Curation of Research Papers Written By PSI 2025 Cohort

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5/28/25

Nanoplastic Exposure in Wild Type Mice Causes Negative Effects on Cardiovascular Health

Hypothesis and Specific Aims

Wild type mice injected with a nanoplastic solution will experience negative side effects to their cardiovascular system. Mice injected with nanoplastic solution will have a buildup of arterial plaque in the form of concentrated lipids**:** The accumulation of nanoplastics within the mice hinders the gene regulation of lipid production. It triggers the activation of the PERK-ATF4 pathway in mice, causing an uncontrolled increase in lipid production. This lipid production outpaces the body's metabolism, leading to an increase in lipid buildup within the arteries. Plaque comes from the buildup of lipids and causes atherosclerosis when there is a buildup of plaque within the arteries. Atherosclerosis causes a blockage in blood flow leading to various diseases related to the cardiovascular system as blood is not being adequately transferred (Yu, 2024). The nanoplastic solution will cause an increase in oxidative stress levels in cardiovascular tissue: Increased exposure of nanoplastics increases the production of reactive oxygen species, which are unstable molecules that are produced during metabolism. Reactive oxygen species are capable of oxidizing the DNA's nucleotide bases, causing mutations and oxidative stress to the cell. Oxidative stress is particularly harmful because it is capable of damaging cell structure, potentially killing it (Wen et al, 2024).

Background

There has been an increasing accumulation of plastics on our earth. It can be found in virtually everything that we use in our day to day lives. It is widely versatile as it can be used in a variety of physical forms while staying easy and cost efficient to produce for companies. Plastic can be found in our homes, devices and even our food. With the increasingly rapid production of plastic, the accumulation of plastic waste only continues to grow. Unlike organic materials that are able to biodegrade and decompose over time, plastic is nearly invincible. If left in a landfill, plastic won't biodegrade unless left for thousands of years. When left to weather over time, plastic continues to break apart into smaller and smaller nanoplastics but never fully degrade (United Nations, (n.d.)). These nanoplastics easily spread throughout our planet and environment, seeping into the soils and waters of our earth. Plants absorb the nanoplastics as they grow, increasing their chemical toxicity. Plastics are made up of a variety of chemicals like forever chemicals and bisphenol A, which are chemicals that are capable of damaging our immune system (Heal-Admin, 2022). This causes a domino effect across animals and humans, increasing the consumption of nanoplastics in the human diet. Past research has demonstrated nanoplastic exposure can induce cellular toxicity within mammals through damage to the cell structure and immune systems response. The severity of damage is directly correlated with the plastics size and length of exposure. Nanoplastics have been a strong point of concern because of their incredibly small size, and therefore ability to damage our bodily systems (Banerjee, 2021). Humans experience prolonged nanoplastic exposure in our diets as everything we consume has nanoplastics in them. Despite this, not much research has been done on the effects of nanoplastic exposure to one of the most crucial systems in our bodies, the cardiovascular system. One team of researchers sought to study the effects of nanoplastics to the cardiovascular system through looking at lipid accumulation, inflammation and oxidative stress in ApoE-/- mice. These three factors are key triggers of atherosclerosis, which is a disease from the buildup of arterial plaque (Wen et al, 2024). This research proved that prolonged nanoplastic exposure has negative effects on the cardiovascular system of ApoE-/- mice, but these results were heightened by the mice's inability to metabolize lipids. ApoE-/- mice lack gene expression for the production of

apolipoprotein E, which is a crucial protein involved in the transportation of lipids throughout your immune system. A lack of these proteins slows the rate of your body's lipid metabolism (Wen et al, 2024). The goal of this research is to examine if the same results can be seen in wild type mice, in order to see if these results from previous research can be validated and translated to the healthy human cardiovascular system. We can predict that we will see negative effects on the cardiovascular system of the wild type mice.

Research Design and Methods

In the study that we are referencing, done by a team of researchers from China, they performed the research experiments on a group of male ApoE-/- mice aged 7 weeks old (Wen et al, 2024). To keep maximum accuracy when comparing our results to that of the study, we are going to be performing these experiments on the same sex and age group of male wild type mice. We are also going to be administering the same concentrations and performing the same experiments to limit the amount of confounding variables when comparing our results to that of this study. We are going to have an overall group of ten mice since this is the number of mice used in the reference study, splitting five between our two different experiments. These mice will be fed the same diet over the span of their 7 weeks growth and kept in the same living conditions to ensure consistency across the mice models. Each mouse will weigh roughly the same, within 5% of their average weight. At 7 weeks old, these mice will be administered a nanoplastic solution through gavage at increasing concentrations of 50, 100, 150, and 200 μg/mL. Each group of five mice will have one control that is administered with no nanoplastic solution and the following four mice will be administered the increasing concentrations until the concentration of 200 μg/mL. We will mark the mice with a color corresponding to the concentration they were administered. The mice will be administered the nanoplastic solution once a day over the span of 3 months. After the last administration, the mice will be fasted for a day before being sacrificed under anesthesia. The aortic tissue and liver of the mice will be collected for study (Wen et al, 2024).

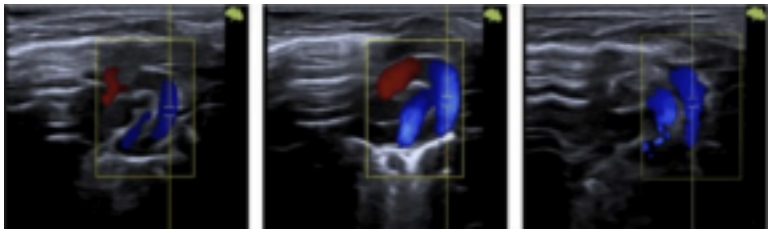

Figure 1: Color doppler flow imaging of mice aortic arch from reference study (Wen et al, 2024)

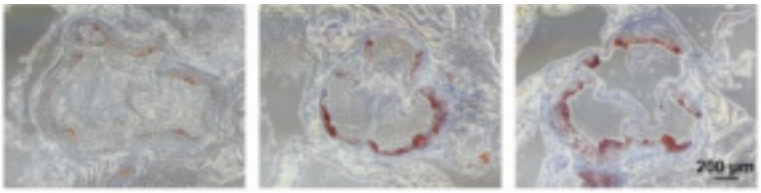

Figure 2: Oil Red O staining of mice aortic arch from reference study (Wen et al, 2024)

To observe the accumulation of lipids within the aortic tissue of the mice, we will use an ultrasound imaging system to obtain ultrasonic biomicroscopy images of the aorta. We will pair these images with color doppler flow imaging to observe the blood flow within the aorta. We want to look at the blood flow as high lipid accumulation can cause a decrease in blood flow within the aorta. Furthermore, we will also slice and stain a portion of the aortic tissue to stain with Oil Red O staining, which will allow us to observe lipid density and accumulation (Wen et al, 2024).

To observe the oxidative stress levels in the mice, we will be looking at their gene expression after exposure to nanoplastics through their liver samples. We are going to focus on the ABCA1 and ABCG1 genes because they are involved in the transportation of cholesterol (Wen et al, 2024). Cholesterol helps metabolize lipids throughout the body. When looking specifically at the liver, cholesterol helps form acids for lipid digestion and metabolism. A lack in the transportation of cholesterol would lead to a molecule imbalance and oxidative stress. Since they are inversely related to each other, looking at cholesterol levels will help us see if oxidative stress is active (Feingold, 2024). We also want to look at the

expression of the SOD gene as it is a gene that helps fight against oxidative stress (U.S. National Library of Medicine, (n.d.)). A lack in the expression of this gene would indicate there is an increase in oxidative stress. We are going to use RT-qPCR analysis to quantify the expression of these genes within the liver samples (Wen et al, 2024).

Summary and Significance

When observing lipid accumulation in the aortic arch of the mice, we expect to see an increase in lipid accumulation as the nanoplastic solution concentration increases. This causes a decrease in blood flow within the aortic arch because of the increasing buildup of lipids, creating a blockage. We can also expect to see an increase in lipid density from the Oil Red O staining of the aortic arch. From the RT-qPCR analysis we predict there to be a decrease in the expression of the ABCA1, ABCG1, and SOD genes. Collectively, a down regulation in these genes will cause a decrease in the transportation of molecules and production of oxidative stress fighting proteins, leading to an increase in oxidative stress. These results will lead us to believe that there is a direct correlation between nanoplastics and the development of atherosclerosis as these are key triggers of the disease. The results we gather from our experiments on wild type mice should align with the results of the reference study on ApoE-/- mice. Some potential limitations that we could encounter are unforeseeable health conditions and premature death within the mice. There were no premature deaths in the comparative study but there is always the possibility with the development of atherosclerosis. We could also end up not seeing any changes within the lipid accumulation and oxidative stress of the mice, meaning that the results in the comparative research were solely due to the usage of ApoE-/- mice. We are also only using male mice in this study so our data isn't completely applicable to female mice. This research would help prove the negative effects of nanoplastic exposure on the cardiovascular system and encourage more research to be done on its correlation to the human body as we are increasingly being exposed to nanoplastics everyday.

References

Feingold, K. R., & Grunfeld, C. (2024, January 14). *Introduction to Lipids and Lipoproteins*. Nih.gov; MDText.com, Inc. https://www.ncbi.nlm.nih.gov/books/NBK305896/ *Health and Environment Alliance | Turning the plastic tide: New HEAL report puts the spotlight on how chemicals in plastic are putting our health at risk*. (2020, September 9). Health and Environment Alliance. https://www.env-health.org/turning-the-plastic-tide-new-heal-report-puts-the-spotlight-on -how-chemicals-in-plastic-are-putting-our-health-at-risk/

Martin, P., Olivares, J., Maass, F., & Valero, E. (2018). Analytic approximations for special functions, applied to the modified Bessel functions I2(x) and I2/3(x). *Results in Physics*, *11*, 1028–1033. https://doi.org/10.1016/j.rinp.2018.10.029

SOD1 gene: MedlinePlus Genetics. (2016, March 1). Medlineplus.gov. https://medlineplus.gov/genetics/gene/sod1/

United Nations. (2021, June). *Plastic Is Forever*. United Nations. https://www.un.org/en/exhibits/exhibit/in-images-plastic-forever

Wan, T., Pan, Y., Du, H., Qu, B., Yi, J., & Chu, D. (2018). Threshold Switching Induced by Controllable Fragmentation in Silver Nanowire Networks. *ACS Applied Materials & Interfaces*, *10*(3), 2716–2724. https://doi.org/10.1021/acsami.7b16142

Wen, J., Sun, H., Yang, B., Song, E., & Song, Y. (2024). Long-term polystyrene nanoplastic exposure disrupt hepatic lipid metabolism and cause atherosclerosis in ApoE-/- mice. *Journal of Hazardous Materials*, *466*, 133583–133583.

https://doi.org/10.1016/j.jhazmat.2024.133583

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Using OCT to investigate potential mechanisms relating to inflammation and loss of residual hearing post-operation of a cochlear implant.

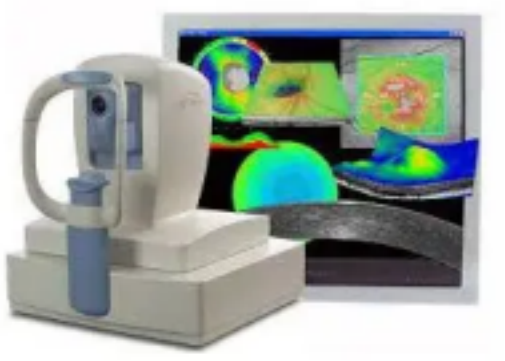
Hypothesis and Specific Aims

Hearing loss affects over 430 million people worldwide, Cochlear Implants are a popular method for combating severe hearing loss as they can be paired effectively with regular hearing aids. Implants using Electric Acoustic Stimulation (EAS) can help stimulate auditory nerves past their natural capacities allowing patients even with severe hearing loss to regain most of their low-frequency hearing. However, some patients experience rapid degradation of their residual hearing following their implant operations. Many studies have attributed this effect to symptoms surrounding inflammation located in the scala tympani. However, the direct consequences of this inflammation and its impact on the ears physiology remains unclear. Recently, Hyzer and colleagues investigated whether fibrosis had potential effect on hearing loss, and found that soft-tissue fibrosis had no effect on hearing loss and it concluded that "there may be alternative mechanisms related to inflammation that contribute to hearing loss" (Hyzer et al., 2024). The objective of this study is to visualize and characterize inflammatory processes within the scala tympani by inducing inflammation in Guinea Pigs similar to postoperative conditions. I hypothesize that inflammation induces structural and microvascular changes that result in hearing loss and that these can be detected and characterized using Optical Coherence Tomography (OCT).

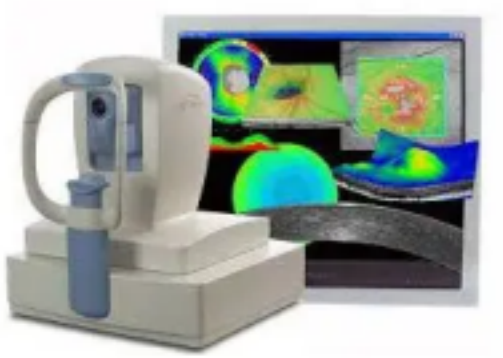
Background

Cochlear implantation often induces an inflammatory and fibrotic response (Rahman et al., 2023). Inflammatory responses can be of two kinds, acute inflammation and chronic inflammation. Acute inflammation is seen immediately following implantation and is characterized by recruitment of neutrophils. This phase is also associated with mast cell degranulation and the release of chemical mediators. Macrophage adhesion to biomaterials then occurs and inflammatory cells release several cytokines and chemokines. These biological occurrences can lead to swelling and pain of the infected site. If the acute phase is unresolved, inflammation turns chronic and a persistent inflammatory environment is established. In this phase macrophages fuse with each other and form foreign body giant cells (FBGCs). The chronic phase is also characterized by recruitment of lymphocytes and their interactions with macrophages (Anderson et al., 2008). Chronic inflammation can lead to changes in tissue and bone growth in the impacted areas.

Both these inflammatory reactions have effects in the cochlea that could make way for residual hearing loss. Hyzer and colleagues recently found that corticosteroids improved hearing and ABR response values. Corticosteroids are a common pharmacotherapeutic used to treat inflammation, providing evidence that inflammation and its mechanisms contributed to the loss of residual hearing loss. This study also found no correlation between tissue fibrosis and loss of hearing,



indicating inflammation, but not fibrosis, may be associated with changes in hearing (Hyzer et al., 2024). Another product of inflammation in the cochlea is the ossification in the inner cochlea structures causing unwanted bone growth and increased bone density. Due to this abnormal growth the hair cells and the organ of Corti may be damaged and eventually sensorineural hearing loss occurs (Tokat et al., 2022). Inflammation is also known to cause pathological consequences that have been observed in animal models of cochlear inflammation. These include degeneration of hair cells of the organ of Corti, disruption of fibrocytes in the spiral ligament, loss of interdental cells of the spiral limbus, swelling of the stria vascularis, and vascular damage (Tan et al. 2013). To observe any structural and microvascular abnormalities we will use OCT. Optical coherence tomography (OCT) is a non-invasive technique for cross-sectional tissue imaging, originally created for eye structures. It typically uses light in the near-infrared spectral range which has a penetration depth of several hundred microns in tissue (Aumann et al., 2019). The backscattered light is measured with an interferometric set-up to reconstruct the depth profile of the sample at the selected location. New trends show the ability of functional OCT to image flow, polarizing properties of tissue and even mechanical properties like elasticity. This feature is extremely helpful in applications of this technology in other areas of the body and in this study the cochlea and inner ear (Aumann et al., 2019).



Picture 1: OCT imaging technology

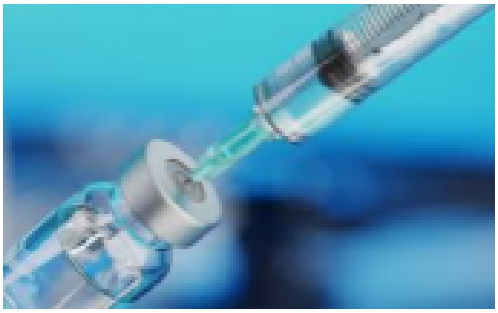
Research design and methods

In this study we will have 3 groups: 2 experimental groups (1 receiving acute inflammation and 1 chronic inflammation) and 1 control group. The inflammatory reaction for chronic inflammation can be induced in animals using a lipopolysaccharide injection (Ma et al., 2022). Each group will consist of 10 Albino Dunkin‑Hartley Guinea Pigs. Both groups will consist of both male and female Guinea Pigs of the same species, of similar weight/size for the respective sex, and are around the same age.

Picture 2: Albino Hartley Guinea Pig

The first experimental group or the acute inflammation group will be implanted with cochlear implants (CIs). This will simulate the effects of acute inflammation following a cochlear implant procedure. Similar to the Hyzer et al. study the animals will be anesthetized initially with ketamine (30 mg/kg IM) and xylazine(3‑5

mg/kg IM), then anesthesia was maintained using isoflurane (dosing: 2%‑3% in 100% oxygen with a flowrate of 3 L/min; increase to 3% during incisions; decreased to 2%‑2.5% after major surgical procedures such as drilling, and dissecting are completed; decreased to 1.5%‑2% during suturing; flushed with oxygen for a few minutes after completion of surgery). A single surgeon will perform all procedures and the CI group will be administered implants following the 2025 American Veterinary Medical Association Guidelines. All animals will have OCT imaging done on their cochlea pre-operatively as well as 10 days postoperatively to monitor any changes in the ear following the inducing of inflammation.



Picture 3: Lipopolysaccharide (LPS) Injection

The second experimental group will not be given cochlear implants but will be administered a Lipopolysaccharide (LPS) injection. This injection will induce inflammation similar to the chronic inflammation found in a patient post operation of a cochlear implant. For the LPS group we will inject them with a dosage of 0.5 mg/kg/day to maintain the effects of chronic inflammation directly in the cochlea. Similar to the acute inflammation group, we will conduct OCT imaging before injection and following the last injection. This will also allow the Guinea pig to experience chronic inflammation sooner than if they received an implant as the inflammation takes place quickly, allowing us to isolate the effects of inflammation from other changes that might occur after the implantation of a cochlear implant.

The control group will not receive any experimental manipulations and will each be scanned twice for normal cochlea activity on the same timeline as the two experimental groups. All of these images will be effectively compared to images taken from the healthy control group. The study will compare the images to look for any abnormalities specifically looking at bone growth or blood vessels and vascular components and correlate any resultant changes to changes in hearing. These comparisons will be performed manually by expert otolaryngologists experienced in evaluating OCT images. Following the OCT imaging, the Guinea pigs will have an ABR test to test for hearing loss of any kind. This test will be administered before the induction of inflammation and after for 10 days. This will directly test that the inflammation induced either by cochlear implants or chronic LPS treatment yields hearing loss. We can then further characterize the physiological consequences of this inflammation using OCT, potentially uncovering important characteristics of inflammation-induced hearing loss. While most of this data is qualitative to begin with, we will quantify this data on a predetermined scale to show growth and changes in the scala tympani. Once comparison has been complete we will hopefully be able to find the mechanisms of an inflammatory response to Cochlear Implantation and its potential relations to the degradation of residual hearing. Ultimately, the goal of this study is to identify the direct link between cochlear implants, inflammation, and degeneration of hearing.

Summary and significance

In summary, during this study, we will be observing the effects of inflammation and its mechanisms of the cochlea and relate it to potential symptoms and residual hearing loss followed by cochlear implant surgery. By observing acute and chronic inflammation and comparing it to a control group we can see how the inner cochlear structures are affected by the different kinds of inflammation and monitor any changes during the transition between these inflammations. Looking for microvascular and structural changes in the cochlea can also help us find appropriate treatments and isolate specific mechanisms that contribute to residual hearing loss. This can help us improve cochlear implantation procedures by creating alternative methods that can combat inflammations. Some limitations include how effective the LPS injections are in simulating chronic inflammation and OCT being used in a cochlear study. LPS injections have been previously tested in mice and have been used to simulate chronic inflammation in various tissues, but is unexplored in the ear. As this is a novel region of testing LPS in Guinea pigs, we will first conduct a dose-response curve to ensure appropriate dosage to simulate accurate effects of chronic inflammation in the scala tympani. OCT was originally invented for optical studies and has not been verified to be used in the ear. The images could be inaccurate or the ear and its structures might not be compliant with OCT imaging guidelines. While past studies on the cochlea have successfully utilized OCT and therefore we are confident in its usage for this experiment, we can also use traditional post-mortem measurements to evaluate changes in anatomical structures.

The goal of this study is to test the direct relationship between inflammation and residual hearing loss following cochlear implantation, and could help researchers and medical professionals identify novel therapeutic approaches to prevent residual hearing loss. This study would also be the next step into looking at inflammatory responses in ears using cutting-edge imaging technology and progress our understanding of the anatomical and pathological complications surrounding inflammation in the inner ear.

References

1. Tokat, Taskin, et al. “Surgical Methods and Auditory Outcomes of Cochlear Implantation in Cochlear Ossification.” The Journal of International Advanced Otology, vol. 18, no. 1, Feb. 2022, pp. 51–56, https://doi.org/10.5152/iao.2022.20146. Accessed 24 Feb. 2023.
2. Muhammad Sabbir Rahman, et al. “Cochlear Implants: Causes, Effects and Mitigation Strategies for the Foreign Body Response and Inflammation.” Hearing Research, vol. 422, Sept. 2022, pp. 108536–36, https://doi.org/10.1016/j.heares.2022.108536.
3. Mizobuchi, Haruka, and Gen-Ichiro Soma. “Low-Dose Lipopolysaccharide as an Immune Regulator for Homeostasis Maintenance in the Central Nervous System through Transformation to Neuroprotective Microglia.” Neural Regeneration Research, vol. 16, no. 10, 2021, p. 1928, https://doi.org/10.4103/1673-5374.308067. Accessed 27 May 2021.
4. Aumann, Silke, et al. “Optical Coherence Tomography (OCT): Principle and Technical Realization.” PubMed, edited by Josef F. Bille, Springer, 14 Aug. 2019, www.ncbi.nlm.nih.gov/books/NBK554044/.
5. Hyzer, Jeffrey M., et al. “Effects of Cochlear Implantation and Steroids on the Aging Guinea Pig Cochlea.” Otolaryngology--Head and Neck Surgery : Official Journal of American Academy of

Otolaryngology-Head and Neck Surgery, vol. 171, no. 2, Aug. 2024, pp. 530–37, https://doi.org/10.1002/ohn.732.

6. Anderson, James M., et al. “Foreign Body Reaction to Biomaterials.” Seminars in Immunology, vol. 20, no. 2, Apr. 2008, pp. 86–100, https://doi.org/10.1016/j.smim.2007.11.004.
7. Tan, Winston JT. “Noise-Induced Cochlear Inflammation.” World Journal of Otorhinolaryngology, vol. 3, no. 3, 2013, p. 89, https://doi.org/10.5319/wjo.v3.i3.89. 8. Ma, Ji-Hyun, et al. “Therapeutic Effect of NLRP3 Inhibition on Hearing Loss Induced by Systemic Inflammation in a CAPS-Associated Mouse Model.” EBioMedicine, vol. 82, Elsevier BV, Aug. 2022, pp. 104184–84, https://doi.org/10.1016/j.ebiom.2022.104184. Accessed 19 June 2024.

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Chemotherapy and Radiation vs Radiation, What is Best for Anaplastic Ependymomas?

Hypothesis and Specific Aims

Pediatric patients diagnosed with Anaplastic Ependymoma have improved survival when treated with chemotherapy and radiation than with radiation alone. The specific aims in this study are to find out the side effects of chemotherapy and radiation (C+R) versus the side effects of radiation alone, the quality of life patients have after respective treatments, and the average time treatment takes compared between the two treatment paths. Many variables could come from the side effects of C+R and conducting an investigative study would prove fruitful as to revealing what they are as well as how much they affect the patient. Quality of life, which could influence treatment duration, adherence, and tolerance, is a topic historically under-researched in pediatric oncologic studies[1]. By investigating these aims, it may provide a clearer understanding of treatment efficacy and what is best for patients—a quicker treatment and much more manageable one? The overall goal of this research is to better understand the treatment course, outcomes, and whether overall survival can be improved with additional treatment modalities.

Background

Relapses occur in 50% of pediatric ependymoma cases and have a poor prognosis[2]. Anaplastic Ependymomas are often hard to treat and tend to recur even after treatment. These particular types of ependymomas are malignant and incredibly fast-growing tumors, consequently being classified as a WHO III grade tumor. Ependymomas account for 6.8% of all gliomas, with the relative frequency being higher in children, thus being a topic of interest[3]. Most frequently, Anaplastic Ependymomas are found in the brain in pediatric patients, as opposed to near the spine, which occurs frequently in adults[3–4]. In a retrospective review from 2019, the mean 5-year survival for pediatric patients with Anaplastic Ependymomas following surgery and radiation is approximately 75%, with a progression-free survival of 56%[5]. Though surgery and radiotherapy are established treatments for ependymomas, chemotherapy has yet to be established and research must be conducted[6]. Since there are few cases, it could be considered unethical to put a patient only on chemotherapy if radiation is the gold standard for treatment of this malignancy. Keeping that in mind, using a combination of chemotherapy and radiation seems plausible to test, if a case-study were to be conducted with a suitable cohort. Realistically, however, this study aims to use methods such as literature review and retrospective data analysis, as cohort sizes are limited due to the rarity of the condition.

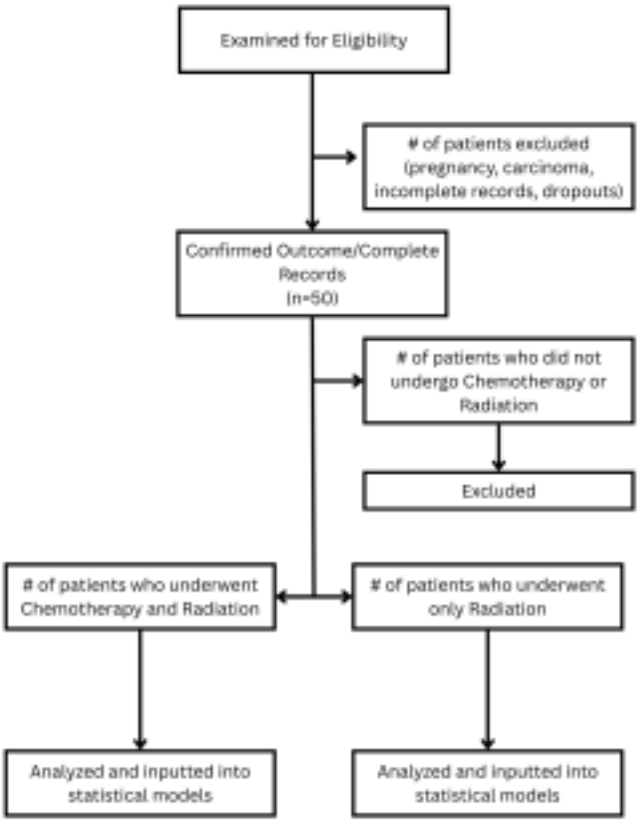

Figure 1 shows the proposed outline of the study: including retrospective chart review using data from several institutions. A tentative data size would be about 50 people, taking into account the rarity of pediatric ependymoma cases. Exclusion criteria consists of pregnant individuals, comorbid malignancies, and incomplete records. After confirming outcomes and complete records, patients who did not undergo chemotherapy or radiation are also excluded. The remaining are then sorted into those who underwent C+R and those who underwent only radiation to be analyzed and put into the statistical models and analysis.

Figure 1: Proposed outline

Research design and methods

The independent variable of this study would be the use of chemotherapy. The dependent variable would be survival rates. Some confounding factors would be radiation dosage or type variation, as it highly depends on the individual's age, sex, ethnicity and frequency of chemotherapy administration. Taking into account demographic factors such as age, sex, and ethnicity could fill in gaps in knowledge of the epidemiology of Anaplastic Ependymoma. Any evidence of homogeneity in a demographic and associated treatment outcomes could provide valuable insight for further research. The study would be primarily quantitative as it is collecting survival rates, treatment duration, and quality of life scores. The participants in this study will be pediatric patients who are under the age of 18 with Anaplastic Ependymoma, and the initial sample size should be about 50 participants for the data to account for dropouts and exclusion criteria. An ethical issue of this study would be waived informed consent, since the study is of very minimal risk. Potential limitations for this study would be the small sample size due to the rarity of Anaplastic Ependymoma, and the fact that this would be a retrospective study, meaning that it is only relying on existing records rather than

gathering new information from the latest patients. A quality of life questionnaire, such as EuroQol 5-Dimensions 5-Levels (EQ-5D-5 L) should be sent to all participants—however, it is not guaranteed all of them will respond.

The study will be conducted in collaboration with institutes across the world as it is best to get a diverse pool of data, as well as increase the chances of getting a suitable sample size. Using the Kaplan-Meier survival analysis, a test to estimate the survival of subjects over a period of time, to determine overall survival rates between patients who underwent C+R and those who only underwent radiation for comparative analysis. To prove a statistical difference between the two curves from the Kaplan-Meier test, we can then input the values into a log-rank test for a qualitative measure (p-value) and into the Cox Proportional Hazards Model (CPHM), a multivariable regression analysis, which would provide a quantitative measure. The CPHM would account for more than one independent variable which would aid in the generalization of data and in turn minimize limitations/weaknesses in the study. It would also provide a Hazard ratio, which gives a relative event rate in the groups[7]. Events are defined as a specific outcome, which would be death in this study. How long a patient lives following treatment would also be measured using the Hazard Ratio. The computer software to be used would be Microsoft Word or Excel in order to increase efficiency in entering data.

In *Table 1*, it illustrates a suitable timeline and gives an ample amount of time for the tasks at hand, and how they will be completed.

Table 1.

Phase	**Tasks**	**Time frame**
1. IRB Approval and Basic Planning	- Submit and receive IRB approval - Plan how to collect and sort data	1-2 months depending on how long the IRB approval takes
2. Data Collection	- Identify eligible patients - Exclude based on criteria outlined above - Find specific and relevant information from medical records - Check for accuracy of data - De-identify data	Months 2-4
3. Data Management	- Clean up data (quality check, inconsistencies) - Finalize data set	Months 5-6

4. Analysis	- Run Kaplan-Meier survival analysis, log-rank, Cox Proportional Hazards Models - Make figures and tables	Months 6-7
5. Interpret Results	- Analyze findings - Identify limitations and weaknesses of the study	Months 7-8
6. Write Report on Findings	- Write about the study and what we found - Organize figures and tables	Months 8-11
7. Submit Report	- Submit to a journal - Revise with feedback	Months 11-12

Summary and significance

In this study, retrospective analysis will be used to compare overall survival for pediatric patients with Anaplastic Ependymomas who received chemotherapy and radiation compared to patients who received radiation alone. The aim of this study is to improve survival rates in pediatric patients with the condition, understand quality of life outcomes, classify side effects of C+R versus radiation, and quantify duration of treatment courses. A potential result would be improved survival rates from patients who had C+R treatments, which could pave the way to implementing it as a primary treatment for Anaplastic Ependymomas and related tumors. However, the combination of chemotherapy and radiation could decrease survival rates, promote intolerable side effects for patients, or demonstrate no difference in overall survival compared to radiation alone. Further research could be aimed toward understanding the effects of chemotherapy monotherapy in the treatment of Anaplastic Ependymomas. A primary limitation of this study would be the small sample size, given the rarity of this malignancy, which could limit this study's generalizability and statistical power. Using only complete outcomes from patient data would significantly skew data to one outcome or the other, such as living for 5 years as opposed to 1 year after C+R treatment. Measuring just chemotherapy and radiation versus radiation rather than studying chemotherapy on its own also limits this study considerably. Without the knowledge of what chemotherapy does on its own, do we know how significantly it contributes any improvement in survival when combined with radiation? Difference in dosage and treatment exposure with C+R is an aspect not explored in this study, and would be the next steps to further future treatments for Anaplastic Ependymomas.

It's important to conduct this study so that children with Anaplastic Ependymomas can experience a better survival outcome and better quality of life.

References

1. Torres VA, Ashford JM, Wright E, Xu J, Zhang H, Merchant TE, Conklin HM. The impact of socioeconomic status (SES) on cognitive outcomes following radiotherapy for pediatric brain tumors: a prospective, longitudinal trial. Neuro Oncol. 2021;23(7):1173-82. doi:
2. Ritzmann TA, Rogers HA, Paine SML, Storer LCD, Jacques TS, Chapman RJ, Ellison D, Donson AM, Foreman NK, Grundy RG. A retrospective analysis of recurrent pediatric ependymoma reveals extremely poor survival and ineffectiveness of current treatments across central nervous system locations and molecular subgroups. Pediatr Blood Cancer. 2020;67(9):e28426. Epub 20200702. doi: 10.1002/pbc.28426. PubMed PMID: 32614133.

3. Gramatzki D, Roth P, Felsberg J, Hofer S, Rushing EJ, Hentschel B, Westphal M, Krex D, Simon M, Schnell O, Wick W, Reifenberger G, Weller M. Chemotherapy for intracranial ependymoma in adults. BMC Cancer. 2016;16:287. Epub 20160423. doi: 10.1186/s12885-016-2323-0. PubMed PMID: 27108407; PMCID: PMC4842281. 4. Anaplastic Ependymoma Bethesda MD: National Institutes of Health, Genetic and Rare Diseases Information Center (GARD); 2025 [updated February 2025; cited 2025 April 22]. Available from: https://rarediseases.info.nih.gov/diseases/10634/anaplastic-ependymoma. 5. Ruangkanchanasetr R, Swangsilpa T, Puataweepong P, Dhanachai M, Hansasuta A, Boongird A, Sirachainan N, Hongeng S. Outcome of postoperative radiation therapy for pediatric intracranial ependymoma: a single-institution review. Childs Nerv Syst. 2019 Aug;35(8):1313-1321. doi: 10.1007/s00381-019-04198-w. Epub 2019 Jun 16. PMID: 31203397.

6. Wu J, Armstrong TS, Gilbert MR. Biology and management of ependymomas. Neuro Oncol. 2016;18(7):902-13. Epub 20160328. doi: 10.1093/neuonc/now016. PubMed PMID: 27022130; PMCID: PMC4896548.
7. Rich JT, Neely JG, Paniello RC, Voelker CC, Nussenbaum B, Wang EW. A practical guide to understanding Kaplan-Meier curves. Otolaryngol Head Neck Surg. 2010;143(3):331-6. doi: 10.1016/j.otohns.2010.05.007. PubMed PMID: 20723767; PMCID: PMC3932959.

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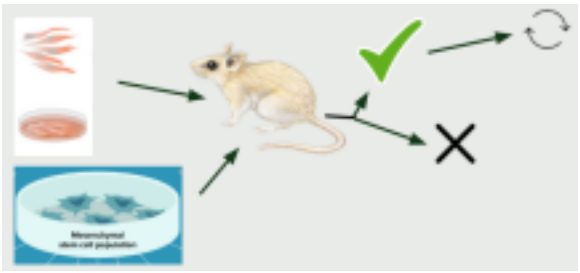
The Effectiveness of MenSCs derived β-cells in-vivo in comparison to IPSCs

Hypothesis and Specific Aims

I propose that Menstrual Stem cells (MenSCs) will allow for less invasive procedures with a greater success rate and provide an exciting niche of stem cell research due to their personalized nature. Through inspecting the effectiveness of MenSCs and Induced-pluripotent stem cells (IPSCs) derived β-Cells in vivo, this study will investigate:



1. The duration of received benefit if successfully implanted
2. The post-acute rejection rate
3. Differences in engraftment rates

Using these endpoints, I will be able to determine if MenSCs can provide a benefit in future treatment of Type 1 diabetes (T1D).

Background

With the ability to self-renew and differentiate into an array of cells that can eventually function as different kinds of tissues[18], stem cells provide a unique opportunity for regenerative medicine. For those with T1D, this points to the opportunity of supplementing in-vitro derived beta cells to help with glucose and insulin regulation.
While stem cells can be derived from a variety of sources a new candidate has emerged, which simultaneously combats social stigma and couples non-invasive procedures with the possibility for more accurate treatment. It has now been found that stem cells can be derived from menstrual blood (known as MenSCs,) which have notably high proliferation rates and offer an exciting course forward[15].

To provide a baseline comparison for the effectiveness of MenSCs, I examined induced pluripotent cells (IPSCs) which have also gained traction and attention for their non-invasive and patient specific qualities[21]. While IPSCs are suspected to be able to differentiate into a wider variety of cells then MenSCs, and both have relatively non-invasive derival aspects, IPSCs have been proven to contribute to tumorgeniracy[13]. This marks the importance of finding alternatively sourced yet equally productive stem cells.

Due to the limited research regarding implementation of MenSCs I found it critical to conduct this trial with non-human subjects. Part of what makes MenSCs so exciting is that they can be easily derived from female patients with T1D, which, after autologous transplantation, could lead to substantially lower rejection rates due to their highly personalized nature. Because of this aspect I decided that the best subject for this trial would be the spiny mouse (acomys cahirinus) due to its similarities during menstruation to humans[2].

While no previous study has studied the duration of effectiveness of MenSC derived beta cells (or truly compared MenSCs and IPSCs), studies have been conducted regarding embryonic stem cells (ESCs), and have found their effective duration to last up to around 8 months in mouse models[17]. It has also been found that IPSC grafts take around 6 months to mature[19], and after examining the two durations, I've determined this

study will last for 9 months to determine superiority over IPSCs.

Research design and methods

This study investigates two main goals, each applied to both MenSCs and IPSCs. The first cohort of mice will look at the successful implantation v.s. rejection rate, as well as duration of received benefit if implanted. The second cohort of mice will examine the difference between success rate and engraftment rate. The third cohort will be the control. While I am using autologous transplantation simply to display the personalized benefits of stem cells, it is imperative for further studies to be conducted with human MenSCs and IPSCs to demonstrate applicability for clinical use.

Each cohort will utilize 10 virgin female spiny mice, which, after nine months, will be euthanized with C02 after being exposed to isoflurane (to anesthetize). Maturation for spiny mice takes around 3 months[8], so all subjects will be taken from the 3 to 4 month range.

All mice will be induced with T1D through streptozotocin, with the goal to destroy beta cells and create insulin deficiency. A marker of induced diabetes is when β-cells make up less than 50% of a blood sample, and all mice must meet this requirement before being used in the experiment[4].

To derive the menstrual blood to MenSCs, the blood will be obtained on the second day of the mices' cycle[20]through a vaginal lavage after exposure to isoflurane. The blood must be kept in a solution based on phosphate buffered saline (PBS) and penicillin[20,5]. The mixture should be processed into the lab within 24 hours, during which it is kept at a cool temperature (<4*C)[5]. Ficol paque is added into the concentration to assist in separation of cells, with centrifusion taking place for around 10 minutes[20,7]. The buffy coat is collected, washed in PBS, and cultured with different nutrients (ex. Dulbecco's modified Eagle's medium/nutrient mixture F12 (DMEM/F12) (GIBCO, USA))[20]in a 20 well plate. Non-adherent cells should be removed by washing with PBS, and remaining cells should remain untouched until over 75% confluency is reached[20]. Derived MenSCs should be tested for containing CD90, CD29, CD105, and CD7 markers and not exhibiting CD34, CD45 and CD133 cell markers (which indicate a lack of differentiation)[1].

Skin fibroblasts are reprogrammed for the IPSCs. These skin grafts are derived from the tail of each spiny mouse, in an area after 2 cm of the tip (with the cut not exceeding 1 cm)[10]. This is imperative, as while spiny mice do experience constant regenerative properties within their cells, the effect is lessened in the tail. To provide the most accurate comparison possible between what this experiment looks like in mice and what it might look like in humans, the skin fibroblasts should not be collected from any other area.
After preparing specimens (through removal of the protective epidermis and mincing the rest,)[9]they should be placed in ten wells and cultured with the Dulbecco's modified Eagle's medium/nutrient mixture and penicillin[20,6]. The Yamanaka factors should be used to induce pluripotency[14], with the reprogramming taking place through the Sendai viral system (specifically through the method discovered by Dr. Akira Kunitomi and co.)[12]. A few days after the fibroblasts receive the reprogramming factors they should be switched to culture conditions on which iPSCs will begin to form colonies over the next couple weeks. Colonies should be expanded with Dulbecco's modified Eagle's medium/nutrient mixture before being tested for OCT4, SSEA3, and TRA-1-60 markers[20, 16]. Successfully differentiated IPSCs should be kept in feeder free systems.

Both groups of stem cells must then be processed into β cells. For IPSCs, cells should be plated onto Matrigel and supplemented with growth support and Activin A Nutrients and ITS should continue to be added, with the addition of Retinoic Acid (RA)[1]. A low glucose base should now be implemented, with adhesion and differentiation supplements[1]. Finally, Nicotinamide should be added, completing the IPSC to β cell differentiation[1].

For MenSCs, cells should be placed on a nutrient solution which supplements growth as well as receive RA[11]. Cells should then be dissociated and placed onto rat-tail collagen coated well plates and

Nicotinamide added (with continued additions of factors to help growth and differentiation)[11]. Finally, Exendin-4 should be implemented to complete the MenSCS to β cell differentiation[11].
Both groups should be stained with Dithizone (DTZ) to identify insulin (as zinc adheres to it), photographed through a microscope, and processed through an image identification system[11]. After training, AI should rank, sort, and determine the staining intensity and efficiency between the two groups of stem cells[11].

This quantitative data should be noted as a base point of initial success, to which further findings can be compared.

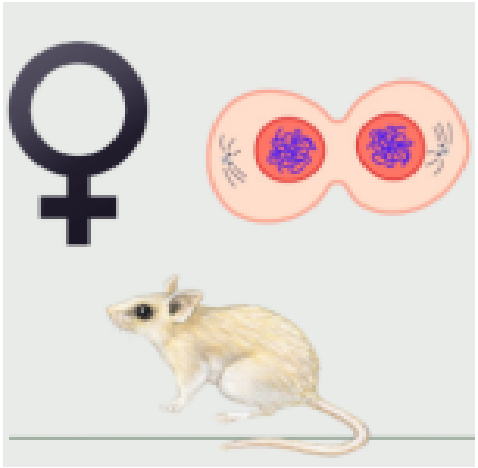
Ten mice will then be injected with β cells derived from MenSCs encapsulated with saline and delivered under the kidney capsule. The other cohort will receive the same treatment but with IPSCs encapsulated with saline, and the control group receiving only saline. Non fasting blood glucose will be checked daily. Once a week, mice will be fasted for 5 hours. After that, an initial glucose test will be conducted before mice are injected with glucose through the tail vein[3]. Glucose will be measured after the first 10 minutes, and then at 30 minutes, 60 minutes, 90 minutes, and 120 minutes. When the initial blood is drawn, a small sample should be diverted for an ELISA test to determine insulin efficiency. The ELISA protocol should also take place on a weekly basis. The duration of impact provides the length of received benefit after successful implementation. No change in insulin would represent a failure of engraftment.

A small section of the pancreas should, directly before euthanasia but after anesthesia, be removed for a histological analysis, in which insulin and proliferation markers must be stained for. Statistical tests and a trained AI program will compare timed glucose levels, insulin secretion, histological markers, staining rates, and engraftment rates for the three cohorts to determine success.

Approval must be obtained from the Institutional Animal Care and Use Committee (IACUC and the Institutional Biosafety Committee (IBC). OHSU does not require approval from the Stem Cell Research Oversight Committee (SCRO), but similar regulations might be applied by an adjacent group.

Summary and significance

The proposed study investigates and compares the efficiency of beta cells derived from IPSCs and MenSCs. Because some concepts of this study are so novel, almost no research has been done that could provide evidence for a solid prediction on whether MenScs or IPSCs might be superior. However, using methods such as timing glucose levels that returned to normal at the quickest rate, an increase in insulin secretion, histological markers showing high proliferation, vibrant stains to indicate zinc and insulin, and limited rejection rates, baseline data could be developed.



But it is important to acknowledge that there are some limitations. Previous research has discovered that spiny mice have the continual ability to regenerate, meaning they don't undergo fibrosis or even scarring for skin and tissues (an ability humans don't have)[8]. Therefore while the organism is important as it successfully models menstruation, it may have more robust regenerative properties than a human would. Additionally, to attempt to prove how personalized medicine and autologous

transplantations can lower rejection rates, all mice used in this study were female. This adds another layer of uncertainty on the widespread implications of the proposed research, constraining all applicable results to only females.

And finally, and perhaps most importantly, to further prove the benefits of autologous transplantation, both MenSCs and IPSCs were derived from the spiny mice. This means that it is crucial for further research with human MenSCs and IPSCs to be utilized (both in experiments using animals and humans) to solidify our understanding of stem cells interactions with different systems.

Despite these limitations, this study opens new doors. It puts emphasis on the gender-based disparities of the medical field; medical properties of menstrual blood have, until recently, not been researched. If MenSCs are able to become a viable and efficient source of treatment, then they could be incredibly effective while fighting social stigma at the same time.

References

1. Alcayaga-Miranda, F., Cuenca, J., Luz-Crawford, P., Aguila-Díaz, C., Fernandez, A., Figueroa, F. E., & Khoury, M. (2015). Characterization of menstrual stem cells: angiogenic effect, migration and hematopoietic stem cell support in comparison with bone marrow mesenchymal stem cells. *Stem Cell Research & Therapy*, *6*(1). https://doi.org/10.1186/s13287-015-0013-5

2. Bellofiore, N., Rana, S., Dickinson, H., Temple-Smith, P., & Evans, J. (2018). Characterization of human-like menstruation in the spiny mouse: comparative studies with the human and induced mouse model. *Human Reproduction*, *33*(9), 1715–1726. https://doi.org/10.1093/humrep/dey247

3. Benedé-Ubieto, R., Estévez-Vázquez, O., Ramadori, P., Cubero, F. J., & Nevzorova, Y. A. (2020). Guidelines and Considerations for Metabolic Tolerance Tests in Mice. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *13*, 439–450. https://doi.org/10.2147/DMSO.S234665

4. Cabrera, S. M., Chen, Y.-G., Hagopian, W. A., & Hessner, M. J. (2015). Blood-based signatures in type 1 diabetes. *Diabetologia*, *59*(3), 414–425. https://doi.org/10.1007/s00125-015-3843-x

5. Chukwu, P. (2025). *Menstrual stem cells and their promise | Signals Blog*. Signalsblog.ca. https://www.signalsblog.ca/menstrual-stem-cells-and-their-promise/

6. Claeys, L., Lidiia Zhytnik, Ventura, L., Wisse, L. E., Elisabeth, Pals, G., Bravenboer, N., Heine, V. M., & Micha, D. (2024). In Vitro Modelling of Osteogenesis Imperfecta with Patient-Derived Induced Mesenchymal Stem Cells. *International Journal of Molecular Sciences*, *25*(6), 3417–3417. https://doi.org/10.3390/ijms25063417

7. Costa-Garcia, M., Moya-Borrego, L., Alemany Bonastre, R., & Moreno Olié, R. (2024). Optimized protocol for culturing menstrual blood-derived MSCs for combination with oncolytic adenoviruses in cancer treatment. *Molecular Therapy: Oncology*, *32*(4), 200907. https://doi.org/10.1016/j.omton.2024.200907

8. Gaire, J., Varholick, J. A., Rana, S., Sunshine, M. D., Doré, S., Barbazuk, W. B., Fuller, D. D., Maden, M., & Simmons, C. S. (2021). Spiny mouse (Acomys): an emerging research organism for regenerative medicine with applications beyond the skin. *Npj Regenerative Medicine*, *6*(1), 1–6. https://doi.org/10.1038/s41536-020-00111-1

9. *Guidelines for Tissue Collection for Genotyping of Mice and Rats*. (2024). https://oacu.oir.nih.gov/system/files/media/file/2024-12/b3-rodent-genotyping.pdf

10. Hare, E., & Hydzik, A. (2018). *Tail Regeneration Depends on Neural Stem Cells | UPMC*. UPMC | Life Changing Medicine. https://www.upmc.com/media/news/081318-neural-stem-cells

11. Kuebler, B., Aran, B., Flores, R., Pérez-Jurado, L. A., Veiga, A., Corominas, R., & Cuscó, I. (2020). Generation of induced pluripotent stem cells (iPSCs) by retroviral transduction of skin fibroblasts from four patients suffering Williams-Beuren syndrome (7q11.23 deletion). *Stem Cell Research*, *49*, 102087. https://doi.org/10.1016/j.scr.2020.102087

12. Kunitomi, A., Hirohata, R., Arreola, V., Osawa, M., Kato, T. M., Nomura, M., Kawaguchi, J., Hara, H., Kusano, K., Takashima, Y., Takahashi, K., Fukuda, K., Takasu, N., & Yamanaka, S. (2022). Improved Sendai viral system for reprogramming to naive pluripotency. *Cell Reports Methods*, 100317. https://doi.org/10.1016/j.crmeth.2022.100317

13. Lee, A. S., Tang, C., Rao, M. S., Weissman, I. L., & Wu, J. C. (2013). Tumorigenicity as a Clinical Hurdle for Pluripotent Stem Cell Therapies. *Nature Medicine*, *19*(8), 998–1004. https://doi.org/10.1038/nm.3267

14. Liu, X., Huang, J., Chen, T., Wang, Y., Xin, S., Li, J., Pei, G., & Kang, J. (2008). Yamanaka factors critically regulate the developmental signaling network in mouse embryonic stem cells. *Cell Research*, *18*(12), 1177–1189. https://doi.org/10.1038/cr.2008.309

15. Nikoo, S., Ebtekar, M., Jeddi-Tehrani, M., Shervin, A., Bozorgmehr, M., Vafaei, S., Kazemnejad, S., & Zarnani, A.-H. . (2014). Menstrual blood-derived stromal stem cells from women with and without endometriosis reveal different phenotypic and functional characteristics. *Molecular Human Reproduction*, *20*(9), 905–918. https://doi.org/10.1093/molehr/gau044

16. Ramirez, J.-M., Gerbal-Chaloin, S., Milhavet, O., Qiang, B., Becker, F., Assou, S., Lemaître, J.-M., Hamamah, S., & De Vos, J. (2011). Brief Report: Benchmarking Human Pluripotent Stem Cell Markers During Differentiation Into the Three Germ Layers Unveils a Striking Heterogeneity: All Markers Are Not Equal. *Stem Cells*, *29*(9), 1469–1474. https://doi.org/10.1002/stem.681

17. Rezania, A., Bruin, J. E., Riedel, M. J., Mojibian, M., Asadi, A., Xu, J., Gauvin, R., Narayan, K., Karanu, F., O'Neil, J. J., Ao, Z., Warnock, G. L., & Kieffer, T. J. (2012). Maturation of Human Embryonic Stem Cell–Derived Pancreatic Progenitors Into Functional Islets Capable of Treating Pre-existing Diabetes in Mice. *Diabetes*, *61*(8), 2016–2029. https://doi.org/10.2337/db11-1711

18. Tian, Z.-Y., Yu, T., Li, J., Wang, T., & Higuchi, A. (2023). Introduction to stem cells. *Progress in Molecular Biology and Translational Science*, *199*, 3–32. https://doi.org/10.1016/bs.pmbts.2023.02.012

19. Wang, X., Gao, M.-X., Wang, Y., & Zhang, Y. (2022). The progress of pluripotent stem cell-derived pancreatic β-cells regeneration for diabetic therapy. *Frontiers in Endocrinology*, *13*. https://doi.org/10.3389/fendo.2022.927324

20. Wu, Q., Wang, Q., Li, Z., Li, X., Zang, J., Wang, Z., Xu, C., Gong, Y., Cheng, J., Li, H., Shen, G., & Dong, C. (2018). Human menstrual blood-derived stem cells promote functional recovery in a rat spinal cord hemisection model. *Cell Death & Disease*, *9*(9). https://doi.org/10.1038/s41419-018-0847-8

21. Youssef, A. A., Ross, E. G., Bolli, R., Pepine, C. J., Leeper, N. J., & Yang, P. C. (2016). The Promise and Challenge of Induced Pluripotent Stem Cells for Cardiovascular Applications. *JACC: Basic to Translational Science*, *1*(6), 510–523. https://doi.org/10.1016/j.jacbts.2016.06.010

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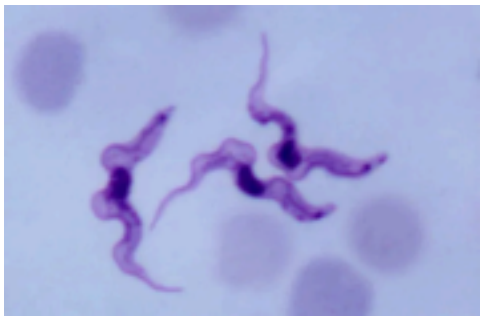
A Conceptual Framework for Treating Chagas Disease During the Chronic Phase Hypothesis and Specific Aims

Image source: Getty Images



Chagas disease caused by *Trypanisoma Cruzi* (*T. Cruzi*), is an infectious disease that has the majority of patients being diagnosed in the chronic phase of *T. Cruzi*. *Benznidazole* is used as a treatment for Chagas disease, but the standard of the 60-day regimen is insufficient with cure rates ranging from 5-20% (Bosch-Nicolau P. et. al, 2024). However, recent studies demonstrate that benznidazole's pharmacokinetic profile, characterized by a half-life sustained tissue exposure, can allow for shorter, lower dose regimens without sacrificing efficacy (Bosch-Nicolau P. et. al, 2024).

The hypothesis of this research proposal is that by modifying the pharmacokinetic exposure of *benznidazole* through reduced dosage, and/or shortened duration regimens, it is possible to maintain a sufficient tissue-level drug concentration to eliminate *T. Cruzi* in individuals. Individuals with the optimized regimens will achieve a non-inferior parasitological clearance compared to the standard in the status quo.

Specific Aim 1. Study consists of evaluating the efficacy of three benznidazole dosage regimens at a standard 60-day, intermediate 30-day, and short 14-day groups in adults with chronic phase Chagas disease using PCR-based diagnostics.

Specific Aim 2. Assess the pharmacokinetics of benznidazole in each regimen, including peak plasma concentration, drug accumulation, and tissue penetration over time. Specific Aim 3. The study will then compare the incidence and severity of adverse drug reactions and treatment discontinuation rates among the three trial groups.

Background

Image Source: Medical News Today

Chagas disease is an infectious disease that is characterized as a condition that stems from the parasite *T. Cruzi*. Patients with Chagas disease typically have been in some form of contact with Triatomine bugs. The two most common ways for patients to contract Chagas disease is either if they've been in Rural parts of the Americas, or if they've had an organ transplant where the donor contracted Chagas disease. Currently, Chagas disease affects more than 8 million people worldwide, with an estimation of 280,000 cases in the US (CDC

N.D.). Other methods of contracting Chagas disease include blood transfusions, organ transplants, and sex linkage (Rassi et. al, 2017).

There are two phases of Chagas disease. The first phase is titled “Acute Phase” where current treatment (*benznidazole)* has been successful in curing Chagas disease. The second phase is titled “Chronic Phase”, where 20-30% of patients in the chronic phase develop serious problems that include heart issues that can lead to heart failure, and digestive problems which can lead to trouble eating or bowel movement (Bosch-Nicolau P. et. al, 2024). The most current and popular treatment for Chagas disease is *benznidazole*, a chemical compound that is taken via oral ingestion. However, current side effects of this medicine include rash, numbness, fever, muscle pain, loss of appetite, and trouble sleeping. Rare side effects include bone marrow suppression, which can lead to low blood cell levels (Andrade, D. V. et. al, 2014).

In the status quo, there is no viable treatment for Chagas disease in the chronic phase, as *benznidazole* has a significant reduction in efficacy once the condition worsens. There is no known viable treatment for successfully treating Chagas disease in the chronic phase at a clinical level. However, current efforts in the world include modifying existing medicine by investigating new regimens to reduce the current side effects of *benznidazole* and to target specific areas that become critical in the chronic phase.

By analyzing pharmacokinetics in individuals that are taking *benznidazole* alongside having *T. Cruzi*, it can enhance the treatment efficacy with the reports pertaining to dosing regimen and systemic exposure. This analysis will help further develop the modified *benznidazole* in the clinical trial.

This study will ultimately prove sufficient by successfully finding a regimen in *benznidazole* that can then be used to treat Chagas disease in the chronic phase by identifying the parasite and the removal of it in the patient’s body. In the status quo, current efforts have been placed in managing the symptoms and complications as the current research shows that Chagas disease provides irreversible organ damage if the patient has Chagas disease for an extended period of time.

Research Design and Methods

This study will be conducted using a randomized, controlled, and an open-label clinical trial to evaluate the efficacy of the three *benznidazole* dose regimens in adults with chronic Chagas disease. The primary goal of the study is to eliminate *T. Cruzi*, determined by PCR-based detection of *T. Cruzi* DNA in peripheral blood samples.

The study population will include adults (16-65 years) with confirmed chronic Chagas disease by doing Enzyme Linked Immunosorbent Assays (ELISAs) and indirect hemagglutination as serologic tests. Those that are pregnant, breastfeeding, or have severe comorbidities will be excluded from the study.

Group A is the control group, consisting of standard-dose *benznidazole* (5mg/day) for 60 days. Group B is the intermediate-dose of *benznidazole* for 30 days. Group C will be the short-course *benznidazole* for 14 days

The sample collection & DNA replication will consist of peripheral blood (5ml) from each participant at the following checkpoints: Baseline (pre-treatment), Day-7 (end-treatment), follow up: 3, 6, & 12 month post-treatment.

Blood will be collected into guanidine-EDTA (GE) buffer tubes to lyse cells and preserve DNA. Samples will then be collected at 4ºC for ≤7 days before processing. Genomic DNA will be extracted using appropriate blood kits. PCR-based detection for *T. Cruzi* consists of highly repetitive satellite DNA of *T. Cruzi* chose high sensitivity in chronic-phase infections. Protocol will follow as a reaction mix (25µL): 2µL template DNA, 0.2 µM of each primer, 200 µM Nucleoside triphosphate (dNTPs), 1.5 mM MgCl2, 1x Taq buffer, and 1.25 U Taq polymerase.

Visualization will consist of amplicons being separated by agarose gel, visualized under UV

illumination. A positive result will indicate detectable parasite DNA in the blood. Negative controls and positive controls (no template & known *T. Cruzi* DNA) will be included in each batch. The primary outcome in the data analysis should show that PCR-negativity at 12-month follow up as a result of the absence of *T. Cruzi* DNA. Alongside treatment adherence, incidence of adverse effects, and dropout rates.

Statistical analysis will include proportional PCR-negative participants at each time point that will then be compared between treatment arms using Chi-Square tests. Kaplan-Meier curves will also model PCR-negativity. Logistic regression will assess associations between treatment response and demographic/clinical factors.

Ethical considerations include informed consent obtained by all participants. These study protocols will be reviewed by institutional ethic boards in the US. All procedures will comply with Good Clinical Practices (GCP) guidelines for the clinical trial.

Summary and Significance

Chagas disease caused by *T. Cruzi* remains one of the most neglected infectious diseases, affecting an estimated 8 million people globally. Many of these individuals are only diagnosed with Chagas disease when they reach the chronic phase of the infection.

While *benznidazole* is the primary anti parasitic treatment, it becomes insufficient in dealing with chronic Chagas disease due to the irreversible organ damage, with cure rates reported as low as 5-20% (Bosch-Nicolau P. et. al, 2024). This research proposal aims to evaluate the safety, tolerability, and parasitological efficacy of modified *benznidazole* regimens.

This study addresses a critical gap in Chagas disease treatment by targeting one of the main barriers to effective care, the poor tolerability of current treatment in the chronic phase. By optimizing *benznidazole* regimens based on pharmacokinetics and patient-centered outcomes, this research has the potential to reshape treatment guidelines and expand treatment access to the thousands of individuals diagnosed with chronic Chagas disease in the US. The proposed use of PCR-based diagnostics provides a highly sensitive and non-invasive method for monitoring parasitological response which proves intrinsic in addressing chronic infections (Hochberg, M.S. et. al, 2021).

Current potential limitations of the study include parasitemia in chronic phase, lack of definitive biomarkers of cure, variability in parasite strains,dropout risk, and limited sample size. However, if successful this study could directly benefit those that are diagnosed with chronic Chagas disease in the US.

References

1. Hochberg, N. S. et. al, (2021). *Chagas disease in the United States: A perspective on diagnostic testing limitations and next steps*. The American journal of tropical medicine and hygiene.
2. Centers for Disease Control and Prevention. (n.d.-a). *Clinical testing and diagnosis for Chagas disease*. Centers for Disease Control and Prevention.
3. Rassi, A. et. al, (2017). *Https://www.sciencedirect.com/science/article/abs/pii/S1047847720300046?via%3Dihub* . ScienceDirect. https://www.med.upenn.edu/pmi/events/https-www-sciencedirect-com-science-article-abs-pii-s1047847720300046-via-3dihub
4. Andrade, D. V. et. al, (2014). *Acute chagas disease: New global challenges for an old neglected disease*. PLoS neglected tropical diseases. https://pmc.ncbi.nlm.nih.gov/articles/PMC4117453/

5. Bosch-Nicolau P. et. al, (2024) *Efficacy of three benznidazole dosing strategies for adults living with chronic Chagas disease (MULTIBENZ): an international, randomized, double-blind, phase 2b trial*. The Lancet Infectious Diseases, Volume 24, Issue 4, pages 386-394.

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Does Precarious Employment Increase the Risk of Cognitive Decline? Hypothesis and Specific Aims

The hypothesis of this study is that precariously employed migrant care workers are at a higher risk of developing Cognitive Decline (CD). This relationship is supported through the positive correlation of chronic stress with CD, which is a common consequence of precarious work. Care workers often work in precarious working conditions, and according to Bagon et al., 2024, care workers in Canada struggle with achieving permanent residency, which places them in positions vulnerable to exploitation by employers. This added stress can ultimately increase the possibilities of chronic stress–potentially leading to CD, highlighting the importance of this study. The aims of this study are (1) to identify the prevalence of CD in care workers, (2) identify the correlation between chronic stress and precarious working conditions (3) to examine whether perceived stress levels in workers can lead to and further exacerbate lower cognitive function, (4) and to determine if loss in cognitive function is reversible.

Background

In the past decade, Precarious Employment (PE), a form of labor characterized by job insecurity, unfavorable working conditions, and limited employee rights, has become widespread in global society (Matilla-Santander et al., 2021). Migrant workers, individuals who relocate for employment opportunities, constitute a large portion of the PE population in Canada(Ornek et al., 2022). Due to their limited opportunities and unstable working conditions, they are at a higher risk of developing health and mental complications. According to a study conducted on the well-being and health of precarious workers (Hsieh et al., 2016a), migrant workers may be at risk of higher stress levels due to factors such as immigration status and language barriers, placing them in positions susceptible to exploitation by employers (Ornek et al., 2022).

Cognitive decline (CD), the progressive loss of thinking skills, memory, and judgment, continues to be a rising concern within migrant care workers. Given the global increase in migrant workers and PE (Bagon et al.,2024), understanding how PE contributes to cognitive function is critical. Such findings will further highlight poor working conditions that impact vulnerable populations, help determine preventative measures to preserve and improve cognitive health, and drive policy reformation in precarious work. Prolonged exposure to stress can activate higher and longer bursts of cortisol levels. When cortisol, also known as the “stress hormone,” is released for longer periods, it can weaken and damage the hippocampus, amygdala, and prefrontal cortex, leading to loss of memory formation, concentration and learning abilities, and emotional processing (Lupien et al., 2018). This places immigrants in PE in extremely vulnerable positions, especially because of their exposure to discrimination, prejudice, and the coerced performance of subordinate tasks through threats of deportation by employers (Hsieh et al., 2016b). The higher amount of job-stressors immigrants have has the ability to further exacerbate stress levels, potentially increasing the probability of lower cognitive function.

Despite these connections, most studies tend to examine the relationship between chronic stress and PE

and other mental health problems (De Looze et al., 2024), but fail to examine the relationship of chronic stress with CD in PE. This study aims to close the gap by examining whether migrant individuals in PE experience loss in cognitive function from the severity of their job-related stressors over a 5-year period, as well as determining whether loss in cognitive function can be regained over time. The study will take place in Canada, due to the escalating number of care workers who are battling immigration status while being forced to undertake precarious working conditions (Kavanaugh, 2024). Despite Canada's pilot programs that were created to establish a pathway to permanent residency for care workers, many care workers have struggled to obtain permanent residency (Kavanaugh, 2024). The execution of this study would provide evidence for a framework for policy reformation in precarious work, as well as educating the public and care workers about the potential long term effects on cognitive function due to precarious work. Therefore, they can seek out early preventative strategies to mitigate stress and engage in stress management strategies to protect their mental and cognitive health.

Research design and methods

This study will utilize a mixed-method longitudinal study design over a 5-year period to study the correlation between chronic stress among immigrant care workers in PE with CD. The study will take place in Canada due to the prevalence of PE and care workers immigration crisis. The independent variable in the study is care worker working conditions. The dependent variables are the development and severity of CD, and perceived stress. CD will be measured quantitatively using the Montreal Cognitive Assessment (MoCA), a validated screening tool assigned to assess mild CD (Dautzenberg et al., 2019). Perceived stress will be measured quantitatively using the Perceived Stress Scale (PSS), a validated questionnaire that assesses the amount of stress one has perceived in recent circumstances (She et al., 2021). Care working environment and stressors will be measured quantitatively using the Job Content Questionnaire (JCQ), a validated questionnaire that assesses the psychosocial aspects of work (Santos et al., 2017). Participants must be migrants who are care workers. Because changes in cognition occur as older age approaches (Matilla-Santander et al., 2021), when the study is first initialized, subjects will be between ages 45 and 60; old enough to develop loss in cognitive function while not having age be the primary factor in the CD. Participants will be recruited through flyers and sign-up sheets at care worker and precarious work support and advocacy groups such as Family Caregivers of BC, Ontario Care Association, and the Vancouver Committee for Domestic Workers and Caregivers Rights. Advertising will also be posted on social media sites such as Facebook and Instagram. The study will include 130 participants and be conducted in a work support or advocacy group with permission granted from the organization.

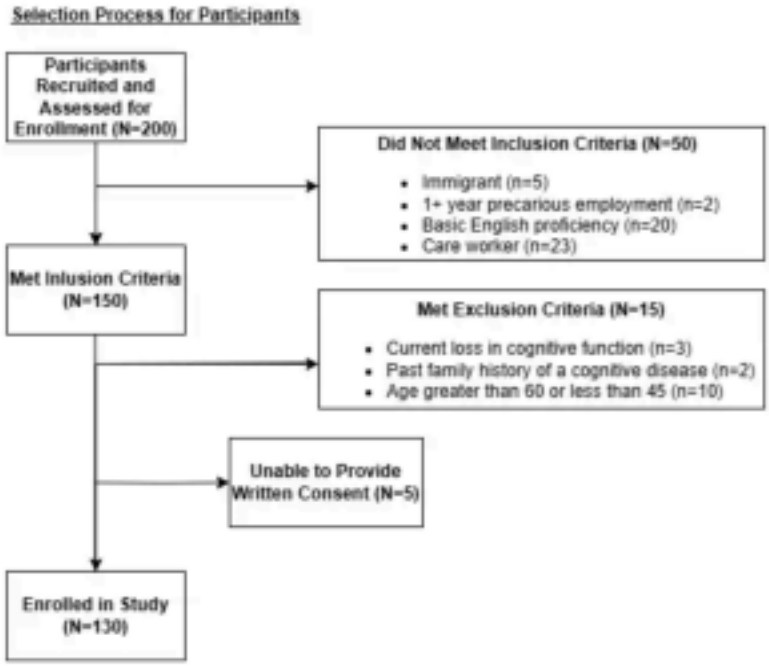


Figure 1: Selection Process for Participants

Quantitative Study

In the quantitative study, to understand the physiological demands and work support participants receive, participants will be asked to complete a Job Content Questionnaire (JCQ), a questionnaire that measures decision latitude, physiological demands, job insecurity, and supervisor and coworker support (Santos et al., 2017). The JCQ includes positively worded and negatively worded questions with answers that range from a scale of 1 (strongly disagree) to 4 (strongly agree) (Santos et al., 2017).

To quantitatively measure the correlation of precarious work and stress, participants will complete the Perceived Stress Scale-14 (PSS), a 14-question questionnaire that assesses a user's perception of their recently stressful circumstances (She et al., 2021). The test consists of positively and negatively worded questions to reduce bias (She et al., 2021), and is answered on a 5-point scale, ranging from responses from never (0) to very often (5). See Appendix A.

To quantitatively measure cognitive function, participants will complete the MoCA, a screening tool designed to assess mild CD. The test assesses visuospatial memory, attention, language, orientation and recall. The test takes approximately 10 minutes and is scored out of 30 points; anything greater than or equal to 26 points being a healthy cognitive function, and less being a low cognitive function (Dautzenberg et al., 2019). To reduce bias, participants with 12 years of education or lower will receive an additional point to take account for education barriers (Dautzenberg et al., 2019). The MoCA will be assigned in the beginning of the study to determine which participants already have CD so they can be excluded to prevent bias in the study's results, and it will also be assessed yearly throughout the 5-year study period to determine the CD participants experienced through their chronic stress.

Qualitative Study:

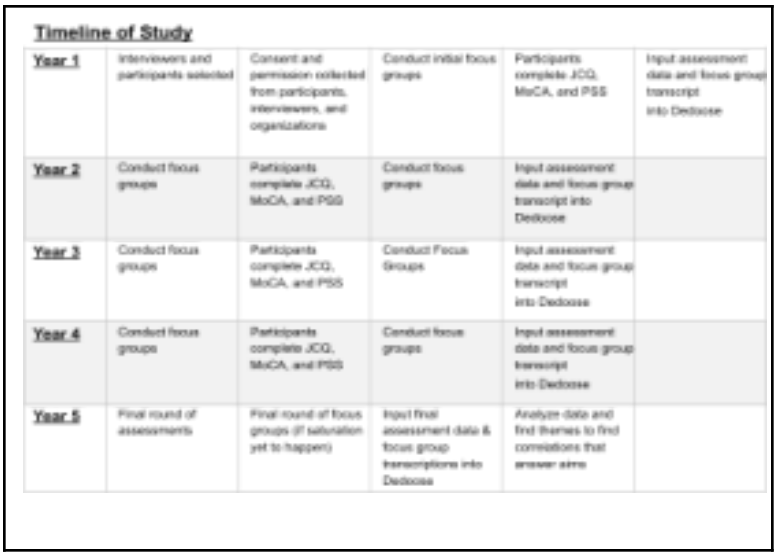
To qualitatively examine the psychosocial stressors care workers endure, recorded focus groups will be conducted throughout the study. Focus groups will first be conducted in the very beginning of the study and then be conducted twice annually until the interviews reach data saturation–a point in time where nothing new is learned from the interviews and all participants share relatively similar experiences with perceived stress and cognitive changes. Participants will be split into groups of 10, and will be asked questions about their current stressors, job-environment, cognitive health, and job-related stressors. Participants will also discuss their immigration status and whether it has contributed to their stress. Although participants will already be taking the PSS, the questions will help interviewers have a deeper understanding on how participants perceive their stress and work life. Two interviewers will be assigned to each focus group to ask and guide participants through the questions. Interviewers will be chosen through care worker advocacy group organizations so participants in the focus groups will be more comfortable sharing their experiences. Interviewers will be required to display proof of their employment and associations with these organizations prior to interviews. To prevent bias occurring during the focus group interpretation, interviewers will be excluded when being selected if they are PE.

Data Analyzation

All quantitative and qualitative data at the end of the study will be analyzed through Dedoose–a web-based application that is designed to analyze mixed-method studies. Quantitative data recorded (PSS, MoCA, JCQ) will be imported along with qualitative transcribed focus group interviews. This information will be analyzed to identify patterns and relationships between cognitive health, perceived stress, and work conditions. This will also be used to examine whether participants with higher perceived stress experienced lower cognitive function and will determine if participants who developed CD at some point throughout the study regained cognitive function.

Ethical Considerations

Permission will be collected from the community and advocacy groups to collect participants and conduct interviews. Written informed consent will be collected from participants, which outlines that participation in the study is voluntary and participants are free to leave any time. Participants will be assigned a subject ID when analyzing survey results to ensure confidentiality.



Timeline of Study

Year 1	Interviewers and participants selected	Consent and permission collected from participants, interviewers, and organizations	Conduct initial focus groups	Participants complete JCQ, MoCA, and PSS	Input assessment data and focus group transcript into Dedoose
Year 2	Conduct focus groups	Participants complete JCQ, MoCA, and PSS	Conduct focus groups	Input assessment data and focus group transcript into Dedoose	
Year 3	Conduct focus groups	Participants complete JCQ, MoCA, and PSS	Conduct Focus Groups	Input assessment data and focus group transcript into Dedoose	
Year 4	Conduct focus groups	Participants complete JCQ, MoCA, and PSS	Conduct focus groups	Input assessment data and focus group transcript into Dedoose	
Year 5	Final round of assessments	Final round of focus groups (if saturation yet to happen)	Input final assessment data & focus group transcriptions into Dedoose	Analyze data and find themes to find correlations that answer aims	

Table 1: Timeline of Study

Summary and significance

This study seeks to examine the lasting consequences of chronic stress experienced by care workers. Using a mixed-method longitudinal design, this study could reveal a correlation between CD and care worker working conditions. Results could also reveal immigration status heightening perceived stress, and loss in cognitive health being regained overtime. These results can open possibilities for driving policy reform in Canada's pilot programs, such as implementing new programs that establish immediate permanent residency for all care workers upon arrival to Canada (Bagon et al.,2024). Results of this study will also spread awareness about the risks of CD in care work so workers could set up preventative strategies to mitigate stress. Limitations that could arise in the study are the results in the JCQ and PSS. Because these tests are self-reported, participants in the study can lie and falsely report answers which could skew the study's results. Another limitation of this study could be the loss of participants throughout the study. If a large percentage of participants drop out of the study, this could create a smaller sample size, which could cause the results of the study to not be enough to represent the majority of the care worker population.

References

Bagon, C., Dominic Calamba, R., Cordero, K., Massie, A., Minh, A., Mũrage, A., Rosario Martinez, N., & Shaw, J. E. (2024). Front Matter A Decade of Migrant Care Worker Programs: Addressing Racism and Precarity in Canada ©.

Matilla-Santander, N., Ahonen, E., Albin, M., Baron, S., Bolíbar, M., Bosmans, K., Burström, B., Cuervo, I., Davis, L., Gunn, V., Håkansta, C., Hemmingsson, T., Hogstedt, C., Jonsson, J., Julià, M., Kjellberg, K., Kreshpaj, B., Lewchuk, W., Muntaner, C., ... Bodin, T. (2021). COVID-19 and

Precarious Employment: Consequences of the Evolving Crisis. International Journal of Health Services, 51(2), 226. https://doi.org/10.1177/0020731420986694

Ornek, O. K., Waibel, J., Wullinger, P., & Weinmann, T. (2022). Precarious employment and migrant workers' mental health: a systematic review of quantitative and qualitative studies. Scandinavian Journal of Work, Environment & Health, 48(5), 327. https://doi.org/10.5271/SJWEH.4019

Hsieh, Y. C., Apostolopoulos, Y., & Sönmez, S. (2016a). Work Conditions and Health and Well-Being of Latina Hotel Housekeepers. Journal of Immigrant and Minority Health, 18(3), 568–581. https://doi.org/10.1007/S10903-015-0224-Y/TABLES/3

Lupien, S. J., Juster, R. P., Raymond, C., & Marin, M. F. (2018). The effects of chronic stress on the human brain: From neurotoxicity, to vulnerability, to opportunity. Frontiers in Neuroendocrinology, 49, 91–105. https://doi.org/10.1016/J.YFRNE.2018.02.001

Hsieh, Y. C., Apostolopoulos, Y., & Sönmez, S. (2016b). Work Conditions and Health and Well-Being of Latina Hotel Housekeepers. Journal of Immigrant and Minority Health, 18(3), 568–581. https://doi.org/10.1007/S10903-015-0224-Y

De Looze, C., McCrory, C., O'Halloran, A., Polidoro, S., Anne Kenny, R., & Feeney, J.(2024). Mind versus body: Perceived stress and biological stress are independently related to cognitive decline. Brain, Behavior, and Immunity, 115, 696–704.https://doi.org/10.1016/J.BBI.2023.10.017

Kavanaugh, J. (2024, September 25). Canada's Broken Promises to Migrant Care Workers—More Pilot Programs Not a Solution— - CCPA. Canadian Centre for Policy Alternatives. https://www.policyalternatives.ca/news-research/canadas-broken-promises-to-migr ant-care-workers-more-pilot-programs-not-a-solution/

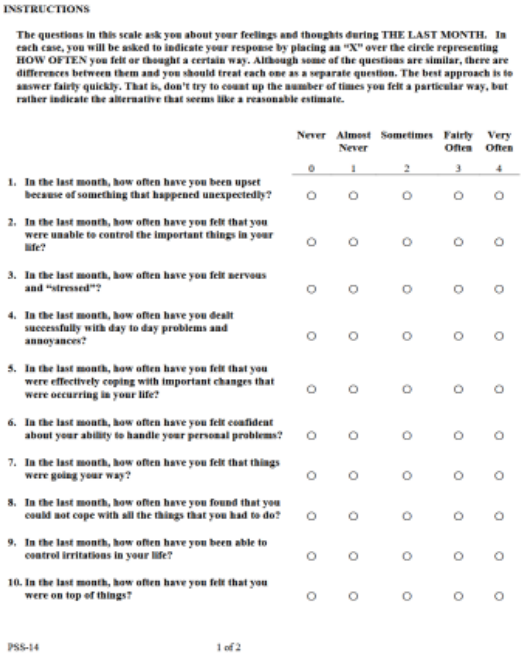
Dautzenberg, G., Lijmer, J., & Beekman, A. (2019). Diagnostic accuracy of the Montreal Cognitive Assessment (MoCA) for cognitive screening in old age psychiatry: Determining cutoff scores in clinical practice. Avoiding spectrum bias caused by healthy controls. International Journal of Geriatric Psychiatry, 35(3), 261.https://doi.org/10.1002/GPS.5227

She, Z., Li, D., Zhang, W., Zhou, N., Xi, J., & Ju, K. (2021). Three Versions of the Perceived Stress Scale: Psychometric Evaluation in a Nationally Representative Sample of Chinese Adults during the COVID-19 Pandemic. International Journal of Environmental Research and Public Health, 18(16), 8312. https://doi.org/10.3390/IJERPH18168312

Santos, K. O. B., Araújo, T. M. De, Carvalho, F. M., & Karasek, R. (2017). The job content questionnaire in various occupational contexts: applying a latent class model. BMJ Open, 7(5),

e013596. https://doi.org/10.1136/BMJOPEN-2016-013596

Appendix A. 8



INSTRUCTIONS

The questions in this scale ask you about your feelings and thoughts during THE LAST MONTH. In each case, you will be asked to indicate your response by placing an "X" over the circle representing HOW OFTEN you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer fairly quickly. That is, don't try to count up the number of times you felt a particular way, but rather indicate the alternative that seems like a reasonable estimate.

	Never	Almost Never	Sometimes	Fairly Often	Very Often
	0	1	2	3	4
1. In the last month, how often have you been upset because of something that happened unexpectedly?	○	○	○	○	○
2. In the last month, how often have you felt that you were unable to control the important things in your life?	○	○	○	○	○
3. In the last month, how often have you felt nervous and "stressed"?	○	○	○	○	○
4. In the last month, how often have you dealt successfully with day to day problems and annoyances?	○	○	○	○	○
5. In the last month, how often have you felt that you were effectively coping with important changes that were occurring in your life?	○	○	○	○	○
6. In the last month, how often have you felt confident about your ability to handle your personal problems?	○	○	○	○	○
7. In the last month, how often have you felt that things were going your way?	○	○	○	○	○
8. In the last month, how often have you found that you could not cope with all the things that you had to do?	○	○	○	○	○
9. In the last month, how often have you been able to control irritations in your life?	○	○	○	○	○
10. In the last month, how often have you felt that you were on top of things?	○	○	○	○	○

PSS-14 1 of 2

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Generating Segmentation to Assist Deep Learning (DL) Models In Becoming Generalizable:

Hypothesis and Specific Aims

Segmentation, the process of delineating structures of interest within an image slice of a certain biological sample[1], in volume electron microscopy (vEM), the process of creating 3D nanoscale images of tissue and cell ultrastructures[1], is one that is often tedious and slow. However, with the introduction of a subsection of machine learning (ML)[2] called deep learning (DL)[2], that enables machines to learn patterns and make predictions based on data they are trained with, researchers have the power to speed up segmentation and get better results faster[1,2]. This is vital in the field of cancer research because vEM images can pick up details that other imaging techniques can't about cell ultrastructures. Thus, my aims and hypothesis are as follows:

Aim 1: Segment structures of interest in various samples of pancreatic cancer tissue in a timely manner in order to give patients the effective treatment they need at the moment.

Aim 2: Train deep learning (DL) models on different sets of data in order to make them generalizable and able to automatically segment structures of interest in other samples.

Hypothesis: By manually segmenting various images of pancreatic cancer tissue samples through vEM, we seek to train deep learning (DL) models to become more accurate, efficient, and generalizable to other pancreatic cancer samples from different patients.

Segmentation of a 90nm cubed block takes about one year, and if we train this DL to be generalizable, meaning that it can segment more than one type of pancreatic cancer sample images automatically, it augments the possibility of personalized and effective treatment for patients. This is the case because from the segmented ultrastructures of the pancreatic cancer tissue images researchers could understand the biology of that cancer better for more efficient treatment.

Background

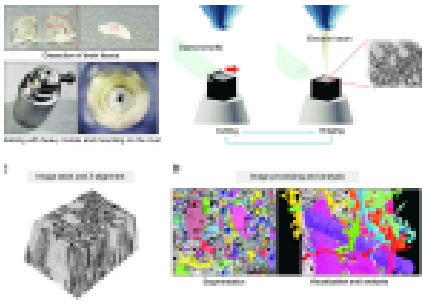
For background information, manual vEM segmentation is the process of manually outlining or delineating structures of interest within an image slice of a certain biological sample[1]; and in this case, we are outlining structures of interest in pancreatic cancer tissue, such as the tumor stroma, which is the tissue surrounding cancer tumors that is made up of malignant cells and normal tissue[3]. This manual segmentation process in vEM can take longer than one year to complete; thus, if researchers needed the information about the cell ultrastructure of a cancer from these images for personalized treatment, they wouldn't be beneficial anymore because the patient's cancer could progress onto the next stage or metastasize.

Thus, with the introduction of DL models in the vEM field, that process data like the human brain with neural networks[2], researchers have the possibility to automatically segment structures of interest within tissue samples and cells. These models for automatic segmentation are still new, but the field of research on them is vastly growing. Hence, training these models to automatically segment data could give cancer researchers the information they need to create personalized treatment for a person's specific cancer. Achieving a generalizable model that can automatically segment images is feasible, as a past study did something similar with DL models and mitochondria. Essentially, in this research project, the researchers trained a DL model to automatically segment stacks of mitochondrial images based on previous segmentation[4]. This project had utilization in discovering the structure of the mitochondria as well, but an issue they did encounter is that it would not work for other cells or tissue samples because DL models like this one are not highly

transferable[1,4,5,6,] or generalizable yet. This is where automatic segmentation with DL models becomes difficult, as they may work really well for one image that has similar data and structures of interest to the one it had been trained upon, and not well for another sample[1,4,5,7,]. However, continuing research in this field can reveal new information about volume electron microscopy, DL models and their transferability, and knowledge about cancer for personalized treatments for patients. Research design and methods:

Step 1: To do this research study, we need to get pancreatic cancer tissue samples from patients. To do this, we would first obtain their informed consent to use their tissue. Then, we would gain approval from an Institutional Review Board (IRB) or ethics committee to ensure our usage of human samples is ethical[8].

Step 2: Following the approval from the IRB, the pancreatic tissue samples need to be fixed, fixation being the process of using solutions like glutaraldehyde to preserve the tissue structures[9], stained, staining being the process of staining the samples with heavy metals to increase contrast in vEM imaging[9,10]; and finally, they need to be embedded into resin to secure and prepare it for sectioning.



Step 3: All of these steps combined creates the block that the volume electron microscope will slice and image[11].This creates the foundation to do a process called serial block face scanning electron microscopy (SBF-SEM)[11]that is continuously slicing 10 nm thick 2D slices from the sample block. This step will be repeated for 6 different pancreatic cancer tissue specimens from 6 different patients, two that are in the early stages (Stages 0 and 1 where the cancer is either at the top of pancreatic duct cells or limited to the pancreas)[12], two that are in the middle stages (Stage 2 and 3 that include a cancer that has grown over the pancreas and to nearby nodes or nearby blood vessels)[12], and two from the later stages of pancreatic cancer (Stage 4, where the cancer has metastasized, or spread, to other parts of the body)[12].

Figure 1: Model of steps done before and after SBF-SEM in vEM (Note: This was done on brain tissue and not pancreatic cancer tissue) (Hyun Kim 2016)[13]

Step 5: Then, we will have a team of researchers segment each of the image slices from the samples from the pancreatic cancer patients in the middle stages of pancreatic cancer, as it is not too far along or too early, and then restack them to see the ultrastructures of the pancreatic cancer tissue.

Step 6: The data from step 5 will then be taken and used to train the DL model, with the goal of creating a DL model that automatically segments and outlines the ultrastructures in these tissues with the data it is given, and other pancreatic cancer tissue images that it was not trained upon, just as in Figure 1.

We will have another team of researchers training the DL model on the data the other team has segmented. And, once the DL model is fully trained on this data, it is possible that it can automatically segment the other 5 samples of pancreatic cancer tissue, if it does in fact turn out to be generalizable. This is the case because the DL model has the power to delineate structures of interest similar to the one it has been trained upon, as it is an AI technology based in discovering patterns and neural networks much like the ones the human brain has[5,6,14,]. Combined with programming and this pre-training, and running other image slices through the DL model, researchers can decipher if it is generalizable or not. Researchers will know if it is generalizable if it can automatically segment and give a comprehensive outlining of the structures of interest in other samples of data that are not the one it has been trained upon. If this is not the case, and the model is not generalizable to other samples, we will repeat the process we did with the pancreatic cancer sample from the middle stages with the early stage and later stage pancreatic cancer tissue

samples, to continuously feed it data to improve it. Then, we will test other samples that have been previously segmented or segmented by other researchers on the model. First, we will see if the model can automatically segment them, and if it does, compare it to the manual work other researchers have done. If not, with approval, we will feed this sample data into the DL model to try and increase its generalizability and transferability to other samples. Experiments similar to ours can be shown through the image in Figure 2.

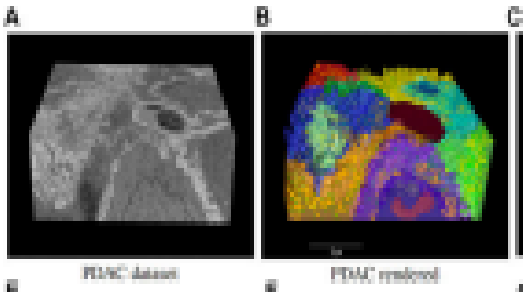


Figure 2: Predictive model of pancreatic cancer ultrastructures using previously segmented data with a DL model (Machiready 2023).[15]

Furthermore, as the experiment goes on, we will also segment more data and continue to train the model to get it as generalizable and efficient as possible. If it does get to that point, its results could be highly beneficial as it will provide feedback about the pathology and ultrastructures of these pancreatic cancer tissues for their patients and their treatments[7,15].

Summary and significance

However, this experiment does have its limitations along with its possibilities. This is an experiment involving manual segmentation which means that it is very timely and costly. It can take up to a year to segment a sample the size of 90nm cubed, as mentioned previously, and paying researchers to do this job for that duration is costly. The research itself on DL models is challenging as well because the model can only be as good as the data it is given, but in this case, it is hard to give the DL model all of the data it needs to segment and predict patterns because that data needs to be manually segmented first. Even if a sample is close to what the DL model is trained upon, it still may be difficult for the model to automatically segment this data accurately.

Even so, this research is still crucial and impactful in this growing field, as it will provide information about what the pitfalls in these DL models are in terms of automatically segmenting different samples of data. If it does work for a sample very similar to one it was trained with, it could provide vital information about what is involved in the pathology and physiology of that stage of pancreatic cancer tissue. Hence, this research is not only crucial for providing information of DL model generalizability and efficiency but information critical for information about pancreatic cancer and tailored treatment.

References

1. Euro-BioImaging Communication. (2022, July 7). *Volume Electron Microscopy: Concepts, Correlations and Computations*.

YouTube.https://www.youtube.com/watch?v=w4qXFvUeklg

2. Federchuk, M. (2022, October 27). *The Difference Between AI, ML and DL*. CENGN.https://www.cengn.ca/information-centre/innovation/difference-between-ai ml-and -dl/

3. Connolly, J. L., Schnitt, S. J., Wang, H. H., Longtine, J. A., Dvorak, A., & Dvorak, H. F. (2003). Tumor Structure and Tumor Stroma Generation. *Holland-Frei Cancer Medicine. 6th Edition*. https://www.ncbi.nlm.nih.gov/books/NBK13447/

4. Conrad, R., & Narayan, K. (2023). Instance segmentation of mitochondria in electron microscopy images with a generalist deep learning model trained on a diverse dataset. *Cell Systems*, *14*(1), 58-71.e5. https://doi.org/10.1016/j.cels.2022.12.00

5. Aswath, A., Alsahaf, A., Giepmans, B. N. G., & Azzopardi, G. (2023). Segmentation in large-scale cellular electron microscopy with deep learning: A literature survey. *Medical Image Analysis*, *89*, 102920. https://doi.org/10.1016/j.media.2023.102920

6. Miltenyi Biotech. (2023, August 8). *Explore the power and benefits of Volume Electron Microscopy*. Bitesize Bio. https://bitesizebio.com/76408/volume-electron-microscopy/

7. 7. A.P.M. Kievits, Lane, R. S., Carroll, E. C., & Hoogenboom, J. P. (2022). How innovations in methodology offer new prospects for volume electron microscopy. *Journal of Microscopy*, *287*(3), 114–137. https://doi.org/10.1111/jmi.13134

8. *Research Using Human Biological Specimens*. (n.d.). Human Research Protection Program (HRPP). https://irb.ucsf.edu/research-using-human-biological-specimen

9. Riesterer, J., Stempinski, E., & Lopez, C. (2019, November 18). *Post-Fixation Heavy Metal Staining and Resin Embedding for Electron Microscopy (EM)*. Protocols.io. https://www.protocols.io/view/post-fixation-heavy-metal-staining-and-resin-embed -36vgre6.html

10. *Embedding in Resin | Electron Microscopy*. (2025). Harvard.edu. https://electron-microscopy.hms.harvard.edu/embedding-resin

11. Courson, J. A., Landry, P. T., Do, T., Spehlmann, E., Lafontant, P. J., Patel, N., Rumbaut, R. E., & Burns, A. R. (2021). Serial Block-Face Scanning Electron Microscopy (SBF-SEM) of Biological Tissue Samples. *Journal of Visualized Experiments*, *169*. https://doi.org/10.3791/62045

12. Lee, S. (n.d.). *Stages of pancreatic cancer*. Canadian Cancer Society. https://cancer.ca/en/cancer-information/cancer-types/pancreatic/staging 13. Kim, G. H., Gim, J. W., & Lee, K. J. (2016). Nano-Resolution Connectomics Using Large-Volume Electron Microscopy. *Applied Microscopy*, *46*(4), 171–175. https://doi.org/10.9729/AM.2016

14. Archana Machireddy, Thibault, G., Loftis, K. G., Stoltz, K., Bueno, C. E., Smith, H. R., Riesterer, J. L., Gray, J. W., & Song, X. (2023). Segmentation of cellular ultrastructures on sparsely labeled 3D electron microscopy images using deep learning. *Frontiers in Bioinformatics*, *3*. https://doi.org/10.3389/fbinf.2023.13087

15. Jadav, N., Velamoor, S., Huang, D., Cassin, L., Hazelton, N., Eruera, A.-R., Burga, L. N., & Bostina, M. (2023). Beyond the surface: Investigation of tumorsphere morphology using volume electron microscopy. *Journal of Structural Biology*, *215*(4), 108035. https://doi.org/10.1016/j.jsb.2023.108035

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Tracking FOXP3-Mediated Immune Dysregulation in Space

Hypothesis and Specific Aims

My hypothesis is that microgravity downregulates FOXP3 expression in regulatory T-cells (Tregs), impairing their ability to control immune responses and contributing to the immune dysregulation observed in astronauts during spaceflight. Human CD4+CD25+ Tregs will be cultured, along with the quantification of FOXP3 mRNA and protein levels through qPCR and Western blot, respectively. Using a rotating wall vessel (RWV) bioreactor to simulate microgravity, Treg behavior in a microgravity environment and in normal gravity will be compared through cell viability assays. This will allow for determination of whether spaceflight conditions alter FOXP3 expression. The consequences of FOXP3 changes on immune regulation will be explored through analysis of expression of FOXP3 downstream targets, particularly Interleukin (IL) 2 and cytotoxic T-lymphocyte associated (CTLA) protein 4, using qPCR and co-culture assays with responder T-cells to gauge functional suppression. These aims construct a proposal to determine whether microgravity conditions impact FOXP3 expression and alter an astronaut's immune regulation in orbit.

Background

The spaceflight environment introduces a range of physiological stressors including microgravity, radiation exposure, and psychological isolation. These stressors collectively disrupt immune homeostasis, compromising the immune responses of astronauts. Previous clinical and experimental data have consistently shown that spaceflight leads to a wide spectrum of immune responses, including reduced T-cell activation, shifts in leukocyte distribution, and reactivation of latent viruses.[1]

Among immune cell types, CD4+CD25+ Tregs are critical for maintaining immune tolerance and suppressing major inflammatory responses.[2] They are defined by expression of the transcription factor FOXP3, which is essential for their development and suppressive function. Therefore, impairment in FOXP3 expression may lead to dysregulated immune responses, as Tregs play a critical role in preventing autoimmune diseases and maintaining immune homeostasis.[3]In the spaceflight environment, microgravity has been shown to reduce T-cell function and alter gene expression.[4]

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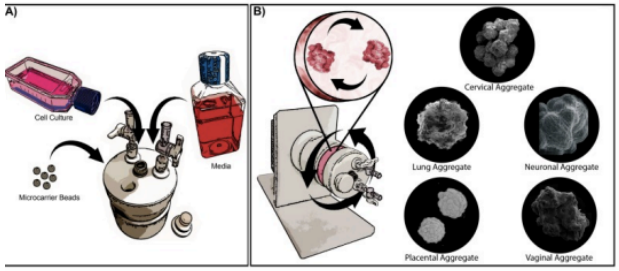

Figure 1. Example of a rotating wall vessel (RWV) bioreactor and the constant rotation motion it is kept in.[7]

Simulated microgravity models, particularly the rotating wall vessel (RWV) bioreactor, have emerged as cost-effective alternatives to in-flight studies.[5] This allows for a controlled environment to study microgravity-induced immune effects, which may have previously been seen as a boundary to learning more about space biology.[6] The RWV bioreactor plays a key role in mimicking the environment in which human Tregs will be impacted.

Despite previous research done on FOXP3 and Treg function, the specific roles in spaceflight-induced dysregulation are yet to be explored. Given the key role of FOXP3 in regulating immune tolerance, studying whether its expression and downstream signaling pathways change under microgravity could reveal a lot about immune dysfunction in space.

This proposed study aims to lay the foundation for a gap in space biology research by identifying whether FOXP3 expression is affected by microgravity. Such findings have the potential to provide information that highlights a more clear relationship between microgravity and astronaut immune system health. By conducting research on FOXP3, the findings can provide insights into the difference between immune tolerance mechanisms in space and on Earth.

Research design and methods

This study will investigate how simulated microgravity affects FOXP3 expression and the functional capacity of Tregs. In vitro methods involving human-derived CD4+CD25+ Tregs with exposure to microgravity using a RWV bioreactor will be used. Experiments will be conducted at three time points: 24, 48, and 72 hours.

Aim 1: Determining how simulated microgravity affects FOXP3 expression in human CD4+CD25+Tregs

The following experimental groups will be established: experimental, control, and functional assay control groups. The experimental group will consist of CD4+CD25+ human Tregs cultured in a simulated microgravity environment using an RWV bioreactor for a set duration. The control group will consist of human CD4+CD25+ Tregs cultured under standard gravity conditions for the same duration as the experimental group.

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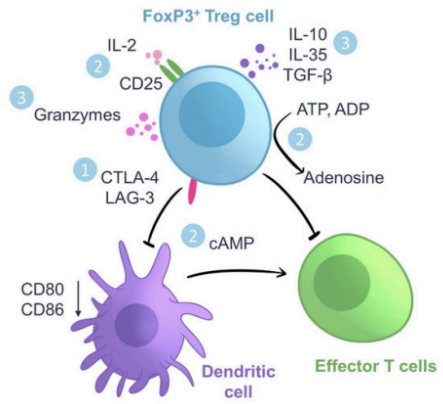

Figure 2. Regulatory T-cell immunosuppression.[8]

Following culture, RNA will be extracted from Tregs using a RNA isolation kit. FOXP3 mRNA levels will be quantified using a PCR thermal cycler and a fluorescent dye. Target genes are FOXP3, CTLA-4, and IL-2. Gene expression will be calculated using the qPCR Double Delta Ct method with housekeeping genes, like GAPDH, as references. Protein will be prepared from each experimental and control group and analyzed for FOXP3 expression using a Western blot. This will validate whether mRNA-level changes will result in a change in protein expression.

Cell viability will be assessed at each time point using trypan blue exclusion assays to confirm that observed changes are not due to differences in survival.

To further explore translational effects of microgravity on FOXP3 expression, a cell-free expression system will be used. FOXP3 mRNA will be translated in vitro under simulated microgravity using the RWV bioreactor. The synthesized proteins will then be visualized via fluorescence and analyzed with a molecular fluorescence viewer. This method allows us to isolate whether microgravity directly interferes with translation mechanisms, independent of cellular stress or regulation. A fluorescence viewer will be used to confirm successful gene amplification under the qPCR and protein synthesis. FOXP3 and other target genes will all be fluorescently labeled so qualitative visualizations can be confirmed.

A power analysis with a biostatistician to determine the minimum number of replicates will be done. Data of expression will be analyzed using the Student's t-test to examine statistical significance. Statistical significance will be defined as $p < 0.05$.

Aim 2: Evaluating whether microgravity-induced changes in FOXP3 expression impair Treg suppressive function

The functional assay control group will consist of CD4+CD25- responder T-cells that will be co-cultured with Tregs from both the experimental and control groups in order to evaluate suppressive function. CD25- responder T-cells represent conventional T-cells whose proliferation is normally inhibited by Tregs. By co-culturing them, the measurement of how well microgravity-exposed Tregs suppress responder T-cell activation can be taken. A loss of suppression would indicate impaired Treg function.

A co-culture suppression assay will be performed to assess whether FOXP3 expression impacts Treg function under microgravity. CD4+CD25- responder T-cells will be labeled with a dye to track cell viability. The responder T-cells will be co-cultured with Tregs from both the microgravity and standard gravity experimental groups at an equal ratio. All experiments will be performed multiple times to make sure it is

reproducible. Samples will be collected at the end of the set exposure period for molecular and data analysis.

This study will involve living human or animal subjects. Therefore, Institutional Review Board (IRB) approval is required and all research will only be done after obtaining this approval. Proper cell handling and biosafety level 2 (BSL-2) precautions will be followed.

Summary and significance

This study investigates how microgravity impacts immune dysregulation by focusing on the transcription factor FOXP3 in regulatory T-cells (Tregs), which are crucial for immune tolerance. Using a rotating wall vessel (RWV) bioreactor to simulate microgravity, we will quantify FOXP3 expression at both mRNA and protein levels and also assess downstream function impacts on immune suppression. Co-culture assays with responder T-cells will be used to determine whether changes in FOXP3 levels correlated with impaired Treg function. Additional assays will be conducted to assess how microgravity influences FOXP3 translation.

Expected results may show that microgravity conditions downregulate FOXP3 expression and impairs the suppressive function of Tregs. This would support the hypothesis that microgravity contributes to immune dysregulation in astronauts in space by reducing Treg activity. This would explain the previously observed increases in autoimmunity during spaceflight. On the other hand, if FOXP3 expression remains unchanged but suppression is still impaired, it means that microgravity affects other regulatory mechanisms.

Limitations of this study would be the use of simulated rather than actual microgravity. This would cause a lack in replicating spaceflight conditions. The RWV model does not account for factors like radiation or psychological stress. In addition, in vitro systems lack the complex model of whole-body immune interactions. To address these issues, data will be analyzed in this context, and future studies could involve in vivo animal models or during spaceflight missions.

Understanding the relationship between microgravity and how it alters FOXP3 expression and Treg function is significant for astronaut health and space exploration. Knowing causes and effects help lay a foundation for future research that can protect immune function during long-term space missions.

References

1. Akiyama, T., Horie, K., Hinoi, E., Hiraiwa, M., Kato, A., Maekawa, Y., Takahashi, A., & Furukawa, S. (2020). How does spaceflight affect the acquired immune system? *Npj Microgravity*, *6*(1). https://doi.org/10.1038/s41526-020-0104-1
2. *NCI Drug Dictionary*. Comprehensive Cancer Information - NCI. (n.d.). www.cancer.gov/publications/dictionaries/cancer-drug/def/cd4-cd25-regulatory-t-cells
3. Rudensky, A. Y. (2011). Regulatory T cells and Foxp3. *Immunological Reviews*, *241*(1), 260–268. https://doi.org/10.1111/j.1600-065x.2011.01018.x
4. Lv, H., Yang, H., Jiang, C., Shi, J., Chen, R., Huang, Q., & Shao, D. (2023). Microgravity and Immune Cells. *Journal of The Royal Society Interface*, *20*(199). https://doi.org/10.1098/rsif.2022.0869

5. Radtke, A. L., & Herbst-Kralovetz, M. M. (2025, January 17). *Culturing and applications of rotating wall vessel bioreactor derived 3D epithelial cell models*. JoVE. https://www.jove.com/t/3868/culturing-applications-rotating-wall-vessel-bioreactor-deriv ed-3d

6. Simons, D. M., Gardner, E. M., & Lelkes, P. I. (2006). Dynamic culture in a rotating-wall vessel bioreactor differentially inhibits murine T-lymphocyte activation by mitogenic stimuli upon return to static conditions in a time-dependent manner. *Journal of Applied Physiology*, *100*(4), 1287–1292. https://doi.org/10.1152/japplphysiol.00887.2005

7. Gardner, J., & Herbst-Kralovetz, M. (2016). Three-dimensional rotating wall vessel-derived cell culture models for studying virus-host interactions. *Viruses*, *8*(11), 304. https://doi.org/10.3390/v8110304

8. Fiyouzi, T., Pelaez-Prestel, H. F., Reyes-Manzanas, R., Lafuente, E. M., & Reche, P. A. (2023). Enhancing Regulatory T Cells to Treat Inflammatory and Autoimmune Diseases. *Int. J. Mol. Sci.*, *24*(9), 7797. https://doi.org/10.3390/ijms24097797

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Determining Early Diagnosis For Pancreatic Cancer Utilizing Combined and Individual DELFI and Galleri Tests

Hypothesis and Specific Aims

I hypothesize that combining the Galleri test to detect methylation patterns with the DELFI test to detect cfDNA fragmentation patterns will significantly increase the confidence in detection accuracy for pancreatic cancer tissue of origin (TOO), as well as the detection accuracy for whether cancer is present. One specific aim of this clinical trial will be to determine the percent accuracy of detecting pancreatic cancer utilizing the DELFI and Galleri in comparison to each test alone. In addition, utilizing a control group of noncancerous patients and non-pancreatic cancer patients, a second aim will be to test the accuracy of the Galleri and DELFI tests, together and alone, at detecting when cancer is and is not present.

Background

Pancreatic cancer is one of the top three leading causes of cancer deaths in the United States.[9] Along with being incredibly invasive and located in a complicated part of the human body, pancreatic cancer has a significant mortality rate because of its consistently late diagnosis.[9] Early diagnosis before the cancer has metastasized has the potential to increase the survival rate of pancreatic cancer up to 6 times, which was found in a study that utilized diabetes mellitus as an indicator of pancreatic cancer when it was still asymptomatic.[1,3] Current early detection techniques are insufficient to impart significant increases in survival time. The most effective early detection methods are genetic tests, imaging techniques, and liquid biopsies. Liquid biopsies have shown promising early detection results for some cancers, and there is great interest in applying these tests to pancreatic cancer.

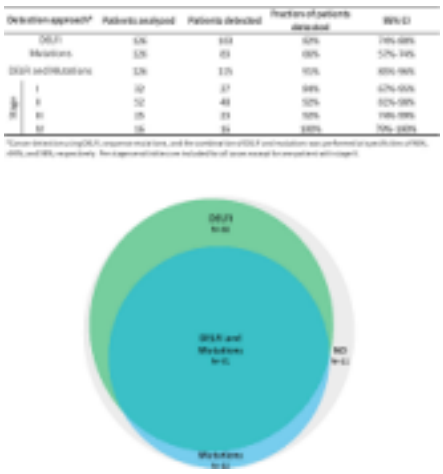

Figure 1: Cancer Detection Using DELFI Test

Different types of blood tests can be utilized for the early detection of cancer and tissue of origin (TOO). Some tests detect methylation patterns in the bloodstream. Methylation patterns are created by chemical modifications that occur when methyl groups are added to cytosines at CpG sites.[8] Cancer affects these methylation patterns and their ability to regulate the modification of gene expression. When cancer is present, abnormal methylation markers are present in the circulating tumor DNA (ctDNA) in the bloodstream. These methylation markers are found using bisulfite sequencing, which converts unmethylated cytosine into uracil. After sequencing the DNA, machine learning analyzes the abnormal methylation markers and compares them with the methylation markers of other cancers to determine TOO.[7]

Along with this technique, fragmentation pattern tests are utilized as an early cancer detection technique. This technique specifically looks at the cell-free DNA (cfDNA) to determine fragmentation signatures, which help to detect cancer early or to determine the TOO.[6]

A particular study by Wenhua Liang et al analyzed DNA methylation sequencing for lung cancer from ctDNA and found that this form of detection has promising results for the
early diagnosis of lung cancer. They tested 288 malignant and benign lung tissue samples along with
132 plasma samples from patients with pulmonary nodules. For the liquid biopsies, they were able to, using 50% of the sample and focusing on comethylation reads, determine that the methylation test had a sensitivity of 79.5% and a specificity of 85.2%[5]. This study demonstrates how methylation blood tests can accurately aid early diagnosis of lung cancer.

Figure 2: Cancer Detection
Using the Galleri test

While methylation tests have shown the ability to detect the TOO by analyzing unique methylation markers, and fragmentation tests have also shown promise in this field through analyzing cancer fragmentation signatures, current technology lacks the ability to detect ctDNA during early cancer stages due to the scarcity of ctDNA in the bloodstream. This makes diagnosis and pinpointing the TOO challenging. However, combining methylation pattern tests with fragmentation tests could generate more consistent and accurate results for the TOO.

Research design and methods

For this experiment, I will utilize 3 different exponential groups. One of which will be a control group of patients who are determined noncancerous through a CT screening process, the second will be a group with other forms of cancer that aren't pancreatic, which will include people with malignant and benign tumors that have gotten biopsied, and lastly a group of patients who have been diagnosed with pancreatic cancer at a variety of stages. With these three groups, I will run the two tests, the Galleri test for methylation patterns and the DELFI to detect fragmentation patterns, on each individual, and compare the rate of accuracy of each test at detecting the TOO for pancreatic cancer and determining if there is cancer present. To detect TOO, I will be utilizing the pancreatic cancer group and testing the rate of accuracy of each test, alone and combined, in determining the TOO. To determine if the tests can detect if cancer is present, I will compare the percent accuracy of the tests alone and combined in determining if there is cancer in the control group and the pancreatic and non-pancreatic cancer groups.

The sample size will be approximately 200 participants, around 100 pancreatic cancer participants and 100 noncancerous and miscellaneous cancer participants, in order for me to have the power to see a statistical significance between the tests. Since I will have to have non-cancerous participants and participants with pancreatic cancer undergo the Galleri test, the DELFI test, and the two tests combined, I will need a large sample size in order to compare the results. This sample size can also account for samples that may be ruled out because of an insufficient amount of ctDNA.

I will limit comorbidities within my pancreatic cancer test group, specifically people with pancreatitis or liver disease. I will also exclude people receiving other cancer treatments, pregnant people, people under 18 or over 80, people recovering from a large injury, and people who have received organ transplants. All of these exclusions are necessary, as including these participants can skew the data because all of these factors impact the cfDNA in the bloodstream.

I will take 21 ml of blood to run both tests on every participant. These participants will all be adults, so this will fit under the restriction of only taking 10.5ml/kg of blood from the participant.[2] For the Galleri test, the cfDNA will be analyzed to determine the methylation patterns present using bisulfite sequencing, then, after the DNA is sequenced, previously developed and trained machine learning will be utilized to determine the TOO. In the DELFI test, the ctDNA is extracted from the liquid biopsy and fully sequenced. It then analyzes the ctDNA fragmentation patterns in the blood and utilizes machine learning to compare the fragmentation patterns against its large data set and match them with a specific type of cancer.

In order to perform this experiment, I will need to get ethical approval from the Institutional Review Board. Along with this, I would need to get approval from GAIL as well as DELFI Diagnostics in order to utilize their tests in my experiment. As resources to aid me in my experiment, I will team up with OHSU's Biostatistics Shared Resource team as well as OHSU 's Cancer Clinical Trials Team. These resources will aid in collecting data, determining statistical power, as well as help conduct the clinical trial.

The data will be presented as percentages, demonstrating the percentage of accuracy each test will show. Out of the participants with pancreatic cancer that underwent each test, I will see how many participants received an accurate diagnosis that they had pancreatic cancer, and compare that with the percentage of participants that received an accurate diagnosis when utilizing both tests. I will also show whether each test had the ability to detect if a participant was cancer-free and whether combining the Galleri and the DELFI tests will increase the accuracy of the results. I am going to present this data in a bar graph, which will display the difference in percent accuracy of each test as well as the tests combined.

Summary and significance

This study will utilize the DELFI test and the Galleri test to determine the TOO for pancreatic cancer patients, and if cancer is present, to see if it yields more accurate results when combining them. I will do this by taking participants with pancreatic cancer, participants with other cancers, and participants who are noncancerous, performing both tests, and comparing the percentage of accuracy each test portrays.

The results will be demonstrated with percent accuracy for cancer detection and TOO detection. Experimental groups include Galleri alone, DELFI alone, and both tests combined. My hypothesis states that I should see an increase in percent accuracy for detecting pancreatic cancer's TOO, as well as overall cancer detection, when the Galleri and DELFI tests are combined. Alternatively, I could potentially see that Galleri is better at TOO, and DELFI is superior for innate cancer detection. My results will be displayed using a bar graph, which visually shows the difference in percent accuracy.

Some limitations to the study are the wide ranges of participants' ages and sex, which can create varied cfDNA patterns and could affect the outcome of the liquid biopsies. Along with this, in the early stages of cancer, there is often not enough cfDNA in the bloodstream for the tests to be able to work accurately, which could potentially cause issues within the experiment. One way to mitigate this issue is to enhance detection sensitivity through pre-analytical techniques, such as timely processing of blood samples and utilizing cell-stabilizing collection tubes. [4,7]

Overall, this study is of vital importance because pancreatic cancers have an incredibly high mortality rate, partly because early detection is so difficult. This experiment could open the doors to the accurate early detection of pancreatic cancer and save thousands of lives each year through early intervention, along with the development of early cancer therapies.

References

1. Aggarwal, G., Rabe, K. G., Petersen, G. M., & Chari, S. T. (2012). New-onset diabetes in pancreatic cancer: A study in the primary care setting. Pancreatology, 12(2), 156–161. https://doi.org/10.1016/j.pan.2012.02.003

2. Blood Draw Guidance | Research A to Z. (2022). Umich.edu. https://az.research.umich.edu/medschool/guidance/blood-draw-guidance

3. Kenner, B. J., Go, V. L. W., Chari, S. T., Goldberg, A. E., & Rothschild, L. J. (2017). Early Detection of Pancreatic Cancer. Pancreas, 46(10), 1238–1241. https://doi.org/10.1097/mpa.0000000000000939 4. Lee, J.-S., Cho, E. H., Kim, B., Hong, J., Kim, Y., Kim, Y., Jang, J.-H., Lee, S.-T., Kong, S.-Y., Lee, W., Shin, S., & Song, E. Y. (2024). Clinical Practice Guideline for Blood-based Circulating Tumor DNA Assays. Annals of Laboratory Medicine, 44(3), 195–209. https://doi.org/10.3343/alm.2023.0389 5. Liang, W., Zhao, Y., Huang, W., Gao, Y., Xu, W., Tao, J., Yang, M., Li, L., Ping, W., Shen, H., Fu, X., Chen, Z., Laird, P. W., Cai, X., Fan, J.-B., & He, J. (2019). Non-invasive diagnosis of early-stage lung cancer using high-throughput

targeted DNA methylation sequencing of circulating tumor DNA (ctDNA). Theranostics, 9(7), 2056–2070. https://doi.org/10.7150/thno.28119

6. Medina, J. E., Dracopoli, N. C., Bach, P. B., Lau, A., Scharpf, R. B., Meijer, G. A., Andersen, C. L., & Velculescu, V. E. (2023). Cell-free DNA approaches for cancer early detection and interception. Journal for ImmunoTherapy of Cancer, 11(9), e006013. https://doi.org/10.1136/jitc-2022-006013 7. Sivapalan, L., Kocher, H. M., Ross-Adams, H., & Chelala, C. (2020). Molecular profiling of ctDNA in pancreatic cancer: Opportunities and challenges for clinical application. Pancreatology. https://doi.org/10.1016/j.pan.2020.12.017

8. Swarup Sonar, Sidhanti Nyahatkar, Ketki Kalele, & Manab Deb Adhikari. (2024). Role of DNA methylation in cancer development and its clinical applications. Clinical and Translational Discovery, 4(1). https://doi.org/10.1002/ctd2.279

9. Why Is Pancreatic Cancer So Deadly? (2019, March 7). University of Utah Health | University of Utah Health. https://healthcare.utah.edu/healthfeed/2019/03/why-pancreatic-cancer-so-deadly

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5TIF4 CAR-T Cell Therapy as a New Treatment for Eosinophillic Esophagitis

Hypothesis and Specific Aims

I hypothesize that the introduction of 5TIF4 cells using CAR-T cell therapy will reduce the histopathological abnormalities, high esophageal eosinophil counts, and elevated levels of IL-4, IL-5, and IL-13 associated with Eosinophilic Esophagitis in a mouse model. Eosinophilic Esophagitis (EoE) is a chronic autoimmune disease caused by increased eosinophils in the esophageal lining. In previous studies, 5TIF4 cells, a type of specialized cell formed of chimeric antigen receptor (CAR) T cells, were engineered and used to treat type 2-high asthma by targeting eosinophils and modulating inflammatory cytokine signaling. In this study, 5TIF4 cells will be introduced into mice with induced Eosinophilic Esophagitis via CAR-T cell therapy. I expect a reduction in esophageal intraepithelial eosinophilia. In addition to lowering eosinophils, I expect this treatment to both reduce histological and immunological markers associated with Eosinophilic Esophagitis, including epithelial and lamina propria thickening, a form of inflammation in the mucosal lining of the digestive tract, and subepithelial fibrosis, characterized by scar tissue buildup in the affected area. The effects of CAR-T therapy in mice with induced Eosinophilic Esophagitis will be assessed by comparing histological analyses of post-mortem esophageal tissue samples between treatment and placebo groups.

Background

Eosinophilic Esophagitis (EoE) is a chronic immune system disease caused by a buildup of eosinophils in the esophageal lining that is usually triggered by acid reflux, allergens, or food. Eosinophils are white blood cells that protect you from allergens, parasites, and other outside organisms. A build-up of eosinophils can cause narrowing, scarring, inflammation, and damage to the esophagus, making it hard to swallow and potentially causing food to become impacted.

Current treatment includes dietary elimination, proton pump inhibitors (PPIs), corticosteroids, and monoclonal antibodies. (Mayo Clinic, 2023). However, these therapies have limited effectiveness. PPIs show a 50% histologic response rate. Corticosteroids range between 68%-77% effectiveness, and dietary elimination varied from 45%-90%, depending on the food restrictions (Syverson & Fox, 2022).

An emerging treatment strategy is Chimeric Antigen Receptor (CAR)-T cell therapy, typically used to treat blood cancers, is now being explored in chronic inflammatory diseases. CAR-T cell therapy re-engineers T-cells into a more efficient version that recognizes and attacks a specific target. (Cleveland Clinic, 2024)

Recently, there was a clinical trial involving CAR-T cell therapy used to target eosinophils as a treatment for asthma. At Tsinghua University, 5TIF4 Cells were created, which, when introduced into the body via CAR-T cell therapy, target eosinophils while inhibiting cytokines, such as IL-4 and IL-13, which contribute to asthmatic inflammation. These cells were engineered by using a TIF program of their invention. They did a genome screening with CRISPR to help edit and eliminate BCOR and ZC3H12A, sending the T cells into an “Immortal-like and Functional T-cell” state. Then, IL-5 cytokine, which acts as the antigen-binding domain, was administered into the TIF program as IL-5 CAR-T cell therapy through gene editing. Afterward, a mutant IL-4 cytokine was introduced into the mix to prevent and inhibit IL-4 and IL-13 long-term, which will help reduce inflammation and tissue damage (Jin et al., 2024).

While 5TIF4 cells were created to become an asthma treatment, I believe they could be adapted to target eosinophils in the esophagus. EoE and Asthma have similar inflammatory pathways. Both conditions trigger a type 2 immune response, which is the overreaction of IL-4, IL-13, and IL-5 cytokines.

Overproduction of these cytokines draws eosinophils to the tissue. Due to asthma and EoE being so similar in that sense, this form of treatment for Eosinophilic Esophagitis could go hand-in-hand.

In preclinical studies, Eosinophilic Esophagitis mouse models are induced with Eosinophilic Esophagitis using oxazolone (OXA), which causes mice to mimic histopathological features of Eosinophilic Esophagitis in humans. It replicates esophageal intraepithelial eosinophilia (the presence of eosinophils within the epithelial layer), epithelial and lamina propria thickening (the thickening of the epithelial tissue and the underlying connective tissue layer), basal cell hyperplasia (an increase in the number of basal cells in the epithelium), and fibrosis (the formation of excess fibrous connective tissue resulting from injury or inflammation) (Dsilva, Avlas, Rhone, Itan, & Munitz, 2024).

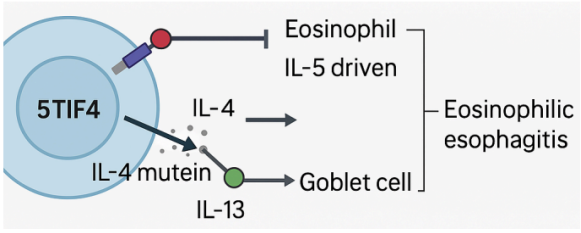

Figure 1 shows a working model of the 5TIF4 fotthe treatment of EoE (adapted from Jin et al)

Research design and methods

In this experiment, there will be four experimental groups. Group 1 will consist of healthy, untreated mice and will be the control group. These mice will be used as the baseline example for regular esophageal histology and eosinophil counts. Group 2 will consist of mice with experimentally induced eosinophilic esophagitis (EoE), but these mice will receive no treatment. Group 2 will provide the baseline for the pre-treatment disease status, this will provide a reference point for both the control and treatment groups. Group 3 will also consist of mice with induced EoE, however, these mice will be treated with the 5TIF4 cells using CAR-T cell therapy. This group will help assess how well the treatment reduces histopathological and immunological changes associated with EoE.

Group 4 will consist of EoE-induced mice that are treated with CAR-T cells. These T cells will be transduced using a scrambled non-targeting guide RNA (gRNA) sequence. These cells will have the same isolation, activation, and transduction steps as the 5TIF4 CAR-T cells, except they will not target the IL-4 Mutein due to the scrambled gRNA. This group serves as a control for the non-specific effects of the CAR-T cell treatment. All 4 groups will be maintained on the same diet and environment to reduce the risk of confounding variables, which could affect the results.

To create the 5TIF4 Cells, we will first isolate T cells from the mice's spleens and lymph nodes. Next, to activate the extracted T Cells, we'll use anti-CD3/CD28 antibodies in the presence of IL-2, IL-7, and IL-15, and then transduce with CAR-encoding lentivirus, with sgRNAs, which will target the IL-4 Mutein construct. Group 4 will follow the same protocol, except that it has non-targeting scrambled gRNAs. Both CAR-T cell treatments will be administered on the same schedule.

After that, we will induce the 3 mouse groups with EoE following the method procedure; On day 0,

apply 15 µl OXA to one or both sides of each of the mice's ears for a total of 60 µl per mouse. On day 7, apply 15 µl of 0.5% OXA to the mice's ears. Repeat this on days 9, 11, 14, and 16. Collect 100 µl of blood from the mice and then spin the blood for 10 min at 3000 × *g*, 4oC. Carefully transfer the serum to a new tube and dilute the serum 1:50 in PBS to measure IgE levels. For the oral gavage portion, modify a plastic feeding tube by making eight holes near the base with a 25G needle. On day 18, prepare a 1% OXA solution by mixing olive oil and 96% ethanol. Vortex the mixture for 10-25 minutes until the powder is dissolved. Use a 1-ml syringe with the modified plastic feeding tube to deliver 200 µl of 1% OXA or vehicle solution through the mice's esophagus. Repeat the garbage three times per week for seven more doses (on days 21, 23, 25, 28, 30, 32, 35) (Dsilva, Avlas, Rhone, Itan, & Munitz, 2024). After this, the mice will receive CAR T cells without conditioning. To assess the Immune responses, euthanize the mouse groups and then use ELISA, flow cytometry (including t-SNE) to study the function and effects the CAR T cell production had, and esophagus histology.

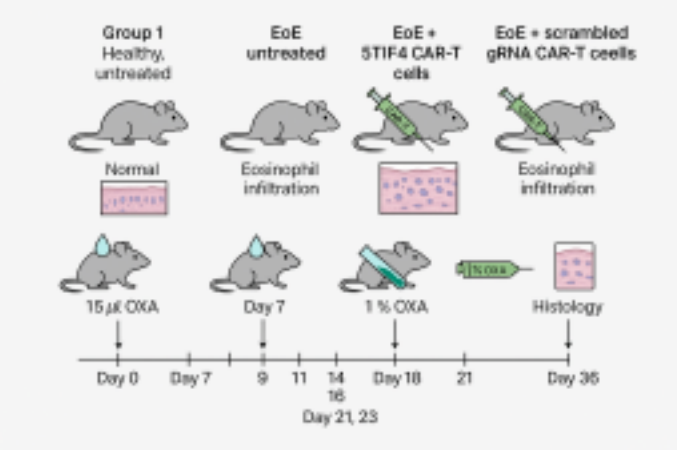

Figure 2 shows the timeline of the experimental design. 4 mouse groups were used to study EoE and CAR-T therapy effects, including controls, untreated EoE, 5TIF4-treated, and scrambled gRNA-treated mice. Key steps include OXA induction, CAR-T cell delivery, and tissue analysis.

Summary and significance

I hypothesize that 5TIF4 cells infused by CAR-T Cell therapy will reduce the histopathological abnormalities and immunological changes associated with Eosinophilic Esophagitis in a mouse model. Specifically, I expect to see reduced esophageal eosinophilia, IL-5, IL-4, and IL-13 levels, reduced intraepithelial eosinophilia, less epithelial and lamina propria thickening, and minimized subepithelial fibrosis. If successful, histopathological analysis of post-mortem esophageal tissue from treated mice should resemble the healthy control group and show significant improvement compared to untreated EoE mice. These results would indicate that 5TIF4 CAR-T therapy is effective in reducing inflammation and immunological damage in the diseased model. This could position 5TIF4 CAR-T cell therapy as a potential treatment for EoE in humans if effectively evaluated. If this experiment were successful, it could introduce another, and possibly more effective, treatment option for the many patients suffering from EoE.

However, certain limitations need to be considered. While EoE mouse models replicate histopathological features in humans, they could struggle to mimic the immune system interactions and other non-histopathological features that could be caused by EoE. Additionally, the study's short observation period may not accurately represent the long-term effects, durability, or recurrence of the disease following the CAR-T treatment. Another limitation is that the study mainly focuses on tissue changes and the cytokine levels for the results, but it doesn't measure how well the mice will be able to swallow or use their esophagus following the treatment, which is important for those with EoE. Finally, the CAR-T cell therapy may not react the same in every mouse, which can affect the results.

References

Mayo Clinic. (2023, July 2). *Eosinophilic esophagitis: Symptoms and causes*. Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/eosinophilic-esophagitis/symptoms-causes/syc-20372197

Cleveland Clinic. (2024, February 14). *CAR T-cell therapy*. Cleveland Clinic. https://my.clevelandclinic.org/health/treatments/17726-car-t-cell-therapy

Jin, G., Liu, Y., Wang, L., He, Z., Zhao, X., Ma, Y., Jia, Y., Li, Z., Yin, N., & Peng, M. (2024). A single infusion of engineered long-lived and multifunctional T cells confers durable remission of asthma in mice. *Nature Immunology, 25*(6), 1059–1072. https://doi.org/10.1038/s41590-024-01834-9

Dsilva, A., Avlas, S., Rhone, N., Itan, M., & Munitz, A. (2024). A Mouse Model for Eosinophilic Esophagitis (EoE). *Current protocols*, *4*(2), e993. https://doi.org/10.1002/cpz1.993

Syverson, E. P., & Fox, V. L. (2022). Update on emerging pharmacologic therapies for patients with eosinophilic esophagitis. *Gastroenterology & Hepatology*, *18*(5), 219–229. https://doi.org/10.1097/MCG.0000000000001592

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The effects of oral supplements on Atopic Dermatitis

Hypothesis

Myself included, millions of people suffer from an immune mediated skin disease called Atopic Dermatitis (AD) worldwide. Though injection and topical AD treatments are available, the focus of this study is to explore an alternative treatment by researching the potential benefits of the interplay of the gut microbiome and systemic immunity to relieve symptoms of AD.[11] Thus, I hypothesize that oral supplemental antioxidants and probiotics will relieve the effects of AD by reducing inflammation and gut microbiome dysbiosis more effectively than topical AD steroids. Mice models of AD will model the effects of the chronic skin disease. The mice will take two supplemental antioxidants, Curcumin and Quercetin, that decrease the production of pro-inflammatory IgE and eosinophils, as well as a probiotic known as Bacillus Subtilis that could reduce the colonization of a skin bacteria known as Staphylococcus Aureus (S-Aureus) which exacerbates AD inflammation.[1-3]The mice on these supplemental measures as opposed to the mice on topical AD steroids are projected to demonstrate less cytokines in their skin, and exhibit less irregularities of the strata of the dermis in skin biopsies. They are also projected to demonstrate less S-Aureus in their stool samples indicating less bodily inflammation. The aim of this study is to determine if such oral supplements could significantly counter the detriments of the prevalent skin disease that is AD, and be more effective than topical AD steroids.

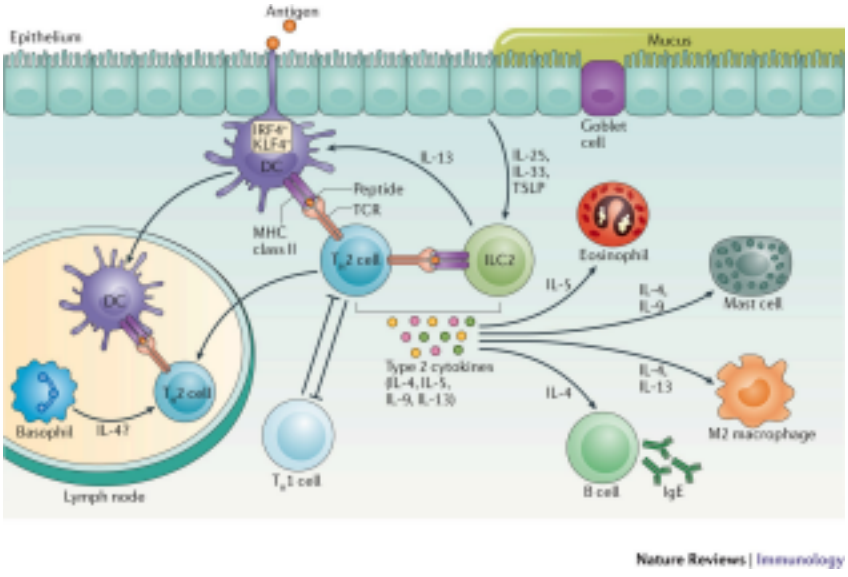

Figure 1: Cytokines of the immune system in response to Atopic

Background:

Atopic Dermatitis also known as eczema is a chronic inflammatory skin disease caused by a compromised skin barrier and immune dysregulation.[4]It is characterized by dry skin, red and itchy inflamed patches of rash and possible pus and oozing.[4] For people with AD, they have less filaggrin and antimicrobial peptides (AMP).[4] Less filaggrin results in the excessive

activation of the Th2 immune response which activates epithelial cell derived cytokines such as IL-4, IL-9, and IL-13, leading to extreme inflammation.[4] Less AMPs lead to decreased ability to fight off unintended bacteria like S-Aureus that colonize 80% of the skin of those with eczema, and only 10% of the skin of those without eczema.

Curcumin is a chemical derived from turmeric that is known for its anti-inflammatory properties.[1] Quercetin is a flavonoid plant pigment that also has anti-inflammatory properties.[2] Both of these antioxidants operate by inhibiting the production of IgE antibodies and T helper type 2 cells (Th2 cells), which release mediators like mast cells and basophils, and eosinophils which release cytokines and damage tissue.[6]

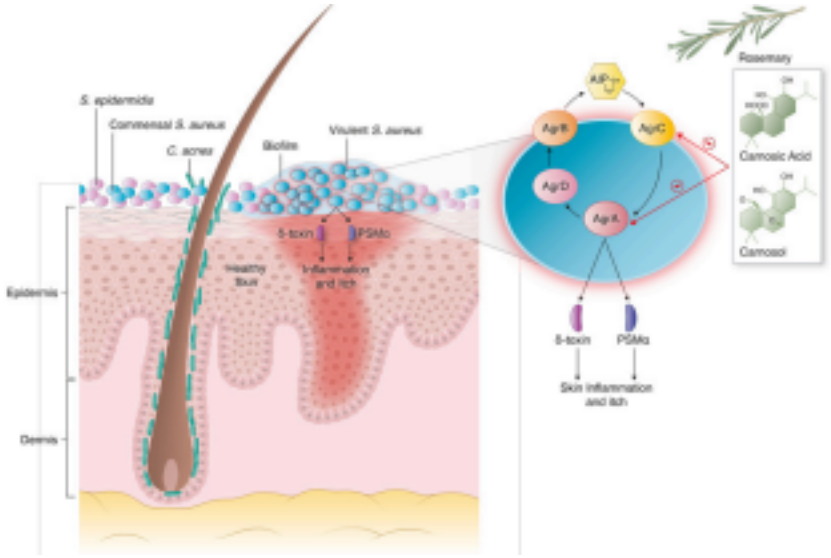

Figure 2: S-Aureus colonization on the epidermis

Bacillus Subtilis is a type of beneficial bacterial probiotic. It is used in this experiment as it has been shown to produce an AMP known as Ba49 that has strong antibacterial properties against S-Aureus.[3]It is used as an oral intervention against S-Aureus, due to how the skin and gut are interrelated, thus skin infections could cause microbial dysbiosis of the gastrointestinal tract by entering the body through the bloodstream.[3] The opposite is also true in how gut dysbiosis affects skin homeostasis which would exacerbate eczema symptoms from S-Aureus.[5]

On the other hand an AD steroid cream like hydrocortisone, are topical drugs that reduce inflammation by preventing the release of Phospholipase A2, an enzyme that breaks down the cell membrane's phospholipids and inflammatory pathways.[7] 1% hydrocortisone is one of most commonly available and used mild topical corticosteroids.

Research design and methods

To model human AD, the Nc/Nga mouse strain, a spontaneously occurring inbred mouse model of AD that demonstrates skin lesions, bred from Nagoya University in 1957, will be used.[8] Mice are required for this experiment in order to perform blood tests, skin biopsies and physical assessments. Prior to the experiment, consultation from the Institutional Animal Use and Care Committee (IAUCC) will be received, and any changes to protect animal welfare will be implemented. During the experiment, the mice will be carefully monitored and taken care of to the maximum extent of comfort, and after the experiment all groups of mice

will be euthanized. There will be two groups with three subgroups of 16 AD mice each three weeks old. Group 1 will consist of non-AD mice and be the negative control. Group 2 will consist of the AD mice and be the experimental pool. Within both groups 1 and 2, there will be the subgroups. Sub 1 will be the mice with no reductive aid, sub 2 will be the mice on the 1% hydrocortisone steroid, and sub-3 will be the mice on supplements of Curcumin, Quercetin, and Bacillus Subtilis. The six groups will be brought up in the same environments for 16 weeks, and after, the results of their AD will be recorded using three tests.

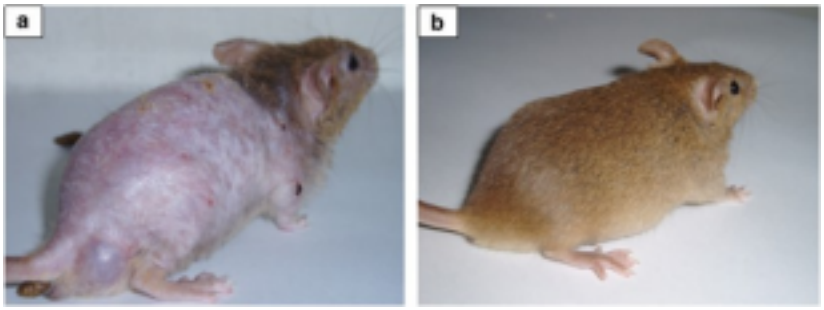

Figure 3: a) Nc/Nga AD model mouse
b) Control mouse

The first test will determine the amount of S-Aureus on the skin by employing the RODAC agar plate technique.[9] All the mice will be prepped by having all their skin shaved off and placed stomach down and back up on a sterilized surface. RODAC plates with convex agar meniscus of type Baird Parker Agar (BPA) that are 25 cm^2 in area will be placed on the mice's right back below the L1 region of their spinal cord. The plate will be then incubated at 35 degrees Celsius for 24 hours. At 24 hours the bacteria, specifically the S-Aureus, will have grown in colonies of the BPA and are easily isolated as they are identified by their distinct black color and white circumference. To quantify the bacteria, the number of colonies will be identified by their appearance, counted, and documented.

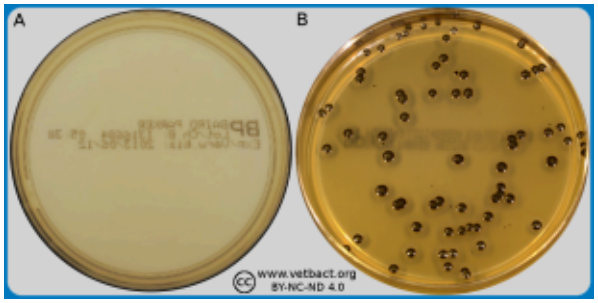

Figure 4: RODAC plates with S-Aureus colonies

The second test will determine the amount of Th2 cells, known for releasing pro-inflammatory cytokines to measure the amount of inflammation caused by AD. Enzyme Linked Immunosorbent Assay (ELISA) will be employed using the mouse Th2 ELISA kit.[10] The blood of the mice will be used as the sample. It will be collected by sanitizing the skin on the lateral side of the right hind leg of the mouse, and a needle will be inserted into the saphenous vein to draw the blood to be kept in a fridge at 6 degrees Celsius until needed. In the ELISA kit, the capture antibodies of the cytokines IL-4, IL-9, and IL-13 coat each of the respective wells of the plates specific to the cytokine. To the plate, 0.2 mL of the blood samples of the three mice groups are piped into 16 wells each. The plate is then incubated at 25 degrees Celsius for one hour before the blood is washed out and detection antibodies are added to the corresponding cytokine wells which are then washed out as well. Finally the enzyme linked solution containing Horseradish Peroxidase (HRP) is added, and the substrate solution that is subsequently added will react to the enzyme to produce a color change. This color change represents the amount of the varying cytokines in the mice blood samples, and this is measured by a microplate spectrophotometer of which the results are graphed.

The third test will be a qualitative examination of the levels of AD of the mice groups by taking

photographs of the mice's skin of the left back below the L1 region of their spinal cord. The size of the skin's dry patches and inflammation will be observed, and the surface area will be measured in cm to compare the extent of AD.

Summary

This study will compare the effectiveness of oral supplemental antioxidants and probiotics compared to topical steroids in treating AD. The Nc/Nga mouse breed models AD in humans and are the subject of the experiment. Effects of AD, such as inflammation by cytokine releasing Th2 cells are measured by Th2 ELISA. Exacerbatory S-Aureus colonies that are prevalent on AD skin are quantitatively examined using RODAC plating. The hypothesis is that the antioxidants, Curcumin and Quercetin, will result in less Th2 incited inflammation and a lower amount of cytokines in the blood of the AD mice, and that the probiotic Bacillus Subtilis will decrease S-Aureus colonies on the skin. It is predicted that the mice on supplements will demonstrate less inflammation and S-Aureus, which will prove that oral treatment is more effective than topical treatment.

However, potential limitations are the time frame, the breed of mice, and that Bacillus Subtillis may not sufficiently target surface S-Aureus colonies. Since the mice are put on the diet for 16 weeks, even if the oral supplements prove to be effective, the experiment does not demonstrate that their effects are immediate. Also, despite how the Nc/Nga mice model inflammation and skin lesions similar to AD in humans, their internal organs, specifically their intestine, differ and could hence absorb or react to the supplements differently than how humans would. Also, though Bacillus Subtillis may circulate throughout the body and help defend against surface S-Aureus marginally, it may mainly target S-Aureus in the gut as it is an oral probiotic.

If oral supplemental antioxidants and probiotics are more effective at treating AD, it could be a big breakthrough in better assisting the millions of people suffering from effects of the disease.

References

1) Peng, Ying, et al. "Anti-Inflammatory Effects of Curcumin in the Inflammatory Diseases: Status, Limitations and Countermeasures." *Drug Design, Development and Therapy*, vol. 15, 2 Nov. 2021, pp. 4503–4525, https://doi.org/10.2147/DDDT.S327378.

2) Li, Yao, et al. "Quercetin, Inflammation and Immunity." *Nutrients*, vol. 8, no. 3, 15 Mar. 2016, p. 167, www.ncbi.nlm.nih.gov/pmc/articles/PMC4808895/.

3) Taggar, Ramita, et al. "Deciphering the Antibacterial Role of Peptide from Bacillus Subtilis Subsp. Spizizenii Ba49 against Staphylococcus Aureus." *Frontiers in Microbiology*, vol. 12, 19 Aug. 2021, https://doi.org/10.3389/fmicb.2021.708712. Accessed 21 Sept. 2022.

4) Afshari, Moeina, et al. "Unraveling the Skin; a Comprehensive Review of Atopic Dermatitis, Current Understanding, and Approaches." *Frontiers in Immunology*, vol. 15, 4 Mar. 2024, https://doi.org/10.3389/fimmu.2024.1361005.

5) Nelson, Steve. "Staph & Eczema: A Skin-Duo You Need to Know About." *National Eczema Association*, 28 Oct. 2022, nationaleczema.org/blog/staph-eczema-a-skin-duo-you-need-to-know-about/. 6) Stone, Kelly D., et al. "IgE, Mast Cells, Basophils, and Eosinophils." *Journal of Allergy and Clinical Immunology*, vol. 125, no. 2, Feb. 2010, pp. S73–S80, www.ncbi.nlm.nih.gov/pmc/articles/PMC2847274/.

7) Gabros, Sarah, and Patrick M. Zito. "Topical Corticosteroids." *PubMed*, StatPearls Publishing, 10 July 2023, www.ncbi.nlm.nih.gov/books/NBK532940/.

8) Suto, H., et al. “NC/Nga Mice: A Mouse Model for Atopic Dermatitis.” *International Archives of Allergy and Immunology*, vol. 120 Suppl 1, 1999, pp. 70–75, pubmed.ncbi.nlm.nih.gov/10529609/, https://doi.org/10.1159/000053599.

9) Li, Yaru, et al. “Comparative Analysis of Sampling Methods for Assessing Bacterial Contamination on Hospital Partition Curtains: Moistened Swabs versus RODAC Agar Plates.” *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*, vol. 29, 23 Sept. 2023, p. e941086, pubmed.ncbi.nlm.nih.gov/37740479/, https://doi.org/10.12659/MSM.941086. 10) Alhajj, Mandy, et al. “Enzyme Linked Immunosorbent Assay (ELISA).” *PubMed*, StatPearls Publishing, 23 Apr. 2023, www.ncbi.nlm.nih.gov/books/NBK555922/. 11) Wiertsema, Selma P et al. “The Interplay between the Gut Microbiome and the Immune System in the Context of Infectious Diseases throughout Life and the Role of Nutrition in Optimizing Treatment Strategies.” *Nutrients* vol. 13,3 886. 9 Mar. 2021, doi:10.3390/nu13030886

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Influence of Different Genres of Music on the Performance of Young Adults

Hypothesis and Specific Aims

We hypothesize that the presence of background music will generally increase a young adult's performance level, with classical music having the most beneficial effect on an individual's performance during a cognitive task due to its slower tempo and rhythm. This study will focus on the influence of different genres of music, or no music at all, when played in the background for an individual performing a task. Specifically, the participants will be separated by exposure to a lack of music, classical music, pop music, rap music, and heavy metal music. The participant's performance levels will be measured through the recorded task completion time and the accuracy of the performed task. Then, through statistical processes, the relationships between various genres of music and the individual's performance will be examined. Therefore, this study aims to identify the genre of music that is the most beneficial to a young adult's performance. The results of this research could have practical implications for educational and work settings, offering guidance on whether and what types of music may be used to optimize performance levels.

Background

As distractions grow continuously with the rise of digital media and electronic devices such as cellphones and laptops, as well as various ways to digital multitask, there is a rising demand for tools that aim to help one focus and increase productivity. Working at cafes and libraries has become increasingly popular, but music is also a prevalent tool used to increase productivity. Often, young adults, particularly high school and college students, utilize music to induce focus when studying. A popular belief among students is that lofi music or classical music can aid in one's studying process by increasing attention and performance. Previous studies have suggested that music is effective at reducing stress and has a positive influence on the attention of the listener1-2. Music can be a difficult medium to evaluate due to its many varieties and subtypes. The specific rhythm, tone, instrumentation, and other features that set it apart from other genres could have unknown impacts on how the brain functions in attention and focus. There have been studies that offer limited insight into the correlations between types of music and performance. One study suggests that music with lyrics had a significant negative impact on the concentration of workers when utilized as background music during work 3. Furthermore, specific genres of music, such as classical music and hip-hop, were found to have positive effects on the performance of medical students when played during standardized exercises4. There also seemed to be a correlation between one's preference for the style and type of music and the effect of the music on the attention and performance of the individual2,5. However, there had been limited studies that examined the relationship between the various general genres of music and its effects on the performance of students. Understanding the rising demand for focus and attention in

today's fast-moving world, this study aims to help students identify the type of music that will be the most effective when it comes to helping them focus and increase attention to achieve better academic success in an academic setting.

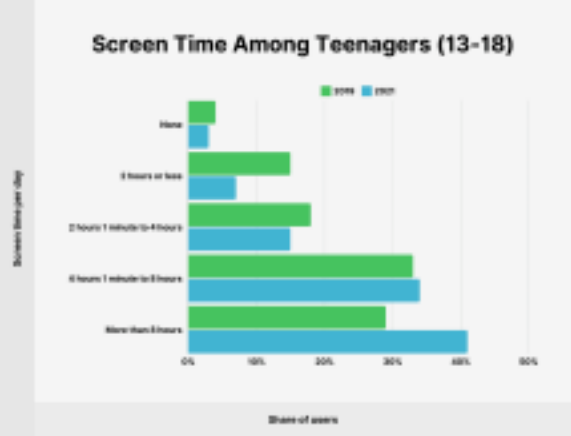

Figure 1: Screen Time exponentially increased from 2019 to 2021

Research design and methods

This study will utilize randomized controlled trials (RCTs) to examine the effect of different genres of music on cognitive performance, defined as solving basic algebra problems.

A total of 200 participants, ranging from ages 16 to 24, will be randomly selected tbased on the following inclusion criteria: currently enrolled in an educational institution and have received a general health check-up within the past twelve months that indicate no significant physical health issues or cognitive impairments. Those who do not fit within the inclusion criteria will not be selected for this study. Participation will be voluntary, and informed consent will be obtained prior to inclusion in the study, although the hypothesis will not be mentioned to the participants to prevent any bias. Participants will also be informed that there is a possibility of exposure to explicit words or songs, and be debriefed afterwards with regards to the study.

The study will involve two major experimental groups, each involving 100 participants. In both experimental groups, participants will be randomly assigned to different conditions in an attempt to avoid selection bias.

Group 1 will include two smaller sub-groups:

Music group (M): Participants will be exposed to a playlist featuring a variety of randomly selected songs from random music genres.
No music group (NM - Control): Participants will complete the task without any background music, serving as the control group and providing comparison to the M group.

Group 2 will explore the genre-specific effects of music, and participants will be randomly assigned to one of five sub-groups: Classical music, Pop music, Rap music, Heavy metal music, and No music (Control).

Group 2 participants of the same sub-group will be exposed to the same song on repeat, as listed below:
Classical music: Eine kleine Nachtmusik by Wolfgang Mozart
Pop music: Style by Taylor Swift
Rap music: Damn by Kendrick Lamar
Heavy metal music: One by Metallica

For both experimental groups, participants will be placed in similar individual rooms, each with a table and a chair. When the timer starts, all participants will be given a pencil to complete the same cognitive task: solving 10 basic algebra problems that do not require calculator use, factoring, and the quadratic formula. At the same time that the timer starts, songs given to the participant's group will begin playing. Throughout the time that it takes for the participant to complete the cognitive task, observations about the participant's behavior will be taken.

The independent variables will be the presence of music in experimental group 1, and the genre of music in experimental group 2. The dependent variable will be task completion time (measured in seconds) and accuracy of the task performed (scored out of 10 based on the correctness of the mathematical answers).

Participants' performance will be evaluated based on the two dependent variables: accuracy and time taken to complete the task. To assess the differences in performance across groups, Analysis of Variance (ANOVA) will be used.

For Experimental Group 1 (M vs. NM), a one-way ANOVA will be conducted to compare the means of the two groups. If the ANOVA yields a p-value < 0.05, this could be interpreted as a statistically significant difference in performance between participants exposed to music and those not exposed to music.

For Experimental Group 2 (classical, pop, rap, heavy metal, and no music), a one-way ANOVA will be used to compare the mean performance across the five groups. If the overall ANOVA yields a p-value <0.05, a Tukey's Honestly Significant Difference test will be performed to identify specifically which genres differ significantly from the others. There will be a focus on whether the group exposed to classical music will outperform the others.

Lastly, this study will be required to be sent to an Instituional Review Board (IRB) in order to be reviewed and approved. The board will review the research proposal and ensure that the human subjects involved in this study will be treated according to the ethical guidelines and regulations.

Summary and significance

This study aims to identify the type of music that is most effective when utilized by young adults as background music to increase performance levels. Through examining the time it takes for an individual to complete the task and the success of task completion, this study reveals the impact of classical, pop, rap, heavy metal, and no music on performance. One potential result that might be obtained from this study is that

classical music will be the most beneficial in terms of increasing an individual's performance levels. This could indicate that non-lyrical music and music that was slow in tempo would demonstrate positive impacts that increase levels of performance when utilized as background music while performing a task. On the other hand, the results obtained from this study may not be generalizable due to the possibility of some individuals having test anxiety that prevents them from performing well on standardized tests, thus producing a lower score regardless of the genre of music in the background. This could be addressed in future studies by adding a variety of ways to test for performance levels that are not just limited to standarized testing. Furthermore, the specific music selected to act as background music could fail to represent an entire music genre, thus limiting the effective and generalizability of the study. This could be addressed in the future by selecting an increased amount of songs to better represent a certain genre. Overall, this study will benefit the educational field by suggesting the most beneficial type of music to listen to while studying, thus increasing the overall academic performance levels of individuals. The results would increase the effectiveness of young adults' study sessions and overall aid them in their academic careers.

Figure 2: This study oung adults enhance the effectiveness of study sessions.

References

Linnemann, A., Ditzen, B., Strahler, J., Doerr, J. M., & Nater, U. M. (2015). Music listening as a means of stress reduction in daily life. Psychoneuroendocrinology, 60, 82–90. https://doi.org/10.1016/j.psyneuen.2015.06.008

Huang, R. H., & Shih, Y. N. (2011). Effects of background music on concentration of workers. Work (Reading, Mass.), 38(4), 383–387. https://doi.org/10.3233/WOR-2011-1141 Shih, Y. N., Huang, R. H., & Chiang, H. Y. (2012). Background music: effects on attention performance. Work (Reading, Mass.), 42(4), 573–578. https://doi.org/10.3233/WOR-2012-1410 Nees, L. K., Grozinger, P., Orthmann, N., Rippinger, N., Hennigs, A., Sohn, C., Domschke, C., Wallwiener, M., Rom, J., & Riedel, F. (2021). The Influence of Different Genres of Music on the Performance of Medical Students on Standardized Laparoscopic Exercises. Journal of surgical education, 78(5), 1709–1716. https://doi.org/10.1016/j.jsurg.2021.03.008 Kiss, L., & Linnell, K. J. (2021). The effect of preferred background music on task-focus in sustained attention. Psychological research, 85(6), 2313–2325.
https://doi.org/10.1007/s00426-020-01400-6

Figure 1: https://backlinko.com/screen-time-statistics

Figure2:https://thestroudcourier.com/2024/01/30/how-music-can-improve-you-the-link-between gpa-and-music/

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Longitudinal progression of subcortical white matter injury in mice

Hypothesis and Specific Aims

Post-ischemic casein kinase 2 (CK2) inhibition using the molecule CX-4945 preserves oligodendrocytes, maintains axonal structure and improves functional recovery against selective white matter ischemic injury.

Aim 1: Investigate the progression of white matter ischemic injury in mice using small-animal magnetic resonance imaging (MRI). We will use imaging analysis software (e.x. ImageJ, 3D Slicer) to quantify and analyze changes in white matter longitudinally after ischemic injury. This will allow for a non-invasive evaluation of lesion development and structural alterations in the brain.

Aim 2: Quantitatively determine molecular changes in white matter following ischemic injury using Polymerase Chain Reaction (PCR). We will analyze the expression of key genes related to oligodendrocyte health, inflammation, and axonal integrity in control mice. This will provide a molecular profile of injury progression and establish a reference for comparing future treatment effects.

Background

White matter injury is a major but often under-recognized contributor to neurological dysfunction across a range of disorders, including stroke, traumatic brain injury, and age-related cognitive decline such as Alzheimer's disease and dementia.[1]

Subcortical white matter is vulnerable to injury due to its distinct vascular supply and high metabolic demands, this sets it apart from cortical gray matter, which has historically received more research attention.[1] A clearer understanding of how white matter injury mechanisms develop and progresses over time is essential for creating effective, targeted therapies.

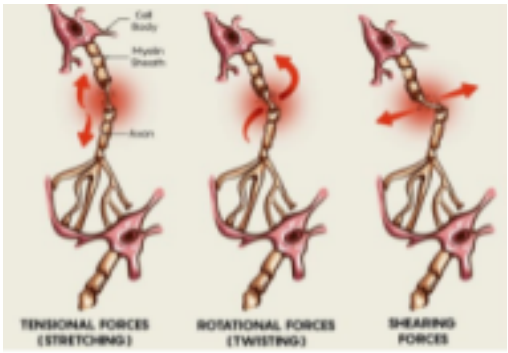

Figure 1. Diffuse Axonal Injury

Despite the clinical relevance, white matter strokes remain underexplored relative to gray matter strokes. A key challenge is that white matter damage involves diffuse axonal injury, where the disruption is more subtle, tearing axonal connections rather than the whole tissue, which makes it harder to detect and characterize with standard methods.[2] This has contributed to the misconception that white matter injuries are less significant, when in fact, they are increasingly recognized as a primary driver of long-term functional impairment.[1, 3] Age compounds this vulnerability. Structural and

functional studies reveal that aging axons exhibit increased diameters, thicker myelin sheaths, and dysfunctional mitochondria characterized by elongation swelling and an overall reduced ATP output.[4] These aging-related changes are also correlated with increasing oxidative stress and compromised calcium homeostasis, primarily due to disrupted mitochondria endoplasmic reticulum interactions. While axonal function may be maintained under normal aging conditions, these adaptations may increase the white matter vulnerability to ischemic injury.[4]

Recent molecular studies identified CK2 as a key mediator in white matter ischemic injury. CK2 via CDK5 and AKT/GSK3β, leading to mitochondrial dysfunction, axonal degeneration and loss of oligodendrocyte support. Inhibition of CK2 with the molecule CX-4945 has been shown to preserve white matter architecture and promote functional recovery post-ischemia. Importantly these protective effects extend to both young and aged brains indicating the therapeutic potential of CK2 inhibitors across different age groups.[3, 5]

Currently, what we know about CK2's role in white matter injury comes from an incomplete picture as opposed to continuous observation. There remains a critical gap in our understanding of how white matter damage evolves over time, particularly in aged brains, and how this correlates with underlying molecular mechanisms and functional decline of the brain.

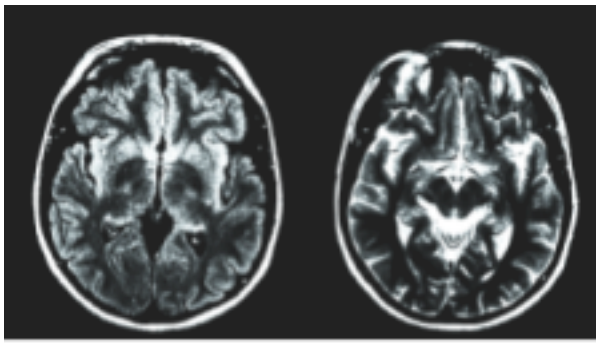

Figure 2: White matter injury progression

This project aims to fill that gap by using high resolution MRI techniques to visualize and quantify subcortical white matter injury overtime through the use of an in vivo selective focal white matter injury model. By integrating advanced imaging analysis tools, we will capture the dynamics of injury progression and potentially link them to biological mechanisms.

Research design and methods

Animal Model:

This study will utilize adult C57BL/6 mice, encompassing both young (3–4 months) and aged (12–14 months) groups to capture age-related differences in white matter response to ischemia. The mice will undergo a selective focal WM ischemia protocol, which involves stereotactic injection of L-NIO into the corpus callosum. This model was chosen for its relevance in studying WM specific injury mechanisms, as highlighted in previous research.[3]

Imaging protocol:

Longitudinal MRI data scans will be used to monitor white matter injury progression. High-resolution scans will be conducted at multiple time points post-injury (days 1, 3, 7, 21, and 28), allowing for a comprehensive temporal assessment of lesion development. This approach aligns with methodologies used in prior studies to evaluate WM integrity over time.[3] These time points will help establish a baseline timeline of injury evolution, which is essential for future studies evaluating therapeutic interventions.

Imaging Analysis:

The MRI datasets will be processed using ImageJ and 3D Slicer software. These platforms enable semi-automated segmentation and volumetric quantification of white matter and help with visualization of the white matter lesions. Quantitative metrics, including lesion volume and signal intensity changes, will be extracted to assess the extent and progression of white matter damage.[3]

Molecular analysis:

At the end of the imaging timeline, white matter tissue, specifically from the corpus callosum, will be collected for molecular testing. RNA will be extracted and analyzed using quantitative PCR to measure the expression of key genes that are related to mitochondria health. (PGC-1α, MFN2), oxidative stress (Nrf2, SOD2), and white matter structure (MBP, PLP1). These genes were selected based on prior studies showing their roles in white matter injury, particularly in relation to the CK2 signaling pathway.[3] Comparing gene expression between young and aged mice, and linking these results to MRI findings, we aim to understand how molecular changes track with the progression of injury. This will help clarify how white matter breaks down after strokes and how age may influence that process.

Statistical analysis

The longitudinal data will be analyzed using repeated measures ANOVA, which is appropriate for tracking changes within the same subjects overtime. Mixed effects models will also be used to account for intra subject variability and to handle the missing data, providing a better statistical framework for the longitudinal design. Correlations between imaging and gene expression results will be explored using Pearson or Spearman tests, as appropriate.[3]

Ethical Considerations:

All of the animal procedures will be conducted in accordance with institutional guidelines and approved by the Institutional Animal Care and Use Committee (IACUC). The use of animal models is essential for studying the complex in vivo interactions involved in white matter injury and recovery, which cannot be fully replicated in vitro.

Summary and significance

This research addresses a critical gap in neuroscience by focusing on the temporal dynamics of subcortical white matter injury in mice. By mapping the progression of ischemic damage using advanced in vivo imaging, this study lays the groundwork for identifying novel therapeutic strategies for ischemic stroke, such as CK2 inhibition. Its findings could advance our understanding of white matter injury and provide therapeutic targets for white matter-related brain disorders. Although white matter's importance in neurological disease is gaining recognition, there is still a lack of clear understanding of how its injuries evolve over time, especially in vivo and within ischemic models. This project directly addresses that gap and has significant therapeutic implication, one example being treating ischemic stroke patients by targeting pathways such as CK2. By establishing a timeline of molecular and structural changes post ischemia, this work lays groundwork for targeted interventions. In particular, it can inform the development and timing of neuroprotective strategies. Using high-resolution MRI and advanced image analysis tools like 3D slicer, we will quantify changes in white matter integrity over multiple time points and correlate them with molecular markers of damage. Focusing on a selective mouse model allows us to capture the natural course of injury in the absence of intervention, which lays the groundwork for future therapeutic studies.

One limitation of this study is its focus on single injury mechanisms and the exclusion of treatment such

as CK2 inhibitors. However, this study allows a clear detailed observation of the injury progression, which is essential before the therapeutic strategies can be meaningfully tested. While the study does not evaluate intervention directly, it sets the stage for future research into protective pathways and pharmacological targets.

Ultimately, this work has the potential to redefine how we study and treat white matter stroke, providing a critical step towards improved clinical outcomes in stroke and other white matter related conditions.

References

1. Marin, M. A., & Carmichael, S. T. (2018). Stroke in CNS white matter: Models and mechanisms. Neuroscience letters, 684, 193–199. https://doi.org/10.1016/j.neulet.2018.07.039

2. Johnson, V. E., Stewart, W., & Smith, D. H. (2013). Axonal pathology in traumatic brain injury. Experimental neurology, 246, 35–43. https://doi.org/10.1016/j.expneurol.2012.01.013

3. Baltan, Selva. (2024). AHA Proposed research plan Sept 1 FINAL. https://docs.google.com/document/d/1PQbdSC6LTowLzQlDuHaiJEh8J9QRVsts/edit?usp=sharing&ouid=109561481514549364708&rtpof=true&sd=true

4. Stahon, K. E., Bastian, C., Griffith, S., Kidd, G. J., Brunet, S., & Baltan, S. (2016). Age-Related Changes in Axonal and Mitochondrial Ultrastructure and Function in White Matter. The Journal of neuroscience : the official journal of the Society for Neuroscience, 36(39), 9990–10001. https://doi.org/10.1523/JNEUROSCI.1316-16.2016

5. Nguyen, H., Zhu, W., & Baltan, S. (2022). Casein Kinase 2 Signaling in White Matter Stroke. Frontiers in molecular biosciences, 9, 908521. https://doi.org/10.3389/fmolb.2022.908521

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Phase III trial: Immunotherapy using Dendritic Cell Vaccines

Hypothesis and Specific Aims

In this experiment, I would like to analyze how Dendritic Cell (DC) Immunotherapy vaccines compare to other treatments on the market when it comes to treating and preventing a recurrence of resected pancreatic ductal adenocarcinoma (PDAC).

My aim of this study is to find whether Dendritic Cell vaccines provide a higher standard of care compared to the standard of care that is currently on the market. Standard of care means that patients should be able to live their lives without a recurrence of cancer. Additionally, I hope to explore patient response and long-term survival rates. My secondary aim is to examine the response of patients based on the different stages of cancer they were in when they first started to receive treatment.

I hypothesize that if the Dendritic Cell vaccines are administered after having a patient's cancer resected, then patients will have a significantly lower chance of having a recurrence of their cancer compared to the normal standard of care.

Background

This research is to further the development of Immunotherapy with Dendritic Cell vaccines. The reason is that it is hard to remove pancreatic cancer in a way where we can be sure we have not left behind other cancer cells (Oberstein, 2013). Because of this, it often causes a recurrence in a person's cancer. This is why treatments like immunotherapy are being developed, in order to be more sure that all cancer cells are removed or attacked.

This study will be a Phase III trial for a study that had previously been completed with a Phase I and a Phase II (van't Land, 2024). It will now undergo a Phase III trial, where it will determine if this treatment is better than others on the market, which is the main objective of this study.

The previous study found that this treatment is safe, and further confirmed that the recurrence of the cancer was much lower. They had demonstrated a 64% recurrence-free survival in 2 years (van't Land, 2024). I have compared it to a value that found the recurrence-free survival rate of 21.3%, with no treatment after resection, which tells us that the immunotherapy vaccines cause a significant increase in recurrence-free survival (Groot, 2018).

To further understand this treatment, we must analyze the current standard of care that is given to patients who have pancreatic cancer now. To make it simpler, we can refer to Figure 1 below.

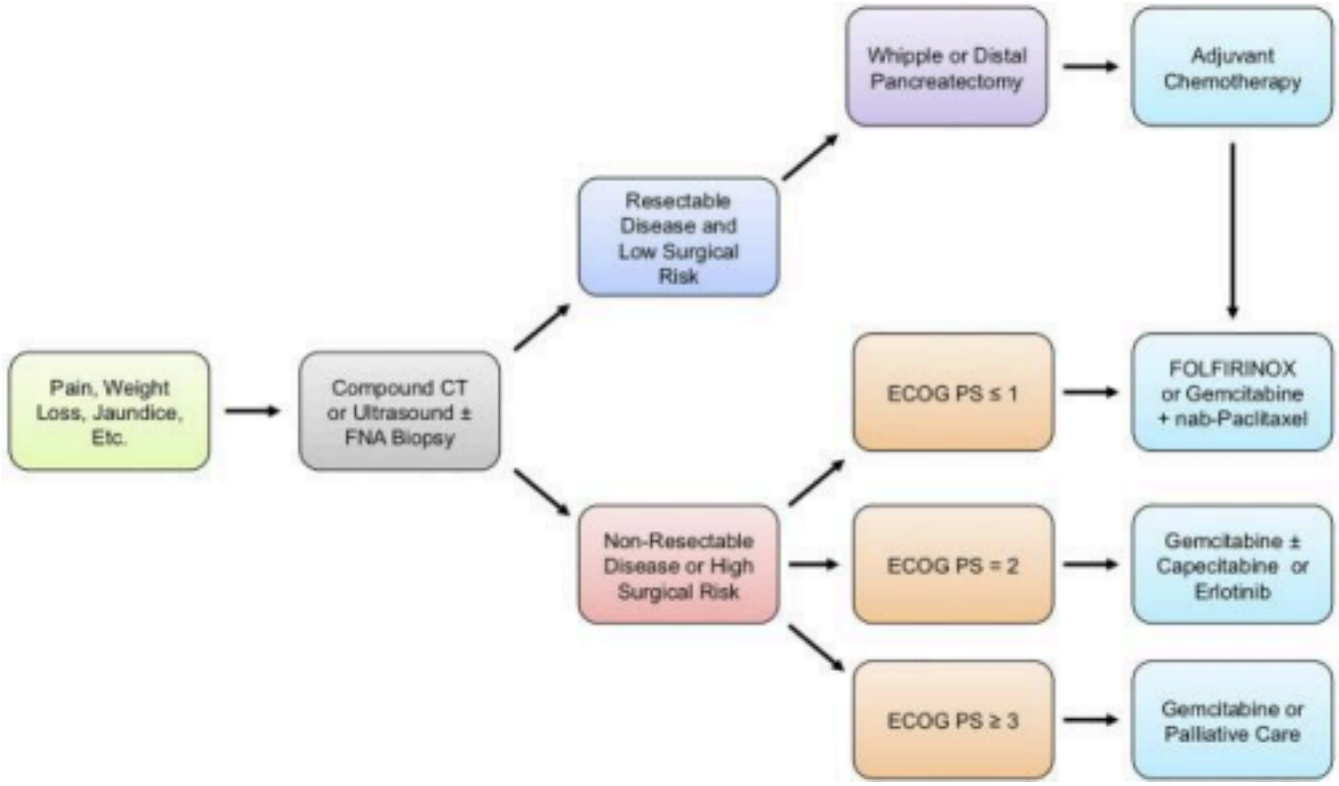


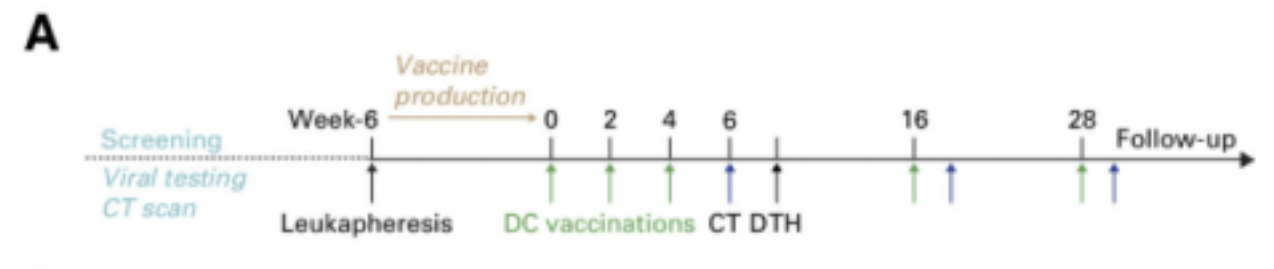
Figure 1: Flow chart showing basic progression from diagnosis to treatment for patients with pancreatic ductal carcinoma (Principe, 2021)

The standard of care is to understand if we can first resect the cancer, which will be the arm we focus on in this case, since we want to focus on cancer that has already been resected. Normally, people with pancreatic cancer will receive a Whipple or distal pancreatectomy, where they will either remove the whole pancreas or the pancreas as well as some surrounding organs (Bergquist, 2017). After that, they will then receive chemotherapy with the appropriate drug. Finally, there will be imaging to make sure the cancer has not recurred. Everyone in our study will go through this first.

Research design and methods

This study will be a randomized, double blind, placebo-controlled trial with the experimental cohort receiving the dendritic cell (DC) vaccine and the control cohort receiving an injection of saline of the same volume. Patients in the experimental group will first undergo imaging to ensure they do not have a recurrence of their cancer. If patients meet the inclusion criteria to continue with the study, we will obtain a blood sample and perform leukapheresis to isolate the dendritic cells. This will occur 6 weeks before the first administration of the vaccine. Dendritic cells will be cultured with antigens specific to cancer cells and then administered in the form of vaccines to the participants at weeks 0, 2, 4, 16, 28 (van't Land, 2024). Doses of the vaccine or saline will be administered to either the experimental or control cohorts, respectively. This will then be followed by follow-ups over a 2-year period, checking if cancer has recurred (van't Land, 2024).

Figure 2: Timeline of process that is described above (van't Land, 2024).



Both groups will receive standard adjuvant chemotherapy using one of the two most common regimens: Gemcitabine with Nab-Paclitaxel or FOLFIRINOX (a combination of 5-fluorouracil, leucovorin, irinotecan, and oxaliplatin), selected based on the patient's individual health status, tolerance, and oncologist recommendation. Chemotherapy will follow standard clinical protocols over a defined cycle and be accompanied by similar follow-up and monitoring procedures as the experimental group (Principe, 2021). After this process, the vaccines or the saline will be administered, with the continuation of monitoring.

Additional data, such as the overall survival rate, will also be observed and analyzed. This will be done by seeing which patients die throughout this process, and will then be reported as a percentage. This approach is intended to provide insight into recurrence rates and the safety, tolerability, and impact of Dendritic Cell vaccines compared to chemotherapy. This will be done by doing a delayed-hypersensitivity skin test. This should be tested as positive, so we know that the vaccine is tolerable, as well as able to be received by patients. As for safety, we want to make sure there are minimal side effects, if any, and they will not be life-altering. Impact will be looked at in the sense of whether the vaccines are working, and positively impacting the patient in terms of having no recurrence.

Since this will be done on human subjects, we will need approval from the IRB. To qualify for participation, individuals must meet specific inclusion criteria. Such as being of age, and histologically confirmed pancreatic ductal adenocarcinoma, who have undergone a successful complete surgical resection of their cancer. Of course, all participants will be required to provide written informed consent. On the other hand, individuals will be excluded from the study if they have received prior chemotherapy or immunotherapy for pancreatic cancer, as this will interfere with the likelihood of the recurrence of their cancer. These criteria help ensure participants' safety and also study the validity of results.

Summary and significance

Overall, the aim of this study is to compare Dendritic Cell immunotherapy vaccines to the current standard of care, which is chemotherapy in patients who have already resected their cancer. This would be done through having a control group, which would be the patients who get chemotherapy, and our experimental group, which would be patients who get the Dendritic Cell vaccines in addition to the prior chemotherapy. The results we will most likely end up with are that the recurrence in patients with cancer who only go through chemotherapy is much higher compared to those who receive the immunotherapy vaccines. This can be interpreted as immunotherapy Dendritic Cell vaccines plus the chemotherapy, resulting in a better standard of care, and should be invested in to start being used in addition to chemotherapy. If the results are not significant enough, we should try to see how we can improve this immunotherapy.

Some limitations of this study are the limited sample size that would be available and the follow-up for

long-term monitoring. Patients are always allowed to drop out of studies, and some patients may even die. Additionally, some might even have a recurrence, and this would take them out of the study. This can cause variability within the study. This can be counteracted by trying to get more than one facility involved with the study. Therefore, there will be a diverse sample size, as well as enough people to give us significant results.

Finally, this study is important as the treatments we currently have for pancreatic cancer are minimal, and often not as effective as we would like them to be. Seeing as we often detect pancreatic cancer in later stages of the disease, having better treatment options can lead to better outcomes for patients.

References

Bergquist, J. R. (2017, January). Type of Resection (Whipple vs. Distal) Does Not Affect the National Failure to Provide Post-resection Adjuvant Chemotherapy in Localized Pancreatic Cancer. *Annals of Surgical Oncology*, *24*. Pubmed. 10.1245/s10434-016-5762-6

Groot, V. P. (2018). Defining and Predicting Early Recurrence in 957 Patients With Resected Pancreatic Ductal Adenocarcinoma. *Annals of Surgery*. Pubmed. 10.1097/SLA.0000000000002734

Oberstein, P. E. (2013, March 07). Pancreatic cancer: why is it so hard to treat? *Sage Journals*. Pubmed. https://doi.org/10.1177/1756283X13478680

Principe, D. R. (2021, July). The Current Treatment Paradigm for Pancreatic Ductal Adenocarcinoma and Barriers to Therapeutic Efficacy. *Frontiers of Oncology*. Pubmed. 10.3389/fonc.2021.688377 van't Land, F. R. (2024, July 01). Dendritic Cell–Based Immunotherapy in Patients With Resected Pancreatic Cancer. *Journal of Clinical Oncology*, *42*(26), 12. Pubmed. 10.1200/JCO.23.02585

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4/22/2025

CRISPR-Cas9 and its use on genetic diseases

Method and Hypothesis:

CRISPR-Cas9 could potentially be used as a method for correcting mutations in genes that cause genetic diseases. It can be used as a long-term cure or treatment for Fragile X Syndrome in humans by editing genomic sequences. The study aims to use CRISPR-Cas9 to correct damaged lab-grown human stem cells. It also aims to test whether the genetic mutation within the cell was nullified. This is checked by sequencing the DNA to verify if the presence of the wild-type gene is active. It also aims to test if the normal function of the lab-grown cells were fully corrected and regained normal cell function.This could be achieved by measuring cellular protein function or the metabolic activity. It can also be measured by testing animals affected by Fragile X Syndrome and determining if there are meaningful behavioral or physical changes to the animals after testing.

Background

DNA is used for copying and storing biological information. It contains the information that living things need for functioning. It is copied with accuracy due to proofreading from DNA polymerase to prevent mutations. Despite this, rare mutations can still occur. They occur during cell division, when copying occurs, as well as exposure to UV radiation and chemicals. These mutations can cause diseases such as sickle cell anemia or cystic fibrosis. These conditions can be passed down from parents or randomly occur because of errors in the DNA sequence. [1]

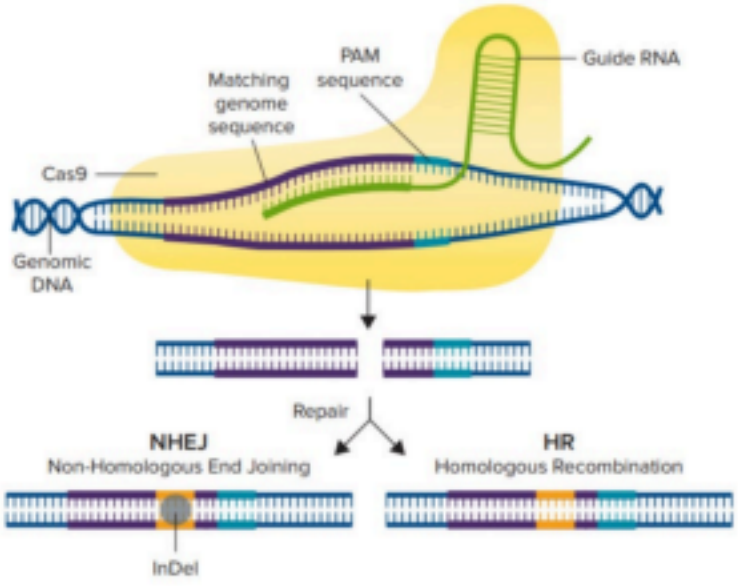

Figure 1. CRISPR-Cas9 use in genomic sequence editing.

One potential method to prevent disease-inducing genetic mutations is through CRISPR-Cas9. CRISPR is a gene editing platform that can precisely modify DNA strands and potentially cure genetic diseases. [2]

Researchers have already tested CRISPR-Cas9 to treat disorders such as sickle cell anemia. [3] Results from short-term is revealed that CRISPR-Cas9 disruption of *HBG1* and *HBG2* gene

promoters was an effective way of reducing the impacts of sickle cell anemia in patients. By editing stem cells, the experiment was able to increase fetal hemoglobin levels. This leads to decrease in sickle cell symptoms for patients. [4] Researchers believe that options like these are likely to become successful within the next 15 years. [3]

CRISPR-Cas9 has also been used to treat Huntington's disease, a fatal disorder caused by mutations in the *HTT* gene. This causes the production of a toxic protein, which can cause death of neurons in the brain. CRISPR-Cas9 has been utilized to target and edit DNA sequences, correcting the *HTT* mutation. This was done by silencing the mutant *HTT* gene. This not only decreased the levels of the mutated gene but also showed visible improvements in cognitive function and longer lifespans. In human-derived cells, CRISPR has been used to excise the expanded CAG repeat region that prevents the expression of harmful proteins. [6]

This study will investigate the efficacy of CRISPR-Cas9 editing when modifying the *FMR1* gene in Fragile X Syndrome (FXS), a common cause of intellectual disabilities.[5] FXS is caused by CGG trinucleotide repeat expansions, this leads to a silencing of the gene. [8]

We will use two different experimental models. One will be human patient derived stem cells with the full mutation of the *FMR1* gene associated with FXS .[8] Mice can also be used to test the CRISPR delivery, behavioral outcomes, as well as life expectancy after treatment. The goal will be to compare the before and after editing of the *FMR1* expression both in living animals as well as stem cells in the laboratory. The research will utilize three experimental groups for stem cells and three groups in mice. Group A will consist of a FXS model with no CRISPR treatment applied. This will become the negative control group. Group B will contain a FXS model treated with CRISPR Cas-9. It will target the *FMR1* promoter regions and this will be the experimental group. Group C will consist of healthy mice or stem cells that are not present with FXS. This will become our positive control group.

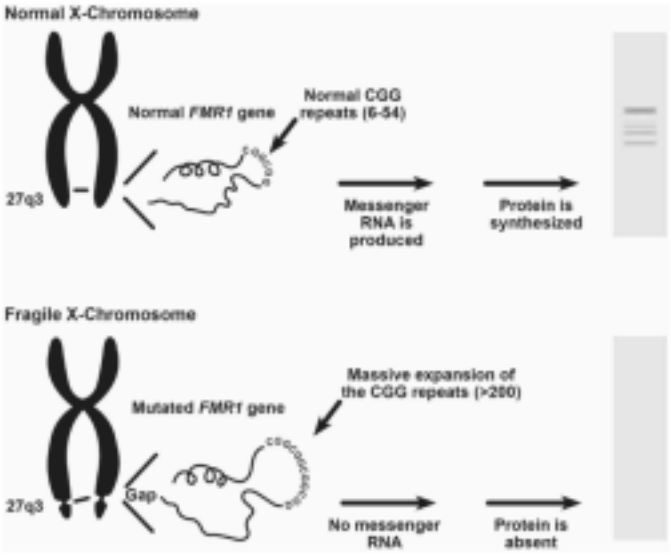

Figure 2. X Chromosomal interaction with FMR1 gene.

The study will utilize stem cells from patients affected by FXS. IRB approval is necessary to ensure patient treatment and safety. A CRISPR Cas-9 system will be employed to cut the CGG repeats. Guide RNAs will be created and then delivered to the stem cells. In mice the guide RNAs are injected, targeting the brain such as the cortex and hippocampus.

Outcomes may be measured in mice through behavior. Unedited FXS mice will be observed, analysing their behavior. This will be compared and contrasted with the CRISPR edited mice to determine the behavioural effects. Behavioural tests may include open field tests and mazes to evaluate behaviours. In stem cells *FMR1* levels may be measured at varied time ranges after CRISPR treatment.

The use of human stem cells will follow IRB approval guidelines. This will protect the rights and welfare of humans being used in research. Animal studies should be conducted with proper care to follow Institutional Animal Care and Use Committee guidelines. The use of FXS mice is necessary as determining the behavioral and health impacts for humans cannot be fully replicated by in vitro systems alone and research using in vivo must be conducted as well.

Summary and Significance

This study utilizes CRISPR-Cas9 editing as a tool to correct *FMR1* gene mutations that cause FXS in humans. FXS causes intellectual disabilities in humans. [5] By targeting the CGG repeats, the aim of this experiment is to correct the *FMR1* expression in human derived stem cells as well as lab controlled mice. If the experiment is successful, the hypothesis is confirmed and the treatment will be successful. This will be verified through cognitive and behavior improvements in mice and increased FMRP protein production in stem cells. Potential results could be the deletion of the CGG repeat region as well as improved neurological function in animals. In the mice it is expected that cognition in tests like mazes and puzzles would be improved when compared to the untreated Fragile X Syndrome mice.

The pitfall of this could be long term health risks when applied to humans. A limitation is the incomplete or improper editing of the human genome, which may result in correction errors, limiting the efficacy of the experiment.

Despite limitations, this study is significant in the field of healthcare as it could provide a cure for

Fragile X Syndrome while paving the path for further research in curing other genetic neurological disorders.

References

afia Samir, Human DNA Mutations and their Impact on Genetic Disorders, Recent Patents on Biotechnology; Volume 18, Issue 4, Year 2024, e031123223098. [1]

Kolanu ND. CRISPR-Cas9 Gene Editing: Curing Genetic Diseases by Inherited Epigenetic Modifications. Glob Med Genet. 2024 Mar 29;11(1):113-122. doi: 10.1055/s-0044-1785234. PMID: 38560484; PMCID: PMC10980556. [2]

Braga LAM, Conte Filho CG, Mota FB. Future of genetic therapies for rare genetic diseases: what to expect for the next 15 years? Ther Adv Rare Dis. 2022 Jun 10;3:26330040221100840. doi: 10.1177/26330040221100840. PMID: 37180410; PMCID: PMC10032453. [3]

Sharma A, Boelens JJ, Cancio M, Hankins JS, Bhad P, Azizy M, Lewandowski A, Zhao X, Chitnis S, Peddinti R, Zheng Y, Kapoor N, Ciceri F, Maclachlan T, Yang Y, Liu Y, Yuan J, Naumann U, Yu VWC, Stevenson SC, De Vita S, LaBelle JL. CRISPR-Cas9 Editing of the *HBG1* and *HBG2* Promoters to Treat Sickle Cell Disease. N Engl J Med. 2023 Aug 31;389(9):820-832. doi: 10.1056/NEJMoa2215643. PMID: 37646679; PMCID: PMC10947132. [4]

Hagerman RJ, Berry-Kravis E, Hazlett HC, Bailey DB Jr, Moine H, Kooy RF, Tassone F, Gantois I, Sonenberg N, Mandel JL, Hagerman PJ. Fragile X syndrome. Nat Rev Dis Primers. 2017 Sep 29;3:17065. doi: 10.1038/nrdp.2017.65. PMID: 28960184. [5]

Alkanli SS, Alkanli N, Ay A, Albeniz I. CRISPR/Cas9 Mediated Therapeutic Approach in Huntington's Disease. Mol Neurobiol. 2023 Mar;60(3):1486-1498. doi: 10.1007/s12035-022-03150-5. Epub 2022 Dec 9. PMID: 36482283; PMCID: PMC9734918. [6]

U.S. Department of Health and Human Services. (n.d.). *Overview - toolkit*. National Center for Advancing Translational Sciences. https://toolkit.ncats.nih.gov/module/clinical-trials-and-fda-review/serving-on-boards-to-review-and-monitor-clinical-trials/overview/ [7]

Stone WL, Basit H, Shah M, Los E. Fragile X Syndrome. 2023 Oct 28. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan–. PMID: 29083768. [8]

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Investigating Brain Activation Patterns in Bilingual Individuals Faced with Impersonal Dilemmas

Hypothesis and Specific Aims

Bilingual individuals make different decisions when presented with impersonal emotionally stimulating ethical scenarios in their native language versus their second language.

Primary Aim: To investigate whether bilingual individuals make different decisions when presented with emotionally stimulating ethical scenarios—such as the Trolley problem—depending whether the situation is presented in their native language or their second language.

Secondary Aims:

1. To investigate and compare the areas of brain activation that are used when decisions are made by bilingual individuals that are presented with an ethical scenario in their native language or their second language.
2. To explore whether implicit bias plays a role in ethical decision-making, specifically if participants have a preference for individuals who speak their native language or their second language. This aim will study potential bias and its influence on ethical choices made by bilingual individuals.

Background

As the number of bilingual individuals continues to rise and multilingualism becomes more common, questions about how language influences implicit bias and moral decision-making prevail. Multiple studies have explored these connections, however, the results are inconsistent.

In “Bilinguals on the footbridge: the role of foreign-language proficiency in moral decision making” by Federico Teitelbaum Dorfman, the moral foreign-language effect (MFLE) was observed at intermediate language 2 proficiency (L2p) levels and at high L2p levels. The MFLE suggests that bilingual individuals make different moral decisions when using their foreign language compared to their native language. In this study, L2p rarely affected how people responded to impersonal dilemmas, however, L2p did influence personal dilemmas. MFLEs are consistent at intermediate L2p levels but not at high L2p levels. Overall, personal dilemmas do not produce MFLE for all levels of proficiency. The results are inconsistent, however, they show how the MFLE seems sensitive to L2p, especially in the case of personal moral dilemmas. “Conversely, the MFLE does seem sensitive to lower L2p in the face of personal dilemmas.

Utilitarian decisions in L2 increase systematically at intermediate L2p levels, with MFLEs emerging in 85% of studies. Yet, this pattern proves inconsistent at high L2p levels, as the MFLE appears in only 50% of studies.” (Dorfman et al., 2024)

Similarly, “Moral Judgement in Early Bilinguals: Language Dominance Influences Responses to Moral Dilemmas” by Galston Wong also found inconsistent results. In this study, participants answered ten different scenarios on a computer screen and used the “1” to “7” keys to respond to the questions (1 = definitely no, 7 = definitely yes). Three slides were presented: they read the information on the first slide, they were given a paragraph that reiterated the given choice of action and asks the participants whether they would commit to it on the second slide, and asked how distressing it was to answer each scenario on the 1 to 7 scale (1 = strongly disagree, 7 = strongly agree) on the third slide. They tested for the main effects of language and dilemma type (personal/impersonal), examined the relationship between participants’ language dominance and moral decisions, and the relationship between emotional intensity and participants' moral responses. “The mixed-factor analyses found no main effect of language across all five different scenarios on the participants' utilitarian ratings, $p > 0.05$, indicating that participants responded similarly across both the English and Mandarin Chinese versions of the task.” (Wong et al., 2018) In their conclusion, they state that alternative explanations may need to account for the role of emotional arousal in the moral foreign language effect (FLe) and language dominance effect (LDe) phenomenon.

Given the inconsistent results across these studies, our research aims to provide specific correlation between bilingual individuals and their choices when presented with impersonal dilemmas in their native language versus their second language. These differences will reflect in recognizable patterns of brain activation.

Research design and methods

This study will use human participants who are selected randomly from local universities and supermarkets through online platforms and surveys. All participants must be bilingual, with at least five years of exposure to their second language. Prior to their participation, individuals will provide a signature on an IRB-approved informed consent form. They will be separated into 3 age groups: 18-30, 31-50 and 51+. Within each age group, participants are categorized into one of two proficiency groups: low proficiency or high proficiency.

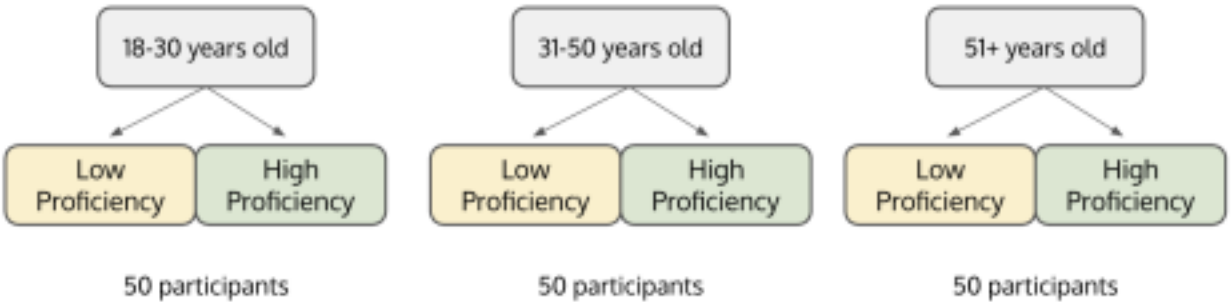


Figure 1: Two subgroups within each age group

Participants will take the STAMP 4S (or STAMP WS for less commonly taught languages) language proficiency assessment to categorize them into the low or high language proficiency groups based on their numerical scores in listening/speaking and writing/reading (Avant

Assessment, 2025). A score of 5 or below will be classified as low proficiency and a score of 6 or higher will be classified as high proficiency. This distinction will ensure a clear definition between the proficiency groups. In total, there will be six experimental groups: ages 18-30 with low proficiency and high proficiency, ages 31-50 with low proficiency and high proficiency, and ages 51+ with low proficiency and high proficiency. Each participant will verbally respond to five moral dilemma questions, including the trolley problem, as a person reads them a paragraph about each situation.

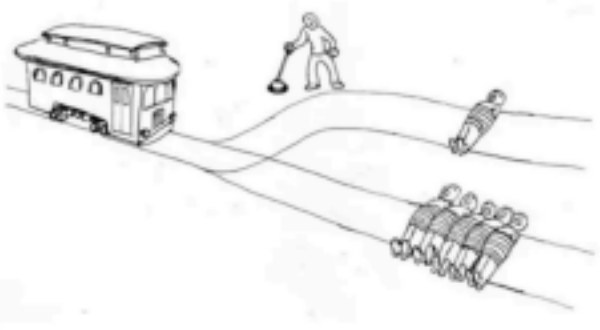


Figure 2: The Trolley Problem

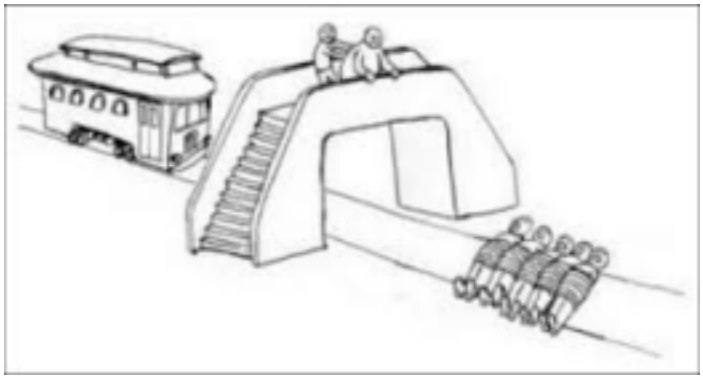


Figure 3: The Footbridge Dilemma 3

"A moral dilemma arises when an agent is in a choice situation in which he/she cannot satisfy the dictates of morality." (Vallentyne, 1989) Moral dilemmas presented to participants will include various types of scenarios such as the Trolley Problem and the Footbridge Dilemma. The time the participants take to respond to each scenario will be involved in the analysis of results. Once participants select their choice, they will not be able to change their decision. Participants will verbally respond to each question with "A" or "B". Each question will be asked in both their native and second language and responses will be recorded. When we ask the trolley problem, on one rail will be a person who speaks the participant's native language and on the other rail will be a person who speaks their second language. Results to these questions will be compared between all six groups using the help of a statistician.

These questions will be presented during functional magnetic resonance imaging (fMRI) scanning to observe brain activation patterns. Participants will be asked to remain still and

respond verbally. fMRI data will be collected using a 3T scanner. For each experimental group, fMRI will be used to collect blood-oxygen-level-dependent (BOLD) signals to determine the activity in different brain regions. We will observe brain regions like the amygdala, medial prefrontal cortex, and ventral prefrontal cortex and their activation levels during each task. To determine whether a brain region is more active, the fMRI detects increased oxygenated blood flow to that region, since neural activity triggers a blood flow response that is measured through the BOLD signal. "The blood-oxygen-dependent (BOLD) signal, detected in fMRI, reflects changes in deoxyhemoglobin driven by localized changes in brain blood flow and blood oxygenation, which are coupled to underlying neuronal activity by a process termed neurovascular coupling." (Hillman, 2015) After getting all of the scans, we will compare and observe the BOLD signal scans between the low proficiency group with the high proficiency group within each age group. When comparing, we will involve statisticians to perform quantitative analysis to identify statistically significant in BOLD activation between each group. This analysis will determine whether language proficiency and age interact to influence neural activity during moral reasoning tasks.

Summary and significance

In this study, we hypothesize that bilingual individuals will make different decisions when presented with emotionally stimulating ethical scenarios in their native language versus their second language. To test this, we will use fMRI scans to observe brain activation patterns as participants are asked ethically challenging questions.

We anticipate that when participants make decisions in their second language, they may have the tendency to exhibit less emotional attachment and less activity in emotion-related brain regions. However, with their native language, we predict that they will have a stronger emotional connection which will increase activity levels in their emotion-related brain regions. These results will provide an insight into the cognitive and emotional contributions when bilingual individuals are presented ethical scenarios in both of their languages.

Most bilingual people have different levels of fluency in both of their two languages which can skew and provide inconsistent results between each of the participants. To address this limitation, in future studies we could assess each participant's language proficiency through the Avant STAMP language proficiency test that provides a numerical value to measure their proficiency in speaking, listening, reading, and writing for both languages. By defining the participant's language proficiency, we would be able to provide results that are more consistent and generalizable. However, this leads us into our next limitation, the STAMP test. Though the STAMP assessment is used to categorize our participants into their subgroups, no test perfectly assesses an individual's language proficiency. We also don't know if everyone will take the test as seriously as others which could skew our results due to inaccurate categorization. The last limitation I found is the different languages participants speak since I did not limit which languages I used in the experiment, there are many differences between languages that could

affect my results.

This study will enhance our understanding of language-contextualized cognition, and highlight the different biases that different cultures may present. It will analyze how languages reflect different cultural values which could influence decision-making and moral reasoning. By examining these connections, this research will promote multilingualism and cultural unity. Ultimately, this study will highlight the importance of linguistic and cultural diversity.

References

1. Bilinguals on the footbridge: The role of foreign-language proficiency in moral decision making | bilingualism: Language and cognition | cambridge core. (n.d.). https://www.cambridge.org/core/journals/bilingualism-language-and-cognition/article/bilinguals-on-the-footbridge-the-role-of-foreignlanguage-proficiency-in-moral-decision-making/C7A25FAC773BB8C2B5EA127CAA250DAB
2. Wong, G., & Ng, B. C. (2025, April 22). *Moral judgement in early bilinguals: Language dominance influences responses to moral dilemmas*. Frontiers. https://www.frontiersin.org/journals/psychology/articles/10.3389/fpsyg.2018.01070/full 3. *Stamp 4S*. Avant Assessment. (2025a, March 24). https://www.avantassessment.com/tests/stamp/4s#:~:text=About%20STAMP%204S,encounter%20in%20the%20real%20world.
4. Vallentyne, P. (1989). Two types of moral dilemmas. *Erkenntnis*, *30*, 301–318. https://klinechair.missouri.edu/docs/two_types_of_moral_dilemmas.pdf
5. Hillman, E. M. C. (2014, July 8). *Coupling mechanism and significance of the bold signal: A status report*. Annual Review of Neuroscience. https://www.annualreviews.org/content/journals/10.1146/annurev-neuro-071013-014111

Skylerr Huston

CRISPR-cas9 treating Scrapie in Sheep

Hypotheses

Scrapie is a fatal, progressive neurodegenerative disease that primarily affects sheep and goats. It belongs to a group of diseases known as Transmissible Spongiform Encephalopathies (TSEs), which are caused by misfolded proteins known as prions. Scrapie has no known cure and is always fatal, making it crucial to explore new treatment methods. More research is needed to understand how to effectively treat and ultimately prevent this disease. This study hypothesizes that the CRISPR-Cas9 gene editing system may offer a potential cure and preventative treatment for Scrapie in sheep. By targeting and editing specific genes associated with prion production, CRISPR-Cas9 may be able to reduce or eliminate the disease's effects. The primary aim of this research is to utilize the CRISPR-Cas9 system to treat, manage, and possibly reverse the symptoms of Scrapie in infected sheep. Another secondary goal is to explore the broader potential of CRISPR-Cas9 as a therapeutic approach for other prion-caused TSEs, including those that affect both animals and humans, such as Creutzfeldt-Jakob disease and bovine spongiform encephalopathy.

Background

A prion is a misfolded protein usually causing neurodegeneration. Scrapie is a fatal neurodegenerative disease (grouped among many other similar diseases called TSEs (Transmissible Spongiform Encephalopathies) that is caused by a misfolded protein called PrP^{Sc} (a prion) which accumulates causing neurodegeneration. Scrapie is usually found among goats and sheep and is transmibble, especially during birth to the lymphoid system and central nervous system. There is now an ongoing eradication program that is ongoing and still needs research.

Scrapie was first detected in Europe in the 18th century and has become an increasing threat for sheep populations. The disease progresses slowly, often years before the first signs show, as the PrP^{Sc} prion builds up in the brain. Sheep begin to show signs such as weight loss, behavioural changes, itching, and loss of coordination. Diagnosing Scrapie is often challenging to diagnose due to the disease exhibiting similar signs to other neurological diseases.

Certain genotypes such as ARR are associated with resistance. Selective breeding methods have been able to reduce the prevalence of Scrapie in sheep flocks, but have failed to fully eliminate the disease and still requires more research.

The PrP^{Sc} gene is resistant to breakdown by enzymes and usually accumulates in places in the lymphoid system and central nervous system. Early on in the infection the prion gathers in lymphoid tissues like the tonsils, spleen, and lymph nodes. As the disease progresses and travels along the nervous system and spinal cord, it damages neurons and supporting cells. This leads to spongiform degeneration, where the brain forms sponge-like holes resulting in the symptoms of the disease.

Research Designs and Method

This project will explore if CRISPR-cas9 can treat a neurodegenerative disease, Scrapie

in sheep. Scrapie is caused by a prion, which is an abnormal protein that folds the wrong way and deposits in the brain causing damage overtime. The overall goal is to use CRISPR to edit the gene that makes this protein, the PRNP gene, to stop the disease from developing.

To study this, the sheep will be split the sheep into three groups:

- One group will not be treated (control group)
- One group will be treated with CRISPR-cas9 to edit the PRNP gene
- One group will be given an inactive treatment to make sure any effects are really from CRISPR and not something else

Sheep are the best animal to use for this research because they naturally get Scrapie, and get it more commonly compared to goats. Testing on sheep will help us see how the disease works in the brain, which cannot be done in a dish or lab. This study will need approval by IACUC (Institutional Animal Care and Use Committee) to make sure the sheep are treated ethically and humanely.

Using the CRISPR-cas9 system will target the PRNP gene, which tells the body how to make the prion protein. If we can cut out and change this gene we may be able to stop the harmful prion protein (PrP^{Sc}) from forming.

First the CRISPR-cas9 will be tested on sheep cells in the lab. The DNA in these cells will be checked to see if the gene was successfully edited using a method called PCR (Polymerase Chain Reaction), which makes copies of the gene to analyze. Results will be separated by gel electrophoresis, which lets us see if the gene is changed.

After successful lab testing the CRISPR will be given to live sheep using a safe delivery method, such as a vector/virus that carries the CRISPR system into the brain. The sheep will be observed for the next few years to look for signs of Scrapie such as problems walking, behaviour changes, or signs of brain damage.

During the study the sheep's brain and tissues will be tested to check for:

- Protein levels, using tests like Western blotting, which can check amounts of prion protein present
- Check for signs that CRISPR gene editing worked
- Check for off-target edits by comparing DNA sequences from treated and untreated animals, to check if CRISPR changed other parts of the sheeps DNA

Data from the experiment will be compared using basic statistics. For example, we will compare how many sheep in each group got sick, how long they stayed healthy, and how much prion protein was found in their brains. A P-value < 0.05, will be used to decide if differences between the groups are statistically significant (not due to chance).

Summary

This study explores if CRISPR-cas9 gene editing can be used to treat Scrapie, a fatal

brain disease found in sheep caused by a folded protein called a prion. Scrapie is one of several neurodegenerative diseases known as TSEs (Transmissible Spongiform Encephalopathies), which affect both animals and humans. The goal of the study is to use CRISPR to target and change the PRNP gene, which is responsible for making the prion protein, in order to prevent the disease from spreading.

If the CRISPR treatment works we expect the treated sheep to:

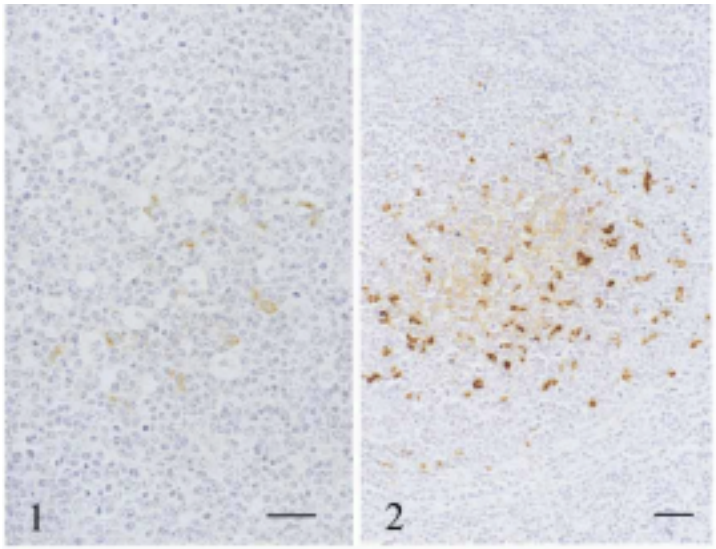
- Show fewer signs of brain damage
- Stay healthier then the untreated ones
- Have lower levels of the harmful prion protein

This would suggest that CRISPR successfully edited the PRNP gene and maybe a possible treatment for Scrapie and other similar diseases. It could also open the door to using gene editing for other brain diseases caused by misfolded proteins.

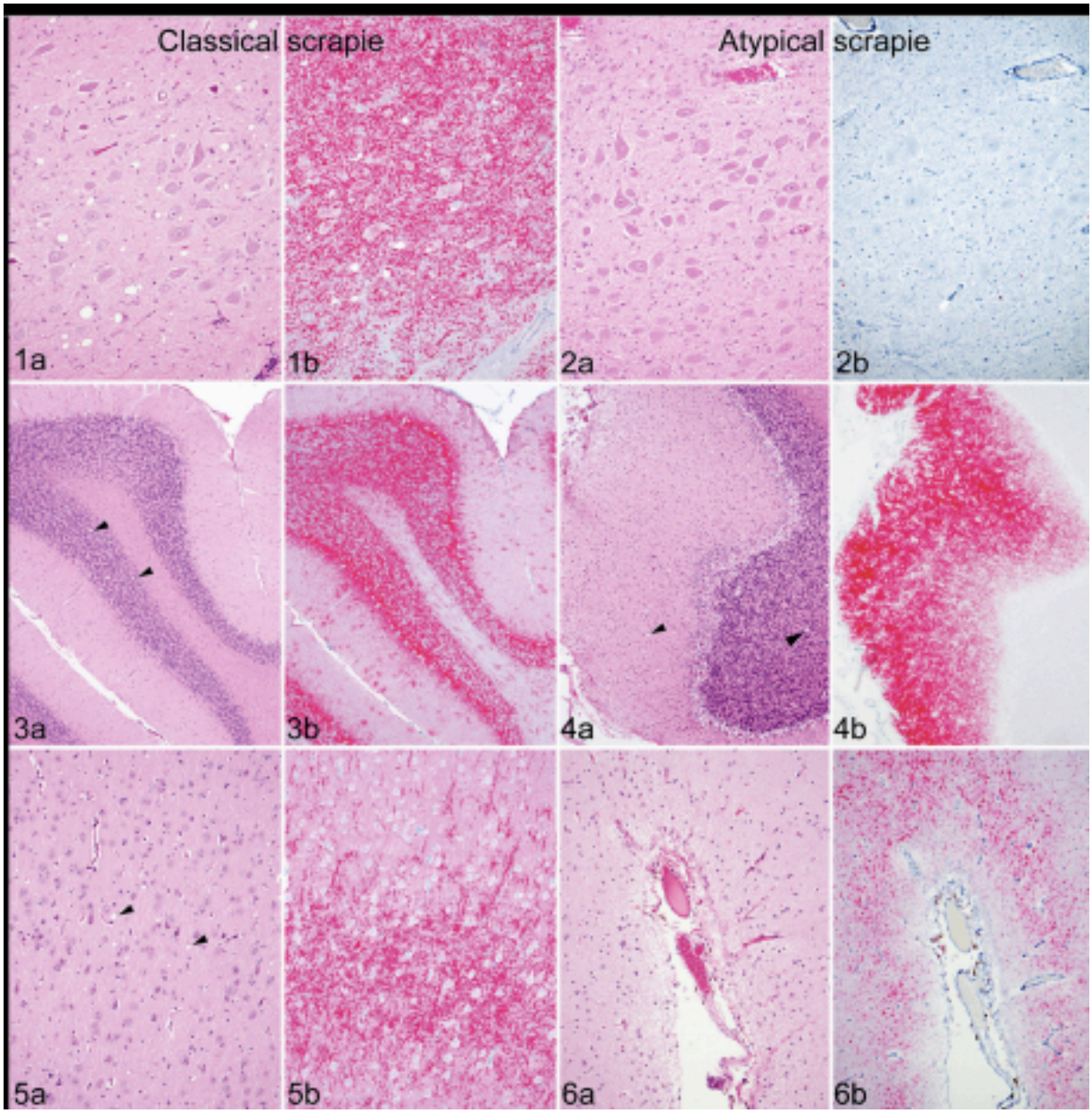
Sudden limitations and challenges include a long-time frame because Scrapie develops slowly, so results might take a while to see. In addition, CRISPR may accidentally edit the wrong part of the DNA which causes off-target effects. Finally, getting the CRISPR system into the body safely presents a challenge.

Scrapie is currently incurable and prion diseases like it are always fatal. - Expand on it By exploring as a treatment this study could lead to new ways to stop these diseases, not just in sheep, but possibly in humans as well. This kind of research could be an important step forward in how we understand and target caused by protein misfolding and neurodegeneration.

Images



2 stages of prion protein buildup in the brain



Various figures displaying prion causing spongiform (holes in the brain) in sheep References

References

- Baylis, M., & Goldmann, W. (2004). The Genetics of Scrapie in Sheep and Goats. Current Molecular Medicine, 4(4), 385-396. https://doi.org/https://doi.org/10.2174/1566524043360672

- Van Keulen, L. J. M., Vromans, M. E. W., & Van Zijderveld, F. G. (2002). Early and late pathogenesis of natural scrapie infection in sheep. *APMIS*, *110*(1), 23–32. https://doi.org/10.1034/j.1600-0463.2002.100104.x

- van Keulen, L. J. M., Schreuder, B. E. C., Vromans, M. E. W., Langeveld, J. P. M., & Smits, M. A. (2000). Pathogenesis of natural scrapie in sheep. In *Prion Diseases* (pp. 57–71). Springer. *Archives of Virology. Supplemental*, 16. https://doi.org/10.1007/978-3-7091-6284-9_6

- Cassmann, E. D., & Greenlee, J. J. (2020). Pathogenesis, detection, and control of scrapie in sheep. *American Journal of Veterinary Research, 81*(7), 600–611. https://doi.org/10.2460/ajvr.81.7.600

- Greenlee, J. J. (2019). Review: Update on classical and atypical scrapie in sheep and goats. *Veterinary Pathology, 56*(1), 6–16. https://doi.org/10.1177/0300985818794247

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Accuracy of Fetal Brain MRI Prediction on Postnatal Neurodevelopmental Outcomes

Hypothesis and Specific Aims

This research proposal hypothesizes that fetal brain magnetic resonance imaging (MRI) can accurately predict postnatal neurodevelopmental disorders, such as autism spectrum disorder (ASD), by identifying abnormalities in brain morphology. One specific aim of this study is to compare the relative functionality of fetal brain MRI with a more commonly used imaging technology, ultrasonography, also known as ultrasound or sonography, by analyzing the strengths and deficiencies of both processes. Additionally, the impacts of environmental factors and maternal health on fetal brain MRI predictions will also be examined, specifically though stress levels, nutrition, and environmental toxins such as heavy metals. Stress levels will be collected through patients' self reports, and nutrition and environmental toxicity will be measured through blood samples testing vitamin D, iron, lead, and mercury levels. Finally, this study seeks to improve early detection of neurodevelopmental disorders by building on the findings of the previous aims, and thus strengthens genetic counseling efforts.

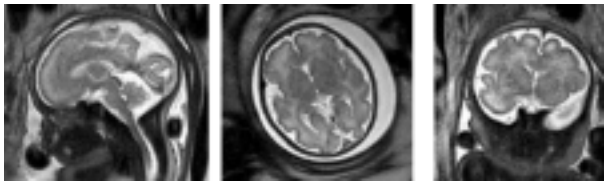


Figure 1. Fetal brain MRI (Nagaraj & Kline-Fath, 2022)

Background

Magnetic resonance imaging (MRI) is a noninvasive imaging technique that can produce detailed images of anatomical structures using magnetic fields and radio waves. The primarily MRI sequences used are ultrafast T1 and T2-weighted scans. Ultrafast T2-weighted techniques such as single-shot fast spin-echo (ssFSE) minimize the effects of fetal motion and can greatly reduce imaging time (Jarvis et al., 2019; Tee et al., 2016). In utero exposure to drugs, environmental toxins, maternal stress, mental health, malnutrition, and more, have been found to affect fetal brain development (De Asis-Cruz et al., 2022). Fetal brain MRI functions as a second-line examination in prenatal care, often performed after abnormalities in fetal development are detected in ultrasonography, also known as ultrasound or sonography (Powers et al., 2022). Scans are usually taken during the second or third trimester, or around after 22 weeks gestation, the time since the first day of the mother's last menstrual period (Glenn, 2010).

Subplate (SP) neurons are one of the first neurons generated in the cerebral cortex and undergo apoptosis after the cortex matures. They can act as biological markers for neurodevelopmental disorders such as schizophrenia and autism spectrum disorder (Serati et al., 2019). As SP neurons are involved in functions during fetal gestation, longitudinal pre- and postnatal imaging studies, such as fetal brain MRI, early detection of SP neurons through fetal brain MRI could help to better predict neurodevelopmental outcomes and allow

for new discoveries concerning developmental trajectories in the SP zone (Serati et al., 2019).

Autism spectrum disorders (ASDs) are permanent neurodevelopmental disorders that can affect communication and behavior, usually recognized within the first three years of life. Approximately 1 in every 100 children worldwide are diagnosed with ASD, and the disorder can be caused by many different genetic abnormalities, including single gene mutations (i.e. CHD8, SHANK3) and deletions or duplications of DNA segments. These abnormalities can result in abnormal cortical development and connectivity.

Ultrasonography is currently more utilized than MRI because of its low cost, high availability, and scientific support. However, MRI does hold some advantages over ultrasonography. For instance, MRI allows for an increased field of view of internal structures, greater image quality through increased detail and contrast, and precise 3D reconstructions (Tee et al., 2016). Additionally, MRI can be useful in diagnosing central nervous system syndromes in the second trimester. The ultrasonographic diagnosis of Joubert syndrome or related disorders may only find general brain abnormalities. However, fetal MRI was found to detect a brain deformity at the pontomesencephalic junction, otherwise known as the molar tooth sign, and diagnose Joubert syndrome within 17-18 weeks of gestation (Saleem, 2013). This demonstrates that MRI use could likely increase the speed of detection for disorders associated with brain deformities, such as autism spectrum disorders. Early detection can allow for greater support postnatally, as well as improve children's developmental outcomes and future quality of life.

Research Design and Method

This study will implement a quantitative, longitudinal approach, using a retroactive cohort study and measure the capability of fetal brains, measured by magnetic resonance imaging (MRI) scans and under the effect of maternal factors, in predicting neurodevelopmental outcomes such as Autism Spectrum Disorder (ASD).

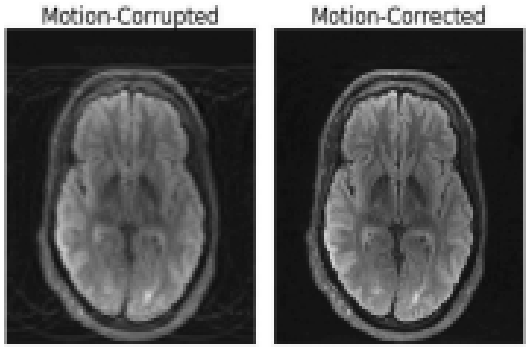
The independent variables in this study include factors affecting maternal health: namely stress, nutrition, and the environment. Stress levels will be self-reported, nutrition levels will measure vitamin D and iron levels through blood samples, and environmental toxins will also be measured through the detection of heavy metals in blood samples. Another independent variable is fetal brain morphology, which will be measured through fetal brain MRI scans, performed on a 1.5 tesla MRI scanner at or after 22 weeks of gestation.

The dependent variable is the prevalence of postnatal neurodevelopmental disorders, measured through cognitive assessments at postnatal follow-ups, such as ADOS (Autism Diagnostic Observation Schedule). Confounding/intervening variables include the socioeconomic status of participants, and background variables encompass the age of mother and fetus, ethnicity, gestational age, and the sex of the fetus.

The study will require 100 participants with the inclusion criteria of single gestation pregnancy and pregnant individuals in the second or third trimester of pregnancy, recruited from participants engaging in routine prenatal ultrasounds. Additionally, the study aims to target patients who have a medical history of ASD and examine whether maternal health factors exacerbate the likelihood of brain abnormalities and a potential ASD diagnosis.

Timeline

Month 1: Obtain IRB (Institutional Review Board) or IEC (Independent Ethics Committee) approval
Month 2: Recruit participants according to inclusion guidelines, gather patient consent forms Months 2-4: Collect data surrounding maternal health factors
Months 4-6: Conduct fetal MRI scans
Months 6-24: Conduct postnatal follow-ups to analyze neurodevelopmental changes Month 24-26: Analyze data and interpret results



There are no particular ethical issues surrounding this study, as data suggests no significant risk to the fetus during MRI, although it is recommended to wait until the second trimester before performing the imaging process. For potential limitations, fetal motion during MRI scans may affect the imaging process by distorting and degrading the images and require repeat scans, delaying the process.

Figure 2. Effects of motion-corruption in MRI scans (Ouyang, n.d)

The setting will be a medical center or hospital with access to both fetal MRI technology and ultrasonography techniques, as to provide a means of comparison. Data will be collected and analyzed through regression models, and machine learning algorithms can be utilized to connect identified biomarkers to possible neurodevelopmental disorders.

Summary and Significance

This study analyzes the extent to which fetal brain MRI scans can accurately predict postnatal neurodevelopmental outcomes, such as autism spectrum disorder (ASD). Variables such as fetal brain morphology and maternal health factors were measured. Potential results include the identification of biological markers such as SP neurons that can aid in early detection. However, several limitations may significantly affect the accuracy of prediction. Firstly, the scope of the study is not large and may not be indicative of a greater population. Confounding factors such as relative location may play a role in influencing the results of the data. Additionally, limitations within the MRI process include low quality or distorted images due to a high water content in the fetal brain, low contrast, and excessive fetal motion. Early detection of neurodevelopmental disorders can help with future actions within genetic counseling, aiding prenatal and postnatal treatment. However, there are also ethical issues surrounding early detection, as acknowledging the likelihood of brain abnormalities and their possible consequences could potentially promote the termination of the pregnancy. Despite these current boundaries, ultimately, MRI has the capacity to become a vital, noninvasive tool in disorder detection. The applications of MRI show potential not only in neurodevelopmental disorders like ASD, but in neurological and psychiatric disorders such as schizophrenia as well.

References

De Asis-Cruz, J., Andescavage, N., & Limperopoulos, C. (2022). Adverse Prenatal Exposures and Fetal Brain Development: Insights From Advanced Fetal Magnetic Resonance Imaging. *Biological psychiatry. Cognitive neuroscience and neuroimaging*, *7*(5), 480–490. https://doi.org/10.1016/j.bpsc.2021.11.009

Glenn O. A. (2010). MR imaging of the fetal brain. *Pediatric radiology*, *40*(1), 68–81. https://doi.org/10.1007/s00247-009-1459-3

Jarvis, D. A., & Griffiths, P. D. (2019). Current state of MRI of the fetal brain in utero. *Journal of magnetic resonance imaging : JMRI*, *49*(3), 632–646. https://doi.org/10.1002/jmri.26316 Nagaraj, U. D., & Kline-Fath, B. M. (2022). Clinical Applications of Fetal MRI in the Brain. *Diagnostics*, *12*(3), 764. https://doi.org/10.3390/diagnostics12030764

Ouyang, A. (n.d.). *MIT researchers combine deep learning and physics to fix motion-corrupted MRI scans*. MIT News | Massachusetts Institute of Technology. https://news.mit.edu/2023/mit-researchers-combine-deep-learning-physics-fix-motion-corrupted-MRI-scans-0817

Powers, A. M., White, C., Neuberger, I., Maloney, J. A., Stence, N. V., & Mirsky, D. (2022). Fetal MRI Neuroradiology: Indications. *Clinics in perinatology*, *49*(3), 573–586. https://doi-org.liboff.ohsu.edu/10.1016/j.clp.2022.05.001

Saleem S. N. (2013). Fetal magnetic resonance imaging (MRI): a tool for a better understanding of normal and abnormal brain development. *Journal of child neurology*, *28*(7), 890–908. https://doi.org/10.1177/0883073813486296

Serati, M., Delvecchio, G., Orsenigo, G., Mandolini, G. M., Lazzaretti, M., Scola, E., Triulzi, F., & Brambilla, P. (2019). The Role of the Subplate in Schizophrenia and Autism: A Systematic Review. *Neuroscience*, *408*, 58–67. https://doi-org.liboff.ohsu.edu/10.1016/j.neuroscience.2019.03.049

Tee, L. M., Kan, E. Y., Cheung, J. C., & Leung, W. C. (2016). Magnetic resonance imaging of the fetal brain. *Hong Kong medical journal = Xianggang yi xue za zhi*, *22*(3), 270–278. https://doi.org/10.12809/hkmj154678

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The use of Vitamin D managing Breast Cancer found in Animals

Hypothesis and Specific Aims

Recent studies have demonstrated the use of vitamin D in the treatment of osteosarcoma, which decreased tumor growth and metastasis in osteosarcoma.[5]In the light of other studies that reveal a connection between vitamin D deficiency and breast cancer development in women, I hypothesize that the use of vitamin D as an additional treatment modality can decrease breast cancer growth and metastasis. The first aim of this study is to test whether regular Vitamin D treatment can decrease the risk of metastasis and slow tumor progression in animal models of breast cancer. Impact of vitamin D on tumor growth will be measured *in vitro* using cell culture assays and *in vivo* by daily tumor measuring using calipers. Metastatic potential will be evaluated *in vitro* by a scratch assay and *in vivo* by measuring metastatic lesions at endpoint analysis. The second aim of the study is to evaluate cellular changes after vitamin D treatment at the RNA and protein level. To observe these changes, we will use scRNA-seq to assess differentially changed transcripts, with a particular interest in genes relating to EMT. We will verify transcriptomic changes with Western blotting. This study predicts that animals receiving Vitamin D will show a slower rate of tumor progression and a reduced risk of metastasis compared to animals that do not. If this study is successful, it may show an accessible supplement with potential therapeutic value in treating breast cancer.

Background

Breast cancer is the malignant transformation of cells within breast tissue.[1]It is one of the most commonly diagnosed cancers worldwide, and a leading cause of cancer-related deaths in women, with a global yearly death toll of 670,000.[1] One of the major contributors to its high mortality rate is metastasis–the spread of cancer cells from breast tissue to other organ sites.[2] Once this occurs, the prognosis worsens significantly and treating it becomes much more difficult and complex. Treatment for breast cancer is typically based on the type of breast cancer and its stage, and includes: surgery, radiation, chemotherapy, hormonal therapy, targeted drug therapy, and immunotherapy.[3] While these treatments can be effective, they often come with many side effects, high costs, and the potential for resistance over time. Due to these factors, there is an urgent need for an [illegible] ver adverse effects.

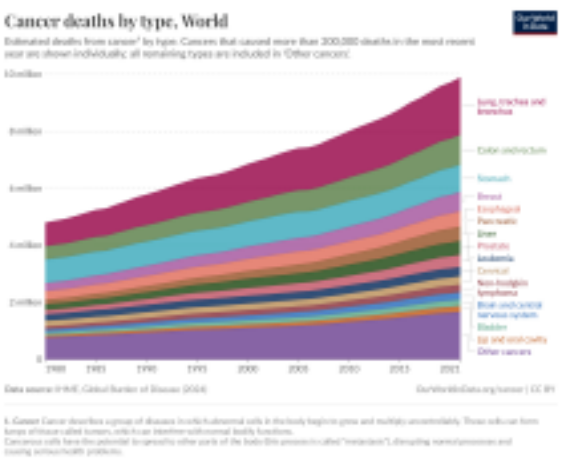

Figure: Cancer deaths by type in the World

674,199 breast cancer related deaths in 2021, shows an increasing trend from the 1980s to 2020s.

Vitamin D, which is a fat-soluble vitamin, plays an important role in a number of body functions such as calcium absorption, bone metabolism, immune function, muscle

function, and cellular regulation.[4]Its deficiency can lead to consequences including hypocalcaemia, bone loss, and muscle weakness.[4]It is now being investigated for its potential anti-cancer properties. Studies have shown that low vitamin D levels are correlated with higher risks of developing breast cancer.[4] Breast cancer patients are also at an increased risk for medical complications associated with vitamin D deficiency such as bone loss, falls, fractures, and infections.[4] Bone loss induced by cancer treatment affects up to 80% of breast cancer patients. The annual loss of bone mineral density in breast cancer patients may also be up to 7 times greater than the annual loss by women without cancer.[4] This loss of bone mineral density produces a significant increase in risk of fractures as opposed to healthy women, which results in increased mortality and negative consequences.[4] As vitamin D plays an important role in bone metabolism, this indicates the use of vitamin D to prevent bone loss.

A preclinical study on osteosarcoma shows promising results. In the study, vitamin D–specifically its active form of $1,25(OH)_2D$--was shown to significantly reduce tumor growth and lung metastasis in murine models of osteosarcoma.[5] Researchers found that vitamin D acted through 2 main mechanisms: downregulation of Snai2, a driver of epithelial-to-mesenchymal transition (EMT), and reprogramming of nonsense-mediated RNA decay (NMD) pathway, which altered gene expression in favor of tumor suppression, immune recognition, and reduced metastatic potential.[5] Also, the vitamin D receptor was shown to directly inhibit pro-metastatic signaling, supporting the role of Vitamin D in preventing cancer cell spreading.[5] This study suggests that Vitamin D's regulatory influence on EMT and RNA surveillance pathways can be relevant to other metastatic cancers such as breast cancer, which spready frequently.[5] Therefore, I hypothesize that vitamin D may act via similar mechanisms to decrease tumor growth and metastatic potential in breast cancer.

Research design and methods

This study aims to investigate the effects of regular vitamin D treatment on breast cancer progression and the risk of metastasis in a murine model. Two groups (each group consisting of 10 mice) of female BALB/c mice will be used as they are commonly chosen for cancer research due to their similarity to humans in terms of anatomy, physiology, and genetics.[6] This study will be conducted under standard laboratory conditions and approved by the Institutional Animal Care and Use Committee (IACUC), ensuring ethical treatments of the animals. Animal models are necessary for this study as they allow us to study both tumor development and effects like metastasis and changes in bone density which cannot be accurately modeled in vitro.

In order to simulate human breast cancer, all mice will be orthotopically injected with 4T1 breast cancer cells into the mammary fat pad.[6] This specific cell line is well known for its ability to spontaneously produce highly metastatic tumors, making it an ideal model for studying tumor growth and the spread of cancer.[7] After injection, the mice will be randomly assigned to either a control group using PBS (ethanol vehicle) or a treatment group using calcipotriol (a form of vitamin D), which is a dose known to not cause hypercalcemia or any deleterious side effects.[5] Treated mice will receive Vitamin D through intraperitoneal (IP) injections of calcipotriol at a dose of 60 μg/kg, which will be administered once daily for 21 days following tumor injection.[5]

Tumor growth will be monitored daily through the use of digital calipers, and tumor volume will be calculated. The survival will be measured by recording the time until natural death or when tumors reach a volume of $2cm^3$, at which point euthanasia will be administered in accordance with the IACUC regulations. To evaluate metastasis, tissue samples from major organs such as the lungs, liver, and bones will be collected and stained (hematoxylin or eosin) to visually count the number and distribution of metastatic lesions.[8]

To evaluate cellular changes, tumors will be excised from both groups following euthanasia and analyzed for gene and protein expression. scRNA-sequencing will be used to evaluate the changes in gene expression between the treated and untreated tumors, with a focus on epithelial-mesenchymal transition (EMT) related genes that were indicated in prior literature, such as CDH1 (E-cadherin), VIM (vimentin), and Snail1. Differential gene analysis will then be performed to identify significantly up or down regulated genes after treatment in an unbiased manner. Additionally, chromatin accessibility will be assessed during ATAC sequencing to determine if vitamin D alters the epigenetic regulation of genes involved in tumor progression. These changes could potentially explain the long term regulatory effects of vitamin D on a genome. In order to validate the RNA sequencing results, protein expression of differentially expressed genes will be measured using Western blotting.

Bone density, which is important due to breast cancer's tendency to metastasize to bone, will be measured using micro-computed tomography. This imaging technique will allow for detailed assessment of bone structure, density, and signs of degradation. The mice within the vitamin D group are expected to show less bone degradation, which indicates a potential protective feature of supplementation. The scans will be taken post-death from common metastatic sites such as the femur or spine. This data will help identify early signs of bone degradation caused by tumor invasion. Also, histological staining may be used on the bone sections to confirm tumor cells within bone marrow cavities, giving more information on metastasis patterns.

In vitro experiments will also help to support the findings from the *in vivo* model. 4T1 cells will be cultured with and without vitamin D treatment, and effects on cell proliferation will be measured through cell counting and proliferation assays using CellTrace. A scratch assay will also be performed to examine the impact of Vitamin D on cell migration, using cultured 4T1 breast cancer cells. A uniform "scratch" will be made down the center of the cell using something to stimulate a wound (e.g. a sharp object), cells will then be treated with Vitamin D and monitored over time using microscopy to measure how quickly the cells migrate to fill the gap. A slower rate of gap closure in the Vitamin D treated group compared to untreated may indicate a reduced migratory ability.

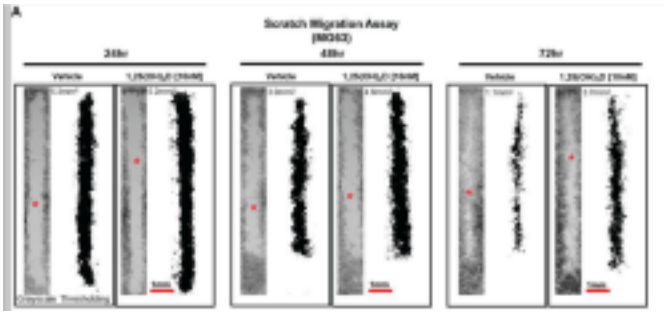

Figure 2A: Scratch migration assay from Vitamin D used in osteosarcoma

Example to show the predicted effect from Vitamin D usage in breast cancer of a slower rate of gap closure in treated group.

All of the data will be statistically analyzed using different tools, such as R and Excel. Tumor volumes, survival data, and gene expression levels will be compared between control and treatment groups using tests such as t-tests or ANOVA for tumor growth. A p-value of less than 0.05 will be considered statistically significant. Overall, this research design combines a multitude of data such as tumor size, survival, metastasis, gene expression, protein analysis, and bone density, in order to explore the hypothesis that Vitamin D supplementation reduces breast cancer progression and metastasis.

Summary and significance

This study aims to evaluate the effects of Vitamin D on breast cancer progression and metastasis using

both *in vivo* and *in vitro* models. Through tumor measurements, metastatic tissue staining, gene and protein expression analysis, and bone density imaging, this research will assess how Vitamin D influences tumor behavior. Based on prior literature, we anticipate that Vitamin D treated mice will show slower tumor growth, fewer metastatic lesions, and preserved bone density as compared to the controls.

There are potential limitations of this study. The 21-day treatment period may not completely represent the long term effects of Vitamin D on tumor progression. Also, the intraperitoneal (IP) injection of calcipotriol may behave differently for oral supplementation in human patients. In order to address these limitations, future experiments can include other breast cancer models, alternative treatment methods, and longer treatment durations. If no significant reduction in tumor growth is observed as a solo therapy, Vitamin D can be combined with other therapies such as chemotherapy, or immunotherapy to see if it enhances therapeutic outcomes.

This work will yield a better insight into the efficacy of vitamin D as a therapeutic treatment and will provide strong precedence for clinical transition if preclinical data supports anti-tumor or anti-metastatic potential. Further investigation can explore using sequencing data such as scRNA-seq and ATAC-seq to determine the down-sequencing pathways altered by the Vitamin D treatment, which can help identify networks related to immune signaling or metastasis. Also, studies using CRISPR can compare effects of different types of Vitamin D such as D2 (Ergocalciferol), D3 (Cholecalciferol), and the active form D1 (calcitriol), to help show which form of Vitamin D has the most potential anti-cancer effects. Overall, these findings may support clinical trials in testing Vitamin D as a safe therapy for breast cancer patients.

References

1. World Health Organization. (2024, March 13). Breast cancer. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/breast-cancer 2. National Cancer Institute. Comprehensive Cancer Information - NCI. (n.d.). https://www.cancer.gov/publications/dictionaries/cancer-terms/def/metastasis 3. American Cancer Society. Breast Cancer Treatment | Treatment Options for Breast Cancer | American Cancer Society. (n.d.). https://www.cancer.org/cancer/types/breast-cancer/treatment.html

4. Peppone, L. J., Rickles, A. S., Janelsins, M. C., Insalaco, M. R., & Skinner, K. A. (2012). The association between breast cancer prognostic indicators and serum 25-OH vitamin D levels. Annals of surgical oncology, 19(8), 2590–2599. https://doi.org/10.1245/s10434-012-2297-3

5. Capobianco, E., McGaughey, V., Seraphin, G., Heckel, J., Rieger, S., & Lisse, T. S. (2023). Vitamin D inhibits osteosarcoma by reprogramming nonsense-mediated RNA decay and SNAI2-mediated epithelial-to-mesenchymal transition. Frontiers in oncology, 13, 1188641. https://doi.org/10.3389/fonc.2023.1188641

6. Zeng, L., Li, W., & Chen, C. S. (2020). Breast cancer animal models and applications. Zoological research, 41(5), 477–494. https://doi.org/10.24272/j.issn.2095-8137.2020.095 7. Gao, Z. G., Tian, L., Hu, J., Park, I. S., & Bae, Y. H. (2011). Prevention of metastasis in a 4T1 murine breast cancer model by doxorubicin carried by folate conjugated pH sensitive polymeric micelles. Journal of controlled release : official journal of the Controlled Release Society, 152(1), 84–89. https://doi.org/10.1016/j.jconrel.2011.01.021 8. Gurina, Tatyana S. "Histology, Staining." StatPearls

[Internet]., U.S. National Library of Medicine, 1 May 2023, https://www.ncbi.nlm.nih.gov/books/NBK557663/ 9. Liu, Fei, et al. "Biomarkers for EMT and Met in Breast Cancer: An Update." Oncology Letters, U.S. National Library of Medicine, Dec. 2016, pmc.ncbi.nlm.nih.gov/articles/PMC5228449

10. Cancer deaths by type. Our World in Data. (n.d.). https://ourworldindata.org/grapher/cancer-deaths-by-type-grouped

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Comparing Oxford Nanopore Sequencing and Antifungal Susceptibility Testing for Rapid and Accurate Identification of Antimicrobial Resistance in the Clinical Fungal Infections of Patients Undergoing Chemotherapy

Hypothesis and Specific Aims

When it comes to technology, is the new really better than the old? This study compares the accuracy and efficiency of antifungal susceptibility testing (AST) and a newer technology, Oxford Nanopore Sequencing (ONT) in identifying antimicrobial resistance (AMR) in clinical fungal infections of patients undergoing myelosuppressive chemotherapy. Patients undergoing myelosuppressive chemotherapy were selected for this study as this group is both more susceptible to severe fungal infections and has a higher mortality rate associated with these infections. This study hypothesizes that ONT will be the more effective of the two technologies.

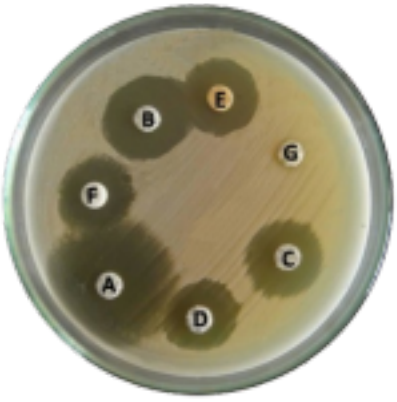
The specific aims of this study are:

1. Comparing ONT and AST in terms of accuracy and efficiency in identifying AMR in clinical fungal infections
2. Comparing the predictive capabilities of ONT and AST in identifying AMR in clinical fungal infections
3. Evaluating the potential impact of ONT on treatment selections and patient outcomes

By addressing these aims, this study could allow clinicians to implement a potentially superior diagnostic tool and ultimately select more effective drugs in a more timely manner, leading to more targeted antifungal therapies.

Background

AMR in fungal infections is an understudied area, yet fungal infections amount to 1.6 million deaths in developing countries and are becoming more widespread in several risk groups including patients undergoing chemotherapy.[1] Efficient identification of AMR in fungal infections allows clinicians to provide more effective treatments. Currently, AST is used in clinical settings to identify AMR, but a newer alternative–ONT–shows promise. This proposed study will compare the two methods.



When a patient has a fungal infection, they are first given an empiric drug chosen based on the provider's experience. Then AST (a phenotypic method) works by taking a sample from the infection and identifying the primary fungus causing the infection by culturing the sample. Fungal isolates are tested with different antimicrobial agents to determine whether the sample has AMR against any of the agents.[2] The length of the process depends on the type of fungi, but it can take anywhere from a few days to eight weeks.[3] Only after this lengthy waiting period can the correct antibiotic be given to the

patient.

Figure 1: Traditional Antimicrobial Inhibition Test (Srinivas, 2018)

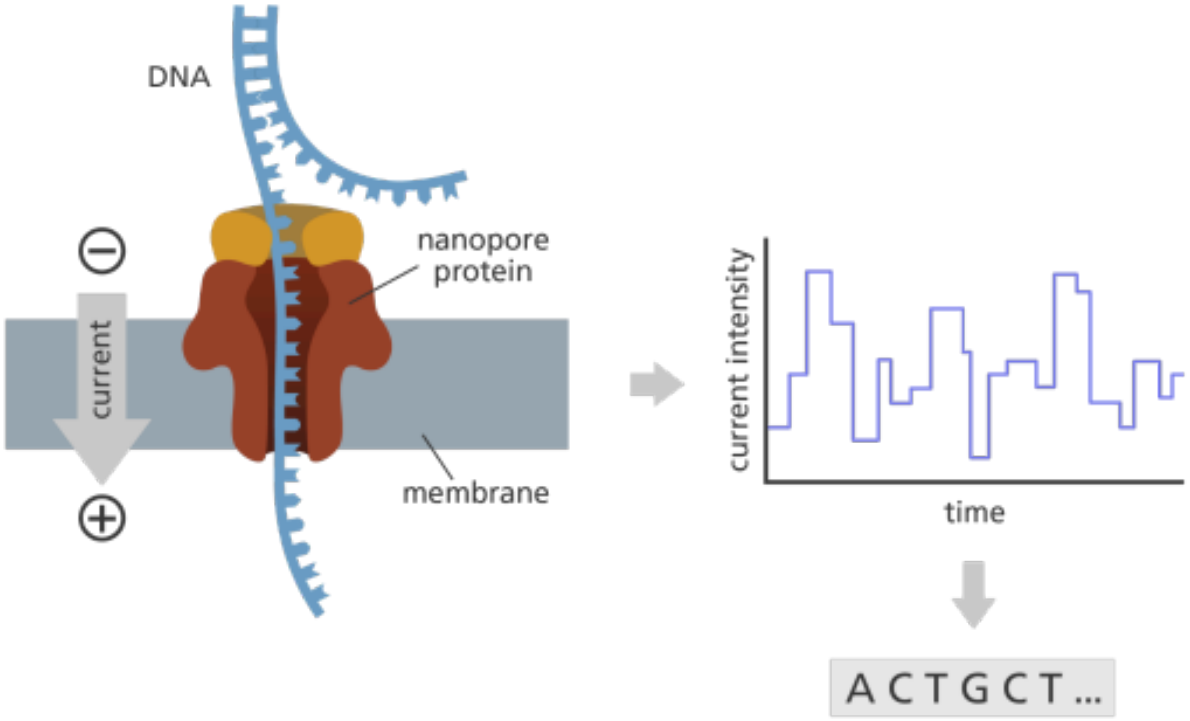


Figure 2: Oxford Nanopore Sequencing (Srinivas, 2018) (Boldú, n.d.)

ONT (a genotypic method) uses flow cells with tiny pores that liquid samples (DNA or RNA molecules) can flow through. A sensor chip measures the amount of current flowing through each pore. Different nitrogenous bases cause different disruptions to the current as they pass through, which the basecalling algorithm is able to turn into a nucleotide sequence. As this happens in real-time, scientists are able to stop whenever they have collected the necessary data.[4] However, whole genome sequencing with adequate depth can take around 48 hours.[5] AMR can be identified with this technology by searching for genes associated with AMR against a specific antifungal agent.

A study published in Nature Communications details a case where an immunocompromised patient was given the antibiotic Meropenem for their bacterial infection. The infection's primary pathogen was identified as a *Klebsiella Pneumoniae* isolate and AST was used to determine that the pathogen was resistant to carbapenems, so the patient was instead given CAZ-AVI. Shortly afterwards, another sample was taken which showed resistance to CAZ-AVI, but susceptibility to carbapenems, so Meropenem was reintroduced, but the patient passed away. The AST process took 52 hours each time. In order to see how ONT would have responded, researchers sequenced both the pre and post CAZ-AVI samples, and found that $bla_{\text{KPC-2}}$ was present in the first sample, confirming Monoporem resistance, and $bla_{\text{KPC-14}}$ was present in the second sample, confirming CAZ-AVI resistance and carbapenem susceptibility. They also found one $bla_{\text{KPC-14}}$ copy in the first

sample. Both samples took fifteen hours to sequence. After sequencing the first sample for an additional two hours, they found a second bla_{KPC-14} copy, showing the potential for CAZ-AVI resistance that AST did not detect.[6] This study demonstrates ONT's ability to accurately and efficiently predict AMR in bacteria. It can be inferred that ONT would perform similarly with fungi.

Research design and methods

This study will begin by randomly selecting fifty males and fifty females undergoing chemotherapy who have fungal infections. As we are using human subjects, we will submit a proposal to the IRB for review, and implement patient consent forms. We chose such a large sample size because some patients may have clinical fungal infections without AMR. It is vital that we test both males and females, because a 2015 study published in PLOS Genetics showed an infection by the smut fungus *Microbrotryum lychnidis-dioicae* had different effects on males and females.[7] Additionally, fungal infections in humans could adapt for males and females differently, so to ensure that our data represents which of the two AMR detection tools (ONT or AST) is better for all chemo patients, we must test both sexes. We chose to study patients undergoing chemotherapy, as a 2010 study published in the Journal of Antimicrobial Chemotherapy stated that from 1995-2001 the mortality rate for chemo patients with invasive fungal infections was 56.9%. Although it decreased to 28.6% from 2001-2006,[8] recent studies have shown that due to AMR and climate change, general frequency, severity, and distribution of fungal infections are on the rise again, especially in risk groups like patients undergoing chemotherapy.[1]

Two samples of the infection will be taken from each patient. We will utilize sample collection methods appropriate for the location of the infection. We chose to look at various infection locations in order to test both technologies (AST and ONT) in as many areas as possible so as to determine which is best overall in a clinical setting. However, during the data analysis process, we will observe whether either technology favored a specific sample type. For superficial infections, we will be using skin scrapings, as nail clippings cannot be used for fungal infections growing on other parts of the body. To collect the scrapings, we will wipe the patch of skin with the most recent lesion using an alcohol wipe. Then, a scalpel held at an angle will be used to scrape the skin-making sure to sample the leading edge of the infection-into the laboratory specimen container.[9] For systemic infections, blood tests are generally considered the best way to get a sample, so for this study patients who have systemic fungal infections will be sampled by through the traditional venous method.[10]

One sample from each patient will be cultured in petri dishes and the primary pathogen will be identified. The pathogen will then be exposed to different antimicrobial agents to see which it is resistant to (which do not inhibit its growth), per standard AST process.[2] The second sample from each patient will be treated with a 2.2% concentration saponin solution, enabling extraction of the host DNA. The fungal DNA will be extracted from the host DNA using mechanical lysis. Finally, the DNA will be purified using AMPure XP solid phase reversible immobilization beads, and the library prepared for ONT GridION sequencing with the Rapid PCR Barcoding Kit. Then it will be run through the ONT GridION with the goal of flagging genes associated with AMR.[11] The patient will be given an antifungal treatment that the infection is not resistant to. The amount of time it takes to confirm which antifungal agents the infection is resistant to will be measured for both tools. The two tools will be operated by different researchers, so results from one experiment will not affect the other.

One important thing to consider is that AMR can manifest in two main ways, and ONT and AST will be tested on their ability to detect both of them, as either of these forms could appear in a clinical setting. Intrinsic AMR is when fungal infections have an innate resistance to an antifungal, usually due to loss of function resistance mutations such as premature stop codons.[1] Acquired AMR is when fungal infections develop resistance after being exposed to an antifungal,[12] and can be due to gain of function resistance mutations where

the regulator that controls the efflux pumps allows them to reduce drug concentration within the cells to ineffective levels.[1] Sometimes one patient will begin with a fungal infection that has intrinsic AMR to one antifungal, but it will develop acquired AMR to another after some time. This is why we will obtain a second set of samples from the one hundred patients seven days after the first and run both AST and ONT again, to see how the infections have adapted.

We will measure the success of each method based on the length of time from when the sample is collected to when AMR is identified, and based on the accuracy of each system. When considering accuracy, we will take into account whether one system detected AMR to an agent that the other did not. If there is a discrepancy like this in the data, we will test the sample for which the discrepancy occurred again using both tools until the results do match up, and we can confirm whether or not the infection was resistant to the agent at that time. In some cases, although neither tool is wrong, one of the two may have enhanced predictive capabilities (for example if one of the tools is able to predict that the Day 1 infection will develop resistance to another antifungal and the sample from Day 8 validates that). This could allow clinicians to prescribe antifungal treatments that will be more effective in the long run, leading to a survival rate better than the standard (which is currently extremely low for patients undergoing chemotherapy, as shown by previous studies).

Summary and significance

This study will compare AST and ONT in their ability to detect AMR in the clinical fungal infections of patients undergoing myelosuppressive chemotherapy. We will look at both speed and accuracy, as both are vital in a clinical setting. Our most straightforward data to interpret will be the data outlining the time it took each tool to detect AMR in each sample. We will also compare technologies on their ability to detect AMR across all the samples and calculate an average deduction in efficiency. Accuracy is more difficult to measure, but we will look at the percentage of the time each technology was able to detect the AMR present in the sample. The technologies will also be compared on their predictive capabilities. One limitation of this study may be the fact that it focuses on patients undergoing chemotherapy-as this may affect the way the infection adapts. Additionally, the amount of patients in the study who end up having infections with AMR may or may not be significant, so there may not be a large enough sample size to holistically compare these two methods. However, this study could potentially demonstrate the potential of ONT to replace AST in a clinical setting. This replacement could have significant clinical implications, including increasingly effective antifungal treatments prescribed in a more timely manner. This would result in improved patient outcomes and a lower mortality rate associated with fungal infections in high risk populations.

References

1. Alastruey-Izquierdo, A., & Martín-Galiano, A. J. (2023). The challenges of the genome-based identification of antifungal resistance in the clinical routine. Frontiers in microbiology, 14, 1134755. https://doi.org/10.3389/fmicb.2023.1134755
2. Boldú, L. O. (n.d.). Oxford Nanopore Technology. Your Genome. https://www.yourgenome.org/theme/what-is-oxford-nanopore-technology-ont-sequencing/ 3. Center for Devices and Radiological Health. (2023, September 28). Antimicrobial resistance and medical devices. U.S. Food And Drug Administration. https://www.fda.gov/medical-devices/products-and-medical-procedures/antimicrobial-resistance-and-medical-devices#:~:text=Antimicrobial%20susceptibility%20tests%20(ASTs)%20are,is%20needed%20to%20be%20effective.

4. Testing.com. (2020, December 18). Antibiotic susceptibility testing - Testing.com. https://www.testing.com/tests/antibiotic-susceptibility-testing/#:~:text=How%20long%20will%20it%20t ake,menu%20in%20clinical%20microbiology%20laboratories.
5. How nanopore sequencing works. (n.d.). Oxford Nanopore Technologies. https://nanoporetech.com/platform/technology
6. Nanopore MinION Sequencing. (n.d.). Molecular and Cellular Imaging Center. https://mcic.osu.edu/genomics/nanopore-minion-sequencing#:~:text=Sequencing%20takes%20approxi mately%2048%20hours,the%20quality%20of%20the%20run.
7. Sauerborn, E., Corredor, N. C., Reska, T., Perlas, A., Da Fonseca Atum, S. V., Goldman, N., Wantia, N., Da Costa, C. P., Foster-Nyarko, E., & Urban, L. (2024). Detection of hidden antibiotic resistance through real-time genomics. Nature Communications, 15(1). https://doi.org/10.1038/s41467-024-49851-4
8. Fassett, C. (2023, August 22). Dangerous fungus is becoming more prevalent. Scientists believe climate change could be to blame | AP News. AP News. https://apnews.com/article/dangerous-fungus-climate-change-new-york-c1b5ab8f4232a5f2320929dae3b 80200
9. Hahn-Ast, C., Glasmacher, A., Mückter, S., Schmitz, A., Kraemer, A., Marklein, G., Brossart, P., & Von Lilienfeld-Toal, M. (2010, January 27). Overall survival and fungal infection-related mortality in patients with invasive fungal infection and neutropenia after myelosuppressive chemotherapy in a tertiary care centre from 1995 to 2006. Journal of Antimicrobial Chemotherapy. https://academic.oup.com/jac/article/65/4/761/824580
10. Collecting specimens for the investigation of fungal infections. (n.d.). https://bpac.org.nz/BT/2011/March/fungal-infections.aspx
11. DermNet. (2023, June 14). Laboratory tests for fungal infection. DermNet®. https://dermnetnz.org/topics/laboratory-tests-for-fungal-infection
12. Street, T. L., Sanderson, N. D., Kolenda, C., Kavanagh, J., Pickford, H., Hoosdally, S., Cregan, J., Taunt, C., Jones, E., Oakley, S., Atkins, B. L., Dudareva, M., McNally, M. A., O'Grady, J., Crook, D. W., & Eyre, D. W. (2022). Clinical Metagenomic Sequencing for Species Identification and Antimicrobial Resistance Prediction in Orthopedic Device Infection. Journal of clinical microbiology, 60(4), e0215621. https://doi.org/10.1128/jcm.02156-21
13. Garcia-Effron, G. (2021). Molecular markers of antifungal resistance: Potential uses in routine practice and future perspectives. Journal of Fungi, 7(3), 197. https://doi.org/10.3390/jof7030197