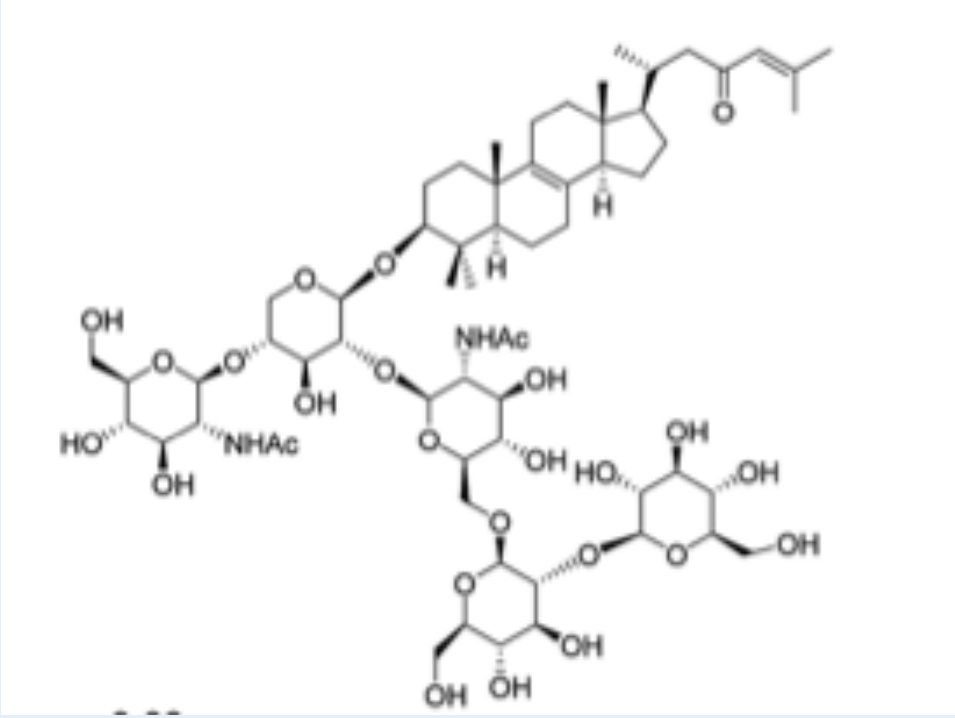
Sarasinoside A1 as a Potential Inhibitor of Chemoresistance



Jennifer Wildi
CAPS 200 Winter 2016

Acknowledgements: Dr. Cal Roskelley, Erin Bell, Dr. Heather Yule

A Critical Issue…

- Breast Cancer – 2nd most common cause of death due to cancer in women [1]
- 30% early-stage breast cancer patients – recurrent disease [1]
- Chemotherapy resistance – most important cause of chemotherapy failure [2]

Recent Findings…

- EMT of breast cancer cells induces chemoresistance [3,4]
- Majority of tumor cells remaining after chemotherapy have mesenchymal phenotype [3]
- Sarasinoside A1 (inhibitor of single-cell invasion) works by re-epithelializing the cells [5]

The Next Steps…

- EMT targeting – hopeful strategy to prevent chemoresistance [6]
- Sarasinoside A1 proved useful in EMT targeting in the context of single-cell invasion [5]
- Useful in the context of chemoresistance?

Question

From a phenotypic approach, does Sarasinoside A1 reduce resistance to Cyclophosphamide in mesenchymal breast cancer cells?

Hypothesis

H_0: No effect on chemoresistance

H_A: Reduces chemoresistance

Specific Aim

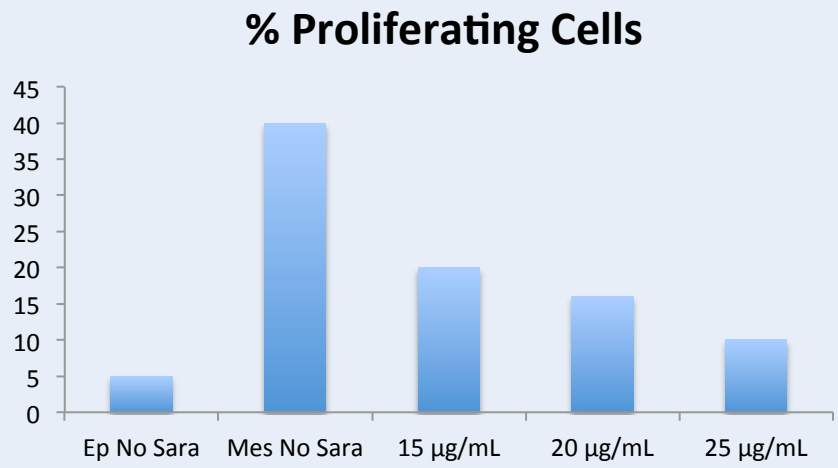
Determine if *in vitro* administration of Sarasinoside A1 prior to Mafosfamide treatment affects sensitivity of mesenchymal breast cancer cells to Mafosfamide

Preliminary Study

Goal: Determine appropriate dose of Mafosfamide.

- Doses of 2, 5, or 10 µg/mL Mafosfamide on monolayer culture of MCF-7 cells. [7,8]
- Measure cell death – Annexin V Detection Protocol. [9]
- Optimal therapeutic dose from death curve.

Methods



- **5 conditions**
  -MCF-7 cells alone
  -MDA-MB-231 cells alone
  -MDA-MB-231 cells with 15, 20, and 25 µg/mL Sarasinoside A1
- **Mafosfamide added** 18 hours post Sarasinoside treatment [5]
- **Chemosensitivity tested** 72 hours post Mafosfamide treatment [7]

  -Proliferation: Ki-67 detection with Ki-67 antibody [10]
  -Apoptosis: Annexin V Detection Protocol [9]
  -Senescence: β-Galactosidase Assay [11]

Expected Outcomes

- In conditions with Sarasinoside A1,
  -Proliferation ↓
  -Senescence ↑
  -Apoptosis ↑

- Why? Re-epithelialization expected to increase chemosensitivity

Significance

- Potential to be administered in conjunction with current chemotherapies
- Forward step in solving problem of recurrent tumors

Future Directions

- Testing for induced collective invasion [5]
- Testing with other chemotherapeutic drugs

References

1. Gonzalez-Angulo, A.M., Morales-Vasquez, F., Hortobagyi, G.N. Overview of resistance to systemic therapy in patients with breast cancer. *Breast Cancer Chemosensitivity*. Springer New York, 1-22.2 (2007)
2. Chen, T. Overcoming drug resistance by regulating nuclear receptors. *Adv Drug Deliv Rev* **62** 1257-1264 (2010)
3. Fischer, K.R., *et al*. Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. *Nature* **527** 472-476 (2015)
4. Zheng X., *et al.* Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer. *Nature* **527** 525-530 (2015)
5. Austin, P., *et al.* The Invasion Inhibitor Sarasinoside A1 Reverses Mesenchymal Tumor Transformation in an E-Cadherin–Independent Manner. *Mol Cancer Res* **11**, 530-540 (2013)
6. Qi, X., Zhang, L., Lu, X. New Insights into the Epithelial-to-Mesenchymal Transition in Cancer. *Trends pharmacol sci* (2016)
7. Goldstein, M., Roos, W.P., Kaina, B. Apoptotic death induced by the cyclophosphamide analogue mafosfamide in human lymphoblastoid cells: contribution of DNA replication, transcription inhibition and Chk/p53 signaling. *Toxicol Appl pharmacol* **229** 20-32 (2008)
8. Graves, M.L., *et al*. The cell surface mucin podocalyxin regulates collective breast tumor budding. *Breast Cancer Res* **18**, (2016)
9. Hingorani, R., Deng, J., Elia, J., McIntyre, C., Mittar, D. Detection of apoptosis using the BD Annexin V FITC Assay on the BD FACSVerse™ system. BD Biosciences (2011)
10. Smith, C. Cell Proliferation Assays: Methods For Measuring Dividing Cells. http://www.biocompare.com/Editorial-Articles/117892-Cell-Proliferation-Assays/ (2012)
11. De Jesus, B.B., Blasco, M.A. Assessing cell and organ senescence biomarkers. *Circ res* **111** 97-109 (2012)
12. Chorawala, M.R., Oza, P.M., Shah, G.B. Mechanisms of anticancer drugs resistance: an overview. *Int J Pharm Sci Drug Res* **4** 1-9 (2012)