Abstract A219

Efficacy of a Spectrum-Selective Cathepsin Inhibitor in a Mouse Model of Bone Cancer

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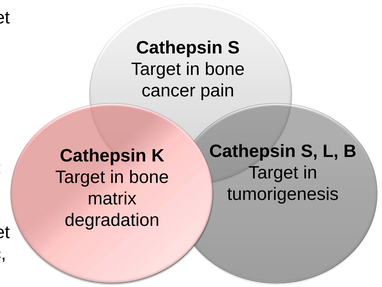
Introduction

Introduction: Increased cathepsin levels and activity have been shown to play a role in a variety of tumors, including lung and breast malignancies, and are correlated with poor patient prognosis. Proteolytic activity of cathepsins S, L, B and K may play a role in degradation of the basement membrane and extracellular matrix allowing tumor cell invasion and facilitating tumor metastasis. Cathepsin K inhibition has been reported to suppress bone resorption both in women with post-menopausal osteoporosis and patients with breast cancer by inhibiting osteoclast function. Studies with cathepsin S inhibitors have demonstrated a reduction in nociception in animals, suggesting a role for cathepsin S in tumor-associated bone pain and neuropathy. We report the efficacy of the spectrum-selective cathepsin inhibitor VBY-825 in a mouse model of metastatic breast cancer.

Cathepsin Biology in Oncology

- Cat K inhibition: Will target osteolytic metastases dependent on osteoclast-specific CatK for bone invasion, matrix degradation
- Cat S, L, and B inhibition: Will target tumorigenesis
- Cat S inhibition: Will target spontaneous, neuropathic, and inflammatory pain



VBY-825 Prototype Compound Profile

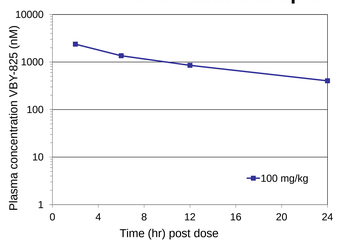
	Enzyme K_i (app) (nM)					
	CatS	CatL	CatB	CatK	CatF	CatV
VBY-825	0.13	0.25	0.33	2.3	4.7	0.25

No inhibition (IC_{50}>10μM) of a panel of cysteine, serine, and aspartyl proteases: caspase 3, chymotrypsin, cathepsin D, cathepsin H, cathepsin X/Z, neutrophil elastase, MMP9, thrombin

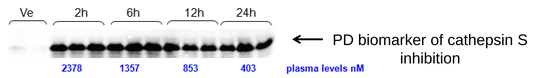
- VBY-825 is a potent, reversible inhibitor of a subset of cysteine cathepsins
- VBY-825 is orally bioavailable and pharmacologically active in a preclinical model of pancreatic islet cancer where it was found to significantly decrease tumor burden and tumor number (Elie et al, 2010).

VBY-825 Prototype Compound Profile

PK/PD Relationship of VBY-825 in Mice



- VBY-825 sustains plasma levels with QD dosing in mice sufficient for cathepsin inhibition and generation of a biomarker of CatS inhibition (accumulation of invariant chain substrate Iip10, in spleen)



← PD biomarker of cathepsin S inhibition

VBY Compound Series

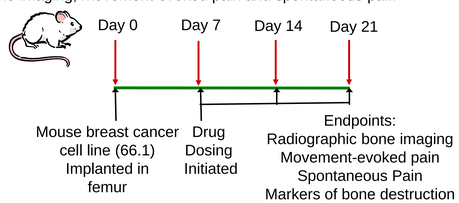
Virobay is developing a series of spectrum-selective cathepsin inhibitors with structural diversity

Inhibitor	Enzyme K_i (app) (nM)					
	CatS	CatL	CatB	CatK	CatF	CatV
VBY-B	0.46	0.29	3.2	0.96	30	<0.25
VBY-C	0.006	<0.25	<0.25	0.06	<0.25	<0.25
VBY-D	0.023	<0.25	2.9	0.38	1	0.25

Results

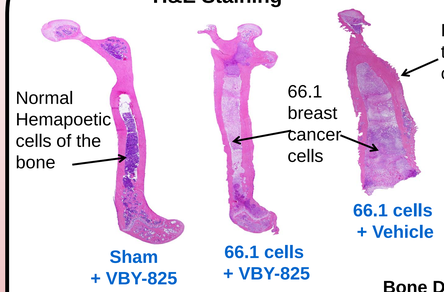
1. VBY-825 is efficacious in mouse model of bone cancer and bone cancer pain

- 66.1 mouse breast cancer line was injected and confined to the intramedullary space of the femur of non-immunocompromised mice.
- Beginning on post surgical day 7, animals received either VBY-825 (100 mg/kg), zoledronic acid (ZA; 100 ug/kg), or 5% dextrose vehicle QD for 14 days.
- Endpoints measured on day 7, 14, and 21 of the study included radiographic bone imaging, movement-evoked pain and spontaneous pain



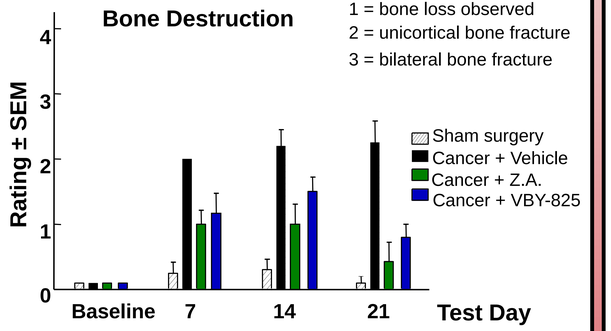
Results

H&E Staining



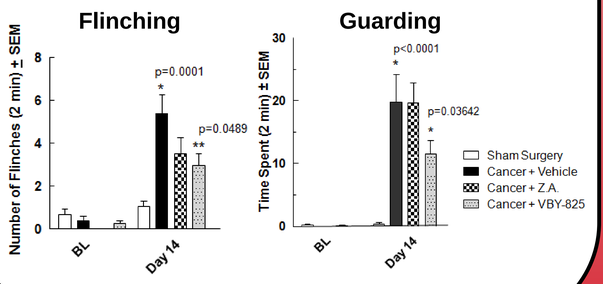
Bone Destruction Rating:

0 = normal
1 = bone loss observed
2 = unicortical bone fracture
3 = bilateral bone fracture



2. VBY-825 demonstrated analgesic activity in a model of bone cancer pain

- VBY-825 reduced behaviors associated with spontaneous pain including flinching and guarding at day 14 of the study

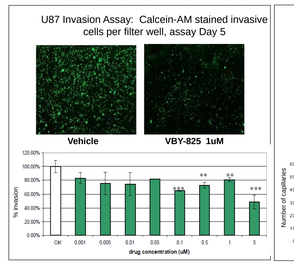


Results

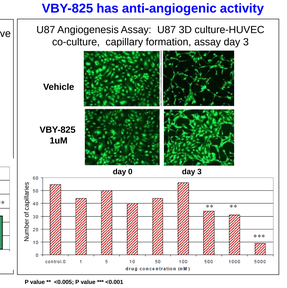
3. VBY-825 inhibits tumor angiogenesis and invasion *in vitro* in a 3D tumor cell line growth assay

- HuBiogel™ is a human-derived biomatrix system that allows for 3D growth and proliferation of tumor cells, invasion, and angiogenesis in a physiologically relevant environment that closely mimics the *in vivo* environment (Vivo Biosciences Inc, Birmingham, AL)
- VBY-825 is a potent inhibitor in 3D HuBiogel™ tumor cell line growth assays assessing
  - Tumor angiogenesis in tumor cell cell-HUVEC co-cultures
  - Tumor Invasion

VBY-825 reduced tumor cell invasion



VBY-825 has anti-angiogenic activity



Conclusions

- The cathepsin inhibitor VBY-825 demonstrated efficacy in a mouse model of metastatic bone cancer and associated pain
- VBY-825 demonstrated both analgesic activity as well as protection from bone matrix loss and bone remodeling
- Cathepsin inhibition reduced angiogenesis and tumor matrix invasion in *in vitro* assays, supporting the role of cathepsins as therapeutic targets in oncology
- These studies support the development of spectrum-selective cathepsin inhibitors in metastatic and primary bone cancer, with a mechanism of action complementary to standard chemotherapy

Reference: Elie et. al., (2010). Identification and pre-clinical testing of a reversible cathepsin protease inhibitor reveals anti-tumor efficacy in a pancreatic cancer model. *Biochimie* **92**, 1618-1624.

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