Investigating the combined effects of natural killer cell enhancement and platelet inhibition on cancer metastasis rates

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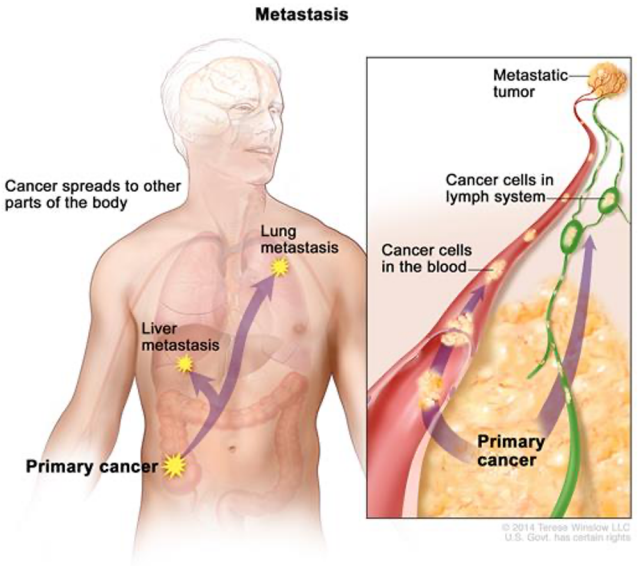
Introduction to Metastasis

- Metastasis is the leading cause of treatment failure in cancer treatments [1]
- There is currently no effective treatment option available for individuals with this advanced stage of the disease [2]



[3]

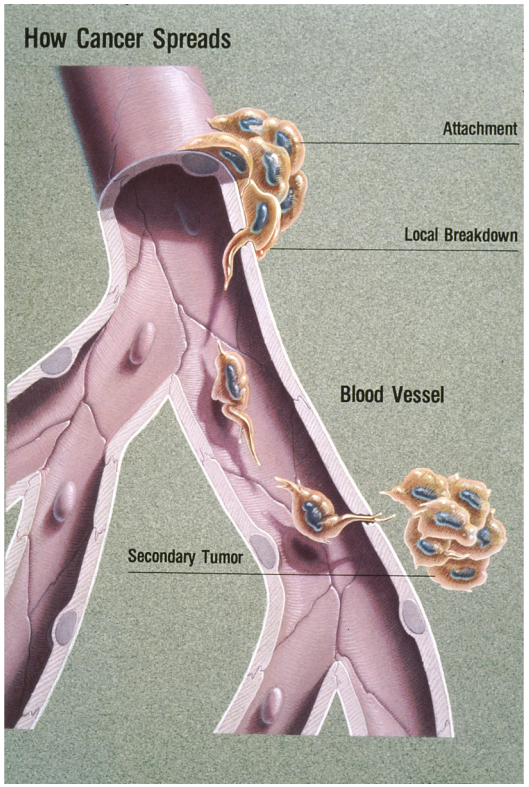
90%
of cancer deaths are a result of **metastatic disease**.

[4]

[1] C. N. Qian, *et al.*, 2017. [2] M. Iizumi, *et al.*, 2008. [3] American Association for Cancer Research, 2015. [4] National Institutes of Health, 2020.

Mechanism of Metastasis

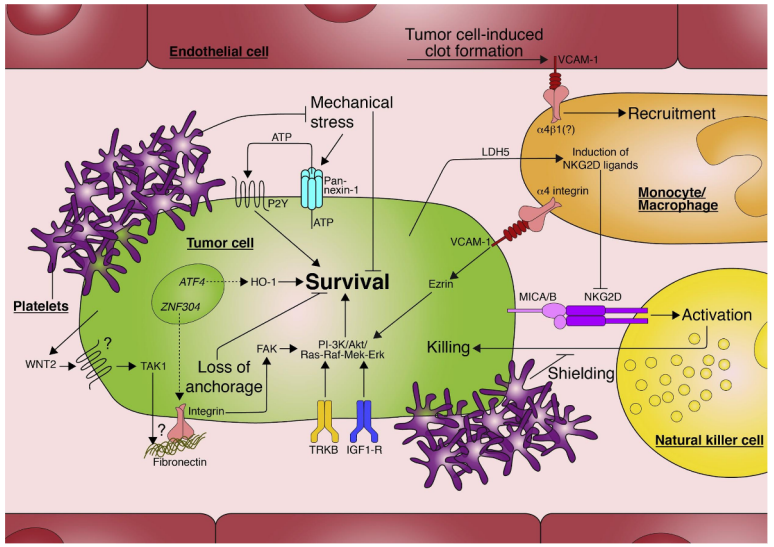
- The majority of metastasis occurs through the bloodstream [5]
- Extravasation of cancer cells into neighbouring tissues is the rate-limiting step of the process [6]



[5] B. Strilic, *et al.*, 2017.
[6] Proceedings of the National Academy of Sciences of the United States of America, 2000.

Metastasis Prevention Studies

- Targeting cancer cells in the bloodstream: activate NK cells [7], inhibit platelet activation [8][9]
- Combination therapy involving the two strategies has yet to be researched [5]



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[5] B. Strilic, *et al.*, 2017. [8] J. S. Miller *et al.*, 2005.
[8] M. Z. Wojtukiewicz, *et al.*, 2017. [9] A. L. Papa *et al.*, 2019.

Hypothesis

Given that the inhibition of platelet activation and enhancement of natural killer cell cytotoxicity have been shown to negatively affect cancer cell growth and metastasis, it is predicted that

inducing both effects will result in a greater tumour cell response than either strategy alone.

Hypothesis

Specifically, it is hypothesized that the addition of interleukin-2 and Aspirin to a culture of KHYG-1 NK cells, MDA-MB-468 breast cancer cells and platelets will result in

decreased MDA-MB-468 cell survival over a predetermined period of time, when compared to the effects of interleukin-2 or Aspirin alone.

Specific Aims/Objectives

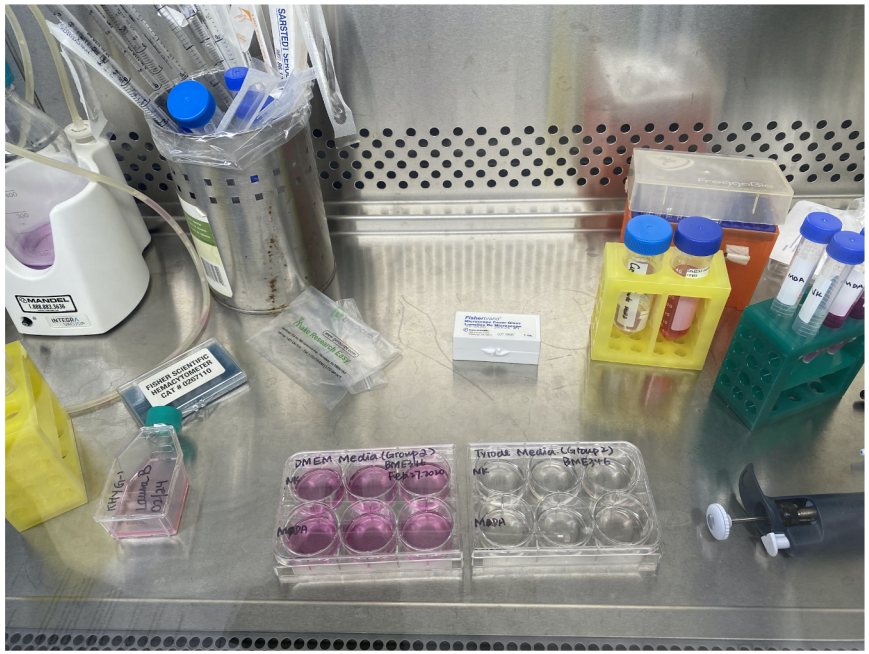
1) Determine a **method for co-culturing** platelets, cancer cells and natural killer cells in a compatible culture medium

2) Determine the **length of time** over which the effects of interleukin-2 and Aspirin occur after being added to the cell culture

3) Determine the **mechanism** by which cancer cell survival is primarily affected

Methods

- Assembly of the culture media
- Experimental design
- Co-culturing
- Experimental techniques
- Experimental plan



Methods - Assembly of Culture Media

- Require a media that does not cause platelet activation (no calcium)
- Assembled a modified Tyrode's buffer solution without $CaCl_2$ and added HEPES buffer, according to literature [9][10]

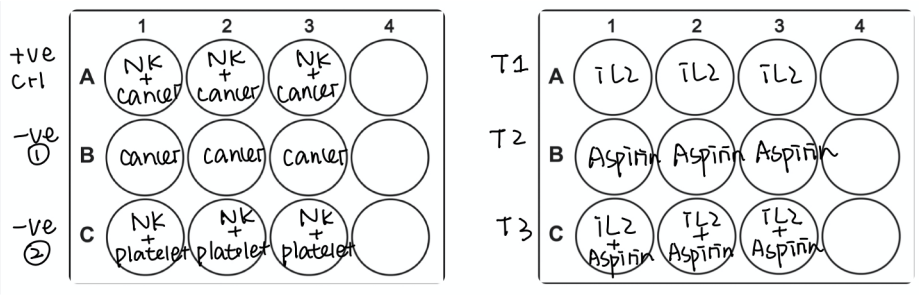
NaCl	137 mM
KCl	2.7 mM
MgCl	1.0 mM
2HPO4	0.2 mM
NaHCO3	12 mM
Glucose	5.5 mM
HEPES	25 mM

[9] A. L. Papa *et al.*, 2019. [10] www.researchgate.net, 2020.

Methods - Experiment Design

Positive Control	Negative Control 1	Negative Control 2	Test Case 1	Test Case 2	Test Case 3
KHYG-1 NK MDA-MB-468	MDA-MB-468	KHYG-1 NK MDA-MB-468 Platelets	KHYG-1 NK MDA-MB-468 Platelets IL-2	KHYG-1 NK MDA-MB-468 Platelets Aspirin	KHYG-1 NK MDA-MB-468 Platelets IL-2 Aspirin

Methods - Culturing

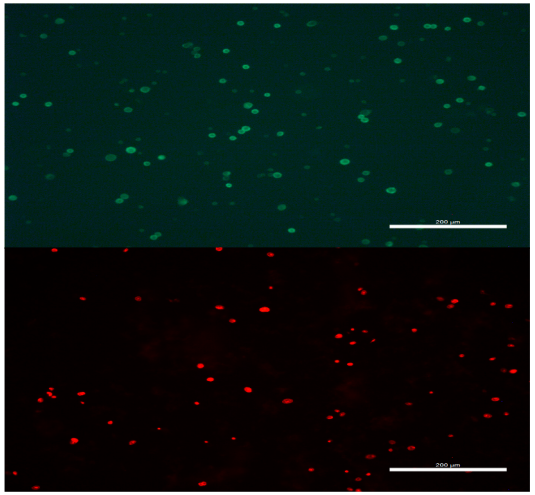


*All test cases are co-culture of MDA-MB-468 cancer cells, KHYG-1 NK and platelets

- Each case was plated in triplicate on a 12-well plate, with 0.5 mL of each cell type at 100 000 cells/mL and 0.1 mL of platelets at stock concentration
- IL-2, Aspirin added in concentrations of 0.01 mg/L and 325 mg/L, respectively

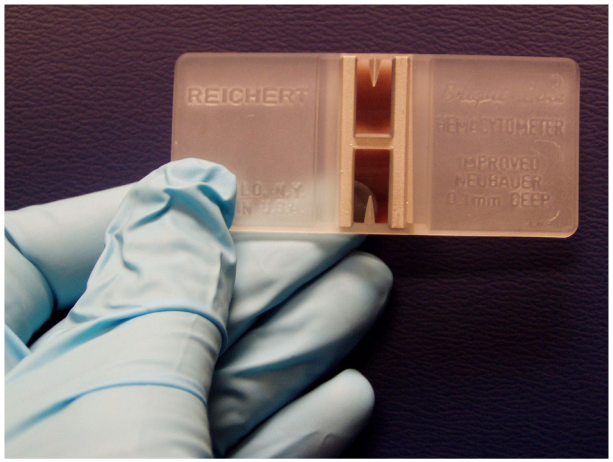
Experimental Technique - Fluorescent Staining and Analysis

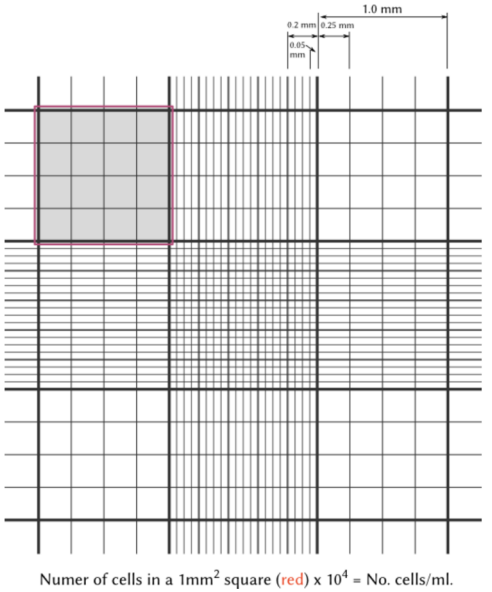
- MDA-MB-468 Cells incubated with CellTracker stain (green) in Tyrode's solution with a concentration of 0.5 mM/ml
- After incubation, ethidium homodimer (EthD-1) added as a dead stain (red) to differentiate between live and dead cancer cells
- Live/dead count used to determine % survival



Experimental Technique - Hemocytometer

- Cell concentration calculations
- Cancer cell survival calculations





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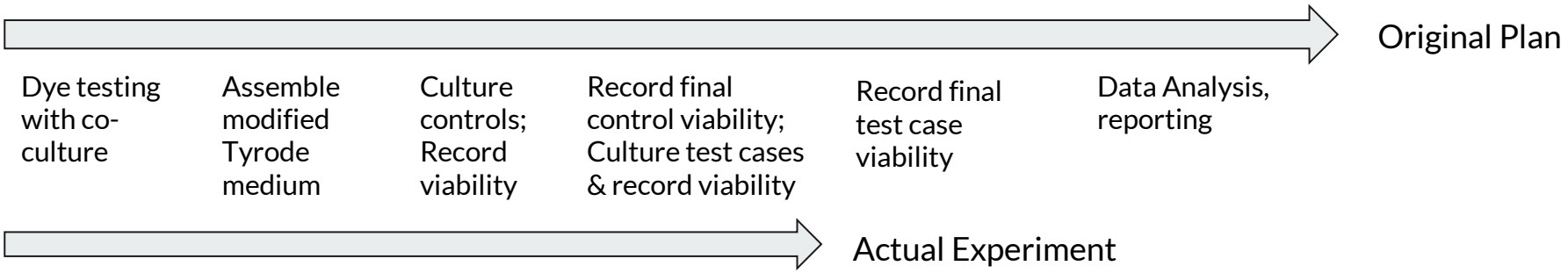
[11] The Privalsky Lab, 2020.

Changes to Experimental Plan

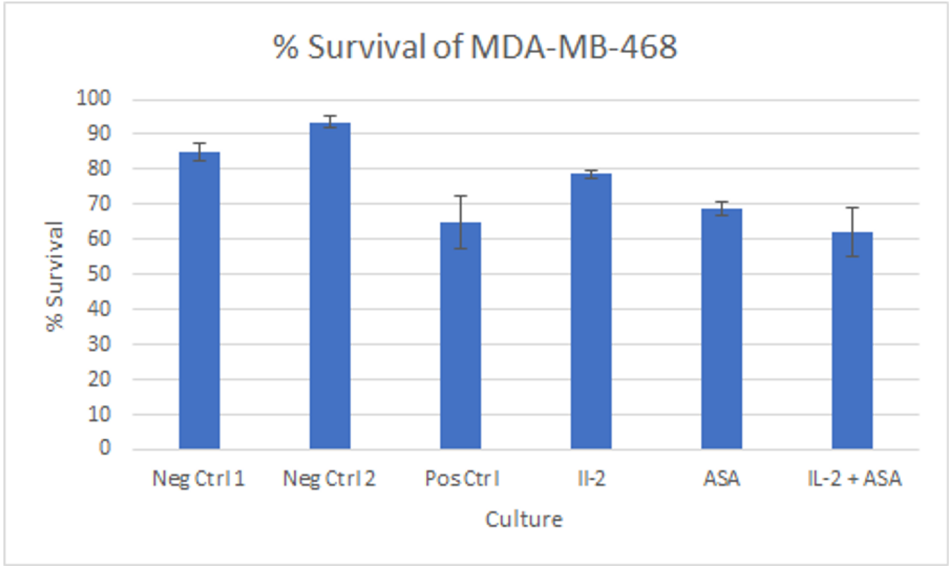


1) During culturing, trypsinization not required for NK cells (KHYG-1)

2) Calcium AM live stain –> CellTracker live stain [12]

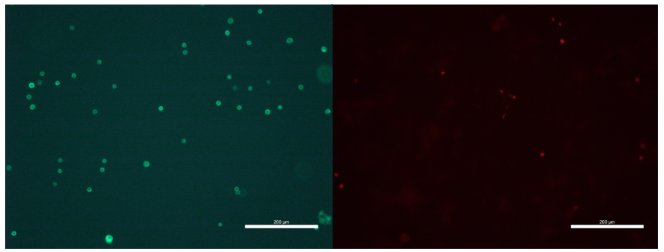
Results

- IL-2 + ASA combination resulted in 20.9% and 9.7% than ASA and IL-2 alone, respectively
- All three test cases resulted in decreased survival compared to negative controls

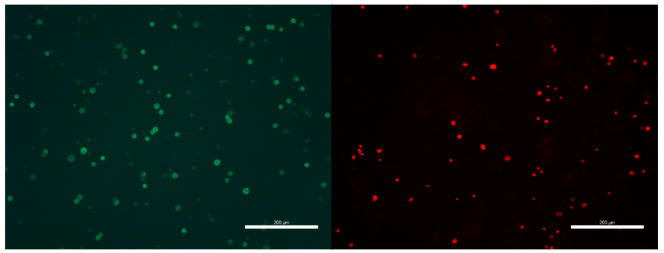


Noteworthy Observations

- Positive control did not give expected results; more cells survived than expected
- Single-drug wells gave more consistent results than the combination therapy



Positive Control



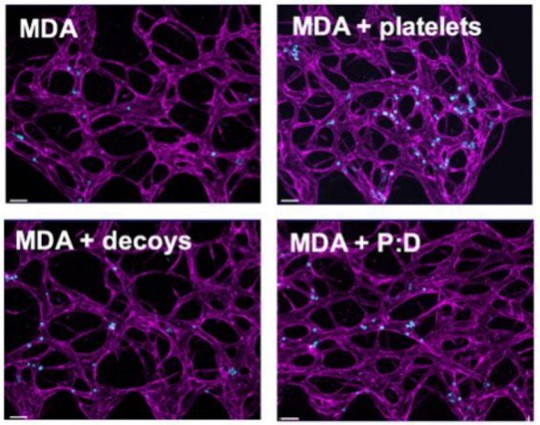
ASA + IL-2 Combination

Conclusion

- Due to the small sample size, no definite conclusions can be made from this experiment
- However, the synergistic effect observed between IL-2 and ASA is an important step in establishing this type of combination therapy as a potential antimetastatic treatment
- This trial justifies future studies to evaluate different doses and types of immune-enhancing and antiplatelet therapies

Future Directions

- Further study of NK-antiplatelet combination therapies with larger sample size for more robust statistics
- Run experiment within blood vessel mimic to directly observe the process of extravasation - HUVEC microfluidic device
- Imaging - more dyes, imaging of platelets and KHYG-1 cells



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[9] A. L. Papa *et al.*, 2019.

Acknowledgements

We would like to thank all of the people who helped make this project possible, especially Gary Hoang, Laura Bahlmann, Dr. Rob Laister, Professor Matsuura, and all of our BME346 classmates.

THANK YOU!

References

[1] C. N. Qian, Y. Mei, and J. Zhang, “Cancer metastasis: Issues and challenges,” *Chinese Journal of Cancer*, vol. 36, no. 1. BioMed Central Ltd., 2017.

[2] M. Iiizumi, W. Liu, S. Pai, E. Furuta and K. Watabe, "Drug development against metastasis-related genes and their pathways: A rationale for cancer therapy", *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, vol. 1786, no. 2, pp. 87-104, 2008.

[3] American Association for Cancer Research. “AACR Cancer Progress Report 2015.” *Clin Cancer Res 2015*, vol. 21(Supplement 1):SI-S128, 2015.

[4] National Institutes of Health. “Metastasis”. *National Cancer Institute,* 2020.

[5] B. Strilic and S. Offermanns, “Intravascular Survival and Extravasation of Tumor Cells,” *Cancer Cell*, vol. 32, pp. 282–293, 2017.

[6] “Mosaic Blood Vessels in Tumors: Frequency of Cancer Cells in Contact with Flowing Blood” *Proceedings of the National Academy of Sciences of the United States of America, v*ol. 97, no. 26 , pp. 14608-14613, 2000. [Online]. Available: https://www-jstor-org.myaccess.library.utoronto.ca/stable/2666343?seq=1#metadata_info_tab_contents. [Accessed: 03-Feb-2020].

References

[7] J. S. Miller et al., “Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer,” *Blood*, vol. 105, no. 8, pp. 3051–3057, 2005.

[8] M. Z. Wojtukiewicz, D. Hempel, E. Sierko, S. C. Tucker, and K. V. Honn, “Antiplatelet agents for cancer treatment: a real perspective or just an echo from the past?,” *Cancer Metastasis Rev.*, vol. 36, no. 2, pp. 305–329, 2017.

[9] A. L. Papa et al., “Platelet decoys inhibit thrombosis and prevent metastatic tumor formation in preclinical models,” *Sci. Transl. Med.*, vol. 11, no. 479, 2019.

[10] “What are the culture conditions and media for blood platelets?” [Online]. Available: https://www.researchgate.net/post/What_are_the_culture_conditions_and_media_for_blood_platelets. [Accessed: 10-Feb-2020].

[11] The Privalsky Lab. “Cell Counting with a Hemocytometer”, *University of California Davis,* 2020.

[12] “CellTracker™ Green CMFDA Dye.” [Online]. Available: https://www.thermofisher.com/order/catalog/product/C7025#/C7025. [Accessed: 08-Apr-2020].