

# Chill out! The impact of stress hormones on cancer cells *in vitro*

## Discussion

### Outcome

- In this project our overall results did not accept our hypothesis
- We decided that our results were inconclusive due to the errors listed

### Errors

- Not checking confluency before splitting cells so we didn't know how many cells were present in each well
- Not mixing the reagent so not every cell was introduced to norepinephrine equally when adding it to the 96 well
- It was done in vitro vs in vivo and we weren't sure how much norepinephrine to add. In vivo the body has high levels of norepinephrine and translating to in vitro we have to do a higher concentration than what would have been in congruence with in the body in order to pipette
- Our cells were different between the first trial with the last 3 trials which is an error because they were later in passage

### Future experiments

- Verifying that confluency is the same through each experiment using a hemacytometer and adding trypan blue to the cells
- Mixing in the reagent using a multipipet after it has been added to assure all cells are introduced to the reagent
- Not allowing our cells to overgrow their environments and die by keeping a more careful watch on the cells

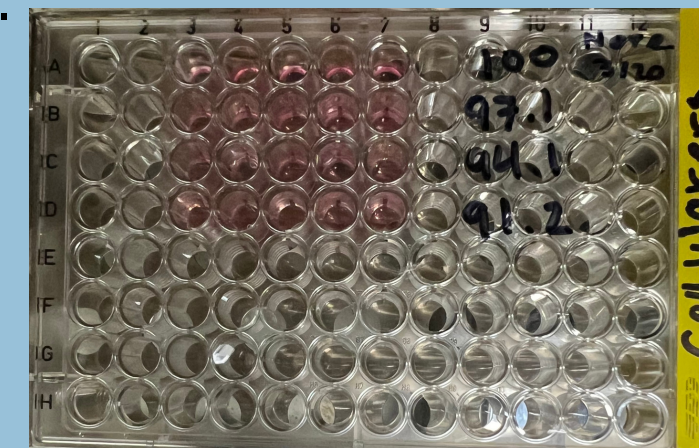
## References

1. Chen, Guoqiang, et al. "Stress Hormones: Emerging Targets in Gynecological Cancer." *Frontiers in Cell and Developmental Biology*, vol. 9, no. 699487, Summer 2021, Frontiers, doi: 10.3389/fcell.2021.699487.
2. Jaber, Nadia. "Study Suggests a Link between Stress and Cancer Coming Back." *National Cancer Institute*, 12 Jan. 2021, Study Suggests Link Between Stress, Cancer Returning - NCI.
3. N, A. "Cancer Statistics." *National Cancer Institute*, N.A., *Cancer Statistics - NCI*.
4. N.A. "Catecholamines - Blood." *ucsfhealth.org*, 6 Oct. 2020, Catecholamine blood test ([ucsfhealth.org](http://ucsfhealth.org)).
5. N, A. "Key Statistics for Cervical Cancer." *American Cancer Society*, 12 Jan. 2023, *Cervical Cancer Statistics | Key Facts About Cervical Cancer*.
6. Patterson, Eric. "Stress Facts and Statistics." *The Recovery Village Drug and Alcohol Rehab*, 5 Sept. 2022, *Important Facts and Statistics About Stress: Prevalence, Impact, & More* ([therecoveryvillage.com](http://therecoveryvillage.com)).

## Methods

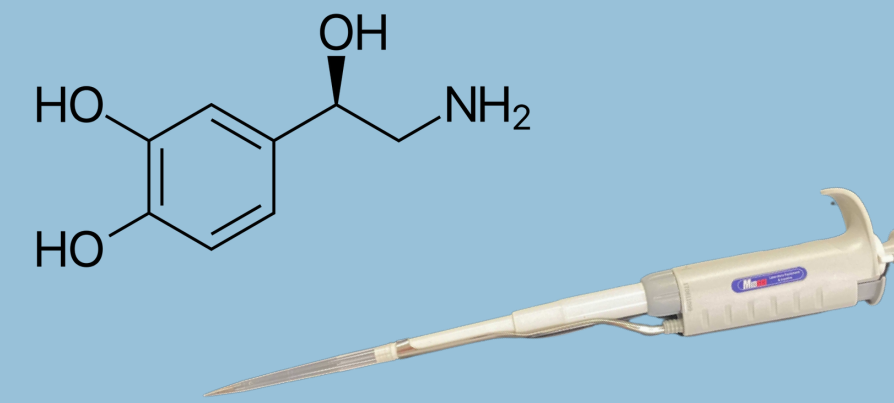
### Plating:

- With the remaining cell mixture after splitting, take a 96-well plate and add the following amounts of mixture to five wells each using a 200µl multi-pipet and 20µl pipet: 100µl (control), 97.1µl, 94.1µl, and 91.2µl.
- Incubate 96-well plate at 37C for 24hrs.



### Adding Reagent:

- Using a 10µl pipet add 2.9µl, 5.9µl, and 8.8µl of norepinephrine to the wells with 97.1µl, 94.1µl, and 91.2µl cell mixture respectively.
- Mix the reagent and cells using a 10µl pipet.
- Incubate 96-well plate at 37C for 24hrs.

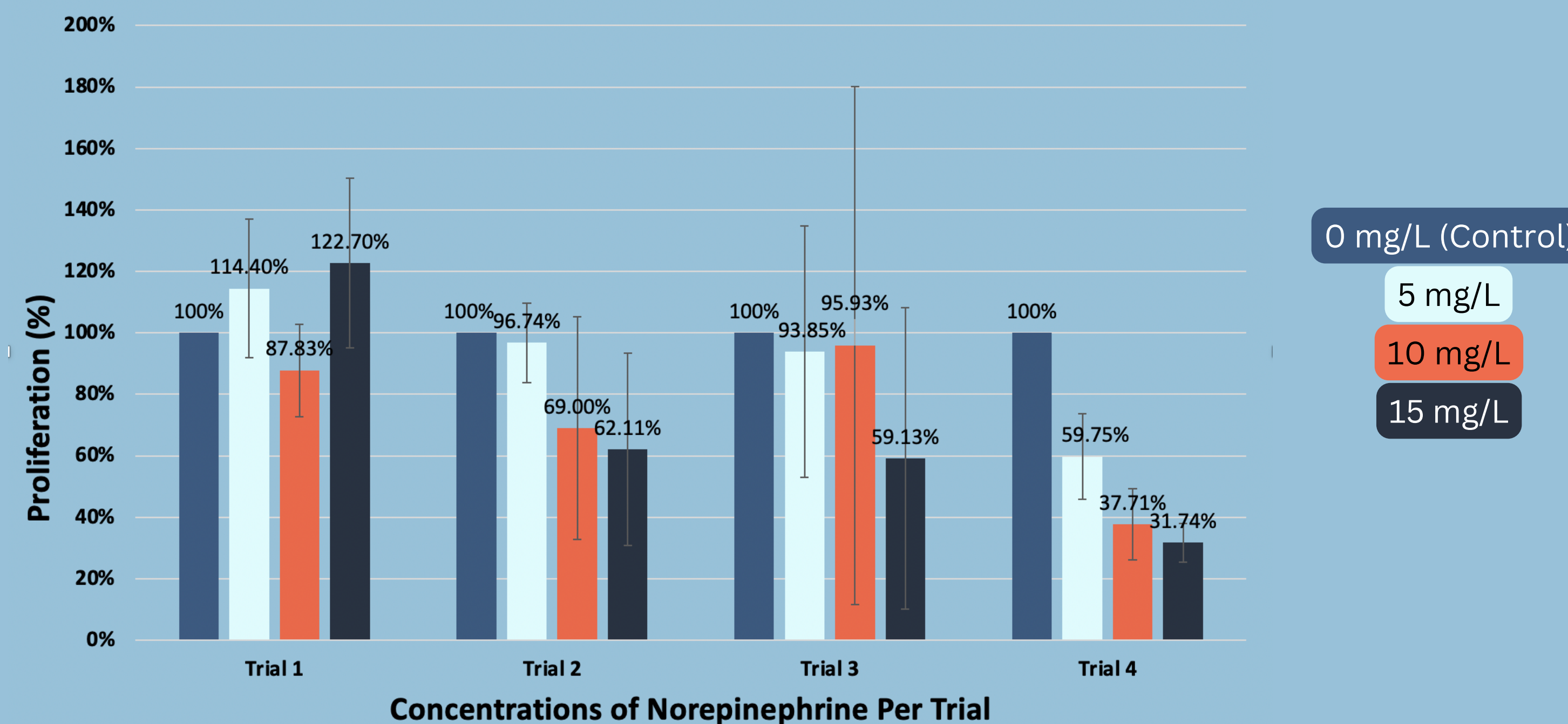


### Cytotoxicity Assay:

- Removed the media using a 200µl multi-pipet.
- Wash cells with 200µl sterile PBS and removed it.
- Added 100µl fresh medium and 20µl CellTiter to each well
- Incubate it at 37C for 1hr.
- Using the microplate reader, read the absorbance at 490nm.



## Results



**Figure 1: Proliferation of HeLa cells after administration of norepinephrine.** Our results gave mixed conclusions in regard to the impact of norepinephrine on HeLa cells in vitro. Trial 1 exhibited a higher proliferation of HeLa cells, whereas Trials 2, 3, and 4 exhibited a decline in cell proliferation

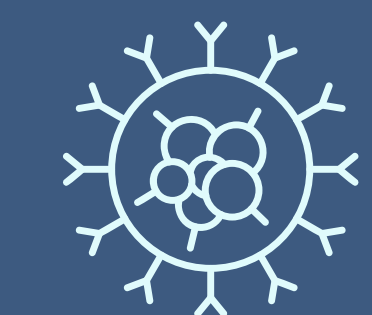
- While trials 2, 3, and 4 had a consistent trend, our results are technically inconclusive due to the possible experimental errors
- Overall, the trend exhibited by trials 2, 3 and 4 show a decrease in the proliferation of HeLa cells when introduced to norepinephrine in vitro
- This trend exhibits conflict with both our hypothesis and the research done in vivo

## Abstract

Norepinephrine, the neurotransmitter responsible for stress response, is known to increase the life span of cancer cells in the human body. By administering norepinephrine directly to HeLa cells in vitro, we were able to track the impact that this neurotransmitter has on the proliferation cancer cells. In our study, we found that norepinephrine actually had a negative impact on the proliferation of HeLa cells in vitro, but our results are deemed inconclusive due to possible experimental errors.

## Background

- Approximately 33% of people have high stress [6], and there is a direct relationship between stress and tumor growth in the human body [2]
- In regards to cervical cancer, the origin of the immortal HeLa cell line, the American Cancer Society estimated that 13,960 new cases will be diagnosed in 2023 [5]
- Norepinephrine is the main chemical contributor to stress, and is released in panic attacks between levels of 70 to 1700 pg/mL. [4]
- HeLa cells are known to have norepinephrine receptors [1], and cancer cells are known to proliferate in the presence of norepinephrine[2]



## Hypothesis

Based on our research, if we administer norepinephrine to HeLa cells, there should be an increase in the cancer cell's proliferation after 24 hours