



# Prostate-Specific Membrane Antigen (PSMA) Activated Prodrug and Imaging Agents



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## Introduction

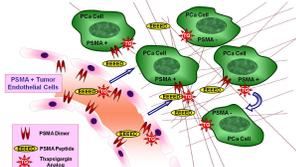
- Antiproliferative Chemotherapies have had limited success in the treatment of metastatic cancers.
- These therapies have significant toxicities that greatly limit the amount and duration of therapy.
- Targeted Therapies attack cancer specific targets while minimizing toxicity.
- Heterogeneity of target expression within tumors results in Outgrowth of Resistant Cells.
- Limits the effectiveness of targeted therapies such as kinase inhibitors and monoclonal antibodies

### An Alternative Targeting Strategy:

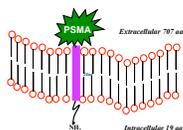
- Identify a critical intracellular protein whose function is required for survival of all cell types
- Create a therapeutic index by targeting an inhibitor of the protein selectively to tumor sites
- Resistance is unlikely as cells need continued expression of the target protein to survive

### The Thapsigargin Prodrug Strategy

- Couple thapsigargin, a potent inhibitor of a critical intracellular protein to a tumor protease specific peptide substrate to create an inactive prodrug.
- PSMA is selectively activated by release of thapsigargin cytotoxin by protease expressed within tumor tissues

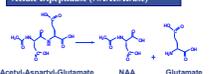


## Rationale for Targeting PSMA

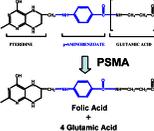


- > High levels of expression in normal prostate and in prostate cancers
- > No Detectable Expression in Normal Endothelium
- > Positive Expression in NEOVASCULATURE of large number of tumor types
- > PSMA expression is upregulated following androgen ablation!
- > Unique Enzymatic Activity

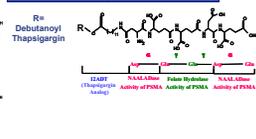
PSMA is an N-Acetylated Alpha-Linked Acidic Dipeptidase (NAALADase)



PSMA is a Folate Hydrolase

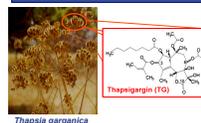


Exploiting the Dual Enzymatic Functions of PSMA



## Thapsigargin as Therapy For Cancer

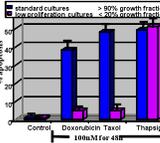
Thapsigargin is a natural product isolated in high yield (1% of seed weight) from *Thapsia garganica*



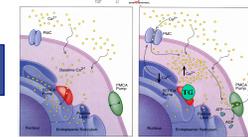
Thapsigargin is a Potent Non-Specific Cytotoxin



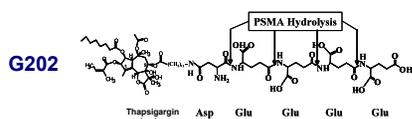
Thapsigargin induces PROLIFERATION INDEPENDENT apoptosis in prostate cancer cells



Thapsigargin irreversibly inhibits a critical intracellular protein, the Sarcolemmal Endoplasmic Reticulum Calcium ATPase (SERCA) pump, causing sustained elevation of intracellular calcium, activation of ER stress response and release of apoptotic factors from mitochondria.



## G202 Kills PSMA-Positive Cells In Vitro

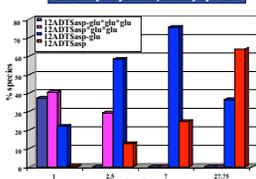


PSMA Prodrug IC50 Values In Vitro

Prodrug/drug	IC <sub>50</sub> Values (nM)		
	SIH	LNCaP	PC9
AMM2: 12ADT <sup>1</sup> Sup <sup>1</sup> gla	529 ± 888	251 ± 4	39
AMM1: 12ADT <sup>1</sup> Sup <sup>1</sup> gla <sup>1</sup> gla	8816 ± 1141	384 ± 36	28
AMM5: 12ADF <sup>1</sup> gla <sup>1</sup> gla <sup>1</sup> gla <sup>1</sup> gla	8541887	239 ± 8	35
AMM7: 12ADF <sup>1</sup> gla <sup>1</sup> gla <sup>1</sup> gla <sup>1</sup> gla <sup>1</sup> gla	4507 ± 880	344 ± 9	25
AMM9: 12ADT <sup>1</sup> Sup <sup>1</sup> gla <sup>1</sup> gla <sup>1</sup> gla	10164 ± 400	191 ± 29	97
AMM3: 12ADT <sup>1</sup> Sup <sup>1</sup> gla <sup>1</sup> gla <sup>1</sup> gla <sup>1</sup> gla	14709 ± 200	1031 ± 698	2

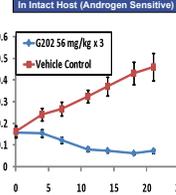
AMM5 is Not hydrolyzed by PSMA

G202 is Hydrolyzed Sequentially by PSMA

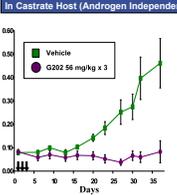


## Efficacy Against Human Prostate Cancer Xenografts

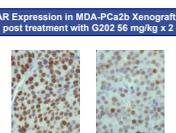
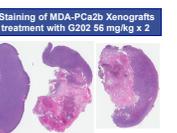
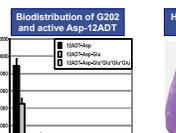
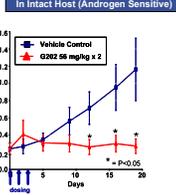
LNCaP Human Prostate Cancer In Intact Host (Androgen Sensitive)



CWR22R-H Human Prostate Cancer In Castrated Host (Androgen Independent)

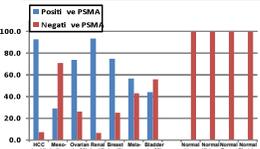


MDA-Pc4 2b Human Prostate Cancer In Intact Host (Androgen Sensitive)

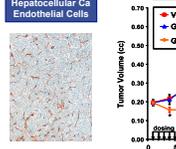


## Efficacy against Human Cancer Xenografts

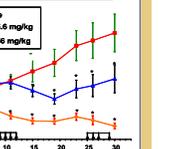
PSMA Expression in a Panel of 340 Human Cancers



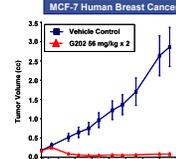
PSMA Staining Hepatocellular Ca Endothelial Cells



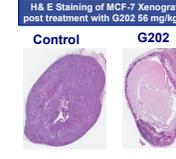
SH2C Human Kidney Cancer



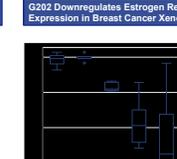
MCF-7 Human Breast Cancer



H&E Staining of MCF-7 Xenografts post treatment with G202 56 mg/kg x 2

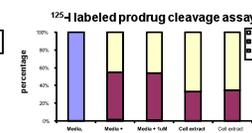
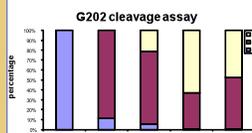


G202 Downregulates Estrogen Receptor Expression in Breast Cancer Xenografts

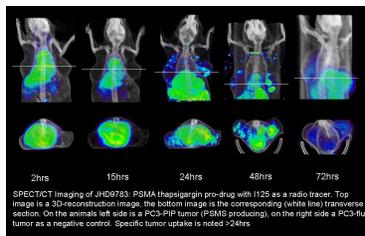


## Radiolabeled PSMA-Activated Thapsigargin Prodrug for Imaging Prostate Cancer

Label



Cleavage assay for PSMA pro-drugs G202 and <sup>125</sup>I labeled prodrug. Metallobranes were detected with LC/MS. Results show that LNCaP cells can cleave both compounds. Highest amount of free-drug was found in the cell extract sample, indicating clear uptake of the activated free-drug.



SPECT/CT imaging of JH07933 PSMA thapsigargin pro-drug with 1125b as a radio tracer. Top image is a SPECT reconstruction image, the bottom image is the corresponding (white line) transverse section. On the animals left side is a PC3-PIP tumor (PSMG producing), on the right side a PC3-Iu tumor as a negative control. Specific tumor uptake is noted x4x4.

## Conclusions

- Preclinical studies demonstrate that G202 is a PSMA-activated prodrug that has broad-spectrum anti-tumor activity against a panel of human cancer xenografts
- G202 licensed to GenSpera, a start-up biotechnology company based in San Antonio, TX ([www.genspera.com](http://www.genspera.com))
- GenSpera has completed GMP manufacture of clinical grade G202
- Toxicology studies completed in rats and monkeys
- IND awarded in September 2009
- Phase I clinical trials began at Johns Hopkins and University of Wisconsin 2010

## G202 Phase I Clinical Trial

Study Schema

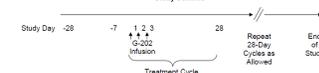


Table 1. G202 Dose Escalation Schedule

Dose Level	G202 Dose (mg/m <sup>2</sup> )
Level 2	0.3
Level 1	0.6
Level 1	1.2
Level 2	2.5
Level 3	5.0
Level 4	10.0
Level 5	17.0
Level 6	25.0

