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Fall 2024 Best Manuscript

Maria Xu

Maria Xu ’27 is a current sophomore at Harvard College, studying Neuroscience with a secondary in Global Health & Health Policy. She currently conducts research in the Valera lab, which studies traumatic brain injury in women who have experienced intimate partner violence; her research focuses on analyzing the structural brain morphology of women who have experienced IPV-related non-fatal strangulation. On campus, Maria is involved with the Harvard College Debate Union and the Harvard College Honor Council; she additionally works as a PACU volunteer at Brigham & Women’s Hospital. Maria's manuscript regarding the role of the church of the province of Central Africa's role in combatting malaria was produced as a research paper for GenEd 1093: “who lives, who dies, who cares: reimagining global health.”

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December 2024

Dear Harvard Community,

It is our pleasure to present the Fall 2024 issue of The Harvard Undergraduate Research Journal (THURJ), a student-run biannual publication dedicated to showcasing exceptional research from Harvard undergraduates of all disciplines and interests.

Founded in 2007, THURJ previously enjoyed broad success on the College campus until 2021, when the COVID-19 pandemic brought the publication to a sudden stop. In the Fall of 2023, THURJ was revived through dedicated work from its General and Executive Boards—in addition to the trust of Harvard's many undergraduate researchers—and published its first print edition in four years. This issue marks one year since THURJ returned from its hiatus, and it provides us with the unique opportunity to reflect on the organization's growth since then.

Since last fall, the THURJ General Board has almost tripled in size, and the Executive Board has experienced a similar change. This expansion has allowed us to establish two new sub-boards within the organization—Internal Relations and External Relations—enabling us to engage more effectively both within the organization and with our external collaborators. Manuscript submissions have tripled in volume, and our online readership has grown by over 150%, underscoring a renewed interest in research journalism at Harvard.

Such growth has been and continues to be fueled by our very own members. We are continually impressed and inspired by the work of these THURJ members who, in addition to their organizational responsibilities, introduce incredible new initiatives every semester. Over the past year, we have relaunched the THURJ tradition of awarding a Best Manuscript Prize and introduced a cover art contest. Additionally, we started a high school research competition to inspire and increase exposure to research journalism among younger students. This semester, we hosted the first-ever Undergraduate Research Journal Summit, with attendees from Stanford, Yale, the California Institute of Technology, and more. Optimization of our internal operations resulted in a new Digital Object Identifier (DOI) system and a partnership with the Harvard Libraries to offer both print and digital copies of the Journal. All of these initiatives aim to further THURJ's mission of publishing and publicizing Harvard undergraduate research and ensuring that THURJ's contributions remain a lasting part of Harvard's scholarly community.

In addition to all THURJ members—from Peer Reviewers to Executive Board—we would like to extend our sincere gratitude to the other individuals and organizations who make our work at THURJ possible. First, thank you to all of our Faculty Reviewers; your insight is critical throughout our rigorous review process. Next, thank you to our advisor, Dr. Andrew Berry, who is ever-engaged with the undergraduate research community. Finally, thank you to the Office of the Dean of Science Education, the Office of the Dean of Harvard Medical School, and the Office of the Dean of the School of Engineering and Applied Sciences, whose generosity allows us to continue our long-running tradition of print publication.

The future of THURJ is indeed bright. We are all incredibly excited and proud to present to the Harvard community the largest-ever issue in the Journal's history, featuring six remarkable works of undergraduate research and eleven fascinating pieces of short-form commentary on the state of research at Harvard and beyond. Please enjoy!

Sincerely,

Ellie Shin
Editors-in-Chief

Hugh Hankenson

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About Us

The Harvard Undergraduate Research Journal (THURJ) showcases peer-reviewed undergraduate student research from all academic disciplines. As a biannual publication, THURJ familiarizes students with the research publication process. This process not only stimulates faculty student collaboration and provides students with valuable feedback on their research, but also promotes collaboration between the College and Harvard's many graduate and professional schools. In addition to publishing original student research papers, THURJ keeps the Harvard community updated on and provides an important forum for discourse on the cuttingedge research that impacts our world today.

About the Cover

Taruna Singh '24 -'25

The cover art visually and conceptually explores the multifaceted idea of "equilibrium," blending its economic and humanistic dimensions. In economics, equilibrium refers to the precise balance where market supply and demand intersect, a state that can be mathematically charted and represented as a fixed point on a graph. This concept is mirrored in human interactions, where equilibrium emerges through moments of connection. These moments, though often imperfect, achieve a fleeting balance that transcends the physical—symbolized here by the pinky promise, this is a gesture of trust and commitment that intersects at the same central point of harmony as the economic graph.

Research

Rebuilding Trust and Connecting Community: The Role of the Anglican Church of the Province of Central Africa in Malaria Prevention, Treatment, and Eradication

Maria Xu
Harvard College '27

Malaria continues to be a leading cause of death in sub-Saharan Africa. In the face of widespread state failure to instate productive antimalarial policy, the Church of the Province of Central Africa (CPCA), an evangelical Anglican church spanning Botswana, Malawi, Zambia, and Zimbabwe, has emerged as a particularly effective organization in lowering disease burden. Critically, the failure of state antimalarial policy in Central Africa and the uniquely powerful position of the CPCA has not yet been sufficiently considered from a *biosocial* scope in the literature, incorporating both biological and social factors. A historical analysis of health policy in sub-Saharan Africa reveals colonial medicine campaigns aimed at eradicating diseases using invasive treatments as a potent force behind present-day mistrust in state healthcare. Further, the declining amount of international aid into antimalarial efforts as well as the inefficient allocation of existing funds can be traced back to the neoliberal ideals (e.g. ideals supporting the free market and deregulation) of bureaucratic rationality—the belief in bureaucracy as a rational means of societal organization—championed by the Reagan and Thatcher administrations. These factors contribute to the woefully inadequate provision and distribution of malaria control equipment, including insecticide-treated nets and antimalarial drugs. In contrast, the CPCA has been able to combat malaria on an unprecedented level; capable of reaching thousands of congregants in remote border regions, it has been instrumental in mobilizing volunteers, distributing resources, and educating citizens. The CPCA notably functions as a "local moral world," a group with shared moral experiences and meanings, capable of community-level integration. Its success reaffirms the necessity of faith-based organizations in combating infectious diseases in low-income countries and improving health outcomes.

Introduction

The persistence of malaria as a devastating issue and leading cause of death in many low-income countries, despite being largely treatable with modern medicine, is one of the greatest failures of both regional and global health policy. In 2021, there were an estimated 247 million cases of malaria worldwide and 619,000 malaria deaths; the African region alone was home to 95% of malaria cases and 96% of deaths (WHO, 2023). In contrast, infectious diseases such as malaria are notably missing from the leading causes of death in more affluent countries, establishing malaria as a marker of stark inequality in global health (Farmer et al., 2013). Various faith-based organizations and non-governmental organizations have stepped in to deliver medical care in regional settings. This paper will specifically focus on the role of the Church of the Province of Central Africa (CPCA) as one such faith-based organization, analyzing where and why the CPCA has succeeded in countering a devastating illness that local governments continue to struggle with.

The CPCA, an evangelical Anglican church, has fifteen dioceses (districts) that cover the countries of Botswana, Malawi, Zambia, and Zimbabwe (Steffenson, 2013). With the exception of Botswana, which has been able to consistently maintain malaria incidence at one case per thousand in the past decade (WHO Africa, 2023), all of these countries continue to deal with high rates of malaria prevalence and mortality. Malawi and Zambia both rank in the 20 countries with the world's highest malaria incidence; in 2021, Malawi accounted for 1.7% of global malaria cases and 1.2% of global malaria deaths, while Zambia carried roughly 1.4% of the global malaria case and death burden (USAID President's Malaria Initiative, 2022). Zimbabwe has also struggled with a high malaria burden, regularly receiving hundreds of thousands of cases per annum; just this year, the country recorded 144,508 positive malaria cases by August (Mugarisi, 2023). These countries are all located in the southern sub-Saharan region of Africa, as pictured below:

Figure 1. Map of Sub-Saharan Africa
Note: The Church of the Province of Central Africa spans the southern African countries of Zambia, Botswana, Zimbabwe, and Malawi (Pew Forum on Religion & Public Life, 2010).

The myriad reasons why governmental health policy in sub-Saharan Africa has largely failed to control or eliminate malaria has been the subject of extensive scholarly work. This research has centered around three main causes: the first is the rise of antimalarial drug resistance against chloroquine and sulfadoxine-pyrimethamine in the late 20th century (Takala-Harrison & Leufer, 2015). The additional causes are a misallocation of meager funds that has led to low-cost efficacy for health policy and a lack of primary infrastructure to distribute resources like insecticide-treated nets (ITNs) and indoor residual sprays (IRS) (Kouyaté et al., 2007; Mtalimanja et al., 2022). The scientific consensus is that the historical and present-day inability for African governments to counter malaria does not stem from a lack of effective treatment options, but rather a lack of funding and infrastructure to manufacture and distribute treatment. For example, the recently developed drug Coartem faces severe supply-side problems, when it could have the possibility to save millions of lives. That is, there is a notable shortage of Coartem to distribute to affected individuals due to a severe lack of global investment into its production (Robertson et al., 2018). This is an important contributing factor to the continued devastating impacts of malaria in the Central African region.

However, it is evident that this wealth of existing scholarly research regarding the failures of anti-malaria policy in Central Africa lacks a sufficient *biosocial* scope, integrating both biological and social factors. For instance, little analysis has been conducted to link the idea of coloniality to present-day mistrust of the state medical system, which is a major impediment to the distribution of health knowledge and treatment. Beyond coloniality, there has been insufficient research regarding the influence of neoliberalism (e.g. ideals supporting the free market and deregulation) and the "affordability"-focused selective primary health care movement on anti-malaria health policy in Central Africa. Definitionally, neoliberalism supports the notion that health is a commodity that ought to be delivered in a market rather than a right for all that the government should provide. Funding from Western sources is critical for malaria interventions in Africa, to the point where about a third of the world's money spent on malaria comes from the United States (Robertson et al., 2018); research findings have pointed towards a lack of funding from foreign donors as a major reason behind ineffective governmental malaria response. However, existing research has not interrogated the cause of this financial situation. This paper will analyze the structural problems with anti-malaria policy funding in relation to the rise of neoliberalism and the push for "affordability" in the mid-20th century; it will draw on Keshavjee and Farmer's historical characterization of the transformation of healthcare to a privatized neoliberal apparatus guided by the priorities of foreign donors (Keshavjee, 2014; Farmer, 2013).

Regardless of the underlying reason, in the face of widespread state failure in combating malaria, the Church of the Province of Central Africa has emerged as a powerful regional force that is dedicated to using community-level interventions to promote health. For the past decade, they have been instrumental in distributing education, health knowledge, and hundreds of thousands of antimalarial insecticide-treated mosquito nets to rural communities. In particular, they have worked with the Cross-Border Malaria Initiative to reach remote communities in Zambia that border Angola and Namibia; these border regions are known for having the highest malaria burden, while also often being the last places to receive support fighting illness (Christian Aid, 2012). With regard to other health interventions, the Anglican church is most well-known for its role in combating HIV/AIDS in South Africa; indeed, the church's response to the HIV/AIDS crisis in South Africa has many parallels to its ongoing malaria response, especially with regards to the way it uses community integration as a mechanism for treatment (Madlala & Khanyile, 2023).

This paper will thus firstly focus on an analysis of the historical factors that have led to the current structural issues within the governments of Central African countries. It will ask two questions: **(1)** What role has colonialism played in the loss of trust in state medical systems in Central Africa? **(2)** How has neoliberalism, bureaucratic rationality, and the "affordability" movement contributed to failure in state medical systems to adequately counter malaria? Subsequently, this paper will discuss the role of the CPCA in the region as a provider of equitable and accessible care for malaria. It will analyze the role of religion and community-level engagement in municipal health systems, and ask a final question: **(3)** How can the CPCA continue to deliver care through the local moral world of religion?

Background: Colonial Medicine & Loss of Trust

"Mistrust does not form in a vacuum"
— Eugene Richardson, *Epidemic Illusions*

Colonial Medical Campaigns in sub-Saharan Africa

In order to properly interrogate why church-affiliated institutions such as the CPCA have become so essential to the facilitation of malaria control, it is important to analyze how colonial legacies have contributed to the loss of trust in state mechanisms for medical care. As Eugene Richardson writes in his book *Epidemic Illusions*, the hierarchical orders imposed by European colonialism continue to shape populations' dispositions towards medicine and health care. As a result, they should be taken into consideration as a causal factor in how populations respond to antimalarial efforts (Richardson, 2020). Indeed, the practices of colonial medicine that facilitated the expansion of French, English, Belgian, and other European settlements in Western and Central Africa left a notable residue of resentment behind them. They often involved campaigns to prevent the spread of infectious diseases like smallpox, sleeping sickness, and tuberculosis; these campaigns would force vaccinations and invasive treatments upon indigenous populations. As a result, these campaigns can be seen as potent examples of Foucault's theory of *biopower*, where Western imperial powers used colonial medical institutions to establish control over the health and social welfare of colonized populations (Farmer, 2013).

Multiple studies have found that these past medical interventions are significantly correlated with lower levels of trust in medicine from present-day citizens. For instance, campaigns aimed at eradicating human African trypanosomiasis in French Equatorial Africa—which included forced lumbar punctures and treatment with aminophenyl arsonic acid—may serve as strong explicating factors for Congolese citizens refusing to seek medical care or accept vaccines during the Ebola outbreak (Richardson, 2020). Similarly, a study examining French colonial medical campaigns for diseases such as sleeping sickness, leprosy, syphilis, and malaria in central Africa (affected regions displayed in **Figure 2**) concluded that areas with higher exposure to these campaigns had reduced trust in medicine and lower vaccination rates.

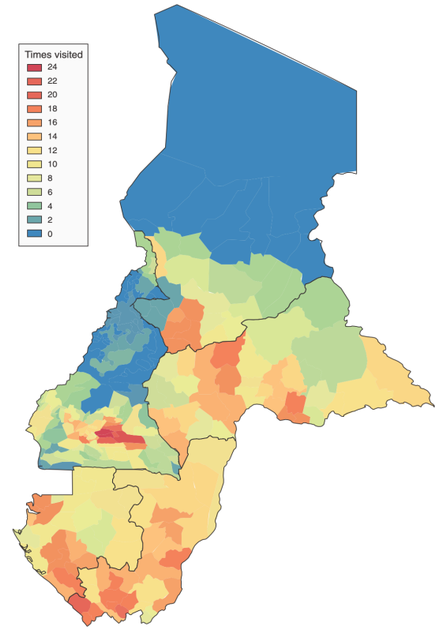

Figure 2. French Colonial Government Medical Campaign Visits in Central Africa Between 1921-1956
Note: A map of Central Africa, in what is present-day Cameroon, Central African Republic, Chad, Republic of Congo, and Gabon. Color-coded by number of visits to indicate regional exposure to French colonial medical campaigns (Lowes & Montero, 2021).

From the years 1921 to 1956, millions of individuals were forced into invasive medical examinations and received injections of medications with severe side effects, including blindness, gangrene, and death. Colonial officers used the drug atoxyl (an arsenic-based drug) that often caused partial or total blindness (in up to 20% of patients). When examining the correlation between medical campaign visits and vaccinations/blood test refusals, there is strong evidence to suggest that the colonial medicine campaigns had negative consequences for vaccinations and consent to a blood test (Lowes & Montero, 2021).

It is important to note that religion too has a deeply controversial history with regards to colonial medicine, with medical missionaries holding up Western civilization and Christianity at large as a solution to illness and a pathway to salvation. Traditional medicine was linked to "heathen" religions, and hospitals became important sites for religious conversion (Farmer et al., 2013). However, in many Central African countries, Christianity has broadly been able to overcome its fraught history of forced religious conversion and invasive medical practices. In fact, many post-colonial scholars and theologians have argued that African theology has been broadly successful in undergoing decolonization and rooting its religious practice in local values (Said, 1976). This idea of community-level integration will be expanded upon later in this paper.

It can be argued that due to the decades of time between the colonial medicine campaigns and the present-day, it is unlikely that it holds such a strong grip in public consciousness. However, the lasting trauma and legacy of colonialism in sub-Sarahan Africa is well-documented. Thus, the context of colonial medicine campaigns with regards to malaria and other infectious diseases may help to explain the rise of church-affiliated institutions within southern sub-Saharan Africa in administering preventative and curative healthcare. It seems logical that religion, which holds weight both in dictating an individual's spiritual beliefs and in forming a strong faith-based community centered around it, would feel more trustworthy in administering medical treatment than the state. This fits into the reality of the present; in Zambia, church-affiliated institutions are responsible for over 50% of the provision of formal health services in rural areas of the country and roughly 30% of the country's overall healthcare (USAID President's Malaria Initiative, 2022).

Background: The Rise of Neoliberalism, Underfunded Medical Systems, & "Cost-Effective" Malaria Control

"Having the idea lying around — and, in this case, endorsed by global health's major donors — keeps the idea alive and adds to its practicability. It becomes 'common sense'"
— Salmaan Keshavjee, *Blind Spot*

Although academics and epidemiologists alike agree that one of the major problems in the global fight against malaria is a critical lack of international funding towards distributing effective solutions, the root of this problem still remains obscure in academic literature. With that being said, the theory of bureaucratic rationalization may have an important relation to decreased international funding for malaria efforts. Bureaucratic rationality refers to the idea of emphasizing efficiency, calculative measures, and teleological ends in bureaucratic institutions, and has resulted in a system where major health organizations such as the WHO are broadly unwilling to expend significant capital on disease treatment for underprivileged people. This can be illustrated in the way that the WHO's cost-effectiveness analysis declared AIDS too expensive to treat amongst poor Americans (Farmer et al., 2013).

Moreover, in the latter half of the 20th century, this idea of affordability and return on investment as king was bolstered by a new framework of neoliberalism ushered in by the Reagan and Thatcher administrations (Farmer et al., 2013). Over time, the acceptance of *neoliberalism* by major institutions has transformed healthcare into a privatized apparatus guided by the priorities of foreign donors. For example, policies like the implementation of revolving drug funds in Malawi (i.e. where drugs provided by donors and international agencies would be sold to communities at marked-up prices to support building healthcare infrastructure) only served to support the overwhelming idea that countries should premise their policy decisions around the *minimization* of public sector health spending (Keshavjee, 2014).

With regards to antimalarial policy in sub-Saharan Africa, bureaucratic rationality and neoliberal ideologies in healthcare have led to two broad outcomes: the first is dwindling amounts of *international* support into anti-malarial efforts. The second is a misallocation of those funds themselves. As mentioned in the introduction, the vast majority of funding for malaria interventions in Africa comes from Western sources, which has created a dangerous system of overreliance (Robertson et al., 2018). A study in Burkina Faso estimated that 2-3 billion USD of external funds would be necessary to properly scale up the response against malaria in sub-Saharan Africa—which would include investments into insecticide-treated nets and artemisinin combination therapy, the current best treatment option for malaria; however,

investments are only present in the range of 100-200 million USD. The same study cited that loans were similarly hard to acquire even from organizations like the Global Fund to Fight AIDS, Tuberculosis, and Malaria (GFATM), the main external funding body for malaria treatment in sub-Saharan Africa—mostly due to those organizations' own financial problems. It is clear that this critical lack of funding stems in part from a reluctance from international donors to expend large amounts of money, an action that falls in line with neoliberal ideals of minimal government spending and interference.

Furthermore, when funding is present, it is rarely used for the most effective policies on the ground, but rather for the most cost-effective policies—that is, policies that will reduce the most amount of malaria burden for one unit of additional investment towards funding. For instance, a study that tracked malaria health disbursements in Zambia found that mass drug administration was largely *cost*-effective (Mtalimanja et al., 2022),even though these kinds of policies rarely allow for drugs to be distributed towards the regions with the highest malaria burden in the country, which tend to be rural border areas. Another example of this is the distribution of insecticide-treated nets. Even though ITNs are associated with a 50% reduction in malaria morbidity and a 20% reduction of all-cause mortality in children, insecticide-treated net coverage in young children still remains unacceptably low in sub-Saharan Africa.

It is important to distinguish that the fault for the ongoing spread and devastation of malaria does not lie solely with international donors. Without the existence of Western aid, there would have been substantially less resources devoted to health infrastructure in sub-Saharan Africa. However, the rise of neoliberal ideals that have directly pushed back against global health aid spending has been greatly detrimental to sustained support in the fight against malaria.

Countering Malaria in the 21st Century

In the past decade, antimalarial drug resistance has proven to be a major problem in central and southern Africa. In the past, resistance to chloroquine and sulfadoxine-pyrimethamine—drugs initially used for the treatment of malaria—received late response, meaning that resistance spread globally before there was an opportunity for containment. Recently, however, resistance to the drug artemisinin in Southeast Asia has emerged, which offers an opportunity to proactively avert spread to malaria-endemic Africa; indeed, the Global Plan for Artemisinin Resistance Containment (GPARC) has been established by the World Health Organization for this very purpose (Takala-Harrison & Laufer, 2015). However, continuous investment is necessary to make this plan a reality if artemisinin resistance is indeed conferred upon citizens who are currently dependent on it as a primary source of treatment. With what is already known about global health, this will require a shift in norms surrounding malaria funding and the allocation of funds towards preventing *future* problems alongside putting out current fires.

Research also suggests that the recent COVID-19 pandemic may have had a significantly detrimental impact on malaria transmission and outbreak. Zimbabwe experienced a significant increase in malaria morbidity and mortality concurrent with detection of the first COVID-19 cases in the country and the resultant efforts to curb the pandemic's spread; the country's total number of malaria cases from January to June of 2020 (221,860) exceeded the expected number by 30,197 cases. Epidemiologists have theorized several reasons for this observation, including disruptions in efforts for malaria prevention and control, limited malaria control equipment, and modification of health-seeking behavior. According to the WHO, less than half of the 22 million insecticide-treated nets that were expected to be distributed globally in 2020 were distributed: less than half of malaria-endemic countries that had planned IRS campaigns in 2020 had completed them. This could also be due to the well-documented economic impact of the pandemic, which would have decreased the amount of health infrastructure spending that was available for antimalarial efforts. With regards to citizen behavior, fear of infection, lockdowns, and other restrictions may have disincentivized access to routine health services (Gavi et al., 2021). Notably, this might also have caused decreased visits to community health facilities or public spaces like schools or churches where malaria education, preventative equipment, and treatment would have been distributed.

Regardless, it is clear that the instability that the pandemic has invited upon countries in sub-Saharan Africa has only exacerbated the problem of malaria, making it even more urgent of an issue to be solved. But with all of the difficulties with instating effective governmental antimalarial policy, the question remains: *who* is best equipped to solve it?

The Anglican Church of the Province of Central Africa: Waging War Against Infectious Disease

"In the first three gospels, the evangelists focus on the bread and wine, inviting us to know that, as we take, bless, break and share bread, we find ourselves in the presence of Jesus. In the fourth and last gospel, the focus shifts to the basin and the towel — and Jesus' challenge to his disciples and us to kneel with him in serving."
— The Right Reverend William Mchombo

The Church of the Province of Central Africa has long made it clear that its mandate aligns with the provision of aid to the most vulnerable. Like most other Anglican Communion-affiliated organizations, it is committed to addressing development issues such as poverty and health alongside practicing the evangelical Christian faith. In recent years, the bishops of the CPCA have pledged particularly to support efforts to halt the spread of infectious diseases (Steffenson et al., 2013). At first, this was centered around the HIV/AIDS crisis, where they provided important resources for prevention and counseling. Now, their focus has been shifted to countering malaria. It, like many other faith-based organizations, sees infectious diseases like malaria as a piece of the early childhood development and aid for the suffering for which they believe they have a requirement to provide holistic care for: this includes other aspects of their mandate including providing nutrition and access to vaccines (Robertson et al., 2018). However, other aspects of the church's religious mandate—in particular, its promotion of proselytization—may raise concerns. Regardless of the practical antimalarial benefits the CPCA provide, it is important not to forget its evangelical nature, which means it may function as a source of religious coercion.

With that being said, the CPCA has been instrumental in distributing education, health knowledge, and insecticide treated mosquito nets to rural communities along border regions in Zambia through the Cross-Border Malaria Initiative. Border regions are often the most difficult places in which to work and can be the last places to receive help fighting disease. By working through the church in remote communities in Zambia bordering Namibia and Angola the Cross-Border Malaria Initiative has distributed education and health knowledge in addition to 100,000 insecticide treated mosquito nets (Christian Aid, 2012). Beyond their own initiatives, the CPCA often receives requests from health centers themselves to work together on medical interventions, with governments acknowledging the church's unique power to mobilize thousands of people into actions like cleaning their surroundings to repel mosquitoes. Health centers in Zambia often reach out to religious leaders that have the greatest amount of control over their communities, tasking them with informing communities on using insecticide-treated mosquito nets and spraying their homes. Churches are also asked to bring in staff and volunteers for the government to train as malaria agents, such that they are able to teach congregants about noticing symptoms of malaria and receiving access to treatment—along with encouraging them to go to health centers to begin with (Robertson et al., 2018). The extensive network, resource mobilization, and strong community trust that religious groups command in addressing public health challenges has been studied and documented in other contexts, such as in Nigeria (Alao et al., 2023).

There are other contributing factors to the CPCA's ability to effectively combat malaria. Churches, who are consistently able to attract a wide diversity of devoted congregants, often have access to a much broader range of translators; this allows them to have a unique leg up on state-run health organizations in overcoming language barriers. This is no small benefit to communication, given that in Zambia alone there are seventy-two unique dialects that are spoken. On the other hand, they are often also able to serve as an accurate source for information collection on behalf of the government: for instance, the Christian Council of Churches of Angola found many communities that the government had said had received mosquito nets but actually had not. Lastly, local religious institutions are not necessarily mutually exclusive with international funding; churches can partner with NGOs and foreign governments such that they implement services with external funding. This allows churches to act as a supplement for the government in two important ways—in understanding the spread of an infection, and in treating the infection itself—all the while circumventing the issues of bureaucracy and mistrust endemic to governments that were mentioned earlier.

Religion as a Local Moral World: Community-Level Malaria Prevention & Treatment

"One, they know that they are faithful; they are going to come, and in big numbers. And they will take the messages seriously."
— Reverend Albert Chama, Archbishop and Primate of the CPCA

While the role of the CPCA with regard to healthcare distribution has been discussed, less analysis has been dedicated to the unique sociocultural context of the church that positions it to improve health outcomes far more than the state. This paper will focus on three aspects of the church in this regard: community-level integration, sensitivity to cultural and religious needs, and power in changing beliefs as a local moral world. Firstly, churches are far better at integrating themselves within local communities, where pastors are able to interact with their congregants regularly and form tight, trusting social relationships. The potential positive effects of social participation and community-level involvement have been studied in other contexts.

For instance, a study in Palencia, a town in Guatemala, notes that it has three different spaces for health participation: the municipal-level health commission (i.e. municipal government, health district), the community-level social development councils, and the community health workers (CHW) program. The people in Palencia benefit greatly from this system, which promotes their engagement in health, from participation in planning to the implementation and evaluation of policies, and thus improves the responsiveness of the health system to their needs (Ruano, 2013). Similarly, in Haiti, trained *accompagnateurs* (community health workers) were the structural backbone of the HIV Equity Initiative, as they were respected in their home communities. It was found that the clinic staff providing psychosocial support and overseeing social projects like housing improvement and potable water projects were effective, both to encourage compliance and to prevent transmission (Behforouz et al., 2004). It should then follow that faith-based organizations would be able to provide better and more holistic care due to being able to directly impact congregants' daily lives by providing sources of food, educational programs, charitable donations, and other integral social services.

Existing research has also supported the idea that healthcare delivery should incorporate local religious practices and beliefs in order to address the cultural contexts of certain areas, which is exactly what the CPCA is able to do. Previous research has emphasized the importance of cultural competence for healthcare providers and systems—i.e. that healthcare professionals work towards understanding and addressing the important cultural, social, and religious needs that patients may have. Without such competence, patients may experience adverse health consequences, receive poor quality care, and be dissatisfied with their treatment (Swihart et al., 2023; Ager & Ager, 2011). For many patients, religion and spirituality are central values to them that cannot be compromised when they are receiving treatment; the CPCA, as a religious organization itself, is thus able to exercise sensitivity with individuals' faith while advocating for effective antimalarial policy.

Lastly, it is important to consider Kleinman's theory of the "local moral world" when considering the role that the CPCA plays in combating malaria. The local moral world can describe a neighborhood, village, hospital, or any other community that has direct influence over someone's moral experience—that is, the shared practices and beliefs over what is at stake in a local setting. In this case, the Church of the Province of Central Africa functions as a local moral world; as a well-respected social institution that commands faith-based credibility, it has the power to change cultural norms around health. This can be immensely impactful, as cultural norms themselves have the capacity to entirely change health outcomes, from garnering support for HIV/AIDS prevention by destigmatizing STDs to increasing the efficacy of treatment programmes for smoking cessation by establishing the harms of smoking (Kleinman, 2010). In this context, the CPCA is able to change cultural norms surrounding malaria prevention and treatment; this means that they are not only able to promote certain practices to repel mosquitoes, but they can in fact encourage congregants to go to health centers by changing norms around *trusting* state medical systems. This is an important step forward, not only in antimalarial efforts, but in reconstructing trust in government-run medicine as part of the decolonization process in postcolonial countries.

However, this does not indicate that there are not potential issues with faith-based care. Although the Anglican church is well known for its work to destigmatize and treat HIV in South Africa and oppose violence against women in the Democratic Republic of Congo, there are also certain groups who have stigmatizing attitudes towards sexuality and HIV and some who do contribute towards violence against women. Some faith activities can also be a cause of concern if they are done as an *alternative* to modern medicine: for instance, healing missions for those with disability and long-term illness. At best, these activities are not at all effective; at worst, they can be actively harmful to the practicing person, as is the case with the ingestion of toxic substances as parts of certain

ceremonies (Tomkins et al., 2015). Although the CPCA, which works closely with major antimalarial organizations and state-run infectious disease departments, can largely be held accountable, it is important to note that this is not the case for many other religious organizations within sub-Saharan Africa.

Conclusion: Working Towards a Malaria-Free Future

"Malaria eradication requires a 100% mind-set of success. There are no 70% or 80% or 90% efforts that pass in malaria control and eradication"

— T.K. Naliaka

Ultimately, malaria continues to be a devastating disease and prescient public health issue in much of southern sub-Saharan Africa. This paper analyzes governmental antimalarial policy with a biosocial model rather than a biomedical model, extending beyond surface-level problems such as drug resistance and insufficient distribution first-line treatment to focus on the role of colonialism, bureaucratic rationality, and neoliberalism in state failures to adequately control and eliminate malaria. Notably, historical colonial medical campaigns have significantly contributed to a loss of trust within the medical system in sub-Saharan African countries. Concurrently, an emphasis on neoliberal and "cost-effective" ideologies in global health have resulted in the crucial lack of international funding behind antimalarial efforts and the insufficient allocation of funding towards distribution of the most effective malaria drugs and preventative equipment.

With that being said, this paper emphasizes the immense importance of religion in combating malaria through the current efforts of the Anglican Church of the Province of Central Africa within Botswana, Malawi, Zimbabwe, and Zambia. This analysis, too, requires a biosocial approach: beyond its ability to strengthen medical interventions by being able to more efficiently distribute equipment like insecticide-treated nets, the CPCA holds a unique role in antimalarial efforts due to its social integration within local communities, allowing it to build trust among community members. This positions it to improve health outcomes far more than the state due to it being able to command faith-based power over cultural and moral norms as a potent social institution as well as go beyond providing medical treatment when providing community-level care. If there is one key point that is reinforced by the CPCA's impressive antimalarial efforts in the past decade, it is that religion does not necessarily have to be a force for evil in post-colonial states, but rather, a potent force for good.

Indeed, religion—and especially, religion that has been reclaimed in local contexts—serves as a primary source of faith and hope for citizens in exploited, poverty-stricken countries where the specter of imperialism still looms large. Harnessing that power to fight against the infectious diseases that continue to exist as shameful marks of inequality in global health should not just be a possibility, but an imperative.

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Refining Sigal et al.'s Model of Cancer Treatments and Heterogeneous Tumor Growth in "Mathematical Modeling of Cancer Stem Cell-Targeted Immunotherapy" to Exhibit Gompertzian Growth

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Tumors are typically heterogeneous, consisting of multiple cell types. A small but aggressive subset are cancer stem cells (CSCs), which are closely related to tumor growth and resistance, making them critical targets for therapeutic intervention. Traditional therapies like chemotherapy often fail to eradicate CSCs, necessitating targeted treatments like immunotherapy. While the interactions of different types of cancer and immune cells, immunotherapy, and chemotherapy have been studied experimentally, mathematical models are needed to better understand why certain treatments fail and how to improve cancer treatment. In this manuscript, I replicate and expand upon a mathematical model developed by Sigal et al. (2019) that employs a system of seven ordinary differential equations (ODEs) to simulate the effects of immunotherapy and chemotherapy on tumors containing both CSCs and non-CSCs (nCSCs). The model incorporates two types of immune cells involved in promising immunotherapies and antitumor response: dendritic cells (DCs) and cytotoxic T-cells (CTCs). I modify the original model by introducing a Gompertzian growth term to better capture long-term tumor growth as the tumor approaches its carrying capacity, addressing the model's current limitations. Future work includes refining parameter values and extending the model to account for tumors composed solely of CSCs or nCSCs. This modified model has the potential to provide valuable insights into the potential efficacy and optimal timing of cancer treatments.

Introduction

Most tumors are heterogeneous, comprising multiple types of tumor cells (Turner & Kohandel, 2012). Cancer stem cells (CSCs) are one such type that are closely related to poor patient outcomes, including tumor growth, resistance, metastasis, and recurrence, making them an important and attractive target of study and treatment.

CSCs are a class of tumor cells able to perpetually self-renew and differentiate into other types of cancer cells. While CSCs typically make up under 10% of cells in a tumor, they exhibit fast and aggressive growth. Moreover, CSCs are resistant to traditional therapies like chemotherapy or radiotherapy. Therefore, immunotherapy, where the patient's own immune system is used to target tumor cells in a highly specific manner, has gained prominence as a treatment. Because of the specificity of this treatment, immunotherapy can even be used to directly target CSCs (Wu et al., 2023).

While the interactions of different types of cancer cells, immunotherapy, and traditional therapeutic methods like chemotherapy have been extensively studied experimentally, mathematical models are needed to better understand why certain treatments fail and how to improve cancer treatment. Previous work modeling the interactions between cancer and immune cells treat tumors as a homogeneous population, overlooking the significant differences between CSCs and non-CSCs (nCSCs, which have less or no capacity for self renewal or differentiation) (Robertson-Tessi et al., 2012; Wilkie & Hahnfeldt, 2013). More recent models distinguish between CSCs and nCSCs (Goldman et al., 2015) and consider their interactions with chemotherapy and the immune system (Mpekris et al., 2017, 2020). However, few address the combined effects of chemotherapy and immunotherapy in a comprehensive framework. Since CSCs play a significant role in driving tumor progression, developing a model that considers the interactions between CSCs, nCSCs, and immune cells under chemotherapy and immunotherapy is crucial for advancing our understanding of cancer treatment.

In a 2019 paper by Sigal et al. entitled "Mathematical Modeling of Cancer Stem Cell-Targeted Immunotherapy," the authors used 7 ordinary differential equations (ODEs) to model the effects of using immunotherapy and chemotherapy alone or in combination on tumors arising from CSCs and nCSCs. Using this model, they tried to seek the best treatment plan.

The authors chose two parts of the immune system involved in promising immunotherapy treatments: dendritic cells (DCs) and cytotoxic T-cells (CTCs) (Sigal et al., 2019). These cells are a critical component of antitumor response, with DCs taking up cancer antigens and presenting them to CTCs so they can recognize and kill tumors (Jhunjhunwala et al., 2021).

In treatments involving these cells, immature DCs are taken from a patient and activated with tumor antigens to make them present antigens specific to CSCs (which express antigens similar to normal stem cells) or nCSCs (Galassi et al., 2021). These mature DCs were then reintroduced into the patient to interact with and activate naive CTCs to target cancer cells that exhibit the same antigen. However, as a consequence, mature DCs presenting antigens can also be targeted and killed by activated CTCs (Ma et al., 2012). While there are mechanisms protecting mature DCs from such attacks, Sigal et al. (2019) make the simplifying assumptions that activated CTCs will only target mature DCs and tumor cells with the same type of antigen, and choose not to account for these protecting mechanisms.

Based on these factors, Sigal et al. (2019) created a system of 7 ODEs (Equation 1) modeling the effects of the above immunotherapy treatments and chemotherapy. The system modeled the populations of CSCs, nCSCs, activated CSC-specific CTCs, activated nCSC-specific CTCs, mature CSC-specific DCs, and mature nCSC-specific DCs. These dependent variables were denoted as S, P, T_S, T_P, D_S, and D_P, respectively. C was used to denote the concentration of chemotherapeutic agents:

$$\frac{dS}{dt} = \alpha_S S + \rho_{PS} P - \rho_{PS} S - \beta_S S T_S - \delta_S S - \Gamma_S S C$$

$$\frac{dP}{dt} = \alpha_P P + \alpha_{SP} S + 2\rho_{SP} S - \rho_{PS} P - \beta_P P T_P - \delta_P P - \Gamma_P P C$$

$$\frac{dT_S}{dt} = K_{T_S} T_S{}^n \frac{D_S}{S_{T_S}+D_S} - \delta_{T_S} T_S$$

$$\frac{dT_P}{dt} = K_{T_P} T_P{}^n \frac{D_P}{S_{T_P}+D_P} - \delta_{T_P} T_P \quad \textbf{(1)}$$

$$\frac{dD_S}{dt} = \gamma_{D_S} D S - \beta_{D_S} D_S T_S - \delta_{D_S} D_S$$

$$\frac{dD_P}{dt} = \gamma_{D_P} D P - \beta_{D_P} D_P T_P - \delta_{D_P} D_P$$

$$\frac{dC}{dt} = -e_C C$$

Descriptions of the parameters and possible values can be found in **Table 1**.

Parameter	Description	Value
Host-specific		
α_S	reproduction rate of CSCs	0.14 - 0.76 day^{-1}
α_{SP}	production of nCSCs through asymmetrical division of CSCs	0.4 - 6 day^{-1}
α_P	reproduction rate of nCSCs	0 - 0.8 day^{-1}
ρ_{PS}	conversion rate of nCSCs to CSCs	fit as needed to keep %CSCs within 1-10%
ρ_{SP}	conversion rate of CSCs to nCSCs	0 - 0.76 day^{-1}
δ_S	natural death rate of CSCs	0 - 0.25 day^{-1}
δ_P	natural death rate of nCSCs	0 - 0.39 day^{-1}
$\delta_{D_S}, \delta_{D_P}$	natural death rate of CSC- or nCSC-specific DCs	0.2 - 0.8 day^{-1}
Non-host-specific		
β_S, β_P	death rate of CSCs or nCSCs due to CTCs	6.2 x 10^{-8} (aCTCs · $day)^{-1}$
$\kappa_{T_S} T_S^n, \kappa_{T_P} T_P^n$	saturated activation rate of CTCs by mDCs multiplied by the population of CSC- or nCSC-specific naïve CTCs	4.5 x 10^4 (aCTCs/μL)/day
s_{T_S}, s_{D_P}	mDC value at which CTC activation rate is half of its maximum possible value	2.5 x 10^4 mDCs/μL
$\delta_{T_S}, \delta_{T_P}$	natural death rate of CSC- or nCSC-specific CTCs	0.02 day^{-1}
$\gamma_{D_S} D, \gamma_{D_P} D$	maturation rate of DCs due to consumption of cancer cells multiplied by the constant D (population of immature DCs)	0.0063 (mDCs/μL) / (day - cancer cell / μL)
β_{D_S}, β_{D_P}	death rate of CSC- or nCSC-specific DCs due to CTCs	6.2 x 10^{-8} (aCTCs/μL · $day)^{-1}$
Drug-specific		
Γ_S	rate of killing of CSCs by the chemotherapeutic agent	7.8 - 14 x 10^{-4} day^{-1} · (μg/mL)$^{-1}$
Γ_P	rate of killing of nCSCs by the chemotherapeutic agent	5.2 - 7.0 x 10^{-3} day^{-1} · (μg/mL)$^{-1}$
e_C	elimination rate of the chemotherapeutic agent	49 - 124 day^{-1}

Table 1. Parameter descriptions and possible values based on Table 2 in Sigal et al. (2019). These biologically relevant ranges for each of parameter were derived using experimental data from other studies (Luo et al., 2014; Ning et al., 2012) and values in other models of cancer and immune cell growth and interactions (Gao et al., 2012; Turner & Kohandel, 2012).

Using their model, the authors reproduced data from several experiments, including from Luo et al. (2014) and Ning et al. (2012), to ensure that the model accurately represented tumor growth for tumors with and without CSCs. They then simulated the efficacy of immunotherapy and chemotherapy when used alone and together.

In this report, I will detail my replications of the model's simulations using Mathematica to confirm that the model is reproducible and accurately portrays CSC and nCSC growth and response to different treatments. I will then explain my modification of the model by introducing a Gompertz term to better reflect the long-term tumor dynamics, where tumor growth slows as it approaches a carrying capacity. Finally, I will explore how the addition of this Gompertz term impacts the model's simulations of immunotherapy's efficacy.

Breakdown of the Model

Tumor Cells

Sigal et al. (2019) mainly focused on graphing the differential equations (DEs) for S and P, which model CSC and nCSC populations, to analyze the tumor size and how it is impacted by treatments. Despite the drastic differences between CSCs and nCSCs, there are mechanisms by which they can differentiate into each other. These are captured by the 2 DEs:

$$\frac{dS}{dt} = \alpha_S S + \rho_{PS} P - \rho_{PS} S - \beta_S S T_S - \delta_S S - \Gamma_S S C$$

$$\frac{dP}{dt} = \alpha_P P + \alpha_{SP} S + 2\rho_{SP} S - \rho_{PS} P - \beta_P P T_P - \delta_P P - \Gamma_P P C \quad \textbf{(1.1)}$$

CSCs can undergo three different types of cell division: symmetric, asymmetric, and interconversion. Symmetric division happens 10-45% of the time. A CSC forms 2 new CSCs at a rate captured by α_S (a daughter cell replaces the parent cell, resulting in the exponential first term of $\frac{dS}{dt}$). Asymmetric division, where 1 CSC and 1 nCSC are formed, occurs 55-80% of the time. In the model, this occurs at a rate of α_{SP} (this only appears in the DE for P as $\alpha_{SP} S$ because the CSC population is conserved). Interconversion, where a CSC forms 2 nCSCs, happens in 0-10% of CSCs at rate ρ_{SP} (corresponding with $-\rho_{SP} S$ and $2\rho_{SP} S$ in the S and P DEs).

Depending on their characteristics, nCSCs can also undergo replication at a rate of α_P (resulting in the exponential $\alpha_P P$ term in $\frac{dP}{dt}$) or dedifferentiate back into a CSC at rate ρ_{PS}. The value of ρ_{PS} is chosen to keep the CSC population within a reasonable range, ensuring CSCs make up under 10% of the total tumor cells. This dedifferentiation process is represented by $\rho_{PS} P$ in $\frac{dS}{dt}$ (the CSCs formed) and $-\rho_{PS} P$ in $\frac{dP}{dt}$ (the nCSCs that differentiated).

The $-\beta_S S T_S$ and $-\beta_P P T_P$ terms model cancer cell death by CTCs, which occurs when CSCs and nCSCs come in contact with an activated CTC that targets them (denoted by T_S and T_P). Meanwhile, the $-\Gamma_S S C$ and $-\Gamma_P P C$ terms model killing by chemotherapy when CSCs and nCSCs encounter chemotherapeutic agents (denoted by C). There are distinct parameters for CSC and nCSC death because CSCs are far more resistant to chemotherapy.

Finally, there is a term modeling natural death for all the tumor cell, dendritic cell, and cytotoxic T-cell ODEs. Each is modeled by an exponential decay term with a δ_I parameter specific to the cell type I. However, because of CSC's ability to proliferate indefinitely, this term can sometimes be removed entirely from the equation for S.

Cytotoxic T-Cells

The DEs for activated CSC-specific and nCSC-specific CTCs (T_S and T_P) have 2 terms:

$$\frac{dT_S}{dt} = K_{T_S} T_S{}^n \frac{D_S}{S_{T_S}+D_S} - \delta_{T_S} T_S$$

$$\frac{dT_P}{dt} = K_{T_P} T_P{}^n \frac{D_P}{S_{T_P}+D_P} - \delta_{T_P} T_P \quad \textbf{(1.2)}$$

The first term represents naive CTC activation by mature DCs. At high concentrations of DCs, this becomes saturated and reaches the value of parameter $K_{T_S} T_S{}^n$ or $K_{T_P} T_P{}^n$ (the saturated activation rate of CTCs multiplied by the population of CSC- or nCSC-specific naive CTCs). The other term represents the natural death rate of activated CTCs.

Dendritic Cells

Meanwhile, the DEs for mature DCs specific to CSCs or nCSCs (D_S and D_P) involve 3 terms and describe how mDCs are produced and the two ways in which they can die:

$$\frac{dD_S}{dt} = \gamma_{D_S} D S - \beta_{D_S} D_S T_S - \delta_{D_S} D_S$$

$$\frac{dD_P}{dt} = \gamma_{D_P} D P - \beta_{D_P} D_P T_P - \delta_{D_P} D_P \quad \textbf{(1.3)}$$

The first term shows how naive DCs (whose population is represented by the constant D) mature when encountering a tumor cell. The second term represents mDC death by activated CTCs because of the antigens that the mDCs are presenting. The last term represents natural death.

Chemotherapeutics

Finally, the DE modeling chemotherapeutic agents (C) has a single term modeling the clearance of these drugs from the body:

$$\frac{dC}{dt} = -e_C C \quad \textbf{(1.4)}$$

Simulations

To validate the model, I began by replicating simulations Sigal et al. performed using data from two experimental studies (Luo et al., 2014; Ning et al., 2012). These studies involved injecting mice with CSCs and nCSCs, as well as giving them immunotherapy targeting either CSCs, nCSCs, both cell types, or providing no treatment.

I first reproduced the authors' simulation of experimental data of CSC and nCSC tumor growth from experimental data in Ning et al. (2012) **(Fig. 1)**. In the experiment, varying amounts of CSCs and nCSCs were isolated and injected into opposite sides of the same immunocompetent mouse. The size of the tumors that resulted (which had both CSCs and nCSCs because of their ability to convert into each other) were monitored over time. Both studies found that the tumors started with CSCs grew much quicker and more aggressively than ones originating from nCSCs, and Sigal et al.'s models were able to capture that.

To replicate the authors' model, I focused on the DEs for S and P. I added S and P together to get the total tumor size in units of mm^3 (assuming there are 10^5 tumor cells per mm^3, as Sigal et al. did). In the graphs, day 0 corresponds with when the tumor cells took root and induced a tumor in the mouse models.

I started by modeling the growth of a tumor without treatment by varying the initial CSC and nCSC populations and setting the population of CTCs, DCs, and chemotherapeutic agents to 0. I first reproduced modeling of tumor size after inoculation with 50,000 CSCs or nCSCs **(Fig. 1a)**. I used host-specific parameters (which depend on the tumor microenvironment and cancer type in each individual host): $\alpha_S = 0.5$, $\delta_S = 0.2$, $\alpha_P = 0.25$, $\delta_P = 0.2$, $\rho_{SP} = 0.15$, $\alpha_{SP} = 1.8$, and $\rho_{PS} = 0.00053$. These values were selected based on the ones used in Fig 2 of Sigal et al. (2019) and the possible ranges for parameters, which can be found, along with their units, in **Table 1**. I also modeled tumor size with initial populations of 5,000, 10,000, and 20,000 CSCs or nCSCs with $\alpha_S = 0.51$, $\delta_S = 0.2$, $\alpha_P = 0.24$, $\delta_P = 0.2$, $\rho_{SP} = 0.15$, $\alpha_{SP} = 1.6$, and $\rho_{PS} = 0.00018$ **(Fig. 1b)**.

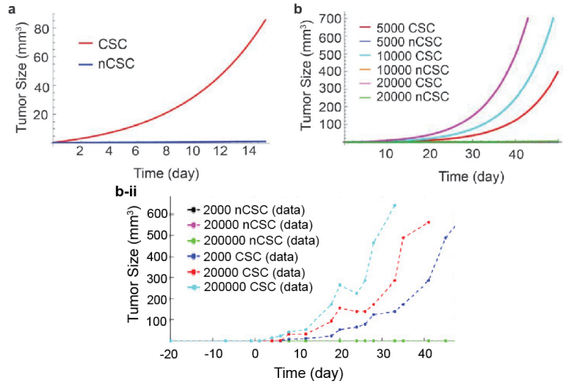

Figure 1. Tumor growth over time with different initial populations of CSCs and nCSCs, with day 0 corresponding to when tumors took root and started growing. **(a)** Initial population of 50,000 CSCs or nCSCs. **(b)** Initial population of 5,000, 10,000, and 20,000 CSCs or nCSCs. Initial populations in this simulation were selected to compare with **(b-ii)** mean tumor size measured experimentally in Figure 2b in Ning et al. (2012) with initial population of 2,000, 20,000, and 200,000 CSCs or nCSCs, plot from Figure 2cii in Sigal et al. (2019).

This simulation successfully captured how CSCs grow far more aggressively than nCSCs, with just 5,000 CSCs being sufficient to form a tumor of substantial size over t = 50 days **(Fig. 1b)**. Furthermore, this simulation showed that varying the size of the initial nCSC population (even with a tenfold increase) doesn't create a significant difference in the size of the resulting tumor **(Fig. 1a, 1b)**. However, while the experimental data in **Figure 1b-ii** shows tumor growth slowing as it is limited by nutrient availability and reaches a carrying capacity, even in tumors starting with far more CSCs, the simulated tumor populations in **Figure 1b** continue to grow exponentially.

Next, I reproduced a model of tumor size after treatments with DCs or CTCs using in vivo experimental data from Figure 4 in Ning et al. (2012) **(Fig. 2)**. Mice were injected with a combination of CSCs and nCSCs and received either immunotherapy targeting CSCs, nCSCs, both cell types (mixed treatment), or no treatment at all. Most of the DCs and CTCs were applied over multiple inoculations before the tumor cells took root in the mice (modeled as day 0), with the treatment schedule shown in **Figure 2a**. I simulated the DC and CTC populations prior to day 0 and used those results as the initial conditions in the system of 7 DEs.

I started by modeling treatment with DCs **(Fig. 2b)**. In the experiment, $3x10^6$ DCs were applied to a mouse over three injections that occurred 22, 15, and 8 days prior to day 0, when a tumor with 10% CSCs and 10^5 total cells took root. For the treatments that targeted CSCs and nCSCs, all of the DCs were either CSC- or nCSC-specific. For the mixed treatment, 10% of the DCs were CSC-specific, and the rest targeted nCSCs, corresponding with the composition of the initial tumor cell population. I used parameters $\alpha_S = 0.7$, $\delta_S = 0.1$, $\alpha_P = 0.2$, $\delta_P = 0.2$, $\rho_{SP} = 0.2$, $\alpha_{SP} = 3.2$, and $\rho_{PS} = 0$ based on the values Sigal et al. (2019) selected.

For CTCs, I modeled how a total of $3x10^6$ CTCs were applied 7 days before, the day of, and 7 days after a tumor with 10^5 cells (5% CSCs) took root **(Fig. 2c)**. Much like with the DCs, treatment consisted of CSC-specific CTCs, nCSC-specific CTCs, or a mix of both (with 5% targeting CSCs). I used parameters $\alpha_S = 0.71$, $\delta_S = 0.2$, $\alpha_P = 0.3$, $\delta_P = 0.2$, $\rho_{SP} = 0.24$, $\alpha_{SP} = 2.8$, and $\rho_{PS} = 0.00013$.

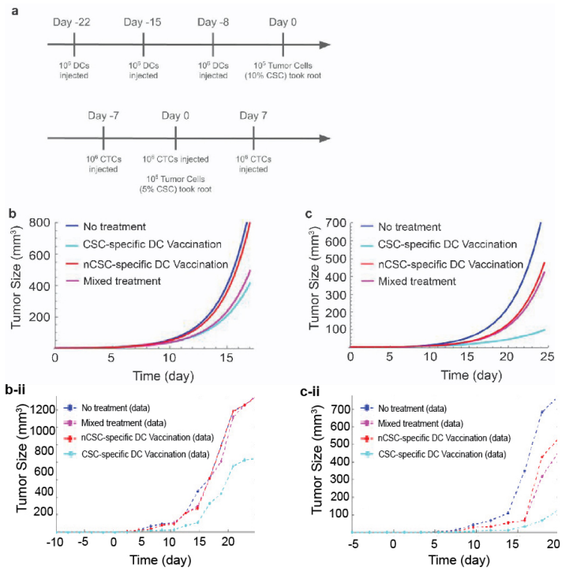

Figure 2. Tumor growth over time after various applications of DCs or CTCs targeting CSCs, nCSCs, both cell types (mixed treatment), or no treatment at all. **(a)** Timeline of treatment with immunotherapy and tumor cells. **(b)** A tumor with 10% CSCs treated with various applications of DCs. Simulation values selected to compare with **(b-ii)** mean tumor size measured experimentally in Figure 4d in Ning et al. (2012), plot from Figure 3b-ii in Sigal et al. (2019). **(c)** A tumor with 5% CSCs treated with various applications of CTCs. Simulation values selected to compare with **(c-ii)** mean tumor size measured experimentally in Figure 4c in Ning et al. (2012), plot from Figure 3c-ii in Sigal et al. (2019).

In **Figure 2**, this model captures the performance of these different treatment regimens relative to each other and to no treatment. The simulation also confirms that immunotherapy targeting CSCs is more effective than treatments targeting nCSCs or both cell types. However, the solution curves modeling tumor growth rates grew far more aggressively than the experimental data, which displays clear sigmoidal growth, with tumor growth slowing and beginning to approach a carrying capacity 20 days after the tumors took root **(Fig. 2b-ii, 2c-ii)**. As a result, Equation 1 failed to model the long-term behavior of the tumors because it assumes tumors obey Malthusian, or simple exponential growth.

Model Modifications

Knowing that tumor growth in vivo is limited by oxygen and nutrient availability, making Malthusian growth unrealistic, I decided to modify the equations for S and P so it can simulate tumors with sigmoidal growth and approaching a carrying capacity. However, the model needed to identify a carrying capacity using the initial tumor population, cell growth rates, and death rates. To account for tumors' slowing growth as they near a carrying capacity, I replaced the exponential Malthusian growth terms with Gompertz terms, which better capture long-term tumor dynamics and allow the sigmoidal growth to approach a carrying capacity without explicitly setting its value. This flexibility is particularly useful for modeling tumor growth.

I selected a Gompertz term to replace the Malthusian terms modeling cell division ($\alpha_S S$ and $\alpha_P P$) and natural death ($-\delta_S S$ and $-\delta_P P$) and got a modified system of the 7 ODEs:

$$\frac{dS}{dt} = \delta_S S \ln\left(\frac{S}{\theta_S}\right) + \rho_{PS} P - \rho_{PS} S - \beta_S S T_S - \delta_S S - \Gamma_S S C$$

$$\frac{dP}{dt} = \delta_P P \ln\left(\frac{P}{\theta_P}\right) + \alpha_{SP} S + 2\rho_{SP} S - \rho_{PS} P - \beta_P P T_P - \delta_P P - \Gamma_P P C$$

$$\frac{dT_S}{dt} = K_{T_S} T_S{}^n \frac{D_S}{S_{T_S}+D_S} - \delta_{T_S} T_S$$

$$\frac{dT_P}{dt} = K_{T_P} T_P{}^n \frac{D_P}{S_{T_P}+D_P} - \delta_{T_P} T_P \qquad \textbf{(2)}$$

$$\frac{dD_S}{dt} = \gamma_{D_S} D S - \beta_{D_S} D_S T_S - \delta_{D_S} D_S$$

$$\frac{dD_P}{dt} = \gamma_{D_P} D P - \beta_{D_P} D_P T_P - \delta_{D_P} D_P$$

$$\frac{dC}{dt} = -e_C C$$

The Gompertz terms for S and P, $\delta_S S \ln\left(\frac{S}{\theta_S}\right)$ and $\delta_P P \ln\left(\frac{P}{\theta_P}\right)$, incorporate tumor cell growth and death rates, as well as the initial population of each type of cancer cell, and are derived from Tier (2003) and (Norton, 1988). The new parameter θ_I is defined as follows for cells of type I (S or P):

$$\theta_I = I_0 e^{\alpha_I/\delta_I} \qquad \textbf{(2.1)}$$

Here, I_0 is a positive, nonzero value that represents the initial population of S or P. The definitions of parameters α_S, α_P, δ_S, and δ_P remain the same.

Since Sigal et al. only provided values for the rate of natural increase, or $\alpha_S - \delta_S$ and $\alpha_P - \delta_P$, I selected a maximum tumor size of 500-700 mm^3 to fit the carrying capacity observed in the empirical data from Ning et al. (2012). This upper limit is based on experimental data and represents an empirical assumption. Keeping all other parameter values constant with the Malthusian version of each simulation, I varied α_S, α_P, δ_S, and δ_P within their biologically relevant ranges to select the optimal values for reproducing experimental data while ensuring the tumor populations approached the observed carrying capacity.

Results

I first used my modified system of ODEs to model tumor growth in the absence of any treatments. I also tried to identify whether the percentage of CSCs making up a tumor creates a significant difference in its growth or whether it can reach the same carrying capacity. Since initial conditions for both CSCs and nCSCs must be greater than 0, I modeled the growth of 1%, 5%, and 10% CSC tumors originating from 50,000 cancer cells over t = 150 days **(Fig. 3)**. I used host-specific parameters $\alpha_S = 0.33$, $\delta_S = 0.075$, $\alpha_P = 0.03$, $\delta_P = 0.025$, $\rho_{SP} = 0.15$, $\alpha_{SP} = 1.8$, and $\rho_{PS} = 0.00053$.

These simulated tumors exhibit Gompertzian growth, starting with exponential growth that then flatten out as they begin to approach an equilibrium of 550 mm^3 at their carrying capacity at around t = 120 days. This matches the trend of the experimental data, especially over longer periods of time. Despite the different percentages of CSCs making up the three tumors, they exhibit similar growth before nearing the carrying capacity at about the same time.

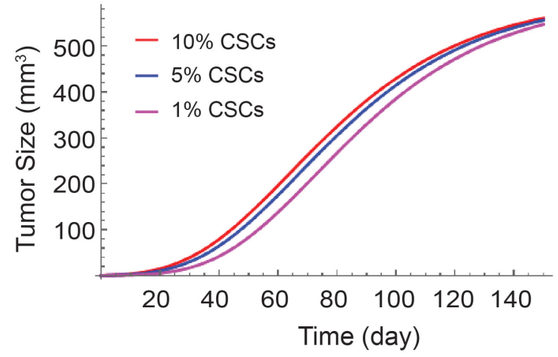

Figure 3. Tumor growth over time using a Gompertz model with an initial population of 50,000 tumor cells, with 1%, 5%, and 10% being CSCs.

I then modeled tumor growth after treatment with DCs and CTCs using this modified system of ODEs to identify whether Gompertzian growth impacts the efficacy of immunotherapy. Parameters ρ_{SP}, α_{SP}, and ρ_{PS}, which model the rate of transitions between CSCs and nCSCs, were kept constant for DCs and CTCs from their counterparts in **Figures 2b** and **2c**. I identified parameters $\alpha_S = 0.675$, $\delta_S = 0.075$, $\alpha_P = 0.04$, and $\delta_P = 0.04$ to model treatment with DCs in **Figure 4a** and $\alpha_S = 0.67$, $\delta_S = 0.06$, $\alpha_P = 0.16$, and $\delta_P = 0.06$ to model the use of CTCs in **Figure 4b**. Using the same dosage and initial conditions as I detailed in **Figure 2a**, I obtained the solution curves shown in **Figure 4**:

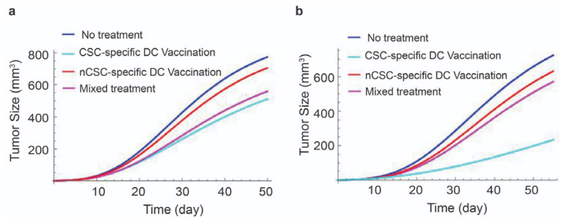

Figure 4. Tumor growth over time after various applications of DCs or CTCs targeting CSCs, nCSCs, both cell types, or no treatment using a Gompertz model. **(a)** A tumor with 10% CSCs treated with various applications of DCs. **(b)** A tumor with 5% CSCs treated with various CTC applications.

Figure 4 shows how the improved system of ODEs qualitatively captures the relative efficacy of the various treatments, comparing each treatment to one another as well as to the no-treatment control. Once again, immunotherapy targeting CSCs performs the best. The curves all show sigmoid Gompertzian growth and flatten out at about t = 50 days at different values, matching with the experimental data **(Fig. 2b-ii, 2c-ii)** and showing more realistic tumor growth than the original model's simulations (Equation 1) **(Fig. 2b, 2c)**. However, if treatment is not continued, the modified model predicts that all of these solution curves will eventually converge at a carrying capacity of about 800 mm^3 (the value the no treatment curves are currently at).

Discussion

In summary, this report presents a modified system of 7 ODEs that models Gompertzian tumor growth and the effects of immunotherapy when used alone and together with chemotherapy, serving as an improvement upon Sigal et al.'s model of exponential tumor growth. This modified model is valuable because it recognizes that there are limits to tumor growth in vivo because of the finite amount of resources available and can simulate sigmoidal growth, with tumor growth slowing as the tumor reaches an equilibrium at its carrying capacity. This allows the solution curves in the model to exhibit much more realistic long-term growth. Moreover, if the growth and natural death rates of CSCs and nCSCs, along with the values of the other host-specific variables governing the conversions between CSCs and nCSCs are known, this model can predict the eventual size of the tumor.

There are a few improvements that could be made to this model. While the model simulated most of the equilibrium values that the tumors in the experimental data approached after receiving immunotherapy or no treatment, there are a few discrepancies. For example, tumor growth in mice receiving CSC-specific immunotherapy was significantly lower in the experimental data than what the model predicts. This can in part be attributed to a difference in the number of tumor cells injected into the mouse and how many actually took root. Of course, tuning specific parameter values, such as β_S, which governs the death rate of CSCs in response to CTCs (and DCs, by extension), could improve the model. This is especially because Sigal et al. set β_S and β_P equal in their model **(Table 1)**, assuming that CTCs are equally effective at killing CSCs and nCSCs, contrary to what the experimental data suggests. Additionally, the model currently makes the simplifying assumptions that activated CTCs will only target mature DCs and tumor cells with the same type of antigen, and chooses not to account for mechanisms protecting mature DCs from such attacks. Modifying the model to consider these could be another point of future studies.

Moreover, it took the modified model two to three times as long to approach equilibrium values: solution curves took about 50 days after tumors took root to do so while the tumors in the experimental data did so in about 15 to 25 days. This difference could be attributed to a need to further tune parameter values and could be a point of future studies.

Currently, this model is unable to simulate initial tumor populations where either CSCs or nCSCs are 0. However, most tumors are heterogeneous and include both CSC and nCSC populations. CSCs typically make up under 10% of a tumor and can differentiate into other cell types, making it unlikely for a tumor to be entirely composed of CSCs. Moreover, tumors composed entirely of nCSCs do not grow aggressively enough to reach a carrying capacity on the relatively short timescale of a few weeks to a few months that I modeled, as seen in **Figure 1**. This makes the case where a tumor is initially entirely CSCs or nCSCs and needs to be simulated using Gompertzian growth unlikely. However, extending the model to simulate this case is another important and interesting point of future study.

This modified model has the potential to provide valuable insights into the potential efficacy of cancer treatments. By incorporating host-specific parameters and tumor composition, it can simulate the long-term behavior of tumors. Additionally, modeling interactions between cancer cells, immunotherapy, and chemotherapeutic agents may provide insights into the optimal timing and efficacy of treatments.

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Peg Solitaire on Caterpillars: Making Them Solvable

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Peg solitaire is a classic game where players aim to remove pegs from a board via a jump move. In the context of graph theory, this game introduces an interesting challenge: for a given graph G, how many edges must be added to make it solvable in peg solitaire? This quantity is known as the minimal solvability number, ms(G). We study the ms(G) specifically for caterpillar graphs, identifying types with known minimal solvability numbers and exploring families, such as caterpillars of length 3, that remain unsolved. For this family, we provide upper bounds on the ms(G) and identify cases where ms(G) = 1.

Overview

This paper is broken down into 5 sections. In section 1, we give an introduction of our topic. This is followed by section 2, which discusses the known minimal solvability number of shorter caterpillars. We discuss their solvability and overall minimal solvability number. We then follow with our main results in section 3, which focuses on giving the upper bounds on the minimal solvability number for caterpillars of length 3. We finally finish our content with section 4, which gives specific examples of caterpillars of length 3 in which we know have minimal solvability number ms(G) = 1. Section 5 discusses possible open questions for further research.

Introduction

Peg solitaire is a traditional one-player game where a player has a board with cells where all but one are filled with pegs. A move in peg solitaire is known as a jump. A jump consists of three adjacent spaces, where the first two vertices have a peg, and the last vertex is a hole. A player would use the first peg to jump over the peg it is adjacent to into the empty hole. A player's objective is, through a series of jumps, to get rid of all the pegs on the board until left with only one. This game is traditionally played on an English cross shaped board (de Wiljes and Kreh, 2022).

Figure 1. A jump move in peg solitaire.

Naturally, one can play on different shaped boards which can be as big or as small as desired. In fact, this combinatorial game can be played on boards that resemble arbitrary connected graphs. In 2011, Beeler and Hoilman (Beeler and Hoilman, 2011) generalized the game of peg solitaire to different graphs. Pegging moves on graphs, focusing on questions like how many pegs must one put on a graph so that, with any given distribution of pegs on any empty vertex v, one can find a sequence of jumps that places a peg at vertex v, were also studied in 2009 and 2006 respectively (Niculescu and Niculescu, 2009; Helleloid et al., 2006). A recent 2022 survey by de Wiljes and Kreh collects many papers in the peg solitaire literature. Similar to the cross board, we can play peg solitaire on a connected graph, $G = (V, E)$, by filling in every vertex but one with a peg. One vertex must be left empty, which we will refer to as the starting hole.

To describe an arbitrary jump, let us say we are given 3 adjacent vertices, a, b, c, where the first two have pegs and the last is a hole. A jump move then uses the peg in a to jump over the second peg in b into the empty hole in c, which we will denote as $a \cdot \overrightarrow{b} \cdot c$. Upon performing this jump, the peg that was jumped over is removed, meaning we are now left with holes in a and b while c now has a peg. With every jump, a player always removes one peg and one peg only. Given a graph with n vertices for $n \in N$, the maximum number of moves a player can perform is $(n - 2)$; by that point, a player would only have one peg remaining, winning the game.

Though the goal of peg solitaire is to remove all but one peg, achieving this isn't possible on every graph due to structural constraints. Some graphs inherently lack a configuration that allows a single remaining peg, making them unsolvable. To systematically study which graphs can be made solvable, we will introduce terminology and concepts developed by de Wiljes and Kreh specifically for peg solitaire on graphs (2022):

When beginning the game, we have a starting state $S \subset V$ of empty vertices, which will always be a single vertex. After playing a game, we end up with a terminal state $T \subset V$ of vertices with pegs and no more jumps are possible. We say T is associated to S if T can be reached from S through a sequence of jumps. With this in mind, we get the following:

Definition 1.1. A graph G is *solvable* if there is some $v \in V$ such that the starting state $S = \{v\}$ has an associated terminal state consisting of a single vertex.

Definition 1.2. A graph G is *k-solvable* if there is some $v \in V$ such that the starting state $S = \{v\}$ has an associated terminal state consisting of k vertices.

Figure 2. Adding an edge to improve solvability.

For the sake of this paper, we will only refer to a graph G as k-solvable if there exists no $j \in N$ where $j <$ k such that the graph is also j-solvable, no matter where the starting hole is assigned.

In other words, the graph cannot be solved with an end state smaller than k. A k-solvable graph will have solitaire number $Ps(G) = k$. Knowing the solvability of graphs is important for determining the following graph invariant. The minimal solvability number $ms(G)$ is the smallest number of edges that have to be added to a graph G in order to make it solvable (de Wiljes and Kreh, 2022) where an 'edge' represents a connection between two pegs.

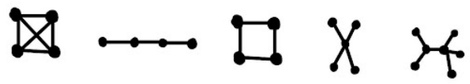

Figure 3. Examples of complete, path, cycle, star, and double star graphs.

The minimal solvability number $ms(G)$ has been found for various graph classes such as complete graphs, path graphs, cycles, stars, and double stars (de Wiljes and Kreh, 2022). De Wiljes and Kreh point out that one "[has] to start with adding edges instead of solving the original graph first," meaning that edges must be appended first before solving the graph (2022). Furthermore, since the complete graph (a graph where all vertices are connected to each other by edges) is solvable and it is possible to add edges to any graph until it becomes complete, we know that the minimal solvability number $ms(G)$ exists for any graph G.

De Wiljes and Kreh pose a natural question about determining the minimal solvability number $ms(G)$ of different classes of graphs. In this paper, we focus on caterpillar graphs, a type of graph consisting of a central 'spine' of vertices with smaller branches, or 'legs,' extending from it. We examine the minimal solvability number $ms(G)$ for various types of caterpillar graphs. Some caterpillars are isomorphic to graphs for which we know the minimal solvability number $ms(G)$; these will be discussed and presented. More importantly, we establish precise upper bounds on the minimal solvability number $ms(G)$ for a previously unknown caterpillar graph – specifically, caterpillars of length 3. These bounds significantly advance our understanding of the minimal solvability number $ms(G)$ for this family. Some caterpillars of length 3 can also easily be solved with only adding one edge, so we also give examples of families of caterpillars of length 3 that have $ms(G) = 1$. The following proposition will be used to help with our results:

Proposition 1.1 [8, Proposition 1.1]. For every connected graph $G = (V, E)$, $ms(G) \leq Ps(G) - 1$.

Known Caterpillar Graphs

A caterpillar graph can be constructed from a path of length n by appending an arbitrary number of pendant vertices to each vertex on the path. The path with pendants attached to each of its vertices will be known as the *spine* of the caterpillar (Beeler et al., 2017). See **Figure 4** below:

Figure 4. A caterpillar of length 3.

We want to categorize different caterpillars and determine their minimal solvability number $ms(G)$. Through the study of the literature surrounding peg solitaire and minimal solvability number $ms(G)$ of graphs, we are able to categorize some caterpillars and assign them a respective minimal solvability number $ms(G)$. One simple category of caterpillars consists of caterpillars of length n with zero pendants attached to every vertex on its spine. Without the caterpillar's pendants, we are only left with the spine of the caterpillar, which by construction is a path of length n. These graphs are isomorphic to the common path graphs, in which we know their minimal solvability number.

Proposition 2.1 [3, Theorem 2.3]. Let P_n be the path on n vertices where $n \in N$. If we have P_{2n} or P_3, then this graph has $ms(G) = 0$. If we have P_{2n+1} for $n > 1$, this graph has $ms(G) = 1$.

This result follows immediately from the fact that paths of even length or length 3 are solvable while paths of odd length for an odd number greater than 3 are 2-solvable (Beeler and Hoilman, 2011). Combined together with Proposition 1.1, we were able to get the given result.

Following this categorization, we now consider caterpillars with pendants attached to its spine since pendants are a notable feature of a caterpillar. For the remainder of this paper, we will be referring to caterpillars of length n. When mentioning said caterpillars, the lengths refer to the length of its spine, not including any extra length that could be considered with any of the caterpillar's pendants.

That being said, let us now consider the smallest length caterpillar: a caterpillar of length one. This caterpillar will have the following notation: $Cat_1(n)$ where n is the number of pendants attached to the single vertex on its spine. These caterpillars are isomorphic to another graph with a known solvability and minimal solvability number, the star graphs. In general, a caterpillar of length 1 with n pendants is at best $(n - 1)$-solvable due to the nature of an empty spine vertex. If the starting hole was in the spine, no moves are possible since we would have no 2 adjacent pegs. If the starting hole was in a pendant, one can at most move from another pendant, over the spine, into the hole. Thus, $(n - 1)$ pegs would still remain, showing it is unsolvable. Because of how unsolvable these caterpillars are, the minimal solvability number $ms(G)$ increases with the number of pendants.

De Wiljes and Kreh determine the minimal solvability number $ms(G)$ of star graphs by appending edges between pendants to form a sort of 'windmill blade.' Due to how they look, we will be referring to edges that connect two pendants as blades edges for the remainder of the paper. De Wiljes and Kreh ultimately prove the following result.

Proposition 2.2 [8, Corollary 2.1]. Let $Cat_1(n)$ be a caterpillar of length 1 with n pendants. If $n \geq 3$, then

$$ms(Cat_1(n)) = \left\lceil \frac{n}{4} \right\rceil.$$

To solve caterpillar graphs of length 1 with additional blade edges, we begin by placing the initial empty hole in a pendant. From here, the solution proceeds in steps:

1. Start by jumping a peg over the central spine into the empty hole.
2. Since there exists blades, we can jump via this blade back into the empty central spine.
3. With another pendant, jump into a pendant that has a blade edge. Depending on the number of pendants in our original caterpillar of length 1, we either have another blade or our graph is solvable.

Through this process, we can achieve solvability by a sequence of systematic jumps. A detailed algorithmic jump sequence is available in (de Wiljes and Kreh, 2022).

Naturally, the next caterpillar we consider is a caterpillar of length 2. This caterpillar will have the following notation: $Cat_2(L, R)$ where L is the number of pendants attached to the left center and R is the number of pendants attached to the right center where $L, R \in N$. This is the final caterpillar in which we know its minimal solvability number since it is also isomorphic to a known graph – a double star graph. The solvability of caterpillars of length 2 is known and proved by the following.

Proposition 2.3 [4, Theorem 3.1]. Let $L \geq R \geq 1$. Then, $Cat_2(L, R)$ is solvable if $L \leq R + 1$.

These caterpillars of length 2 by construction have $ms(G) = 0$, and the following result is known about its general minimal solvability number $ms(G)$.

Proposition 2.4 [8, Proposition 2.5]: Let $L \geq R \geq 1$. Then,

$$ms(Cat_2(L, R)) = \left\lceil \frac{L-R-1}{4} \right\rceil.$$

A full explanation on why this works is outlined by de Wiljes and Kreh (2022). In essence, the authors consider appending edges in between left pendants and perform a specific algorithmic jump set according to whether $L - R \equiv 0, 1, 2,$ or $3 \bmod 4$. So far, we have considered a variety of different caterpillars and have given the minimal solvability number based on its construction. We considered the number of pendants and total length of the caterpillar. Up to now, the given caterpillars were isomorphic to graphs in which we knew their minimal solvability number $ms(G)$. Next, we are going to showcase properties of the minimal solvability number $ms(G)$ of an unknown graph.

A New Caterpillar Graph

After determining the minimal solvability number $ms(G)$ of caterpillars of length 1 and 2, we are now interested in finding the minimal solvability number $ms(G)$ of caterpillars of length 3. Unlike the previous caterpillars we have seen, caterpillars of length 3 are not isomorphic to a graph with a known minimal solvability number $ms(G)$, meaning it cannot simply be presented as we have previously been doing. This raises a natural question: what is the most effective method for appending edges to caterpillars of length 3 in order to be able to solve them? Determining an optimal strategy for their placement becomes essential. To address this, we need to consider both the structure of the caterpillar and the specific arrangement of pendants, as these factors influence which configurations are solvable. We explore approaches for appending edges that minimize the number of edges needed.

In order to distinguish the vertices of a caterpillar of length 3, we are going to assign a specific name to each of the vertices, depending on where they are. Suppose you are given a caterpillar of length 3. This caterpillar will have the notation $Cat_3(L, C, R)$ where L is the number of pendants attached to the leftmost spine vertex, C is the number of pendants attached to the center spine vertex, and R is the number of pendants attached to the rightmost spine vertex. Along with the 3 spine vertices, this caterpillar has a total of $L + R + C + 3$ vertices. For the vertices on the spine, we will refer to the leftmost spine, center spine, and rightmost spine vertices at S_L, S_C, and S_R, respectively. The L pendant vertices will be named $l_1, l_2, ..., l_L$, the R pendant vertices will be named $r_1, r_2, ..., r_R$, and the C pendant vertices will be named $c_1, c_2, ..., c_C$.

With terminology for the vertices of the caterpillar established, we now turn to determining the minimal solvability number $ms(G)$ for caterpillars of length 3. The simplest case occurs when $ms(G) = 0$, indicating that the caterpillar is naturally solvable without adding edges. This leads us to a central question: under what conditions is a caterpillar of length 3 solvable?

While there is no study specifically focused on the solvability of caterpillars of length 3, we can still draw conclusions about their solvability by examining trees with diameter 4. Beeler and Walvroot (2015) demonstrate that any tree of diameter 4 can be constructed by appending pendant vertices to a star graph with n pendants. The structural relationship between caterpillars of length 3 and trees of diameter 4 allows us to leverage known results about these trees to make deductions about caterpillar solvability. In particular, the following proposition specifies the conditions under which a caterpillar of length 3 is solvable. This result was specialized for graphs with diameter at most 4.

Proposition 3.1 [5, Theorem 3.1]: Let $L \geq 2$ and $L \geq R \geq 1$. Then, $Cat_3(L, C, R)$ is solvable if

$$(L + R) - 2 \leq C \leq (L + R) + 1.$$

Furthermore, the graph is k_1–solvable, where $k_1 \leq L + R - C - 1$ if $C \leq (L + R) - 3$. Also, it is k_2–solvable, where $k_2 \leq C - L - R$ if $C \geq (L + R) + 2$.

We then can see that if $(L + R) - 2 \leq C \leq (L + R) + 1$ where $L \geq 2$ and $L \geq R \geq 1$, then the caterpillar of length 3 has $ms(G) = 0$. When approaching a caterpillar of length 3, one should first consider if C is within these bounds in order to see if it is originally solvable. More importantly in regard to the minimal solvability number $ms(G)$, these caterpillars are unsolvable if $C \leq (L + R) - 3$ or if $C \geq (L + R) + 2$.

Before presenting our results, we introduce a key tool for analyzing the solvability of caterpillars of length 3. Defined by Beeler and Walvoort (2015), this tool involves configuring pegs and holes so that a specific sequence of jumps removes certain pegs while others stay constant. This setup, termed a package, is associated with an elimination process called a *purge*, which selectively reduces pegs on the graph. Among the various purges, the *Double Star Purge* is particularly valuable because it enables a systematic reduction of pendants, thereby simplifying the caterpillar's structure.

In the Double Star Purge, which applies to double star graphs (caterpillars of length 2), if the starting hole is positioned in either the left or right center, we can reduce the number of pendants on each side by $k \in N$, provided $k \leq \min(L, R)$. For example, if the hole starts in the right center, we perform the Double Star Purge by first jumping from a left pendant over the left center into the hole in the right center, followed by a jump from a right pendant over the right center into the left center. After these jumps, the number of pendants on each side is reduced by 1, and the hole returns to its original position.

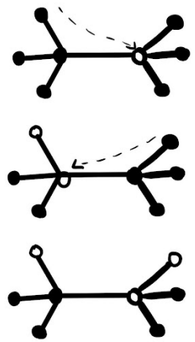

Figure 5. A Double Star Purge.

For caterpillars of length 3, we can leverage the Double Star Purge by focusing on specific sides of the caterpillar. For example, we can consider only the L left and C center pendants along with the center vertices S_L and S_C so that we work with a double star. Then, we can apply the Double Star Purge to systematically simplify this side of the caterpillar. The same principle applies if we consider the R right and C center pendants, as well as S_R and S_C. This technique allows us to progressively reduce the number of pendants and approach solvability.

Also, we will consider appending an edge connecting both S_L and S_R. This edge is one of the most efficient ones we can add since a player is able to reduce the L and R pendants by using this edge to perform Double Star Purges. Because of how it makes graphs more efficiently solvable, we will refer to the edge connecting S_L and S_R as the *Double Star Edge*.

We will also provide an argument as to why blade edges and the double star edge are the most efficient edges to append and are the only edges we consider in this paper. Given an unsolvable caterpillar of length 3 means that $C \leq L + R - 3$. One strategy when appending edges is to append the edges that guarantee reducing the most amount of left and right pendants with the effort of getting C outside of this bound, which would make our graph solvable. Whereas any other edge would reduce the number of pendants by 1, blade edges and the double star edge reduces the number of left and right pendants by more than one. For blades, when used correctly, it reduces the number of left or right pendants by 2 when the player jumps into S_L or S_R. Furthermore, with the double star edge, a player is able to reduce the number of pendants from both the left and right side of the caterpillar by k given that $k < R$. Doing these strategies correctly ensure that our graph becomes more solvable, showing why they may be the best strategy when appending edges and getting an accurate minimal solvability number ms(G) of caterpillars of length 3.

For the most part, caterpillars with a minimal solvability number ms(G) = 0 tend to be balanced, meaning they have a relatively equal number of left and right pendants. This pattern suggests that the closer a caterpillar is to balanced, the lower its minimal solvability number is likely to be. To better understand this relationship, we begin with the caterpillars that are most likely to have the highest minimal solvability number ms(G): those with the most extreme imbalance between left and right pendants. By establishing an upper bound in this highly unbalanced scenario, we can work closer to finding a true equality. Let us consider a caterpillar where $L = n$, $R = 1$, and $C = 0$, which is the most unbalanced that a caterpillar can get.

Theorem 3.1. Let $L \geq R \geq 1$ and $n \in N$. Given $\text{Cat}_3(n, 0, 1)$, it has $\text{ms}(\text{Cat}_3(n, 0, 1)) \leq 1+\lceil \frac{n-3}{4} \rceil$.

Proof. If $n = 1$, we have P_5, which has ms(G) = 1 according to Proposition 2.1. Now, let $n \geq 2$. Put the starting hole in S_C and append the double star edge. Perform the following 2 jumps: $l_n \cdot \vec{S}_L \cdot S_C$ and $S_R \cdot \vec{S}_C \cdot S_L$, where we jump from a left pendant over the left spine into the hole in the center spine and jump from the right spine over the center spine into the left spine. The remaining pendants, peg in S_L, hole in S_R and double star edge create a sub-graph that is a caterpillar of length 2 where $L = n - 1$ and $R = 1$. By Proposition 2.4, this sub-graph has $ms(G) = \lceil \frac{(n-1)-1-1}{4} \rceil = \lceil \frac{n-3}{4} \rceil$. Since we also added the double star edge, the number of edges we added to make the caterpillar solvable was $1 + \lceil \frac{n-3}{4} \rceil$, suggesting that this caterpillar has $ms(G) \leq 1+ \lceil \frac{n-3}{4} \rceil$. ■

We, however, find that this is only an upper bound for the minimal solvability number ms(G) of this unbalanced caterpillar since we can append edges to these caterpillars a different way and get a lower ms(G). Take for instance $\text{Cat}_3(4, 0, 1)$. This caterpillar actually has ms(G) = 1 since we only append a blade edge, and it makes the entire graph solvable. We will see how in the proof below but note that there was no need to add the double star edge. In fact, through extensive case work where we played the game numerous times to the point where the number of left pendants ended up being 12, we find that we can make the upper bound tighter for these unbalanced caterpillars.

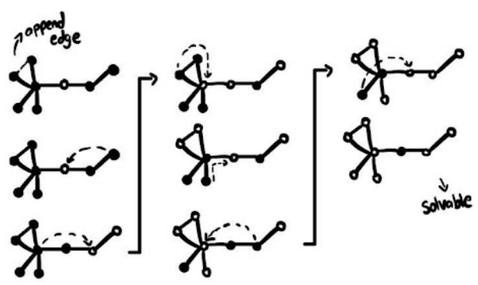

Figure 6. A game solving $\text{Cat}_3(4, 0, 1)$.

Theorem 3.2. Let $L \geq R \geq 1$ and n $\in$ N. Given $\text{Cat}_3(n,0,1)$, it has $ms(\text{Cat}_3(\text{n}, 0, 1)) \leq \lceil \frac{n}{4} \rceil$.

Proof. If $n = 1$, we have P_5, which has $ms(G) = 1$ by Proposition 2.1. If $n = 2$, add an edge connecting l_1 and l_2 and put the starting hole in S_R. Perform the following 2 jumps: $S_L \cdot \vec{S}_C \cdot S_R$ and $r_1 \cdot \vec{S}_R \cdot S_C$. Using the newly appended blade edge, jump $l_1 \cdot \vec{l}_2 \cdot S_L$ and solve the graph with $r_1 \cdot \vec{S}_R \cdot S_C$. Now, let $n \geq 3$. Append $\lceil \frac{n}{4} \rceil$ edges connecting l_1 and l_2, l_3 and l_4, l_5 and l_6... so that we make separate blades. Note that we considered appending $\lceil \frac{n}{4} \rceil$ because this is the number of edges we appended to the caterpillar of length 1. We noticed that an unbalanced caterpillar of length 3 closely resembles a caterpillar of length 1. Put the starting hole in S_C and perform the following 2 jumps: $r_1 \cdot \vec{S}_R \cdot S_C$ and $S_L \cdot \vec{S}_C \cdot S_R$.

Next, using the first appended blade, perform the following jump: $l_1 \cdot \vec{l}_2 \cdot S_L$. The remaining pegs then form a caterpillar of length 2 with $n - 2$ left pendants and one right pendant where we treat S_L and S_C as the respective left and right centers. If $n < 5$, the caterpillar is solvable by Proposition 2.3.

If $n \geq 5$, more work is required. Using a non-blade pendant (like l_n), perform $l_n \cdot \vec{S}_L \cdot l_1$. This jump leaves a hole in S_L, allowing us to use our second blade and jump $l_3 \cdot \vec{l}_4 \cdot S_L$. If $n = 5$ or 6, the remaining pegs leave a solvable caterpillar of length 2, in which we solve our graph accordingly.

If $n \geq 7$, we still have an unsolvable caterpillar of length 2. In this case, however, we can use the first blade to our advantage. Jump from another non-blade pendant (like l_{n-1}) and perform $l_{n-1} \cdot \vec{S}_L \cdot l_2$. We previously added a peg in l_1, meaning out blade where l_1 and l_2 are connected is full again. Using this blade again, jump $l_1 \cdot \vec{l}_2 \cdot S_L$. If $n = 7$ or 8, the remaining pegs leave a solvable double star in which we can solve accordingly. If $n \geq 9$, we iterate the same steps we took above using our additional blades plus jumping into l_1 and l_2 to make more blades and reduce the number of left pendants. The steps we iterate are the following:

1. Using a non-blade pendant, jump into l_1.
2. Use the next blade we have not used yet to jump into S_L.
3. If still solvable, jump from a non-blade pendant over S_L into l_2.
4. Jump $l_1 \cdot \vec{l}_2 \cdot S_L$. If the graph still is not solvable, repeat from step 1. until our graph is reduced to a solvable caterpillar of length 2.

Once we do enough iterations, our graph will reduce to a solvable caterpillar of length 2. After appending $\lceil \frac{n}{4} \rceil$ edges to our caterpillar, we are able to solve our graph, suggesting that $ms(\text{Cat}_3(n, 0, 1)) \leq \lceil \frac{n}{4} \rceil$. ■

Since $\lceil \frac{n}{4} \rceil \leq 1+ \lceil \frac{n-3}{4} \rceil$, we successfully provided a tighter upper bound for this unbalanced caterpillar. Having found a tighter upper bound suggests that there may exist better strategies for adding edges, so the original algorithm provided in Theorem 3.1 is only an upper bound for unbalanced caterpillars of length 3. By this point, we have seen balanced caterpillars and very unbalanced caterpillars and gave bounds on its minimal solvability number $ms(G)$. Of course, there are also caterpillars of length 3 that are neither balanced or very unbalanced, which raises the question of whether one should append only blades or append also the double star edge to these caterpillars. Through more case work, we've decided that if we have $\text{Cat}_3(L, C, R)$ where $R > 1$, then the best method to solving these unbalanced caterpillars is by appending the double star edge and then edges based on the solvability of the caterpillar of length 2 that is sub-graphed.

In summary, the double star edge has helped us create solvable caterpillars, especially by enabling us to transition to sub-caterpillars like $\text{Cat}_2(L - 1, R)$. Then, we can use what we know to find the minimal solvability number $ms(G)$. In cases like $\text{Cat}_3(L, 0, 1)$, the process of quickly eliminating the single right pendant allowed us to simplify the problem to solving the remaining caterpillar of length 2, demonstrating the effectiveness of the blade approach in these highly asymmetric scenarios. However, as we consider caterpillars with $R > 1$, such as $\text{Cat}_3(5, 0, 2)$, our analysis suggests that relying on blades alone becomes less effective. Although we can append only a single blade edge connecting either two left or two right pendants, this configuration often leaves us with isolated pegs on one side, thus failing to solve the caterpillar.

Given these observations, it appears that once $R > 1$, the double star approach is likely more advantageous. This is supported by findings from caterpillars of length 2, where an increase in R correlated with a decrease in the minimal solvability number $ms(G)$. Thus, while further analysis may refine these conclusions (perhaps by exploring alternative edges beyond blades), the current evidence favors adopting the double star method in cases with larger values of R, as it consistently aligns with lower minimal solvability numbers.

Before proving our main result, we need to introduce the concept of hairy complete graphs. A hairy complete graph is denoted as $K_n(a_1, ..., a_n)$ where n is the number of vertices in the complete graph and a_i for $i = 1,...,n$ is the number of pendants attached to the kth vertex of the complete graph, for $k < n$. We will now explore the solvability of hairy complete graphs. Using the notation, we get the following.

Proposition 3.2 [1, Theorem 2.1]. For a hairy complete graph $K_3(x, y, z)$ where $x \geq y \geq z$, this graph is solvable if and only if $x \leq y + z + 2$.

Beeler and Gray provide a complete discussion, but what is most important about this graph is that a caterpillar of length 3 is actually a hairy complete graph with one less edge (2016). In fact, this is why the double star edge was most helpful edge to add since caterpillars of length 3, with the addition of the double star edge, become hairy complete graphs.

We will now give an upper bound on the minimal solvability number of a general caterpillar of length 3 where $C \leq L + R - 3$. The reason we introduce the concept of $L - C - R$ is because we know that a hairy complete graph is solvable if $L \leq C + R + 2$. Thus, if $L > C + R + 2$, then it has a minimal solvability number $ms(G)$ upper bound greater than 1. We then consider if L is one more greater than this value, two more greater, and so on. So, we end up considering $L = C + R + 2 + 1$, $L = C + R + 2+ 2$, ..., $L = C + R + 2 + n$. Moving numbers around, we get the concept of $L - C - R = n$, where n is the given difference. Thus, if $n \geq 3$, then we know that the double star edge is not enough to solve our graph and that we need to append more edges. If n is anything less, we only need to append the double star edge.

Theorem 3.3. Let $L \geq R \geq 1$. Given $\text{Cat}_3(L, C, R)$ where $C \leq L + R - 3$ and $L \geq C$. If $L - C - R = n$ where $n \in N$, then this caterpillar can be reduced to a case where $C = 0$ and $L' - R' = n$. This reduced caterpillar has:

$$ms(\text{Cat}_3(L', R', 0)) \leq 1 + \lceil \tfrac{L'-R'-2}{4} \rceil.$$

Proof. We begin this proof with an argument as why the starting hole in S_C is minimal solvability number of caterpillars of length 2. It is important to distinguish that any caterpillar with center pendants is always reducible to a caterpillar with no center pendants. We achieve this using double star purges and by having the hole in S_C. We want to be able to perform double star purges with both the left and right pendants to be able to reduce our caterpillar so that $C = 0$. Since $C \leq L + R - 3$ and $L \geq C$, we guarantee that we are going to be left with some number of right and left pendants once we reduce the number of center pendants to 0. Along with the double star edge, the remaining pegs form a caterpillar of length 2. Apart from this advantage, we can see that if the starting hole was not in S_C, we never end up with a caterpillar of length 2 with a lower minimal solvability number. Due to the nature of the minimal solvability number of caterpillars of length 2, we

want to reduce the number of left pendants we have while maintaining all of our right pendants. When the hole is in S_C, we can jump $l_1 \cdot \vec{S}_L \cdot S_C$ and $S_R \cdot \vec{S}_C \cdot S_L$ to end up with $\text{Cat}_2(L-1, R)$. If the starting hole is in S_R, we at best get the same result, $\text{Cat}_2(L-1, R)$. If the starting hole is in S_L, we get a worse caterpillar in $\text{Cat}_2(L-1, R-1)$. If the hole is in one of the left or right pendants, we again only end up with at best $\text{Cat}_2(L-1, R)$ where some terminal states do not end up being double stars, which are useless in our algorithm. Therefore, you cannot get a better terminal state with a hole elsewhere and the hole in S_C allows for the initial double star purges to be done to both the center, left, and right pendants. It is thus the most efficient starting hole.

Now, suppose you have $\text{Cat}_3(L, R, C)$. Append the double star edge and have the starting hole in S_C. We know that this caterpillar is unsolvable by construction, so it has $ms(G) > 1$. If $C > 0$, we first want to reduce the caterpillar so that we end up with $C = 0$. Take the difference $L - C - R$ and call that number n. The number n lets us reduce our original caterpillar to a case where $C = 0$ and $L' - R' = n$, leaving us with $ms(\text{Cat}_3(L', R', 0))$. We can further reduce using the optimal hole in S_C and 2 jumps previously discussed to reduce our caterpillar to look like $\text{Cat}_2(L'-1, R')$. By Proposition 2.4, this caterpillar of length 2 has $ms(\text{Cat}_2(L'-1, R')) = \left\lceil \frac{L'-1-R'-1}{4} \right\rceil = \left\lceil \frac{L'-R'-2}{4} \right\rceil$. Therefore, with the addition of $1 + \left\lceil \frac{L'-R'-2}{4} \right\rceil$ edges, our graph becomes solvable, suggesting that $ms(\text{Cat}_3(L', R', 0)) \leq 1 + \left\lceil \frac{L'-R'-2}{4} \right\rceil$, in which $\text{Cat}_3(L', R', 0)$ was reduced from $\text{Cat}_3(L, R, C)$. ■

We will end our discussion of the minimal solvability number of general caterpillars of length 3 by providing an upper bound for the second unsolvable case. Recall from Proposition 3.1 that a caterpillar is also unsolvable if $C \geq L + R + 2$. If this is the case, the number of center pendants is always greater than the sum of the left and right pendants, meaning that double star purges would effectively rid of all the left and right pendants, leaving only center pendants. With all these double star purges, the remaining graph becomes a caterpillar of length 1, so we get the following.

Theorem 3.4. Let $L \geq R \geq 1$. Given $\text{Cat}_3(L, R, C)$, where $C \geq L + R + 2$
$ms(\text{Cat}_3(L, R, C)) \leq \left\lceil \frac{C-L-R+2}{4} \right\rceil$.

Proof. Start with the hole in S_L. Jump $c_1 \cdot \vec{S}_C \cdot S_L$. We are then left with $\text{Cat}_3(L, R, C-1)$ with a hole in S_C. From here, perform L left purges, getting rid of all the left pendants. Then, perform R right purges, also getting rid of all the right pendants. In this case, however, do not make the final jump from a center pendant over S_C as we want the hole to remain in S_R. Performing all those purges leaves us with a caterpillar of length 1 with $C - L - R + 2$ pendants. According to Proposition 2.2, this caterpillar has $ms(G) = \left\lceil \frac{C-L-R+2}{4} \right\rceil$. Thus, our graph becomes solvable after adding $\left\lceil \frac{C-L-R+2}{4} \right\rceil$ edges, suggesting that $ms(\text{Cat}_3(L, R, C)) \leq \left\lceil \frac{C-L-R+2}{4} \right\rceil$. ■

We have now provided a number of upper bounds for the minimal solvability number of caterpillars of length 3. However, through some case work of some unsolvable caterpillars of length 3, we find that a number of these caterpillars only need an addition of one edge to make the graph solvable. In this next section, we will give necessary parameters needed to determine if a caterpillar of length 3 has $ms(G) = 1$.

Specific Caterpillars of Length 3

We will now consider an infinite number of caterpillars that have $ms(G) = 1$, where the additional edge that was added is a blade edge or the double star edge. This will show that many unsolvable graphs become solvable with the addition of just one edge. Our main focus will be the first unsolvable case where $C \leq (L + R) - 3$. We will first consider blade edges, which was the approach we discussed for appending edges to caterpillars of length 1 and 2. Through extensive case work and referencing the k-solvability of these caterpillars, we are able to show the following:

Proposition 4.1. Let $L \geq 2$ and $L \geq R \geq 1$. A caterpillar G of length 3 such that $L + R - C = 3$ is unsolvable and has $ms(G) = 1$. Furthermore, we will show that adding an edge that connects two left pendants makes our caterpillar solvable.

Proof. Recall from Proposition 3.1 that this caterpillar is m–solvable where $m \leq (L + R - C - 1)$ since we have that $C \leq L + R - 3$, meaning it is at best 2-solvable. Thus, they have $Ps(G) = 2$, and according to Proposition 1.1, we know that this graph has $ms(G) \leq 1$. Since these graphs are unsolvable, we have that $0 < ms(G) \leq 1$, meaning it has $ms(G) = 1$. We will now work out the first 5 cases; in each of the cases, add an edge between l_1 and l_2. Later, we will show that an arbitrary caterpillar satisfying the proposition can be reduced to one of these 5 cases:

1. $L = 2, R = 1, C = 0$
   Start with a hole in S_R and complete the following jumps: $S_L \cdot \vec{S}_C \cdot S_R$, $r_1 \cdot \vec{S}_R \cdot S_C$, $l_1 \cdot \vec{l}_2 \cdot S_L$, $S_L \cdot \vec{S}_C \cdot S_R$. One peg remains, so our graph is solvable.
2. $L = 2, R = 2, C = 1$
   Start with a hole in S_R and complete the following jumps: $c_1 \cdot \vec{S}_C \cdot S_R$, $r_2 \cdot \vec{S}_R \cdot S_C$, $S_L \cdot \vec{S}_C \cdot S_R$, $l_1 \cdot \vec{l}_2 \cdot S_L$, $r_1 \cdot \vec{S}_R \cdot S_C$, $S_L \cdot \vec{S}_C \cdot S_R$. One peg remains, so our graph is solvable.
3. $L = 3, R = 1, C = 1$
   Start with a hole in S_C and complete the following jumps: $l_3 \cdot \vec{S}_L \cdot S_C$, $c_1 \cdot \vec{S}_C \cdot S_L$, $r_1 \cdot \vec{S}_R \cdot S_C$, $S_C \cdot \vec{S}_L \cdot l_3$, $l_1 \cdot \vec{l}_2 \cdot S_L$, $l_3 \cdot \vec{S}_L \cdot S_C$. One peg remains, so our graph is solvable.
4. $L = 3, R = 2, C = 2$
   Start with a hole in S_C and complete the following jumps: $r_1 \cdot \vec{S}_R \cdot S_C$, $c_1 \cdot \vec{S}_C \cdot S_R$, $r_2 \cdot \vec{S}_R \cdot S_C$, $c_2 \cdot \vec{S}_C \cdot S_R$, $l_2 \cdot \vec{S}_L \cdot S_C$, $S_R \cdot \vec{S}_C \cdot S_L$, $l_3 \cdot \vec{S}_L \cdot l_2$, $l_1 \cdot \vec{l}_2 \cdot S_L$. One peg remains, so our graph is solvable.
5. $L = 3, R = 3, C = 3$
   Start with a hole in S_C and complete the following jumps: $r_1 \cdot \vec{S}_R \cdot S_C$, $c_1 \cdot \vec{S}_C \cdot S_R$, $r_2 \cdot \vec{S}_R \cdot S_C$, $c_2 \cdot \vec{S}_C \cdot S_R$, $r_3 \cdot \vec{S}_R \cdot S_C$, $c_3 \cdot \vec{S}_C \cdot S_R$, $l_2 \cdot \vec{S}_L \cdot S_C$, $S_R \cdot \vec{S}_C \cdot S_L$, $l_3 \cdot \vec{S}_L \cdot l_2$, $l_1 \cdot \vec{l}_2 \cdot S_L$. One peg remains, so our graph is solvable.

Now, let $n, m \in N$ such that $n \geq 3$ and $m \geq 1$. Consider an arbitrary caterpillar of length 3 such that $L = n$, $R = m$, and $C = n + m - 3$. Append an edge connected l_1 and l_2 and put the starting hole in S_C. Then, do double star purges on the left and center pendants until $(n-3)$ purges are done. This eliminates $(n-3)$ pendants adjacent to S_L and $(n-3)$ pendants adjacent to S_C. Next, if $m \leq 3$, do no double star purges. If $m > 3$, do double star purges on the right and center pendants until $(m-3)$ purges are done, effectively ridding of $(m-3)$ pendants adjacent to S_R and S_C. In either case, we reduce to one of the base cases we showed above, meaning it is still solvable. Thus, any case where we have a caterpillar of length 3 where $(L + R) - C = 3$ is unsolvable with $ms(G) = 1$. ■

We follow a similar idea for the following proposition. We group them together since they both have one extra step at the end when reducing to smaller cases.

Proposition 4.2. Let $L \geq 2$ and $L \geq R \geq 1$. A caterpillar of length 3 such that $L + R - C = 4$ or 5 is unsolvable and has $ms(G) = 1$. Adding an edge that connects two left pendants makes our caterpillar solvable unless $L + R - C = 5$ and $L = 3$; this latter case requires us to add an edge connecting two right pendants in order to make the graph solvable.

Proof. $L + R - C = 4$:

Similarly to Proposition 4.1, let us work out the first 4 cases and then show that an arbitrary caterpillar reduces to one of these 4 cases; in each of the cases, add an edge between l_1 and l_2:

1. $L = 2, R = 2, C = 0$
   Start with a hole in S_C and complete the following jumps: $r_1 \cdot \vec{S}_R \cdot S_C$, $S_L \cdot \vec{S}_C \cdot S_R$, $l_1 \cdot \vec{l}_2 \cdot S_L$, $r_2 \cdot \vec{S}_R \cdot S_C$, $S_L \cdot \vec{S}_C \cdot S_R$. One peg remains, so our graph is solvable.
2. $L = 3, R = 1, C = 0$
   Start with a hole in S_C and complete the following jumps: $r_1 \cdot \vec{S}_R \cdot S_C$, $S_L \cdot \vec{S}_C \cdot S_R$, $l_1 \cdot \vec{l}_2 \cdot S_L$, $l_3 \cdot \vec{S}_L \cdot S_C$, $S_R \cdot \vec{S}_C \cdot S_L$. One peg remains, so our graph is solvable.
3. $L = 3, R = 2, C = 1$
   Start with a hole in S_C and complete the following jumps: $r_1 \cdot \vec{S}_R \cdot S_C$, $S_L \cdot \vec{S}_C \cdot S_R$, $r_2 \cdot \vec{S}_R \cdot S_C$, $c_1 \cdot \vec{S}_C \cdot S_L$, $l_3 \cdot \vec{S}_L \cdot S_C$, $l_1 \cdot \vec{l}_2 \cdot S_L$, $S_L \cdot \vec{S}_C \cdot S_R$. One peg remains, so our graph is solvable.
4. $L = 3, R = 3, C = 2$
   Start with a hole in S_C and complete the following jumps: $r_1 \cdot \vec{S}_R \cdot S_C$, $c_1 \cdot \vec{S}_C \cdot S_R$, $r_2 \cdot \vec{S}_R \cdot S_C$, $S_L \cdot \vec{S}_C \cdot S_R$, $r_3 \cdot \vec{S}_R \cdot S_C$, $c_2 \cdot \vec{S}_C \cdot S_L$, $l_3 \cdot \vec{S}_L \cdot S_C$, $l_1 \cdot \vec{l}_2 \cdot S_L$, $S_L \cdot \vec{S}_C \cdot S_R$, $r_2 \cdot \vec{S}_R \cdot S_C$. One peg remains, so our graph is solvable.

Now, let $n, m \in N$ such that $n \geq 4$ and $m \geq 1$. Consider a caterpillar of length 3 such that $L = n$, $R = m$, and $C = n + m - 4$. Append an edge connected l_1 and l_2 and put the starting hole in S_C. Then, do double star purges on the left and center pendants until $(n-4)$ purges are done. This eliminates $(n-4)$ pendants adjacent to S_L and $(n-4)$ pendants adjacent to S_C. Next, if $m \leq 4$, do no double star purges. If $m > 4$, do double star purges on the right and center pendants until $(m-4)$ purges are done, ridding $(m-4)$ pendants adjacent to S_R and S_C. In either case, we reduce to one of $L = 4, R = k$, and $C = k$ for $k = 1,2,3,4$. If $k = 1$ do one more purge on the left, and if $k \neq 1$, do 2 more purges; one on the left and one on the right. We will always reduce to one of the base cases provided, meaning our graph is solvable. Thus, any case where we have a caterpillar of length 3 where $L + R - C = 4$ has $ms(G) = 1$.

$L + R - C = 5$:

Again, we will work out the first 6 cases and show that an arbitrary caterpillar will reduce to one of these 6 cases. For the first two cases when $L = 3$, add an edge between r_1 and r_2:

1. $L = 3, R = 2, C = 0$
   Start with a hole in S_C and complete the following jumps: $l_1 \cdot \vec{S}_L \cdot S_C$, $S_R \cdot \vec{S}_C \cdot S_L$, $l_2 \cdot \vec{S}_L \cdot S_C$, $r_1 \cdot \vec{r}_2 \cdot S_R$, $S_R \cdot \vec{S}_C \cdot S_L$, $l_3 \cdot \vec{S}_L \cdot S_C$. One peg remains, so our graph is solvable.
2. $L = 3, R = 3, C = 1$
   Start with a hole in S_C and complete the following jumps: $r_3 \cdot \vec{S}_R \cdot S_C$, $c_1 \cdot \vec{S}_C \cdot S_R$, $l_1 \cdot \vec{S}_L \cdot S_C$, $S_R \cdot \vec{S}_C \cdot S_L$, $l_2 \cdot \vec{S}_L \cdot S_C$, $r_1 \cdot \vec{r}_2 \cdot S_R$, $S_R \cdot \vec{S}_C \cdot S_L$, $l_3 \cdot \vec{S}_L \cdot S_C$. One peg remains, so our graph is solvable.

Now, we will consider the next 4 cases. In these, add an edge between l_1 and l_2:

1. $L = 4, R = 1, C = 0$
   Start with a hole in S_C and complete the following jumps: $l_3 \cdot \vec{S}_L \cdot S_C$, $S_R \cdot \vec{S}_C \cdot S_L$, $l_4 \cdot \vec{S}_L \cdot S_C$, $l_1 \cdot \vec{l}_2 \cdot S_L$, $S_L \cdot \vec{S}_C \cdot S_R$, $r_1 \cdot \vec{S}_R \cdot S_C$. One peg remains, so our graph is solvable.
2. $L = 4, R = 2, C = 1$
   Start with a hole in S_C and complete the following jumps: $r_1 \cdot \vec{S}_R \cdot S_C$, $c_1 \cdot \vec{S}_C \cdot S_R$, $l_4 \cdot \vec{S}_L \cdot S_C$, $S_L \cdot \vec{S}_C \cdot S_R$, $r_2 \cdot \vec{S}_R \cdot S_C$, $S_R \cdot \vec{S}_C \cdot S_L$, $l_1 \cdot \vec{l}_2 \cdot S_L$, $S_L \cdot \vec{S}_C \cdot S_R$. One peg remains, so our graph is solvable.
3. $L = 4, R = 3, C = 2$
   Start with a hole in S_C and complete the following jumps: $l_3 \cdot \vec{S}_L \cdot S_C$, $c_1 \cdot \vec{S}_C \cdot S_L$, $l_4 \cdot \vec{S}_L \cdot S_C$, $c_2 \cdot \vec{S}_C \cdot S_L$, $r_1 \cdot \vec{S}_R \cdot S_C$, $S_L \cdot \vec{S}_C \cdot S_R$, $r_2 \cdot \vec{S}_R \cdot S_C$, $l_1 \cdot \vec{l}_2 \cdot S_L$, $S_L \cdot \vec{S}_C \cdot S_R$, $r_3 \cdot \vec{S}_R \cdot S_C$. One peg remains, so our graph is solvable.
4. $L = 4, R = 4, C = 3$
   Start with a hole in S_C and complete the following jumps: $l_3 \cdot \vec{S}_L \cdot S_C$, $c_1 \cdot \vec{S}_C \cdot S_L$, $r_1 \cdot \vec{S}_R \cdot S_C$, $c_2 \cdot \vec{S}_C \cdot S_R$, $l_4 \cdot \vec{S}_L \cdot S_C$, $c_3 \cdot \vec{S}_C \cdot S_L$, $r_2 \cdot \vec{S}_R \cdot S_C$, $S_L \cdot \vec{S}_C \cdot S_R$, $r_3 \cdot \vec{S}_R \cdot S_C$, $l_1 \cdot \vec{l}_2 \cdot S_L$, $S_L \cdot \vec{S}_C \cdot S_R$, $r_4 \cdot \vec{S}_R \cdot S_C$. One peg remains, so our graph is solvable.

Let $n, m \in N$ such that $n \geq 5$ and $m \geq 1$. Consider a caterpillar of length 3 such that $L = n$, $R = m$, and $C = n + m - 5$. Append an edge connected l_1 and l_2 and put the starting hole in S_C. Then, do double star purges on the left and center pendants until $(n-5)$ purges are done. This eliminates $(n-5)$ pendants adjacent to S_L and S_C. Next, if $m \leq 5$, do no double star purges. If $m > 5$, do double star purges on the right and center pendants until $(m-5)$ purges are done, ridding $(m-5)$ pendants adjacent to S_R and S_C. In either case, we reduce to one of $L = 5, R = k$, and $C = k$ for $k = 1,2,3,4,5$. If $k = 1$ do one more purge on the left, and if $k \neq 1$, do 2 more purges; one on both the left and right. We will always reduce to one of the base cases provided, meaning our graph is solvable. Thus, any case where we have a caterpillar of length 3 where $L + R - C = 5$ has $ms(G) = 1$. ■

This fact is useful to know since we are able to reduce down caterpillars that are massive and still only append one edge in order to make it solvable. This pattern unfortunately stops working when $L + R - C = 6$. Through an analysis of appending blade edges to a caterpillar where $L = 3$, $R = 3$, and $C = 0$, we see that appending one blade is not enough, no matter where the starting hole is. Checking adding a blade on either the left or right side was simple due to the symmetry of the graph. Of course, blades are not the only edges that one can add to a caterpillar of length 3, so we must consider appending edges elsewhere before concluding it has $ms(G) > 1$. Testing out edges like an edge connecting l_L and S_C, l_L and r_R, or r_R and S_C showed little improvement, and it still gave the graph appear to have $ms(G) > 1$. However, adding the double star edge helped us out tremendously. If this edge is added, then a caterpillar of length 3 where $L = 3$, $R = 3$, and $C = 0$ has $ms(G) = 1$. The following steps show the sequence of moves needed to solve the caterpillar with the addition of only that edge connecting S_L and S_R: Start with a hole in S_C and complete the following jumps: $l_3 \cdot \vec{S}_L \cdot S_C$, $r_3 \cdot \vec{S}_R \cdot S_L$, $l_2 \cdot \vec{S}_L \cdot S_R$, $r_2 \cdot \vec{S}_R \cdot S_L$, $l_1 \cdot \vec{S}_L \cdot S_R$, $r_1 \cdot \vec{S}_R \cdot S_L$, $S_L \cdot \vec{S}_C \cdot S_R$.This sequence of jumps effectively rids of all the pegs, but one, showing the solvability of the graph. For most of the jumps, the player ended up using double star purges between the left and right pendants since the edge connecting S_L and S_R ended up making a double star (or caterpillar of length 2) sub-graph within this caterpillar of length 3.

In fact, we can generalize the number of pendants that are on the left and right side as long as we keep $C = 0$ for now. Thus, we can get the following.

Proposition 4.3. Let $L \geq R \geq 1$. A caterpillar of length 3 such that $L = n$, $R = n$, and $C = 0$ for $n \geq 2$ has $ms(G) = 1$, where the additional edge added is the double star edge.

Proof. Start hole at S_C and add the double star edge. Jump $l_n \cdot \vec{S}_L \cdot S_C$. Using the double star edge, the pendants on the left and right produce a sub-graph $\text{Cat}_2(n-1, n)$, which is solvable by

Proposition 2.3. Solving the sub-graph using double star purges leaves a peg in S_L. Finally, jump $S_L \cdot \vec{S}_C \cdot S_R$. ■

In fact, due to Proposition 2.3 and following a similar algorithm to the previous proposition:

Corollary 4.1. Let $L \geq R \geq 1$. Consider the following caterpillars of length 3:
1. $L = n$, $R = n - 1$, $C = 0$ for $n \geq 2$
2. $L = n$, $R = n - 2$, $C = 0$ for $n \geq 3$

Both of these caterpillars have $\text{ms}(G) = 1$ where we only append the double star edge.

Proof. $L = n$, $R = n - 1$, $C = 0$:

Start hole at S_C and add the double star edge. Jump $l_n \cdot \vec{S}_L \cdot S_C$. Using the double star edge, the pendants on the left and right produce a sub-graph $\text{Cat}_2(n - 1, n - 1)$, which is solvable by Proposition 2.3. Solving the sub-graph using double star purges leaves a peg in S_R. Finally, jump $S_R \cdot \vec{S}_C \cdot S_L$ to solve the graph.

$L = n$, $R = n - 2$, $C = 0$:

Start hole at S_C and add the double star edge. Jump $l_n \cdot \vec{S}_L \cdot S_C$ and $S_R \cdot \vec{S}_C \cdot S_L$. Using the double star edge, the remaining pendants leave a sub-graph $\text{Cat}_2(n - 1, n - 2)$ which is solvable by Proposition 2.3. Solving the sub-graph using double star purges leaves a single peg in S_R. ■

Furthermore, using our previous discussion of the solvability of hairy complete graphs, we are able to deduce the following.

Proposition 4.4. Let $L \geq 2$ and $L \geq R \geq 1$. A caterpillar of length 3 with L, R, and C left, right, and center pendants, respectively, where $C \leq (L + R) - 3$ has $\text{ms}(G) = 1$ if
1. $L \geq C$ and $L \leq C + R + 2$
2. $L < C$ and $C \leq L + R + 2$

In both cases, we only append the double star edge.

Proof. Appending the double star edge to this caterpillar gives us a hairy complete graph. We thus consider 2 cases. If $L \geq C$, then $L \leq C + R + 2$ for our graph to be solvable. If $L < C$, then $C \leq L + R + 2$ for our graph to be solvable. Both follow immediately from Proposition 3.2. In either case, we start the hole in S_C and reduce to a case where $L = n$, $C = 0$, and $R = n$, $n - 1$, or $n - 2$. This is possible since in either case, $C \leq (L + R) - 3$, $L \geq 2$ and $R \geq 1$. Since we only added one edge to solve the graph, this caterpillar has $\text{ms}(G) = 1$. ■

In fact, we can extend the hairy complete graph argument to the second unsolvable case of caterpillars of length 3.

Proposition 4.5. Let $L \geq R \geq 1$. A caterpillar of length 3 with L, R, and C left, right, and center pendants, respectively, where $C = L + R + 2$ has $\text{ms}(G) = 1$. We only append the double star edge.

Proof. Since for the second unsolvable case $C \geq L + R + 2$, we only consider the case where $C \geq L$. According to Proposition 3.2, by appending the double star edge, we get a hairy complete graph that is solvable if $C \leq L + R + 2$. Thus, we have that $L + R + 2 \leq C \leq L + R + 2$, meaning it is only solvable if $C = L + R + 2$. The way we solve this is by putting the starting hole in S_L and jumping $c_1 \cdot \vec{S}_C \cdot S_L$. The resulting caterpillar is $\text{Cat}_3(L, R, L + R + 1)$. By performing double star purges getting rid of the L left pendants and R right pendants, leaves us with a sub-graph with pegs in S_L, S_R, and c_{L+R+2}. Because we have the double star edge, we can perform the following jumps to solve the graph: $S_L \cdot \vec{S}_R \cdot S_C$ and $c_{L+R+2} \cdot \vec{S}_C \cdot S_R$. ■

These results show the utility of the double star edge in graph solvability and offers new pathways for investigating solvable structures in similar graph classes. By extending the framework to encompass additional pendant arrangements, this work lays the groundwork for further exploration into caterpillars of varying lengths. Moving forward, these insights could be particularly valuable when finding the minimal solvability number of other graphs that have caterpillars of length 3 as subsets.

Possible Future Work and Broader Implications

We end with some possible open questions for further research. Though we gave many upper bounds, we are interested in knowing the actual minimal solvability number $ms(G)$ of a general caterpillar G of length 3. Of course, once that is found, how can we use that to find the $ms(G)$ of caterpillars of length 4, 5, 6, etc. Also, one might work in proving that a certain move set/sequence of jumps is the most efficient. How do we know that an algorithm we get is the best algorithm? Moreover, one can expand and look for the minimal solvability number $ms(G)$ of other unknown graphs like trees of diameter 4 or asters.

In retrospect, having a tight upper bound for caterpillars of length 3 furthers the study of the $ms(G)$. Graphs can take any size and shape, and it is important to group them in families when considering this invariant. Doing so allows us to work on further graphs that have as a subset known familiar graph. In fact, this is how we were able to find the $ms(G)$ of caterpillar graphs of length 1 and length 2. The strategies in this game can further be applied to other versions of peg solitaire. We only considered the 'jump' move, but the $ms(G)$ might change if we consider both the 'jump' and 'merge' move of peg solitaire. Having these results gives us a starting point for this other version of the game. Lastly, we touch on results and algorithmic approaches of playing a game efficiently, and we can further take these strategies on other combinatorial games. We hope these findings contribute to any regard.

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A Multifaceted Insight into Addiction Treatment Programs in the Midwest—Identifying Factors Influencing Substance Use Disorder Treatment Participation and Retention

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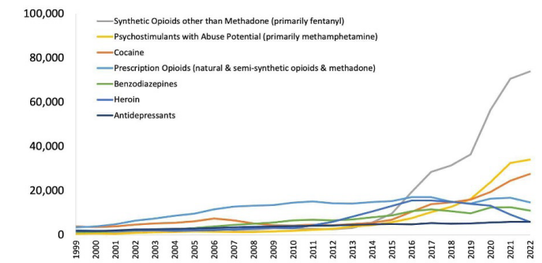
In the past decade, the US government and other key interest groups have taken significant steps to address the addiction epidemic. Despite these efforts, 16.7% of Americans age 12+ suffer from a substance use disorder. Unfortunately, only 23% of those seek treatment with even less than 8.4% of clients completing it successfully and staying sober for more than five years (SAMHSA 2019). Consequently, this study aims to investigate the specific factors that influence treatment participation and retention. Four key domains of influence were identified and studied: confidence, hopelessness, neglect, and misalignment between clients and counselors on treatment needs. The study surveyed 60 clients and 23 counselors from established, licensed substance addiction treatment programs across the Midwest. The hopelessness of clients was determined using the Beck Hopelessness Scale. The survey identified a lack of confidence in the treatment program(s), a lack of confidence in their ability to complete the treatment program, and a lack of finances as prevailing influences in the hesitancy to seek treatment. However, after joining treatment, the effect of client hopelessness seems to have exacerbated these factors, playing a major role in treatment retention, as around 90% of non-hopeless clients see their needs being met compared to only 37% of hopeless clients ($p < 0.05$). This effect perpetuates the perception of neglect in clients, further isolating them from seeking or participating in treatment. Further, considerable discord has been identified between clients and counselors on the perception of those needs and challenges of clients, illuminating critical information that can improve client participation and adherence to treatment programs, significantly improving our approach to the epidemic of substance addiction.

1. Introduction

Substance use disorder is a growing epidemic across the nation, especially in the Midwest. With 54.2 million Americans aged 12 and older suffering from a substance use disorder in 2023 alone (Substance Abuse and Mental Health Services Administration (SAMHSA) 2024, **Figure 1**), treatment for substance addiction is critical for the progress of our nation. Existing treatment options, however, are insufficient in addressing this adversity. Out of 54.2 million Americans who require treatment for their substance use disorder, only 23% receive treatment. Unfortunately, this trend extends beyond just participation, as out of the 23% who receive care, 70-80% discontinue treatment prematurely within 3-6 months (SAMHSA 2024). Similarly, maintaining sobriety is often unsuccessful, as the average individual who achieves long-term sobriety—defined as five years sober—typically undergoes treatment five times before achieving lasting success (Kelly, Bergman, Hoeppner, Vilsaint, & White 2017).

Therefore, it is crucial to identify and address the reasons behind the poor rates of success of addiction treatment programs. In this regard, the study has two major aims: a) to identify the comprehensive reasons why clients do not join treatment, and b) to identify the comprehensive reasons behind why clients prematurely discontinue treatment. This study investigates hopelessness, and lack of confidence, both in treatment programs and in self, and client-counselor discord as major factors influencing these aims. In psychological recovery, particularly from depressive disorders—a common co-occurring condition with substance addiction—hopelessness plays a significant role (Papakostas et. al. 2005, Wilson 2010, Shaygan et. al. 2021). In addition, self-confidence, confidence in the treatment program(s), and confidence in a future outlook are critical for psychological recovery (Shi et. al. 2022, Kadden 2011). Unfortunately, with present research on addiction, especially regarding the treatment of addiction,



*Includes deaths with underlying causes of unintentional drug poisoning (X40–X44), suicide drug poisoning (X60–X64), homicide drug poisoning (X85), or drug poisoning of undetermined intent (Y10–Y14), as coded in the International Classification of Diseases, 10th Revisior Source: Centers for Disease Control and Prevention, National Center for Health Statistics. Multiple Cause of Death 1999-2022 on CDC WONDER Online Database, released 4/2024.

Figure 1. U.S. Overdose Deaths, Select Drugs or Drug Categories, 1999-2022. NIDA 2023.

there is a distinct lack of scholarship on the effects of hopelessness and confidence on substance use disorder treatment participation and retention. The outcomes will provide valuable insight in determining the future course of action for maximizing rates of participation, retention, and success in treatment programs.

2. Methods

2.1: Problem Identification and Outreach

The epidemic of substance addiction in the United States is highly pervasive and dynamic, with the landscape of threats changing daily. In recent years, the rise of synthetic opioids like fentanyl has exacerbated the opioid epidemic, hitting rural areas, particularly in the Midwest, the hardest (Dombrowski et al. 2016, American Chemical Society 2023, DeWeerdt 2019). The rapid expansion of synthetic opioids, particularly fentanyl, has dramatically changed the nature of substance abuse, leading to higher overdose rates and presenting new challenges for treatment centers. The combination of economic hardships and limited access to healthcare in these regions has made them particularly vulnerable to the devastating effects of the opioid crisis. Therefore, the focus of outreach in this study was on substance addiction treatment centers in the Midwest region, as defined by the U.S. Census Bureau in 2024 (U.S. Census Bureau 2024). Outreach efforts included emails, phone calls, and other website-integrated contact. Additionally, extensive shadowing and volunteering at local addiction treatment facilities were conducted to gain a well-rounded understanding of the field. These experiences, combined with insights from previous research and literature, informed the development of a comprehensive survey with participants from seven treatment organizations.

2.2: Overview of Addiction Treatment Programs

Addiction treatment programs are designed to help individuals overcome substance use disorders, lead healthier lives, and ensure long-term sobriety. The physiological, behavioral, and sociological consequences of substance addiction are incredibly complex, however. Therefore, programs often vary drastically in approach, length of treatment, and, consequently, outcomes. Typically, programs start with detoxification (detox), focusing on safely managing withdrawal symptoms under medical supervision and guidance. The detox process is especially crucial for stabilizing individuals physically and emotionally before further treatment. Following detox, clients may enter inpatient rehabilitation, a structured, full-time program where clients live at the treatment facility. This approach, though intensive, includes valuable medical care, therapy, and life skills training, making it suitable for those with severe addiction issues or those who have struggled with outpatient programs in the past. For those who require less intensive support, outpatient rehabilitation programs offer greater flexibility and independence, allowing clients to attend therapy sessions—often held in small peer groups—while continuing to live at home. Intensive outpatient programs and partial hospitalization programs provide intermediate options based on client needs.

Despite program variability, established approaches tend to have key similarities that make them effective. This study has surveyed four different types of treatment approaches/stages: Intensive Outpatient (IOP) care, Aftercare, Neuro-linguistic Programming (NLP), and Drug Court. Often highly structured and curriculum-based, each common approach provides a universal guide to the treatment process, lending itself well to thorough study. Established curricula such as the Cognitive Behavioral Interventions for Substance Abuse (CBISA), the 12-step program approach, and the Universal Treatment Curriculum (UTC) often prevail as effective, evidence-based treatment approaches (NIDA 2020). Although alternative treatment programs, most commonly faith-based programs, have shown great success in the past, they often vary greatly and lack consistency across programs and regions (Yeung 2022). Therefore, for this study, the programs surveyed are solely those that are licensed under common, curriculum-based treatment approaches (CBISA, 12-step, UTC).

2.3: Survey Design

Throughout the project, two separate surveys were distributed, a client survey and a service provider survey. Survey recipients include clients and active counselors of all age ranges above 18 years. The client surveys were physically distributed during a group treatment session, requiring 25-35 minutes of participation for 64 total questions. The counselor survey was distributed on paper and online, requiring 10-20 minutes of participation. Individuals undergoing substance addiction treatment are referred to as "clients" in order to identify them as individuals rather than by their disease.

2.3.1: Ethics

Both client and service provider surveys were administered on a completely voluntary basis. Participants were required to sign informed consent forms detailing all information necessary for participation in the survey. Participants could exit the survey and omit questions at any time. Age, gender, treatment type/time in treatment, and group identification (client v.s. counselor) are the only identifying factors of each of the surveyed, thus the data on individual identifications were not collected. Consent forms were handed separately in a shuffled manner to ensure complete confidentiality, even to the researcher, and collected anonymously. All data was stored on a secured, password-protected home computer with only the researcher and the treatment facility in charge having access to the data. After the study, comprehensive data and results were shared with requesting treatment facilities to provide feedback for further improvement. The study, including all surveys, data collection, data storage, and analysis was conducted following IRB documentation and approval.

2.3.2: Risk Assessment

Participation in the study had minimal associated risks. Possible risks included potential discomforts for individuals including time commitment and minimal emotional stress from recollecting past events. Additionally, time commitment could have been a hindrance to participants; however, the impact was greatly minimized, as for service providers, the survey may be taken at any time. For clients, the survey was administered during a treatment session, requiring a minimal time commitment. Finally, emotional discomfort was a potential risk for participation; however, this discomfort was minimized through the option for participants to skip any of the questions, and the inclusion of minimal questions requiring the recollection of any potential traumatic events in participants' lives. Although there was no direct benefit to the clients or counselors, the results of this study allow treatment facilities to identify and address areas of discord, if any. This benefits both clients and providers over time by offering a comprehensive view of their interactions and contributing to the betterment of the program.

2.3.3: Assessing Hopelessness

The prevalence and degree of hopelessness in clients were assessed using the Beck Hopelessness Scale, a scientifically verified method for identifying hopelessness (Beck, Weissman, Lester, & Trexler 1974; Steed 2001; Pretorius & Padmanabhanunni 2024). The Beck Hopelessness Scale consists of 20 questions pertaining to the self-perception of individuals and how they see themselves in the future. Scores ranging from 0 to 3 are considered within the normal range, 4 to 8 identify mild hopelessness, scores from 9 to 14 identify moderate hopelessness, and scores greater than 14 identify severe hopelessness (Beck & Steer 1988; Pretorius & Padmanabhanunni 2024).

2.4: Demographics

Demographics were collected solely for client participants to minimize unnecessary identifiers such as race, socioeconomic status, and geographic area. These were excluded to ensure privacy and protection for these participants. Client survey demographics were collected at the beginning of the survey. Client demographics for gender and age represent the overall population in addiction treatment programs as depicted in **Table 1**. **Table 1** additionally shows a survey focused on drug addiction outpatient treatment programs. 60 clients and 23 counselors were surveyed across 5 states from the Midwest region.

2.5: Data Analysis and Statistical Methods

Data analysis and statistics were conducted using Microsoft Excel, Wolfram Mathematica, and MATLAB. Data was analyzed through Microsoft Excel using statistical analysis methods such as unpaired, 2-tailed student's T-test ($p < 0.05$), Z-test, and regression analysis ($R^2 > 0.70$). Figures were generated using Wolfram Mathematica and MATLAB.

3. Results

Interactions with client treatment groups, as well as numerous counselors, physicians, and law enforcement, joined with extensive literature review and previous study, informed the development of a comprehensive survey identifying and assessing major factors that likely contributed to treatment participation hesitancy as well as discontinuation among clients. These initial survey results revealed the key domains of the study discussed below, especially emphasizing the role of hopelessness on the influence of these factors. Therefore, this study reveals the specific factors influencing treatment participation hesitancy; the effect of hopelessness on client needs, the perception of neglect, the influence of a lack of information, and the influence of a lack of self-confidence.

3.1: Factors Influencing Treatment Participation Hesitancy

Individuals with substance use disorder in need of addiction treatment often do not seek help, despite its availability. Such a trend has been observed across the nation; however, there exists a lack of substantial literature for identifying the precise reasons behind this resistance. Therefore, this study sought to determine the factors behind client resistance to seeking help **(Figure 2)**.

Demographics	N	%
Overall	60	
Gender		
Male	28	53%
Female	24	45%
Non-Binary	0	0%
Age, year		
18 - 24	10	19%
25 - 34	20	38%
35 - 44	13	24%
45 - 54	9	17%
55 - 64	0	0%
65 - 74	0	0%
75+	0	0%
Addiction Types (Multiple)	12	14%
Opioids	43	49%
Other I.D.	25	29%
Alcohol	3	3%
Gambling	3	3%
Other		
Treatment Type		
IOP	22	39%
After Care	16	29%
NLT	16	29%
Drug Court	2	4%

Table 1. Client Demographics with respect to gender, age, types of addiction, and treatment program strategy.

Clients were differentiated and compared on the time it took for them to seek treatment after they realized they needed it (< 1 year, quick responders v.s. > 1 year, hesitants). **Figure 2** shows some of the major reasons for resistance in clients, with a lack of confidence in the treatment program(s), a lack of confidence in their being able to complete the treatment program, and a lack of finances being the most prevalent. From the data, it is clear that the deficiency in information dissemination didn't have a significant influence. Indeed, only ~22 % of hesitants believed that lack of information influenced their decision not to join the treatment programs. Lack of confidence, both in the treatment program and in themselves, has a major impact on clients' resistance or hesitancy to seek treatment(s) for the first time. Correlation analysis further develops this finding, as a lack of confidence in the treatment program(s) is strongly positively correlated with a lack of self-confidence ($R^2 = 0.717$). A lack of self-esteem is known to play a significant role in the tendency towards addiction and use of addictive substances (Alavi 2011; Chebli et. al. 2023), further affirmed by survey results indicating that clients with poor self-confidence also believed their lack of self-confidence played a

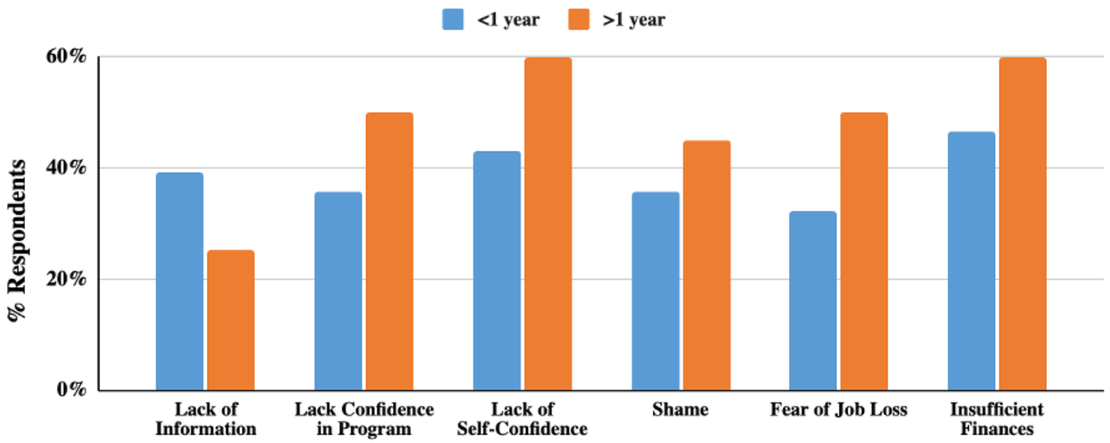


Figure 2. Major Factors Influencing Treatment Participation Hesitancy. Clients were asked, "After you had realized that you needed help for the first time, how influential were the following reasons for your resistance to seeking help? (1 – Not at all Critical; 5 – Very Influential)." Answers 4 and 5 were categorized as seeing each factor as influential to their hesitancy with seeking treatment and plotted. Results were compared between those who took less than one year to join a treatment program and those who took greater than one year.

significant role in their decision not to seek treatment, correlating strongly ($R^2 = 0.712$). In addition, their lack of self-confidence continued despite participation in treatment, serving as a significant barrier to continued participation ($R^2 = 0.719$). Financial concerns seem to be a critical factor that influences the decision to seek help. However, financial insecurity remains a highly complex, multifaceted issue that encompasses socioeconomic status, job market, education status, treatment expenses, and more. Without further investigation into the details of financial insecurity and its role in treatment participation, the collected data is insufficient to conclude results at this time; however, it warrants a separate comprehensive study.

Taken together, confidence seems to be a critical factor in joining the addiction treatment process, both confidence in the program(s) and self-confidence. With both being closely related, it is more important than ever to not overlook the significance of confidence-building programs in mainstream treatment processes.

3.2: Hopelessness

The study identified 17% of clients surveyed to have moderate to severe hopelessness as determined using the Beck Hopelessness Scale (Beck & Steer 1988; Pretorius & Padmanabhanunni 2024). Hopeless clients and non-hopeless clients were then differentiated and compared against each other across various domains. Although the survey has determined 17% of clients to have moderate to severe hopelessness, it is likely this is a significant underestimate, as participants include only those currently in treatment programs. This excludes those for whom hopelessness has deterred their participation.

3.2.1: Treatment Programs Addressing Client Needs: The Effect of Hopelessness

The study determined that 86% of clients felt that their needs were being met by their current addiction treatment program. However, when hopeless clients are differentiated from non-hopeless clients, a different trend emerges. Around 90% of non-hopeless clients see their needs being met while only 37% of hopeless clients feel the same ($p < 0.05$, data not shown in the figure). This clearly showed a significant disparity in the perception of the clients that are positively diagnosed using the Beck Hopelessness Scale. Broadly, addiction treatment programs are found to be highly effective in addressing specific individual needs; however, unfortunately, clients with moderate to severe hopelessness are being left behind in their recovery regimen.

3.2.2: Hopelessness: Investigating the Role of the Perception of Neglect

Substance use disorders are the most frequent behavioral consequences of childhood abuse and neglect (Schäfer et. al. 2017). In adulthood, the perception of societal neglect simply perpetuates the effects of onset in childhood. Critically, the perception of neglect, whether it may be from society or the family, often serves to isolate individuals, deterring them from joining treatment programs or getting the help they need. Therefore, it is crucial to better understand the factors contributing to the perception of neglect in treatment programs. When surveyed, 32% of clients from the non-hopeless group felt that they were neglected by society. However, this number significantly increased to 64% ($p < 0.05$) for clients with moderate to severe hopelessness. In contrast, fewer clients from both groups felt that they were neglected by their family as compared to society, though the trend of greater perceptions of neglect in hopeless individuals continued (27% of hopeless clients vs 15% of non-hopeless clients, $p < 0.05$) as seen in **Figure 3**.

The relationship between hopelessness and neglect is incredibly complex; however, it continues to have major implications in the world of psychology and behavioral science. This relationship is especially amplified in developmental stages, as childhood familial

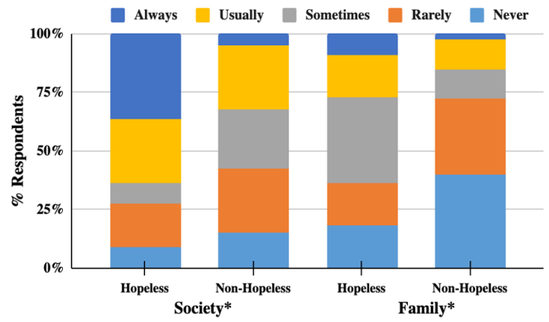


Figure 3. Neglect by Society vs Neglect by Family comparing hopeless with non-hopeless individuals. Clients were asked, "How often do you feel neglected or ignored by society/family because of your situation?" respectively.

neglect has been found to cause depression and associated hopelessness and lack of self-confidence among teens (Glickman, E., Choi, K., Lussier, A., et. al. 2021; Paterniti, S., Sterner, I., Caldwell, C. et. al. 2017). A similar trend seems likely regarding the relationship between hopelessness and neglect in addiction treatment programs. Those who feel neglected by their families or society likely feel hopeless for their future and lack self-esteem as they often stagnate in the addiction recovery process.

3.2.3: Lack of Information

Lack of information on addiction recovery can play a major role in individuals who need treatment not seeking it (Generes, W.M. 2022). This knowledge gap can lead to delayed or missed opportunities for intervention, particularly among those who are already struggling with feelings of hopelessness. Therefore predictably, when clients were differentiated on hopelessness, a significant disparity was noted between hopeless and non-hopeless clients on the impact of the lack of information on the availability of resources for help before joining the treatment program (80% vs. 21.5% respectively, $p < 0.05$). After joining the treatment program, however, the influence of the lack of information on resources on treatment participation is reduced in both groups **(Figure 4)**. Despite slight reductions in this influence, **Figure 4** shows the disparity between hopeless and non-hopeless groups continued (60% v.s. 20%; $p<0.05$), underscoring the need for targeted treatment for these individuals.

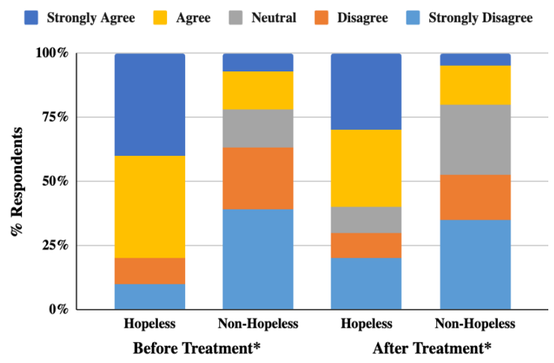


Figure 4. Effect of Hopelessness on the Impact of Lack of Information in Treatment Participation and Retention. Clients were asked to assess whether the lack of information was a significant barrier to them joining treatment as well as whether it remains a barrier to retention. These results were then compared between hopeless and non-hopeless individuals.

3.2.4: Lack of Self-Confidence

Lack of self-confidence is a major dissuader in both joining a treatment program and maintaining attendance and progression through treatment. For many individuals, this lack of confidence stems from the often repeated failed attempts to quit or negative self-perceptions and stigmas tied to their substance addiction.

Consequently, they often feel incapable of achieving long-term recovery, attributing failure to their self-worth and self-efficacy. Unfortunately, this lack of self-confidence for many individuals with substance abuse disorder is common. While many mainstream addiction treatment programs focus on rebuilding self-confidence as part of the recovery process, the study has found that such efforts are often ineffective for individuals who suffer from moderate to severe hopelessness **(Figure 5)**. This finding underscores the need for alternative, specialized approaches when addressing the unique challenges faced by clients experiencing profound hopelessness. Both hopeless and non-hopeless groups are statistically similar in terms of the role of lacking self-confidence as a barrier for them joining the treatment (33% vs 37%, $p = 1.00$). However, after joining the treatment program, none of the clients from the non-hopeless group felt that a lack of self-confidence influenced them to leave the program without completion. This number improved significantly for the hopeless group compared to before joining the program (33% to 9%, $p < 0.05$). Although the overall perception is improved in the hopeless group after joining the treatment program, the difference in perception still exists compared to the non-hopeless group (10 % vs. 0%, $p < 0.05$). This disproportionate benefit from treatment programs has unfortunately left hopeless individuals behind, trapping them in a cycle of worsening hopelessness and diminished self-confidence, further hindering their recovery progress.

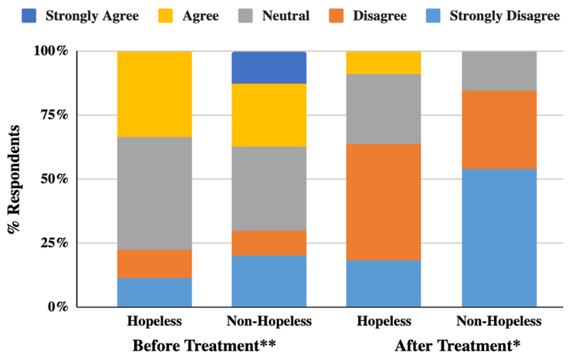


Figure 5. Effect of Hopelessness on the Lack of Self-Confidence on Treatment Participation and Retention. Clients were asked to assess whether the lack of self-confidence was a significant barrier to them joining treatment as well as whether it remains a barrier to retention. These results were then compared between hopeless and non-hopeless individuals. *$p < 0.05$, ** $p = 1.00$.

3.3: Client-Counselor Discord: Misalignment on Client Needs

Counselors, as well as other relevant service providers, are often incredibly compassionate and dedicated to the betterment of their clients. However, this same dedication and compassion can potentially blind policymakers and curriculum designers from ensuring efforts and resources are allocated in alignment with the real, on-ground needs of client cohorts. Most often, discord on the needs of clients and the perception of those needs often arises from misunderstandings or mischaracterizations around what clients truly need during their addiction recovery process. Clients may have specific, immediate concerns—such as relief from withdrawal symptoms, support with housing or employment, or managing comorbid mental health issues—that they feel are critical to their recovery. On the other hand, counselors may often focus more on long-term goals like complete sobriety, behavioral change, or adherence to a structured treatment plan, which may not align with the client's immediate priorities.

This discord can manifest in several ways. For instance, clients may feel their basic needs are being inadequately addressed, or that their voices are being overlooked in shaping their treatment plan. In efforts for logistic efficacy and simplicity, programs often, quite understandably, create and apply a universal approach to recovery, leading to client frustration and eventual disengagement from the program. On the other hand, an overcompensation on treatment and prioritization of certain individual needs without addressing the broader structure of addiction recovery can also create discord. Overemphasis on certain needs leads to an underemphasis on others—understanding the hierarchy of prioritization is critical for ensuring overall treatment success. If counselors focus too heavily on catering to certain personal preferences—such as allowing too much flexibility in attendance or overlooking essential steps in the recovery process—it can lead to a lack of accountability and structure, crucial aspects of long-term success. In such cases, clients may feel supported in the short term but lack the necessary tools and discipline to maintain progress, ultimately undermining their recovery journey.

A survey of counselors on need prioritization, in alignment with questions asked to clients, revealed a trend characterizing the latter manifestation (**Figure 6a**, **Figure 6b**). Counselors have significantly overestimated the prevalence of almost all assessed influencers of poor participation and retention in the program. In other words, clients often feel more positive about themselves than counselors' perceptions of clients, both before joining treatment (**Figure 6a**) and after (**Figure 6b**). This misalignment between counselor perceptions and client self-assessments can result in discordant priorities within outreach and retention programs,

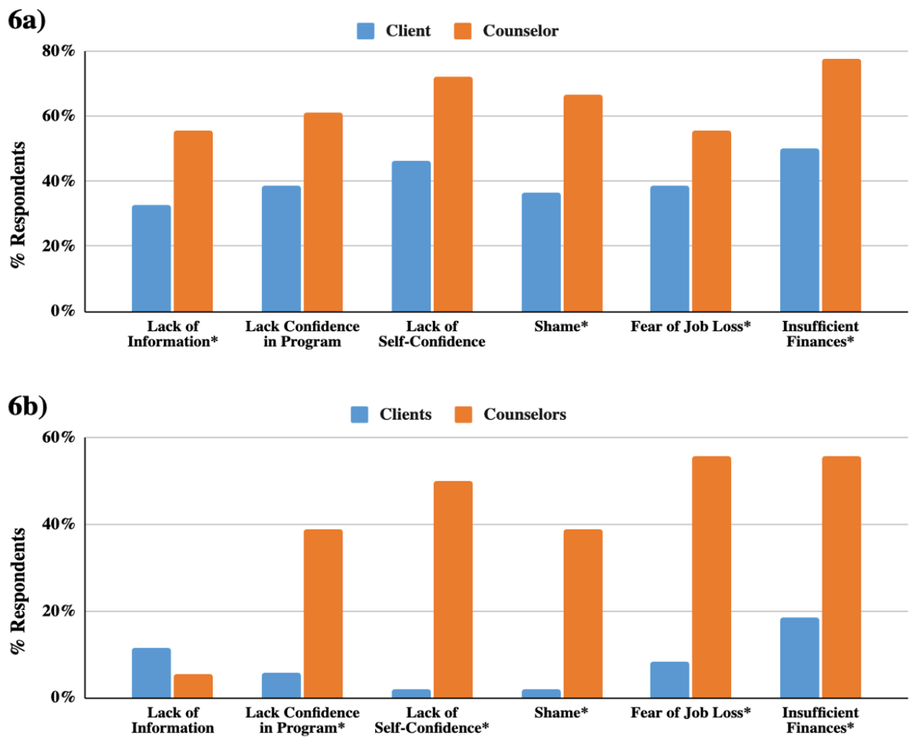


Figure 6. Discord Between Clients and Counselors on the Needs of Clients. a) Before Treatment: clients were asked to assess whether each factor was a significant barrier to them joining treatment. b) After Joining Treatment: clients were asked to assess whether each factor was a significant barrier to them staying in the treatment program. Counselors were asked to assess the significance of each factor on client behavior. These results were then compared between clients and counselors. Total respondents that agreed or strongly agreed are plotted. *p < 0.05.

leading to strategies that overlook the most impactful factors driving client participation while over-prioritizing factors with less influence. By accurately identifying and addressing the most influential factors, programs can better support clients in their recovery, maximizing participation, minimizing dropout rates, and improving overall success. This alignment not only strengthens the therapeutic alliance but also enhances the long-term sustainability of treatment efforts and its ability to adapt to the changing priorities of client cohorts.

4. Discussion

In this study, a systematic review of addiction treatment programs in the Midwest region was conducted to identify the specific reasons behind poor client participation and retention. The study identified, among many other factors, three major influencers behind both participation and retention: hopelessness, lack of confidence, and client-counselor discord. Cultivating hope in psychological treatment is crucial for long-term, effective outcomes. Hope Theory, as proposed and established by (Snyder, C. 2002), has encouraged future research with regards to accurately enhancing hope in medical feedback. Helping people pursue medical goals for which they are best suited can improve medical outcomes (Long, Katelyn N.G., Kim, Eric S., Chen et. al, 2020; Zhang, X., Zou, R., Liao, X., et. al. 2020; Olsman E. 2020). However, addiction treatment programs vary greatly in their approaches, which often leaves certain groups—particularly those struggling with hopelessness and low self-confidence—disproportionately underserved.

The findings from this study can inform the development of targeted treatment programs to prevent the exclusion of certain individuals. For example, a pre-treatment survey, such as the one provided by the study, can be used to assess the specific individual needs of clients within their treatment program cohort. Assessing the level of hopelessness and lack of confidence clients have before treatment can more efficiently and effectively help treat clients more holistically. A treatment regimen that treats hopeless individuals with different approaches and prioritizations than non-hopeless individuals likely can improve rates of participation, retention, and especially success rates of treatment.

Motivation for individuals seeking addiction treatment often stems from deeply rooted personal experiences and critical turning points in their lives. Among surveyed individuals, backgrounds of childhood trauma, abuse, neglect, and isolation were common. After years of addiction, it was the feeling of "hitting rock bottom" that has motivated so many individuals to seek treatment to be a "circuit-breaker in their lives." Individuals are forced to reassess their lives and make meaningful changes (Chen G. 2010; Prangley, T., Pit, S.W., Rees, T. et. al. 2018). When clients in the study were asked, in an open-ended question, about the factors that motivate them to continue treatment, almost 60% of individuals cited family, especially children, as their motivation. Interestingly, the family of an individual has been found to have a dual influence on addiction treatment and its success. Familial and relationship issues have been found to be among the leading inducers of relapse and stress, a conclusion affirmed by most current treatment programs. Consequently, current treatment processes isolate individuals from their families, seeing only the neglect and abuse the family provides within clients' lives. However, family, especially children, for many individuals are the largest motivator for completing treatment. This identified dual influence presents great potential for treatment programs to amplify connections with supportive family members, destigmatizing the treatment process and strengthening post-treatment relationships. A sense of isolation often is the reason for using addictive substances; harnessing the existing relationships in a client's life, rather than using isolation, can prove to be more effective in the long run and lead to better treatment, and even societal outcomes.

5. Future Study

Unfortunately, due to the COVID-19 pandemic, organizational participation rates were very low. This resulted in less than 10% of organizations responding to requests for participation. This may skew data towards greater representation for more established organizations and treatment facilities. Future studies may be done to increase the representation of various regions of the United States, beyond the Midwest, and even internationally. Additionally, further study may be conducted to increase the number of participants to improve statistical validity and conclusions. Further study may be done on various groups of service providers involved in the addiction treatment process such as law enforcement and physicians to gain a different perspective on the treatment process and further identify discord between clients and various service providers.

6. Conclusions

The study identified the unique reasons behind clients not participating and completing addiction treatment, investigating the effects of hopelessness, lack of confidence, and client-counselor discord as contributing factors. Minimizing the influence of factors that may deter individuals from joining treatment is crucial to improving rates of success with treatment; however, amplifying the factors that motivate and drive individuals to join treatment for the first time is paramount (Brorson et. al. 2013). The findings will be valuable in making improvements to the current treatment programs in order to improve rates of treatment participation, retention, and long-term success.

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Author's Note

Data collection, analysis, and interpretation were conducted independently. There are no outstanding or significant conflicts of interest to be declared. All research was funded internally and independently.

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DETICKT IT: A Machine Learning-Based Application for Real-Time Tick Identification and Spatiotemporal Disease Risk Assessment

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There is an alarming increase in the population of ticks and tick-borne diseases (TBDs), with 475,000 cases reported annually, some of which are fatal. Due to limited training, healthcare providers and the public cannot always accurately identify ticks and their associated infections, leading to delayed diagnoses and treatments. Additionally, the prevalence rates of different disease-causing pathogens vary based on geographic locations. To facilitate the identification process and provide an expedited risk assessment of TBDs, a machine learning-based iOS application, DETICKT IT was created. The app features a ResNet50V2 (transfer learning) deep convolutional neural network (CNN) built in Python for combining real-time tick-species identification with a location-based tick-risk assessment by embedding the Centers for Disease Control and Prevention's (CDC's) spatiotemporal tick and pathogen surveillance statistics. With DETICKT IT, users can now receive an immediate and accurate analysis to determine whether they are at risk of contracting a certain TBD. The app is able to accurately identify the ten most common tick species in North and South America: American dog tick (*Dermacentor variabilis, D. similis*), Asian longhorned tick (*Haemaphysalis longicornis*), Brown dog tick (*Rhipicephalus sanguineus*), Eastern blacklegged tick (*Ixodes scapularis*), Western blacklegged tick (*Ixodes pacificus*), Groundhog tick (*Ixodes cookei*), Gulf Coast tick (*Amblyomma maculatum*), Lone star tick (*Amblyomma americanum*), Rocky Mountain wood tick (*Dermacentor andersoni*), and soft tick (*Ornithodoros*). The overall accuracy is 97% with precision, recall, and F1 score metrics of 0.96, 0.97, and 0.96, respectively. This freely accessible app shows promise in assisting tick bite victims with their decision to seek further medical assistance, particularly those with underlying health conditions.

Introduction

The Centers for Disease Control and Prevention (CDC) reported that 50,000 people are diagnosed with tick-borne diseases (TBDs) in the United States each year, including Lyme Disease (LD; Marx et al., 2021). However, underreporting discovered by Kugeler et al. (2021) suggests that the real incidence rate is much higher, closer to ~500,000. Due to underdiagnosis and asymptomatic clinical manifestations (Madison-Antenucci et al., 2020; Lantos et al., 2020), the scope of this public health threat is difficult to assess (Welc-Falęciak et al., 2023). Moreover, in recent decades, the number of cases has doubled, in part due to climate change (Campbell-Lendrum et al., 2023).

Ticks are ectoparasites and their range and tick-borne pathogens are increasing worldwide, with warmer winters in certain regions (Odgen et al., 2021; Kilpatrick et al., 2017) leading to phenological changes (MacDonald et al., 2019; Newman et al., 2018). While LD caused by *Borrelia burgdorferi sensu stricto* (Burgdorfer et al., 1982) remains one of the most prevalent tick-borne pathogens in North America, Europe, and Asia (Kugeler et al., 2015; Grochowska et al., 2020), other diseases have emerged and become pervasive in recent decades (Eisen et al., 2017). Madison-Antenucci et al. (2020) reports that the foci of endemicity or new locations of ticks is expanding and so are the pathogens and bacteria being transmitted which vary greatly by species.

The Eastern blacklegged tick (*Ixodes scapularis*) is one of the most clinically important ticks in the United States and abroad due to its transmission of diverse pathogens (Tokarz et al., 2019), such as *Anaplasma phagocytophilum* (anaplasmosis), *Babesia microti* (babesiosis), *Borrelia miyamotoi* (hard tick relapsing fever), *Borrelia mayonii* (LD), and *Ehrlichia muris eauclairensis* (ehrlichiosis; Eisen & Eisen, 2018). Other important ixodid vectors include the Asian Longhorned tick (*Haemaphysalis longicornis*; Egizi et al., 2020), Brown dog tick (*Rhipicephalus sanguineus*; Little et al., 2022), American dog tick (*Dermacentor variabilis, D. similis*; Newman et al., 2018), Eastern blacklegged tick (*I. scapularis*; Eisen et al., 2018), Lone star tick (*Amblyomma americanum*; Thangamani et al., 2023), Gulf Coast tick (*A. maculatum*; Paddock & Goddard, 2015), Groundhog tick (*I. cookei*; Ferreira et al., 2023), Western blacklegged tick (*I. pacificus*; MacDonald et al., 2019), Rocky Mountain wood tick (*D. andersoni*; Ebel et al., 2023), and soft tick (*Ornithodoros*; Chitimia-Dobler et al., 2023). These species have been associated with certain TBDs, including *B. mayonii* (transmitted by the *I. scapularis / pacificus*), *B. miyamotoi (I. scapularis / pacificus)*, tick-borne encephalitis (TBE), Crimean-Congo hemorrhagic fever (*R. sanguineus*), human granulocytic anaplasmosis (HGA) caused by *Anaplasma phagocytophilum (I. scapularis / pacificus)*, babesiosis (*I. scapularis / pacificus*), human monocytic ehrlichiosis (*A. americanum, D. variabilis*), severe fever with thrombocytopenia (SFTS; *H. longicornis*), Heartland virus disease (*A. americanum*), *E. ewingii* infection (*A. americanum*), Powassan encephalitis (*I. scapularis / cookei*), and Bourbon virus disease (*A. americanum*; Madison-Antenucci et al., 2020). Tick exposure and bites are also associated with other diseases and co-infections (Lantos et al., 2020), such as Rocky Mountain Spotted Fever (RMSF; Dahlgren et al., 2017), Southern Tick-Associated Rash Illness (STARI; Belisle et al., 2017), Alpha-Gal Syndrome (AGS; Karim, et al., 2019), a red-meat allergy

associated with bites from the Lone star tick (*A. americanum*), Gulf Coast tick (*A. maculatum*), American dog tick (*D. variabilis, D. similis*), and Eastern blacklegged tick (*I. scapularis*), among other TBDs (Madison-Antenucci et al., 2020). The Asian longhorned tick (*H. longicornis*), indigenous to Asia (Beard et al., 2018), has recently been associated with the transmission of thrombocytopenia syndrome (SFTS) and viral hemorrhagic fevers (VHFs; Luo et al., 2015), *Rickettsia japonica* [Japanese spotted fever (JSF); Mahara, 1997], *Anaplasma, Babesia, Borrelia, Ehrlichia*, and *Rickettsia* (Beard et al., 2018).

Despite existing antibiotic treatments, diseases such as LD can become chronic if not treated immediately (Eisen et al., 2017). Moreover, LD affects more than two million people in the U.S. (DeLong et al., 2019), with many cases remaining undiagnosed due to atypical clinical manifestations or the absence of an otherwise characteristic "erythema migrans" skin rash (Nathavitharana & Mitty, 2015). Moreover, the economic burden of TBDs and LD is significant – estimated between $345 million and $968 million per year – with an average of $1,200 per treatment, and with later stages of disease development being more costly (Hook et al., 2022). Thus, there is a need for diagnostic capabilities to be improved, and machine learning-based approaches may offer potential solutions (Rich et al., 2022; Akbarian et al., 2021; Omodior et al., 2021; Sanchez-Galan et al., 2023).

The pathogens and diseases transmitted are dependent upon both the tick species and the geographical location of the tick; therefore, the classification of the tick species is critical to formulating an accurate diagnosis (Otranto et al., 2012). Traditional diagnostic methods have used tick species classification to inform whether a person is at risk of contracting a TBD, primarily LD (Nieto et al., 2018). However, there is no direct correlation between a certain species of tick and the TBD that the species carries, since the risk factors vary greatly for both tick lifecycle and geographic location (James et al., 2006; Madison-Antenucci et al., 2020). Clinical studies of patients presenting with a tick bite in Lyme-endemic areas have shown that the prevalence of LD in all species of ticks fluctuates, including the notorious biting arachnid Eastern blacklegged tick (*I. scapularis*). Moreover, Poland et al., (2001) concluded that the abundance of host-seeking infected ticks is not linearly correlated with the probability of disease transmission of the LD spirochete *Borrelia burgdorferi sensu stricto* (Eisen et al., 2016). Rather the proportion of ticks infected with *B. burgdorferi s.s.* informed the risk of human LD infection (Hinckley et al. 2016). With this degree of uncertainty, it is necessary to couple real-time identification with location-specific incidence rate data. Connecting these datasets in one platform is beneficial, since the general public and healthcare professionals alike find tick recognition challenging due to the large number of different species known and intra-species variability (Madison-Antenucci et al., 2020). Thus, morphological identification of tick species is imperative for accurate TBD risk assessment (Nieto et al., 2018). Moreover, some ticks are often difficult to identify using only morphological traits because of the allopatric nature of certain species (Chilton et al., 2013), and thus the geographic location of the ticks can inform the probability of species (Riggs et al., 2015).

Correct identification requires in-depth entomological training due to feature-wise minute differences that are difficult for the general public to identify (Nieto et al., 2018). Computer vision-based approaches to tick identification have been successful, with Rich et al. (2022) creating a convolutional neural network (CNN) that can accurately identify the American dog tick (*D. variabilis, D. similis*), Eastern blacklegged tick (*I. scapularis*), and the Lone star tick (*A. americanum*) with 99.5% accuracy on a given test dataset when transfer learning is used. Other deep learning algorithms feature the three major human-biting tick species (Akbarian et al., 2021; Omodior et al., 2021) with the highest reported accuracy of 93% (Sanchez-Galan et al., 2023). These machine learning-based approaches can proficiently classify images as noted in multiple studies where deep learning models were implemented for insect identification (Rich et al., 2022).

Given that human experts are the limiting factor for diagnostic capacity in this field as well as the pressing health concerns over TBDs worldwide, deep learning-based models hold promise for the instantaneous assessment of TBD risks based on specimen determination and prevalence analytics.

Materials and Methods

Project Overview

The goal of this project was to create a machine learning-based mobile application that could be used to identify the species of a tick and provide disease risk analysis based on the location of the user. The three most pathogenic ticks are the Eastern blacklegged tick (*I. scapularis*), Lone star tick (*A. americanum*), and American dog tick (*D. variabilis, D. similis*) and were thus used in previous versions of this study. After fine-tuning the model and adjusting the hyperparameters, ten species in total were identified: American dog tick (*D. variabilis, D. similis*), Asian longhorned tick (*H. longicornis*), Brown dog tick (*R. sanguineus*), Eastern blacklegged tick (*I. scapularis*), Western blacklegged tick (*I. pacificus*), Groundhog tick (*I. cookei*), Gulf Coast tick (*A. maculatum*), Lone star tick (*A. americanum*), Rocky Mountain wood tick (*D. andersoni*), and soft tick (*Ornithodoros*; **Fig. 1**).

For the first iteration of the system, a learning model that could accurately identify the aforementioned three most disease-producing tick species was created. Inception v3 (a CNN) was used in a previous version of the app, reaching an accuracy of 80%; however, after closer examination, the model was overfitting and misclassifying ticks and non-ticks. Thus, a different CNN, Xception, was

Figure 1. A taxonomic representation of the ten species used for tick identification. Photos of the American dog tick (*D. variabilis, D. similis*), Asian longhorned tick (*H. longicornis*), Brown dog tick (*R. sanguineus*), Eastern blacklegged tick (*I. scapularis*), Western blacklegged tick (*I. pacificus*), Groundhog tick (*I. cookei*), Gulf Coast tick (*A. maculatum*), Lone star tick (*A. americanum*), Rocky Mountain wood tick (*D. andersoni*), and soft tick (*Ornithodoros*) are featured.

chosen to detect whether a tick was present or not as well as determine the species of tick. After fine-tuning the subsequent version of the model that could identify ten species, a risk assessment was created based on the CDC's U.S. tick-borne surveillance data. For user accessibility, an iOS mobile application with other useful features was deployed onto the Apple App Store.

Validation, Testing, Dataset Preparation, and Data Preprocessing

For the three-species-model, a dataset was curated. To collect the image sets, web scrapers were utilized to collect photos of each class of ticks from readily available datasets on Google Images, iNaturalist, and other image platforms. Manual cleaning of noise and removing photos of mislabeled species was implemented to ensure the dataset used was morphologically correct (**Fig. 1**). Most images were also from databases that genetically confirmed their species classification using molecular assays (Luo et al., 2022). Images were then cropped and resized to 500 ppx to prevent object (tick) occlusion. The cleaned dataset included 3,123 photos: 776 "non-ticks," 743 American dog ticks, 807 Eastern blacklegged ticks, and 797 Lone star ticks. Each class equally represented 25% of the set, and model weights were adjusted accordingly to balance the set.

After the three-species model was built, the training, validation, and testing datasets were built for the ten-species neural network. This dataset featured 709 "non-ticks," 579 American dog ticks, 849 Blacklegged ticks, 809 Lone Star ticks, 409 Brown Dog ticks, 612 Rocky Mountain ticks, 644 Gulf Coast ticks, 683 Asian longhorned ticks, 780 Groundhog ticks, and 312 soft ticks (**Fig. 1**). Of note, the Eastern and Western blacklegged tick were combined into one class, because the features were too similar for a neural network to differentiate, and hence a heuristic algorithm was applied which will be discussed later. For classes such as soft ticks where there was a lack of available photos, the class weights were adjusted to compensate for imbalance and impact during the training process.

The data pipeline for both iterations was comparable. To prevent data leakage between classes, a two-step dataset split process was performed. The first split divided the dataset into training, validation, and testing subsets. The second split, exclusively applies data augmentation to the training set and splits the rest of the dataset into training, validation, and testing. Following the data preprocessing and pre-cropping to 500 ppx, data augmentation was implemented to increase the data variability by creating three copies of all the photos each with different orientations. The testing set was isolated from the training pipeline to safeguard against data contamination and biased model performance evaluation. By presenting the testing data, or the "unseen" and new data, to the model, the unbiased performance could be evaluated.

Ticks are very small ranging from 3-5 mm; therefore, their varying appearances are difficult to detect by the human eye as well as for some neural networks (Rich et al., 2022). The Xception model is known for its ability to pick up small features (Chollet, 2017), however, small ticks were originally a challenging task. To overcome this problem, tiny black squares were placed on the data set to generate noise and enable the model to pay closer attention to minor feature changes, such as color, distinctive dots and shapes, and tick shape (**Fig. 2**). After applying the black dots, the model showed an ability to generalize, which was later used for the ten ticks.

Figure 2. Sample selection of photos of female and male *I. scapularis, A. americanum, D. variabilis, D. similis,* and a "Non-tick" class that were used for training with the "Black Dot" method. Black dots were used to induce noise and allow model generalization.

Model Architecture and Selection

For the task of tick image classification, a CNN was chosen (Rich et al., 2022). The architecture of the model, however, would be crucial to the performance, so different CNNs were tested and compared for different iterations and number of species detected as well as their computational efficiency and ability to generalize.

The models tested were ResNet50V2, ResNet152V2 (an improvement of ResNet50), and Xception. ResNet50V2, from the ResNet architecture (He et al., 2016), has a 50-layer deep architecture that is able to identify shortcut connections to improve gradient flow and ease of training. It is often pre-trained on large datasets to employ transfer learning, allowing it to be suitable for complexity and performance. ResNet152V2 is more complex than ResNet50V2 with a 152-layer deep architecture. It is also often used for transfer learning due to its pre-trained weights. Xception, or "Dense Extreme Inception," builds on the Inception architecture and replaces traditional convolutions with depth-wise separable convolutions for better feature extraction (Chollet, 2017).

Each model was trained for 10 epochs or periods of time, and the performances were compared (**Fig. 3-5**). ResNet50V2 ended with a loss of 0.2225 (**Fig. 3A**) and accuracy of 0.922 (**Fig. 3B**). The model performed well across the loss and accuracy metrics, however, the high testing and validation accuracy indicated the model was overfitting on the training and validation datasets, while not performing well on the test dataset. ResNet152V2 ended with a loss of 0.3125 (**Fig. 4A**) and an accuracy of 0.844 (**Fig. 4B**). Similar to ResNet50V2, the training and validation sets displayed high accuracy, the test set did not perform well. Last, Xception was tested, ending with a loss of 0.2708 (**Fig. 5A**) and accuracy of 0.8937 (**Fig. 5B**). Unlike the other models tested, Xception performed well and was not overfitting as seen in the consistency of the training, validation, and testing accuracy metrics. The dotted line, denoting the testing accuracy, was around the training and validation accuracy (**Fig. 5B**), while the other models' training and validation accuracies were well above the dotted line (**Fig. 3B, 4B**).

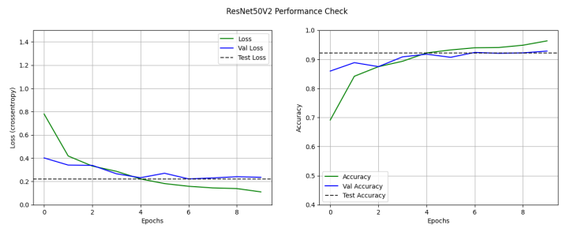

Figure 3. Model performance of ResNet50V2. **A)** Train, Validation, and Test loss metrics. **B)** Train, Validation, and Test accuracy metrics. The dotted line is the test accuracy (B), which indicates overfitting due to the high train and validation accuracy and lower test accuracy.

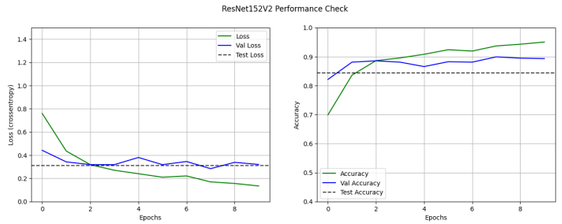

Figure 4. Model performance of ResNet152V2. **A)** Train, Validation, and Test loss metrics. **B)** Train, Validation, and Test accuracy metrics. Similar to ResNet50V2, the dotted line is the test accuracy (B), which indicates overfitting due to the high train and validation accuracy and lower test accuracy.

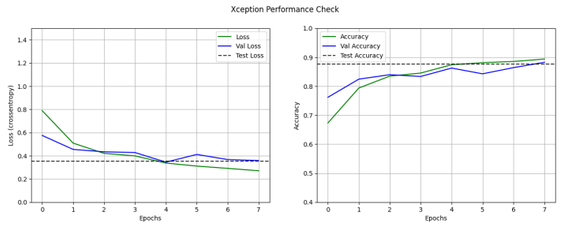

Figure 5. Model performance of Xception. **A)** Train, Validation, and Test loss metrics. **B)** Train, Validation, and Test accuracy metrics. Unlike the ResNets, Xception was not overfitting, which can be seen as the three train, validation, and test accuracy metrics are consistent.

Thus, Xception was the appropriate architecture for the tick classification CNN due to its feature extraction and depth, as seen in the model parameters (**Table 1**). The CNN consisted of multiple layers, pooling, and fully connected layers designed for the challenging task of identifying minute differences for tick species identification.

Model	Xception
Trainable parameters	21,086,252
Non-trainable parameters	54,528
Total parameters	21,140,780

Table 1. Xception model architecture and parameters for identification of three species.

While the Xception model worked well for three species, the architecture had to be reevaluated for the implementation of ten-species classification. The same networks ResNet152V2 (**Fig. 6A**), Xception (**Fig. 6B**), and ResNet50V2 (**Fig. 7B**) were tested and evaluated with the addition of MobileNetV2 (**Fig. 7A**), a lightweight architecture often used in mobile applications (Howard et al., 2017). It is resource-efficient in constrained environments, which is less relevant in this study since high accuracy is prioritized. Both ResNet152V2 and Xception are computationally intensive and have similar results (**Fig. 6A-B**) as ResNet50V2 (**Fig. 7B**). ResNet50v2, an improved version of ResNet50 (He et al., 2016), balances efficiency and performance with architectural features, pre-activation, bottleneck structures, and pre-trained weights that make it effective for feature extraction and stable training. It consistently outperformed the other models in terms of its validation accuracy and loss (**Fig. 7B**). Thus, ResNet50V2 was chosen (**Table 2**), as highlighted in **Figure 7B,** for the task of identifying the ten species due to its robust initial performance and potential for future facile fine-tuning.

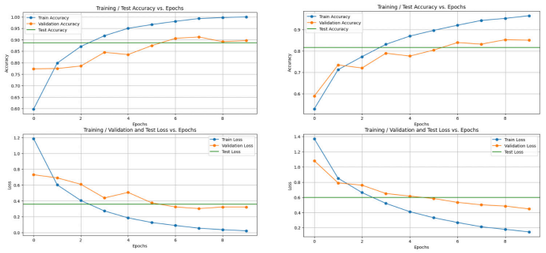

Figure 6. Model performance of ResNet152V2 and Xception. **A)** ResNet152V2 Train, Validation, and Test loss metrics. **B)** Xception Train, Validation, and Test accuracy metrics.

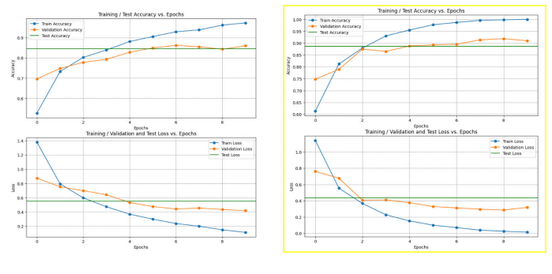

Figure 7. Model performance of MobileNetV2 and ResNet50V2. **A)** MobileNetV2 Train, Validation, and Test loss metrics of MobileNet. **B)** ResNet50V2 Train, Validation, and Test accuracy metrics.

Model	ResNet50V2
Trainable parameters	23,782,922
Non-trainable parameters	45,440
Total parameters	23,828,362

Table 2. ResNet50V2 Model Architecture and Parameters For Identification of Three Species.

Model Finetuning

After Xception was chosen for the three ticks, 13 hyperparameters, such as batch size, learning rate, and dropout rate, were adjusted to fine-tune the model in order to reach peak performance and create a robust learner, preventing the model from overfitting (**Table 1**). Similar hyperparameter optimization was implemented for the ten species with ResNet50V2 (**Table 2**) since Xception was no longer suitable as more species were added. For the ten species, the model adjustments took ~19 hours to train and ended with a validation accuracy of 97.2%.

The "Window Algorithm"

While the validation accuracy, after running the model with the test dataset, was relatively high, a collection of real-world

Figure 8. Novel Window Algorithm implementation. **A)** A zoomed-out photo was given to the model with a tiny tick surrounded by a noisy background. **B)** Model zoomed in. **C)** Model cropped again. **D)** Final cropped image that allowed for more than 95% confidence that a tick was present and the species was correct.

tick photos that the model would newly encounter yielded only an accuracy of 46%. The testing data were structurally different from the training and validation sets because they often featured occluded tick images and other realistic yet sometimes unusual user-generated photo scenarios. Most of the issues were related to the model detecting "non-tick" when, in fact, there was a tick present. To overcome this, the "window algorithm," was designed and implemented. The window algorithm continuously cropped the photo until over 95% confidence was reached by the model with regard to the species classification or whether a tick was present. If the model reached 95% confidence on a species, then the predicted class was presented to maintain computational efficiency. If the model reported over 95% confidence that there was no tick present, the window algorithm would also be run.

In **Figure 8**, the model was given a photo of a zoomed-out tick, which was a Lone star tick by its true label. When the zoomed-out photo was tested, the model predicted "non-tick" with 0.9999323 confidence (**Fig. 8A**). After running the window algorithm once, the model once again predicted "non-tick" with 0.9998989 confidence (**Fig. 8B**). The second time, the CNN predicted a "Lone star tick" with 0.7222055 confidence (**Fig. 8C**). While the model eventually predicted the true class, 72% confidence was too low to report to users. Thus, the algorithm was run again, and the CNN predicted "Lone star tick" with 0.998703 confidence (**Fig. 8D**). This demonstrated the network's ability to use real-world examples despite the small size challenge of ticks. If the model does not reach 0.95 confidence that there is a tick in the photo, the user will receive a "tick not found" notification, which allows them to re-center the tick for optimal results.

Location-Based Heuristic Algorithm Approach

While the window algorithm significantly improved the model performance for three types of ticks, as more species were added, the neural network misclassified certain types, such as the Eastern and Western blacklegged ticks, whose inter-species differences are highly refined. However, their geographic ranges are different. While all blacklegged ticks are morphologically similar feature-wise, it is rare to find an Eastern blacklegged tick in California or a Western blacklegged tick in Connecticut (Nieto et al., 2018). Utilizing the CDC's tick surveillance data by state, the probability of a certain tick found in a given geographic region could be ascertained. When the user takes a photo of a tick with the app, their geographic coordinates will be assigned to a respective state (or country if outside the U.S.).

Tick-Borne Disease Risk Assessment

Information for the spatiotemporal risk assessment was used from the CDC's Tickborne Pathogen Surveillance data. The CDC's dataset displayed the prevalence of the following vectors in the U.S. by species: LD, Tularemia, Spotted Fever Rickettsiosis (SFR), Anaplasmosis, *Ehrlichia chaffeensis, E. ewingii, babesiosis, Powassan encephalitis*, hard tick relapsing fever, Bourbon virus disease, and Southern Tick-Associated Rash Illness (STARI).

Of note, because of the COVID-19 pandemic (Boyce et al., 2023), some of the TBD datasets only go back to 2019; and due to lack of available data and privacy issues, the risk assessment currently works only in the U.S. and South America. The ticks most documented by the CDC are the Lone star tick, American dog tick, and the Eastern blacklegged tick. The CDC's National Notifiable Diseases Surveillance System (NNDSS) releases a dataset including pathogen prevalence by geography and displays the annual incidence of diseases in the U.S.. For example, the I. scapularis set reports the following pathogens: *Borrelia burgdorferi sensu stricto* (responsible for LD), *B. mayonii, B. miyamotoi, Anaplasma phagocytophilum, E. muris eauclairensis* (EME), *Babesia microti*, and the Powassan (POW) virus. Furthermore, due to the lack of information available in some areas, location-based weighting was applied to the CDC dataset based on population size and the proportion of infected individuals from tick bites to predict the risk of infection if a user in a given region is bitten. Moreover, since there is some known underreporting of cases, tick risk analysis must take into account that actual risks may inevitably be higher or lower, respectively.

For easier public use, a gradient scale from "low level" to "high level" of danger was implemented (**Fig. 9E**). However, a user of the app can also view the full risk breakdown of the pathogen prevalence by selecting "view risks," which displays more risk details associated with notifiable TBDs. Prior to taking the photo of the tick, the user fills out a form that provides ground information for their risk assessment. In the form, the user is asked if the tick has bitten someone or an animal and later asks if the tick was engorged or enlarged when found (**Fig. 9C**). The risk will only be considered "high level" if a tick bite was reported.

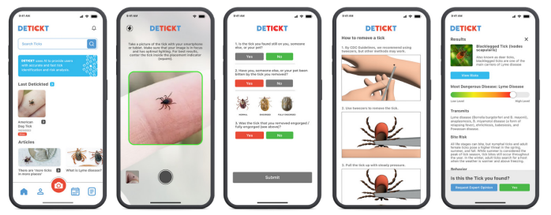

Figure 9. DETICKT IT UI / UX Schematic. **A)** The DETICKT IT homescreen where users can see ticks that they have identified. **B)** The camera feature to take the photo of the tick. **C)** After users take the photo, they will be prompted to fill out the risk background form which will further inform the risk assessment. **D)** If the user selects that they still have a tick attached, a tutorial on how to remove a tick based on CDC guidance will appear. **E)** After prior steps, the user will receive the tick species as determined, their potential risk of a tick-borne infection, and more information about the tick.

iOS Application User Interface (UI) / User Experience (UX) Design and Features

DETICKT IT was originally developed in Xcode with Swift within a service-oriented architecture. After the CNN model (Figure 7B) was ported from a local environment to Amazon Web Services (AWS), open application programming interfaces (APIs) were created for functions, such as specimen identification, location finder, and risk assessment. Using this approach, DETICKT IT is able to scale through AWS Lambda functions, thereby delivering results to the app's users in mere seconds. The code was later migrated to Flutter because it serves as a codebase for both iOS and Android – with a view toward eventually increasing availability to Android users as well.

DETICKT IT has an active "tick-bite" data pipeline, which allows users who have been bitten or are interested in the definitive identification of a tick they have found to upload their images (Figure 9). There are other features, such as a "Learn More" (Figure 9E), where users can learn about other ticks and track their symptoms after a tick bite. Figure 9 outlines the prominent features of the mobile application.

Results

Model Performance Metrics

The model was first evaluated for three species and later for ten. After using the Xception CNN and implementing the window algorithm, an accuracy of 97% was reached for the three most prevalent ticks. After optimization with the ResNet50V2 model, the accuracy for ten ticks was also 97%. Prior to implementing the window algorithm, the confusion matrices, which display the model's performance in terms of true labels versus the predicted label (**Fig. 10**), indicate few false positives and false negatives in both the validation and the test sets. However, it is important to evaluate the model with unfamiliar datasets containing images that feature partially occluded ticks and other real-world scenarios (including less-than-ideal photographic settings). This was a challenge for the model and resulted in only 46% accuracy, but the novel window algorithm provided a feasible approach to dealing with realistic, user-produced images.

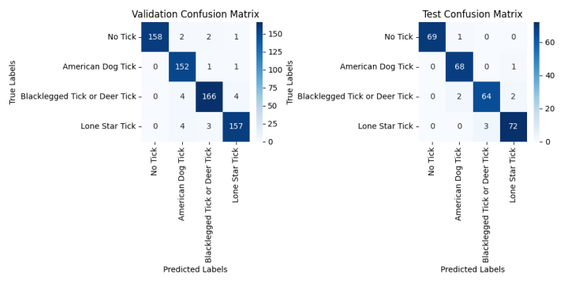

Figure 10. Test and validation confusion matrices prior to implementing the Window Algorithm. **A)** Validation and **B)** Test Confusion Matrices. The implementation of the window algorithm decreased the errors of the model due to feature-based classification.

After applying the window algorithm for N = ~45 in each class, there were only very minor classification errors (**Fig. 11**). Of note, the errors occurred when the model reported that there was no tick present when in fact there was. This indicates that the model was not confusing the species, but rather having difficulty locating the tick in the photo. Therefore, after refining the window algorithm, the accuracy of the model was increased to 97%. In one notable instance, during preprocessing, an image was misclassified by human error but was correctly identified by the model, another display of the CNN's ability to generalize.

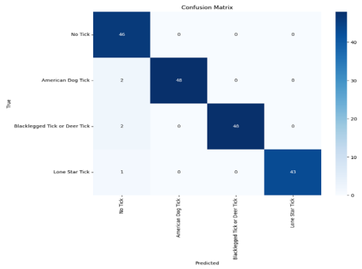

Figure 11. Confusion matrix after implementing the Window Algorithm. Minimal errors are occurring, as seen in the distribution. The model was not confusing species but rather could not always detect a tick in a photo.due to feature-based classification.

F1, accuracy, recall, and precision scores were calculated for the three ticks (**Table 3**). These reflect the model's performance and ability to generalize. The eventual model ended up performing overall with 0.98 precision, 0.97 recall, and 0.97 F1 score.

The three-species model worked well as seen in the confusion matrices (**Fig. 11**), so gradually more species were added until classification of ten species was reached. In **Figure 12**, confusion matrices for six ticks are shown. The classification report indicated that the network struggled with classifying the Rocky Mountain wood tick with only 0.47 precision. The CNN's overall performance had 84% accuracy after the addition of three new types of ticks. Due to the morphological similarities between the Rocky Mountain wood tick, the American dog tick, and the Gulf Coast tick, the aforementioned location-based heuristic algorithm was applied. This greatly increased the model's accuracy to 93% (**Fig. 12B**). **Figure 12B** is the confusion matrix post-algorithm utilization with fewer classification errors committed.

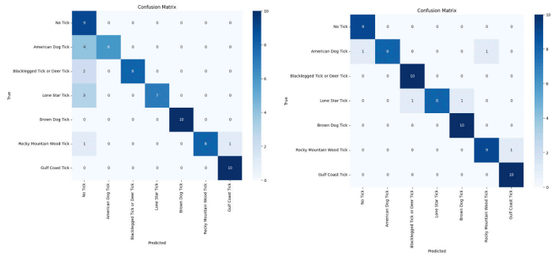

Figure 12. Confusion matrices for six ticks with Window Algorithm pre- and post-Location Heuristic Feature. **A)** Confusion matrix without the location feature. **B)** Confusion matrix post-location feature algorithm applied, greatly improving the performance of the model.due to feature-based classification.

After 93% accuracy was achieved and the location-based algorithm was used as well as after changing the model to ResNet50V2, the ten-species model was tested. While adding these three species, the Asian longhorned tick, the Groundhog tick, and the soft tick, they all had distinct features which were not challenging for the model to identify. **Figure 13** displays the validation and test confusion matrices for the ten species. There is a very minimal error rate for both matrices, which is indicative of the model's high performance (**Fig. 13**).

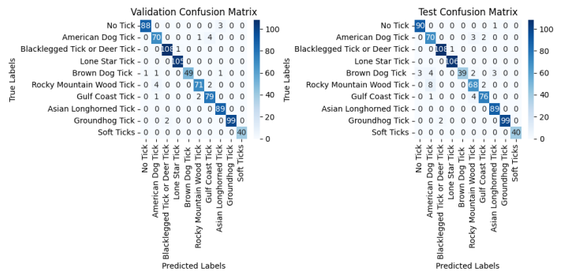

Figure 13. Confusion matrices after implementing the Window Algorithm. Minimal errors occurred, as seen in the distribution. **A)** Validation confusion matrix. **B)** Test confusion matrix.

After using random photos not contained in the training set as well as applying both the window and location algorithm, the confusion matrix in **Figure 14** was produced. There are not many classification errors, and the remaining challenges relate to interspecies similarities. Overall, the model for ten species ultimately reached an accuracy of 97%, precision of 96%, recall of 97%, and F1 score of 96%. After applying these innovative algorithms in concert, the accuracy of this latest model is exhibiting real-world viability.

Model	Precision	Recall	F1 score	Overall Accuracy
Three Species	0.98	0.97	0.97	0.97
Ten Species	0.96	0.97	0.96	0.97

Table 3. Performance Metrics of Both Models: Precision, Recall, and F1 Scores.

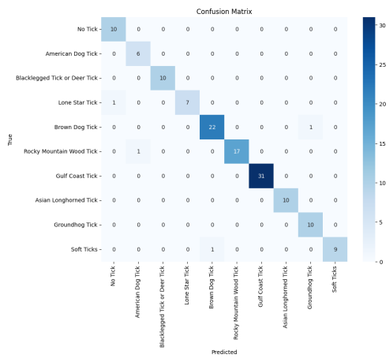

Figure 14. Confusion matrix for ten species and "non-tick" control class after implementing the Window Algorithm and Location-Based Heuristic Algorithm. Minimal errors are seen in the distribution with a relatively high true positive rate.

Discussion

The performance metrics indicate that the neural network can suitably generalize and classify ticks at a relatively more accurate (97%) and certainly faster rate than the general public (Nieto et al., 2018). The gradual expansion to ten species allowed for the meticulous optimization and refinement of the CNN architecture. Moreover, the implementation of the location-based heuristic and novel window algorithms represents an innovative approach to achieving real-world applicability and scalability. The window algorithm can also be applied to other problems, such as medical imaging (Müller et al., 2021) or computer vision tasks where minute object features are difficult to detect or discern.

While a machine learning-based approach to tick identification has been explored, this novel mobile app can identify the ten most common and disease-causing species and predict the risk posed by each tick (by connecting to the CDC's tick-borne disease prevalence database). Based on the literature, this is the first implementation of a machine learning-based app that can identify ticks with high (97%) accuracy as well as provide a qualified assessment of the potential risk of infection.

There are current limitations with the availability of data in regard to tick photos and tick-borne disease surveillance databases. Due to the lack of available data, transfer learning was also utilized to compensate for any data disparities. Future versions of the app include expanding the geographic regions to other countries (data availability permitting) to provide this service more globally to tick bite victims.

One of the most challenging aspects of tick species classification is the minute appearance differences between species. While 97% is relatively high and due to the model's reported performance, which is indicative that the network isn't overfitting, there is still uncertainty with the quality of user-produced images. Since this mobile device- and user-centric approach is highly dependent on the quality of the photos received, during training and testing, real-world scenarios of occluded, relatively blurry, and zoomed-out photos of ticks were used, yet these approaches still may not have accounted for all user-produced errors.

The risk of tick-borne diseases varies by stage of the tick and sex (Eisen et al., 2016); therefore, a viable computer vision-led approach to determining disease risk must be able to account for these differences. With certain species, the male ticks look distinct from their female counterparts – visual differences an ML model can learn to distinguish. In future iterations, the model would incorporate training a female and male class for each species, whereas at present no delineation is made between male and female species during training. Once again, accurate photographic data availability / accessibility remains a significant issue for further improving overall performance with the incorporation of this new feature.

This mobile app has the potential to significantly outperform medical professionals' reported ability to accurately classify ticks (Nieto et al., 2018). Moreover, the embedded tick risk assessment feature allows users to gain awareness of the risks associated with a given tick (bite) in a certain geographic area and in real time. The knowledge and confidence gained will facilitate diagnostic accuracy and shorten critical time-to-treatment, enabling affected individuals to seek medical attention. DETICKT IT is currently available on Apple's App Store and is free, with ~3,000 downloads (as of 09/01/24).

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How *Bostock v. Clayton* County Protects Harvard University's Final Clubs

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This essay analyzes how the Supreme Court's decision in *Bostock v. Clayton County* on sex discrimination applies to Harvard University's lawsuit with the Final Clubs. Other Supreme Court cases supporting the right to intimate association for private clubs are also considered in the context of the lawsuit. Ultimately, *Bostock* determines that members of the Final Club are protected from sanctions, reaffirming their right to intimate association.

The issue first takes context within the history of Harvard, the Final Clubs, and their recent relations, most importantly Harvard's sanctions and the subsequent lawsuit. Next, this analysis of the U.S. Supreme Court case *Bostock v. Clayton County* establishes the concept of "but-for" causation for sex-based discrimination. This standard is then applied to Harvard's case to determine that Harvard's sanctions did indeed constitute sex discrimination.

Harvard's final clubs are also compared to and contrasted with other private clubs, such as Princeton's eating clubs and Boston's Somerset Club, and Harvard's lawsuit is further evaluated according to legal proceedings regarding private clubs, such as *Roberts v. U.S. Jaycees and Board of Directors*, *Rotary International v. Rotary Club of Duarte*. These cases provide the right of private clubs to intimate association, and comparisons to other clubs allow specific conclusions to be drawn about when a private club has that right.

Finally, the discussion explores the implications of these findings for unrecognized student organizations and students' personal relationships more broadly.

Introduction

As ancient, secretive clubs with elite alumni, Harvard University's single-sex final clubs have naturally stimulated much curiosity about what happens inside and how to join. Just as naturally, the exclusive clubs have also inspired conflict. Recent events highlight the interest and controversy surrounding the Final Clubs, such as the recall of Harvard College's student body co-president after a Final Club expelled him, bringing allegations of misconduct to light (Srivastava and Tan, 2024). Harvard's struggle lies in the fact that it cannot directly regulate the clubs, which lack recognition from Harvard and do not seek it, which has led the university to try influencing club members indirectly through sanctions. Thus, the question of how Final Clubs, other unrecognized groups, and their members are legally protected from Harvard's regulatory authority remains of great importance.

Harvard first attempted to sanction members of the single-sex Final Clubs in 2016, promptly receiving a lawsuit from some of the clubs. During the course of the case, the Supreme Court decided *Bostock v. Clayton County* (2019), making a landmark legal determination of sex discrimination by employing a "but-for" causation test: a method to determine if an outcome would have occurred if only one factor, such as sex, had been different. Fascinatingly, Harvard interpreted *Bostock* as protecting the sex-discriminating Final Clubs. Applying *Bostock*'s "but-for" test to Harvard's sanctions reveals that the sanctions themselves discriminated on the basis of sex. Therefore, the Final Club members are protected from sanctions by *Bostock*, reaffirming their right to intimate association. Ultimately, students have a right to organize and associate privately, even if it is odious to the university.

This essay first contextualizes Harvard's historical relationship and recent lawsuit with the Final Clubs, which ended shortly after the *Bostock* decision. Next, *Bostock*'s "but-for" test is analyzed to clarify how sex discrimination is determined. *Bostock*'s reasoning is then extended to Harvard's lawsuit to show that Harvard's sanctions discriminated according to sex. Other court decisions regarding discriminatory private clubs are also identified as protecting Final Club members' right to intimate association. Finally, how the protections for Final Clubs apply to other student organizations is considered.

Harvard's History, Sanctions, and Lawsuit with the Final Clubs

While they are sometimes compared to fraternities, Harvard's final clubs are a special type of undergraduate social organization due to their age, selectivity, and elusiveness. Therefore, when judging the legal merit of Harvard's sanctions, the Final Clubs must be evaluated according to their unique history and qualities.

As of 2021, there are six all-male Final Clubs (Scherer, 2021). Most of these are at least one hundred years old, date to "the 19th century and have had Kennedys, Roosevelts, and an endless procession of politicians, writers, and businessmen as former members" (Greenbaum, 2018). The Fly Club, for example, is almost two hundred years old, and in order to become a member, a student must first be invited to the "punch," similar to a fraternity's rush. Of the approximately one hundred students "punched," about twenty become members (Horton, 1992). The "punch" process produces a highly selective membership featuring "the wealthiest and connected individuals" (Scherer, 2021). Once students become members, they have access to the "clubhouses usually including dining areas, libraries, and a game room" (Scherer, 2021). The Final Clubs are also notably secretive. Some clubs have "strict rules about speaking with the press", and the Porcellian Club, for example, does not allow non-members to enter its clubhouse (Greenbaum, 2018). In short, the Final Clubs are longevous, opaque, and exclusive.

One might then compare the Final Clubs to Harvard for much of its history and would not be alone in doing so. Drew Faust, Harvard's president at the time of the sanctions, wrote that "the final clubs in particular are a product of another era, a time when Harvard's student body was all male, culturally homogenous, and overwhelmingly white and affluent." Some members have countered that the Final Clubs are now racially and economically diverse (Greenbaum, 2018). Nevertheless, six Final Clubs remain all-male, and that is the root of the university's conflict with them.

Given their historical similarities, there was a time when Harvard had a congenial relationship with the Final Clubs. Harvard even used to "give preference to final club students for a special scholarship given to upperclassmen" (Horton, 1992). However, the university ended its official connections with the Final Clubs in 1984 because they "refused to admit women" (Horton, 1992). While Harvard's conflict has historically been with the all-male Final Clubs, there are now all-female Final Clubs as well (Scherer, 2021). In fact, one motivation for Harvard's sanctions was tied to a report that stated "a Harvard College woman is half again more likely to experience sexual assault if she is involved with a Club than the average female Harvard College senior," whether that was because she went to parties at all-male Final Clubs or she was in an all-female Final Club (Greenbaum, 2018). Another motivation was that the Final Clubs' exclusive selection practices contradicted Harvard's purported values of "gender, race, and socioeconomic" diversity (Greenbaum, 2018). Thus Harvard, in an attempt to combat discrimination, promote equality, and prevent sexual assault, developed sanctions against the Final Clubs.

In 2016, Harvard announced its sanctions against students who join "unrecognized single-gender social organizations." The sanctions would prevent students in these organizations from holding leadership positions in Harvard-recognized organizations and make them ineligible to receive the university endorsements necessary for scholarships such as the Marshall and Rhodes (Greenbaum, 2018). The sanctions applied to both all-male and all-female Final Clubs, as well as fraternities and sororities, and as a result, some clubs became gender-neutral in order to avoid the sanctions (Scherer, 2021). Other clubs, though, did not acquiesce, instead filing suit.

In *Kappa Alpha Theta Fraternity, Inc. v. Harvard University*, three fraternities, two sororities, and three students sued Harvard, alleging that its sanctions violated Title IX of the Educational Amendments of 1972. While no Final Clubs were directly involved in the lawsuit, they were considered alongside fraternities and sororities. When Harvard motioned to dismiss the lawsuit, the U.S. District Court for the District of Massachusetts only agreed that the two sororities and one of the students lacked standing, denying the rest of Harvard's motion (Kappa Alpha Theta Fraternity, 2019). In the eyes of then-Harvard President Lawrence Bacow, the District Court's denial seemed to signal that it "would ultimately grant judgment in the plaintiffs' favor" (Knieriem and Schumer, 2020). Before the case could be decided, however, Harvard dropped its sanctions following *Bostock v. Clayton County*, a Supreme Court decision on sex discrimination under Title VII of the Civil Rights Act of 1964. Since Harvard dropped its sanctions, the legality of the sanctions was never decided by a court (Knieriem and Schumer, 2020). Thus, the legal protection of Harvard's final clubs remains an open question, and one that must first be considered where it ended: with *Bostock*.

Sex Discrimination as defined by *Bostock v. Clayton County*

To determine whether employment discrimination because of sexuality or transgender identity constitutes sex discrimination, *Bostock* employs a "but-for" test to isolate the sex of the individual. Such a test reveals that sex can be an implicit reason for discrimination even if the discrimination ostensibly targets something else. Therefore, any factor in which sex plays a role is protected from discrimination under Title VII.

Bostock establishes "but-for" causation to prove when sex discrimination has occurred, even if sex is not the obvious or direct cause. While sexuality, for example, is not equal to sex, the Supreme Court tested whether it depended on sex by "chang[ing] one thing at a time and see[ing] if the outcome changes" (Bostock, 2020). To clarify the role that sex plays in sexuality so that sex alone can be changed, an employment policy against homosexuality can be described as one against men who are attracted to men. Then, to determine whether such a man has faced discrimination, the Court, using the "but-for" test, changes one aspect (the sex of the man) and questions whether a woman who is attracted to men would have been treated the same. Since a woman who is attracted to men is not homosexual, she would have been treated differently, and thus, policies against homosexuality necessarily discriminate based on sex. Essentially, it is discriminatory for employers to penalize "men for being attracted to men and women for being attracted to women" but not women for being attracted to men and men for being attracted to women (Bostock, 2020).

The Court rejects several counterarguments, affirming that anything that depends on sex is protected. Firstly, "a defendant cannot avoid liability just by citing some other factor" (emphasis in original) (Bostock, 2020). Even if homosexuality is an employer's primary concern and the employer does not care if it discriminates against men or women, sex is still a fundamental component of sexuality and thus the employer's concerns. Additionally, it is not redeeming that an employer "discriminates against both men and women because of sex" (Bostock, 2020). For example, a policy against homosexuality is not allowed even though it applies to both men and women because it discriminates against men attracted to men and women attracted to women, which is still sex-based. Furthermore, a policy can be discriminatory at the individual level "even if the scheme promotes equality at the group level," such as a pension plan that tries to account for life expectancy by requiring women to pay more (Bostock, 2020). It makes no difference whether an employer has other intentions, be it prejudice against homosexuality or a goal for equality, if the employer's treatment depends in any part on the sex of the individual.

The Court also rejects the notion that changing someone's sex in the "but-for" test requires changing other factors, grounding discrimination in the sex of the individual alone. To test whether a homosexual man has faced discrimination, the "but-for" test does not "just change his sex. Along the way, we change his sexual orientation too (from homosexual to heterosexual)" (Bostock, 2020). However, the behavior in question (being attracted to men) remains the same, and the Court rejects the idea that the test should change "both his sex and the sex to which he is attracted" (Bostock, 2020). Doing so obfuscates the fact that an employer opposed to homosexuality tolerates attraction to men in women but not in men. Discriminating against other factors that depend on sex is not allowed, and if the "but-for" test changes a factor, that factor must depend on sex.

However, *Bostock* acknowledges significant limitations on the decision. Rather than accepting broad consequences in the ruling, the decision specifies that "[u]nder Title VII, too, we do not purport to address bathrooms, locker rooms, or anything else of the kind" (Bostock, 2020). Thus, *Bostock* is a ruling that only firmly addresses the issue of sex discrimination in employment. Although the decision declines to make any sweeping judgments, it does not place any limits on its reasoning for what is sex-based. Instead, the decision emphasizes that what separates this case from others is the definition of discrimination, which, "[a]s used in Title VII… refers to 'distinctions or differences in treatment that injure protected individuals'" (Bostock, 2020). Thus, while sex-segregated bathrooms are indeed sex-based, it is not necessarily an injurious difference in treatment to require men to use one bathroom and women to use another. While *Bostock* confines its decision on what constitutes discrimination to Title VII, it does not limit the "but-for" test for determining what is sex-based.

Despite limiting its judgment to Title VII, the *Bostock* decision still establishes an expansive definition of sex-based discrimination. While it may not be immediately clear why factors tangentially related to sex–such as same-sex relationships–are protected in the same way as sex itself, the Court's ruling clarifies that whenever changing someone's sex would change another characteristic, that characteristic is considered sex-based and is protected as such.

Bostock v. Clayton County as applied to Harvard's Lawsuit with the Final Clubs

The principles established in *Bostock* are relevant to Harvard's lawsuit with the Final Clubs because both cases rest upon determining if certain actions constitute sex discrimination, as recognized by Harvard itself (Knieriem and Schumer, 2020). Using *Bostock*'s "but-for" test, Harvard's sanctions against single-sex organizations are shown to discriminate according to sex in the same way that employment policies against single-sex relationships would.

It is first worth considering whether *Bostock*'s limited Title VII decision is directly applicable to Harvard's case. As a federally-funded educational institution, Harvard is subject to Title XI, which also prohibits discrimination based on sex (Kappa Alpha Theta Fraternity, 2019). In *Kappa Alpha Theta Fraternity, Inc. v. Harvard University*, the ruling on Harvard's motion to dismiss the lawsuit, the U.S. District Court for the District of Massachusetts pairs Title VII to Title XI since "Courts in the First Circuit cite cases from the Title VII context in analyzing the scope of Title IX" (Kappa Alpha Theta Fraternity, 2019). Thus, while *Bostock* is partially restricted to cases of employment under Title VII, it can nonetheless be fully applied to Harvard's lawsuit, which falls under Title IX. While *Bostock*'s reasoning for what is sex-based would be applicable regardless, the connection between Title VII and Title IX means that *Bostock*'s definition of discrimination extends to Harvard's case as well. Harvard's sanctions did not allow students in single-sex groups to hold official leadership positions or receive scholarships requiring school endorsement. As such, Harvard was "treating that individual worse than others who are similarly situated" (Bostock, 2020). The *Bostock* decision is directly applicable, and Harvard was treating Final Club members discriminately.

Furthermore, *Bostock*'s "but-for" test reveals that Harvard's sanctions against Final Club members were sex-based. A sanction against members of single-sex organizations inherently depends upon the sex of the member, whether they are male or female. For example, Harvard's sanctions applied to male students who joined all-male Final Clubs, but not female students who joined all-male clubs. While this example may seem self-defeating since a female student could never join an all-male club, it follows the Supreme Court's "but-for" test, where only one aspect can be changed.

One may object that if a female student did join an all-male club, it would no longer be all-male, and that is why Harvard's sanctions would not apply. One may then suggest that a man in an all-male Final Club instead be compared to a woman in an all-female Final Club. However, *Bostock* rejects the notion of changing other factors alongside sex. When considering whether a homosexual man faces sex discrimination, he must be compared to a woman who is also attracted to men, not a woman who is attracted to women (Bostock, 2020). Regarding protection from sex discrimination, single-sex clubs and single-sex relationships are therefore analogous.

Additionally, according to *Bostock*, Harvard's sanctions cannot be justified by other means since they have "but-for" causation. While Harvard's direct intention may not have been to discriminate according to sex, it is irrelevant if "other factors" motivated Harvard (emphasis in original) (Bostock, 2020). Ironically, Harvard commits sex discrimination when it sanctions single-sex clubs even if it was attempting to foster "equality at the group level" (Bostock, 2020). Thus, Harvard's sanctions are not justified even though they apply to both men and women. Sanctioning women in all-female clubs and men in all-male clubs is comparable to "an employer who fires both lesbians and gay men equally [which] doesn't diminish but doubles its liability" (Bostock, 2020). Despite Harvard's other motivations, the sanctions do not withstand *Bostock*'s "but-for" test.

Even Harvard seemingly acknowledged that their sanctions would not pass the "but-for" test by ending them, making the lawsuit moot. Scherer correctly points out that Harvard's choice to drop its sanctions "is not binding on other colleges and universities" (Scherer, 2021). Harvard's choice is not binding for itself either, and Harvard may have dropped the sanctions before there was a court decision to avoid being "legally barred" from creating similar policies in the future (Knieriem and Schumer, 2020). The legal avenue for sanctions against single-sex Final Clubs remains open, at least technically. However, Scherer also properly notes that "having such an elite university interpret a Supreme Court decision this way will deter other colleges and universities from enacting similar policies" (Scherer, 2021). Instead of sanctions, Scherer proposes two options for other universities to regulate fraternities and sororities, either unrecognizing all Greek life or keeping it on campus so as to regulate it (Scherer, 2021). Harvard, though, has no such choice, unless the unrecognized single-sex Final Clubs seek recognition.

A specific comparison of *Bostock* to *Kappa Alpha Theta* reveals that Harvard's sanctions against members of Final Clubs constitute sex discrimination. While there may be no legal method for Harvard to regulate single-sex Final Clubs, perhaps Harvard could sanction unrecognized organizations "without using the words man, woman, or sex (or some synonym)," such as by sanctioning all exclusive social clubs (Bostock, 2020). Even then, however, Final Clubs may be protected as intimate associations in the same way that personal relationships are.

Other Court Cases on Discriminatory Private Clubs

Harvard's final clubs are certainly atypical, but there are similar organizations that have inspired informative litigation prior to the *Bostock* decision, creating additional relevant case law. While regulations on other organizations have been upheld, the Final Clubs remain protected because they are non-public, non-business intimate associations.

The Final Clubs are unique but still have their Ivy League peers. Perhaps unsurprisingly for exclusive universities, Brown, Columbia, Cornell, Dartmouth, the University of Pennsylvania, and Yale have "secret collegiate societies" of their own (Scherer, 2021). Princeton's eating clubs are an especially enlightening example, both because they faced lawsuits and because of their key differences from the Final Clubs. The New Jersey Supreme Court ruled in *Frank v. Ivy Club* (1990) that the discriminatory policies of the Eating Clubs were not protected because the clubs have an "integral connection and mutual benefit" with Princeton, since most upperclassmen used the clubs and the university "depended on the clubs to provide meals to students" (Horton, 1992). The Final Clubs, conversely, do not provide any service for most students or the university and as such, they do "not fall under the state public-accommodations law" (Horton, 1992). They cannot, then, be regulated on the same grounds as organizations that provide a public benefit.

Non-public clubs can still be regulated if they have professional purposes, however. Take, for example, the Somerset, a Boston social club considered the "most prestigious of clubs." After the Boston Licensing Board threatened to revoke "the food and liquor licenses of clubs that have more than 100 members, are used for business or professional purposes and choose members on the basis of sex, race, color or religion," the Somerset agreed to admit women (Hornblower, 2000). The "business or professional purposes" are the key distinction between the Somerset and the Final Clubs. Likewise, the Supreme Court has recognized in *New York State Club Association v. New York City* (1988) that cities can pass laws that limit sex discrimination in "organizations which are 'commercial' in nature" (Horton, 1992). The City of Cambridge has had past conflicts with the Final Clubs and may be willing to pass a law regulating them (Edwards and Montgomery, 2024). However, it does not seem that undergraduates represent "the city's business and professional community," nor that there is "business and commerce transacted at the clubs" (Horton, 1992). Since the Final Clubs are non-business organizations, they are protected from regulation under *New York State Club Association*.

The Supreme Court has recognized the rights of non-public, non-business clubs before. Both *Roberts v. U.S. Jaycees* (1984) and *Board of Directors, Rotary International v. Rotary Club of Duarte* (1987) held that organizations have a right to intimate association if they have "a relatively small size, a high degree of selectivity in decisions to begin and maintain the relationship, seclusion from others in critical aspects of the relationship, and purpose of the relationship" (Horton, 1992). Therefore, discrimination in private clubs can be regulated in some cases, such as if a club is large, serves a public purpose, or is commercial in nature. The Final Clubs, as small, selective, and secretive social clubs, are protected as intimate associations.

Discussion

Under the "but-for" test in *Bostock v. Clayton County*, the Final Clubs are protected from Harvard's sanctions on the grounds that the sanctions against single-sex clubs implicitly discriminate according to sex. Even if Harvard attempted to regulate the Final Clubs for reasons other than their single-sex status, the clubs are protected by the right to intimate association recognized by *Roberts* and *Rotary International*. Thus, Harvard's final clubs are protected from University sanctions or regulation. While any sanctions on the Final Clubs would affect a relatively small number of students, the legal protections for Final Clubs are not without their implications for the greater student body.

The Final Clubs, as unrecognized student organizations, are merely intimate associations in the same way as other relationships between students. The protections for Final Clubs, therefore, extend to all private relationships among students, whether that be friendships or unofficial student groups. Given that Harvard would not recognize any new organizations during the 2023–2024 academic year, the importance of protections for unofficial student groups has greater relevance (Jones and Peña, 2023). Additionally, Harvard has no obligation to recognize student groups. While *Healy v. James* (1972) recognized "a 'heavy burden' of justification on a public college or university seeking to deny a student organization recognition and the concomitant benefits," it did not establish such a standard for private universities like Harvard (Hauser, 1990). Since Harvard can limit which organizations are recognized, the rights of unrecognized organizations become paramount.

The protections for the Final Clubs under *Bostock, Roberts,* and *Rotary International* mean that students are protected in their personal relationships from University regulation. In the same manner that Final Clubs are protected from sanctions, no student can be sanctioned merely for belonging to any intimate association. Thus, students are protected in their private actions, whether that be joining a Final Club, personal relationships, or other private groups, regardless of what the university thinks of those actions.

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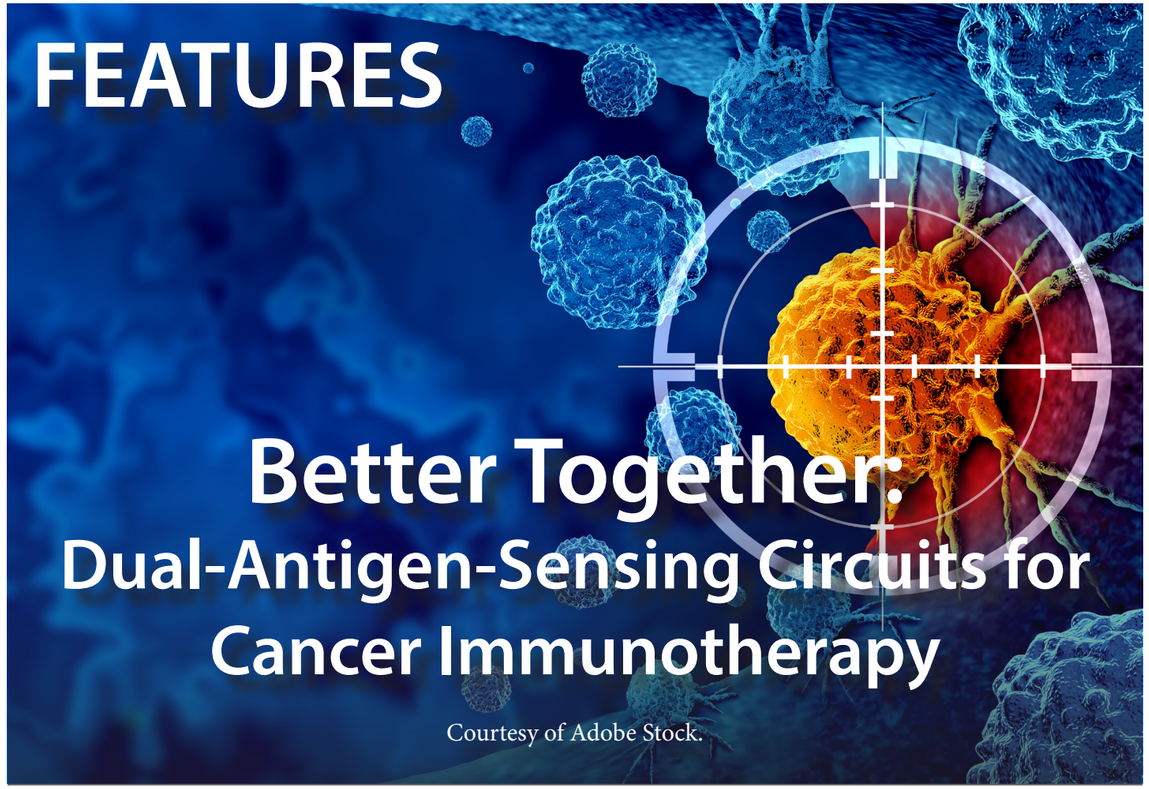
Features



Courtesy of Adobe Stock.

Allison Liu ’28

(figures courtesy of Lale Baylar ’28)

Introduction

Cancer is the second leading cause of death worldwide (Weiss, 2021). To address this global challenge, researchers have developed a toolkit of varied treatment options including chemotherapy, which uses drugs to kill cancer cells; radiation therapy, which directs high-energy rays at tumors; hormone therapy, which inhibits tumor growth; and surgery, which removes tumors directly. One particularly promising and versatile approach to treatment, immunotherapy, harnesses the patient’s own immune system to fight cancer. Cellular immunotherapy—also known as adoptive immunotherapy or T lymphocyte (T cell) transfer therapy—involves engineering the patient’s immune cells to make them better at recognizing and attacking tumors. Cellular immunotherapy is a “living drug” that can potentially remain in the body for years, continuing its anti-tumor activity if relapse occurs (Kumar et al., 2021).

Cellular immunotherapy relies on cytotoxic T cells, a class of white blood cells that kill foreign, infected, and cancerous cells in the body (Richard et al., 2023). T cells identify targets using specialized receptors that recognize specific proteins—called antigens—found on threatening cells (Waldman et al., 2020). Antigen recognition triggers complex networks of signaling proteins and transcription factors within the T cell that mediate its gene expression, thereby activating the T cell and enabling it to kill the antigen-presenting cell.

The immune system naturally kills most spontaneous cancer cells, keeping them from growing into tumors. But cancer is elusive, and the selective pressure exerted by the immune system can cause cancer cells to adapt to present less and less of the nonessential antigens that T cells use as targets, a process called antigen loss (Mishra et al., 2024). If the target antigen has been lost, tumors can escape detection by the immune system. Cellular immunotherapy attempts to address tumor escape by engineering T cells to recognize custom antigens of the designer’s choosing—for instance, essential proteins that tumors are less likely to repress—thereby reducing reliance on default nonessential antigen targets. Scientists equip patients’ own T cells with genes encoding custom-designed synthetic receptor proteins that recognize a particular tumor antigen target. These engineered receptors direct the T cells to attack cancer.

Successes and Challenges

Antigen Specificity

A major advantage of cellular immunotherapy over chemotherapy and radiation therapy is that it targets tumors with cellular-level precision, minimizing off-target damage to healthy cells. T cells’ engineered receptors require antigen detection to activate cell-killing responses, so non-cancerous tissue is only harmed if the chosen tumor antigen target also appears on healthy cells—a situation called “on-target, off-tumor” toxicity (Flugel et al., 2023). Still, some safe-to-use immunotherapies involve manageable amounts of on-target, off-tumor toxicity, such as treatments for B cell cancers that target both malignant and healthy B cells.

Tumor-specific antigen targets (i.e., antigens found only on cancer cells) are desirable because targeting them poses no risk of on-target, off-tumor toxicity. Ideally, targets should be exclusively and uniformly expressed by cancer cells. Without exclusivity, T cells could attack healthy cells that coincidentally presented the antigen; without uniformity, a subset of cancer cells that lacked the antigen could escape undetected. However, discovering true tumor-specific antigens is difficult, so some immunotherapies instead target tumor-associated antigens expressed at high levels in cancer cells and lower levels in healthy cells (Okarvi & AlJammaz, 2019).

Furthermore, antigen loss remains a challenge even though the antigens that cellular immunotherapy targets are often harder to lose than the antigens that T cells naturally target. As long as an antigen is nonessential, tumor cells may downregulate or remove it (Mishra et al., 2024).

Solid Tumors

Cellular immunotherapy has successfully been used to treat liquid cancers, but it demonstrates decreased efficacy against solid tumors such as sarcomas, carcinomas, and glioblastomas (Dagar et al., 2023; Guzman et al., 2023). Solid tumors are particularly difficult to treat because they are surrounded by a network of extracellular molecules and non-cancerous tissue that supports tumors, collectively termed the tumor microenvironment (Tormoen et al., 2018). This tumor microenvironment hinders T cells by suppressing their activity, depriving them of nutrients, and physically limiting their access to tumors. Researchers are actively developing and testing new strategies to overcome the challenges posed by the microenvironment.

Introducing Two Synthetic Receptors: SynNotch and CAR

The ongoing challenges of antigen specificity and the solid tumor microenvironment necessitate novel approaches in cellular immunotherapy. A recent innovation combines the activity of two particularly promising receptor proteins—the chimeric antigen receptor (CAR) and the synthetic Notch (synNotch) receptor—in order to more precisely target tumors. Both of these proteins can recognize tumor antigen targets and activate therapeutic responses in T cells, but each has a distinct function when combined.

Chimeric Antigen Receptors

One of the most well-researched synthetic receptor proteins used in cellular immunotherapy is the CAR. The first approved human “gene therapy” in the United States was a type of CAR T cell therapy, Kymriah, that was approved by the FDA in 2017 to treat acute lymphoblastic leukemia (Awasthi et al., 2023). CARs enable the activation of T cells’ built-in cell-killing mechanisms in response to user-defined antigen targets (**Fig. 1**). Lodged in the cell membrane, CARs are proteins that comprise (1) an extracellular receptor region that recognizes the tumor antigen target; (2) a transmembrane region that anchors the protein and relays signals from the receptor to the inside of the cell; and (3) intracellular signaling regions that induce T cell activation (Irvine et al., 2022).

Synthetic Notch Receptors

SynNotch receptors, developed more recently than CARs, are a new generation of engineered synthetic

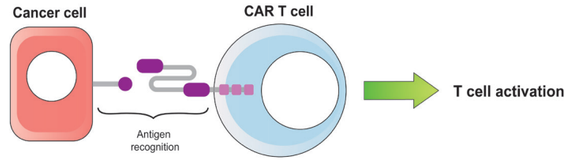


Figure 1. An antigen-presenting cancer cell binds to a CAR T cell, triggering T cell activation.

receptor proteins with exciting implications for the future of immunotherapy. Like CARs, synNotch receptors are transmembrane proteins that recognize user-defined antigen targets and trigger cellular responses. But unlike CARs, antigen recognition by a synNotch receptor activates the expression of customizable, potentially non-native genes. When the antigen target binds to the extracellular region of the synNotch receptor, the intracellular region releases a transcriptional regulator into the cytoplasm that traffics to the nucleus and activates DNA transcription (**Fig. 2**) (Roybal et al., 2016b).

What makes synNotch receptor proteins so promising is their ability to trigger the expression of customizable genetic response programs that are not naturally found in T cells. Researchers choose which genes the receptor

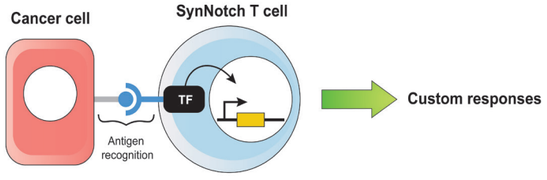


Figure 2. An antigen-presenting cancer cell binds to a synNotch T cell, triggering custom therapeutic responses (adapted from Roybal et al., 2016b).

controls by inserting chosen DNA into the T cell's nucleus to be transcribed upon receptor activation. These genetic programs have the potential to perform a wide variety of functions, such as increasing T cell toxicity against tumors (Bonamino et al., 2022; Golubovskaya & Wu, 2021), inducing T cell proliferation (Li et al., 2022), and helping T cells navigate the solid tumor microenvironment (Foeng et al., 2022).

Combining Receptors in Dual-Antigen-Sensing Circuits

CARs and synNotch receptors each play important roles in the toolkit of cellular immunotherapy, recognizing tumor antigens in order to either activate T cells or trigger the expression of custom genetic programs with diverse functions. However potent these proteins are alone, recent studies have shown that they may be even more effective when combined.

Two of the most significant hurdles in CAR T cell therapy for the treatment of solid tumors are (1) ensuring that only cancer cells are targeted and (2) avoiding antigen loss and tumor escape (Choe et al., 2021). In an attempt to overcome these challenges, Dr. Wendell Lim's laboratory at the University of California, San Francisco engineered T cells that express both synNotch receptors and CARs in order to recognize two different tumor antigen targets. Together, synNotch receptors and CARs form a molecular AND-gate circuit that activates T cells only when both antigens are present (Roybal et al., 2016a).

In dual-antigen-sensing T cells (**Fig. 3**), the synNotch receptor has been engineered to recognize a particular antigen: antigen A. If antigen A is present on a nearby cancer cell, the antigen binds to the synNotch receptor, activating the expression of genes encoding a CAR. This CAR has been engineered to sense a different antigen: antigen B. If the cancer cell that presented antigen A also presents antigen B, then the newly expressed CAR will recognize antigen B and induce T cell activation to kill the cancer cell (Roybal et al., 2016a). SynNotch-CAR circuits broaden the range of targets for cellular immunotherapy by opening the doors to antigens that may not be tumor-specific on their own but that become specific when combined with a second target (Choe et al., 2021).

In vivo mouse studies have demonstrated that synNotch-CAR T cells spare non-cancerous cells that express a single antigen (i.e., only antigen A or only antigen B) while successfully killing tumors that express both antigens at the same time (Roybal et al., 2016a). Additional studies found that synNotch-CAR T cells were better at controlling

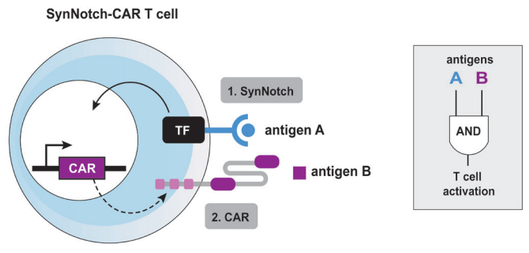


Figure 3. SynNotch-CAR T cells recognize two antigen targets in order to induce T cell activation (adapted from Roybal et al., 2016a).

solid tumors in mice than traditional CAR-only T cells and that the synNotch-CAR T cells did not damage healthy tissue (Hyrenius-Wittsten et al., 2021; Choe et al., 2021).

Conclusion

Uniting CARs and synNotch receptors to create T cells that require two antigen targets for activation is a powerful new strategy already being used to treat solid tumors in mice. SynNotch-CAR circuits help overcome the challenge of antigen specificity by enabling precise AND-gate antigen recognition, allowing researchers to choose from a wider variety of antigen targets that may reduce the risk of on-target, off-tumor toxicity. Since the particular antigens that synNotch receptors and CARs target are up to the engineer, this technology could potentially be applied to many different types of cancer. Although further research, clinical testing, and approval are still necessary before this technology can be used to treat disease in humans, synNotch-CAR circuits are a promising innovation in the ongoing fight against hard-to-treat solid cancers. This genetic engineering-based technology may further enhance cellular immunotherapies when combined with other approaches such as protein, chemical, biological, or materials engineering (Irvine et al., 2022).

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Exploring miRNA Therapies for Neurogenesis: Therapeutic Potential and Bioethical Considerations

Courtesy of Adobe Stock.

David Kim ’27

Introduction

Traumatic brain injuries (TBIs) impact approximately 190 individuals each day and highlight a substantial need for effective neurological therapies to treat these injuries (Centers for Disease Control and Prevention, 2024). While the body is capable of cell regeneration following some injuries—for example, epidermal skin cells are fully replaced every month—neurons have historically been considered a notable exception. The reigning belief for much of the 20th century was that humans were born with a fixed number of neurons, limiting neurological treatment strategies to symptom management rather than neural regeneration. However, evidence from recent decades reveals that neurogenesis—the process of creating new neurons—can occur in specific regions of the adult brain such as the hippocampus, which is primarily associated with memory function (Costa et al., 2015). These findings have opened new avenues in neurodegenerative research and indicate the potential for full recovery after severe brain injury and degeneration.

In parallel, the discovery of microRNAs (miRNAs) has revolutionized the understanding of gene regulation. Filling critical roles in post-transcriptional gene regulation, these noncoding RNA molecules affect processes such as cellular proliferation and differentiation, which are important to human growth and development (O’Brien et al., 2018). Due to their ability to influence multiple genes simultaneously, miRNAs have shown promise in helping create biotechnological therapies for neurological disorders, especially those caused by dysregulation in gene expression (Broderick & Zamore, 2011). miRNAs significantly affect cellular processes like programmed cell death and inflammation in response to toxic substances, which are central to neurodegenerative and injury-induced neuropathies, suggesting that they may also play an essential molecular role in facilitating neural repair and regeneration.

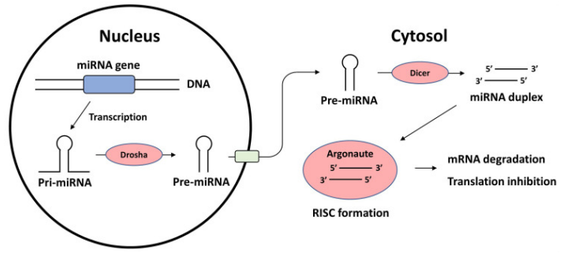


Figure 1. miRNA biosynthesis and mechanism in regulating gene expression (Jung et al., 2021)

Advancements and Delivery Mechanisms of miRNA Therapies

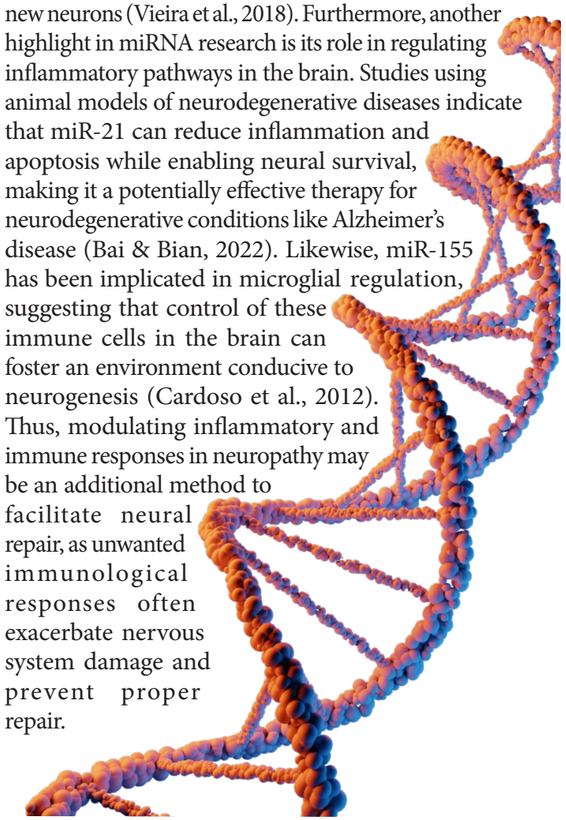
Although miRNAs offer significant potential for neuronal repair, their primary therapeutic limitation is the lack of appropriate delivery mechanisms (Segal and Slack, 2021). Delivering miRNA molecules directly to target tissues, especially the brain, presents several challenges such as ensuring the stability and specificity of the miRNA molecules and minimizing the immune response to these foreign particles (Lee et al., 2019). To this end, researchers have been particularly excited by two delivery systems: adeno-associated viral vectors (AAVs) and nanoparticle-based approaches (Han et al., 2011).

Discovered in the late 20th century, AAVs are small, non-enveloped DNA viruses used as a delivery mechanism for gene therapy—a medical treatment that utilizes genes to treat or prevent diseases—due to their ability to precisely transmit genetic information without causing disease (Issa et al., 2023). AAVs are especially advantageous for neurological therapeutics because they are able to cross the blood–brain barrier, which is a natural membrane that protects the brain from toxins in the blood but consequently limits effective drug delivery to the brain. Furthermore, AAVs reach their delivery sites with precision and are able to selectively target neural cells with minimal immune activation (Wang et al., 2019). Similarly, nanoparticles, which are ultrafine particles spanning the nanometer range, can encapsulate miRNAs as a means of transduction, allowing for spatial and temporal control over release (Lee et al, 2019). This enhances cellular uptake rates of the intended information and reduces unintended degradation.

By combining these miRNA delivery methods with stem cell therapies, neural stem cells may more effectively differentiate into neurons. For instance, miRNAs such as miR-21 and miR-124 have demonstrated potential in promoting neuronal survival and integration within damaged neural circuits, thus creating synergistic effects that facilitate neurogenesis and functional recovery (Liu et al., 2021). This combined approach could be particularly useful for treating TBIs and neurodegenerative diseases that are typically characterized by progressive neural loss and dysfunction, such as subarachnoid hemorrhages and Alzheimer’s disease.

Current Research on miRNA in Neurogenesis and Neuropathy

The application of miRNAs to neurogenesis research proves their therapeutic versatility. For instance, miR-124 and miR-9 have been found to facilitate neural stem cell differentiation into mature neurons, promoting both neurogenesis and the assembly of functional neural circuits (Xue et al., 2016). These characteristics are particularly valuable in regenerative medicine, as they present potential strategies for restoring neural functions lost in injury or disease as well as the possibility of inducing the formation of new neurons (Vieira et al., 2018). Furthermore, another highlight in miRNA research is its role in regulating inflammatory pathways in the brain. Studies using animal models of neurodegenerative diseases indicate that miR-21 can reduce inflammation and apoptosis while enabling neural survival, making it a potentially effective therapy for neurodegenerative conditions like Alzheimer’s disease (Bai & Bian, 2022). Likewise, miR-155 has been implicated in microglial regulation, suggesting that control of these immune cells in the brain can foster an environment conducive to neurogenesis (Cardoso et al., 2012). Thus, modulating inflammatory and immune responses in neuropathy may be an additional method to facilitate neural repair, as unwanted immunological responses often exacerbate nervous system damage and prevent proper repair.



However, optimizing miRNA specificity and reducing its off-target effects remain critical areas of study to this day. Because miRNAs regulate multiple genes, the potential for unintended and harmful gene interactions is significant (Hua et al., 2006). Sequencing efforts to develop miRNA analogs specific to target pathways could potentially mitigate these effects, but more research is needed—particularly in brain applications where even small changes in gene expression can significantly impact cognition and behavior. Furthermore, manufacturing miRNA delivery mechanisms requires immense funding and is currently not very productive in delivering nucleotides or adjusting for proper dosages, prolonging the length of neurogenesis research (Zhang et al., 2021).

Bioethical Considerations of miRNA-Based Therapies

While a standardized, usable model of miRNA therapies in the context of neurogenesis may be physiologically beneficial, its ethical implications warrant scrutiny. The traditional view in cognitive science is that the brain is an indispensable part of identity. Therefore, any regeneration therapies, including miRNA-mediated stem cell therapy, could introduce unfamiliar entities that affect the behaviors and actions of the individual, thus potentially changing a part of the patient's identity. However, these therapies have the potential to heal chronic pain and injury in life-altering or life-threatening situations. As a result, these therapies raise critical philosophical questions about the extent to which it is acceptable to prioritize physical well-being over self-identity.

For example, one central philosophical debate is the continuity of the self, referenced in the famous Greek analogy of the Ship of Theseus (The Editors of Encyclopædia Britannica, 2024). If all parts of the ship are gradually replaced, then would it still be the same ship? If not, when would it become a different ship? Similarly, if individual neurons within the brain are continually regenerated or altered through miRNA-induced neurogenesis, could this influence aspects of an individual's identity? While the body naturally replaces cells like skin cells, which are not directly involved in cognitive processes, research has identified neurons as unique in encoding memories and self-perception. As such, artificial neurogenesis raises unique considerations regarding potential shifts in memory, personality, and behavior—more so than other non-neuronal cells of the body—and how changing the physical neurons might affect our fundamental identities.

Manipulating neural circuits using miRNA therapies also inherently brings the risk of misuse, particularly in the realms of autonomy and patient consent. For instance, a potential concern is that these therapies may impact thoughts and behaviors in ways that compromise individual free will. In model organisms like Drosophila melanogaster (fruit flies), scientists have observed that specific neurons control distinct behaviors: for instance, Moonwalker Descending Neurons can be optogenetically activated to induce the involuntary action of walking backward, suggesting that future research could uncover similar structures in humans (Sen et al., 2017). Thus, specific human behaviors may be attributed to specific circuits or characterizable networks as the scientific literature grows, and their changes in conjunction with neurogenesis may affect free will, either explicitly through misuse by bad actors or implicitly through bias. In the past year, studies have found that neural activity could be used to accurately predict specific behaviors or speech patterns, a discovery that holds great potential in the advancement of brain-computer interfaces (Doctrow, 2024).

Conclusion

Based on current research and its implications, miRNA therapies may serve as a promising strategy for promoting neurogenesis and neuroregeneration, offering hope for patients with TBIs and neurodegenerative conditions. By modulating apoptosis, inflammation, and neural differentiation, miRNAs could provide a means of treating neural damage at a cellular level, moving beyond symptomatic management toward true regeneration. However, as these treatments advance, it is crucial to address the accompanying ethical and philosophical issues, particularly those pertaining to personal identity, autonomy, and the potential for unintended behavioral effects. Only by addressing these complexities can miRNA therapies be developed responsibly with an emphasis on patient welfare and a nuanced understanding of the profound implications they hold for the human experience.

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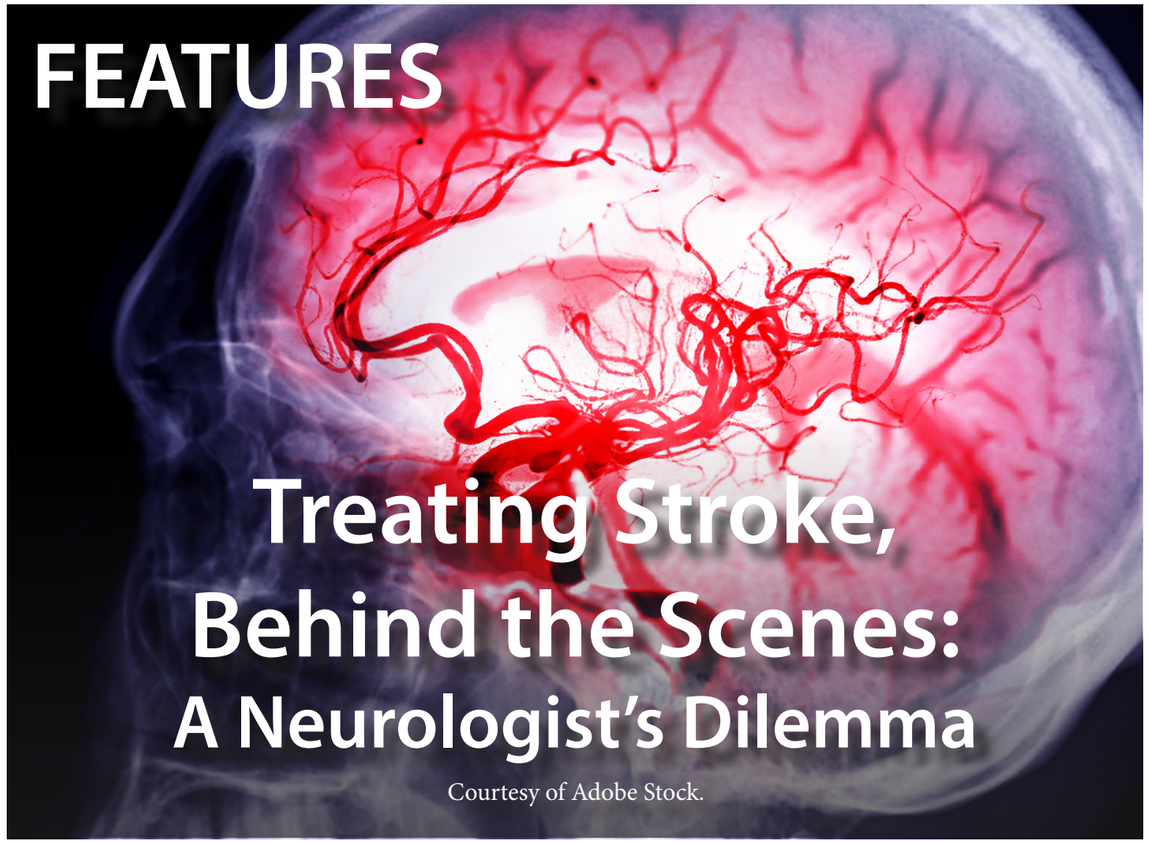


Treating Stroke, Behind the Scenes: A Neurologist's Dilemma

Courtesy of Adobe Stock.

Ambika Grover '27

(figures courtesy of Bridgette Pehrson '27)

Introduction

What is a disease without pain?

Many people have experienced a familiar headache after a long day at work, but those are easily relieved with a painkiller. Maybe they've felt a sudden wave of dizziness but brushed it off as dehydration or low iron levels. And what of the slurred speech episode of a dementia patient already struggling with communication? This, too, is easily dismissed as just another symptom. Why seek medical care just to incur a hefty bill?

"What is a disease without pain?"

These seemingly harmless "nuisances" can, in fact, be symptoms of stroke, a life-endangering brain injury to the central nervous system attributable to a lack of blood flow or burst blood vessels. Each year, about 795,000 Americans will have a stroke (CDC, 2024), and every four minutes, someone will die because of it (AHA, 2024).

When we think of life-threatening conditions, an acute cardiac arrest often comes to mind—a sudden collapse marked by loss of consciousness, with symptoms so unmistakable that they are recognizable even by the untrained eye. This is why many states require CPR training in schools—to equip a new generation with the skills to save lives in those crucial minutes before paramedics arrive (AHA).

Meanwhile, the less obvious conditions escape notice and sufficient preparation. Stroke's common symptoms—facial drooping, dizziness, aphasia (difficulty speaking or understanding speech), blurred vision, headache, and loss of balance—are often brushed off as a momentary impediment that can be self-treated. Its unremarkable nature renders it a complex case needing time in the limelight.

Portrait of Dr. Leung. Image courtesy of Tufts Medicine.

As Dr. Lester Y. Leung, M.D., M.Sc., a vascular neurologist and Harvard College '06 alumnus, illustrates, "Often, what happens is that people sit on their symptoms for a while. They try to see if they can self-treat or go to sleep." He continues to explain that, alternatively, some patients experience loss of function, rendering them unable to speak or to call for help. Dr. Leung—who directs the Comprehensive Stroke Center and Stroke and Young Adults Program at Tufts Medicine and serves as an Associate Professor at Tufts University School of Medicine—plays a critical role in identifying stroke patients, facilitating prompt treatment, and increasing community awareness. His contributions have been instrumental in advancing stroke care throughout the Boston area.

Unfortunately for healthcare professionals, the challenge of administering quality care often begins beyond the reach of hospitals and emergency care systems. "There is a narrow window where people can receive treatment, but there are a million reasons why people can't go [to the hospital]. There are a lot of sociodemographic factors: whether or not you have a doctor or an available adult, child, or coworker [as well as] whether or not your community and family have the knowledge [needed]. There is a financial disincentive if people don't know right off the bat that they are at risk,"

"There is a narrow window where people can receive treatment, but there are a million reasons why people can't go to the hospital."

Dr. Leung describes.

Indeed, the average emergency room (ER) visit in the US costs between $2,200 and $2,600 for uninsured individuals (American Family Care, n.d.). Previous poor experiences in the hospital can also discourage someone from considering the ER an option, particularly if a visit may be a false alarm. Combined, the information gap and access gap serve as a barrier to stroke care before the patient has even had a chance to see the doctor. The bottom line then becomes that stroke isn't always severe in how symptoms appear—there can be a gradual, slow onset that escapes notice. This can make receiving care difficult.

Into the Hospital

The question returns to time for patients who do make it to the hospital. The gold standard treatment for ischemic stroke—which comprises

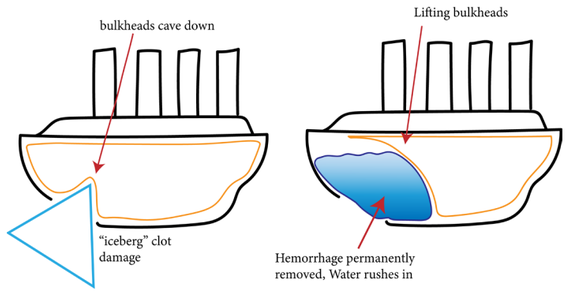


Figure 1. Illustration of the Titanic metaphor depicting how hemorrhage can occur in the surrounding tissue following the formation and removal of a clot.

approximately 85% of all cases and in which a blood clot restricts blood flow to the brain—has a three-hour limit under which it must be administered for patient safety (CDC, 2024). Blood typically flows from the heart, acting as a pump, and is then directed to supply different brain areas. Dr. Leung describes blood flow in the brain as analogous to a tree. "There are four large arteries, which are the trunk of the tree. [...] At the base of the brain, they branch out. Off [of] each branch, there are smaller branches," he explains. Blood clot blockages can also be understood using this analogy. If one of the largest "trunks" is blocked, it is called a large vessel occlusion (LVO). A blockage at the "branch" extending from the trunk can be referred to as a small or medium occlusion, which often cannot be detected in scans.

In many cases, a thrombolytic treatment—also known as a clot "buster"—is used to restore blood flow. According to FDA standards, the administration of the thrombolytic should be within 3 hours of stroke symptom onset (Hughes et al., 2023). The reality is up to individual doctors, hospitals, or field consensus; Tufts Medical Center, for example, authorized a 24-hour window for thrombolytic administration in light of delays caused by the COVID-19 pandemic.

However, restoring blood flow to the site of the brain injury may cause existing damage to worsen, possibly even leading to another type of stroke via a process known as "hemorrhagic transformation," or a brain bleed. In these cases, prognosis is exceedingly poor, with approximately half of patients dying within the subsequent 30 days. Dr. Leung describes why administering thrombolytic agents is time-sensitive: "It is like the Titanic, where the iceberg is tearing through different chambers in the hull of the ship. Bulkheads, the inner barriers within the hull, go down automatically when water rushes in. The idea is to prevent the whole ship from sinking. When part of the brain is sinking, the bulkheads shut down. But if we restore blood flow too soon, it can make things worse." Preemptively dislodging a blood clot—the "iceberg"—may have unintended consequences, explaining the complexity behind thrombolytic administration decisions.

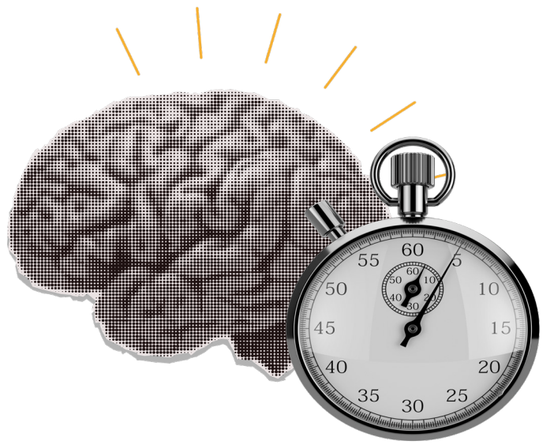
At Tufts Medical Center, about half of the patients are brought in via ambulance, where paramedics can communicate with hospital staff to provide a preliminary assessment and indicate if the incident is likely to be a stroke. Dr. Leung describes this as the ideal scenario, giving hospital and neurology teams time to prepare and be in the bay when patients arrive. The end goal is to reduce the time to treatment and recognition to zero, "changing the process from serial to parallel." Other times, patients enter through the waiting room, where the description of symptoms to staff determines whether a red flag is raised.

The coordination of hospital efforts to get potential stroke patients seen as promptly as possible is another task that requires careful preparation and high-stakes decisions. Stroke does not necessarily manifest identically across people; different people can exhibit unique symptoms, making it hard to establish a uniform framework for diagnosis. For example, research has suggested symptoms can differ based on factors including sex and ethnicity (Hosman, et al). "I tell my trainees that there are 100 different symptoms, but there are 6–7 greatest hits. Those are the ones we teach to our physicians, but also what we teach our colleagues in non-neurology specialties," Dr. Leung explains.

Unlike diabetes or heart disease, stroke does not have a blood test to identify it. Instead, neurology teams must work to "triangulate" around the disease, leveraging tools at their disposal. For instance, Magnetic Resonance Imaging (MRI)—a type of diagnostic test that can create detailed images of internal parts of the body—is highly useful, but most hospitals cannot offer it within the first

"But if we restore blood flow too soon, it can make things worse."

hour of a patient's arrival. Herein lies another challenge in administering quality care: hospitals are not created equal, and the resources largely determine what care patients can receive. A community hospital affiliated with a medical school likely has better facilities and more neurologists, resulting in a much larger reach than that of



a single, rural hospital. Once the patient is in the hands of emergency response teams, it is up to emergency care units to diagnose, assess, and treat the stroke as quickly as possible.

Gambling with Time

An elderly patient arrives at the emergency room, where they are promptly admitted and placed under the care of a team of neurologists due to a suspected stroke. But what is the time since the patient started exhibiting symptoms? Unknown—a caretaker arrived this morning to find them slurring their speech and unable to move the right side of their body. Neurologists suspect a medium-sized blockage but are worried that symptoms may have started the previous day. And, while administering a thrombolytic could restore blood flow, it could also lead to another bleed, particularly given the patient's age.

Determining the time since stroke, which is crucial for the administration of time-sensitive thrombolytic treatment, is often an unclear process. Neurology teams must ask family members to answer difficult questions about the onset of symptoms. If there was a delay in admitting the patient from the emergency room, that also must be considered. Unclear time windows are a recipe for tight calls, meaning that the choice to administer the double-edged treatment falls on the neurologist.

In ambiguous situations, giving a patient a thrombolytic at the end of the window can be risky. But risk aversion in and of itself has the potential to harm, too. Dr. Leung highlights doctors' conundrum: "There are certain principles or Hippocratic oath[s] you subscribe to. [...] We often uphold ["do no harm"] as a core principle, but people can go too far, do nothing, and not take risks. It's a false dichotomy because, if you choose not to treat something, you are potentially withholding treatment that could have significantly benefited the patient."

Given the choice, neurologists often closely consult the family about the benefits and drawbacks of treatment. For instance, administering a thrombolytic may expedite recovery but may also increase the risk of death if things do not go as planned; a doctor might take the time to explain the situation to a close family member, ensuring they understand the associated risks. Establishing a shared understanding reduces the likelihood of conflict.

Ultimately, the decision from there varies from neurologist to neurologist—rather than following strict guidelines, choosing to administer the drug comes down to risk thresholds. "Our brains are not always built to think scientifically when thinking about how to make decisions. Everyone is a little different," Dr. Leung remarks. Negative experiences, such as a lawsuit or an unsuccessful treatment can leave a lasting imprint on a neurologist, making them unwilling to take risks in the future, even if it could help the patient. Left unchallenged, such an experience can influence a neurologist's outlook forever. According to Dr. Leung, the way to correct that is "to anchor your experience with assessing patients at different time points, data, [and] probability while also connecting with patients on a personal level." Administering stroke treatment is a complex, high-stakes puzzle that demands careful precision, but with the right interventions, recovery from stroke is possible—physical therapy, speech therapy, and other programs can help survivors reclaim full independence over time.

"At the center of it all is a simple lesson: every second counts. When the time window is unclear, it falls on the neurologist and families to make swift decisions, aiming to set treatment in motion before it's too late."

At the center of it all is a simple lesson: every second counts. When the time window is unclear, it falls on the neurologist and families to make swift decisions, aiming to set treatment in motion before it's too late.

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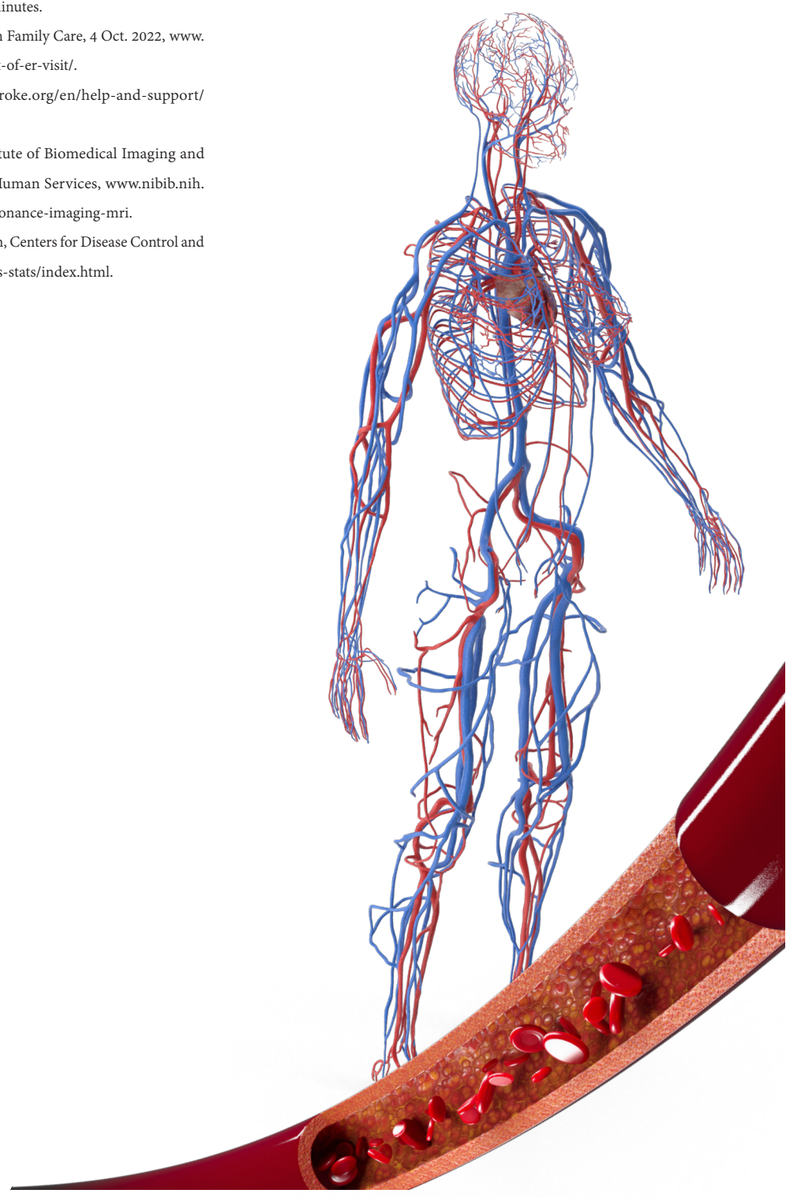

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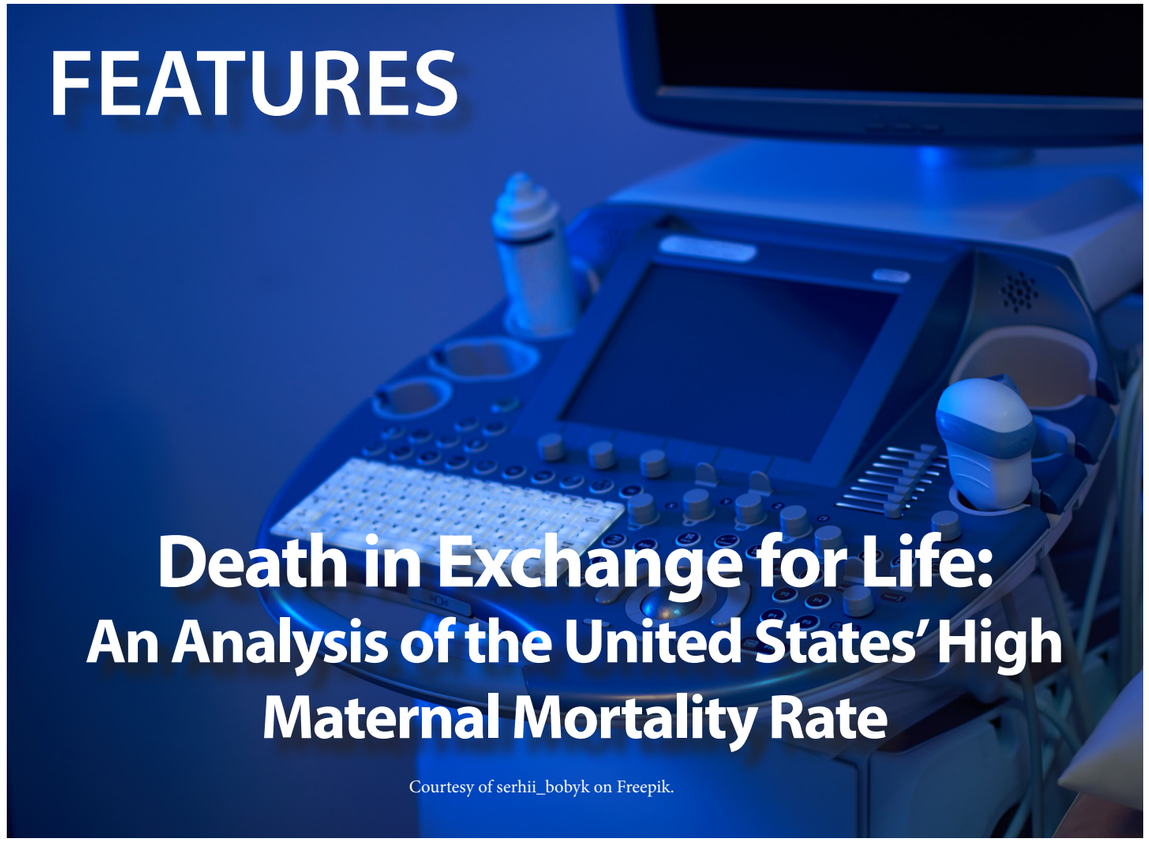

Death in Exchange for Life: An Analysis of the United States' High Maternal Mortality Rate

Courtesy of serhii_bobyk on Freepik.

Lara Rahman '28

Introduction

Every day, expectant mothers in the United States face the daunting reality of a high rate of maternal mortality. These maternal deaths can be attributed to induced abortion, miscarriage, embolism, obstetrical bleeding, hypertensive disorders, sepsis, and more (Say et al., 2014). The unfortunate truth is that many of these deaths are preventable and may not have occurred elsewhere in the world.

The World Health Organization (n.d.) defines maternal mortality as deaths "related to or aggravated by pregnancy or its management (excluding accidental or incidental causes)." From the 1930s to 1970s, maternal mortality rates in high-income countries decreased rapidly, largely due to advances in healthcare (Center for Disease Control, 1999). For example, the clinical introduction of antibiotics, such as penicillin and sulfonamides, and aseptic techniques in the 1940s successfully prevented and treated many infections associated with childbirth. Another critical advancement was the widespread practice of the Cesarean section (C-section), a procedure that offers a surgical alternative in situations where traditional childbirth poses risks. In the U.S., the overall rate of C-sections increased from 2.5% to 5.1% of births between 1932 and 1963. Moreover, C-sections are safer for not only mothers but also babies; the mortality rate for newborns decreased from 9.8% to 2.9% during these same three decades (Antoine & Young, 2020).

However, while the trend of decreased maternal mortality has persisted in other high-income countries, the U.S. has recently observed a rise in its rate; the current maternal mortality rate is comparable to that of the 1970s (**Fig. 1**). In 2022, the U.S. recorded 22 maternal deaths for every 100,000 live births—more than double the rate in most other high-income countries (Hoyert, 2024). A 2020 analysis conducted by the Commonwealth Fund found that half of the ten high-income countries they

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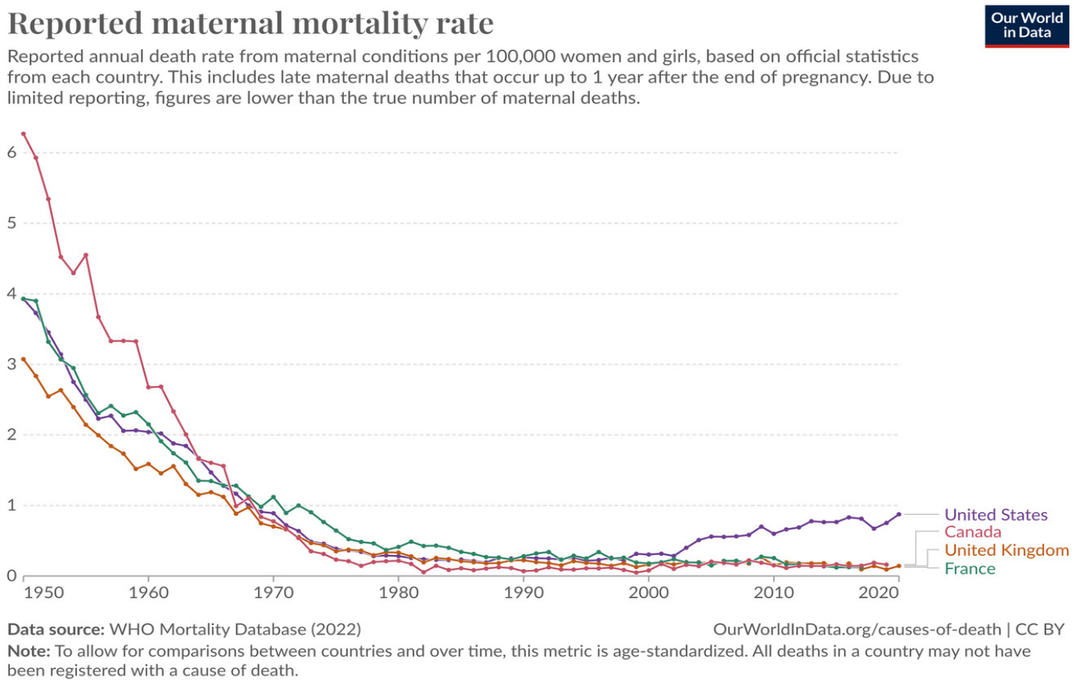

Figure 1. Annual maternal mortality rates for the U.S., Canada, United Kingdom, and France according to data from the World Health Organization (Our World in Data, 2024).

studied had a maternal mortality rate of fewer than five maternal deaths per 100,000 live births. The healthcare system of the United States—a global leader in medical innovation—differs from those of peer countries in various ways that contribute to divergent outcomes in maternal mortality, including healthcare access, midwife assistance, and maternal leave.

Healthcare Access

Most high-income countries manage healthcare access through a system known as universal healthcare. In these systems, most patients are guaranteed equal access to quality healthcare services with minimal financial strain or out-of-pocket payments. However, the U.S. takes a different approach as the only high-income country without near-universal healthcare coverage (Gunja et al., 2023). Instead, health insurance is predominantly provided through private employers or public programs funded by the state and federal governments, such as Medicaid and Medicare (Tikkanen, 2023). A patient's income and age level will largely determine the public coverage programs for which they are eligible. With this multi-payer structure comes a higher probability of patients in the United States having to pay out-of-pocket for their medical care (Gunja et al., 2023). These out-of-pocket payments contribute to the finding that U.S. adults with lower to average incomes are more likely to have cost-related issues accessing healthcare than their counterparts in peer countries (Tikkanen, 2023). Throughout pregnancy, out-of-pocket costs could deter lower-income patients from seeking adequate maternal care, increasing their risk of adverse events (Gunja et al., 2024). Furthermore, under a model in which healthcare workers are paid by insurance companies, healthcare workers may choose to reject a patient with Medicaid due to the program's lower reimbursement rates (Decker 2012). This practice further restricts healthcare access for some lower-income patients and leaves many disadvantaged mothers at risk of complications.

Another aspect of the U.S. healthcare system that differs from other countries is access to abortion. Abortion access can safeguard a mother's life and lower the maternal mortality rate when carrying a pregnancy to term poses serious health risks. In Canada, the United Kingdom, and many European nations, abortion is legalized within specific term limits. For example, in the United Kingdom, abortion is available up to 23 weeks and six days into pregnancy (MSI Reproductive Choices, n.d.). This means that an expectant mother can choose—often due to a physician's recommendation—to undergo a medically safe abortion. Contrastingly, in many U.S. states, abortion is heavily restricted and even criminalized, regardless of the mother's health risks. For example, Chapter 170A of the Texas Health & Safety Code (n.d.) currently mandates that physicians who perform an abortion be subjected to a civil penalty of at least $100,000 in addition to the possibility of a first- or second-degree felony charge. Structural designs such as these contribute to the difficulties expectant mothers face in accessing healthcare in the U.S.

Midwives

Another factor affecting the U.S.'s maternal mortality rate is the maternal care workforce, the size and capability of which frequently determine how much time and attention each expectant mother will receive. Due to the global shortage of maternal care providers, many high-income countries have turned to midwives to fill the gap in care that pregnant women face (Commonwealth Fund, 2023). Midwives, who are trained to manage lower-risk, non-surgical pregnancies, are a common alternative to obstetrician-gynecologists (OB-GYNs), who typically focus on higher-risk pregnancies and surgical procedures. Together, they help more women give birth in safe environments by implementing safe birthing practices, ultimately decreasing the maternal mortality rate.

The U.S., Canada, and Korea are the few high-income countries whose maternal care workforces do not primarily consist of midwives **(Fig 2)**. In the U.S. specifically, this can be attributed to three primary factors: federal laws that limit midwife scope of practice, differing opinions between physicians and midwives, and the late 19th-century shift from community-based to hospital-based care (Niles & Zephyrin, 2023). Due to the small number of midwives in the U.S., most insurance plans do not cover their services; as a result, the ratio of licensed healthcare professionals to mothers in need of aid is exceptionally low at only 16 providers per 1,000 live births. A smaller maternal care workforce can make it more difficult for mothers to receive the care they require for safe, low-risk pregnancies.

Maternal Leave

While the factors influencing maternal mortality rate primarily concern the period before and during pregnancy, maternal leave after birth also plays a crucial role (Jou et al., 2018). In 2019, the WORLD Policy Analysis Center calculated an average length of 29 weeks of paid leave among the countries with a national paid maternity leave **(Fig. 3)**. However, seven countries worldwide do not mandate paid maternity leave: the U.S., Palau, the Marshall Islands, Micronesia, Papua New Guinea, Nauru, and Tonga (Miller, 2021).

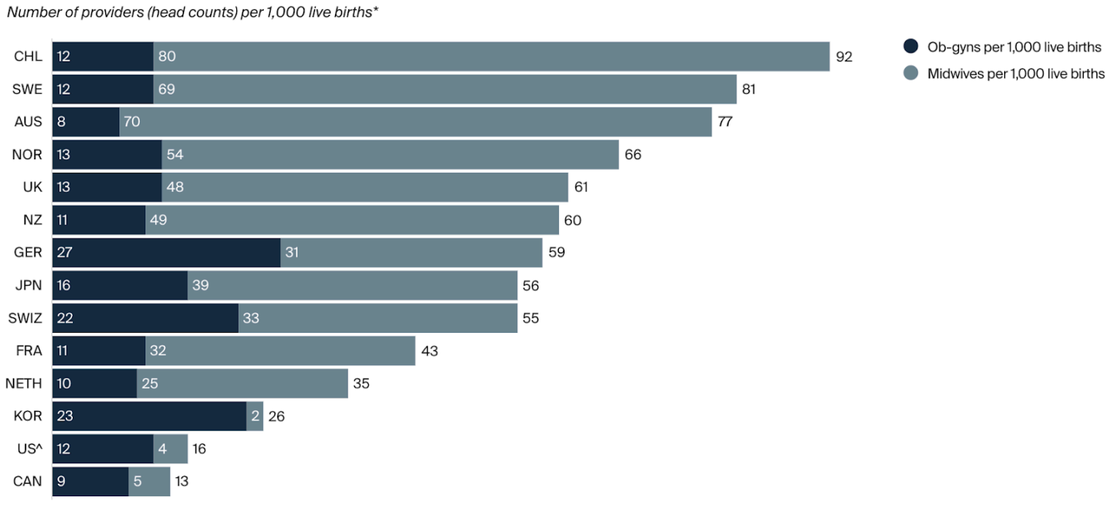

Figure 2. 2023 ratio of OB-GYNS to midwives in the maternal care workforce for various high-income countries (The Commonwealth Fund, 2024).

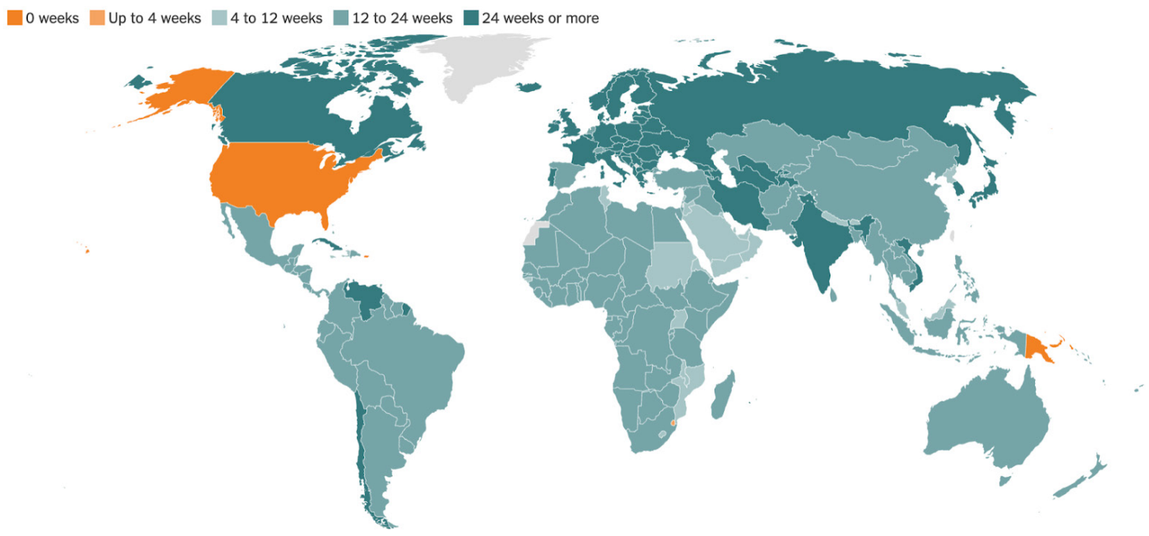

Figure 3. Worldwide paid leave for mothers (New York Times, 2021).

The absence of guaranteed maternity leave has multiple potential impacts on mothers; for example, many mothers return to work earlier than is healthy due to financial and career burdens. Maternal mortality includes deaths that occur up to 42 days after giving birth (World Health Organization, n.d.), some of which are caused by mental or physical overextension. This ultimately impacts mothers from disadvantaged backgrounds the most, compounding their already challenging circumstances; for instance, lower-income mothers may feel obligated to return to work early in an attempt to establish financial stability for their newborns, thereby placing their own health at further risk.

Conclusion

Most high-income countries have succeeded in maintaining low maternal mortality rates through policies expanding healthcare access, midwives, and maternal leave. If the U.S. were to implement similar policies, its maternal mortality rate may decrease once again.

For example, the Netherlands offers a child-raising assistance program called kraamzorg, which operates through the Dutch national healthcare insurance program (ACCESS NL, n.d.). Kraamzorg provides in-home support for individuals who assist with baby care, household tasks, and health monitoring for both mother and child. Mothers are eligible for up to 49 hours of the program over ten days immediately after giving birth. Kraamzorg alleviates many newfound stressors (e.g., childcare and home cleanliness) and may decrease maternal mortality rates by advising healthy lifestyle habits and assisting with tasks that could burden a postpartum body.

Another potential policy model for the U.S. is Australia's Mother Baby Units (MBUs). Though offered only by select hospitals, stays in MBUs are covered under Australian national healthcare. In these hospital units, from late pregnancy until the baby takes their first steps, specialized care is offered for mothers with mental health concerns. Mothers participate in various treatments including individual counseling, group therapy, and nursing care (Department of Health, Western Australia, 2024). This approach of recognizing and addressing maternal mental health may have the potential to significantly reduce maternal mortality in the U.S.

Continued research on the causes and factors of the maternal mortality rate is key to creating more effective policies in the U.S. By addressing root causes, investigators and policymakers can develop lasting solutions, decreasing the maternal mortality rate to its previous low. Without policy changes, the maternal mortality rate may continue to increase, and pregnancy will remain a dangerous process in the U.S., a reality with wide-reaching impacts on countless families and communities. Motherhood is considered one of the most beautiful journeys on Earth—it should not also come with deadly consequences.

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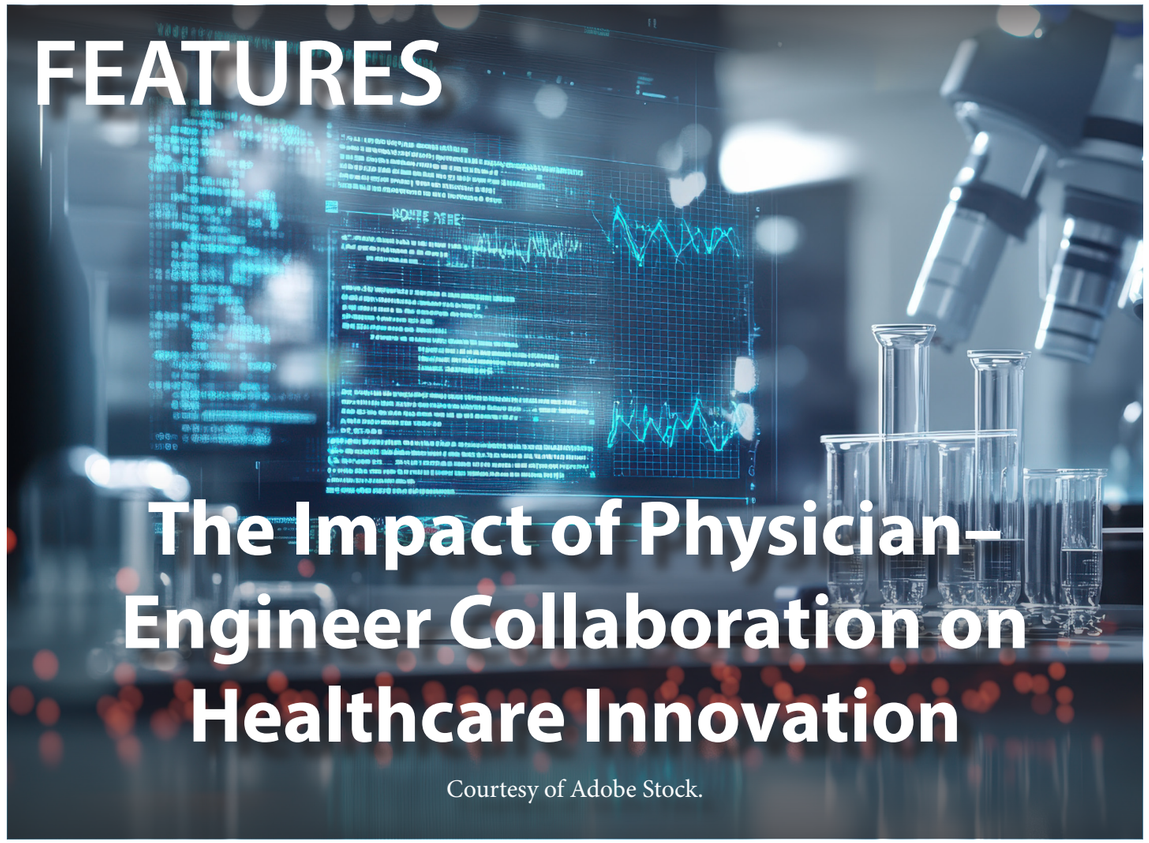

The Impact of Physician–Engineer Collaboration on Healthcare Innovation

Courtesy of Adobe Stock.

Wafiqah Zubair ’26

Introduction

Bioengineering—also known as biomedical engineering—is the interdisciplinary study that applies engineering principles in biological contexts to uncover new knowledge, improve existing systems, and solve issues in healthcare and other biologically relevant areas. Bioengineering is a rapidly growing field. The percentage of Harvard College undergraduates concentrating in engineering, many of whom are interested in healthcare applications, has grown from six to 19 percent from 2007 to 2017 (Lee & Xie, 2017). Beyond Harvard, 13,222 bachelor’s degrees were awarded in biomedical engineering in 2022 (Data USA, n.d.).

More and more students are choosing to study this discipline for various reasons, including career potential. According to the Bureau of Labor Statistics, bioengineering jobs in the U.S. are estimated to grow seven percent in the next ten years, faster than the four percent projection for all occupations (U.S. Bureau of Labor Statistics, 2024a; U.S. Bureau of Labor Statistics, 2024b). The industry’s growth is not limited to the United States—the global bioengineering technology market is currently valued at $320 billion, an almost $40 billion increase from the $282 billion valuation in 2023 (The Business Research Company, 2024). Market valuation is estimated to continue growing at a compound annual rate of 13.5% (The Business Research Company, 2024). Furthermore, healthcare and biotechnology continue to be highly innovative fields, producing 3D-bioprinted devices and new cancer therapy drugs and spurred by the ever-present need for medical remedies.

Despite the rapid growth of bioengineering, healthcare professionals have been slower to embrace emerging technologies designed for their area of work. A 2018 study by German researchers found that doctors were less enthusiastic about adopting novel digital health technology than patients and other medical staff, and they cited several reasons (Safi et al., 2018). For one, they felt that these technologies directly challenged their relationships with patients and their independence in diagnosing and treating patients. Also, doctors expressed that their proficiency in technology development was low and could be supplemented by IT support and training (Safi et al., 2018). This suggests that doctors may feel uncomfortable using new technology simply because they do not have enough knowledge or input in its design.

Even when doctors accept newly-engineered technologies, it is often a transaction of a final product between the designers and consumers, as opposed to a collaborative design process. This can lead to a dissonance between the engineers’ design and the appropriate solution for a patient’s medical needs, resulting in inefficient innovations and sometimes disastrous consequences.

Björk-Shiley Convexo-Concave Heart Valve

One example is the Björk-Shiley Convexo-Concave (C/C) Heart Valve. A heart valve is a flap that opens and closes pathways for blood flow through the heart, strictly controlling blood pressure and flow timing. Diseased heart valves lead to insufficient flow of oxygenated blood to cells and often need to be replaced with prosthetic heart valves (Fielder, 1995). In 1979, Shiley Inc. introduced the 60° C/C valve, which involves inlet and outlet struts to hold a disc in place as it swings open and closed to mimic normal valve function **(Fig. 1)**. Clinical trials showed that the valve significantly decreased blood clotting, causing doctors to transplant it into approximately 85,000 patients in the late 1970s and 1980s (Birkmeyer, 1992). However, the outlet strut fractured in many implanted valves, dislodging the disc from the rest of the device and leading to uncontrolled blood flow (Fielder, 1995). This complication led to patient death within minutes to hours. Shiley, and later Pfizer, voluntarily recalled C/C valves starting in 1980, and several patients elected to have their Björk-Shiley valve explanted or replaced, but the damage had been done. By 1993, 386 patients had died due to fractured heart valves (Fielder, 1995). Even today, patients with the Björk-Shiley valve weigh the risks between its tendency to fracture and the expenses, time, and risks involved with another invasive valve replacement (Birkmeyer, 1992).

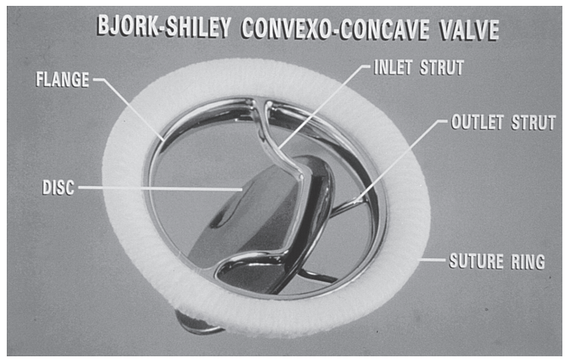

Figure 1. Björk-Shiley 60° convexo-concave heart valve, with labeled parts (Walker, 1995).

According to Dr. J.H. Fielder of Villanova University, Shiley may have altered the valves and sent them out to be implanted in patients without adequately informing surgeons and patients of their risk (1995). Had surgeons been aware of the true quantification of complications, they could have opted to implant other prosthetic heart valves in their patients. “By working on changes while still marketing the valve, Shiley was treating surgeons and patients simply as a means to its marketing program and not acknowledging their right to make informed choices about participating in it” (Fielder, 1995). This highlights the consequences of a lack of rigorous collaborative safety checks and clear communication between engineers and cardiac surgeons, further emphasizing the importance of collaboration between physicians and engineers at every level of the design process, from conceptualization to implementation.

The Living Heart Project

Some professionals within the healthcare innovation field are pioneering the way for such collaboration. Steve Levine is an engineer spurred by his daughter’s heart condition to found The Living Heart Project, which offers analytic modeling of an individual patient’s heart (Levine, 2024). The process starts with a CT or MRI scan of the heart for diagnosis. These scans can be utilized by virtual twin technology—an innovation used in the aerospace and automotive industries—to create 3D simulations of a heart complete with a heartbeat profile and blood and oxygen flow **(Fig. 2)** (Levine, 2024).

Over the past decade, the Living Heart Project has grown steadily, attracting numerous collaborators like Dr. David Hoganson, a pediatric cardiac surgeon at Boston Children’s Hospital (Russell, 2023). He works with engineer Peter Hammer and his team to envision his young patients’ hearts and to determine the best course of action during his surgeries. The engineering team

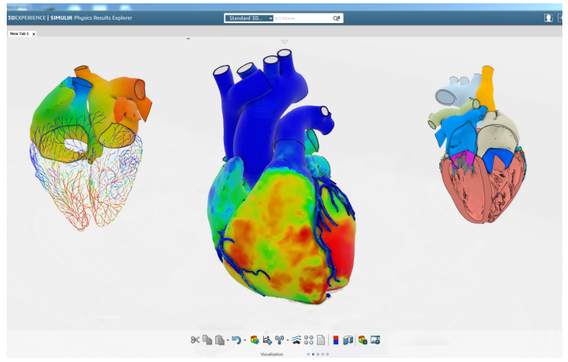

Figure 2. Example digital human heart model on 3DEXPERIENCE platform (Dassault Systèmes, n.d.).

uses the 3DEXPERIENCE platform to create a real-time connection between the real and virtual world, which can be easily displayed on a screen or in virtual reality. This allows them to simulate the problem and potential solutions using real-life scientific principles and under different contexts (Russell, 2023).

During one case, Dr. Hoganson was presented with a child whose heart had holes that needed to be filled with patches to restore proper oxygenated and deoxygenated blood flow throughout the heart and body (Russell, 2023). Although he had patch material, he had to determine the proper size and shape of the patch to withstand the high blood flow pressures within the heart. Hammer and his team worked to simulate the young patient's heart, ultimately creating a workflow using the mechanical properties of the patch material to test whether different patch configurations would be able to close the holes safely and effectively. Thus far, the team of engineers has created more than 900 models to help Dr. Hoganson and to make patients' families feel more involved (Russell, 2023). This consistent collaboration between physicians and engineers has ensured the success of many difficult surgeries and better lives for many patients.

Personal Protective Equipment (PPE) for the COVID-19 Pandemic

The importance of teamwork between doctors and engineers is heightened in times of crisis. During the first surge of the COVID-19 pandemic in 2020, decreased manufacturing due to lockdown policies and the increased risk of exposure to the virus for healthcare workers led to an unmet demand for PPE (Brownell, 2020). Engineers and facilities with 3D printers sought to address this need, printing and distributing as many face masks as possible to healthcare workers. Unfortunately, the combined efforts of industrial labs, college makerspaces, and 3D printing enthusiasts were insufficient to provide enough masks for the healthcare force.

Dr. James Weaver, a Research Associate at Harvard, aimed to design an effective mask that could be mass-produced at the regional scale (Brownell, 2020). He interviewed clinicians and other healthcare professionals on their face mask material preferences and their opinions on convenience, gathering information and inspiration to guide PPE design. Then, he collaborated with Wyss Core Faculty member Dr. Jennifer Lewis, other Wyss members, researchers from Columbia University, and a local plastic food packaging company called Lacerta Group, Inc. to bring the face mask designs to fruition. His efforts resulted in the Dome Shield, a full-coverage, anti-fogging face mask made of a single food-grade material and priced at only $0.75 **(Fig. 3)**. More importantly, the team was able to produce 400,000 masks a day using Lacerta Group's manufacturing facilities and distributed over seven million masks over four months to frontline healthcare workers and the rest of the community (Brownell, 2020).

Dr. Weaver attributes the team's openness to collaboration for the success of the Dome Shield initiative. According to a Wyss Institute article, he reported that

Figure 3. Dome Shield, designed and produced by Wyss Institute and Lacerta (Brownell, 2020).

the designers, researchers, physicians, hospitals, and industrial partners "committed time, effort, and resources into helping develop these face shields [...], saying 'yes' when they could have easily said 'no.'" (2020). The joint effort allowed engineers and researchers to quickly create enough masks while meeting the crucial needs of frontline workers, emphasizing the positive impact that healthcare professionals' clear communication can have on creating effective medical solutions.

Future Directions

To date, the potential of collaboration between physicians and engineers is only beginning to be uncovered. Some examples of healthcare issues that are still prevalent are antibiotic resistance, chronic disease management, and mental health treatment access. Clinicians have an insider perspective of how antibiotic resistance plays out in an infected patient, what challenges exist for patients living with chronic diseases like diabetes, and why mental health care may still be inaccessible to many patients. Their insights can offer engineers invaluable guidance on what medical problems require solutions and how to solve them, creating a streamlined and effective workflow.

One way to promote this collaboration is to introduce engineering principles, as well as the resulting notion of innovation, early on in medical education. According to physicians from the University of California, Los Angeles and Tufts University, design thinking is "directly transferable to the practice of clinical medicine when troubleshooting a broad range of issues, such as improving health outcomes, standardizing clinical processes, and reducing costs" (Rambukwella et al., 2021). The physicians further corroborate that a deeper understanding of technological principles would increase their comfort in adapting to new technological innovations throughout their clinical practice. In response, Texas A&M's EnMed Program at the School of Engineering Medicine, the Harvard-MIT Program in Health Sciences and Technology (HST), and other programs are integrating the practices of innovation, design, and collaboration, all characteristics distinctive to engineering.

Effective communication and collaboration between physicians and engineers play a key role in improving patient care and driving medical innovation. Engineers working on health applications can gain valuable perspectives from physicians in order to design and iterate solutions that address real-world clinical needs. Similarly, informed by their first-hand experience with patients during daily rounds or consultations, physicians can collaborate with engineers to bridge gaps in the healthcare system. By working together, doctors and engineers can drive meaningful progress in healthcare, shaping safer, lower-cost, and more effective treatments to improve patient lives.

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You Reap What You Edit:
Synthetic Gene Circuits in Plants

Courtesy of Adobe Stock.

Sohum Sukhatankar '28

Introduction

A sunflower turns to track the sun, a Venus flytrap snaps shut at a slight touch, and a desert plant responds to minute changes in humidity. Though plants cannot "see" or "hear" in the conventional sense, they nonetheless possess the ability to detect environmental stimuli including humidity, acidity, heat, and more (**Fig. 1**). Advances in molecular biology are now enabling scientists to harness plants' environmental responses by integrating them with systems of synthetic gene circuits, which continuously update gene expression via logic gate-like structures.

Historically, genetic plant modification has relied on transgenes—genes from other species inserted into a plant's genome to enhance traits like nutritional value or pathogen resistance. For instance, golden rice has genes from the soil bacterium *Agrobacterium tumefaciens* that improve its nutritional content (Tang et al., 2009). However, transgenic plant creation is not without its flaws. There is no guarantee that a transgene will express consistently at the desired level or in the right cellular location, as transgenic plants express these transgenes continuously in all cells. Synthetic gene circuits offer a solution by enabling plants to dynamically modulate gene expression in response to specific environmental conditions through a network of logic gates. Unlike transgenes, synthetic gene circuits can function by causing epigenetic modifications rather than altering DNA itself, minimizing the risk of gene transfer to wild plants and reducing the chance of unintended effects.

Plants as Genetic Sensors, Processors, and Actuators

Clarifying plant genetics is critical for understanding the construction of environment-sensitive genetic synthetic circuits. Unlike bacteria—in which prototypical gene circuits were developed—plants lack operons, or groups of linked genes that can modulate the activity of other genes based on various input signals. Instead, they have a variety of signaling components (e.g., protein cascades, which serve to amplify signals) that connect across pathways to create a complex network of related information (Andres et al., 2019). The inner workings of plants can be harnessed by coupling these mechanisms to three synthetic gene circuit components: sensors, processors, and actuators, with the bulk of the customizability arising from the processor design.

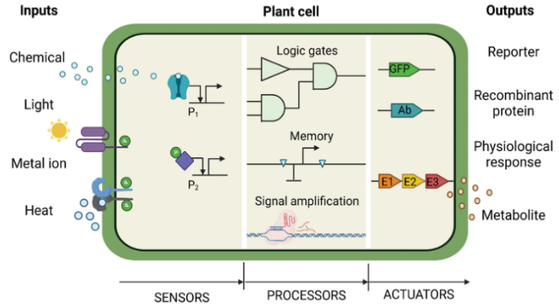

Figure 1. An overview of the structure of synthetic gene circuits, wherein a variety of inputs pass through a combination of plant and human-engineered components to produce a desired output (Vazquez-Vilar et al., 2023).

Sensors

Synthetic gene circuits can better measure external stimuli via sensor modules, which record environmental signals like light level, chemical concentration, or humidity, converting these signals into transcriptional outputs. A well-studied example is the auxin sensor, which couples green fluorescent protein (GFP) activity to the amount of auxin, a plant hormone produced in response to changes in light or soil pH (Vazquez-Vilar et al., 2023). By combining sensors for different plant hormones and enzymes to produce distinct outputs—such as fluorescence—researchers can assess the effects of a plant's environment on its behavior.

Processors

While sensors detect external signals, processors transform these signals into transcriptional outputs, amplifying sensor signals and linking them to normally unrelated genes. Processors achieve this by coupling two protein domains to form synthetic transcription factors (TFs), which are proteins that regulate gene expression. One domain regulates specific genes, while the other binds to particular DNA sequences. This "chimerizing" of TFs—combining sequences from different plants or species—resembles traditional transgenic plant processes but influences gene expression rather than editing the content of genes. CRISPR technology has further simplified this synthesis by enabling the DNA-binding domain of TFs to pair with the dead Cas9 (dCAS9) protein (Piatek et al., 2015). Unlike Cas9, which cleaves DNA, dCas9—directed by guide RNA—only binds to a target gene's promoter sequences to decrease further transcription.

A recent advance of CRISPR interference in synthetic gene circuits involves two binding sites (A and B) for dCas9 in the luciferase gene's promoter sequence (Khan et al., 2022). Each site is targeted by a guide RNA triggered by a distinct sensor input, independently recruiting dCas9 to one of the sides only. The binding of dCas9 to either site represses the promoter, meaning that the promoter is active *if and only if* neither guide RNA is expressed. This system can be modeled by the logical operator NOR, where an output of TRUE means that the promoter has been activated by guide RNA information. To illustrate, imagine charging a computer: it will charge (NOR is true) *only if* both the charger is plugged into the computer's port (A is false) and the wall outlet (B is false). Conversely, if either connection is unplugged (A or B is true), the computer won't charge (NOR is false). NOR(A, B) is true *if and only if* both A and B are false **(Fig. 2)**. As it turns out, the NOR operator is "functionally complete," meaning that all logic gates can be expressed as a combination of NOR gates. If this approach can be extended to other genes or gene combinations with suitably chimerized activators and repressors, researchers could theoretically engineer optimal gene expression for plants based on true/false responses to an unlimited array of environmental factors.

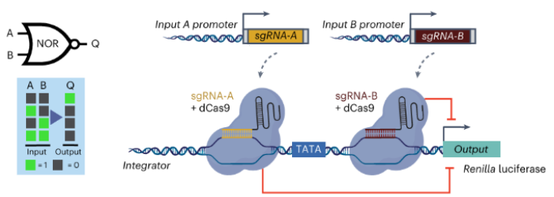

Figure 2. The circuit synthesized by Khan et al. (2022). The expression of the Renilla luciferase gene (the output) is only activated when neither promoter (A, B) is bound. Only when both outputs are False (A = 0, B = 0) does the output become true (Q = 1).

Actuators

Processor outputs are directed to a series of actuators—genes responsible for regulating various plant traits including metabolic enzymes, ion channels, and defense proteins. Actuators can also take the form of fluorescent proteins. An experiment by Vazquez-Vilar et al. (2017) quantified the level of transcription via the relative amount of the fluorescent protein luciferase in different regions of the plant at different times. Measuring this activity allowed the circuit to be fine-tuned to achieve the desired reporter protein expression levels.

Flaws in Current Methods and Timeline for Implementation

Significant challenges remain before synthetic gene circuits can be implemented in plants on a large scale. Since this method requires a series of logic gates—each of which should return either TRUE or FALSE—to activate specific cells, intermediate levels of expression due to imperfect orthogonality can make the truth value of a gate ambiguous, potentially producing the opposite of the desired value for all subsequent gates.

Additionally, adapting these circuits across different species requires specific tailored modifications. Most research has focused on thale-cress (*Arabidopsis thaliana*), a preferred model organism due to its small genome size and short generation time (Vazquez-Vilar et al., 2023). Transient expression via the bacteria *Agrobacterium*, which works extremely well in transforming *A. thaliana*, has been the method of choice for observing the effects of gene circuits, but stable crops other than A. thaliana will likely need alternative hosts and methods to retain the circuits over the long term.

Other Features and Applications of Synthetic Gene Circuits

In synthetic gene circuits, maximizing specificity and orthogonality—minimizing cross-interaction between synthetic and endogenous plant genes—is crucial. This design prevents interference, both between natural responses to stimuli and circuits as well as between different circuits. High orthogonality allows for more predictable responses and reduces the risk of harmful cross-talk within host cells. One strategy for orthogonality is to incorporate components from non-plant sources such as the bacteria-derived dCas9 or the firefly-derived luciferase. Another strategy is the randomization of the DNA elements within a processor (Belcher et al., 2020). While past studies combined DNA elements within the same organism for the processor design, researchers found that the use of DNA elements from both yeast and plants had the potential to produce both a wider range of transcriptional strength and a more orthogonal circuit.

Some researchers are working to adapt synthetic gene circuit technology for use in humans. The mammalian immune system is generally more accessible than that of plants, opening possibilities for applications like chimeric antigen receptor T cell therapy (CAR-T therapy), where chimerized T cells express antigen-targeting receptors on their surfaces (Li et al., 2023). These receptors can then help trigger anti-cancer responses in affected areas of the body. Much like in plants, mammalian circuits prioritize orthogonality to avoid interference with the body's natural systems. However, early trials of CAR-T therapy have occasionally led to adverse effects and even death. In one case, inadequate orthogonality of chimeric T cells led to a "cytokine storm," as the patient's immune system mistakenly attacked the patient (Morgan et al. 2010). For synthetic gene circuits to be safely and broadly implemented, researchers must ensure near-total predictability in circuit behavior, especially given the complexity of potential interactions with thousands of endogenous human genes.

Conclusion

The potential impact of synthetic gene circuits in agriculture is immense. If integrated across various crops at a large scale, these circuits could increase plant resilience towards pathogens and severe weather events by upregulating genes that control immune responses and other defenses precisely when needed. At the same time, scientists have the agency to make sure any of these changes do not affect the plant's environment if something goes awry - they can simply inactivate the circuit by reprogramming the involved guide RNAs (Khan et al, 2022).. By combining existing genes within the plant with a highly customizable system of circuits, researchers are optimizing the world around us via computer logic in a sustainable and eco-friendly way. As gene circuit technology develops and is integrated into more complex organisms, these systems may be harnessed to make many aspects of human life easier, starting with crop engineering.

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Volume 15 Issue 1 | Fall 2024 FEATURES

Drugs for Speech: Could Pharmacological Treatment of Speech Disorders Displace Speech Therapy?

Courtesy of atlascompany on Freepik.

April Keyes ’26

Introduction

We can all picture speech therapy. A smiling therapist sits with their patient, likely a young boy, showing him pictures of colors and trying to get him to say the word “blue.” He struggles with the introductory “buh” sound, and the therapist repeats it to him, exaggerating their facial movements in an effort to help him do the same. In this scenario, the child likely struggles with stuttering, either repeating the “buh” sound or being unable to produce it at all.

This depiction of speech therapy is common and demonstrates a large truth: adolescent boys are the largest demographic affected by stuttering (Howell, 2007). However, speech therapy serves a much broader purpose—people from all ethnicities, all age groups, and all gender identities have speech disorders and seek therapy as a result. In the United States alone, children who experience speech problems receive speech therapy at rates of around 66–67% (HHS, 2015). Additionally, stuttering is far from the only speech disorder that is treated in speech therapy. Speech disorders are complex and range from aphasia—which impairs language expression and comprehension—to dysphonia—which causes pitch and volume issues—to apraxia of speech, which disrupts lip, tongue, and jaw movements.

Speech disorders can arise for a myriad of reasons. Genetics, such as the genes GNPTAB and GNPTG, have been found to play a large role in the etiology, or cause, of stuttering (Frigerio-Domingues & Drayna, 2017). Twin studies demonstrate a higher correlation in stuttering frequency among monozygotic (identical) twins when compared to fraternal twins (Dworzynski et al., 2007). Independent of genetics, neurodegenerative conditions such as Parkinson’s disease and Alzheimer’s, as well as traumatic brain injuries, are associated with speech impairment by damaging the brain areas responsible for speech processing and production. Physical conditions, such as impaired vocal cords or nerve damage, can also impact speech (Penn Medicine, 2022).

Beyond affecting different demographics and resulting from various etiologies, speech disorders can range significantly in both severity and impact. It is no secret that impaired communication abilities can have serious consequences for those afflicted by them, including but not limited to mental health issues, impaired social relationships, and missed job opportunities (Wilmot et al., 2024). Studies have found negative correlations between speech-language disorder severity and college attendance as well as evidence that adults with speech-language disorders are less likely to participate in the workforce. When they do, they are more likely to work in jobs involving unskilled manual labor and are more likely to face termination than their fluent coworkers. Furthermore, school-age children who stutter are up to six times more likely to suffer from social anxiety disorder and are around seven times more likely to have generalized anxiety disorder than their fluent classmates (Foster et al., 2023). While stuttering does not encompass all speech disorders, its impacts can be reasonably assumed to extend to children with other impairments.

Because there is so much variance between disorders, one question arises: how can speech therapy effectively treat all of them? What distinctions exist between therapeutic approaches to various disorders, and, if some aren’t effective, where else can we look for therapeutic impact?

The Current State of Speech Therapy

As a current practice, speech therapy is multifaceted. Speech therapy sessions are tailored to the needs of each patient, yet standard practice uses many of the same techniques. These practices most often include exercises for breathing and swallowing, recall exercises for those struggling with word identification, and sound formation exercises (Santos-Longhurst, 2019). Speech therapy sessions are typically held frequently for about 30–60 minutes each, and many patients who have persistent disorders remain in therapy for years at a time (NLM, 2022).

Speech therapy has helped millions deal with the physical and social difficulties that result from speech disorders. Patients have learned to better produce coherent sentences, overcome inabilities to articulate certain sounds, redevelop speaking ability after strokes, and more. However, the average cost of a speech therapy session in the United States is between $100 and $250 per hour, and not everyone may be willing or able to pay this cost and/or sacrifice their time (Geller, 2024). Whether a working dad or a college student with classes, a potential beneficiary of speech therapy may not always be able to access it.

Furthermore, speech therapy is not a permanent solution for some patients. Without continued treatment, relapse into old and less fluent ways of speaking is possible, especially for patients who invest extra time and effort into a new form of communication (Tichenor & Yaruss, 2020). These methods often emphasize breathing and word-forming techniques that are unnatural to the speaker (Lowe et al., 2021). Without motivation and practice after one's therapy ends, regression—whether it be large or small—is relatively common. In extreme cases, when a patient’s “new” way of speaking is too difficult to maintain and expends too much mental energy, full relapse can occur.

Drug-Induced Stuttering: What We Know and Implications

When speech therapy fails to have lasting effects or is unfavorable to someone’s socioeconomic circumstances, attention can shift to the possibility of medication. While there are very few known drugs to alleviate speech disorders, and there are no FDA-approved medications specifically for stuttering, clinical data has provided insight into potential neurological targets (Maguire et al., 2020). Observations of induced speech disorders have been recorded from clinical trials and standard medications regularly given to patients. This question then remains: what are these medications targeting, and could a common factor between them be applied to the therapeutic treatment of speech disorders?

Several pharmaceuticals have resulted in side effects involving stuttering, particularly antidepressants, antiepileptics, and antipsychotics, all of which are involved in altering neuronal signals (Nikvarz & Sabouri, 2022). Neurons fire action potentials, or electrical signals that travel down the length of the axon after receiving information, and these medications impact action potential frequency, which can influence the strength of a neurological signal.

Interestingly, neurological pathway interference has not been observed to impact stuttering in all patients

identically, with some drugs causing different effects in different patients (Brady, 2020). Risperidone, an antipsychotic that balances dopamine and serotonin levels, has been observed in multiple cases to both cause and alleviate stuttering presentation (Atay et al., 2014). Clozapine, another serotonin and dopamine balancing antipsychotic used for schizophrenia, has displayed induced stuttering, even influencing severity according to medication dosage (Jaguga, 2021). Haloperidol, a dopamine antagonist antipsychotic used in similar schizophrenic cases, has displayed fluency-improving effects for many years (Murray et al., 1977; Andrews & Dozsa, 1977). With these conflicting impacts on stuttering displayed in a snapshot of the many medications shown to impact speech fluency, it is difficult to progress toward a unified pharmaceutical treatment. However, seemingly conflicting stuttering presentations can still provide great insight into speech disorder etiology and potential treatment targets.

Dopaminergic Pharmaceuticals: Impact and Function

One overwhelmingly common factor within the current therapeutics shown to impact speech ability is dopamine (Maguire et al., 2020). Drugs that have displayed treatment impacts in stuttering, Tourette's syndrome, and generalized fluency issues include vesicular monoamine transporter 2 (VMAT2) inhibitors—which deplete dopamine—and serotonin-dopamine antagonists (SDAs) (Makhoul & Jankovic, 2023.; Lavid, n.d.).

Dopamine-related medications that influence speech are either agonists or antagonists—meaning that they either activate or block dopamine receptors—and therefore stimulate or inhibit neuronal activation. These medications are often used in the treatment of mental health disorders such as depression and schizophrenia that are associated with dopamine dysregulation, such as the deficit of dopamine in the ventral tegmental area and the hyperactivity in the subcortical brain associated with major depressive disorder and schizophrenia respectively (Conn et al., 2020; Belujon & Grace, 2017). This coexisting impact of dopamine agonists and antagonists on these disorders and speech has led to one theory surrounding speech disorders (independent of those caused by physical injury): similar to the hyperactivity associated with these mental health conditions, speech disorders such as stuttering and aphasia may be associated with unusual activity levels in dopaminergic pathways within the

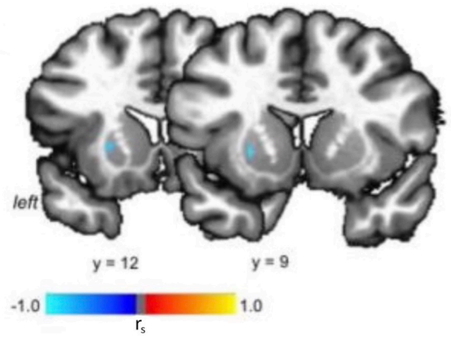

Figure 1. Speech induced brain activation and dopamine coupling is shown. This occurs in the ventromedial portion of the left hemisphere, providing evidence for a linkage between dopamine expression levels and human speech. The color bar depicts Spearman's correlation coefficients (rs), which measure direction and strength of variable association. These range from -1 to 1. The light blue color shown indicates a strong association between dopamine expression and brain activity (Simonyan et al., 2013).

brain, leading to either hyper- or hypoactivity that inhibits the proper synthesis and production of speech (Turk et al., 2021).

Many studies have demonstrated clear connections between dopamine and speech production. A recent study has provided the first concrete evidence of endogenous dopamine expression during human speech production, showing expression in the ventromedial portion of the lateral striatum in the left hemisphere, or the brain region most strongly associated with motor and reward (**Fig. 1**, Simonyan et al., 2013). The basal ganglia is the center for dopamine production under normal conditions, after which the neurotransmitter spreads to the frontal cortex, midbrain, and brainstem. Dopamine is largely accepted as crucial to motor function, as its modulation has proven very important to speech production and other types of vocalizations. Despite the large scientific acceptance of dopamine as crucial to motor control within the brain, not much is understood about the chemical basis of this impact on speech (Turk et al., 2021).

Recent studies have implicated astrocytes—star-shaped glial cells that support the nervous system—in speech regulation because of their close connection to the dopaminergic pathways in the brain and impact in involved conditions such as Parkinson's or stuttering (**Fig. 2**). Another indicative finding is that calcium levels, which dictate glial cell function, often change in unison with dopamine levels in relevant brain areas (Turk et al., 2021).

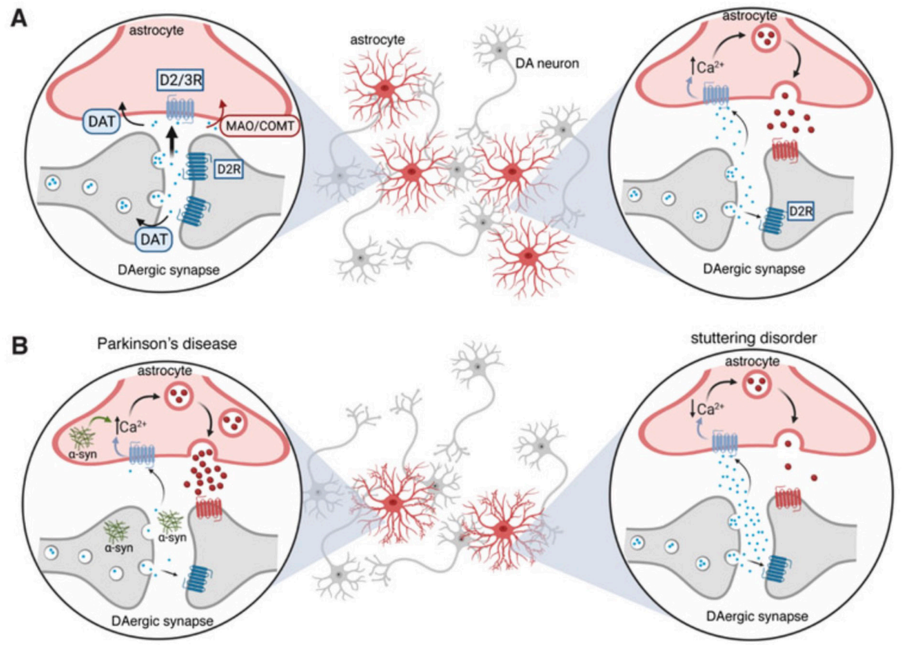

Figure 2. [A] Dopaminergic circuits involving astrocytes in the basal ganglia are depicted. In each of the four pictures, the presynaptic dopaminergic neuron (right gray) releases dopamine into the synaptic cleft that is detected by postsynaptic dopamine receptors on the postsynaptic neuron (right gray). Astrocytes (red) play a role in recycling dopamine, as after they detect dopamine their intracellular calcium levels increase, leading to the release of gliotransmitters(chemicals that influence neuronal function and communication). **[B]** Two different diseased synapses are shown. Parkinson's disease results in excessive release of gliotransmitters. In the proposed stuttering model, excessive dopamine release actually results in decreased calcium levels in the astrocytes, leading to decreased gliotransmitter release (Turk et al., 2021).

These areas include the substantia nigra pars compacta (SNc), which is a brain region comprised of dopaminergic neurons associated with motor and emotional regulation as well as speech, and the ventral tegmental area (VTA), which also mediates dopamine levels and plays a crucial role in reward processing. Lastly, the nucleus accumbens (NAc) is also implicated, which is associated with speech and emotional behavior. An apparent connection between emotionality and speech production can be observed when considering brain region functionality, which further supports the evidence surrounding dopamine, normally used to treat many emotionally driven disorders, in speech disorder treatment.

Further, dopamine has even displayed an impact on neurodegenerative speech conditions such as Parkinson's disease (PD). In PD, characteristic loss of laryngeal muscle activity is thought to derive from a loss of binding at dopamine receptors, an effect that was propagated in a study that utilized dopamine antagonists to further inhibit dopamine activity (Feng et al., 2009). This led to severe vocal inability, demonstrating an etiology stemming from dopamine-associated underactivity as opposed to the hyperactivity usually thought to cause disorders like stuttering.

Differential Drug Use: Treating the Source or the Symptoms

The success of dopaminergic pharmaceuticals as well as the apparent linkage between emotional brain centers and those responsible for speech production raises important questions: what aspect of speech disorders are these drugs targeting? Are these dopaminergic therapeutics truly targeting the speech pathways, or potentially targeting the emotional pathways to which they are connected? As it is known that anxiety and depression levels have positive correlations with most speech disorder severities, could it be that these drugs are just having a typical therapeutic effect, calming the nerves to help fluency (Bernard & Norbury, 2023; Lockett, 2021). If this is true, would it make the therapeutic

pursuit of these agents less valid? These questions can only be answered by more research. Ultimately, the purpose of therapeutic development is to offer speech disorder patients alternative pathways to obtain the therapeutic effect they are pursuing.

Dopaminergic drugs also pose safety risks, as do many commercial therapeutics if used in the wrong dosages or with unknown conflicting medical conditions. The most common side effects include dizziness, headaches, and arrhythmia (irregular heartbeat); in older patients, psychiatric disturbances such as anxiety, depression, and hallucinations can become more common. These risks may be well worth it to a patient with severe social and workplace repercussions for their speech disorder and must be evaluated on a case-by-case basis.

Conclusion: Where are we now?

Therapeutic development is no easy task, and the conflicting presentations of speech disorders in patients are another barrier. If FDA approval is not guaranteed or even feasible, will speech disorder medications be offered on a case-by-case, experimental basis? As of now, the future of therapeutic development for speech disorders remains both uncertain and largely patient-based. Patients willing to experiment with different medications for the sake of their eventual fluency will advance our records and eventually improve their social efficacy, but will this advance therapeutic development? The fundamental complexity of speech is daunting, and time will reveal whether such individual efforts can bridge the gap in our understanding.

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Leah Lourenco '26

Introduction

Intense abdominal pain, crippling fatigue, and incessant visits to the bathroom plague a growing subset of patients daily. This is a reality for an estimated 2.8 million people in the US and 10 million worldwide, with no cure in sight (CDC, 2024; World IBD Day, n.d.). These individuals live with Inflammatory Bowel Disease (IBD), an umbrella term composed primarily of Crohn's Disease (CD) and Ulcerative Colitis (UC) **(Fig. 1)** (Crohn's & Colitis Foundation of America, 2014).

IBD is an autoimmune condition in which the patient's immune system mistakenly attacks healthy cells throughout the gastrointestinal system (CDC, 2024). In healthy individuals, the intestinal epithelium is the primary barrier between the food passing through the system and the rest of the human body. This barrier, sealed by intercellular connections, protects the body from infiltration of any harmful microorganisms. In patients with IBD, this epithelium is incomplete due to intercellular junction disconnections resulting from innate dysfunction or severe inflammation (McDowell, 2024). This

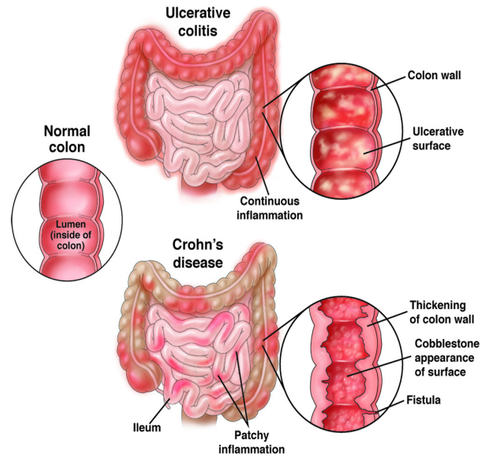

Figure 1. Comparison of CD vs. healthy vs. UC colons (American Gastroenterological Association, 2021).

compromised epithelial barrier increases patients' risk of infection from food-borne microbes.

Patients using conventional IBD treatments may experience a broad range of medication efficacy, leaving many unsatisfied

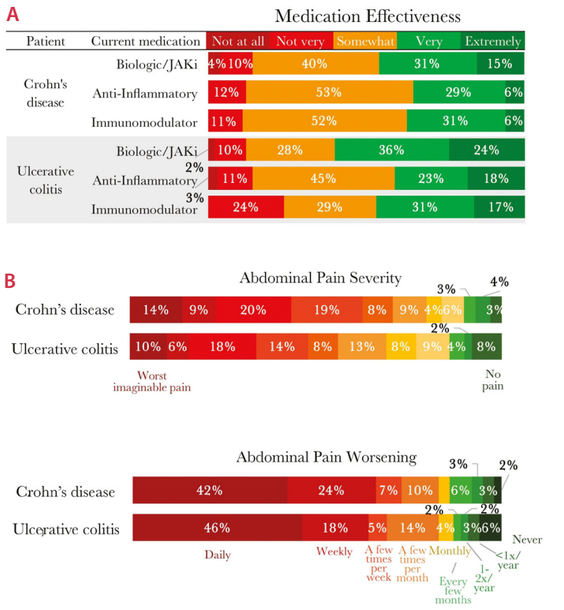

Figure 2. A. Patient-observed medication efficacy (sample size: CD, N = 189; UC, N = 87). B. Patient-rated severity of abdominal pain (Charabaty et al., 2022).

with their treatment (**Fig. 2A**). Furthermore, patients with both CD and UC continue to experience both severe or worsening abdominal pain daily (**Fig. 2B**). There is a need for novel and improved solutions for these patient populations. To develop novel solutions, it is critical to understand the CD and UC therapies developed and administered in the past.

Therapy of the Past

IBD was first reported by Matthew Baillie in 1793; ever since, researchers have made great strides in understanding the disease's function and best treatment practices. In the early 1900s, practitioners could only treat IBD patients by prescribing bed rest and performing colon irrigations, a procedure that involves flushing the bowel with fluid (Actis et al., 2019). This procedure can often cause symptoms to worsen and even damage the intestinal lining (Mayo Clinic, n.d.).

Between the 1950s and the early 1990s, a variety of drugs were used to treat and manage UC and CD. This included corticosteroids, aminosalicylic acids, and thiopurines (Actis et al., 2019). Thiopurines are immunosuppressants used to prevent T cells from causing inflammation in the body (Neurath, 2010). Although these therapeutics could improve IBD symptoms, their lack of specificity resulted in a weakened immune system throughout the body, putting patients at risk of opportunistic infections.

In 1998, more modern treatments entered the gastroenterology clinic space **(Fig. 3)**. Infliximab, commonly known as Remicade, was the first modern therapy approved for CD, achieving FDA approval in 1998 (Actis et al., 2019). Infliximab functions by binding to and inhibiting the inflammatory tumor necrosis factor (TNF) produced by several types of immune cells (Fatima et al., 2024). However, infliximab contains rodent-derived components, which led to adverse immune responses in patients. To resolve this challenge researchers developed adalimumab, also known as Humira—a fully human monoclonal antibody that functions similarly to infliximab—which was approved for IBD treatment in 2007 (Drugs, n.d.; Venaa & Cassano, 2007).

A different class of monoclonal antibodies known as IL-12 and IL-23 inhibitors acts to prevent immune response in IBD by selectively inhibiting the immune system (Actis et al., 2019). The most common drug of this class is ustekinumab—also known as Stelara—and it was approved for IBD in 2016 (Drugs, n.d.). These therapies act throughout the body and do not focus solely on the gut. Therefore, side effects from ustekinumab can manifest anywhere. Vedolizumab, another monoclonal antibody, was a breakthrough therapy in 2014 that addressed this issue, serving as the first localized treatment for IBD (Drugs, n.d.; Actis et al., 2019). Given that each of these therapies is biological in nature, it is common for patients undergoing such treatments to experience negative immune responses. When this happens, patients must be taken off of the medications, necessitating alternative therapeutic options.

Due to the patient-specific nature of IBD, scientists have experienced difficulty in developing an all-encompassing therapy. Many patients are still using non-selective immunosuppressive therapies that are over a decade old and often result in numerous adverse side effects. As a result, there is a great need for a new era of innovation in the IBD space.

Current Therapeutic Landscape

Three main pathways are currently being explored in IBD treatment: drug delivery strategies, cell-based therapies, and precision medicine.

Novel approaches to drug delivery strategy in IBD are dominated by nanotechnology. Nanotechnology is advantageous due to its highly localized nature, which reduces systemic immunologic reactions in patients. It promises to be particularly useful in IBD applications because, as inflammation becomes more severe, the colon becomes more permeable. This allows nanoparticles to permeate exclusively in the locations where inflammation is present. Furthermore, when the colonic epithelium is damaged, positively charged

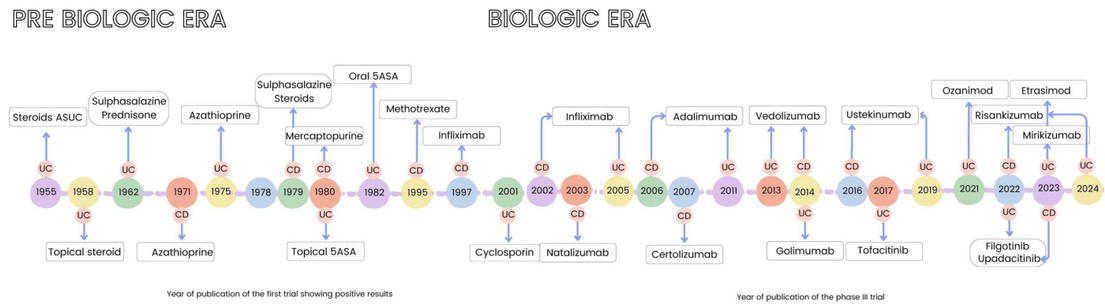

Figure 3. Approval timeline of IBD drugs since 1955 (IBD-EII, n.d.).

proteins accumulate around the inflammation. By negatively charging the surfaces of the nanoparticles, researchers can further direct their localization to sites of inflammation (Yasmin et al., 2022). Researchers have also identified a variety of other ways to control nanoparticles such as inflammatory surface marking and redox sensitivity.

Another drug delivery strategy is the use of prodrugs, in which drug release is controlled through exposure to specific activating enzymes (Yasmin et al., 2022). For example, cyclosporine, a prevalent immunosuppressant, has been successfully studied as a prodrug candidate in vitro. Researchers developed a material complex that stores cyclosporine and only allows the cyclosporine to be released when the complex comes in contact with the phospholipase A2 (PLA2) enzyme, which is concentrated in the inflamed intestine (Markovic et al., 2022). This approach provides a localized method of delivering cyclosporine, an established treatment for IBD.

Cell-based therapy primarily focuses on healing the soft intestinal lining and preventing inflammation. These aims have been approached using both immune and human stem cell therapies. T regulatory cells (Tregs) and tolerogenic dendritic cells (Tol-DCs) are both involved in maintaining immune homeostasis (Hossein-Khannazer et al., 2021), with Tregs inhibiting immune responses by secreting anti-inflammatory cytokines (Leung et al., 2010). Additionally, by controlling Tol-DC maturity and antigen expression, researchers can engineer them to prevent adverse immune responses (Hossein-Khannazer et al., 2021).

Two main stem cell types have been explored for IBD treatment: hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) (Hossein-Khannazer et al., 2021). HSCs are characterized by their ability to differentiate into blood and immune cells. Scientists are able to utilize this characteristic to heal tissues damaged by IBD. However, this approach is significantly limited by the immune response caused by HSC administration. MSCs, conversely, can differentiate into any cell type other than embryonic cells. They are capable of both targeted tissue regeneration and immune regulation. The most common stem cell therapy for IBD is MSC transplantation to supplement tissue healing and immune control (Tian, 2023). MSCs have low immunogenicity, so the body does not engage in an immune response against them. However, MSC therapies are currently limited because MSCs are also capable of developing pro-inflammatory phenotypes due to their high differentiation potential.

Finally, precision medicine is perhaps the most promising yet difficult-to-execute therapeutic direction being researched today. IBD affects each patient differently, with varying triggers, symptoms, and even treatment efficacies. The aim of precision medicine is to specifically calibrate the therapeutic approach to each patient. By looking at a patient's genes, microbiome, molecular pathways, and other markers, researchers hope to provide a personalized therapeutic approach (Annese &

Annese, 2023). One of the many potential applications of precision medicine in IBD is in predicting an individual's response to a certain therapy. In one case study, researchers sprayed anti-TNF markers on the intestinal surface during endoscopy to quantify cells expressing TNF. Only patients with high TNF initially responded well to anti-TNF drugs (Annese & Annese, 2023). Using strategies such as these, researchers may be able to predict patient response to treatment and thereby avoid long periods of ineffective therapy.

Dr. Ashwin Ananthakrishnan, M.P.H., M.B.B.S., M.D, an expert in IBD research and practicing gastroenterologist at Massachusetts General Hospital provided insight on the current outlook of IBD treatment. Dr. Ananthakrishnan studies the environmental factors that contribute to IBD in the general population and develops predictive models for IBD development and response to treatment. Additionally, Dr. Ananthakrishnan's group is seeking to "identify if biomarkers in the tissue, blood, or stool can identify which treatment is most likely to work for a given patient and thus help make treatment selection less of a random choice." Successful identification of these biomarkers would allow practitioners to make more informed decisions about their patients' treatment plans.

Dr. Ananthakrishnan stated that "while no treatment has promised 100% effectiveness, during the past 10 years, we have seen the approval of over ten different medications including four different medication classes." He further emphasized the need for a more holistic understanding of the treatment timeline, patient monitoring, and incorporation of more non-invasive techniques. Also promising is the "role of the microbiome in IBD and how it can be beneficially modified" to improve IBD treatment. Dr. Ananthakrishnan also commented on the impact of COVID-19 on emerging IBD therapies and referenced the "substantial decline in in-person clinical contact." This decreased patient involvement in clinical trials and in-person assessments as well as blood, tissue, or stool collection over the last several years. Clinical trial enrollments have increased once more, but researchers are still facing a reduction in involvement due to trial complexity, treatment availability, administrative burden, and trial requirements.

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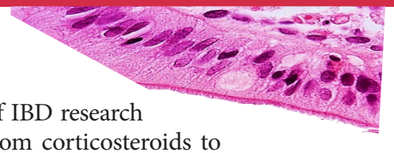

Conclusion

The modern history of IBD research is short, and progress from corticosteroids to monoclonal antibodies to small molecules has been quick. Now, patients are looking towards new solutions and improvements to existing treatments. Current therapies are accompanied by a host of side effects that often result in immunocompromisation for patients. It is time for a breakthrough in IBD therapy research. While cell-based therapies and personalized medicine are exciting innovations, they are challenging both to research and also to implement practically. Potentially the most promising practical research areas are in improving and optimizing pre-existing treatments.

For treatment to have come this far since the 1950s is an impressive feat. Moving forward, patients, researchers, and clinicians have a hand in the future of IBD treatment. With improved patient involvement in clinical trials and researcher–practitioner collaboration, it seems certain that research and technology will only continue to provide better options for the 10 million people around the world who face their IBD diagnosis daily.

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Citizen Science and the Democratization of the Climate Crisis

Courtesy of the National Parks Service / Renata Harrison.

Sophie Gao ‘28

Introduction

The question of how to contend with the rapidly warming Earth is immense in scope. The implications of the climate emergency affect each and every individual on the planet—whether in the form of increased food scarcity, extreme weather events, or negative health impacts. Scientists warn that since 1982, our planet has been warming three times faster than it was in the 1800s and that the past decade has been the warmest on record (Dahlman & Lindsey, 2024). An immense amount of scientific data is required to understand this uniquely global crisis, allowing us to combat climate change by mitigating its fundamental causes, including greenhouse gas emissions and deforestation, and by adapting communities, ecosystems, and socioeconomic structures to its impacts.

Though traditional perceptions of scientific research and data collection may involve white-coated academics gathered around a bubbling flask or typing out lines of code, citizen science—the collection and analysis of natural data by the general public—may aid in the large-scale data collection required to improve understanding of the climate crisis. Public involvement in the production of scientific knowledge is not only a way of accelerating data collection far beyond what might be accomplished by a handful of researchers but also gives participants agency in developing solutions for this increasingly dire crisis.

A Brief History of Citizen Science

Members of the general public have been recording their observations of the world around them for centuries. Science was not professionalized until the 19th century with the proliferation of scientific societies and journals; instead, early climate science was largely done by amateur experts and non-scientific professionals tracking the world around them for cultural or agricultural means (Le Treut et al., 2007). For instance, court diarists in Japan have logged the dates of cherry blossom blooms for over a thousand years, while the oldest organized datasets on American climate were compiled not by scientific or government officials but by farmers documenting sowing and harvesting seasons (Miller-Rushing et al., 2012).

Even after science was professionalized, citizens have been continuously collecting data on the natural world, often in amateur science groups. A study at Portland State University compared recent observations of plant leafing and flowering to data collected between 1826 and 1872. This data was collected by a network of over 500 observers organized by the New York State Regents and the Smithsonian Institution’s national network of meteorological observers, which would later become the National Weather Service. The investigators concluded that, as a result of changing temperatures and exponentially growing industrialization, plants now leaf and flower earlier than they did two hundred years ago (Battle et al., 2022).

Citizen science was not formally named until the 1990s, when it was conceptualized independently by Alan Irwin, a British sociologist who defined citizen science as “developing concepts of scientific citizenship which foregrounds the necessity of opening up science and science policy processes to the public,” and American ornithologist Rick Bonney, who defined citizen science as projects where amateur scientists contributed to scientific data (Riesch & Potter, 2013). The current ideal of citizen science as defined by the National Parks Service is top-down, with scientific researchers introducing a project that they recruit citizens to participate in. There are also bottom-up projects, which are created by citizens to address problems in their own communities, with scientific researchers stepping in to aid in the more technical aspects of the work—an approach referred to as “community science” (National Parks Service, 2024).

Since the 1990s, citizen science efforts have helped describe the changing climate and inform responses to natural disasters. A 2019–2022 European Union-sponsored study, TeRRIFICA, surveyed citizens across six European countries to create maps describing climate change impacts on specific regions, demographics, and environments with the ultimate goal of informing policy suggestions (TeRRIFICA, 2022). “One important aspect of citizen science is that it includes knowledge and expertise to which researchers do not usually have easy access," said Norbert Steinhaus, TeRRIFICA’s project coordinator in a promotional video. He continued, “It’s cultural knowledge. It’s individual knowledge” (Global University Network, 2022). Another successful implementation of citizen science involved the rapid characterization of earthquakes in Haiti using data from individual seismometers. Following the devastating earthquake that struck Haiti in 2010, researchers spent days determining the ruptured fault that had caused the earthquake and worked for months after to characterize the event. In 2019, French researchers distributed inexpensive, handheld seismometers to citizens to complement national seismometer networks. When another earthquake struck Haiti two years later, researchers were able to calculate the quake’s strength, epicenter, and geometry within hours using data collected from these citizen seismometers (Corbet et al., 2022).

Personal Impact of Involvement in Citizen Science Work

The act of participating in citizen science can also help create a more informed populace. By decentralizing and democratizing the practices of scientific knowledge generation and data collection, participation in citizen science projects can raise awareness of climate issues and incentivize the general population to advocate for governmental change. For instance, a Brazilian study calling on individual citizens and local schools to help map natural disaster risks found that the experiment helped those communities build technical data analysis skills and improved their understanding of risk-management strategies (Albagli & Iwama, 2022). Similarly, participation in citizen science projects raises awareness of the issue being investigated. In the case of climate change, this newfound awareness correlates with increased environmental stewardship and likelihood that a community will be prepared for natural hazards (Walker et al., 2020). A third study noted that 95% of those surveyed who participated in at least one citizen science project per month reported a better understanding of the importance of environmental monitoring and the role humans can play in that process. 73% of those in the same

study noted that they felt more motivated to protect the environment (Jones et al., 2013).

Citizen science may also help alleviate climate anxiety, a recent phenomenon defined as distress related to climate change. According to a study conducted at the University of Kent, participants in citizen science conservation projects are likely to do so because they are concerned about wildlife welfare—in other words, participation in these projects empowers them with the opportunity to make an impact in larger issues (Maund et al., 2020). Aldona Czajewska, who coordinated the University of British Columbia's team for Climate Action Engagement & Outreach, noted that participation in citizen science projects such as species identification using apps "allows people to appreciate the life around them and the importance of the species we share the world with" instead of only worrying about the climate crisis that shapes our world (Wahking, 2024). Citizen science participation also benefits participant well-being, with participants reporting positive outcomes related to their mental or physical well-being in focus group discussions (Eichholtzer et al., 2023).

Pitfalls of Citizen Science & Attempts to Rectify Them

However, participants in citizen science may not always accurately reflect demographic realities. According to a 2022 study conducted at North Carolina State University, 96% of participants in the Audubon Christmas Bird Count, which invites layman volunteers across the country to tally all birds seen or heard over a 24-hour period to determine the health of that population, were white (Allf et al., 2022). These findings, said Dr. Caren Cooper, a professor who worked on the study, demonstrated that only a narrow demographic was engaging with citizen science efforts (Moore, 2023). Similarly, a Pew Research Center poll noted that younger and more educated individuals are more likely to participate in citizen science activities (Thigpen & Funk, 2020).

Mismatches between reporting populations and the populations that stand to benefit from citizen science have marked effects. For instance, popular apps like iNaturalist and eBird—which encourage users to share animal and plant sightings with researchers to better understand the biodiversity of local environments and species distribution—are more likely to have observations concentrated in predominantly white areas. This lopsided user distribution means that the maps produced by these apps are more likely to emphasize certain areas when reporting wildlife sightings. To help reduce bias, researchers at Washington University in St. Louis called on their peers to conduct outreach with diverse populations, for instance by translating project materials into different languages (Ogliore, 2024). A group at the University of Haifa also encouraged researchers to account for demographic bias in their statistical models and make specific engagement goals for target populations (Arazy & Malkinson, 2021).

Citizen science is also uniquely susceptible to politicization and subjugation to commercial interests. For instance, in 2019, Airbnb announced that it would send five citizen scientists on an Antarctic cruise to collect snow samples to be tested for microplastics. However, these projects are often avenues for companies to demonstrate social responsibility and direct attention away from the environmentally-harmful impacts of their ventures; Antarctic cruises have recently been noted as major sources of environmental pollution impacting climate patterns and habitats (Blacker et al., 2022).

Conclusions

As questions about adapting to and remedying a rapidly-changing climate grow increasingly pressing, it is critical to leverage all possible methods of large-scale knowledge generation to understand the world around us. Ongoing citizen science projects aim to do just that, leveraging new and accessible technologies to monitor water quality, document species distributions, and inform disaster relief responses.

These projects not only aim to round out our knowledge of the climate crisis but also to give participants a sense of agency in a global crisis. By leveraging inexpensive, easy-to-use technologies, a bicycle ride could become a method of tracking air quality, or a garden could be a microenvironment-monitoring station. As we enter an era where the study of climate science is simultaneously more accessible and more pressing than ever before, it is vital to recall the impact collective action has had on our centuries-long effort to understand the natural world and to consider how we can leverage the power this collaboration between researchers and the public holds to mitigate the effects of the climate crisis.

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Courtesy of UC Davis Health.

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Introduction

Communication remains a priority for people with speech disorders from neurologic conditions such as stroke and amyotrophic lateral sclerosis (Card et al., 2024). Amyotrophic lateral sclerosis (ALS) is a neurodegenerative condition that affects how the brain and spinal cord's nerve cells communicate with muscles. This condition damages motor neurons that regulate voluntary muscle movements, causing symptoms of muscle weakness that progressively worsen and can lead to dysarthria, a motor speech disorder characterized by difficulty in forming and pronouncing words ("Amyotrophic Lateral Sclerosis," n.d.; "Dysarthia," n.d.). This lack of communication not only increases a diagnosed individual's risk of isolation, depression, and decreased quality of life but also largely influences whether they pursue or withdraw from life-sustaining care in ALS (Katz et al., 1992; Lulé et al., 2009; Bach et al., 1993). While augmentative and assistive communication technologies like eye-tracking devices are available, these instruments experience low rates of information transfer and become increasingly difficult to use as patients lose voluntary muscle control with the progression of ALS symptoms (Fager et al., 2019). Ongoing efforts to develop speech neuroprostheses have culminated in a major breakthrough at the University of California Davis, where scientists have successfully created a novel device to translate brain activity into speech from an ALS patient (Card et al., 2024). This device possesses tremendous positive implications in the role of brain–machine interfaces and treatment strategies for irreversible neurologic conditions like ALS.

"Lou Gehrig's Disease"

Tackling novel treatment options requires an understanding of ALS. Cases of this disease can be divided into "sporadic" and "familial" types of ALS, with 90% of ALS diagnoses being sporadic—appearing randomly and not inherited from family members—and about 10% being caused by mutations from one or both biological parents during conception. Individuals are likely to develop symptoms for ALS between the ages of 55 and 75, with the condition most commonly affecting white, non-Hispanic people assigned male at birth ("Amyotrophic Lateral Sclerosis," n.d.). Most famously, New York Yankees baseball player Lou Gehrig's tragic battle against ALS left behind a legacy of strength to future generations, and the condition was formerly called "Lou Gehrig's disease." Gehrig was committed to playing his beloved sport despite suffering from the disease until he was diagnosed in June 1939 and subsequently retired ("Lou Gehrig and the History of ALS," n.d.). Similarly to Gehrig, who passed away in 1941 at the young age of 37, people diagnosed with ALS experience mean survival times of approximately three to five years after diagnosis and this prognosis worsens rapidly as symptoms progress (Francis, n.d.; "Amyotrophic Lateral Sclerosis," n.d.). Moreover, the development of depression and anxiety due to the inability to communicate from complications with dysarthria further exacerbates this tragic outlook ("Amyotrophic Lateral Sclerosis," n.d.).

Dysarthria

Dysarthria is caused by damage to the brain via stroke, brain tumor, or other acquired brain injuries. The manifestations of the motor speech disorder vary based on the location of the damaged neurons within the brain. Flaccid dysarthria, for example, results from lower motor system damage and causes breathy and nasally speech. Contrastingly, spastic dysarthria is caused by damage to upper neurons in the brain and leads to strained or harsh tones in speech. Yet, all types of dysarthria negatively impact muscles with speech functions, preventing full control over body parts that control speech and resulting in speech that is difficult to understand. Despite the potential for possible improvement in communication through speech therapy, dysarthria remains largely irreversible due to its relation to chronic neuromuscular conditions, neurological trauma, strokes, and irreversible diseases like ALS. Notably, up to 30% of individuals diagnosed with ALS have dysarthria ("Dysarthia," n.d.). The lack of adequate and effective treatment has encouraged researchers to uncover more possibilities and strategies for rehabilitation, particularly using brain–machine interfaces.

Existing Literature

In ongoing efforts to develop speech neuroprostheses, a useful resource for comparison is provided by the many studies that analyzed the speech of able-bodied speakers under electrophysiological monitoring in clinical settings. In addition to this valuable wealth of data, successful cases of brain–computer interfaces being utilized in real-time to restore lost speech with implanted electrocorticographic arrays or intracortical multielectrode arrays have further inspired this area of research (Moses et al., 2021; Metzger et al., 2022; Metzger et al., 2023; Luo et al., 2023). In particular, two recent studies of established "brain-to-text" speech performance inspired the researchers at UC Davis to begin their research and development of neural prostheses. In these studies, cortical neural signals generated during attempted speech were decoded into phonemes, the "building blocks of words" that allow researchers to assemble the neural signals into words or sentences (Card et al., 2024). With a median word error rate measured as the percentage of words that were incorrectly decoded, the studies reached an error word rate of 25.5 percent from a 1,024-word vocabulary and 23.8 percent from a 125,000-word vocabulary (Metzger et al., 2023; Willet et al., 2023).

Methods

The team enrolled Casey Harrell, a 45-year-old man with ALS whose symptoms had begun five years before the study (Yehya, 2024). At the time of enrollment, he was non-ambulatory—depending entirely on others for everyday functions such as transportation, dressing, eating, and hygiene—and experienced limited orofacial movement with mixed upper- and lower-motor neuron dysarthria. Harrell's speech was largely unintelligible as he communicated at a mean rate of 6.8 correct words per minute when speaking

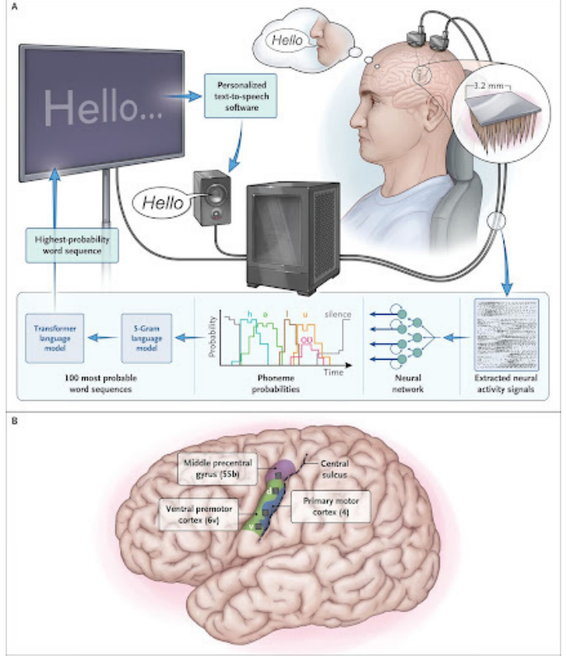

Figure 1. A diagram showing the locations of electrode and speech-decoding setup. Image courtesy of Card et al. (2024).

to expert listeners as compared to the rate of conversational English, which is approximately 160 words per minute. The severity of Harrell's dysarthria remained constant during the study period (Card et al., 2024).

The team of researchers implanted four microelectrode arrays into Harrell's left precentral gyrus, a cortical region necessary for the coordination of motor activities related to speech. The decision to target the speech motor cortex was informed by a previous study in which two arrays implanted in a participant's ventral premotor cortex provided informative signals for speech decoding (Card et al., 2024; Willet et al., 2023). Before implanting the microelectrode arrays, the researchers used functional MRI (fMRI) and standard clinical fMRI tasks like sentence completion, silent word generation, object naming, and others to confirm that the participant was left-hemisphere language dominant. Each microelectrode array was roughly 3.2 by 3.2 millimeters and was implanted through a five-by-five centimeter craniotomy on the left side under general anesthesia. Careful to avoid placing microelectrode arrays through large vessels on the cortical surface, the team inserted 664 electrodes in an eight-by-eight grid with each electrode designed to record signals from a small number of cortical neurons. Next, pedestals were secured to the skull with titanium screws for recording activity at 256 sites and then connected by detachable connectors that used HDMI cables to transmit data to computers. The researchers used signal-processing systems to acquire signals from the two connector pedestals that were sent to computers for real-time signal processing and decoding (**Fig. 1**) (Card et al., 2024).

Decoding Speech

Over 32 weeks and 84 sessions, the team collected data in Harrell's home. The participant used the implanted system in two ways: to complete an instructed-delay copy task, where words were shown on a computer screen and Harrell was asked to attempt to say the words after a visual or an audio cue, and in a participant-paced conversation mode that allowed him to participate in unstructured conversation. In both applications, cortical activity at four microelectrode arrays was recorded and decoded as the participant attempted to speak. As the sessions progressed, the team worked to calibrate the system to grow a more complex vocabulary and better adapt to Harrell's speech by predicting his words through neural networks. The neuroprosthesis was also able to punctuate sentences automatically and eventually send them to Harrell's personal computer similarly to a Bluetooth keyboard, allowing for activities like writing emails (**Fig. 2**) (Card et al., 2024).

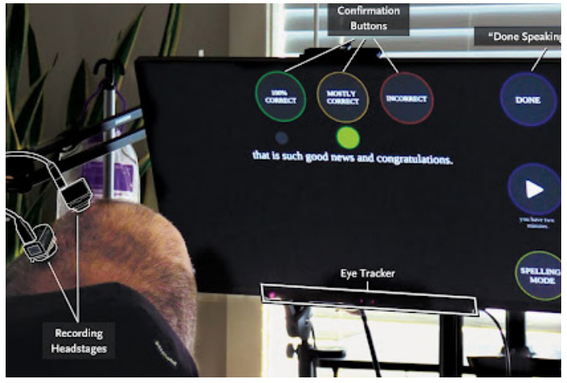

Figure 2. An image of the conversation-mode user interface. Image courtesy of Card et al. (2024).

Results

By the end of the study, the communication rate of the neuroprosthesis exceeded the participant's standard means of communication and could decode words with which it was not explicitly trained. Affirming a lack of acoustic or vibration-related contamination in the recorded neural signals, the researchers applied open-source language models to translate the predicted sequence of words into the most likely English sentence. In the first session, the team decoded Harrell's speech-induced cortical activity in real-time as the neuroprosthesis interpreted attempted speech with a word error rate of 0.44%, which served as an estimate of overall communication activity and was calculated as the ratio of the total number of words to the number of words expected to be decoded (**Fig. 3**). Errors were defined as any need for insertion, deletion, or substitution to match the decoded sentence to the intended sentence. Moreover, in the second research session, neuroprosthesis' expanded vocabulary from 50 to over 125,000 words, which encompasses most of the English language, allowed the participant to communicate freely. Within 16 hours of use, the neuroprosthesis incorrectly identified only 2.5 percent of attempted words (Card et al., 2024) in comparison to the five percent presented by most forms of advanced English automated speech recognition (Tüske et al., 2021). Furthermore, able-bodied speakers have a word error rate of approximately one to two percent when reading text aloud (Thomson et al., 2013). The participant in this study could converse at a rate of 32 words per minute, and the indicated that the system's voice even resembled his own. Upon completion of the study, the system decoded Harrell's attempted speech with an error rate of 9.8 percent—allowing the participant to use the conversation mode to fluidly perform activities ranging from talking to his research team, family, and friends to engaging in his occupation by participating in

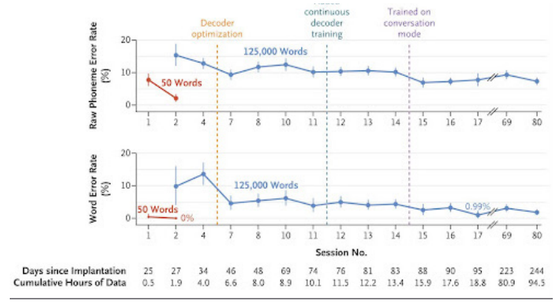

Figure 3. A graph displaying the improved performance of online speech decoding after the implantation. As shown in the graph, the neuroprosthesis was eventually able to decode the participant's attempted speech with a copy-task word error rate of 9.8% (Card et al., 2024).

videoconferencing meetings, writing documents, and more (Card et al., 2024).

Conclusions

While the development of this technology is radical in augmentative and assistive communication technologies, future investigation is necessary since the replication of similar results in future users with similar or different disorders is unknown (Card et al., 2024). Some limitations could include costs and access, as a surgical procedure is required for the neuroprosthesis. Also, since English serves as the foundation for most speech-to-text technologies, decoding other languages (e.g., Sino-Tibetan or Afroasiatic languages) could also present an obstacle due to the structural and lexical differences between language families. Yet, while expanding the vocabularies of brain–machine interfaces beyond the initial language of English would be more difficult, making this effort would be a commendable step towards ensuring more widespread access and inclusivity in globalized treatment plans. Overall, the study marks a significant accomplishment in restoring natural communication for individuals suffering from ALS. The profound accomplishment of this neuroprosthesis in achieving near-natural speech could drastically improve individuals' quality of life, as it may allow users to engage in real-time conversations with a regained sense of autonomy. Furthermore, the technological advancements of this study may encourage the development of advanced speech neuroprostheses that can decode more complex brain signals and ultimately benefit other conditions that also affect communication such as stroke or paralysis. More broadly, this innovative device may revolutionize and redefine approaches to disabilities, restoring lost functions through personalized and inclusive solutions with novel treatments tailored to the patient's needs.

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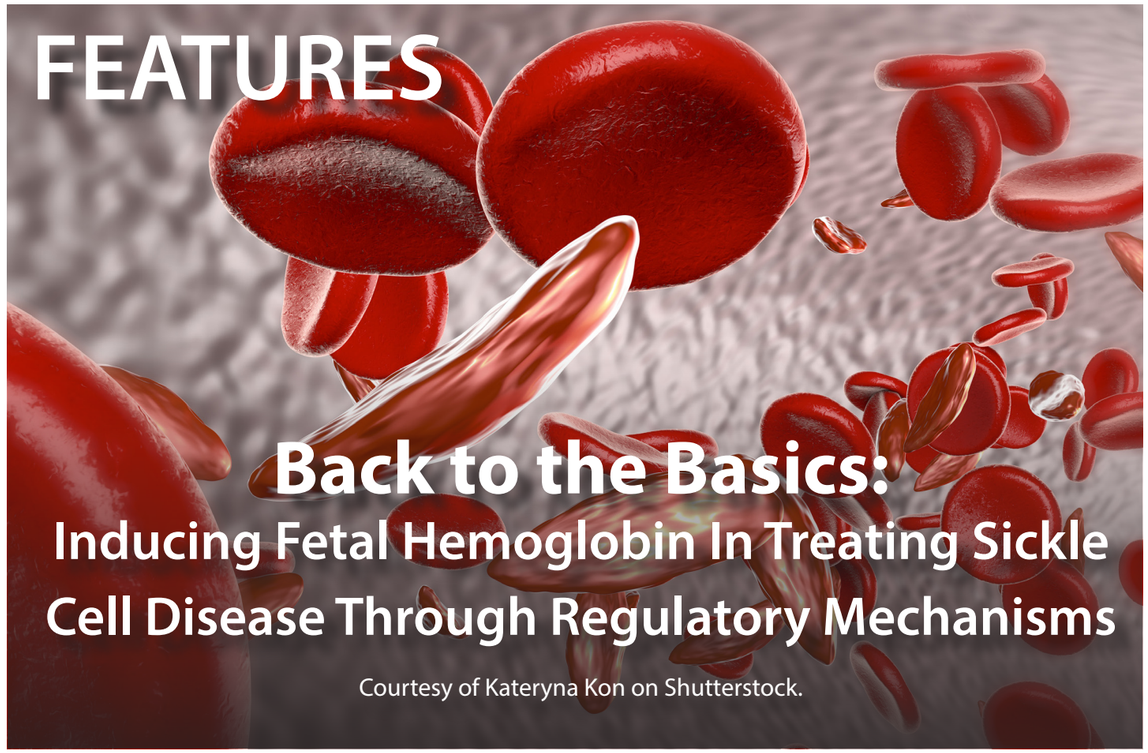

Dalevyon Knight '27

Introduction

Sickle cell disease (SCD) is a group of inherited blood disorders that affect many individuals around the globe. SCD is an autosomal recessive disorder, meaning individuals must inherit the recessive allele from both parents to have SCD. Sickle cell disease can cause anemia, which is when the body fails to produce enough healthy red blood cells. In particular, SCD turns healthy, biconcave disc red blood cells into a sickle shape, which leads to a lack of oxygen in the body. SCD can cause a wide range of symptoms, with one of the most common and severe symptoms being painful episodes known as crises. Crises can last for days and cause pain in areas such as the chest, back, and legs. Additionally, due to the lack of oxygen delivery from the sickle-shaped cells, fatigue and shortness of breath are also common symptoms.

In the United States, more than 90% of individuals who have sickle cell disease are African American, as SCD occurs in about 1 of every 365 Black or African American births (CDC). Furthermore, approximately one in every thirteen Black or African-American babies is born with the sickle cell trait, meaning that they only inherit one recessive allele from a parent (CDC). While those with the sickle cell trait typically show little to no symptoms, the recessive allele can still be passed on to the next generation, potentially leading to the development of sickle cell disease in offspring. Globally, sickle-cell disease (SCD) primarily affects populations in Sub-Saharan Africa as well as those in developing countries (CDC). **Figure 1** shows the distribution of SCD worldwide in 2015, with similar rates still prevalent today (Ashorobi and Bhatt, 2019).

Sickle Cell Disease Treatments

There is only one well-known procedural cure for sickle cell disease: a bone marrow transplant. A common type is the allogeneic (or donor) transplant, which replaces diseased blood cells with healthy ones (Smith). While this process is conceptually straightforward, a bone marrow transplant is a complex and lengthy process.

The transplant process begins with chemotherapy, which destroys the patient's existing blood-forming cells—hematopoietic stem cells—including those that produce sickle-shaped blood cells. Chemotherapy also weakens the immune system, reducing the chance that the body rejects new stem cells when they are introduced (Smith). Following

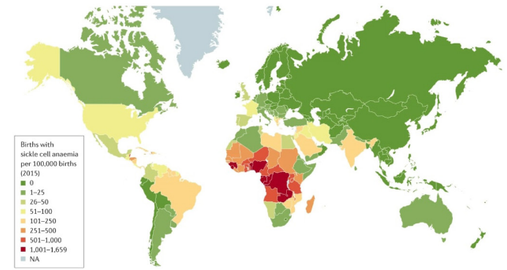

Figure 1. Global Breakdown of Sickle Cell Anemia Birth Rates (Kato, Gregory J., et al. 2015)

chemotherapy, healthy cells from a donor are injected into the patient intravenously("Sickle Cell Disease | NMDP", n.d). These donor cells travel to the bone marrow, where they begin producing healthy cells including normal red blood cells. Finally, the patient enters a recovery period, spending several weeks in the hospital before continuing to recover at home, during which they may take medication and must follow specific rules to prevent infection ("Sickle Cell Disease | NMDP"). Doctors closely monitor the pain and symptoms of the patient to ensure everything post-transplant runs smoothly. During recovery, there are risks of fever, infection, bleeding, anemia, and dietary problems for the patient.

Bone marrow transplants have demonstrated notable success rates: A transplant from a matched related donor has an 85–90% success rate, while a transplant from a sibling or family member has a 95% success rate ("Investigating Bone Marrow Transplants: A Cure for Some Sickle Cell Disease Patients"). Despite this, there are many associated risks with a bone marrow transplant. One of the most common complications is graft-versus-host disease, in which immune cells produced by the transplanted cells attack the patient's own body, potentially leading to permanent and severe health impacts ("BMT for Sickle Cell Disease"). Other associated risks include infection, rejection of the new cells, infertility post-transplant, organ damage, or even death ("Yale Medicine"). Approximately five percent of those who receive the transplant die ("Stem Cell Transplant for Sickle Cell Disease"). Given these risks, many patients rely on symptom management to alleviate the effects of SCD. Fluids, over-the-counter supplements, medical care, and nutritional supplements like zinc can help manage symptoms (Johns Hopkins Medicine).

Beyond a bone marrow transplant, the drug hydroxyurea has made significant strides in providing symptom relief for SCD patients. Hydroxyurea can help treat SCD by preventing the formation of sickle-shaped red blood cells. While primarily used as a treatment for cancer, the intake of hydroxyurea is considered safe as doctors prescribe a lower dose to treat SCD than to treat cancer (*Hydroxyurea for Sickle Cell Disease*). Hydroxyurea reduces the number of crises, episodes of acute chest syndrome, blood transfusions, and organ damage.

While this drug has made progress in the cure for sickle-cell anemia, the global impact has been mixed. Obtaining hydroxyurea in regions such as Sub-Saharan Africa may prove difficult. Barriers such as cost and the amount of hydroxyurea an individual can receive produce a paradox: hydroxyurea is life-changing, but it is not the ideal drug for sickle-cell disease. Thus, ongoing research aims to develop safer and more effective treatments for SCD.

Ongoing Research in SCD: A Case Study

Rather than focusing solely on stem cell transplants, current research is exploring how the induction of certain types of hemoglobin—a protein in red blood cells that carries oxygen from the lungs to the rest of the body—can improve SCD treatment. Fetal hemoglobin (HbF), a form of hemoglobin with a greater oxygen-binding affinity than adult hemoglobin (HbA) is one candidate for these hypothesized therapeutics (Kaufman and Lappin, 2020). Normally, adult hemoglobin completely replaces fetal hemoglobin by 12 months of age (Kaufman and Lappin, 2020). Researchers believe that inducing fetal hemoglobin in adults could be a gateway to sickle cell disease treatment due to advancements in gene editing.

Dr. Pamela Ting and her team at Novartis have pioneered a method for inducing fetal hemoglobin through the degradation of a key transcription factor known as WIZ, a repressor of HbF (Ting, et.al). Transcription factors are proteins that control whether or not a gene is expressed by binding to DNA. The inverse relationship between WIZ and fetal hemoglobin (**Fig. 2**) suggests potential for applying WIZ degradation to SCD treatments. As a result, Dr. Ting and her team began looking at cereblon (CRBN)-dependent degraders of transcription factors (Ting, et.al). The team screened 2,814 CRBNs to test for induction of fetal hemoglobin. While 7.4% of the CRBN-dependent degraders increased the fetal hemoglobin fraction, only one increased the percentage of hemoglobin dramatically while sparing

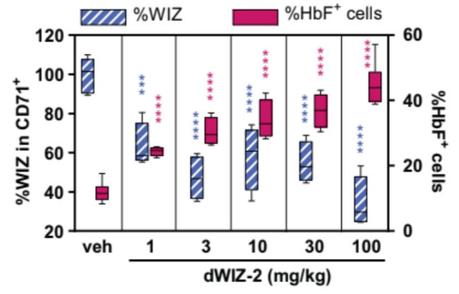

Figure 2. Inverse Relationship between WIZ and Fetal Hemoglobin (Ting et al., 2024).

erythroblast (pre-developed red blood cell) proliferation and differentiation: Compound C (Ting, et.al). Moreover, HbF induction was not observed in erythroblasts deficient in CRBN, meaning that Compound C acts through a protein degradation mechanism to induce HBF (Ting, et.al). After this finding, Compound C was renamed to DWIZ-1.

DWIZ-1 operates by degrading the WIZ transcription factor post-transcriptionally. Sequence analysis and modeling showed that WIZ is composed of 11 Zinc finger (ZF) proteins (Ting, et.al). WIZ (ZF7) is the primary ZF involved in the formation of a ternary complex, which is a direct binding mechanism of three different molecules. The researchers concluded that DWIZ-1 induces HbF expression by directly binding to CBRN and recruiting a complex of enzymes that degrade WIZ through WIZ (ZF7) (Ting, et.al). A second assay revealed that there was a high binding affinity of WIZ (ZF7) and the DWIZ-1 complex and that WIZ (ZF7) and DWIZ-1 bind through hydrogen bonding (Ting, et.al). Therefore, the researchers hypothesized that this affinity is essential to the effectiveness of DWIZ-1.

In addition to DWIZ-1, the team discovered a chemical analog, DWIZ-2, in which a methyl group of DWIZ-1 is removed. Early tests show that DWIZ-2 also works as a CRBN-dependent degrader, depleting only a small number of additional proteins. DWIZ-2 also increased fetal hemoglobin in patients with SCD from 17% to 45% of the total hemoglobin (Ting, et.al). The research team studied the in vivo activity of DWIZ-2 in human hematopoietic stem and progenitor cells (HSPCs) transplanted into mice and subsequently found that mice treated with DWIZ-2 averaged 65% WIZ degradation (Ting, et.al). Moreover, in primary human erythroblasts treated with DWIZ-2 in two different time intervals: 24 hours or seven days, in total, 407 genes were differentially expressed and 293 of these genes were upregulated, which aligns with the hypothesis that WIZ functions as a repressor (Ting, et.al). The team has further shown that WIZ degradation does not exhibit toxicity by investigating the effect of DWIZ in non-human primate models. Based on all their findings, the research team ultimately believes in the potential for WIZ degradation applications in treating human SCD.

Conclusion

Despite advancements in sickle cell disease research, researchers are still discovering ways to provide a safe and accessible cure for all those affected with SCD. Bone marrow transplants are expensive and often limit access to sickle cell disease treatment. Because sickle cell disease affects minority populations, limited access to life-saving care creates an additional barrier for these populations and leaves a detrimental impact on their health. Furthermore, access to treatment in developing countries is constrained by multiple factors including limited numbers of healthcare workers and equipment, lack of educational awareness, and poor infrastructure. However, through dedicated SCD clinics, point-of-care screening, and regional multilevel treatment guidelines, access to SCD treatments has begun to improve (Novartis). The cure for sickle cell disease is challenging at the molecular level, but the shift from stem cell transplant to new molecular modalities in research is promising. Scientists are currently focusing on in vivo gene editing— directly altering a patient's cells' genetic material inside the body—instead of ex vivo gene editing, where cells are removed from the body, treated, and then reinserted. Such an investment in new research and new technology will take time to develop. Many scientists like Dr. Ting's team are working daily to offer a holistic approach to treatment that combines scientific innovation with policy-driven accessibility. This approach will be essential in creating comprehensive solutions that benefit all individuals affected by SCD.

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