Effect of *Cornus officinalis* (CO) on a Model for Triple Negative Breast Cancer

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Study Rationale-I

- The triple negative breast cancer (TNBC) lacks estrogen receptor-α (ER-α), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2) expression (1).

- The treatment of choice for TNBC includes cytotoxic Anthracyclin/Taxol based chemotherapy or selective PARP, PI3K and mTOR small molecule inhibitor based targeted therapy (2, 3).

- The tumor suppressive function of RB via the Cyclin D-CDK4/6-p-RB pathway is compromised in therapy resistant basal-like and TNBC molecular subtypes (4, 5).

- The conventional treatment options for TNBC are associated with long-term systemic toxicity, acquired tumor resistance and emergence of drug resistant cancer stem cells, compromising therapeutic efficacy (5, 6).

- Minimally toxic natural phytochemicals and herbal extracts may represent testable alternative for therapeutic efficacy of TNBC (7).

Study Rationale-II

- Nutritional herbs represent treatment of choice in traditional Chinese medicine for breast cancer (8, 9).

- Non-fractionated aqueous extracts from several mechanistically distinct nutritional herbs have documented growth inhibitory efficacy in a model for the Luminal A molecular subtype of clinical breast cancer (10, 11).

- Non-fractionated aqueous extract from *Cornus officinalis* (CO) exhibit growth inhibitory efficacy via down-regulation of proliferation specific genes, up-regulation of apoptosis specific genes and modulation of cellular metabolism of 17β-estradiol, generating anti-proliferative metabolites (12).

Study Objectives

- To examine the growth inhibitory efficacy of *Cornus officinalis* (CO) in breast carcinoma-derived MDA-MB-231 cells representing a model for triple negative molecular subtype of clinical breast cancer.

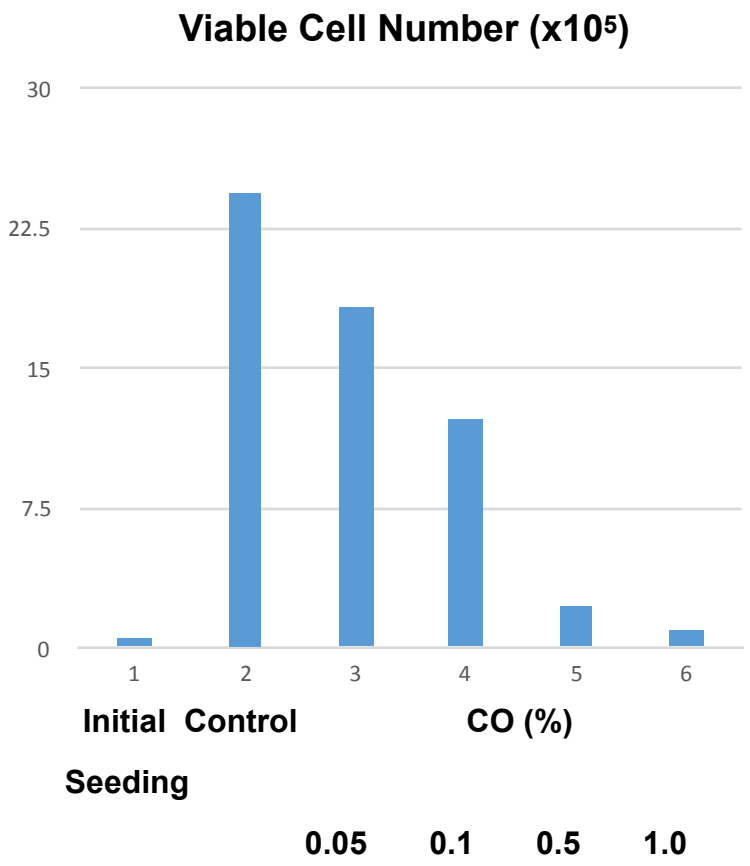
- To identify mechanistic leads and potential molecular targets for the efficacy of CO.

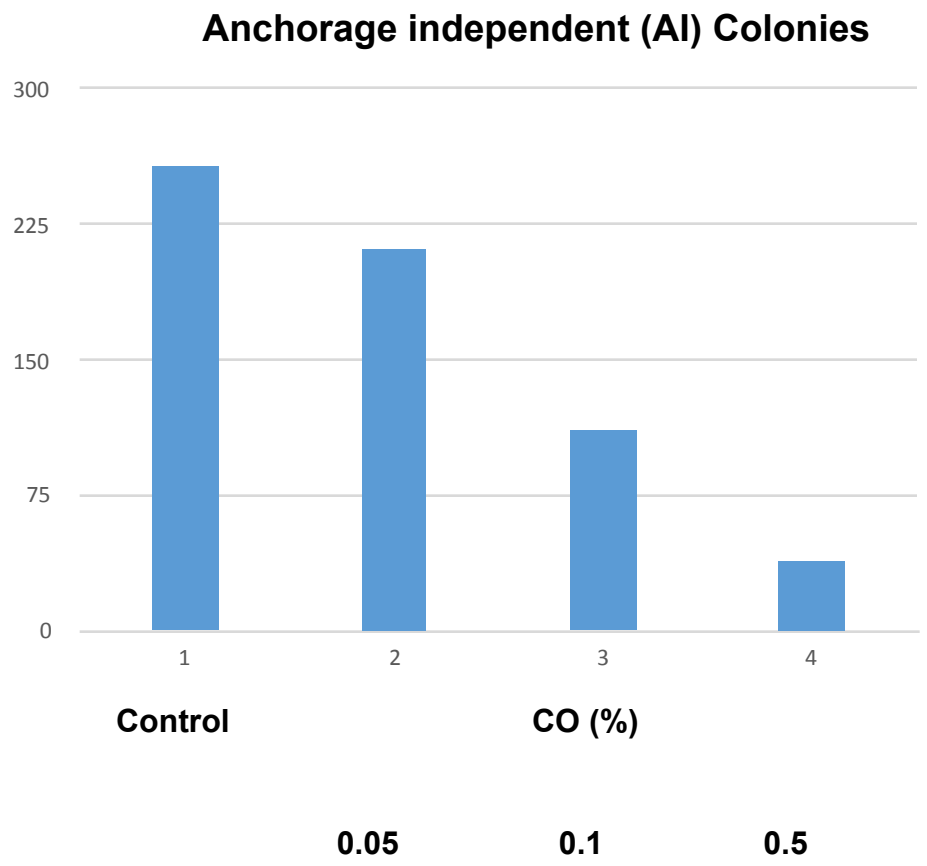
Characterization of Triple Negative MDA-MB-231 Cells

End Point Biomarker	Triple Negative Breast Cancer (TNBC) Model MDA-MB-231[a]
Population Doubling (hr)	15.0±2.2
Saturation Density ($x10^5$)	32.9±2.3
G_1: $S+G_2/M$ Ratio	0.6±0.3
Anchorage independent (AI) Colonies	257±57
Tumor Development (Incidence)	10/10
Tumor Latency (weeks)	3-5

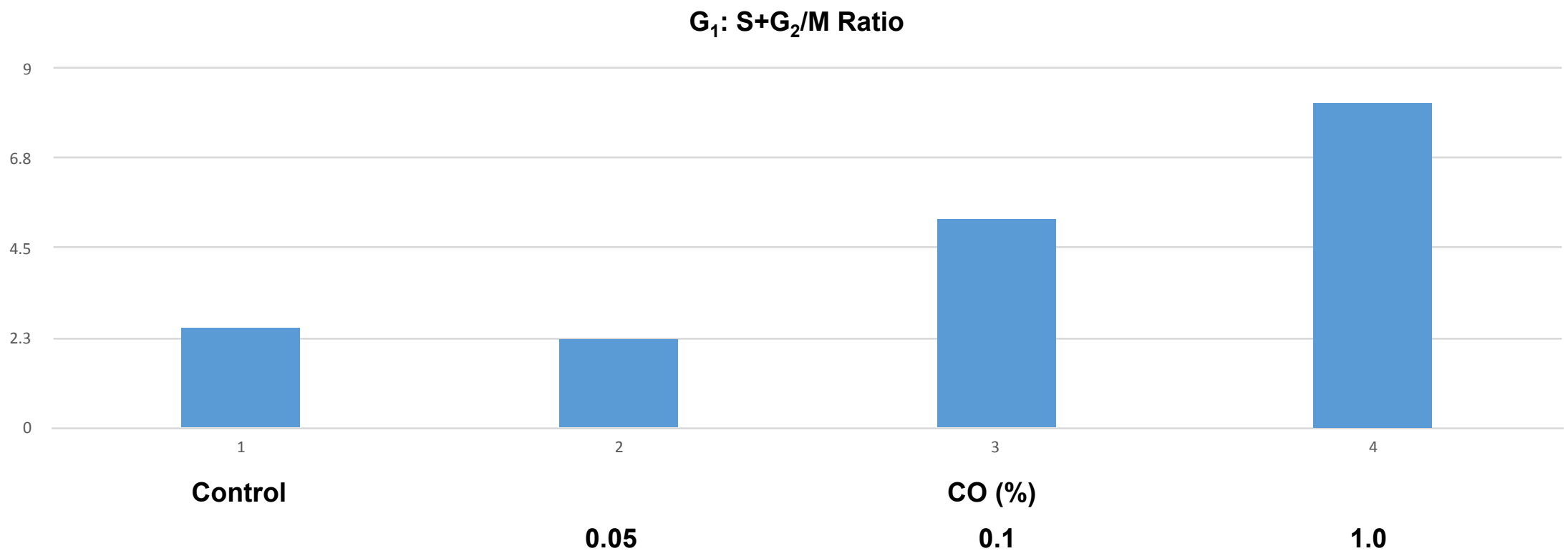
[a] ER-α negative, PR negative, HER-2 negative

Dose Response of *Cornus officinalis* (CO)

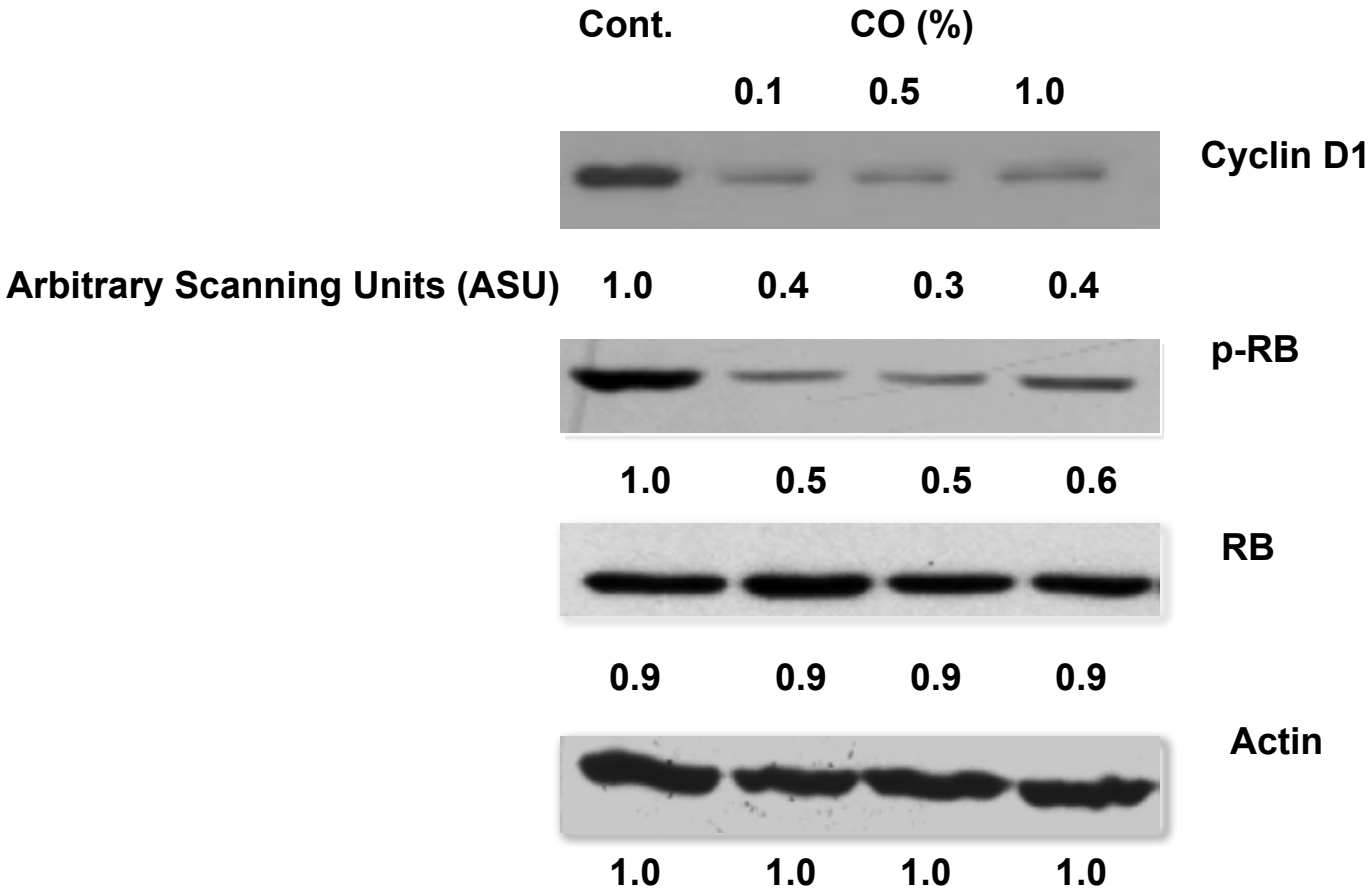




Effect of *Cornus officinalis* (CO) on Cell Cycle Progression

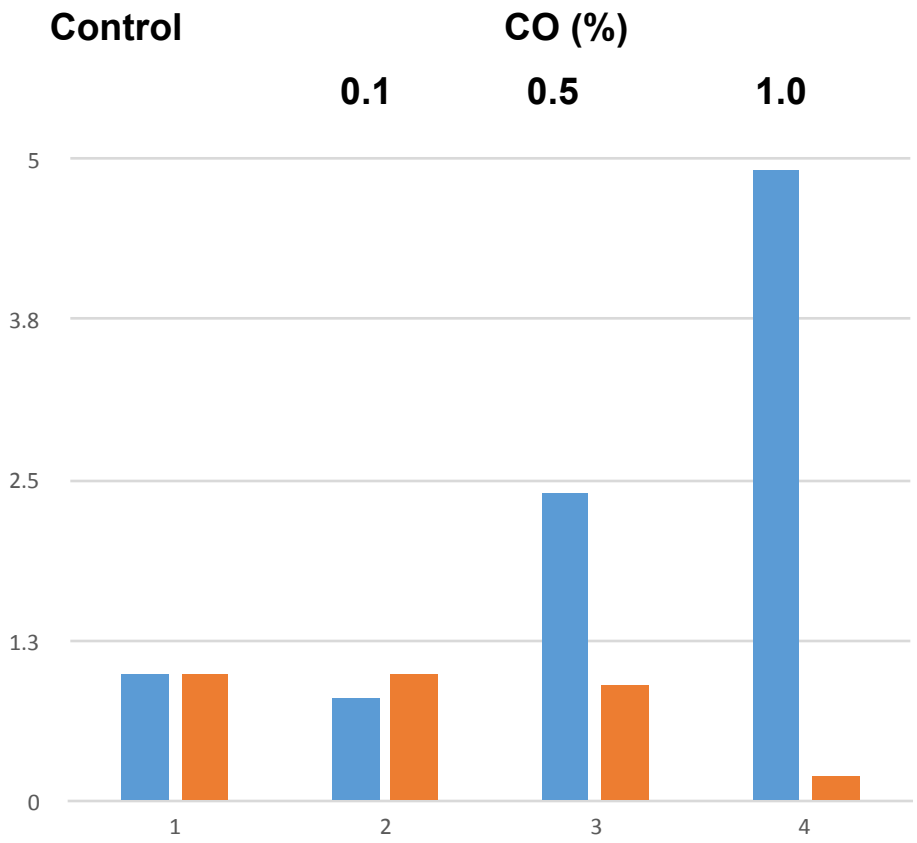


Status of Cell Cycle Regulatory Proteins



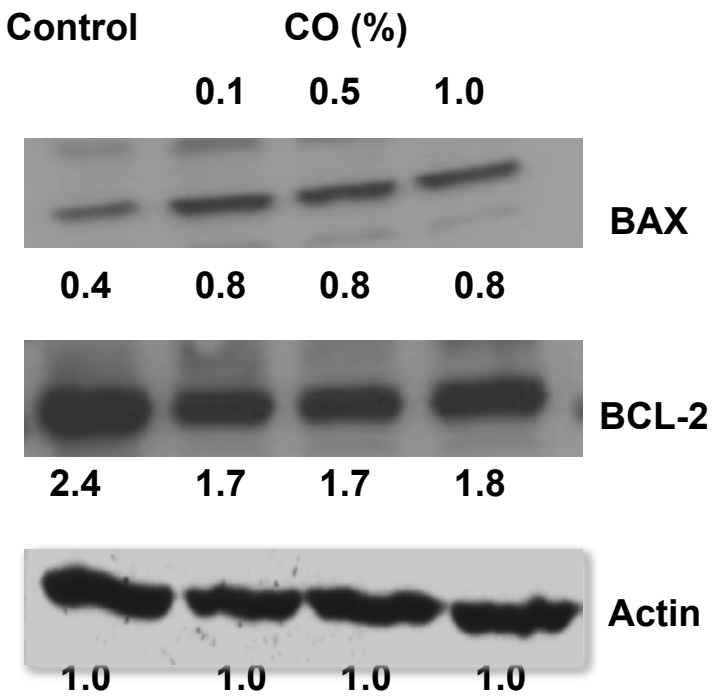
Status of Apoptosis Specific Genes

Relative Gene Expression (σσ Ct)

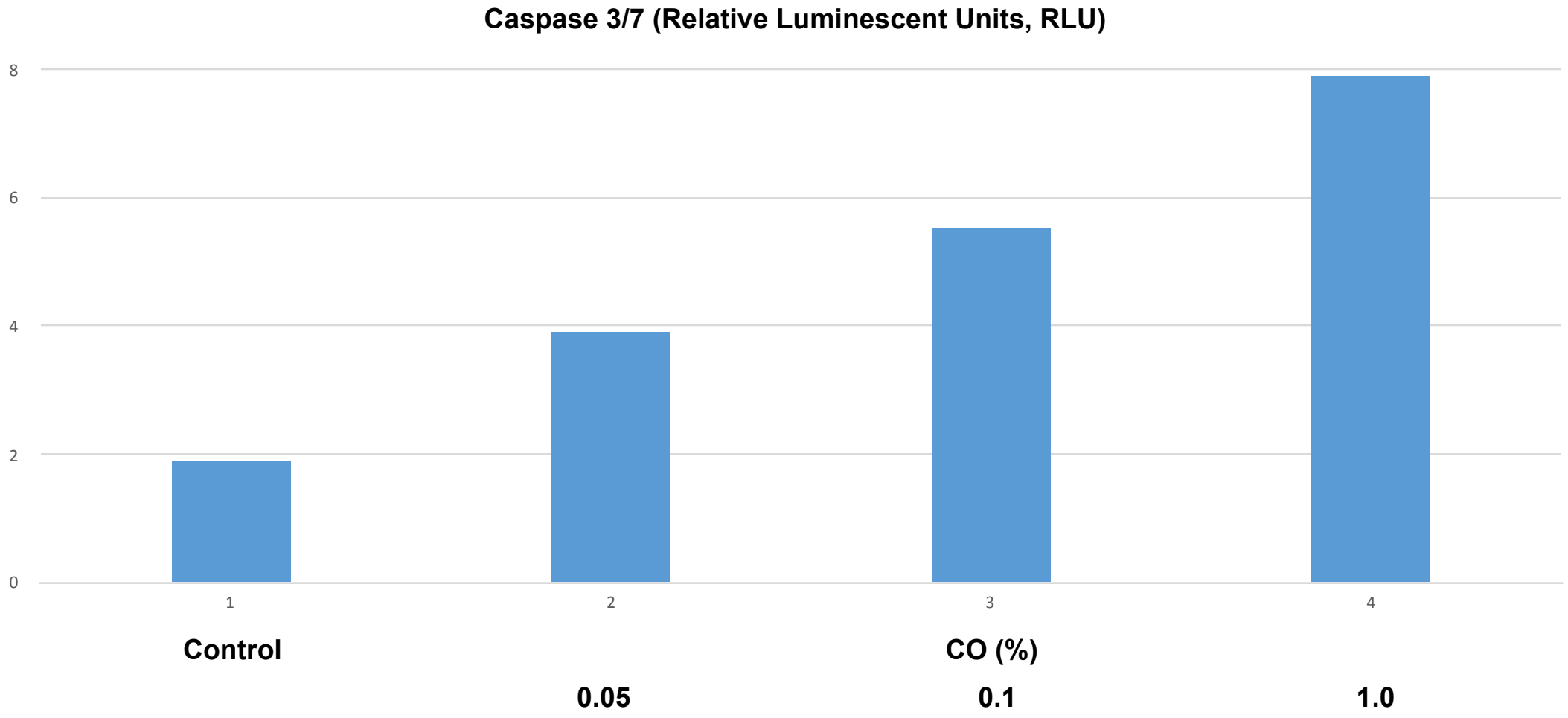


Blue: BAX, Orange: BCL-2

Protein Expression (Arbitrary Scanning units, ASU)



Status of Caspase Activity



Study Outcome-I

Dose response of *Cornus officinalis* (CO)

- Non-fractionated aqueous extract from CO exhibited a dose dependent growth inhibition of MDA-MB-231 cells. within the cytostatic range (IC_{25} : 0.05%, IC_{50} : 0.1% and IC_{90} : 0.5%).

- CO treatment at a concentration of > 1.0% induced a cytotoxic response.

- CO treatment induced a dose dependent reduction in the number of anchorage independent (AI) colonies.

Study Outcome-II

Effect of *Cornus officinalis* (CO) Cell Cycle Progression and Cellular Apoptosis

- Treatment with CO inhibited G_1 to S phase transition in a dose dependent manner, leading to progressive increase in the G_1: $S+G_2/M$ ratio.

- Treatment with CO inhibited Cyclin D1 and p-RB expression, modulating the RB pathway.

- Treatment with CO modulated the apoptosis specific intrinsic BAX-BCL-2 pathway, and induced pro-apoptotic Caspase 3/7 activity.

Study Conclusions

- *Cornus officinalis* (CO) inhibits anchorage dependent growth and anchorage independent colony formation of MDA-MB-231 cells.

- CO mediated inhibition of cell cycle progression is associated with modulation of the RB pathway.

- CO mediated pro-apoptotic effects are associated with modulation of the BAX-BCL-2 pathway and induction of Caspase activity.

- These data provide mechanistic leads for efficacy of CO and thereby, validate an experimental approach to prioritize efficacious herbal extracts for treatment of triple negative breast cancer.

Acknowledgements and Dedication

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This study is dedicated to the memory of Laurie Mezzalingua (1968-2009). During this period Laurie selflessly and generously devoted herself to helping many others suffering from breast cancer.

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