Undergraduate Thesis Prospectus

Industrial Scale Production of R21/Matrix-M Malaria Vaccine for Sub-Saharan Africa

(technical research project in Chemical Engineering)

Stubborn Enemy: The Persistence of Malaria in Sub-Saharan Africa

(sociotechnical research project)

by

Sierra Giles

May 11, 2023

technical project collaborators:

Anupama Jayaraman
Ian Lucas
Jacob Wilkins
William Wonsik

On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

Sierra Giles

Technical advisor: Eric W. Anderson, Department of Chemical Engineering

STS advisor: Peter Norton, Department of Engineering and Society

Sierra Giles
STS 4500
May 11, 2023

General research problem

How can death from preventable disease in children be reduced?

Preventable and treatable diseases still annually kill millions worldwide. Children under five years old are especially vulnerable. Barriers to preventative and curative care include poverty and a lack of education and infrastructure, as low income countries bear the greatest burden of child mortality (WHO, 2020). In particular, Sub-Saharan Africa has the greatest mortality rate of children under five: 1 in 13. Malnutrition is a great contributor, making children susceptible to common afflictions like diarrhea, pneumonia and malaria. Availability of food, clean water and healthcare services including vaccination would prevent or treat these ailments before they cause death. Some countries are decreasing rates of childhood death, but many are not; their current strategies for managing these deaths must be reevaluated.

Industrial Scale Production of R21/Matrix-M Malaria Vaccine for Sub-Saharan Africa

How can R21 malaria vaccine production be optimized to create an adequate supply of vaccines?

Five students, Sierra Giles, Anupama Jayaraman, Ian Lucas, Jacob Wilkins and William Wonsik will design a pilot scale operation of the R21 malaria vaccine as a capstone project overseen by Eric Anderson in the Department of Chemical Engineering. They must determine target yield and develop a process to meet the demand of at-risk populations in Africa.

In 2019, there were 228 million reported cases and 405 thousand malaria-related deaths, which remains one of the leading causes of morbidity and mortality in the developing world. In particular, Sub-Saharan African countries carry the majority of the malaria cases caused by

Plasmodium falciparum (Figure 1), the parasite implicated in over 90% of world mortality due to malaria.

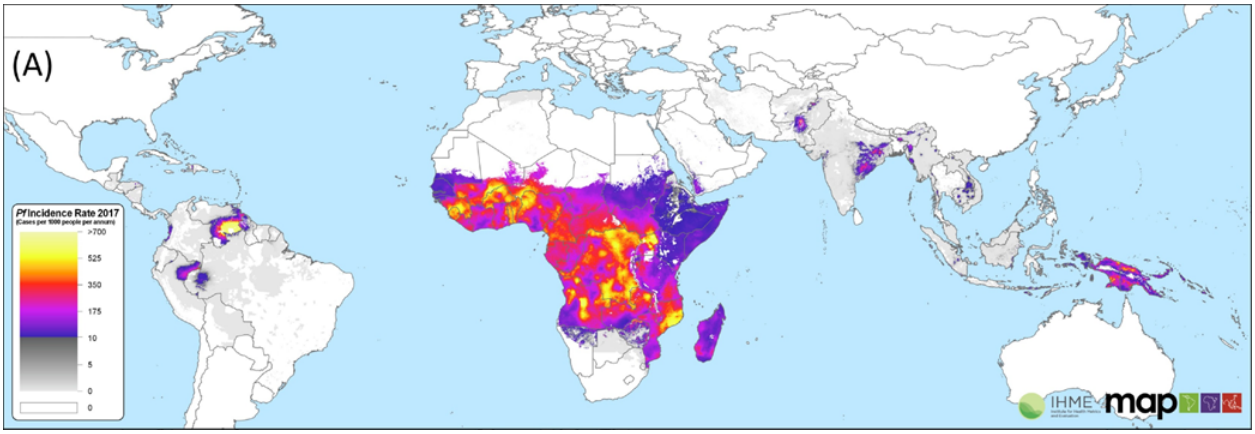

Figure 1. *P. falciparum* malaria incidence, 2017 (Price et al., 2020)

The disease is spread via the *Anopheles* mosquito vector, which allows the parasite to enter the bloodstream and lyse host red blood cells during replication (Talapko et al., 2019). The infection results in high fevers, nausea, and muscle pain, among other symptoms. Severe malaria cases may result in severe anemia, comas, and respiratory distress. Although the drug artemisinin can completely eradicate the infection from the blood, drug resistance allows *P. falciparum* to persist in the blood asymptomatically, causing recrudescence and further parasite spread (Cowman et al., 2016). As a result, there is a need for an effective malaria vaccine.

Currently, the RTS,S/AS01 vaccine, developed by GlaxoSmithKline, is the only approved vaccine for malaria that was recommended for widespread use in endemic regions (D'Souza & Nderitu, 2021). The vaccine is a virus-like particle (VLP) that presents circumsporozoite protein (CSP), a protein on *P. falciparum* that is critical for infecting cells, by linking it to an unrelated antigen. AS01, a saponin-based adjuvant, is added to enhance vaccine efficacy (Nadeem et al., 2022). Unfortunately, clinical trial data demonstrate that at 48 months following the initial three-dose vaccination, the vaccine has only a 36% efficacy in children (5-

17 months at receipt of vaccine) and a 26% efficacy in infants (6-12 weeks at receipt of vaccine). The efficacy further declines over time (Olotu et al., 2016).

Recently, scientists at Oxford University developed the first vaccine to meet the World Health Organization's goal for 75% efficacy. Known as R21/Matrix-M, the vaccine is a pre-erythrocytic malaria vaccine that improves the RTS,S/A01 vaccine design. By modifying vaccine synthesis to increase the proportion of CSP, Oxford scientists were able to develop a more immunogenic VLP (Collins et al., 2017). Recent clinical trial data shows that 24 months after initial vaccination, the R21 vaccine has an 80% efficacy against malaria in children (5-17 months at receipt of vaccine) when mixed with Matrix-M, another saponin-based adjuvant (Datoo et al., 2022). Additionally, R21/Matrix-M is easier to develop than RTS,S/A01, due to its cheaper and more modern design (Mandavilli & Cheng, 2022). Currently, the vaccine is manufactured by the Serum Institute of India, allowing for the production of R21 to be nearly 30 times greater than RTS,S (Ledford, 2022). Although the vaccine is still undergoing clinical trials to confirm efficacy, R21/Matrix-M is a promising candidate for widespread use.

In anticipation of vaccine approval, this project aims to develop a cost-effective process to manufacture single-doses of R21/Matrix-M for use in preventing malaria infections in Sub-Saharan Africa. The process will involve industrial scale upstream, downstream, formulation and fill-finish stages (Figure 2). Upstream processing will include batch fermentation with *Pichia pastoris*, which is critical for R21 production. In downstream processing, the yeast cells will first be lysed using chemicals and bead vortexing, allowing the CSP fusion proteins to self-assemble into VLPs. Subsequently, the lysed material will go through centrifugation, followed by depth-filtration, and then two cycles of ultracentrifugation and size-exclusion chromatography to purify the particles (Collins et al., 2017). For formulation and fill-finish, the R21 protein particle will be

mixed with Matrix-M, which will be acquired from Novavax, at a 1:10 ratio (Datoo et al., 2022), resulting in the final product. The project will conclude with an economic and feasibility analysis.

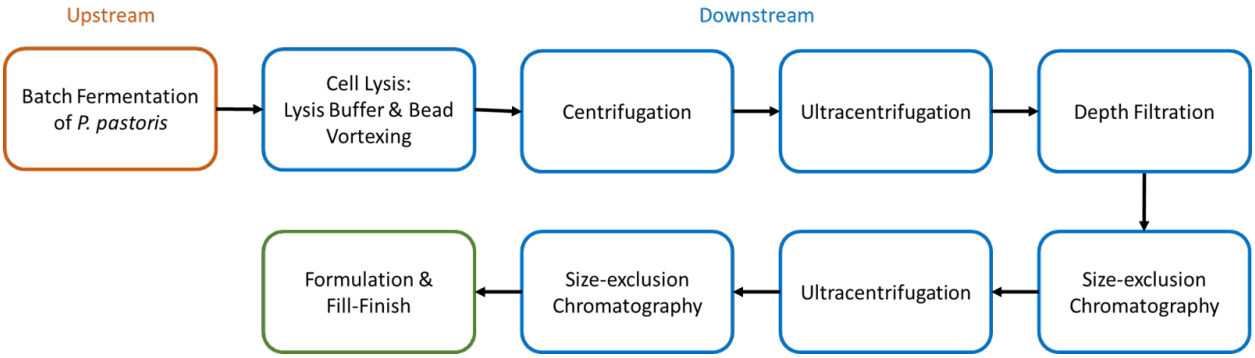

Figure 2. Simple Process Flow Diagram of Proposed R21/Matrix-M Manufacturing Process

Our team will complete the design project over two semesters in CHE 4474 and CHE 4476. We plan to meet weekly to review progress and assign future work. Additionally, we will meet biweekly with Professor Eric Anderson, the chemical engineering capstone faculty advisor, to receive feedback and guidance. We will also consult Professor Michael King, an industry expert on vaccines, and Professor Giorgio Carta, a leader in bioseparations, for further advice. As R21/Matrix-M is a new vaccine still in clinical trials, there is limited available data on its large-scale manufacturing. As a result, our team will consult Collins et al., which documents the methodology for lab-scale vaccine production, and documentation on the production of VLP vaccines that use technologies similar to those in R21.

Empty Efforts to Manage Malaria

Since the year 2000, how have international NGOs, national health agencies, and local caregivers strived to reduce the incidence of malaria in Sub-Saharan Africa?

Over 90% of worldwide malaria deaths occur in Africa, mostly among children under five (Taylor, 2022). In 2020, global spending to fight malaria had risen to $2.5 billion dollars annually, but malaria rates have not wavered (Roberts, 2022). Efforts to control mosquitoes have been effective in some regions but not universally. What methods have been employed to prevent the spread of disease?

Those with a stake in the war on malaria include international non-governmental organizations (NGO), national health agencies, local caregivers, and people (particularly children) living in areas with a high malaria prevalence. The World Health Organization (WHO) is a United Nations organization acting internationally to further "the attainment by all peoples of the highest possible level of health" (United Nations, n.d.). WHO has drafted a global strategy to fight malaria, the *Global technical strategy for malaria 2016-2030* (2021). This strategy instates a goal of 90% reduction in malaria cases from 2015 to 2030. In its early stages, the plan showed some success, reducing malaria deaths by 22% but malaria incidence by only 3% from 2015 to 2020. The first malaria vaccine, 'RTS,S', was approved and recommended by WHO in 2021. Following this recommendation, a promotional video by WHO shared a Ghanian mother's testimony to the importance of the vaccine (WHO, 2022).. She shares that her infant son contracting malaria affected more than just him. The time required to travel for healthcare preented her from caring for her healthy children too. This story not only appeals to emotion but encourages trust by featuring a woman with whom the target group may identify. By agreeing to appear in the video, this woman backs WHO's promotion of the vaccine as safe and invaluable in protecting children.

Though the approval of the first malaria vaccine was a significant success, its efficacy is only 36% in children aged 5-17 months (Datoo et al., 2021). A better vaccine is on the horizon:

the Oxford R21 vaccine. Vaccinologist Adrian V. S. Hill explains why the R21 vaccine trumps the RTS,S vaccine: the R21 vaccine is half the cost, has 30 times greater production capabilities, and an efficacy of over 70% (Ledford, 2022). Researchers like Hill use their position to prevent illness or death anywhere their medical advancements can reach. They take on the responsibility of communicating the importance of their findings and innovations.

Without widespread healthcare workers, not all local people can access preventative or curative care. Furthermore, many healthcare workers are emigrating to further their career opportunities (Poppe et al., 2014). Some cannot even enter a training program because there are not enough spaces to accommodate all those who wish to enter the field. Others who leave fear their safety because of political instability and ethnic divide across the continent. One physician explained the situation that triggered him to flee from Africa to Europe: "I was on duty. And the person who sat in front of me was a soldier of another ethnic group. … he said: 'well, if you don't save my child, I kill you.' It's like that that I fled the hospital" (Poppe et al., 2014). Political instability not only poses a danger but prevents healthcare workers from achieving their career or education aspirations, contributing to a lack of widespread healthcare across Sub-Saharan Africa.

Doctors Without Borders (MSF) is one NGO that dedicates its efforts to treat malaria in over two million people each year (MSF, 2022). They deploy mobile clinics where healthcare is otherwise inaccessible, treating manageable cases and transporting extreme cases to hospitals. These mobile clinics also offer training and additional supplies to local healthcare providers. MSF employs mosquito larvicide spraying and distribution of bed nets to prevent cases. In 2012, MSF began the Seasonal Malaria Chemoprevention (SMC) program to anticipate malaria spikes based upon seasonal weather changes to pre-emptively distribute anti-malarial pills. In 2015,

MSF passed these responsibilities off to individual National Health Ministries. These efforts further MSR's mission of achieving diversity, equity, inclusion and doing no harm (MSF, n.d.).

The Mozambique National Health Ministry is a government organization that utilizes MSR's methods. Their program is dubbed the National Malaria Control Programme (NMCP) and prioritizes children under five and pregnant women. The NMCP aims to equip health clinics to accurately diagnose and treat malaria while encouraging people to seek healthcare at their first sign of sickness (Ministry of Health, 2006). While they set goals for distribution of preventatives like bed nets, they continue to be unmet because they do not have the infrastructure to provide aid far and wide like MSF (Roberts, 2022).

Effectiveness of these strategies depends heavily on trust. A lack of vaccine literacy is associated with vaccine hesitancy as the risks are overblown and the benefits of the vaccine are not understood (Engelbrecht et al., 2022). Without understanding the importance of the vaccine, Sub-Saharan Africans have been reported to refuse vaccines because of their religion or a fear of needles (Dzinamarira et al., 2022). Government trust is a large factor in rejection of vaccines and other anti-malaria efforts (Engelbrecht et al., 2022). Not only is indoor insecticide spraying invasive and smelly, some local people fear there is a sinister motivation behind the method (Roberts, 2022). Trust in the healthcare providers is also key, as colonial medicine can incite a fear of being exploited as test subjects by high-income countries (Mutombo et al., 2022). Increasing accessibility of healthcare and other anti-malarial methods is a wasted effort if local people aren't willing to utilize them.

References

Collins, K. A., Snaith, R., Cottingham, M. G., Gilbert, S. C., & Hill, A. V. S. (2017). Enhancing protective immunity to malaria with a highly immunogenic virus-like particle vaccine. *Scientific Reports*, *7*(1), Article 1.

Cowman, A. F., Healer, J., Marapana, D., & Marsh, K. (2016). Malaria: Biology and Disease. *Cell*, *167*(3), 610–624.

Datoo, M. S., Natama, H. M., Somé, A., Bellamy, D., Traoré, O., Rouamba, T., Tahita, M. C., Ido, N. F. A., Yameogo, P., Valia, D., Millogo, A., Ouedraogo, F., Soma, R., Sawadogo, S., Sorgho, F., Derra, K., Rouamba, E., Ramos-Lopez, F., Cairns, M., … Tinto, H. (2022). Efficacy and immunogenicity of R21/Matrix-M vaccine against clinical malaria after 2 years' follow-up in children in Burkina Faso: A phase 1/2b randomised controlled trial. *The Lancet Infectious Diseases*.

MSF (2022, Jan 26). Doctors Without Borders USA. Malaria. https://www.doctorswithoutborders.org/what-we-do/medical-issues/malaria

MSF (n.d.). Doctors Without Borders USA. Who we are. https://www.doctorswithoutborders.org/who-we-are

D'Souza, J., & Nderitu, D. (2021). Ethical considerations for introducing RTS,S/AS01 in countries with moderate to high Plasmodium falciparum malaria transmission. *The Lancet Global Health*, *9*(12), e1642–e1643.

Dzinamarira, T., Nachipo, B., Phiri, B., & Musuka, G. (2021). COVID-19 Vaccine Roll-Out in South Africa and Zimbabwe: Urgent Need to Address Community Preparedness, Fears and Hesitancy. *Vaccines*, *9*(3). Multidisciplinary Digital Publishing Institute.

Engelbrecht, M., Heunis, C., & Kigozi, G. (2022). COVID-19 Vaccine Hesitancy in South Africa: Lessons for Future Pandemics. *International Journal of Environmental Research And Public Health*, *19*(11), 6694.

Ledford, H. (2022). Malaria vaccine booster prolongs protection. *Nature*.

Mandavilli, A., & Cheng, K.-C. (2022, October 4). At Long Last, Can Malaria Be Eradicated? *The New York Times*. https://www.nytimes.com/2022/10/04/health/malaria-vaccines.html

Mutombo, P. N., Fallah, M. P., Munodawafa, D., Kabel, A., Houeto, D., Goronga, T., Mweemba, O., Balance, G., Onya, H., Kamba, R. S., Chipimo, M., Kayembe, J.-M. N., & Akanmori, B. (2022). COVID-19 vaccine hesitancy in Africa: A call to action. *The Lancet Global Health*, *10*(3), e320–e321.

Nadeem, A. Y., Shehzad, A., Islam, S. U., Al-Suhaimi, E. A., & Lee, Y. S. (2022). Mosquirix™ RTS, S/AS01 Vaccine Development, Immunogenicity, and Efficacy. *Vaccines*, *10*(5), Article 5.

Olotu, A., Fegan, G., Wambua, J., Nyangweso, G., Leach, A., Lievens, M., Kaslow, D. C., Njuguna, P., Marsh, K., & Bejon, P. (2016). Seven-Year Efficacy of RTS,S/AS01 Malaria Vaccine among Young African Children. *New England Journal of Medicine*, *374*(26), 2519–2529.

Poppe, A., Jirovsky, E., Blacklock, C., Laxmikanth, P., Moosa, S., De Maeseneer, J., Kutalek, R., & Peersman, W. (2014). Why sub-Saharan African health workers migrate to European countries that do not actively recruit: a qualitative study post-migration. *Global Health Action*, *7*, 24071.

Price, R. N., Commons, R. J., Battle, K. E., Thriemer, K., & Mendis, K. (2020). Plasmodium vivax in the Era of the Shrinking P. falciparum Map. *Trends in Parasitology*, *36*(6), 560–570.

Ministry of Health (2006, July). Ministry of Health, Republic of Mozambique. Strategic Plan for Malaria Control in Mozambique. World Health Organization Regional Office for Africa.

Resch, S., Ryckman, T., & Hecht, R. (2015). Funding AIDS programmes in the era of shared responsibility: An analysis of domestic spending in 12 low-income and middle-income countries. *Lancet Global Health*, *3*(1), E52–E61.

Roberts, L. (2022). Lingering Fever. *Science,* 377(6611). https://www.science.org/content/article/battle-malaria-africa-stalled-can-research-mozambique-explain

Talapko, J., Škrlec, I., Alebić, T., Jukić, M., & Včev, A. (2019). Malaria: The Past and the Present. *Microorganisms*, *7*(6), 179.

Taylor, D.A. (2021, July 13). West African Scientists Are Leading the Science Behind a Malaria Vaccine. *Smithsonian Magazine.* https://www.smithsonianmag.com/innovation/west-african-scientists-are-leading-science-behind-malaria-vaccine-180978134/

United Nations. (n.d.). World Health Organization. https://www.un.org/en/academic-impact/who

World Health Organization. (2020, September 8). Children: Improving survival and well-being. https://www.who.int/news-room/fact-sheets/detail/children-reducing-mortality

World Health Organization. (2021) Global technical strategy for malaria 2016–2030, 2021 update. https://www.who.int/publications/i/item/9789240031357

World Health Organization. (2022, April 22). Lifesaving malaria vaccine: Harnessing innovation to fight an ancient killer. [Video] YouTube. https://youtu.be/N1D6BTw0i9I