (Rituxan)
- Humanized (mouse CDRs, human framework and constant regions)(Herceptin)
- Human

Chimeric 1G8 and Fragments

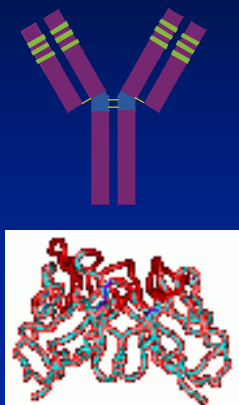


- ◆ Z. Gu (Reiter lab) and Sherie Morrison lab developed chimeric 1G8
- ◆ **Biologically active**
- ◆ Poor expression (1-2 $\mu\text{g/ml}$)
- ◆ Prone to precipitation

Chimeric 1G8 Antibody Has Greater Anti-tumor Activity Than Murine Parent



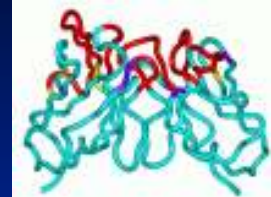
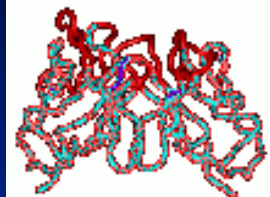
Engineering Anti-PSCA Antibodies: Humanized 2B3 and Fragments



Humanized 2B3

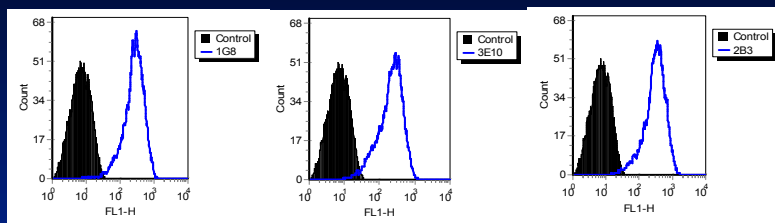
- ◆ Modeled and designed CDR-graft (M. Sherman)
- ◆ Assembled by complete gene synthesis/PCR
- ◆ Expressed intact humanized Abs at 10-30 $\mu\text{g/ml}$
- ◆ Purified mg quantities by Protein G

Engineering Anti-PSCA Antibodies: Humanized 2B3 and Fragments



- ◆ Re-modeled 1G8 (based on new structures available in PDB)
- ◆ Identified six additional supporting residues
- ◆ Back-mutation in progress
- ◆ Exploring affinity maturation by yeast display

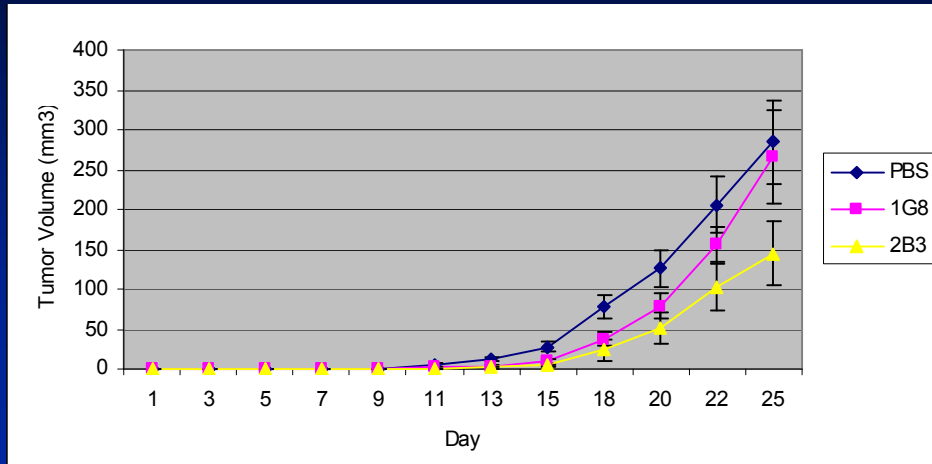
Engineering Anti-PSCA Antibodies: Humanized 2B3



LNCaP-
PSCA

- ◆ Two versions made; 2B3 expresses better and binds better than 3E10
- ◆ **Biologically active**
- ◆ 2B3 diabodies and minibodies constructed and transfected for expression (J. Leyton)
- ◆ Affinity reduced (10^8)

Humanized PSCA mAB 2B3 Greater In Vivo Activity Than Murine 1G8



MicroPET Imaging using Humanized I-124 Labeled Anti-PSCA mAb

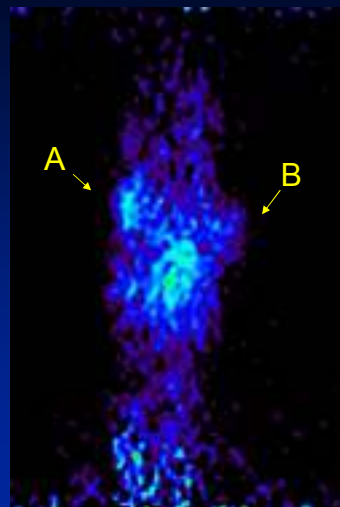
Athymic mice bearing PSCA-PC3 (human prostate; A) and C6 (rat glioma; B) xenografts.

Injected with 136 μ Ci of I-124 2B3 mAb (labeling efficiency 94%, immune reactivity 2%)

Imaged on Focus microPET at ~ 48 hours.

Biodistribution at time of sacrifice (~ 94 h) (n = 4)

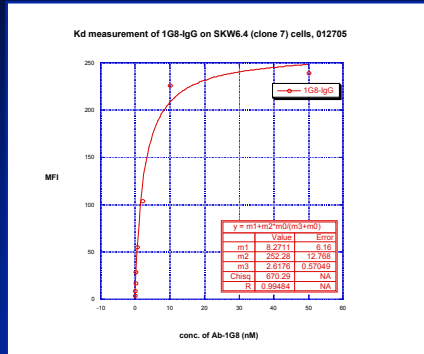
PSCA-PC3:	12.7(1.6)% ID/g
C6:	7.6(2.8)% ID/g
Liver:	4.8(1.1)% ID/g
Kidney:	4.6(0.8)% ID/g



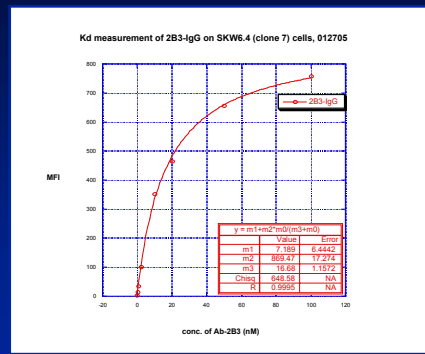
Coronal

Tove Olafsen, Ph.D

Affinity Measurements by Flow Cytometry 1G8 and 2B3



1G8 2.6 nM

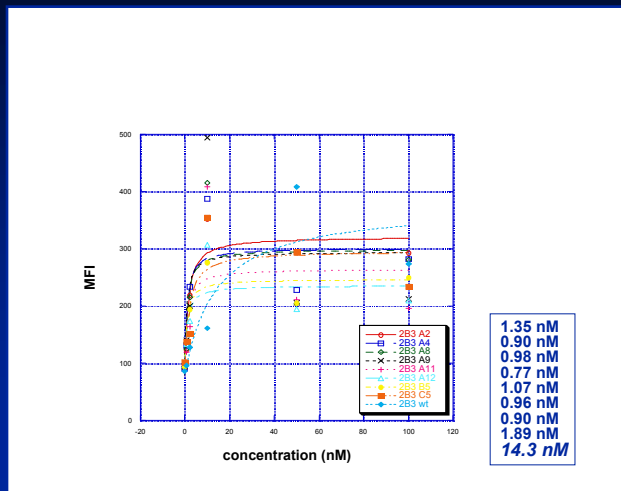


2B3 16.7 nM

J. Marks lab

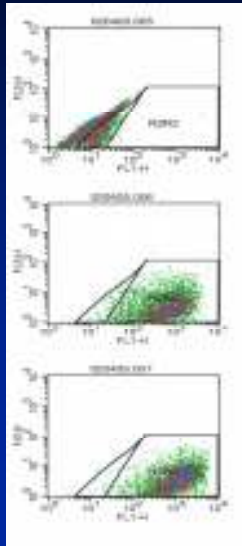
Affinity maturation of 2B3 by yeast display

- Error-prone PCR to generate diversity
- Display scFv library on yeast
- Select by flow cytometry
- Picked 7 candidates after third round; 1 after fourth round
- Affinity determined by flow cytometry



E. Lepin, J. Marks lab

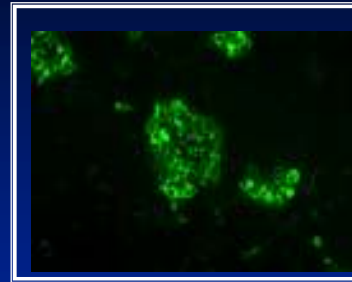
2B3 Minibody: Binding to PSCA-positive cells



Negative ctrl

Minibody

2B3 Intact

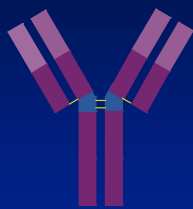


2B3 Minibody

2B3 Diabodies and minibodies have been expressed and purified for *in vivo* imaging studies

J. Leyton, T. Olafsen

Human PSCA: AGS-PSCA



1. Fully human antibody produced in Abgenix SCID-hu mice
2. High affinity– 1.6×10^{-10} M
3. Pre-clinical activity— a) prevents metastasis and leads to tumor regression in 50% + of mice bearing LAPC-9 xenografts b) synergy with Taxotere causing complete remissions
4. Co-development agreement with Merck
5. Epitope?? Mechanism of action??

AGS-PSCA

- ◆ Phase I trial
- ◆ Dose escalation
- ◆ Metastatic prostate cancer
- ◆ Pharmacokinetics, safety
- ◆ Currently open at Memorial Sloan Kettering (Scher) and Johns Hopkins (Carducci)

Acknowledgements

- ◆ Zhennan Gu
- ◆ Joyce Yamashiro
- ◆ Evelyn Kono
- ◆ Anna Wu
- ◆ Tove Olafson
- ◆ Owen Witte
- ◆ Sherrie Morrison
- ◆ Jonathan Said
- ◆ David Seligson
- ◆ Massimo Loda (DFCI)
- ◆ Mark Ruben (DFCI)
- ◆ Robert Jenkins (Mayo)
- ◆ Robert Vessella (Washington)
- ◆ Mark Sherman (CoH)
- ◆ Jeff Ravetch (Rockefeller)