Novel Enzyme Prodrug Therapy of Breast Cancer Designed for Effective Delivery to the Tumor

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ABSTRACT

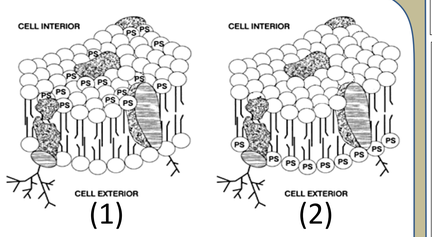
A new anticancer therapy for solid tumors is being developed to eliminate both the primary tumor and distant metastases. This approach uses an enzyme prodrug system targeted to the solid tumor vasculature, specifically to exposed phosphatidylserine. Two recombinant exogenous enzymes (*L-methioninase* & *cytosine deaminase*) were linked separately to human annexin V protein to form fusion proteins (FPs). Annexin V will specifically target both the outer membrane of endothelial cells in tumor vasculature and cancer cells. The resulting therapeutic effect is (1) death to tumor endothelial cells leading to vessel clotting and oxygen deprivation, and (2) death to surrounding tumor cells due to leaky capillaries and the EPR effect. Cytotoxicity experiments have verified the ability of the FPs to produce therapeutic levels of methylselenol and 5-fluorouracil *in vitro*. Preliminary tests in nude mice using targeted L-methioninase and selenomethionine prodrug gave promising results.

INTRODUCTION

The aim of this project is to develop a new enzyme prodrug therapy for treating breast cancer in which the enzyme is targeted to phosphatidylserine (PS) exposed on the tumor vasculature. PS has previously been found to be exposed on the surface of the vasculature endothelium of blood vessels in tumors but not on normal endothelium. Because it binds to anionic phospholipids such as PS, annexin V was used in two fusion proteins, each with a different enzyme that converts a prodrug to an anticancer drug.

PHOSPHATIDYLSERINE

- Target for this treatment
- Abundant in cell membrane
- Normal healthy cells (1)
- Tumor cells (2)



HUMAN ANNEXIN V (36 kDa)

- Ca^{2+} dependent binding
- High affinity for membrane phospholipids ⟹ *Phosphatidylserine*

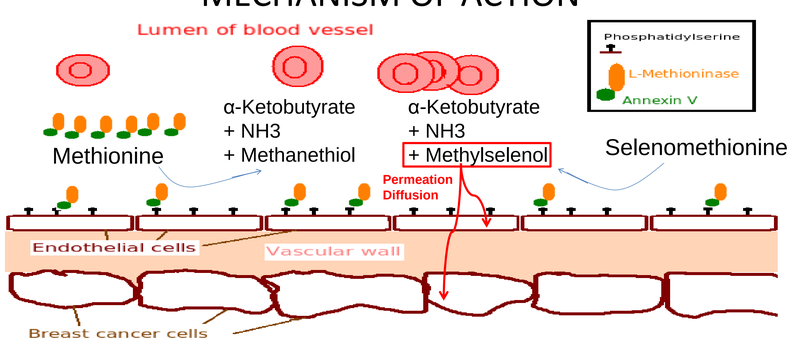
L-METHIONINASE (43 kDa)

- *Pseudomonas putida,* homotetramer
- Selemomethionine (SeMet) conversion to toxic methylselenol

CYTOSINE DEAMINASE (17 kDa)

- *Saccharomyces cerevisiae,* dimer
- 5-Fluorocytosine (5-FC) conversion to toxic 5-fluorouracil (5-FU)

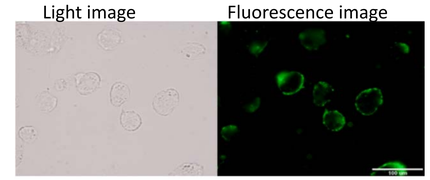
MECHANISM OF ACTION



BINDING ASSAY: To determine the strength of annexin V binding to exposed PS

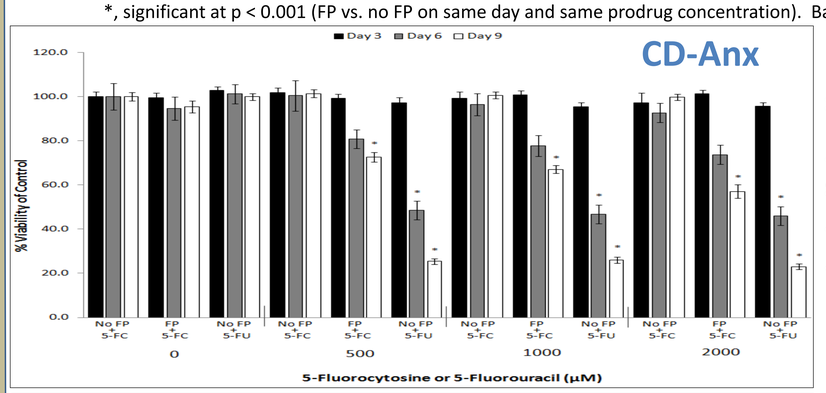
Protein	HAAE-1 Endothelial Cells	MCF-7 Cancer Cells	MDA-MB-231 Cancer Cells
Meth-Anx:	K_d = 1.7 ± 0.6 nM	K_d = 1.9 ± 1.2 nM	K_d = 2.2 ± 1.2 nM
CD-Anx:	K_d = 1.5 ± 0.2 nM	K_d = 0.6 ± 0.4 nM	K_d = 4.2 ± 1.8 nM

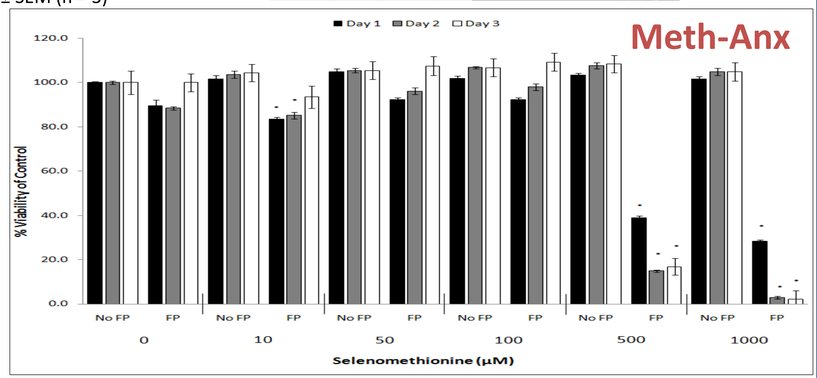
FLUORESCENCE MICROSCOPY:
CD-Anx binding to surface of MDA-MB-231 cells

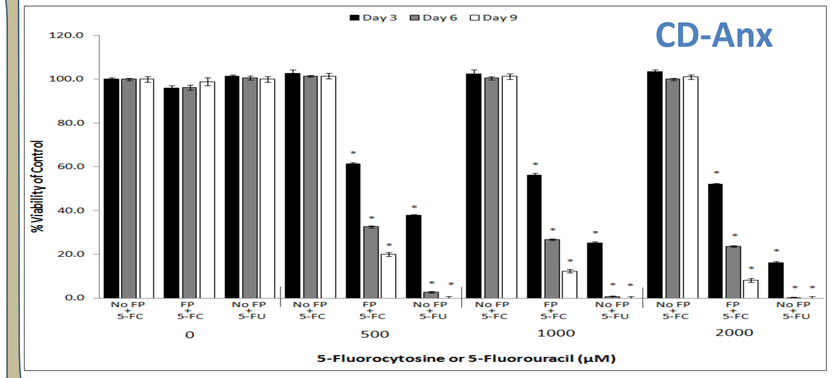


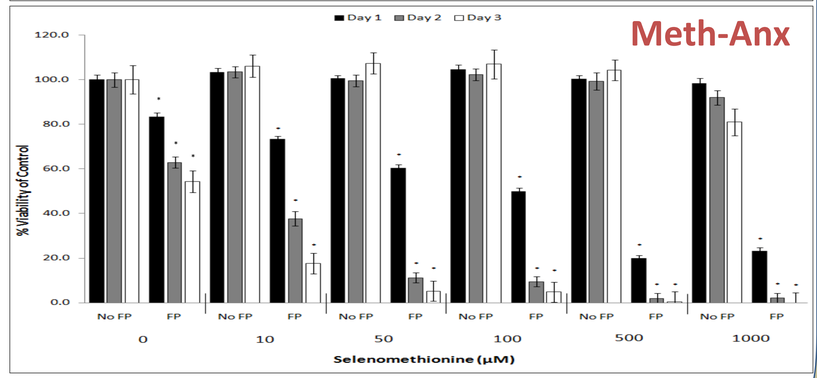
CYTOTOXICITY ASSAY: To test the effect of the enzyme prodrug on cells *in vitro*

*, significant at p < 0.001 (FP vs. no FP on same day and same prodrug concentration). Bars, ± SEM (n = 3)







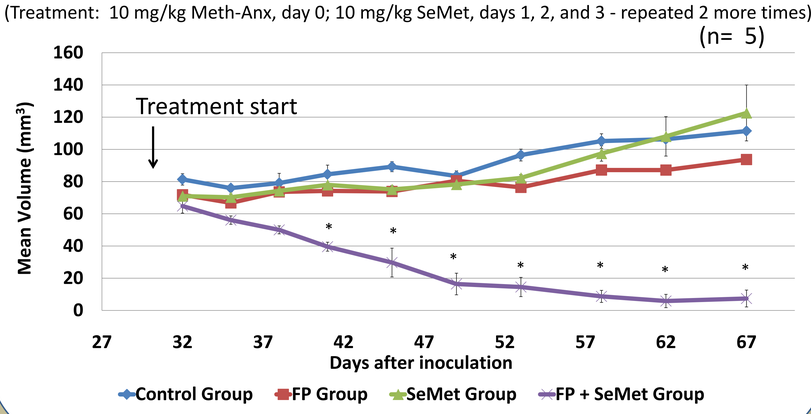


Van Rite et al, Cancer Letters 307 (2011) 53–61

Van Rite et al, Cancer Letters 301 (2011) 177-184

TREATMENT of MDA-MB-231 CELLS in NUDE MICE

(Treatment: 10 mg/kg Meth-Anx, day 0; 10 mg/kg SeMet, days 1, 2, and 3 - repeated 2 more times)
(n= 5)



*, p < 0.001, significant compared to control group

CONCLUSIONS

- Meth-Anx + SeMet produces cell killing in 1 day *in vitro*
- CD-Anx + 5-FC produces cell killing in 3 days *in vitro*
- No significant effect on cells *in vitro* with prodrug present and FP absent
- 3 rounds of Meth-Anx + SeMet produce significant reduction in MDA-MB-231 breast tumor size in nude mice

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