



# The Effect of Cigarette Smoke Condensate on Acute Myeloid Leukemia

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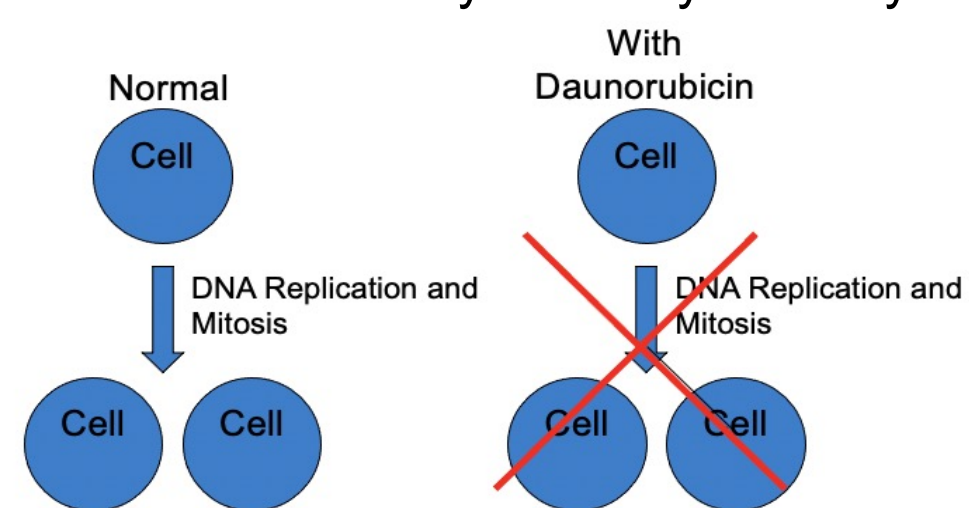
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## Introduction

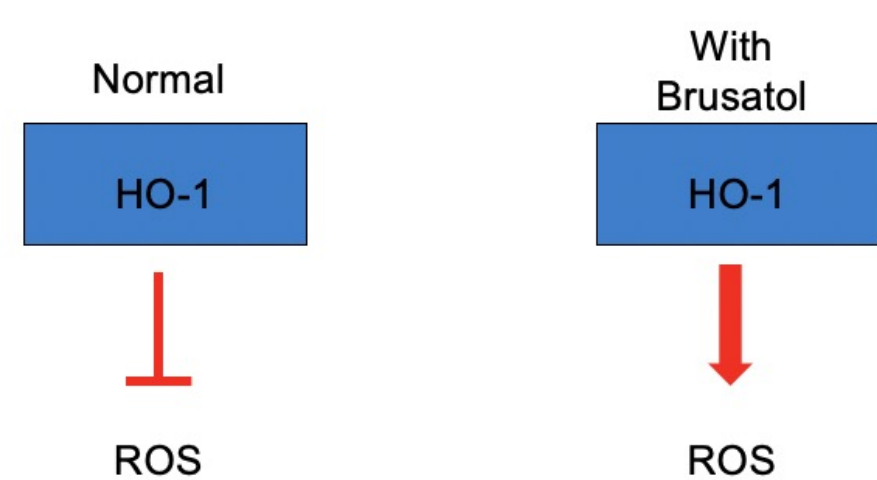
- Modifiable behaviors, such as smoking, can increase cancer risk; data shows that smoking influences survival in acute myeloid leukemia (AML) patients.
- Little is known about the mechanism by which smoking impacts cancer treatment or the expression of proteins that regulate redox reactions, such as Heme Oxygenase-1 (HO-1).
- We hypothesize that cigarette smoke condensate (CSC) treated leukemia cells would increase HO-1 expression due to increased reactive oxygen species (ROS), and lead to resistance to treatments which could be reversed by an HO-1 inhibitor.

## Materials and Methods

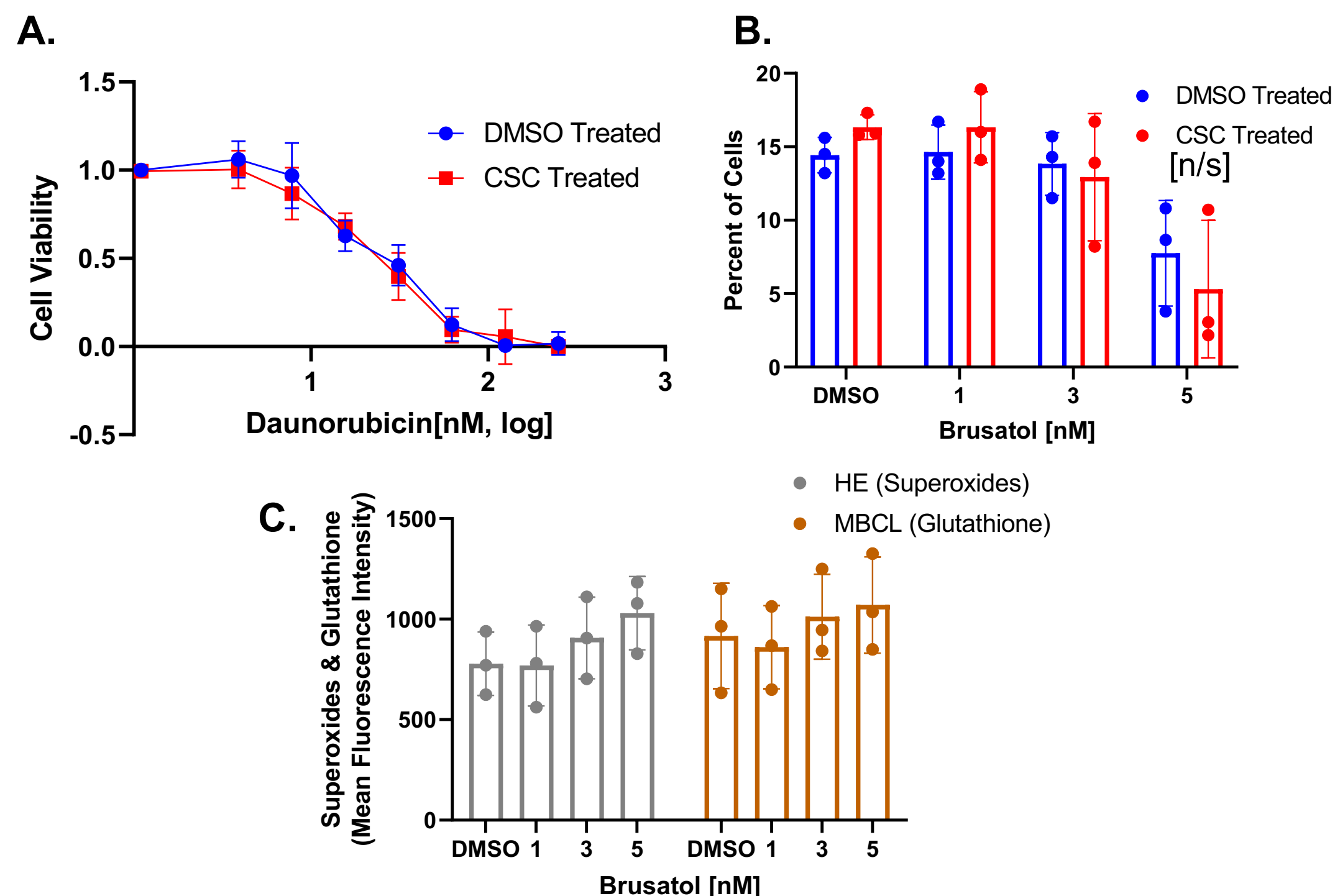
1. Treated human AML MOLM13 and MOLM14 with CSC to chemically mimic cigarette smoke exposure or with dimethyl sulfoxide (DMSO) as the control for two weeks.
2. Visualized cell proliferation from Daunorubicin treated cells with an alamarBlue assay. PI staining for Brusatol to analyze cell cytotoxicity.



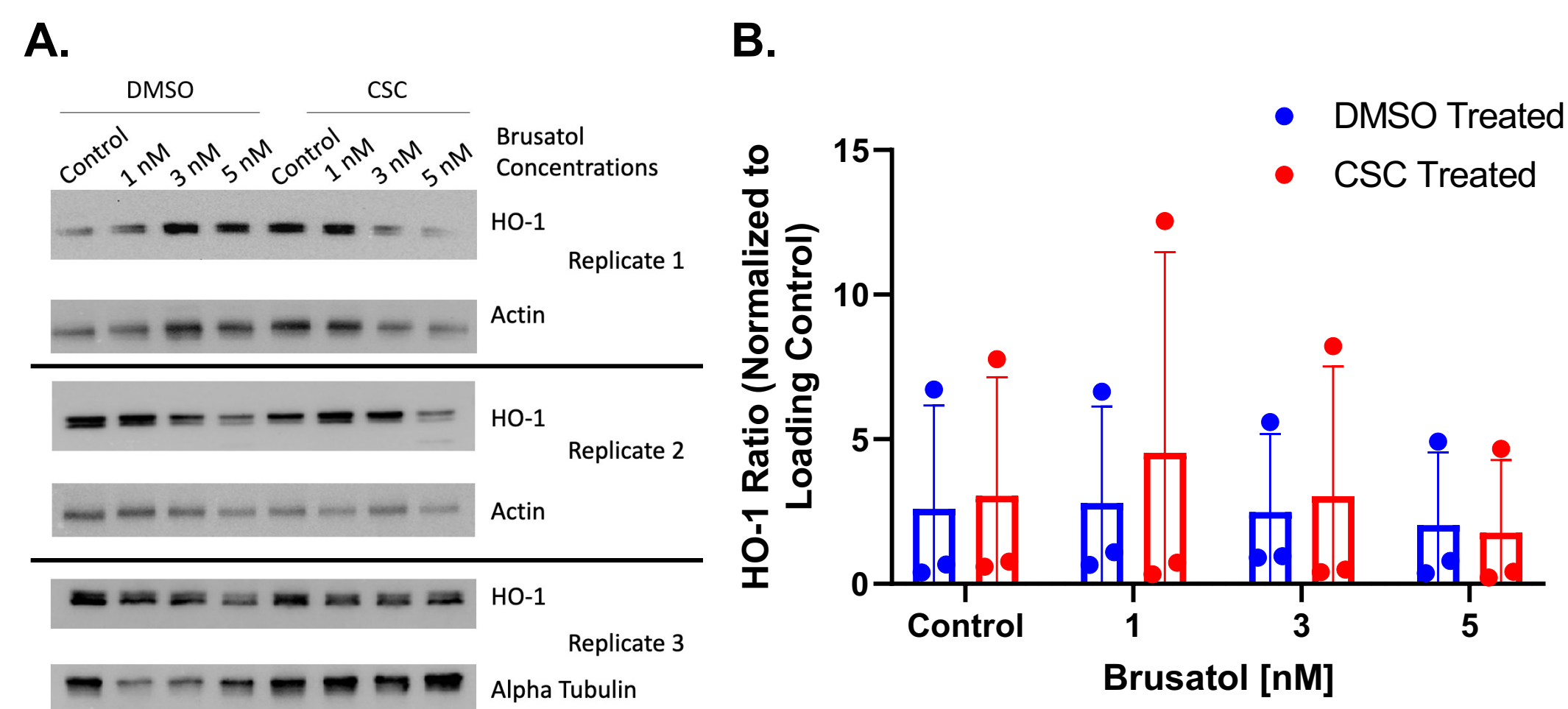
3. Assessed HO-1 expression with Brusatol treated cells by western blot analysis and analyzed superoxide (ROS) and antioxidant (glutathione) levels with flow cytometry.



## Results



**Fig. 1. Effect of Daunorubicin and Brusatol on Cell Viability, ROS Expression, and Antioxidant Activity.** (1A.) Treated 50,000 CSC and DMSO MOLM14 cells with Daunorubicin ranging from 250 nM to 3.9 nM. Three days later, added 10X alamarBlue to create a 1X concentration of alamarBlue. Viability was normalized to the control wells. No statistically significant changes in cell proliferation between DMSO and CSC treated AML cells were seen (N=3, P=0.05). (1B.) Treated CSC and DMSO MOLM14 cells with Brusatol. Little cell death at low concentrations according to PI staining analysis. (1C.) There is a trend of increased ROS and antioxidant activity, although the results are insignificant. n/s: no statistical significance



**Fig. 2. Effect of Brusatol on HO-1 Expression.** Treated CSC and DMSO MOLM14 cells with Brusatol. Resuspended Brusatol in DMSO. Added DMSO to the control wells. Three days later, made cell lysates and analyzed HO-1 expression with a loading control (actin or alpha tubulin) by western blot analysis (2A., 2B.). Although the results are statistically insignificant, two of the three replicates showed that HO-1 expression increased in CSC treated cells and one showed a decrease in HO-1 expression in CSC treated cells with Brusatol (N=3, P=0.05). Found a trend of increased HO-1 with Brusatol treatment in CSC.

## Conclusions

- Despite previous *in vivo* experiments indicating enhanced proliferation with cigarette smoke exposure, this was not the case for our *in vitro* experiments.
- Our setting does not encompass the tumor microenvironment, which may be contributing to AML growth from smoking, so it may be necessary to use *in vivo* models to mimic human smoking.
- Brusatol seems to modulate HO-1 expression at low concentrations that do not cause cell death.

## Future Directions

- We will test the modulation of CSC responses to both Daunorubicin and Brusatol on cell viability in AML cells as a potential treatment option.

## Acknowledgements

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## References

- 1) Figueroa, Mary and Chandra, Joya. Cellular Redox Status and Modifiable Behaviors. *Springer Nature Singapore Pte Ltd*, 2021.