Assessing Marine Bivalves as Novel Invertebrate Models for Targeting Anti-apoptotic BCL-2 Family Proteins in Blood Cancer

The *in vivo* Effects of Ursolic Acid and Gossypol Acetic Acid on Tumor Cells in *Mercenaria mercenaria*

Background

Current limitations of animal testing

Ethics
3 R's of animal research
- Replace, Reduce & Refine

Effectiveness
Mammals: Clinical trial prediction accuracy of **<10%**

Efficiency
Studies often limited in sample size → less accuracy

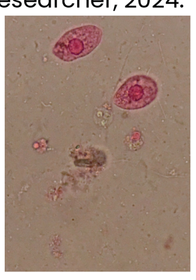
Disseminated Neoplasia (DN)

A **contagious cancer** that is **naturally occurring** in the hemolymph fluid (blood) of marine bivalves

p53 and mortalin have been linked to tumorigenesis & several **Bcl-2 family members** have been cloned

Currently, there exists **little research** concerning the ***in vivo* effects of human cancer treatments on tumor cells in bivalves.**

Image taken by student researcher, 2024.



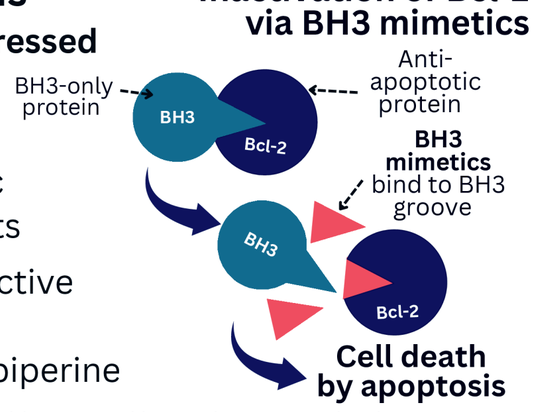
Leukemia and Bcl-2 family proteins

Bcl-2 anti-apoptotic proteins are **overexpressed** in around **half of all human cancers**

Gossypol acetic acid (AT101) is a widely studied pan-Bcl-2 inhibitor & BH3 mimetic
- Limited use due to toxic side effects

Ursolic acid is proposed to have high selective binding affinity with Bcl-2 proteins
- Low bioavailability → bioenhancer piperine

Inactivation of Bcl-2 via BH3 mimetics



Graphics created by student researcher in Canva, 2025.

Research Questions

Are *Mercenaria mercenaria* an effective alternative *in vivo* model for Bcl-2 protein pathways in blood cancers?

&

Does pairing ursolic acid and piperine effectively target Bcl-2 related pathways as a chemotherapeutic combination?

Methodology

Identifying DN in *M. Mercenaria*

Image taken by student researcher, 2024.

Commercially harvested clams examined for **>20% neoplastic cells**

Acute exposure *in vitro*

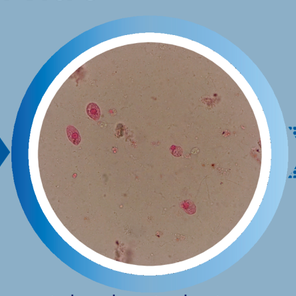


Image taken by student researcher, 2024.

Direct exposure to hemolymph samples for **60h**

Treatment effects *in vivo*

Image taken by student researcher, 2024.

50-day exposure to:
- AT101; Ursolic acid & piperine
- Cell viability, lipid & protein assays

Automated Image Processing

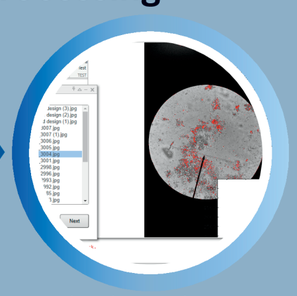


Image taken by student researcher, 2024.

Ilastik ML program trained on **280+ images** to count & classify cells

Sample Collection *in vivo*

Hole clipped into shell & labeled → Hemolymph fluid & muscle tissue samples extracted → ***in vivo* sample staining**

Erythrosin B: viability stain for hemolymph fluid

High cell viability

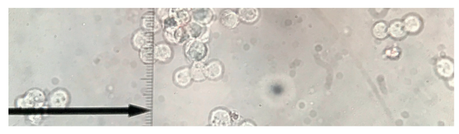


Image taken by student researcher, 2024.

Low cell viability

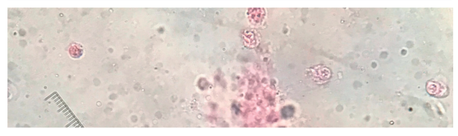


Image taken by student researcher, 2024.

Sudan III: lipid stain for wet tissue

Normal tissue

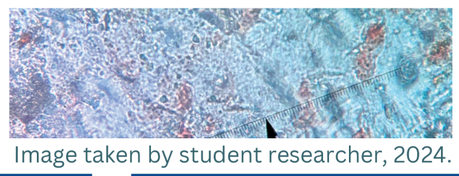


Image taken by student researcher, 2024.

Neoplastic tissue

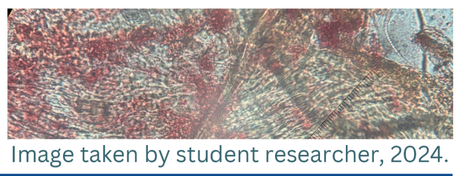
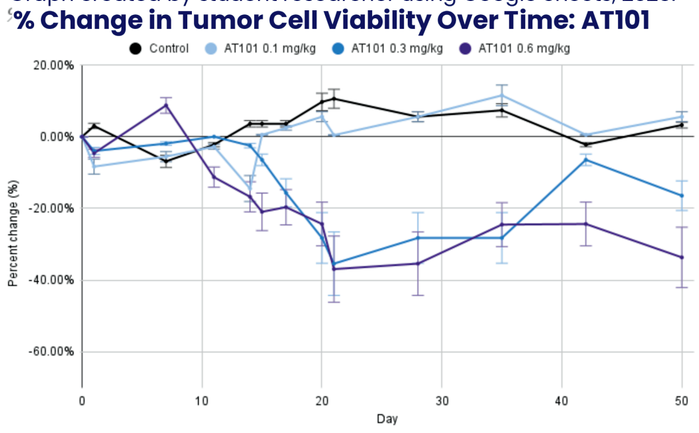


Image taken by student researcher, 2024.

Data Analysis & Results

Graph created by student researcher using Google Sheets, 2025.

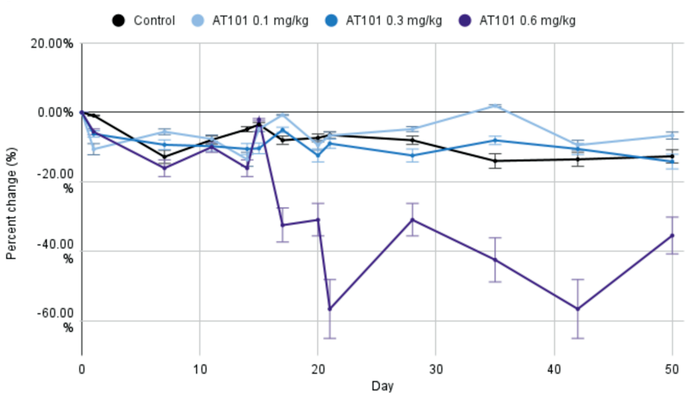
% Change in Tumor Cell Viability Over Time: AT101



Graph created by student researcher using Google Sheets, 2025.

Significant cancer cell viability decrease **(p<0.05)** in groups treated with AT101 at concentrations **≥0.3 mg/kg**

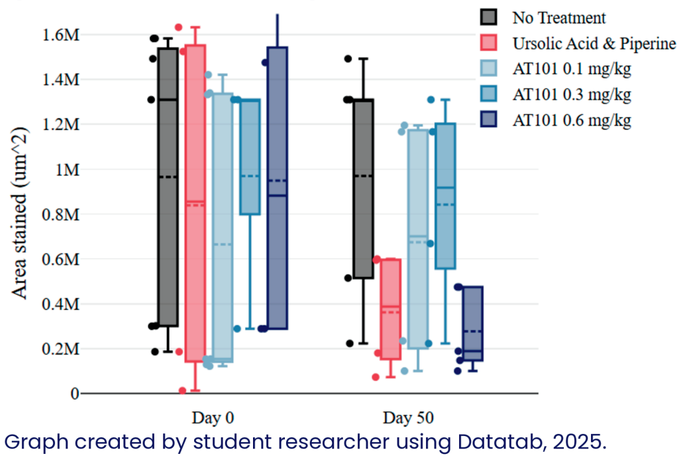
% Change in Normal Cell Viability Over Time: AT101



Decrease in normal cell viability for AT101 at **0.6 mg/kg**

Suggests **high** dose-dependent concordance **(p = 0.0476)**

Lipid Stained Area in Neoplastic Tissue (um^2)

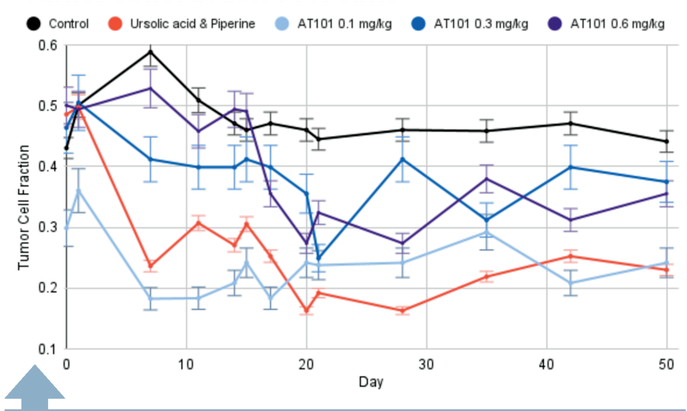


Graph created by student researcher using Datatab, 2025.

Significant reduction in lipid area in ursolic acid & piperine **(p=0.0102)** and AT101 at 0.6 mg/kg groups **(p=0.0022)**

Graph created by student researcher using Google Sheets, 2025.

Tumor Cell Fraction Over Time

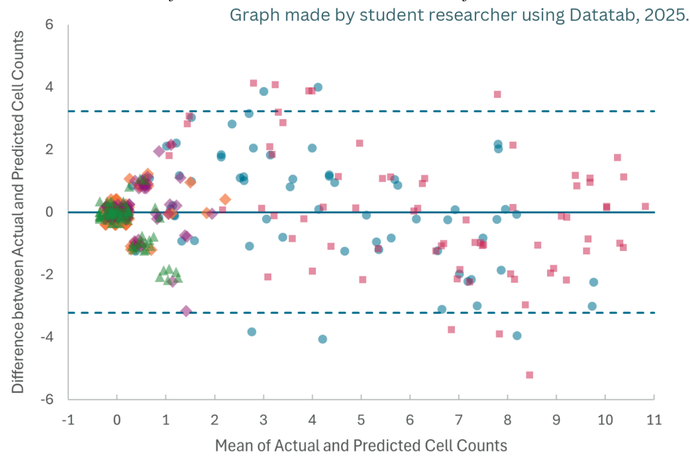


Combined treatment **slows tumor progression (p = 0.0195)**

Ilastik Automated Cell Identification

*Bland-Altman Plot for Actual vs. Predicted Cell Classification - Added Noise**

Graph made by student researcher using Datatab, 2025.



Accuracy	Precision	Recall
90.81	78.82	80.36

SDS-PAGE Protein Assay Two-tailed P-values

Comparing protein-lane staining ratio at 26 kDA between untreated and treated hemocytes

Treated cancerous clams vs. untreated control	**0.0209**
Healthy clams vs. untreated control	0.0613

Conclusions

Interpretation

AT101 at concentrations ≥0.3 mg/kg has **anticancer effects with moderate cytotoxicity**

Treatment with **ursolic acid + piperine** successfully **reduced tumor growth** and decreased lipid droplet production with **minimal cytotoxicity to normal cells**

Treatment responses in *M. mercenaria* occur at **very similar dosages** to humans

Conclusions

M. mercenaria shows strong potential as an **accurate & accessible leukemic model system**

Ursolic acid + piperine can be further explored as a **minimally toxic combination treatment**

Applications

Ursolic acid & Piperine as a Chemotherapeutic Combination
Pairing alongside conventional chemotherapies

Minimizing Vertebrate Testing
Can be utilized as an initial "screen" in drug discovery

Reducing Costs per Specimen
Commercially available, low maintenance & easily transmissible

Key References

Barber, B. J. (2004). Neoplastic diseases of commercially important marine bivalves. *Aquatic Living Resources*, *17*(4), 449–466. https://doi.org/10.1051/alr:2004052

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Wang, F.-Y., & Ching, T.-T. (2021). Oil Red O Staining for Lipid Content in Caenorhabditis elegans. *Bio-Protocol*, *11*(16), e4124. https://doi.org/10.21769/BioProtoc.4124

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