Beta Amyloid Proteins and Alzheimer's Disease Treatments

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Abstract

Alzheimer's Disease is a degenerative disorder of the brain that results in the deterioration of memory, language, and behavior. The accumulation of a protein called amyloid beta is responsible for the development of Alzheimer's Disease. This protein forms plaques in the brain, obstructing communication between neurons and playing a crucial role in the progression of the disease. Potential treatments aiming to reduce the presence of beta amyloid plaques include immunotherapy (both active and passive), enzymes that degrade beta amyloid, and inhibitors of beta secretase. Unfortunately, effective treatments for Alzheimer's Disease are currently unavailable due to the severe side effects and limited effectiveness of existing options. As Alzheimer's Disease is influenced by various factors including but not limited to amyloid beta proteins, a comprehensive study that includes clinical trials becomes vital in finding an effective treatment. Clinical trial data suggests that beta amyloid degrading enzymes show the most promise as a future treatment, as they have successfully reduced amyloid beta plaque levels in the brain with minimal side effects compared to the other treatments. This review aims to provide a basic understanding of Alzheimer's Disease by exploring the interactions between neurons, amyloid beta proteins, and other internal factors that contribute to the disease's development. Additionally, it highlights three potential treatments and their approaches to preventing or reducing amyloid beta proteins, supported by relevant clinical trial data. Finally, the review discusses the side effects and limitations of current treatment options while considering the potential of becoming viable future treatments.

Keywords: Alzheimers, Beta Amyloid Protein, Beta Secretase

1. Introduction

The initial identification of Alzheimer's Disease (AD) dates back to 1906 when German Physicist Alois Alzheimer first reported a case. Alzheimer's is the most prevalent cause of dementia, a clinical syndrome characterized by memory loss, impaired judgment, and difficulties in daily activities (Lopez et al., 2019). The accumulation of amyloid beta and hyperphosphorylated tau protein are the two primary factors known to contribute to the development of Alzheimer's disease. While much attention has been focused on these proteins, their build-up leads to the deterioration of synapses and affects dendritic spines. This loss of signal transmission between neurons ultimately results in apoptosis or cell death, causing the brain to shrink in size. AD can be categorized into two types based on different age groups: Familial Alzheimer's (early-onset), affecting individuals between the ages of 30-65, often influenced by genetic and environmental factors, and Sporadic Alzheimer's (late-onset), diagnosed in individuals over the age of 65 (Athar et al., 2021). Familial Alzheimer's represents a smaller percentage, around 5-10%, of all Alzheimer's cases and is considered rarer compared to Sporadic Alzheimer's. When it comes to Familial Alzheimer's the scientific community primarily focuses on identifying early signs of the disease to enable earlier diagnosis and better management of symptom severity. However, more emphasis is placed on Sporadic Alzheimer's since it is more common and would effect a wider population of people with Alzheimer's. The treatments discussed in this review

primarily target Sporadic Alzheimer's and aim to reduce and prevent the accumulation of beta amyloid protein. Beta amyloid protein contributes to the development of Alzheimer's by disrupting the balance between protein clearance and production, leading to the build-up of Aβ between neurons and hindering neuronal signaling. Another contributor to Alzheimer's is the formation of Neurofibrillary Tangles, caused by the tau protein. Tau protein assists in maintaining microtubules, internal structures that facilitate the transportation of nutrients and molecules from the cell body to the axon and dendrites. In Alzheimer's, chemical changes cause tau to detach from microtubules and adhere to other tau molecules, resulting in the formation of tangles and disrupting synaptic transmission between neurons. These factors lead to neuronal damage or death and contribute to the shrinkage or atrophy of the brain, a prominent clinical feature of Alzheimer's cases. Although both beta amyloid protein and tau protein are of importance, this review primarily focuses on treatments that specifically target beta amyloid protein. A correlation has been discovered, where an increase in beta amyloid in the brain is associated with an increase in tau. However, further research is required to fully understand this relationship. Recent clinical findings suggest that beta amyloid leads to the aggregation of tau, making it more harmful (Rajmohan & Reddy, 2017). Hence, the focus of this review is on targeting beta amyloid plaques, which significantly contribute to the progression of the disease. Multiple potential treatments are currently under investigation and have shown promising results in certain clinical trials. These treatments encompass Immunotherapy, Beta Amyloid Degrading Enzymes, and Beta Secretase Inhibitors, all of which aim to reduce or prevent the formation of amyloid beta plaques (Weller & Budson, 2018).

2. Role of Amyloid Beta Protein in Alzheimer's Disease

Amyloid beta proteins play a significant role in the development of Alzheimer's disease (AD) by accumulating between neurons and forming amyloid beta plaques. These plaques disrupt neuronal signaling, leading to cell death, brain atrophy, and ultimately dementia (Chen et al., 2017). Amyloid Precursor Protein (APP) is a transmembrane protein that undergoes enzymatic breakdown, particularly by b-secretase, resulting in fragments with different functions. The cleavage between b-secretase and APP produces Aβ monomers. Aβ serves various roles in the brain, including neuroprotection, memory and synaptic plasticity regulation, cholesterol transport regulation, and acting as a transcription factor (Gouras et al., 2015). The Amyloid Hypothesis describes a cascade of events that explains the dynamic equilibrium of amyloid beta protein. This equilibrium relies on the balance between the clearance and production of Aβ. However, disturbances caused by oxidative stress or other factors can lead to an increased accumulation of Aβ and the formation of amyloid beta plaques. The increased Aβ levels trigger an inflammatory response by the immune system, resulting in cell death and the progression of AD (Folch et al., 2018).

3. Immunotherapy

Immunotherapy is a highly focused area of research in the treatment of Alzheimer's disease (AD). It involves activating the immune system by introducing specific antibodies to elicit an immune response. There are two types of immunotherapy: active and passive immunization. Active immunization involves administering synthetic Aβ fragments as a vaccine to activate the immune system's T-cells and B-cells, which can help reverse the deteriorating conditions caused by AD. On the other hand, passive immunization involves injecting externally produced monoclonal antibodies that target amyloid (Blasko & Loebenstein, 2003). While immunotherapy has shown promise in animal trials by reducing Aβ levels, there is limited human clinical trial data available. Some animals treated with active immunization experienced encephalitis (inflammation of the brain) and vasogenic edema (disruption of the blood-brain barrier causing fluid accumulation), while those treated with passive immunization showed amyloid-related imaging abnormalities (Wisniewski & Goni, 2014). These side effects raise concerns about the safety and effectiveness of immunotherapy in humans. Furthermore, passive immunization is considered short-term and costly compared to active immunization, which is more long-term and cost-effective. In a clinical trial involving mice, different vaccines were tested, and while they showed reduction in Aβ levels in the mice's brains, there were unwanted side effects due to immune responses. Researchers estimate that the vaccine may only be effective for a subset of patients (20-30%) due to genetic and immune response differences. Based on the available clinical data, it is evident that immunotherapy

as a treatment for AD is complex and carries risks (Valiukas et al., 2022). Modifications are necessary to minimize side effects and ensure its effectiveness for the entire population. Ongoing research aims to address these challenges and improve the potential of immunotherapy in treating AD.

4. Beta Amyloid Degrading Enzymes

Extensive research has been conducted on Beta amyloid degrading enzymes (ADE) due to their highly effective role in degrading Aβ. These enzymes are naturally present in the brain and are responsible for breaking down small amounts of intracellular and extracellular Aβ. Neprilysin, Endothelin-Converting Enzyme, Insulin-Degrading Enzyme, and Plasmin are among the potential candidates for Aβ degradation, and their connection with Aβ deposition has been established. However, during the progression of AD, a decrease in protein expression and oxidative stress disrupts the function of ADE, leading to the formation of beta amyloid plaques (Schenk et al., 2012). In a clinical trial involving cultured cells of Chinese hamster ovaries and mice, Neprilysin was found to be a crucial enzyme correlated with amyloid beta deposition. Reduced levels of this enzyme were associated with higher percentage deposition. Endothelin-Converting Enzyme demonstrated a 90% reduction in extracellular Aβ concentration, although this varied depending on different controls and levels. Nevertheless, this enzyme plays a significant role in Aβ clearance, requiring further research. Insulin-Degrading Enzymes exhibited gene-dose dependent effects on Aβ deposition, showing different outcomes in transgenic mice compared to heterozygous mice. On the other hand, Plasmin, despite its involvement in various neuronal functions, did not show a direct impact on Aβ levels in plasminogen deficient mice (Wang et al., 2006). Gene therapy, a method of reconstructing genetic material for desired effects, has shown potential in increasing ADE expression. However, ethical concerns and limited knowledge hinder its application in humans. Gene delivery of certain degrading enzymes to APP has been experimented on transgenic mice, resulting in reduced amyloid beta levels. As an alternative, pharmacological agents have been explored to increase ADE expression in a more efficient and ethical manner compared to gene therapy (Sikanyika et al., 2019). Further research on enzyme activation holds promise for the development of new drugs to treat AD.

5. Beta Secretase Inhibitors

Beta Secretase is an enzyme responsible for cleaving the transmembrane protein APP, which triggers the production of Aβ. Enzyme inhibitors are molecules that bind to this enzyme, reducing its activity and preventing it from catalyzing the reaction. BACE inhibitors have shown promise as a valid treatment for AD by blocking beta-secretase and halting the production of Aβ (Kumar et al., 2018). However, the development of these inhibitors faced a challenge with the Blood Brain Barrier (BBB), which restricts the passage of large or hydrophilic molecules into the brain's extracellular fluid. Scientists have since discovered methods to overcome this barrier. Some BACE inhibitors have reached phase I trials, but they were terminated due to liver abnormalities or issues with blood plasma (Lukiw, 2013). Gamma secretase is another enzyme involved in amyloid cleavage, and combining gamma secretase modulators with BACE inhibitors has shown effectiveness in reducing Aβ with minimal side effects (Madav et al., 2019). Clinical trials involving the beta secretase inhibitor MK-8931 demonstrated significant reductions in Aβ levels in the Cerebrospinal Fluid (CSF). In a separate one-week trial with 32 patients having mild to moderate AD, 84% of Aβ levels were decreased in the CSF. However, it is important to note that BACE inhibitors may not have a significant impact on patients with moderate AD, as the amyloid beta plaques would have already formed (Menting & Claassen, 2014). Further research is necessary to determine the potential of BACE inhibitors as a future treatment option. Additionally, managing side effects and addressing the limitations of this treatment approach are crucial areas for improvement.

6. Conclusion

The key findings of this literature review indicate that three treatments, namely immunotherapy, beta amyloid degrading enzymes, and beta secretase inhibitors, all aim to target and reduce the formation of beta amyloid plaques,

which are known to be one of the main causes of AD. Immunotherapy involves the use of antibodies that target different forms of amyloid, and can be administered through passive or active immunization. While immunotherapy seems to be successful in targeting amyloid, further investigation is necessary due to the severe side effects, such as encephalitis and vasogenic edema. Passive immunization is unlikely to be a viable treatment option due to numerous disadvantages such as increased cost, severe side effects, and being a short term treatment. Moreover, data from active immunization clinical trials suggest that this treatment can only benefit a limited percentage of the population (20-30%) due to specific genetic factors, making future modifications complex and time-consuming (Valiukas et al., 2022).

Beta amyloid degrading enzymes directly act upon the beta amyloid plaques in the brain. Many scientists believe this approach has the potential to become the leading treatment for Alzheimer's in the future. However, this treatment faces a major roadblock, as it requires gene therapy to increase enzyme expression. Scientists are cautious about gene therapy as in the past there were problems such as targeting the wrong cells, creating an unwanted immune response, and other infections, so they are moving in a slow pace to understand more about gene therapy and apply that to beta amyloid degrading enzymes as a possible treatment for AD. Beta amyloid degrading enzymes have shown promising results in clinical trials with Endothelin-Converting Enzymes degrading 90% of extracellular Aβ. Proper management of side effects and successful results in clinical trials will pave the way for advancement in this treatment modality.

Lastly, Beta secretase inhibitors have demonstrated good results in clinical trials by directly blocking the enzyme beta-secretase from initiating the production of Aβ. In clinical trials, beta-secretase inhibitors have successfully lowered Aβ levels in cerebrospinal fluid (Menting & Claassen, 2014). However, the main drawback of this treatment is its inefficiency in patients already presenting with amyloid beta plaques, as it primarily stops the production of Aβ. Additionally, it can have some side effects such as liver abnormalities or problems with blood plasma. Therefore, scientists are hesitant on exploring more on this treatment option as it will not have a major effect on people already present with mild to severe AD.

It should be kept in mind that AD is caused by numerous additional factors, but after considering the advantages and disadvantages of all treatment options and the data analyzed in this literature review, beta amyloid degrading enzymes show the most promise for being a future treatment option. This is based on the positive outcomes that showcase significant reduction in Aβ levels. Gene therapy appears to be a roadblock for this treatment as it is still evolving, but scientists are gaining better control over it and are moving closer to conducting clinical trials. Moreover, making the treatment more affordable and accessible to a broader population is equally important. It is crucial to acknowledge that Alzheimer's disease (AD) is caused by various factors, beyond just beta amyloid plaques. Therefore, the treatment strategy should encompass comprehensive research into all these contributing factors to achieve the most effective results.

References

Athar, Teeba, et al. (2021). Recent Advances on Drug Development and Emerging Therapeutic Agents for Alzheimer's Disease. *Molecular Biology Reports*, vol. 48, no. 7, 2021, pp. 5629–5645, doi:10.1007/s11033-021-06512-9.

Blasko, I., & Grubeck-Loebenstein, B. (2003). Role of the Immune System in the Pathogenesis, Prevention and Treatment of Alzheimer's Disease. *Drugs & Aging*, 20(2), 101–113. https://doi.org/10.2165/00002512-200320020-00002

Chen, G. S., et al. (2017). Amyloid beta: structure, biology and structure-based therapeutic development. *Acta Pharmacologica Sinica*, 38(9), 1205–1235. https://doi.org/10.1038/aps.2017.28

Folch, J., et al. (2018). Review of the Advances in Treatment for Alzheimer Disease: Strategies for Combating β-Amyloid Protein. *Neurología (English Edition)*, 33(1), pp. 47–58, doi:10.1016/j.nrleng.2015.03.019.

Gouras, Gunnar K., et al. (2014). B-Amyloid Peptides and Amyloid Plaques in Alzheimer's Disease. *Neurotherapeutics*, 12(1), pp. 3–11, doi:10.1007/s13311-014-0313-y.

Kumar, Devendra, et al. (2018). Secretase Inhibitors for the Treatment of Alzheimer's Disease: Long Road Ahead. *European Journal of Medicinal Chemistry*, 148, pp. 436–452, doi:10.1016/j.ejmech.2018.02.035.

Lukiw, W. J. (2012). Amyloid beta (Aβ) peptide modulators and other current treatment strategies for Alzheimer's disease (AD). *Expert Opinion on Emerging Drugs*, 17(1), 43–60. https://doi.org/10.1517/14728214.2012.672559

Madav, Y., et al. (2019). Recent Therapeutic Strategies Targeting Beta Amyloid and Tauopathies in Alzheimer's Disease. *Brain Research Bulletin*, 146, pp. 171–184, doi:10.1016/j.brainresbull.2019.01.004.

Menting, C., et al. (2014). b-secretase inhibitor; a promising novel therapeutic drug in Alzheimer's disease. *Frontiers in Aging Neuroscience*, 6, https://doi.org/10.3389/fnagi.2014.00165.

Rajmohan, R., and Hemachandra R. P. (2017). Amyloid-Beta and Phosphorylated Tau Accumulations Cause Abnormalities at Synapses of Alzheimer's Disease Neurons. *Journal of Alzheimer's Disease*, 57(4), pp. 975–999, doi:10.3233/jad-160612.

Schenk, D., et al. (2012). Treatment Strategies Targeting Amyloid -Protein. *Cold Spring Harbor Perspectives in Medicine*, 2(9), doi:10.1101/cshperspect.a006387.

Sikanyika, N. L., et al. (2019). Powering Amyloid Beta Degrading Enzymes: A Possible Therapy for Alzheimer's Disease. *Neurochemical Research*, 44(6), pp. 1289–1296, doi:10.1007/s11064-019-02756-x.

Lopez, S., and Jose A., et al. (2019). Alzheimer's Disease. *Handbook of Clinical Neurology*, pp 231–255, doi:10.1016/b978-0-12-804766-8.00013-3.

Valiukas, Z., et al. (2022). Immunotherapies for Alzheimer's Disease—A Review. *Vaccines*, 10(9), p. 1527, doi:10.3390/vaccines10091527.

Wang, D., et al. (2006). Beta-Amyloid Degratiaon and Alzheimer's Disease, *Journal of Biomedicine and Biotechnology*, 2006(3), pp. 1–12, doi:10.1155/jbb/2006/58406.

Weller, J., & Budson, A. (2018). Current understanding of Alzheimer's disease diagnosis and treatment. *F1000Research*, 7, 1161. https://doi.org/10.12688/f1000research.14506.1.

Wisniewski, T., & Goni, F. (2014). Immunotherapy for Alzheimer's Disease. *Biochem Pharmacol,* 88(4), 499–507. https://doi.org/10.1016/j.bcp.2013.12.020.

Determinants of a Firm's Sustainable Competitive Advantages under Supply Chain Disruptions

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Abstract

This study examined the significance and mechanisms of the roles of the determinant factors play in gaining sustainable competitive advantage. The factors include supply chain resilience, performance, and awareness of potential disruptions. The investigation was conducted within manufacturers in the US in the context of their supply chain operations under disruptions. Employing a linear regression on the four factors, this empirical examination shows that supply chain resilience, performance, and awareness of potential disruptions exert positive effects on the improvement of sustainable competitive advantage. Additionally, this study investigated the role of market volatility, which plays a negative role in building sustainable competitive advantage and the role is marginal. The findings of this study offer practical implications to supply chain managers. Limitations of this study suggest future research directions. First, the regression analysis was based on a dataset with small sample size, which might constrain the generalizability of the findings of the investigation. Further research might include a substantial sample size to better represent the manufacturers in the US. Second, inventory management can impose disruptions on supply chain operations and generate ripple effects in a supply chain. However, it is not considered in the study. Future research is expected to assess its role in building sustainable competitive advantage.

Keywords: Sustainable competitive advantage, Supply chain resilience, Performance, Market volatility, Awareness, Disruption.

1. Introduction

Supply chain disruptions are unanticipated events that interrupt usual operations of a supply chain and put supply chain firms at risk financially and operationally. During unprecedented supply chain disruptions, traditional supply chain practices such as globalization and lean operations could increase supply chain vulnerability because of the imbalance of product supply and customer demand. For instance, during the Covid pandemic, highly increased demand for masks and medical products resulted in severe supply shortage and automobile companies had to switch to produce masks and personal protective equipment to cope with the unexpected changes in the market. Such vulnerability leads to more disruptions in supply chain processes including planning, sourcing, manufacturing, delivery, and return. The disruptions' ripple effect adversely influences supply chain parties from suppliers, manufacturers, distributors, to customers. To survive and recover from supply chain disruptions, it is crucial for firms to gain sustainable competitive advantages through supply chain management. Prior studies suggested that building supply chain resilience (e.g., Zhao, et al., 2023) and fostering awareness of potential disruptions (e.g., Stephens, et al., 2022) can be effective ways to cope with the adversities. However, research on the link between supply chain resilience or awareness of potential supply chain disruptions and sustainable competitive advantages is scarce. Furthermore, market volatility has not been considered as an explanatory factor for sustainable competitive advantages.

Lastly, improved supply chain performance might help gain a firm's sustainable competitive advantages under supply chain disruptions, which has not been discussed in the literature. This study aims to fill these research gaps by examining the determinant roles of both internal and external factors in building sustainable competitive advantages. To explore the determinant factors of sustainable competitive advantage, we will conduct an empirical investigation within the manufacturers in the US in the context of supply chain operations to evaluate the significance and mechanism of the roles of both internal and external factors including supply chain resilience, performance, market volatility, and awareness of potential supply chain disruptions. The anticipated findings of this study would be significant impact of the four factors, with internal factors exerting positive impact and external factor (market volatility) exerting negative impact on sustainable competitive advantage.

2. Literature Review

Prior studies have empirically investigated determinant factors of achieving sustainable competitive advantage in different contexts and regions. It is suggested that knowledge management and dynamic capabilities foster sustainable competitive advantages through a survey conducted in Iran (Beigi et al., 2023). This survey was conducted from Iranian knowledge firms in the context of general business environment. Another study performed by Lee and Yoo (2021) assessed the role of marketing and innovation capability in enhancing sustainable competitive advantage within small business enterprises in Korea. The results show both capabilities are contributing factors to the improvement of sustainable competitive advantage. Two studies were conducted respectively in Indonesia. Suryantini et al. (2023) examined the five factors of achieving sustainable competitive advantage including technology adoption, intellectual capital, strategic flexibility, open innovation, and business performance within small and medium enterprises. The other study by Astuti et al. (2023) suggested that sustainable competitive advantage is dependent on three types of capitals (human, relational, and structural). Strategic flexibility is also a determinant of sustainable competitive advantage in a study of Malaysian hotels (Hossain et al., 2022).

The empirical studies on the determinants of sustainable competitive advantage in the relevant literature focused on eastern cultures in the context of general business management. Since global supply chain operations were largely disrupted during the Covid pandemic, to achieve sustainable competitive advantages, they need to be restored (resilience), maintain efficient operations (performance), and disruption-oriented (awareness of potential disruptions) in turbulent markets (market volatility). In particular, the three capabilities of readiness, response, and recovery of supply chain resilience cope with three phases of supply chain disruptions respectively including before, during, and after disruptions (Ali et al., 2022; Zhao et al., 2023). The capabilities embedded in supply chain resilience equip firms with dynamic capabilities in different phases of disruptive events. Post Covid, it is of paramount importance to examine the factors determining sustainable competitive advantages in the context of supply chain management within manufacturers in the US. Thus, this study assesses the significance and mechanism of the roles of the factors including supply chain resilience, performance, market volatility, and awareness of potential supply chain disruptions in the context of supply chain operations within manufacturers in the US.

3. Research Methodology

3.1 Dataset

The dataset was collected through a web survey. A list of emails were selected from D&B Hoovers based on functional groups in the US. We chose the following screening criteria for the email list: manufacturing and operations related functional groups, industry index of 31, 32, and 33 indicating manufacturing industry, contact levels at vice presidents, senior vice presidents, and executive vice presidents, job functions as manufacturing executive, operations executive, and purchasing executive, employees (single site) more than 100, revenue more than 1 million. A link to the survey was sent to each identified respondent. After two rounds of data collection, we received 83 responses in total. The industries include Materials and Industrial Machinery, Construction and Engineering, Electronic Products

and Telecommunications, Chemical and Pharmaceutical/Healthcare, Energy, Automotive and Transportation, Food and Beverages, Textiles and Clothing, Automotive and Transportation, and others.

3.2 Missing Value Analysis and Non-Response Bias

There are different ways to cope with a missing value in a dataset. Scholars can either delete an observation with missing values or replace missing values with the mean, median, or mode. Data selection bias might be introduced when the observations with missing values are all deleted. We choose not to delete an observation if the missing values are less than 25%. 2 out of 83 collected observations were removed due to more than 25% values missing. For the rest of the 81 observations, we replaced the missing value of categorical variables with the mode and replaced the missing value of quantitative variables with the median. As a result, for the rest of the data analyses, 81 samples were included in the study.

Since the data were collected through two rounds of efforts, we need to check the non-response bias by comparing the respondents in the two rounds. ANOVA was performed for the analysis of comparison between the two rounds of data collection. The results show that the difference is ignorable at $p \leq 0.05$ in terms of the firm's size, age, and annual sales. Thus, there is no evidence for non-response bias in the data collection efforts.

3.3 Scale Development for Independent and Dependent Variables

In this study, we included one dependent variable and four independent variables. In particular, for the four independent variables, a firm's supply chain resilience, performance, and its awareness of potential disruptions are internal factors and market volatility as an external factor.

All scales used in the survey were either adopted, adapted, or newly developed from previously published articles in an extant literature review. In particular, sustainable competitive advantage (ca) is a dependent variable, which is measured by six items in the questionnaire to reflect the level of recognition from customers, reputation of products, differentiation in products, loyalty of customers and connections with them, and just-in-time delivery in the face of disruptions. This measure was from Abeysekara et al. (2019).

Four independent variables in this study include supply chain resilience (resi), performance (scp), market volatility (vola), and awareness of potential disruption (aware). The scale for supply chain resilience was gauged by three questions developed through an extant review of the literature. There are many resilience scales in the literature, however, few of them reflect the capabilities of supply chain transformability, adaptability, and agility. In this study, the measure of supply chain resilience (resi) taps the degree to which the respondent firms' transformability, adaptability, and agility in under disruptions. Supply chain performance (scp) is measured by the degree of reduction in costs and lead time and by the level of process flexibility and customer satisfaction. Market volatility (vola) is measured by capturing the degree of changes in a respondent firm's business environment and its products, customers' needs, supplier capabilities, and competitors' products. For the measure of awareness of potential supply chain disruptions (aware), we adapted from Bode et al. (2011). It includes six items in the questionnaire about a firm's supply chain disruption experience, alertness and awareness of potential disruptions, improvement for coping with potential disruptions, measures to reduce the disruption impact, and disruption analyses.

3.4 Descriptive Statistics

In this study, we used uniform and consistent scale measurement from 1 (strongly disagree/not at all/very stable) through 5 (strongly agree/a very large extent/very volatile). There is no issue for consistency and normality for the scales. Descriptive statistics shown in Table 1 include minimum and maximum value

Table 1. Detailed Statistics

	N	Min	Max	Mean	Std. Dev.
ca	81	1.00	5.00	3.5523	.75711
resi	81	1.00	5.00	3.4846	.74304
scp	81	1.00	5.00	3.5185	.80891
vola	81	1.00	4.75	2.8323	.71133
aware	81	1.33	5.00	3.6144	.67016
Valid N (listwise)	81				

for each variable, and their respective mean and standard deviation. The mean of the five variables included in the study ranges from 2.83 to 3.61 with 81 valid samples entered in the model.

3.5 Correlations

Correlation matrix shows the relationships between the variables which are included in the study. The coefficient of correlation is from 0 (no correlation) to 1 (perfect correlation). The significance of the correlation between two variables reflects whether a correlation is significant. The following criteria is used for significance testing:

When the significance level is less than 0.005, it is strongly significant;

When the significance level is between 0.005-0.01, it is significant;

When the significance level is between 0.01-0.05, it is moderately significant.

Table 2. Correlations

		ca	tran	scp	vola	aware
Pearson Correlation	ca	1.000	.771	.704	.023	.655
	resi	.771	1.000	.708	.025	.706
	scp	.704	.708	1.000	.142	.543
	vola	.023	.025	.142	1.000	-.003
	aware	.655	.706	.543	-.003	1.000
Sig. (1-tailed)	ca	.	<.001	<.001	.420	<.001
	resi	.000	.	.000	.412	.000
	scp	.000	.000	.	.104	.000
	vola	.420	.412	.104	.	.489
	aware	.000	.000	.000	.489	.

As shown in Table 2, sustainable competitive advantage is significantly correlated with supply chain resilience, performance, and awareness of potential disruptions.

The correlations matrix in Table 2 also shows that there exists significant correlation between several predictor variables, we need to check for multicollinearity to see whether the correlations are severe enough to affect the regression and results interpretation.

3.6 Reliability Test of the Scales

Reliability of the scales refers to the internal consistency of the items in the scale measurement. It is tested using SPSS to show the Cronbach's alpha coefficient for each scale in the analysis. The coefficient indicates whether the measurement items are internally consistent and reliable. When a coefficient has a value greater than 0.70, the scale has acceptable reliability. In this study, we tested reliability for each variable. Tables 3-7 show the results of

Table 3: Reliability statistics for sustainable competitive advantage

Alpha	Alpha with Standardization		N	
.897	.898		6	
Statistics for Item-Total				
	Mean with Deletion	Variance with Deletion	Correlation	Squared Correlation	Alpha with Deletion
ca1	17.67	14.400	.768	.678	.872
ca2	17.63	13.811	.830	.756	.862
ca3	17.81	15.378	.627	.435	.893
ca4	17.81	14.178	.783	.645	.870
ca5	17.68	14.346	.776	.671	.871
ca6	17.81	15.403	.562	.385	.904

reliability test for the five variables respectively. The alpha coefficients are from 0.80 to 0.90, exceeding the threshold 0.70, indicating good reliability for each scale. In particular, Cronbach's alpha for sustainable competitive advantage

Table 4. Reliability statistics for resilience

Alpha	Alpha with Standardization		N	
.803	.803		4	
Statistics for Item-Total				
	Mean with Deletion	Variance with Deletion	Correlation	Squared Correlation	Alpha with Deletion
resi1	10.58	5.447	.560	.404	.779
resi2	10.52	4.803	.697	.516	.712
resi3	10.25	4.988	.642	.433	.741
resi4	10.32	5.146	.571	.379	.776

is 0.89 (Table 3), resilience is 0.80 (Table 4), supply chain performance is 0.85 (Table 5), market volatility is 0.83 (Table 6), and awareness of potential supply chain disruption is 0.86 (Table 7). The last column in the table shows the Cronbach's alpha coefficient if an item is deleted. We remove an item from the measurement for a scale when the coefficient increases significantly after

an item is removed. We also remove an item when the coefficient becomes greater than 0.70 after the removal. Consequently, we don't need to remove any item in this study based on the outcomes of the reliability testing.

Table 5: Reliability statistics for supply chain performance

Alpha	Alpha with Standardization		N	
.846	.848		4	
Statistics for Item-Total				
	Mean with Deletion	Variance with Deletion	Correlation	Squared Correlation	Alpha with Deletion
scp1	10.60	6.370	.733	.618	.787
scp2	10.74	6.044	.711	.620	.793
scp3	10.51	6.050	.686	.491	.804
scp4	10.41	6.397	.612	.424	.836

Table 6: Reliability statistics for market volatility

Alpha	Alpha with Standardization		N	
.831	.832		4	
Statistics for Item-Total				
	Mean with Deletion	Variance with Deletion	Correlation	Squared Correlation	Alpha with Deletion
vola1	8.33	4.500	.665	.446	.785
vola2	8.54	5.026	.636	.406	.796
vola3	8.49	4.778	.687	.472	.774
vola4	8.59	4.819	.651	.424	.790

Table 7: Reliability statistics for awareness of potential supply chain disruption

Alpha	Alpha Standardization		N	
.856	.858		6	
Statistics for Item-Total				
	Mean with Deletion	Variance with Deletion	Correlation	Squared Correlation	Alpha with Deletion
aware1	18.37	12.722	.431	.236	.871
aware2	17.97	12.281	.659	.538	.830
aware3	17.94	11.547	.684	.505	.824
aware4	18.09	11.364	.731	.562	.815
aware5	18.09	11.159	.694	.583	.822
aware6	18.09	11.184	.689	.583	.823

3.7 Multicollinearity Test

In regression analyses, multicollinearity exists when independent variables are correlated and these variables cannot offer unique information as they are supposed in the model. With multicollinearity, issues could be caused in modeling fitting and result interpretation. To avoid multicollinearity, we used mean-centered value for both dependent and independent variables (Aiken and West, 1991). As shown in Table 8, the collinearity tolerance values for the variables are from 0.35 and 0.97, which exceed 0.10. The threshold value is 0.10 for collinearity tolerance in the regression model (Hair et al., 1998).

At the same time, we also checked the variance inflation factor (VIF) in the output of the regression model to see whether the correlations between the independent variables are strong. The threshold value for VIF is 5. When VIF is greater than 5, a potential strong correlation between independent variables would cause multicollinearity, which results in unreliable outcomes of a regression model. Table 8 shows that the VIF of the independent variables ranges from 1.04-2.85, indicating that multicollinearity between the independent variables is at a low degree and can be ignorable.

4. Linear Regression and Results

We performed linear regression using SPSS by entering sustainable competitive advantage as dependent variable and the other four as independent variables including supply chain resilience, performance, market volatility, and awareness of potential disruptions. The regression results are presented in Tables 8 and 9. Standardized coefficients (Beta) and significance level for the t-value indicate whether a predictor variable is significant and has explanatory power for the dependent variable.

The results show that out of the four predictors, three are significant and one is non-significant. Sustainable competitive advantage is dependent on three factors including supply chain resilience, performance, and awareness of potential disruptions. However, market volatility only plays a marginal role in improving sustainable competitive advantage. As expected, the results show a negative impact on sustainable competitive advantage with a coefficient Beta of -0.03. Although it is not significant, they are related in a negative way. That is, a high level of market volatility will lower the level of sustainable competitive advantage. For the other three predictor variables, they all have positive coefficient Beta, indicating positive effects on sustainable competitive advantage. When supply chain resilience, performance, and awareness of potential disruptions increase, sustainable competitive advantage will be improved. Table 9 shows the linear regression model is significant at the significance level less than 0.001, which means that the predictor variables explain a great amount of variance in sustainable competitive advantage. The value of R square of 0.64 in Table 9 means 64% of variance in sustainable competitive advantage can be explained by the regression on the four predictor variables.

4.1 The linear regression equation in the model is as follows

Sustainable competitive advantage =
0.36+0.43×supply chain resilience
+0.29×supply chain performance
-0.03×market volatility
+0.22×awareness of potential supply chain disruptions

4.2 The interpretation of the results are as follows

The intercept is the value of the dependent variable when explanatory variables take on the value of zero. This is not always possible in some cases. The intercept is only meaningful when the independent variables can hold a value of zero, otherwise, the intercept can only determine the position of the regression line in the graph. In this study, the intercept of the regression model is 0.36, which is not meaningful since the four predictor variables cannot have a value of zero. For example, it is barely possible for supply chain resilience, performance, market volatility, awareness of potential disruptions to have a value of zero.

4.3 At the significance level of 0.05,

On average, holding other predictor variables constant, when supply chain resilience increases by one-unit, sustainable competitive advantage will be improved by 0.43 unit.
On average, holding other predictor variables constant, when supply chain performance is enhanced by one-unit, sustainable competitive advantage will be improved by 0.29 unit.
On average, holding other predictor variables constant, when market volatility increases by one-unit, sustainable competitive advantage will be reduced by 0.03 unit.
On average, holding other predictor variables constant, when awareness of potential supply chain disruptions is boosted by one-unit, sustainable competitive advantage will be improved by 0.22 unit.

Table 8. Coefficients[a]

Model		B	Std. Error	Beta	t	Sig.	Interval of Confidence (0.95) for B		Collinearity	
							Lower	Upper	Tolerance	VIF
1	(Constant)	.361	.347		1.041	.301	-.330	1.052		
	resi	.426	.114	.418	3.731	<.001	.199	.654	.351	2.845
	scp	.288	.090	.308	3.208	.002	.109	.467	.480	2.085
	vola	-.033	.072	-.031	-.456	.649	-.176	.110	.967	1.035
	aware	.217	.106	.192	2.041	.045	.005	.429	.497	2.012
a. Dependent Variable: ca										

Table 9: Summary of the Regression Model

Model	R	R Square	Adjusted	Std. Error	Statistics for Change				
					Change in R Square	F Change	df1	df2	Sig. F Change
1	.815[a]	.664	.647	.44999	.664	37.616	4	76	<.001
a. Predictors: (Constant), aware, vola, scp, resi									

5. Managerial Implications

This study assessed the roles of four factors (supply chain resilience, performance, market volatility, and awareness of potential disruptions) play in developing a firm's sustainable competitive advantage under unanticipated supply chain disruptions. The investigation was conducted within manufacturers in the US in the context of supply chain operations. The findings of this empirical investigation provide several implications for supply chain professionals. First, managers are suggested to focus on building resilience to cope with adversities. In particular, supply chain firms should be capable of (a) restoring and reforming processes to overcome adversities induced by disruptions; (b) reconfiguring both external and internal resources to restore normal operations during and after disruptions; (c) ensuring continuity of restored operations; (d) meeting evolving market demand. Second, it is crucial for supply chain managers to lower costs, shorten lead time, enhance process flexibility, and improve customer satisfaction. Third, supply chain firms are suggested to bolster marketing capabilities to rapidly cope with the unpredictable changes in customer requirements and supplier capabilities as well as competitors' products. Fourth, managers are encouraged to promote a risk management culture and awareness of potential risks so that disruption orientation can be fostered within an organization.

6. Conclusion

This study examined the determinants of a supply chain firm's sustainable competitive advantage under supply chain disruptions employing a linear regression on supply chain resilience, performance, market volatility, and awareness of potential supply chain disruptions. It contributes to the literature and offers practical implications for supply chain managers. Three factors play significant and positive roles in improving sustainable competitive advantage except for market volatility, which has marginal explanatory power with a negative association with sustainable competitive advantage. The findings of this study offer practical implications for supply chain professionals that internal factors including supply chain resilience, performance, and awareness of potential disruptions play vital roles in improving sustainable competitive advantage, while external factor such as market volatility is less significant in bolstering sustainable competitive advantage. This study contributes to the existing literature by considering both internal and external factors in assessing the role of determinant factors of sustainable competitive advantages. This study also helps us better understand the mechanism by which supply chain resilience, performance, awareness of potential disruptions, and market volatility exert impact on the improvement of sustainable competitive advantage.

The limitations of this investigation lie in the fact that the regression analysis was based on a dataset with small sample size, which might constrain the generalizability of the findings of the investigation. Further research might include a substantial sample size to better represent the manufacturers in the US. Furthermore, inventory management can impose disruptions on supply chain operations and generate ripple effects in a supply chain. However, it is not considered in the study. Future research is expected to assess its role in building sustainable competitive advantage.

References

Abeysekara, N., Wang, H., and Kuruppuarachchi, D., (2019). Effect of supply-chain resilience on firm performance and competitive advantage: A study of the Sri Lankan apparel industry. *Business Process Management Journal*. 25(7): 1673-1695.

Aiken, LS., and West, SG., (1991). *Multiple Regression: Testing and Interpreting Interactions*. Sage, Newbury Park, CA, USA.

Ali, I., Sadiddin, A., and Cattaneo, A., (2022). Risk and resilience in agri-food supply chain SMEs in the pandemic era: a cross-country study. *International Journal of Logistics: Research and Applications*. DOI: 10.1080/13675567.2022.2102159

Astuti, PD., Luh, KD., and Chariri, A., (2023). Understanding the antecedents and consequences of sustainable competitive advantage: Testing intellectual capital and organizational performance. *Economies*, 11(4): 120-134.

Beigi, S., et al. (2023). A Framework for firm performance under the influence of knowledge management and dynamic capabilities: Examining the mediating role of sustainable competitive advantage. *Iranian Journal of Management Studies*, 16(1): 205-227.

Bode, C., et al. (2011). Understanding responses to supply chain disruptions: insights from information processing and resource dependence perspectives. *Academy of Management Journal*, 54(4): 833-856.

Hair, JF., et al. (1998). *Multivariate Data Analysis*. Prentice Hall, NJ, USA.

Hossain, MS., et al. (2022). Determinants of sustainable competitive advantage from resource-based view: implications for hotel industry. *Journal of Hospitality and Tourism Insights*, 5(1): 79-98.

Lee, S., and Yoo, J., (2021). Determinants of a firm's sustainable competitive advantages: Focused on Korean small enterprises. *Sustainability*, 13(1): 346-362.

Stephens, AR., Kang, M., and Robb, CA., (2022). Linking supply chain disruption orientation to supply chain resilience and market performance with the stimulus–organism–response Model. *Journal of Risk and Financial Management*, 15(5): 227.

Suryantini, NPS., et al. (2023). The sustainable competitive advantage of SMEs towards intellectual capital: The role of technology adoption and strategic flexibility. *Intellectual Economics*, 17(1): 30-56.

Zhao N., et al. (2023). Impact of supply chain digitalization on supply chain resilience and performance: A multi-mediation model. *International Journal of Production Economics*, 259 (May): 108817.

The Evolution of HIV/AIDS and an Overview of Current Advancement Efforts: Treatments, Cures, Vaccines

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Abstract

Since 1981, HIV/AIDS has existed as a prominent global epidemic. Throughout the years, research on the disease has greatly improved and expanded as technology has advanced. Because a latent reservoir of dormant viruses persists in the T cells of HIV patients, eliminating these infected cells is a primary goal and challenge for research on the virus. Over the past 40 years, there has been significant research on a variety of potential solutions, yet a functional cure remains elusive. Current antiretroviral treatments can effectively ensure the inactivation of the latent reservoir, but patients must take these medications for life. Therefore, researchers are actively searching for a cure that can either eliminate dormant viruses or assist the immune system in eradicating them once treatment is stopped. The most promising methods include the "kick and kill" strategy to reveal the latent reservoir and remove it and gene editing methods to create immunity to the virus in patients. However, both of these techniques require further study to improve their effectiveness. Based on an evaluation of the current research, a combination of approaches may lead to more effective results. This review also covered efforts to create HIV/AIDS vaccines and improve treatment methods. Due to ethical concerns around testing experimental cures in human patients, the scope of HIV/AIDS research has been limited. Many trials contain relatively small sample sizes and primarily include Caucasian men as research subjects. A resurgence in research efforts and greater inclusion of women and people of color in research may provide greater insights in finding effective solutions.

Keywords: HIV/AIDS, Antiretroviral therapy, Treatment, Cure, Vaccine, bnAbs, mRNA

1. Introduction

Human Immunodeficiency Virus, HIV, is an infectious disease that spread from chimpanzees to humans and arrived in Hispaniola from sub-Saharan Africa in the 1960s (Darrow, 2021). From there, the virus spread to North America and has since infected millions worldwide. Its last and most deadly stage, Acquired Immunodeficiency Syndrome (AIDS), became a global epidemic in the 1980s. The World Health Organization (WHO) and the Joint United Nations Programme on HIV/AIDS (UNAIDS) estimate that there are currently nearly 40 million people living with HIV (WHO, 2023). HIV is a sexually transmitted disease, spread through bodily fluids like blood, semen, and breast milk. In its early years, it predominantly affected gay men (Darrow, 2021). However, despite misconceptions, HIV can impact people of all sexual orientations.

HIV is a retrovirus with a diameter of 100-130 nm that primarily targets CD4+ T cells (Floderer et al., 2018). CD4+ T cells, also known as helper T cells, are important white blood cells that assist the body in fighting infection by triggering the immune system to attack foreign particles. The HIV retrovirus encodes 3 structural genes: Gag, Pol, and Env. Two important molecules on the HIV envelope that form the spike protein are gp120 (the external glycoprotein) and gp41 (the transmembrane protein). During infection, gp120 binds to the CD4 receptor and a

coreceptor (either CCR5 or CXCR4) on the T cell and is endocytosed by the T cell (Simon et al., 2006). The virus then releases its viral core into the cell, where the genetic material is incorporated into the cell's own DNA by the virus's reverse transcriptase enzyme. The cell is tricked into using its own protein production mechanisms to produce more of the HIV virus. Since HIV is able to infect and replicate within immune cells, it is very hard to detect and kill the virus. Soon, the population of CD4+ T cells depletes, leading to severe immune dysfunction. HIV is constantly evolving, as it displays the highest-ever recorded biological mutation rate, which poses a challenge to treatment and intervention efforts everywhere (Andrews and Rowland-Jones, 2017).

Additionally, the 1980s AIDS epidemic was heavily characterized by discrimination and homophobia that impacted the public health policies regarding HIV/AIDS. In America, for example, the silence and inaction of President Ronald Reagan in the face of the epidemic resulted in thousands of preventable deaths (Ortiz, 2023). The early discriminatory attitude of governments towards the disease lessened the progress of research during the AIDS epidemic. In the 40 years since the virus first emerged, researchers have worked through countless obstacles to provide treatments for patients and find an effective cure. Many new methods have been proposed, including therapeutic vaccines and antibody techniques. However, despite consistent efforts, there is still no method of eradicating the virus and support for research is waning. This review aimed to contextualize advancements and ethical debates surrounding HIV/AIDS, address gaps in current research and suggest new approaches, and provide a comprehensive source of foundational knowledge as a basis for future HIV/AIDS research.
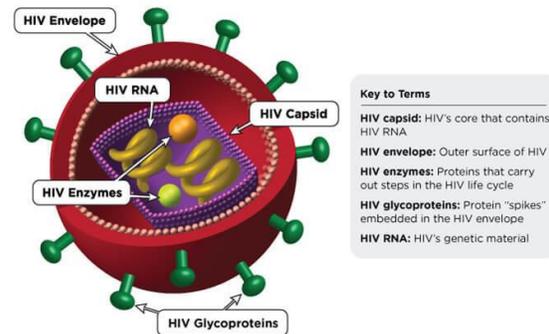

Figure 1. Diagram of the HIV Virus. Adapted from "The HIV Life Cycle," 2021, *National Institutes of Health*. https://hivinfo.nih.gov/understanding-hiv/fact-sheets/hiv-life-cycle

2. Treatments

2.1 Evolution of Treatments

When researchers first developed a treatment for HIV, they tried a monotherapy strategy using just the antiretroviral drug zidovudine (Cohen et al., 1996). Although antiretroviral therapy (ART) showed promise, this 1996 clinical trial proved that implementing only zidovudine was ineffective. Results showed that even if CD4+ T cell counts increased on monotherapy drug treatment, this change was not statistically significant and zidovudine alone showed no clinical benefit. As researchers worked towards finding a viable treatment, they experimented with combining multiple antiretroviral drugs into a treatment cocktail. A four drug ART therapy was found to strongly reduce viral load and replication in people with HIV (Tamalet et al., 1997). This research suggested that this combination could have a clinical benefit related to decreases in HIV RNA levels and increases in CD4+ cells. Because a decrease in the viral load had been found to correlate with a slower progression of disease, this treatment had the potential to greatly extend the lives of HIV patients. This eventually led to the development of ART as it works today.

2.2 How ART Works

Antiretroviral drugs inhibit HIV replication after the virus enters the cell but before its DNA can integrate with the host cell DNA. There are many different cocktails that can be prescribed depending on the patient's condition. Factors include other diseases the patient may have, medications the patient is taking, drug resistance, side effects, convenience, and monetary concerns (National Institutes of Health, 2021). The objective of ART is to bring levels of the HIV virus to an undetectable status within the bloodstream, which generally takes 3 to 6 months, because it is commonly known that a person cannot transmit HIV when their viral load is undetectable. However, HIV/AIDS is a

chronic disease with no cure, so patients must take ART for the rest of their lives, as although ART halts viral replication, it cannot eliminate the latent viral reservoir (Uldrick et al., 2022). With consistent, daily, implementation of ART, infected cells remain inactive and do not produce the virus. Instead, they maintain the potential to become reactivated and make HIV transmissible again. If ART is not taken as prescribed, the virus reemerges due to the activation of these infected cells. While ART has greatly improved the lifespan of patients with HIV, there are still many requirements and complications imposed on the patient, which is why finding a cure is so necessary.

2.3 Streamlining ART

Due to the effectiveness of ART, many governmental programs have been implemented to help people with HIV gain access to ART. New York City's JumpstART program, for example, studied patients who received access to HIV treatment rapidly following diagnosis (Pathela et al., 2021). JumpstART patients were given ART the day of diagnosis and linkage to care within 30 days. They found that the median time for viral suppression within JumpstART patients was just 31 days, compared to 95 days in non-JumpstART patients. Additionally, achieving viral suppression early disrupts HIV transmission because patients with an undetectable viral load cannot spread the disease. Another similar initiative was the DO ART program in Africa (Gilbert et al., 2021). The UNAIDS target of ending the AIDS epidemic by 2030 has called for viral suppression in 95% of people on ART. In Africa, where AIDS is most prevalent, 65% of patients have achieved this. However, there is difficulty linking people with HIV to the proper care. Many are either unable to access or afford ART. The DO ART program aimed to deliver community-based ART, so people living with HIV did not have to travel to faraway clinics to refill their medication. Fixed, closer clinics greatly improved access to care as patients didn't need to pay for transportation and lose a workday. Globally, access to ART is continuing to become more streamlined so that all patients can be treated.

2.4 Drug Resistant Patients

However, as with any drug treatment, ART is not perfect. It often includes side effects such as nausea, fatigue, and other problems. In addition, some patients are infected with mutated HIV strains that are resistant to many ART drugs. Because HIV is able to replicate and mutate quickly, patients with multidrug-resistant strains need new antiretroviral agents for treatment without resistance or harmful drug-drug interactions. A group of researchers studied ibalizumab, an antibody that blocks the entry of HIV into the cell by binding to the cell's CD4 receptor (Emu et al., 2018). This was successful, as significant antiviral activity was observed in patients where medications had previously failed.

Researchers have also been exploring the concept of capsid inhibitors. Capsid inhibitors are a class of drugs that disrupt the HIV capsid, the protein shell which protects the genetic information and other necessary material of the virus (National Institutes of Health, n.d.). These medications may interfere with multiple processes of the viral life cycle). Capsid inhibitors prevent viral uncoating, interacting directly with the HIV capsid to disrupt proper viral development and in turn, infection.

Another study explored the effect of broadly neutralizing antibodies (bnAbs) on HIV (Julg et al., 2022). bnAbs are antibodies that can recognize and block a variety of HIV strains from infecting healthy T cells. They have been shown to robustly reduce plasma viremia, the detection of residual virus in the plasma of the blood after the cells are removed, in people with HIV who are not on ART. Plasma viremia was found to be a very sensitive marker of HIV infection in the blood of patients (Coombs et al., 1989). Because single or dual bnAb treatments have been relatively unsuccessful, researchers in the 2022 study tested a triple bnAb therapy (Julg et al., 2022). However, they found that even with this, viral rebound, a renewal in infection, occurred quickly. Although the initial decline in viremia was rapid, the response was not as robust as current treatments. They concluded that at least a fourth bnAb would be necessary for this technique to be effective and that further study is needed. Researchers are testing new combinations of treatments to assist as many people as possible. However, the prevalence of drug-resistant HIV strains only further emphasize the need for a functional cure.

3. Cures

3.1 Challenges in Curing HIV

As some scientists strive for ART-free virologic control in patients, others are working on finding a viable cure for HIV. The challenges of curing HIV lie in the latent reservoir. Because the inactive infected T cells have the potential to become active, lifelong ART must currently be administered. In a patient with undetectable viral load, viral rebound will occur quickly if ART is interrupted. Although researchers have found success with reducing the time it takes for viral rebound to happen, there is still no viable cure. Cells that contain the inactive virus do not identify themselves, so finding a way to selectively kill these cells is difficult. But without eliminating the latent reservoir, there is no way to be completely HIV-free.

3.2 Stem Cell Transplants

So far, only a small group of people have been cured of HIV. In most of these cases, the patients received life-saving transplants to treat cancers that they developed along with HIV. The blood of their donors contained an abnormality in the CCR5 co-receptor of HIV (CCR5Δ32/Δ32). This rare mutation, occurring in about 1% of the population, makes hosts immune to strains of HIV that bind to the CCR5 co-receptor (Gupta, et al. 2019). Some of the cured patients have been off of ART for years with no viral load detected in their blood (Tebas et al., 2021). However, this stem cell treatment is not a viable cure. Due to its high toxicity, it is only used as a last resort for anyone who is already facing a potentially fatal blood cancer or other health condition. This method proved the possibility of an HIV cure, however, it cannot be used in most HIV patients and the hunt for a truly viable solution continues.

3.3 "Kick and Kill"

The primary approach in HIV cure research is the "kick and kill" strategy. The idea is that if scientists can reveal the infected cells that contain HIV and boost the immune system to selectively kill them, patients may be cured. Latency reversing agents (LRAs) have been proposed in order to expose the dormant infected cells (Wei et al., 2014). LRAs can activate the latent reservoir of infected cells and induce transcription of the virus so the immune system can identify cells that contain the virus by the antigens they present. This is the "kick" component of the strategy. In the "kill" step, the immune system is able to find the newly activated HIV cells that formerly made up the latent reservoir, so a strong method to eliminate these cells is necessary. Because histone deacetylase enzymes play a key role in stopping expression of the virus, histone deacetylase inhibitors (HDACi) have been proposed as potential LRAs (Søgaard et al., 2015). HDACi treatment promotes gene transcription and disrupts HIV latency. The most potent of the FDA-approved HDACis is a drug called romidepsin (RMD). RMD has been shown to induce HIV expression in patients on ART, however, is so far unable to reduce the size of the latent reservoir. But in 2016, RMD was used in conjunction with Vacc-4x, a therapeutic HIV vaccine for infected patients meant to boost the "kill" step of this cure approach (Leth et al., 2016). Therapeutic vaccines are given to patients who already have HIV. These vaccines boost the immune system and prime it against HIV viruses, making them especially helpful in eliminating HIV viruses that were previously dormant. Although the amount of HIV DNA decreased overall over the course of this study, the size of the latent reservoir did not.

More recently, researchers combined RMD with the MVA.HIVconsv vaccine (Mothe et al., 2020). This vaccine was meant to boost the immune system and help it attack the infected T cells RMD revealed. It induce bursts of viral replication and increase T cell activation. The study recorded prolonged viremic control in a promising 23% of participants after ART interruption. However, later assessment of this study demonstrated that patients experienced a decrease in both CD4+ and CD8+ T cells after every dose of RMD, suggesting a possible cumulative toxic effect (Moltó et al., 2021). When RMD was combined with the bnAb 3BNC117, none of the experimental groups had significant reductions in the HIV reservoir despite the amount of total HIV DNA declining slightly (Gruell et al., 2022). Unfortunately, HDACi treatments such as romidepsin haven't worked consistently enough to be completely feasible and more research is needed. Perhaps due to the very small, homogenous sample sizes in a majority of these

studies and the lack of concrete evidence as to the safety of RMD, these methods have still not produced a clinically viable cure. Larger-scale studies which include women and patients of color would help provide more solid evidence on the effectiveness of RMD and the "kick and kill" strategy. Additionally, as shown by the effectiveness of a combination of therapeutic vaccines and RMD compared to just RMD, as seen in Table 1, suggests that a combination of cure approaches may be the most viable solution.

3.4 CRISPR and Gene Editing

Another technique to curing HIV is to use gene editing technology. In a 2018, scientist He Jiankui illegally modified the genes of two twin babies before birth to induce HIV immunity (Cyranoski and Ledford, 2018). He edited the DNA of the embryos to mutate the CCR5 gene, which could lead to HIV resistance. Since this was the first instance of genetically edited babies, the news sparked international outrage. In addition, not all HIV strains rely on the CCR5 receptor for entry, and there is no way to confirm if the children actually became immune. Because gene editing has not been studied enough to determine if there will be negative effects on the children in the future, this experiment was very premature. However, CRISPR/Cas9 gene editing could be a potential HIV cure. In one study, patients were given strong antiretroviral drugs long-acting slow-effective release ART (LASER ART) (Dash et al., 2019). The combination of LASER ART and CRISPR-Cas9 gene editing technology to cut the viral DNA from infected cells eliminated the latent reservoir of HIV from humanized mice. Out of the 7 mice who received the LASER ART and CRISPR combination, 2 mice did not experience viral rebound after LASER ART was stopped. Overall, the CRISPR method shows promise in curing HIV and requires further study before it may be tested in humans. However, the results of the study prove that it is possible to eliminate the latent reservoir, and this approach combined with the therapeutic vaccines in the "kick and kill" strategy may yield more consistent results. Additionally, greater research on prevention of viral genetics from integrating with the T cell genome so the latent reservoir cannot re-establish itself before the immune system eliminates infected cells may benefit future cure- related experiments.

Table1. Comparing the primary experimental methods of curing HIV

Cure Method	Insights	Improvements Needed
Romidepsin: HDACi, "kick and kill"	-Romidepsin can induce viral transcription (HIV RNA in the blood of 5/6 patients increased from undetectable to detectable) -Romidepsin is safe to use -Romidepsin increased T cell activation during the period of administration	-No significant reduction in the viral reservoir, must improve the "kill" component -Small scale study, only included Caucasians
Vacc-4x and romidepsin: Immunization followed by latency reversal, "kick and kill"	-Total HIV DNA in patients declined by 40% -Side effects were mild	-Integrated HIV DNA did not significantly decrease (latent reservoir) -Small sample size
MVA.HIVconsv and romidepsin: Immunization followed by latency reversal, "kick and kill"	-Mostly mild side effects frequently due to MVA.HIVconsv -Induced bursts of viral transcription -Reduced overall HIV DNA levels -Some participants experienced prolonged control of the virus after ART was stopped -Vaccine elicited a strong "kill" response and increased T cell activation	-One serious side effect possibly related to romidepsin -Romidepsin must be administered frequently -Overall effect on latent reservoir was modest -Small sample size
bNAb and romidepsin: neutralizing antibodies followed by latency reversal, "kick and kill"	-Total HIV DNA declined slightly -Romidepsin and bNAb combination was safe overall	-Changes in latent reservoir were not significant -Did not reduce time for virus to reemerge after ART was stopped -Small sample size
LASER ART and CRISPR-Cas9: antiretroviral treatment followed by genetic editing	-Two mice who received both LASER ART and CRISPR-Cas9 did not display viral rebound even after treatment was discontinued -Restoration of CD4+ T cells after LASER ART -Combination treatment was most effective	-Experiment was done in mice which produced human T cells -Requires much more study

4.   Vaccines

4.1 PrEP and PEP

Pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP) are drugs used as preventative measures for HIV. They are antiretroviral medications that when taken, stop the replication of HIV within the body by blocking the reverse transcriptase enzyme, which converts RNA into DNA and is essential for replication (Bavinton and Grulich, 2021). PrEP is meant for patients at risk of contracting HIV, such as people whose partners have the disease. It is very effective in stopping HIV, with a 99% success rate. PEP is given to patients who have already been exposed to HIV. However, this treatment is only able to stop a small number of HIV cells from replicating and must be taken shortly after exposure. These two prevention methods are helpful in decreasing HIV cases and transmission, but global access to these drugs is limited. One of the main UNAIDS goals is to increase the number of people on PrEP and PEP. Current PrEP and PEP targets are not being met and these goals must take higher priority to have a substantial effect on HIV cases.

4.2 Antibody Vaccines

Antibody vaccines allow the host immune system to specifically target elements of virus or infected cells. One goal of HIV vaccine research is to induce bnAbs, antibodies capable of neutralizing HIV by targeting the HIV Envelope (Env). Scientists have mapped areas of vulnerability in the HIV Env for bnAbs to target. Some studies have aimed to focus the immune system response on the fusion peptide, a crucial component of the viral entry machinery (Walsh and Seaman, 2021). The fusion peptide is targeted because its N-terminus is poorly immunogenic and usually hidden from the immune system. In mice, the immunogens produced created antibodies that were capable of neutralizing 31% of the 208 HIV strains tested (Xu et al., 2018). If research can induce the production of bnAbs in people with HIV, this could also potentially work as part of a cure. Research has shown that patients who received infusions of bnAb 3BNC117 at the time of ART initiation were able to maintain long-term CD8+ T cell responses (Rosás-Umbert et al., 2022). However, it is difficult to create bnAbs that can neutralize a diverse range of HIV strains and this technique runs into many challenges. Although these vaccines have been improving, they are often difficult to create in labs and vaccinology is moving towards more efficient vaccine techniques. Therefore, this approach may not be the most viable.

4.3 mRNA Vaccines

mRNA vaccines became widely used during the COVID-19 pandemic and could be applied to HIV. Lab-created mRNA coding for a piece of a protein is given to patients (Centers for Disease Control and Prevention, 2023). This allows for the production of antibodies specific to that protein epitope, which prevents future sickness from the virus. In an mRNA vaccine, the RNA does not need to enter the nucleus and can make proteins in the cytoplasm. Although vaccinology hasn't been able to consistently induce bnAbs by vaccines in people with HIV, researchers found that an mRNA vaccine was able to yield a 79% risk reduction per exposure in mice, offering a promising approach to HIV mRNA vaccines (Zhang et al., 2021). It was also able to induce a robust CD4+ T cell response. Although this is a fairly new technique, its early successes, especially in relation to the COVID-19 pandemic, suggest this may be a valuable approach to explore more deeply and could be instrumental in ending the HIV epidemic.

5.   Discussion

HIV/AIDS research has been a consistent effort since the 1980s epidemic, and while in America it has now become more chronic illness than death sentence, other countries are less fortunate. Accessibility to lifelong ART, prevention measures such as PrEP, and linkage to medical providers are all issues imposed upon those diagnosed with HIV. Marginalized communities are most impacted by high drug prices, access to transmission prevention methods,

as well as effective healthcare. In countries in Africa, these issues have dominated HIV transmission, making progress towards UNAIDS goals difficult. Ending the global AIDS epidemic requires continuing to promote accessible treatments with both healthcare and therapeutic access. As with any yet-incurable disease, the objectives remain to develop a cure for HIV. Currently, improving treatment, prevention, and vaccine options remain priorities to ease the burdens of HIV patients. ART is improving so that the drug cocktail needed to control the viral load is less vulnerable and dependent upon the practice of implementation by patients. Therapeutic vaccines demonstrate promise in assisting a patient's immune system to eliminate the virus, aiming to remove the dependence upon ART and keep patients undetectable. Many cure strategies are being promoted to find an effective mechanism for eliminating the latent reservoir. Advances in vaccinology have furthered the options for prevention against HIV. The mRNA vaccine technology, as recently seen and approved for the COVID-19 pandemic, is being actively researched for HIV. Additionally, the bnAb approach could be boosted. An HIV vaccine would greatly slow the number of new infections and is an essential part of ending the epidemic. As a disease with not only epidemiologic but also social history, it has set a precedent for the role of politics in health. HIV/AIDS has impacted our world for decades. Healthcare research has greatly improved since the epidemic first began, and the world has learned a lot from this disease.

6. Conclusion

As the search for an HIV/AIDS cure and functional vaccine continues, the scientific field must come to a consensus on the most viable methods and urge for a renewal of research and discovery. The evidence collected in this review suggests that, through a comparison of the primary cure methods, it may be most beneficial to the future of HIV/AIDS research to approach these techniques from a more collaborative viewpoint, rather than attempting to perfect one single method. The past 40 years of HIV research has yielded much new information and innovation, and combining this knowledge could lead to greater results than the discoveries of current. The main goals of further study should be to test novel, combined approaches on larger sample sizes of participants, including women and people of color, to understand the impact of the virus on all people who are susceptible. It is also suggested that research on the prevention of the latent reservoir from forming may be beneficial to curing HIV, ending the global epidemic, and creating a distributable vaccine. This review should provide a basis for future studies on treating, curing, and immunizing patients with the HIV virus through its comprehensive collection of the current status of HIV/AIDS research.

References

Andrews, S. M., & Rowland-Jones, S. (2017). Recent advances in understanding HIV evolution. *F1000Research*, *6*, 597. https://doi.org/10.12688/f1000research.10876.1

Bavinton, B. R., & Grulich, A. E. (2021). HIV pre-exposure prophylaxis: scaling up for impact now and in the future. *The Lancet, Public health*, *6*(7), e528–e533. https://doi.org/10.1016/S2468-2667(21)00112-2

Centers for Disease Control and Prevention. (2023). Understanding How COVID-19 Vaccines Work. *Centers for Disease Control and Prevention*. https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines/how-they-work.html

Cohen, O. J., et al. (1996). Antiretroviral monotherapy in early stage human immunodeficiency virus disease has no detectable effect on virus load in peripheral blood and lymph nodes. *The Journal of Infectious Diseases*, *173*(4), 849–856. https://doi.org/10.1093/infdis/173.4.849

Coombs, R. W., et al. (1989). Plasma viremia in human immunodeficiency virus infection. *The New England Journal of Medicine*, *321*(24), 1626–1631. https://doi.org/10.1056/NEJM198912143212402

Cyranoski, D., & Ledford, H. (2018). Genome-edited baby claim provokes international outcry. *Nature*, *563*(7733), 607–608. https://doi.org/10.1038/d41586-018-07545-0

Darrow W. W. (2021). The First 40 Years of AIDS: Promising Programs, Limited Success. *AIDS and behavior*, *25*(11), 3449–3471. https://doi.org/10.1007/s10461-021-03497-1

Dash, P. K., et al. (2019). Sequential LASER ART and CRISPR Treatments Eliminate HIV-1 in a Subset of Infected Humanized Mice. *Nature*, *10*(1), 2753. https://doi.org/10.1038/s41467-019-10366-y

Emu, B., et al. (2018). Phase 3 Study of Ibalizumab for Multidrug-Resistant HIV-1. *The New England Journal of Medicine*, *379*(7), 645–654. https://doi.org/10.1056/NEJMoa1711460

Floderer, C., et al. (2018). Single molecule localisation microscopy reveals how HIV-1 Gag proteins sense membrane virus assembly sites in living host CD4 T cells. *Sci Rep 8*, 16283. https://doi.org/10.1038/s41598-018-34536-y

Gilbert, H. N., et al. (2021). How community ART delivery may improve HIV treatment outcomes: Qualitative inquiry into mechanisms of effect in a randomized trial of community-based ART initiation, monitoring and re-supply (DO ART) in South Africa and Uganda. *Journal of the International AIDS Society*, *24*(10), e25821. https://doi.org/10.1002/jia2.25821

Gruell, H., et al. (2022). Effect of 3BNC117 and romidepsin on the HIV-1 reservoir in people taking suppressive antiretroviral therapy (ROADMAP): a randomised, open-label, phase 2A trial. *The Lancet. Microbe*, *3*(3), e203–e214. https://doi.org/10.1016/S2666-5247(21)00239-1

Gupta, R. K., et al. (2019). HIV-1 remission following CCR5Δ32/Δ32 haematopoietic stem-cell transplantation. *Nature*, *568*(7751), 244–248. https://doi.org/10.1038/s41586-019-1027-4

Julg, B., et al. (2022). Safety and antiviral activity of triple combination broadly neutralizing monoclonal antibody therapy against HIV-1: a phase 1 clinical trial. *Nature Medicine*, *28*(6), 1288–1296. https://doi.org/10.1038/s41591-022-01815-1

Leth, S., et al. (2016). Combined effect of Vacc-4x, recombinant human granulocyte macrophage colony-stimulating factor vaccination, and romidepsin on the HIV-1 reservoir (REDUC): a single-arm, phase 1B/2A trial. *The Lancet. HIV*, *3*(10), e463–e472. https://doi.org/10.1016/S2352-3018(16)30055-8

Moltó, J., et al. (2021). Pharmacokinetic/pharmacodynamic analysis of romidepsin used as an HIV latency reversing agent. *The Journal of antimicrobial chemotherapy*, *76*(4), 1032–1040. https://doi.org/10.1093/jac/dkaa523

Mothe, B., et al. (2020). HIVconsv Vaccines and Romidepsin in Early-Treated HIV-1-Infected Individuals: Safety, Immunogenicity and Effect on the Viral Reservoir (Study BCN02). *Frontiers in Immunology*, *11*, 823. https://doi.org/10.3389/fimmu.2020.00823

National Institutes of Health. (n.d.). Capsid Inhibitors. *National Institutes of Health*. https://clinicalinfo.hiv.gov/en/glossary/capsidinhibitors#:~:text=Capsid%20inhibitors%20are%20a%20class,and%20enzymes%20needed%20for%20replication.

National Institutes of Health. (2021). The HIV Life Cycle. *National Institutes of* Health. https://hivinfo.nih.gov/understanding-hiv/fact-sheets/hiv-life-cycle

National Institutes of Health. (2021). What to Start: Choosing an HIV Treatment Regimen. *National Institutes of Health*. https://hivinfo.nih.gov/understanding-hiv/fact-sheets/what-start-choosing-hiv-treatment-regimen

Ortiz, Jacqueline A. (2023) Silence From the Great Communicator: The Early Years of the AIDS Epidemic Under the Reagan Administration. *Swarthmore Undergraduate History Journal*, *4*(2), 76-99. https://works.swarthmore.edu/suhj/vol4/iss2/6

Pathela, P., Jamison, K., Braunstein, S. L., Borges, C. M., Lazar, R., Mikati, T., Daskalakis, D., & Blank, S. (2021). Initiating antiretroviral treatment for newly diagnosed HIV patients in sexual health clinics greatly improves timeliness of viral suppression. *AIDS*, *35*(11), 1805–1812. https://doi.org/10.1097/QAD.0000000000002937

Rosás-Umbert, M., et al. (2022). Administration of broadly neutralizing anti-HIV-1 antibodies at ART initiation maintains long-term CD8+ T cell immunity. *Nature Communications*, *13*(1), 6473. https://doi.org/10.1038/s41467-022-34171-2

Simon, V., Ho, D. D., & Abdool Karim, Q. (2006). HIV/AIDS epidemiology, pathogenesis, prevention, and treatment. *The Lancet*, *368*(9534), 489–504. https://doi.org/10.1016/S0140-6736(06)69157-5

Søgaard, O. S., et al. (2015). The Depsipeptide Romidepsin Reverses HIV-1 Latency In Vivo. *PLOS Pathogens*, *11*(9), e1005142. https://doi.org/10.1371/journal.ppat.1005142

Tamalet, C., et al. (1997). Quantification of HIV-1 viral load in lymphoid and blood cells: assessment during four- drug combination therapy. *AIDS*, *11*(7), 895–901. https://doi.org/10.1097/00002030-199707000-00009

Understanding Northeast Indigenous Tribes: Twelve Thousand Year Survival with Nature, Knowledge of Medicinal Plants, Agricultural, Food and Dwelling Practices.

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Abstract

This review paper focuses on indigenous tribes who inhabited the northeastern part of United States for over ten thousand years prior to European contact in 1620. Our research, which involved consulting various databases such as government and museum sites, archaeological journals, research papers, articles and books on northeast indigenous tribes, aimed to gain an understanding of how these tribes managed to survive for centuries. These tribes acquired knowledge and skills that allowed them to adapt to the changing climate and environment of the northeastern United States which had been evolving significantly since the post ice-age era, beginning approximately 11,700 years ago. These tribes utilized natural resources including native plants and herbs for medicine and developed unique dwelling techniques in construction of their houses in order to survive harsh winters. They used natural flora and fauna such as wild berries, animals and aquatic life for food and other necessities. They developed farming and gardening methods which supported soil conservation and crop interdependencies. These tribes preserved and stored food for winter by utilization of smoking and drying techniques. This research helps to conclude that the indigenous tribes of the Northeast (Algonquins and Iroquois) were advanced societies. They were self-sufficient, and had an impressive amount of understanding about their surrounding natural environment. The age-old techniques and practices used by these tribes can further benefit modern societies including the development of effective therapeutics and support for climate initiatives.

1. Introduction

Before the arrival of Europeans in 1620, North America had been inhabited by different indigenous tribes for over ten thousand years. There are two major groups of indigenous tribes of Northeast America as categorized based on the language they spoke: Algonquian and Iroquoian.

The Algonquins lived in the area of New England and included the indigenous groups Abenaki, Micmac, Nauset, Narragansett, Nipmuc, Pennacook, Pequot, Wampanoag, and Woronoco. These groups spoke the same language and had a very similar culture. The livelihood of northern New England was dependent on fishing, trade, hunting, and gathering Farming is prevalent in southern New England, where tribes lived in small villages near fields due to favorable climatic conditions. The family responsibilities of all these indigenous tribes that lived 12,000 years ago was very similar. Women were responsible for gathering nuts and berries from accessible forests and harvesting corn, beans, and squash from their fields with assistance of their children. Men provided food from fishing and hunting.

The Iroquois people had lived in Ontario and upstate New York. The Iroquois Confederacy mainly included the Haudenosaunee, Mohawk, Oneida, Onondaga, Cayuga, Seneca, and Wenrohronon Indian tribes. Similar to the Algonquins, the Iroquois also spoke the same language and shared similar cultures. The Iroquois Confederacy used kinship to organize their society. Woman in Iroquois societies played an important role in decision-making including food distribution. Iroquois family structure was matriarchal and when tribe members were married, they moved into the mother's longhouse (Wallenfeldt, 2023). Iroquois cultivated crops including corn, beans, and squash and relied on hunting and fishing for livelihood. "Haudenosaunee (Iroquoian) were farmers who supplemented their diet with hunting and fishing" (Ngapo et al., 2021, p.2).

This research will provide insight on indigenous tribes migration to the north eastern part of the United States and evolution of these initial inhabitants as food gatherer using primitive tools living in scattered groups during the Paleo-Indian Period to those living semi-nomadic village life and development of better tools and utensils during the Archaic Period. Lastly, there was a gradual emergence of agricultural societies who used advanced horticulture practices and advancement in fishing and hunting techniques during the woodland periods. This paper will delve into the knowledge of indigenous tribes Algonquins and Iroquois of their natural resources including different parts of native plants to be used for medicinal purpose and treatment of different disease and construction different types of dwellings (wigwams, wetus, and longhouses). We will study common agricultural practices (including The Three Sister crops, controlled burn and permaculture), fishing techniques and food preservation methods used by the northeastern indigenous tribes. Understanding contribution made by indigenous tribes in natural therapies, farming methods, tools, housing, and survival in the face of the harsh northeast cold climate can inspire our society to utilize available natural resources in a sustainable manner, thereby promoting the well-being of both the ecosystem and humanity.

2. Indigenous Gene Pool Diversity

The genetic exploration of indigenous gene pools provides substantial insight into their origins. The ancient populations crossed via Bering Strait into Alaska from Asia. More recently, it has been found that the passage was not only in one direction. Instead, the Bering Sea region was a place of intercontinental connection, and people had routinely sailed back and forth over a time span of thousands of years (Price, 2023). Recent studies of ancient DNA have indicated that indigenous tribes split from Siberians and East Asians 25,000 years ago and entered the American continent through Beringia, the land and marine area between Russia and Canada. This information was gathered from the discovery of sixty-four newly sequenced samples of DNA from individuals inhabiting various regions in America approximately 13,000 years ago. Based upon the discovery of DNA and data obtained in two independent studies, scientists have inferred that ancient populations expanded rapidly across America beginning approximately 13,000 years before present (Wade, 2018). Individuals had traveled east from Alaska, around the Great Lakes, and eventually settled along the Atlantic coast including the northeastern region of North America(O' Brien and Kiffel-Alcheh, n.d). These individuals were the ancestors of the indigenous individuals who came to be known as the Algonquin and the Iroquois.

3. History of New England Indigenous and their Adaptation to the New England Environment

Existence of indigenous tribes in the Northeast region dated back to the Paleo-Indian Period, spanning from 12,500-9000 years BP. The Mashpee Wampanoag Tribe (part of the larger Algonquins group, and also known as the "People of the First Life") inhabited the land of current day Massachusetts and Eastern Rhode Island since approximately 12,000 years ago (Sgouros, n.d.). During the Paleo-Indian Period, communities had lived in small, scattered groups, relying on hunting mammoths, caribou, mastodons, and other large grazing animals that inhabited the tundra and boreal habitats (Ritchie and Funk, 1973). Their toolkits were limited, primarily consisting of chipped stone tools, with the fluted spear or javelin point being the main item. Additionally, they gathered plant-based food. "The early Native Americans were all hunter/gatherers, living off the abundance of plants and animals they found nearby" (Park et al., 2016, p.173). The communities who lived in this period camped along waterways as rivers provided water, food source, transportation, protection from other tribes, and also supported trade.

In this period, the sea levels had dropped by 100 meters and the continental shelf had protruded above sea level by about 100 kilometers. Remains of now-extinct species including mammoth and mastodon have been discovered by modern fisherman (Ritchie and Funk, 1973). The warming climate caused the melting of glaciers at a gradual rate, leading to significant changes in the environment that occurred between the time period of 10,000 to 5,000 years ago in the northeast region of the United States. It also caused the growth of deciduous trees including walnuts, chestnuts, and butter nuts. Other food options including fruits, seeds, and nutritious roots had become more available. These changes in the environment greatly influenced indigenous tribes' culture and livelihood and signal the beginning of the Archaic period.

The Archaic Period, occurring from 9,000-2,500 years BP, was characterized by semi-nomadic villages situated near tidal bays that facilitated the harvest of shellfish. These settlements were surrounded by forests rich in various seeds, berries, nuts, and wildlife including deer, wild turkeys, raccoons, box turtles, woodchucks, and squirrels for hunting. "The age of the Late Archaic was a period in which enterprising skills were advanced far beyond those of the proceeding, highly nomadic Early Archaic hunting-fishing culture" (Fowler, 1975, p.8). This period witnessed an expansion in material culture and the advancement of technology, such as dug-out canoes. Furthermore, in their daily life, women utilized wooden bowls, grinding stones, mats, bone drills, and awls (Ritchie and Funk, 1973). Stone utensils including steatite bowls were developed to cook liquid food (Fowler, 1975).

During the Woodland Period (2,500 years BP to 15th century), native communities experienced significant developments in their technology. We see evidence of the emergence of the use of pottery, copper tools, smoking pipes, and the bow and arrow for hunting. During this period hunting, fishing, and gathering of wild food and shellfish were prevalent. The horticultural system of cultivating corn, beans and squash was developed during this period. Indigenous tribes had also increased their reliance on sea resources. Native tribes, especially those living in the Long Island region along the coastline of the Atlantic Ocean, captured migratory fish and other marine organisms including whales as important food resources (Ritchie and Funk, 1973).

4. Medicinal Plants Used by Northeastern Indigenous Tribes

Over many centuries, northeastern indigenous communities used different varieties of native nonvascular plants including liverworts and mosses as well as vascular seed-bearing plants including both flowering angiosperm and conifers (gymnosperm) for medical and healing therapies. Plants produce secondary metabolites which serve as a protective mechanism against pathogens and herbivores, and have several therapeutic properties (Wink, 2015). Indigenous tribes used therapeutic properties of native plants to treat diseases throughout their history.

Medical knowledge was passed down through generations over thousands of years through a strong oral tradition. Indigenous medical philosophy stresses the interconnection of humanity, nature, and the spiritual realm (Park, 2004).

Modern medicine now recognizes the efficacy of some indigenous medical treatments. Indigenous tribes used Podophyllum peltatum, found in deciduous forests of the northeast region of the United States for treatment of different conditions such as cataracts and rheumatism and as an insecticide (Moerman, 2009). In modern medicine resin from the root of this plant is called podophyllin used in treatment for Condylomata Acuminate (venereal warts). "*Podophyllum peltatum* is also the basis for the production of etoposide, a semisynthetic derivative of podophyllotoxin, a chemical found in mayapple. Etoposide is widely in the treatment of several forms of cancer" (Moerman, 2009, p. 14).

Several tribes including Algonquin and Iroquois used an infusion of Gaultheria procumbens L. (Ericaceae) to treat headaches, colds, arthritis, rheumatism, and lumbago (Lawson, 2021). G. procumbens naturally ranges in eastern North America and the essential oil from this plant has significant amount (96.6–99.8%) of methyl salicylate (Nikolic et al., 2013). This oil has wintergreen flavor and is used in chewing gums, toothpaste, and mouthwashes. "It is also used in the treatment of cellulites, a bacterial infection that causes the skin to become inflamed" (Nikolic et al., 2013, p. 562). "The natural products have the advantage of being readily absorbed through the skin, and for that reason they are often used as the active ingredients in sport creams for stopping muscle pain" (Moerman, 2009, p. 15).. Table 1 lists examples of medicinal native plants parts used by Algonquin and Iroquois to treat different diseases (Moerman, 2009).

Approximately 40% of pharmaceutical products come from nature and traditional medicine including indigenous medicine [according to the World Health Organization (WHO) report published in August 2023]. WHO recognized the role of traditional medicine to improve public health. The further research is required to understand the effectiveness and efficacy of different natural compounds extracted from northeast native plants and indigenous therapeutic methods. It can help to shape health policies and modern medicine.

Table 1. Therapeutic Categories and Medicinal Plant

Use	Medicinal Plant and Description	Tribe
Analgesic	Infusion or crushed leaves of *Comptonia peregrina* was used to treat headaches. A leaves infusion of *Prunella vulgaris* was used to treat fever.	Algonquin
	Powdered roots of *Aesculus Hippocastanum* was used for relief of chest pain. The leaves of *Collinsonia canadensis* were applied to the forehead to relieve headaches. *Mitchella repens* has analgesic properties - a compound decoction was used in treating pain from urination; an infusion of roots and barks was used to treat back pain, and its berries were used to treat labor pain.	Iroquois
Anticonvulsive	Infusion of *Asarum canadense* was given to children for treatment of convulsions.	Algonquin
	Cold infusion or decoction of powdered roots of *Claytonia virginia* was given to children with convulsions. Compound decoction of *Lobelia cardinalis* taken by women for epilepsy. Infusion of crushed dried berries and leaves of *Medeola virginiana* given to babies with convulsions. Compound decoction of *Nupbar lutea* taken by men with epilepsy.	Iroquois
Antidiarrheal	Infusion of rhizomes of *Coptis trifolia* was used to treat diarrhea.	Algonquin
	Decoction of *Anthemis cotula* plant, bark and roots used for diarrhea. Compound decoction *Ulmus americana* used for diarrhea and cramps.	Iroquois
Antiemetic	Infusion of *Lobelia inflata* and compound decoction *Ulmus americana* was used to treat vomiting; a compound decoction of *Sanicula marilandica* plants was taken to induce vomiting in order to counteract a poison.	Iroquois
Antihemorrhagic	Compound decoction of roots and barks of *Acer spicatum* and *Actaea rubra* was taken for internal hemorrhage. Compound decoction of *Ulmus americana* with smashed twigs was used in treatment of internal hemorrhage.	Iroquois
Antirheumatic	Root infusion of *Actaea rubra* was used as a wash for treating rheumatism, an infusion of *Polystichum acrostichoides* used to treat rheumatism in leg.	Iroquois
Burn Dressing	Compound infusion of dried leaves and roots of *Physalis heterophylla* was used as wash for scalds and burns.	Iroquois
Cold Remedy & Cough Medicine	Boiled roots of Coptis trifolia was used to treat serious cold; a poultice of wetted, inner bark of *Pinus resinosa* (red pine) was applied to the chest for treatment of a cold.	Algonquin
	Lobelia siphilitica plant was used as a gargle for coughs. Decoction of stems of *Phytolacca americana* were used for chest cold treatment and expectorant.	Iroquois
Dermatological Aid	A poultice of gum of *Abies balsamea* was applied to open sores, insect bites, boils and infections; a poultice of boiled root chips of *Acer spicatum* (mountain maple) applied to wound and abscesses. The leaves of *Toxicodendron radicans was* rubbed on skin affected by poison ivy.	Algonquin
	Compound of *Asarum canadense* used to treat boils. Infusion of smashed *Lobelia kalmaii* plants used as drops for abscesses. Decoction of *Mitchella repens* plants given to babies with rashes. Infusion of mashed roots or poultice of *Sanguinaria canadensis* used to treat poison ivy, plant juice of this plant is used as wound medicine, poultice of this plant is applied for drawing thorns or treating leg sores.	Iroquois

Diuretic	Leaves of *Diervilla lonicera* (northern bush honey suckle) were used as a diuretic	Algonquin
	Infusion of *Argentina anserina* leaves used as diuretic.	Iroquois
Ear Medicine	Poultice of chewed roots of *Aralia nudicaulis* was applied for ear ailments.	Algonquin
	Infusion of one smashed leaf of *Balsamita major* (Costmary) and *Lobelia kalmaii* plants used as drops for earache.	Iroquois
Eye Medicine	Root infusion of *Coptis trifolia* used as drops for sore eyes. Decoction of *Pyrola elliptica* plant used as drops for sore eyes and inflamed eye lids.	Iroquois
Heart Medicine	Branches and leaves infusion of *Corylus cornuta* used for heart problems. Medicinal tea using *Polypodium virginianum* used to treat heart disease.	Algonquin
	Root decoctions of *Collinsonia canadensis* used for heart diseases. Compound decoction of roots of *Sanguinaria canadensis* taken to regulate heart rate.	
Kidney Aid	Root infusion of *Cypripedium acaule* used to treat kidney problems in children	Algonquin
	Root decoctions of *Collinsonia canadensis* used for kidney trouble. Decoction of *Equisetum byemale* used to treat kidney and urinary troubles and used for excessive urination.	Iroquois
Laxative	Needles of *Abies balsamea* (Balsam fir) used to make laxative tea, infusion of *Sambucus canadensis* bark scraped downward and used as laxative.	Algonquin
	Compound decoction of twigs of *Sambucus canadensis* given to children as a laxative. Compound decoction of *Sagittaria latifolia* taken for constipation.	Iroquois
Oral Aid	*Salix sericea* used to treat mouth and throat abscesses. Bark or leaves of *Syringa vulgaris* chewed for sore mouth, poultice of *Toxicodendron pubescens* plant applied to infectious sores on lips. Flower stem of *Taraxacum officinale* chewed to prevent tooth decay.	Iroquois
Orthopedic Aid	Decoction of *Phytolacca americana* root applied as poultice to sprains, bruises and swollen joints. Poultice of *Polystichum acrostichoides* applied to back and feet for spinal trouble. Compound infusion of *Taraxacum officinale* roots and barks taken for back pain.	Iroquois
Psychological	*Cardamine concatenate* used as hallucinogenCompound decoction *Smilax herbacea* plants used to treat loss of senses.	Iroquois
Pulmonary and Respiratory	Achillea millefolium was used to treat respiratory disorders; an infusion of *Trifolium pratense* was taken to treat whooping cough.	Algonquin
	In infusion of bark of *Acer saccharum* was used to treat shortness of breath.Roots of *Triosteum perfoliatum* were used to treat pneumonia	Iroquois
Sedative	Compound infusion of *Prunella vulgaris* plants given to babies for excessive crying. *Toxicodendron pubescens* given to treat nervousness in children.	Iroquois
Urinary Aid	Infusion of roots of *Cypripedium acaule* was used to treat urinary tract problems.	Algonquin
	Root decoction of *Actaea pachypoda*, used in treating blood in urine. Compound decoction of *Pteridium aquilinum* taken by men to prevent frequent urination	Iroquois

5. Indigenous Unique Dwellings Techniques

The Algonquins and the Iroquois lived in different types of dwellings. The Algonquins lived in wigwams or wetus, while Iroquois lived in longhouses.

The term 'wigwam' originates from the Algonquian language and means "to dwell". A wigwam was a cone or dome-shaped house typically made of wood. These dwellings could hold from 10 to 12 people. The floors of the

wigwam were covered with tree boughs and blankets made from animal hides. There was a single fire located in the center of the wigwam for cooking and to provide warmth throughout the structure (Gadacz, 2008).

The Wampanoags, who belong to the Algonquin group, typically lived in huts called "wetus" during their growing season. These were built near the coast of the Atlantic Ocean. One wetu could generally accommodate 10 to 20 families or 40 to 50 tribal members at one time. They were typically 50 to 60 feet in length. Saplings were used in construction of the frame of the house and wide sheets of bark from large older trees were used to cover the frames. Double-sided mats were made from cattails plants gathered from the marshes were sun-dried and laid over the frame of house for protection against rain and snow, and kept the inside of the structure dry. The Wampanoags had to survive the harsh winters in which the temperatures dropped to -40 degrees Farenheight. The technological ingenuity of the structure maintained its temperature to as high as 80 degrees Farenheight. The mats helped keep the inside of the dwelling warm, as did the round shape of the dome as this shape helped circulate heat from the fire effectively. Wetus had thicker walls and only one small window for the purpose of retaining heat. All the resources used in the dwelling of the wetus were biodegradable in stark contrast to the construction of modern-day houses. The Wampanoag were technologically skilled in the construction of their houses and were knowledgeable in effectively using renewable resources like saplings and bark (as described in article *Building A Home* by The Plimoth Patuxet Museums).

Iroquoian tribes living in the northeastern region of North America (primarily Ontario and upstate New York) resided in longhouses (or "long houses"), long and narrow structures constructed from resources available in nearby forests. A longhouse is single-room building whose dimensions ranged from thirty to several hundred feet in length, 22 to 23 feet in width and a height of approximately 20 feet. The length of a longhouse was determined by the size of the extended family that would live in it. As the size of the extended family grew due to marriages, the building was enlarged to make room for the expanding family. The interior of the house was divided into sections or compartments which were separated by a central aisle. Two families lived in one compartment separated by the central aisle which extended through the length of the long house. Each section contained a central fire pit which was used for cooking and providing heat and light. Smoke was vented through a small opening in the roof. The structure was primarily made out of wood from strong flexible trees used for the framework of the longhouses. Strips of bark and wooden panels were also used for the exterior. There were openings on both ends of the structures that served as doors, which were covered with animal skins to prevent the cold from entering (as referred in article *Mohawk Haudenosaunee (Iroquois) Longhouse* by The New York State Museum).

Dwellings of indigenous tribes were constructed in different ways using available natural materials, and there were some similarities in the way they built their dwellings. Common features are the use of natural resources, the suitability of the structures to the climate of the region, and the effectiveness by which the dwellings met the needs of the inhabitants.

6. Farming Methods for Soil Conservation and Biodiversity Preservation

Indigenous tribes implemented various farming methods that aided soil conservation and biodiversity preservation. They intercropped corn, beans, and squash, which due to the complementary characteristics of the vegetables, bolsters the health of soil, and the productivity of crops. This is known as the 'Three Sisters' method and is extremely proficient since these three vegetables nurture each other in a way that increases their nutritional value. Interplanting crops helped in controlling pest and creating a uniform stand of corn. The corn plants served as a support for the beans, and the squash covers the soil and control weeds (Ngapo et al., 2021).

Tribes intentionally set planned fires in forests and land used for agricultural purposes at controllable levels. This practice was known as controlled burning and had several benefits. By clearing the land, more agricultural space was created. The subsequent planting of the land with nitrogen-fixing legumes provided increased arability. The forest became more habitable for people because controlled burning reduced the number of pests, offered better defense against threats including other tribes, increased production of berries, and allowed for the easier collection of firewood, and efficiency of travel. The burning of forests also assisted in hunting, since it helped attract the types of animals that they hunted including bears (as described in *Native American Prescribed Burns* from Connecticut Department of Energy & Environmental Protection).

Tribes practiced permaculture. They planted species such as legumes, sunflowers, and other nitrogen-fixing crops to accumulate more fixed nitrogen and vital nutrients to the soil. This practice offers many benefits and is recognized to improve soil health. (Engels, 2020).

These multiple strategies employed by indigenous people supported soil conservation and biodiversity preservation. These practices promoted sustainable and renewable use of natural resources in a way that supported the needs of the population while preserving and improving the resources for use by future generations. In the last two centuries due to colonization and policy changes, historically land inhabited by indigenous tribes reduced to 98.9% in cumulative coextensive lands and 93.9% in non-coextensive lands, direct correlation on its impact to climate (increase heat and decrease precipitation) and loss in soil mineral (as shown by Farrell et al., 2021). "Present-day lands endure nearly two additional extreme heat days per year compared with their historical lands" (Farrell et al., 2021, p.4). The average annual precipitation has reduced by nearly 23% in the present-day lands compared to historical lands owned by indigenous people Additionally, land appropriation, colonization, and intensification of land use which were inhabited by indigenous people lead to loss of biodiversity. "Global land use history confirms that empowering the environmental stewardship of Indigenous peoples and local communities will be critical to conserving biodiversity across the planet" (Ellis et al., 2020, p.1). Introduction of indigenous inter-cropping farming methods including Three Sisters' method in small modern farms and communities will promote sustainable organic farming and eliminate the needs of using chemical fertilizers and weedicides. This will further help to maintain ecosystem and biodiversity.

7. Day to Day Survival, Food and Lifestyle

Prior to the Archaic Period, Native Americans were hunters and gatherers who survived on the available plants, animals, and fish. They hunted animals such as mastodons and giant ground sloths using a sling-like weapon know as an atlatl. Their diet included wild berries such as blueberries, black raspberries, and cranberries. Fishing was prevalent among tribes living close to the Atlantic Ocean and rivers.

Populations of mastodons and giant ground sloths became extinct approximately 10,000 - 12,000 years ago. Hunting then focused on deer and bear, and smaller animals including turtles, turkeys, and grouse. Different types of weapons that were used for hunting could also be used for warfare. In addition to the atlatl used to hunt large game, spear, bow and arrow were used to hunt antelope, turkey and deer (Park et al., 2016). These tools indicate that the indigenous tribes were advanced in their invention of weapons purposed for hunting and warfare.

Agriculture was first practiced during the Woodland Period. Primary crops were the Three Sisters (crop consisting of corn, beans, and squash). "It was the dominant food plant association of every nation practicing agriculture in the northeast USA" (Ngapo et al., 2021, p.2). During the woodland period, northeast indigenous tribes lived in small camps, however, much larger villages were established due to the introduction of agriculture (Ritchie and Funk, 1973). Other plants, including potatoes, peppers, and tomatoes, were also harvested. Their gardens consisted of companion planting of different plants in one area instead of dividing gardens into sections containing simply one type of plant. Troughs were also dug between the mounds purposed to collect water for gardens (as mentioned in Park et al., 2016, p. 174). All of these methods demonstrate that the gardening techniques they used were highly efficient.

Fishing was a major source of food particularly for tribes who were living in regions closer to large streams and water sources. They had many methods for catching fish however, using a spear (also known as gigging) was the most common method. They also used nets made out of vines for trapping, as well as fish hooks"A novel fishing method was using ground black walnut hull to spread into pools, which would temporarily stun the fish and cause them to float to the surface where they could easily be captured" (Park et al., 2016, p. 175). The different traditional methods of fishing reveal that indigenous tribes were highly skilled and experienced when it came to capturing fish.

Women primarily did all of the agricultural work including growing and harvesting various crops. They also helped prepare the food the men collected from hunting and gathering. The different cooking methods women learned included baking, deep frying, frying, boiling and roasting over an open fire (Park, 2016).

Food preservation: The Iroquois in New York, particularly the Haudenosaunee, developed effective methods to preserve food. These tribes were reliant on natural techniques such as drying food over fire or sun and wind to preserve fruits, vegetables, and animal meat. They grew specific flint corn suitable for drying and grinding into meal. This

could be stored in granaries to prepare soup for future use. Moreover, the powder from flint corn had high value and was crucial in trading for other necessary high value products. Another preservation technique the Haudenosaunee developed was to prepare pemmican (mixture of dried meat, bone marrow, dried fruit, etc.) with the other food they consumed. Preparing pemmican was essential to survive during winter and for trading for other food not commonly found in the New York region. Pemmican made their foods last longer and be more sustainable (Dealy, 2013).

8. Conclusion

Genetic studies have revealed that the ancestors of Native Americans had migrated across the Bering Strait 25,000 years ago and expanded rapidly across the North American continent. The ancestors of the Algonquin and Iroquois peoples inhabited the northeast region of United States during the Paleo-India, Archaic, and Woodland periods. Over this time period, they acquired skills and developed techniques to adapt to the geographic and environmental changes that were occurring. They successfully transitioned from hunting mammoths during the Paleo-Indian Period to hunting for deer and whales and to gathering diverse plant-based sources of food. They developed technology such as bows for hunting, smoking pipes, copper tools, and pottery for utensils.

Native American tribes had a nuanced understanding of the medicinal properties of native plants. Their medical practices and knowledge were passed down through generations. Infusion of different parts including roots, branches, sap, leaves and bark of native plants were used in the treatment of various ailments including rheumatism and cataracts. Many of the medicinal plants have proven helpful in treating ailments in modern times such as cancer and venereal warts.

Indigenous communities in the Northeast used innovative techniques to construct wigwams, wetus, and longhouses by gathering resources such as bark, wood and saplings. These dwellings were designed to maintain a warm temperature even in the coldest snowstorms, and for communal living. They showcase that these tribes used advanced technology, had remarkable adaptability, and understood resources within their respective landscapes.

At the time of European contact, the three major subsistence techniques were agriculture, hunting, and fishing/gathering. Tribes cultivated the Three Sisters along with other plants. They incorporated controlled burn and permaculture techniques into their agricultural practices which helped replenish the soil. They made their tools, clothing, baskets, and dwellings using available resources including animal skins and bones, vines, wood, and plants. Food preservation techniques, such as the drying of pemmican by Haudenosaunee, were vital.

The Native Americans that resided in the northeast region of the United States were able to effectively adapt to an environment that had changed dramatically over their early years of habitation. The harsh climate required the development of shelters designed to withstand severe cold. Knowledge of plants and their medicinal properties was equally important for survival. The changing climate required changes in subsistence strategies and in the development of tools related to subsistence and other activities. They effectively used natural resources in a way that encouraged ecological balance, sustainability, and biodiversity. It will help modern communities to adopt some of the practices used by indigenous people including agricultural and therapeutic methods to deal with current environment and health challenges.

References

Dealy, A. (2013, August 30). *Early Food Preservation In The Finger Lakes*. Historic Geneva.https://historicgeneva.org/food-and-cooking/early-food-preservation-in-the-finger-lakes/.

Ellis, E. C., et al. (2020). People have Shaped Most of Terrestrial Nature for at least 12,000 years. *PNAS*, 118 (17), 1-8. https://doi.org/10.1073/pnas.2023483118.

Engels, J. (2020, September 29). *Ancient Gardens of The North America*. The Permaculture Research Institute. https://www.permaculturenews.org/2020/09/29/ancient-gardens-of-the-north-america.

Farrell, J., et al. (2021). Effect of Land Dispossession and Forced Migration on Indigenous Peoples in North America. *Science*, 374 (6567), 1-8. DOI: 10.1126/science.abe4943.

Fowler, W. (1975). The Diagnostic Stone Bowl Industry. *Bulletin of the Massachusetts Archaeological Society*, 36 (3 and 4), 1-9.

Gadacz, R. R. (2008, August 11). *Wigman*. The Canadian Encyclopedia. https://www.thecanadianencyclopedia.ca/en/article/wigwam

Lawson, S. K., et al. (2021). The Volatile Phytochemistry of Seven Native American Aromatic Medicinal Plants. *Plants (Basel)*, 10(6), 1061. DOI: 10.3390/plants10061061

Moerman, D. E. (2009). *Native American Medicinal Plants- An Ethnobotanical Dictionary*. Timber Press.

Ngapo, T. M., et. al. (2021). Historical Indigenous Food Preparation Using Produce of the Three Sisters Intercropping System. *Food*, 10 (3), 524. DOI: 10.3390/foods10030524

Nikolic, M., et al. (2013). Chemical composition and biological activity of *Gaultheria procumbens* L. essential oil. *Industrial Crops and Products*, 49, 561-567. https://doi.org/10.1016/j.indcrop.2013.06.002

O' Brien, C., & Kiffel-Alcheh, J. (n.d*). Native People of the American Northeast*. National Geographic https://kids.nationalgeographic.com/history/article/native-people-of-the-american-northeast

Parks, C. (2004, November 05). *Ancestors of Science: Native American Medicine*. Science. https://www.science.org/content/article/ancestors-science-native-american-medicine.

Park, S., et al. (2016). Native American foods: History, culture, and influence on modern diets. *Journal of Ethnic Foods*, 3 (3), 171-177. DOI:10.1016/j.jef.2016.08.001

Price, M. (2023, January 12). *Native Americans—And Their Genes—Traveled Back To Siberia, New Genomes Reveal*. Science. https://www.science.org/content/article/native-americans-and-their-genes-traveled-back-siberia-new-genomes-reveal.

Ritchie, W. A., & Funk, R. E. (1973). *Aboriginal Settlement Patterns in the Northeast*. University of the State of New York, State Education Department.

Sgouros, R. (n.d). *12,000 Years Of Local History.* Natick Historical Society https://www.natickhistoricalsociety.org/12000-years.

Wade, L. (2018, November 08). *Ancient DNA confirms Native Americans' deep roots in North and South America*. Science. https://www.science.org/content/article/ancient-dna-confirms-native-americans-deep-roots-north-and-south-america.

Wallenfeldt, J. (2023, December 12). *Iroquois | History, Culture, & Facts*. Britannica. https://www.britannica.com/topic/Iroquois-people

Wink, M. (2015). Modes of Action of Herbal Medicines and Plant Secondary Metabolites. *Medicines (Basel)*. 2(3), 251-286. https://doi.org/10.3390/medicines2030251

Building A Home. (2021, June 09). Plimoth Patuxet Museums. Retrieved December 17, 2023 https://plimoth.org/for-students/homework-help/building-a-home.

Connecticut Department of Energy & Environmental Protection. (2023). *Native American Prescribed Burns*. https://portal.ct.gov/DEEP/Forestry/Native-American-Use-of-Prescribed-Fire.

Mohawk Haudenosaunee (Iroquois) Longhouse. (2022, November 30). The New York State Museum. Retrieved December 17, 2023, https://www.nysm.nysed.gov/exhibitions/ongoing/native-peoples-new-york/mohawk-longhouse.

World Health Organization. (2023). *Traditional medicine has a long history of contributing to conventional medicine and continues to hold promise.* https://www.who.int/news-room/feature-stories/detail/traditional-medicine-has-a-long-history-of-contributing-to-conventional-medicine-and-continues-to-hold-promise

Mathematical Modeling of Cancer Chemotherapy

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Abstract

The complexity of the tumor system presents a formidable challenge in developing an optimal cancer treatment. Mathematical modeling and computer simulation are increasingly being utilized to predict tumor growth and investigate the efficacy of cancer therapeutic strategies, such as chemotherapy, in suppressing its growth. Along with laboratory experiments, this approach has been utilized to develop an optimal treatment for individual patients. This paper presents a system of ordinary differential equations consisting of the Gompertz and exponential decay models that describe the dynamics of tumor growth and chemotherapy drug concentration. A numerical method was utilized to simulate and solve the model and was further used to examine the effectiveness of various treatment schedules and dosages in suppressing overall tumor growth. The results suggested that frequent smaller dosages of the chemotherapy drug are more efficient than less frequent larger dosages. This study illustrates the use of mathematical model as a predictive tool to help guide laboratory experiments in developing an optimal cancer treatment.

Keywords: Math model, Differential equations, Cancer, Chemotherapy, Numerical simulation

1. Introduction

According to the World Health Organization, cancer is the leading cause of death worldwide, causing about 10 million deaths annually. More than 40% of these deaths could have been prevented through routine screening, earlier detection, and effective treatment (World Health Organization, 2022).

Cancer is caused when normal cells transform into cancerous cells through uncontrollable cell division and the creation of gene mutations. Genetic factors and carcinogens interact to induce these transformations (Matthews, 2022). Groups of cancerous cells are classified as malignant tumors, meaning they can spread rapidly and invade other parts of the body through the process of metastasis. In metastasis, cancerous cells spread into surrounding tissue and organs through the lymphatic system and the bloodstream, making it more difficult to treat (Cancer Research UK, 2020).

To this date, scientists are still searching for a cure for cancer. Innovative research has fueled the development of new medications and treatments. One of the most well-known cancer treatments is chemotherapy. Paclitaxel is a specific type of chemotherapy drug that aims to eliminate cancer by preventing the cell division of cancerous cells, shrinking the malignant tumors significantly. A protein known as tubulin polymerizes into microtubules, which are responsible for cell division. Paclitaxel stabilizes and boosts the polymerization of tubulin and thus the production of microtubules. The microtubules formed by paclitaxel are exceptionally unstable and dysfunctional, promoting apoptosis or cell death of the cancerous cells (Meštrović, 2023). Although paclitaxel appears to be one of the most promising cancer therapeutic drugs, its clinical toxicity and side effects cannot be ignored, especially when given at high doses. Thus, it is important that the anticancer drug is administered strategically to ensure its efficiency while at the same time minimizing its toxicity (Rowinsky, 1993; Marupudi, 2007).

Laboratory experiments for cancer research can be costly and are often quite challenging to perform as each experiment can only be done for a specific type of cancer and cannot be easily modified to investigate others (Errington, 2021). Interdisciplinary research that combines cancer biology, mathematical modeling, and computer simulation has been initiated and done in the last decade. Various modeling techniques, such as cellular automaton, agent-based, and Potts model, have been used to simulate cancer growth (Stott, et al., 1999; Kansal, et al., 2000; Sherratt and Chaplain, 2001; Jiang, 2005). Other math modeling works conducted by (Gevertz, 2011; Sun, et al., 2012; Hendrata and Sudiono, 2019; Lai and Friedman, 2019) focused on investigating the effect of therapeutic strategies, such as antiangiogenic treatments, chemotherapy, TKI treatment, and cell-based therapy, on tumor growth. This interdisciplinary approach has become popular as it can provide additional insights into the tumor system that would complement the experimental results and aid the development of cancer treatments.

Although computational models for cancer published in literature have been able to qualitatively mimic cancer growth and development, such models are generally extensive and may be limited in their clinical applicability as they require parameter values and input data that are impossible to obtain from experiments. Moreover, the simulation of such complex models requires large-scale computing power which may not be easily accessible. Simpler mathematical models with fewer parameters that also exhibit good agreement with experimental observations can potentially be used as a more accessible predictive tool to assess the efficacy of therapeutic drugs and help design an optimal treatment schedule.

The objective of this research is to develop a computational model for tumor growth with few but physically relevant parameters to investigate the efficacy of treatment schedules under various dosages of the chemotherapy drug paclitaxel to determine the most effective approach to reducing tumor growth. The model consists of a system of differential equations which can be simulated using a numerical method implemented in accessible software, such as Excel or Python. The simulation results suggested that frequent smaller dosages of the chemotherapy drug are more efficient than less frequent higher dosages, which agree with the observations in many clinical experiments.

This paper is organized as follows: Section 2 discusses the mathematical model that is used to illustrate the dynamics of the tumor cell population along with the chemotherapy drug (paclitaxel) concentration that influences the growth. The derivation and implementation of Euler's method that is used to obtain the numerical solution to the proposed model are also discussed in this section. The results of this study are presented and discussed in Sections 3 and 4, respectively. Section 5 concludes the paper.

2. Materials and Methods

The two main components in the proposed model are the tumor cell density and the paclitaxel drug concentration in the tumor system. Both dynamics can be represented by first order differential equations described below.

2.1 Tumor Growth Model

Several classical mathematical models have been developed and used to predict how population changes over time. The simplest of such models is the exponential growth model. In this model, the rate of change of the population is assumed to be proportional to the current population size. The differential equation for the exponential growth model is given by

$$\frac{dc}{dt} = rc,$$

where $c(t)$ represents the population size at time t and r is the growth rate of the population. Using the technique of separation of variables, one can derive the analytical solution

$$c(t) = c(0)\, e^{rt},$$

where $c(0)$ denotes the initial population size. In regard to cancer, the exponential growth model can be used to model

the initial growth of cancer, where the cells undergo cell division continuously regardless of the tumor size. However, as nutrients and oxygen deplete over time due to cells' consumption, the growth eventually slows down, making this model physically unrealistic.

An alternative to this model is the Gompertz model. This model was first developed in 1825 by Benjamin Gompertz and later became one of the most widely used models for tumor growth (Gerlee, 2013; Laird, 1964). In the Gompertz model, the population does not grow indefinitely, but its growth slows down as population size increases. The population size eventually plateaus at a certain value known as the *carrying capacity*. One can think of the carrying capacity as the maximum population size that the environment can sustain. The Gompertz model can be described by the following differential equation

$$\frac{dc}{dt} = -rc \ln(c/K), \tag{1}$$

where r and K represent the growth rate and the carrying capacity, respectively. To see why this model is indeed more realistic, first note that when the tumor size is small, that is, when $c(t) < K$, the ratio $c/K < 1$ and the rate of change $dc/dt > 0$. This implies that tumor size $c(t)$ is increasing. However, as the tumor grows and reaches the carrying capacity, that is $c(t) = K$, the rate of change $dc/dt = 0$ and the tumor stops growing. Lastly, if the tumor size $c(t)$ is bigger than the carrying capacity K, the ratio $c/K > 1$, and the tumor size will decrease as $dc/dt < 0$.

2.2 Drug Decay Model

An ordinary differential equation that describes the rate of change of drug concentration over time can be employed to study how chemotherapy drug, such as paclitaxel, affects tumor growth. It is assumed that once administered intravenously, drug diffuses into the tissue and decays naturally as time progresses. A simple exponential decay model

$$\frac{dD}{dt} = -aD$$

can be used to describe the changes in drug concentration in the tumor tissue. In this equation, $D(t)$ represents the concentration of the drug at a time t and a is the decay rate. In many cancer treatments, chemotherapy drugs are administered periodically to maintain their effectiveness in suppressing cancer growth. To incorporate periodic drug administration, the exponential decay model can be modified by adding the drug injection term as follows:

$$\frac{dD}{dt} = -aD + I(t). \tag{2}$$

Here $I(t)$ denotes the amount of drug administered at each injection.

Lastly, to incorporate the effect of chemotherapy drug on tumor growth, the interaction term bcD needs to be subtracted from equation (1). Here b can be interpreted as the killing rate of the cancer cells due to the chemotherapy drug. Therefore, the tumor growth equation becomes

$$\frac{dc}{dt} = -rc \ln\left(\frac{c}{K}\right) - bcD. \tag{3}$$

2.3 Euler's Mothod

Many differential equations that model a real-life situation can be too complicated to solve analytically or do not even have an exact solution. In such cases, numerical methods, such as Euler's method, can be employed to approximate the solution to the initial value problem (IVP) of the form $y' = f(t, y)$, $y(t_0) = y_0$.

The Euler's method computes the approximation to the solution at discrete time in the interval $[t_0, t_f]$. The method starts by dividing this time interval into n subintervals, each of which has length h. That is, $t_0 < t_1 < \cdots < t_f$, where

$h = t_n - t_{n-1}$. Euler's method uses the tangent line to the solution curve $y(t)$ at t_0 to approximate the value $y(t_1)$. Recall that the equation of the tangent line to the curve $y(t)$ at the point $(t_0, y(t_0))$ is given by:

$$y - y(t_0) = y'(t_0)(t - t_0).$$

Evaluating this equation at t_1 gives the approximate value of $y(t_1)$:

$$y(t_1) \approx y(t_0) + y'(t_0)(t_1 - t_0).$$

Since $y'(t_0) = f(t_0, y_0)$ and $t_1 - t_0 = h$, the above equation can be written as

$$y(t_1) \approx y(t_0) + h\, f(t_0, y_0).$$

Repeating this process, the approximate value at t_2 can be obtained by

$$y(t_2) \approx y(t_1) + h\, f(t_1, y_1)$$

and in general, $y(t_n) \approx y(t_{n-1}) + h\, f(t_{n-1}, y_{n-1})$.

If y_n denotes the approximation to the actual value $y(t_n)$, Euler's formula can then be defined recursively as

$$y_n = y_{n-1} + h\, f(t_{n-1}, y_{n-1}) \tag{4}$$

2.4 Implementation

The tumor-paclitaxel model discussed in Sections 2.1 and 2.2 consists of a coupled system of two ODEs:

$$\frac{dc}{dt} = -rc \ln\left(\frac{c}{K}\right) - bcD \tag{5}$$

$$\frac{dD}{dt} = -aD + I(t) \tag{6}$$

where $I(t)$, the amount of paclitaxel drug administered periodically, is the control parameter that can be varied to test the efficacy of different dosages. For example, if a certain amount I_0 of paclitaxel is administered weekly, then $I(t) = I_0$, when $t = 7, 14, 21, \ldots$, and $I(t) = 0$ otherwise.

In the model simulation, dimensionless quantities for the parameters and variables were used to compare the relative influence of the drug concentration in suppressing tumor growth. Implementing Euler's method (4) to approximate the solution to the tumor-paclitaxel model (5)-(6) would yield the following recurrence relations:

$$c_n = c_{n-1} + h\left(-r\, c_{n-1} \ln(c_{n-1}/K) - b\, c_{n-1} D_{n-1}\right) \tag{7}$$

$$D_n = D_{n-1} + h\left(-a\, D_{n-1} + I_{n-1}\right) \tag{8}$$

with initial tumor volume $c(0) = c_0$ and initial drug dosage $D(0) = D_0$. The values of c_n and D_n generated by the above recurrence relations were computed using Excel.

Table 1. Parameter values used in the model simulation.

Parameters	Description	Dimensionless value used in baseline simulation
r	Tumor growth rate	0.2
K	Tumor carrying capacity	1
b	Drug killing rate	0.5
a	Drug decay rate	0.1

Table 2. Simulation scenarios performed in this study.

Scenario	Description	Initial conditions	Dosing schedule
1	Tumor growth without chemotherapy	$c(0) = 0.1$, $D(0) = 0$	$I(t) = 0, t > 0$
2	Tumor growth under single high dose of drug	$c(0) = 0.75$, $D(0) = 0.75$	$I(t) = 0, t > 0$
3	Tumor growth under moderate drug dose administered every 3 weeks	$c(0) = 0.75$, $D(0) = 0.25$	$I(t) = \begin{cases} 0.25, for\ t = 21, 42 \\ 0, otherwise \end{cases}$
4	Tumor growth under small drug dose administered weekly	$c(0) = 0.75$, $D(0) = 0.083$	$I(t) = \begin{cases} 0.083, for\ t = 7, 14, …, 56 \\ 0, otherwise \end{cases}$

Using the Euler's method with step size size $h = 0.2$ and parameter values listed in Table 1, four scenarios were simulated for a period of 60 days as shown in Table 2. The first scenario was tumor growth simulation without chemotherapy. In this simulation, the initial tumor volume $c(0) = c_0 = 0.1$, initial drug dosage $D(0) = D_0 = 0$, and the amount of drug administered $I(t) = I_n = 0$ for all n.

The second scenario was tumor growth simulation under a high dose of paclitaxel administered once. As cancer needs to reach a certain size to be detected and for chemotherapy treatment to be performed, the initial tumor volume was set to $c(0) = c_0 = 0.75$. The paclitaxel dosage was set to be $D(0) = D_0 = 0.75$ administered once at $t = 0$ and $I(t) = I_n = 0$ for all n.

The third scenario was tumor growth simulation under a moderate dose of paclitaxel administered every 3 weeks. The initial tumor volume was set to $c(0) = c_0 = 0.75$. The paclitaxel dosage was set to be $D(0) = D_0 = 0.25$ and $I(t) = 0.25$ for $t = 21, 42$ and $I(t) = 0$ otherwise. Such dosage was chosen to standardize the total amount of drug administered throughout the 60-day period in all simulation scenarios.

The last scenario was tumor growth under a small dose of paclitaxel administered weekly. Likewise, the initial tumor volume was set to $c(0) = c_0 = 0.75$. The paclitaxel dosage was set to be $D(0) = D_0 = 0.083$ and $I(t) = 0.083$ for $t = 7, 14, … , 49, 56$ and $I(t) = 0$ otherwise. This dosage was chosen so that the total amount of drug administered during the 60-day period would not be higher than 0.75.

Sensitivity analysis needs to be performed to determine which parameter plays the most critical role in affecting the model output. Tumor carrying capacity $K = 1$ was used to normalize the tumor volume so that $0 \leq c(t) \leq K$ for all t. To perform sensitivity analysis of the tumor growth rate r, the simulation of tumor growth without chemotherapy (scenario 1) was done with the growth rate r modified by ±10% while keeping the other parameters at their baseline values. For the sensitivity analysis of the other two parameters, the drug killing rate b and drug decay rate a, the simulation of tumor growth under a single drug dosage (scenario 2) was performed with one parameter value increased or decreased by 10%, while keeping the other parameters at their baseline values.

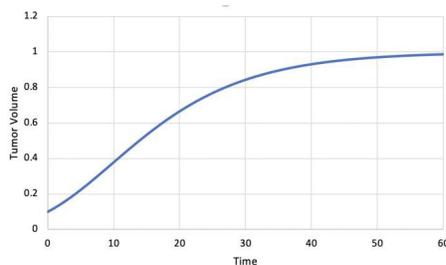## 3. Results

3.1 Tumor Growth Simulations

Figure 1. The Gompertz model simulation for tumor growth without chemotherapy.
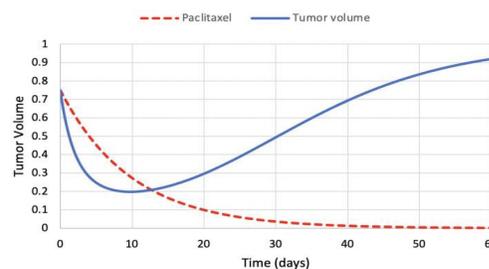

Figure 2. Tumor growth under paclitaxel treatment with a high dose administered once.
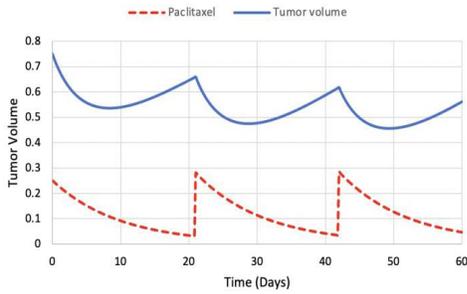
Figure 3. Tumor growth under paclitaxel treatment at a moderate dose administered every three weeks.
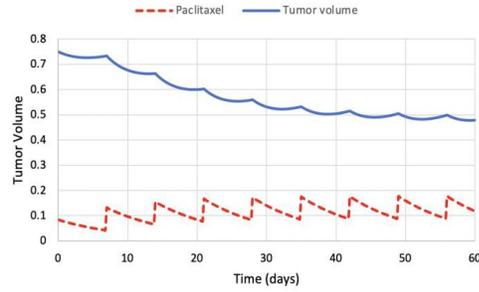

Figure 4. Tumor growth under paclitaxel treatment at a small dose administered weekly.

3.2 Sensitivity Analysis

Table 3. Sensitivity analysis of tumor growth rate r.

Percentage used	Tumor growth rate	Final tumor volume	% change
90%	0.18	0.9795	-0.84%
100%	0.2	0.9878	
110%	0.22	0.9928	0.51%

Table 4. Sensitivity analysis of drug killing rate b.

Percentage used	Drug killing rate	Final tumor volume	% change
90%	0.45	0.9269	0.84%
100%	0.5	0.9192	
110%	0.55	0.9116	-0.83%

Table 5. Sensitivity analysis of drug decay rate a.

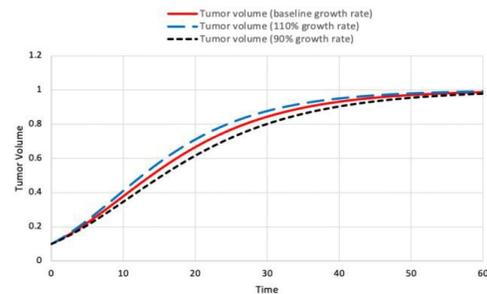| Percentage used	Drug decay rate	Final tumor volume	% change
90%	0.09	0.8951	-2.62%
100%	0.1	0.9192	
110%	0.11	0.9361	1.84%

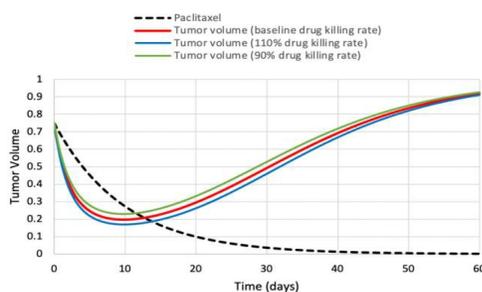Figure 5. Sensitivity analysis plot for tumor growth rate r.

Figure 6. Sensitivity analysis plot for drug killing rate b.

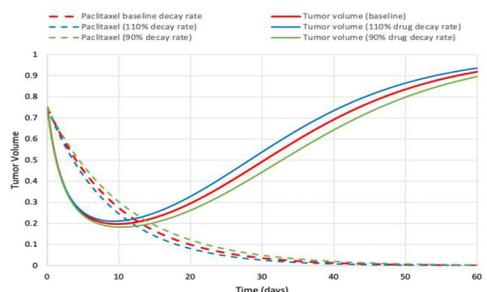
Figure 7. Sensitivity analysis plot for drug decay rate a.

4. Discussion

In all four simulation scenarios described in Section 2, Euler's method was employed to solve the model by computing the equations (7)-(8) recursively with the given initial conditions c_0 and D_0.

In the first simulation without chemotherapy (Figure 1), the initial conditions $c(0) = c_0 = 0.1$ and $D(0) = D_0 = 0$. This corresponds to a relatively small tumor that can quickly grow due to uncontrollable cell division and plenty of nutrients and oxygen in the tumor tissue. As the tumor volume got bigger, nutrients eventually started to deplete and competition among tumor cells increased. The growth eventually slowed down, and the tumor size reached its carrying capacity $c(t) \approx K = 1$, as shown in Figure 1.

In the second simulation (Figure 2), the effect of a single dose of paclitaxel in reducing tumor size was simulated. As cancer needs to reach a certain size to be detected and for chemotherapy treatment to be performed, the initial tumor volume was set to $c(0) = c_0 = 0.75$. A high dosage of paclitaxel $D(0) = D_0 = 0.75$ was administered once at the beginning of the simulation. Figure 2 showed that paclitaxel can reduce the tumor volume from 0.75 to 0.2 in about 10 days. However, as paclitaxel concentration decreased, its efficacy significantly decreased and the tumor could grow again, reaching a volume that is greater than 0.9 at the end of 60 days.

The third and fourth simulations were performed to investigate whether a repeated dose of paclitaxel is needed to suppress tumor growth for a longer period. Starting with the same initial tumor volume of 0.75 as in the previous scenario, Figure 3 showed the simulation result when paclitaxel was administered in a moderate dose of 0.25 every 3 weeks. During the 60-day period, the drug was administered three times (day 0, day 21, and day 42). The dosage of 0.25 was chosen so that the total dosage of drug administered during the 60-day period would be 0.75, which was consistent with the second simulation scenario. It showed that although the tumor size fluctuated and got smaller rather slowly, this chemotherapy regimen could reduce the tumor volume to below 0.6 by the end of 60 days. However, this 3-weekly treatment schedule was still partially ineffective because the growth of the tumor drastically increased every 20 days.

In the last scenario (Figure 4), a weekly administration of paclitaxel at a small dose of 0.083 was simulated. This dosage was chosen so that the total amount of drug administered during the 60-day period would not be higher than 0.75. As shown in Figure 4, the tumor size in general decreased steadily and was around 0.5 at the end of 60 days. Moreover, the fluctuations in tumor size, although still visible, were not as prominent as in the previous scenario, making this treatment schedule by far the most effective. These results were in alignment with those analyses obtained through laboratory experiments performed in (Lin, et al., 2022).

Since the parameters used in the model were estimated, there was a degree of flexibility and uncertainty in their values which remained to be explored. Sensitivity analysis can help determine which model parameter is the most important in controlling the model output. This can help determine where future research and efforts in laboratory experiments would be most beneficial in suppressing tumor growth.

Table 3 and Figure 5 showed that when the tumor growth rate r was varied by ±10% from the baseline value of 0.2 while all other parameters remained fixed, the changes in final tumor volume were less than 1%. Likewise, Table 4 and Figure 6 showed that when the drug killing rate b was increased or decreased by 10% from its baseline value of 0.5, the changes in final tumor volume were also less than 1%. However, when the drug decay rate a was varied by ±10% from its baseline value of 0.1, the final tumor volume changed by more than 2%, as shown in Table 5 and Figure 7. This indicated that the drug decay rate is the parameter that affected the result the most. Periodic drug administration is a way to prevent the drug concentration from being too low due to drug decay. This aligns with the simulation result that suggested that more frequent administration is the most effective in suppressing tumor growth.

5. Conclusion

In this project, a system of ordinary differential equations consisting of the Gompertz model and the exponential decay model were employed to describe the dynamics of tumor growth and chemotherapy drug concentration in the tumor tissue, respectively. Euler's method was implemented to numerically solve the system and simulate the growth of the tumor with and without chemotherapy treatment. The treatment using paclitaxel with different dosages and schedules were simulated to examine their efficacies in suppressing the overall growth of the tumor. The simulation results suggested that weekly administration of paclitaxel with a small dosage is more efficient than either one-time administration with a high dosage or 3-weekly administration with a medium dosage.

The model presented in this study can be applied to any type of cancer and chemotherapy drug if the parameter

values specific to the cell type and drug are adjusted accordingly. Techniques, such as the least squares method, can be performed to estimate these parameter values from the available experimental data to obtain a more accurate and reliable prediction for how a chemotherapy drug affects a specific type of cancer. This project illustrated how mathematical modeling and computer simulation can be used as a tool to investigate the effectiveness of certain treatment regimens and can potentially help develop an optimal treatment strategy.

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References

Cancer Research UK. (2020, July 1). *How cancers grow.* Cancer Research UK. Retrieved September 1, 2023, from https://www.cancerresearchuk.org/about-cancer/what-is-cancer/how-cancers-grow.

Errington, T. M., et al. (2021). Reproducibility in Cancer Biology: Challenges for assessing replicability in preclinical cancer biology. *eLife*. 10:e67995. https://doi.org/10.7554/eLife.67995.

Gerlee, P. (2013). The model muddle: in search of tumor growth laws. *Cancer Research.* 73(08), 2407-2411.

Gevertz, J. L. (2011). Computational Modeling of Tumor Response to Vascular-Targeting Therapies – Part I: Validation. *Computational and Mathematical Methods in Medicine,* Article ID 830515, https://doi.org/10.1155/2011/830515.

Hendrata, M., & Sudiono, J. (2019). A hybrid multiscale model for investigating tumor angiogenesis and its response to cell-based therapy. *In Silico Biology,* 13(1-2), 1-20.

Jiang, A. Y., et al. (2005). A multiscale model for avascular tumor growth. *Biophysical Journal,* 89(6), 3884-3894.

Kansal, R., et al. (2000). Simulated brain tumor growth dynamics using a three-dimensional cellular automaton. *Journal of Theoretical Biology,* 203(4), 367-382.

Lai, X., & Friedman, A. (2019). Mathematical modeling in scheduling cancer treatment with combination of VEGF inhibitor and chemotherapy drugs. *Journal of Theoretical Biology,* 462, 490-498, https://doi.org/10.1016/j.jtbi.2018.11.018.

Laird, A. (1964). Dynamics of tumor growth. *British Journal of Cancer,* 13, 490-502.

Lin, S., et al. (2022). Comparison of one-week versus three-week paclitaxel for advanced pan-carcinomas: systematic review and meta-analysis. *Aging*. 14(4), 1959-1982. doi: 10.18632/aging.203919.

Matthews, H. K., et al. (2022). Cell cycle control in cancer. *Nature Reviews Molecular Cell Biology,* 23, 74-88.

Marupudi, N. I., et al. (2007). Paclitaxel: a review of adverse toxicities and novel delivery strategies. *Expert Opinion on Drug Safety,* 6(5), 609-621. doi: 10.1517/14740338.6.5.609.

Meštrović, T. (2023, May 13). *How Paclitaxel Works*. News Medical Life Sciences. www.news-medical.net/health/How-Paclitaxel-Works.aspx.

Rowinsky, E. K., et al. (1993). Clinical toxicities encountered with paclitaxel (Taxol). *Seminars in Oncology*. 20(4), 1-15. PMID: 8102012.

Sherratt, J. A., & Chaplain, M. A. (2001). A new mathematical model for avascular tumour growth. *Journal of Mathematical Biology*, 43(4), 291–312.

Stott, E. L., et al. (1999). Stochastic simulation of benign avascular tumour growth using the Potts model. *Mathematical and Computer Modelling,* 30(5-6), 183-198.