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ARTICLE COMMENTARY

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Syphilis vaccine development: Aligning vaccine design with manufacturing requirements

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ABSTRACT

Syphilis, caused by *Treponema pallidum* subsp. *pallidum*, is a global health concern with increasing rates worldwide. Current prevention strategies, including screen-and-treat approaches, are not sufficient to resolve rising infection rates, emphasizing the need for a vaccine. Developing a syphilis vaccine necessitates a range of cross-disciplinary considerations, including essential disease-specific protection, technical requirements, economic feasibility, manufacturing constraints, public acceptance, equitable vaccine access, alignment with global public vaccination programs, and identification of essential populations to be vaccinated to achieve herd immunity. Central to syphilis vaccine development is prioritization of global vaccine availability, including access in low- to middle-income settings. Various vaccine platforms, including subunit, virus-like particle (VLP), mRNA, and outer membrane vesicle (OMV) vaccines, present both advantages and challenges. The proactive consideration of both manufacturing feasibility and efficacy throughout the pre-clinical research and development stages is essential for producing an efficacious, inexpensive, and scalable syphilis vaccine to address the growing global health burden caused by this disease.

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Introduction

Syphilis, caused by the extracellular bacterium *Treponema pallidum* subsp. *pallidum*, is a multi-stage infection that persists for a lifetime without antibiotic treatment.[1] Syphilis remains prevalent in low- and middle-income countries; in 2019 there were an estimated 49.71 million cases of syphilis globally.[2] In high-income countries, including the US, Canada and Europe, syphilis rates have been rising at an alarming rate over the last decade, with the highest increase in cases observed in populations of men who have sex with men (MSM), transgender women, and cisgender women.[3–7] *Treponema pallidum* can cross the placental barrier and cause congenital syphilis, which is estimated to affect 1 million pregnancies annually and result in approximately 661,000 cases of congenital syphilis and 355,000 adverse birth outcomes.[8] These numbers may be an underestimation since accurate determination of the burden of congenital syphilis is challenging, due to country-specific variation in access to antenatal screening, syphilis testing during pregnancy, and reliable surveillance data.[9] To address rising congenital syphilis rates, in 2021 the WHO launched a global initiative to eliminate mother-to-child transmission of syphilis,[10] with the aim of reducing global syphilis incidence by 90% by 2030.[11]

Infectious and congenital syphilis rates are highest in low- and middle-income countries (LMICs).[2] As a result, countries with a low sociodemographic index exhibit the highest age-standardized rates (ASR) and disability-adjusted life years (DALYs) due to syphilis, underscoring health disparities in both disease prevalence and disease impact on individuals.[2] In addition to the deleterious physical health outcomes from syphilis, it is essential to consider the psychosocial, quality of life, and economic impacts a syphilis diagnosis can have on individuals. Accordingly, achieving a successful reduction in the incidence of syphilis is predicted to have the highest impact on reducing DALYs of all the curable STIs.[12,13]

Increasing syphilis rates suggest that screen-and-treat public health strategies alone may not effectively reduce disease incidence, underscoring the importance of developing a syphilis vaccine. Modeling predicts a syphilis vaccine with 80% efficacy would significantly reduce both infectious and congenital syphilis over 20 years,[14] and when paired with public health initiatives geared to raise disease awareness and reduce disease incidence the goal of syphilis elimination may be possible. Given that syphilis rates and associated DALYs are highest in LMICs, and that access to screening and effective treatment can be challenging in resource-limited settings, it is crucial to prioritize the design of a syphilis vaccine that is suitable for use in LMIC settings. Developing an effective syphilis vaccine to address global need requires a careful assessment of needed product performance characteristics (PPCs) aligned with requirements for delivering effective protection against infection and disease (within an individual) and spread (within the population), balanced with feasibility of manufacturing. By designing a vaccine where ideal vaccine PPCs and manufacturing requirements are considered early in the design pipeline and revisited at all stages of

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development, vaccine researchers can proactively avoid concerns from industry, regulatory, and decision-making partners regarding manufacturing cost, feasibility, performance, and suitability for use in remote or resource-limited settings. This approach ensures maximum market potential, increases the chances of industry partner support, and enhances global vaccine equity and access. Here, we outline the optimal molecular and biophysical properties, as well as important economic and manufacturing considerations, for a syphilis vaccine and consider the suitability of various platforms including subunit-, virus-like particle (VLP)-, mRNA-, and outer membrane vesicle (OMV)-based vaccines.

The status of syphilis vaccine development

Treponema pallidum presents unique biological challenges for vaccine design, including the minimal complement of outer membrane proteins that are expressed on the host-interacting pathogen surface, and the extensive antigenic variation that is found in select outer membrane proteins, including the *T. pallidum* repeat (Tpr) protein family.[1,15–17] Individuals are also susceptible to reinfection with heterologous strains.[18,19] Although details regarding the immune correlates of protection are limited, current evidence indicates that protection is dependent, at least in part, upon the generation of a delayed-type hypersensitivity-like TH1 immune response that activates interferon-gamma (IFN-γ)-secreting T-cells, and antibodies that opsonize and neutralize *T. pallidum* organisms.[20,21]

Syphilis vaccine development has been designated by vaccine development oversight committees as being between the basic and pre-clinical stages on the vaccine development continuum.[22] The host interfacing outer membrane proteins present on the *T. pallidum* surface have long been known to be important targets for vaccine design; computational analyses performed over two decades ago identified a complement of candidate outer membrane proteins in *T. pallidum*,[23] and more recent analyses targeting the "OMPeome" have advanced this line of investigation.[24] Current high priority syphilis vaccine candidates that are in the pre-clinical assessment stage target known or suspected *T. pallidum* outer membrane proteins and, to date, all have been pursued as protein subunit vaccines. These include proteins such as Tp0751,[25] Tp0136,[26] Tp0326,[27] Tp0633,[28] Tp0856,[29] and Tpr family members including TprK (Tp0897).[30,31] An important consideration from a manufacturing standpoint is that only two of these vaccine candidates have been successfully produced as soluble and stable recombinant proteins (Tp0751 and Tp0136); the other vaccine targets being pursued are beta barrel-containing integral membrane proteins, which require complex re-folding and additional quality control (QC) measures that are not conducive with vaccine manufacturing requirements. Since current syphilis vaccine candidates being pursued have elicited partial protection at best,[25,30,31] it is apparent that an effective syphilis vaccine will require induction of an immune response against multiple *T. pallidum* proteins, an expensive endeavor from a manufacturing standpoint.

Overall, the complexity of *T. pallidum* biology, unknown correlates of protection, limited vaccine-targetable proteins, and inherent need to induce immune responses to multiple proteins pose significant challenges for syphilis vaccine design. To overcome these limitations and ensure compatibility with manufacturing requirements, researchers are focusing on characterizing the immunogenicity and protective capacity of regions of the proteins that correspond to surface-exposed extracellular loops, identifying peptides containing T- and B-cell epitopes to ensure effective *T. pallidum* neutralization and clearance,[29,32–37] and pursuing platforms that can incorporate these essential regions from multiple *T. pallidum* proteins into a single chimeric polyvalent vaccine candidate.[38–40]

Product performance characteristics (PPCs) for a syphilis vaccine

Previous commentaries have outlined the investment case for development of a syphilis vaccine and the economic impact of vaccine implementation.[41–43] The World Health Organization (WHO), in collaboration with the National Institute for Allergy and Infectious Diseases (NIAID), Centers for Disease Control and Prevention (CDC), and STI experts, have proposed a roadmap for advancing STI vaccine development.[22,44] Prior reviews have comprehensively documented the challenges, safety requirements, and needed correlates of protection associated with development of a syphilis vaccine.[20,41,45–47] The current commentary focuses upon aligning the critical requirements for development of an effective syphilis vaccine with industry requirements for vaccine manufacturing. For the syphilis vaccine development pipeline, meaningful and early engagement with industry, regulatory, and advisory agencies will maximize the chances of development of vaccine candidates that offer protection against all stages of disease in a format that aligns with standard industry manufacturing requirements.

Syphilis disease progression includes development of a characteristic chancre at the initial site of infection (primary disease stage), followed by a disseminated rash and general malaise (secondary disease stage). In the absence of treatment, the infection becomes latent and persists for an individual's lifetime. Approximately one-third of individuals infected with *T. pallidum* develop symptoms associated with tertiary disease, including gumma and central nervous system and cardiovascular involvement. Asymptomatic infections and varied disease presentations can also occur.[1,19] An ideal syphilis vaccine would provide protection against chancre and secondary lesion formation, as well as bacterial shedding at other body sites, to prevent infection transmission between individuals. Also necessary for a vaccine is protection against bacterial dissemination across the placental, endothelial and blood-brain barriers, thus preventing congenital syphilis and disease symptom development within an individual. Although achieving complete protection against population spread and disease symptoms via vaccination is desired, it is understood to be a challenging goal due to the complexity of *T. pallidum* infection. A more achievable vaccination goal may be establishment of partial protection against chancre and secondary lesion development and treponemal shedding, which would presumably result in decreased syphilis transmission at the population level and attenuated symptom development at the individual level. However, induction of partial protection against infection, while expected to decrease *T. pallidum* burden within an

individual, may still place infected individuals at risk of developing tissue and organ damage as well as transmitting congenital infection to their developing fetus. For this reason, simultaneous with syphilis vaccine development it will be imperative to develop a direct syphilis diagnostic test that can accurately detect active infection in a vaccinated individual and successfully differentiate from previous infection(s).

To provide broad protection against clinical *T. pallidum* strains, and to protect against reinfection with heterologous strains, vaccine candidates should target *T. pallidum* proteins or peptides that are invariant and shared across all circulating strains of *T. pallidum*. Further, candidate vaccine constructs should mimic the endogenous state of *T. pallidum* proteins to ensure an effective immune response is generated.[39] Additional vaccine considerations include the need for a vaccine that can be safely administered to pregnant individuals at all stages of gestation, individuals who are HIV+ and/or taking PrEP/PEP,[41,48] and individuals who have had a previous *T. pallidum* infection. Induction of long-term protection through vaccination would be ideal, optimally striving for protection that lasts 10–15 years to match the WHO objectives for STI vaccines, including *Neisseria gonorrhoeae* and Herpes Simplex Virus vaccines.[49,50]

The process of vaccine manufacturing is expensive, long-term, and technically challenging. Thus, vaccine candidates that show promise in pre-clinical studies frequently fail at the manufacturing stage.[51] To maximize the likelihood of a syphilis vaccine candidate progressing through manufacturing, implementation, and market introduction phases, it is essential that researchers consider vaccine candidate features that align with manufacturing requirements (including ease of production, scalability, and long shelf-life) and global and public expectations for performance (including reasonable dosing schedule, induction of long-term protection, convenient route of immunization, ease of storage, and low cost). Further, early consideration of strategies for cost-effective vaccine production includes designing vaccines that can utilize existing vaccine manufacturing infrastructure and are compatible with current Good Manufacturing Processes (cGMP)[52] production. By designing a vaccine that avoids the need for complicated manufacturing strategies, can be produced using existing manufacturing systems, and prioritizes optimal production characteristics from the early stages of development, researchers reduce the probability of late-phase vaccine product failure and increase the likelihood that a vaccine can be produced at scale.[51]

Current vaccine platforms

Protein subunit vaccines

Protein subunit vaccines, which consist of recombinantly produced, pathogen-originating proteins, are a well-established and highly effective vaccine platform. Subunit vaccines offer advantages such as consistency, safety, and established production infrastructure.[53] To produce a subunit vaccine for syphilis, researchers must identify infection-relevant proteins that can be recombinantly expressed at large scale and are stable, soluble, and do not require downstream processing steps such as refolding in order to be representative of the natural state of the protein found in the pathogen. Since each protein contained within a subunit vaccine requires an independent production line, production costs are commensurate with the number of antigens.[51] To minimize these constraints, an ideal syphilis vaccine candidate will be a single antigen that is soluble and retains native epitope conformation without requiring additional refolding or other laboratory-based, non-automated manipulations. Further, protein vaccine candidates should be able to be produced with reproducible quantity and quality by commonly used biomaterial production organisms. For example, subunit vaccine production pipelines using *Escherichia coli, Saccharomyces cerevisiae*, or *Pichia pastoris* are generally less expensive due to lower material costs and well-established manufacturing facilities and procedures.[53,54] However, use of these organisms may not be compatible with complex vaccine product requirements, including vaccines dependent upon the incorporation of *T. pallidum*-specific post-translational modifications that mimic the natural state of the protein(s), highlighting the importance of considering vaccine design constraints early and throughout the development pipeline.[39]

According to the WHO vaccine preferred product characteristics,[55] subunit vaccines should be stable at standard cold-chain temperatures (2° to 8°C), tolerate a freeze-thaw cycle, retain stability for several hours after removal from cold storage, and tolerate temperatures up to 40°C for short durations.[55] To address this need, the stability and efficacy of candidate subunit vaccines following long-term storage should be evaluated to ensure consistency between production and delivery.

Due to the complex pathogenesis of *T. pallidum* infection, and the fact that individual subunit vaccine trials to date for syphilis vaccine development have resulted in partial protection at best,[25,30,31] it is expected that an effective syphilis vaccine will need to comprise multiple prioritized *T. pallidum* vaccine candidates. To avoid increased manufacturing costs associated with producing multiple antigens, one strategy being pursued by researchers is to develop protein scaffolds that can be decorated with B- and T-cell epitopes derived from multiple *T. pallidum* proteins.[40] By pairing with an adjuvant, these scaffolds can elicit specific and robust immune responses toward each included epitope, inducing high titers of neutralizing and/or opsonic antibodies and a robust cell-mediated immune response. Optimally, these scaffolds would originate from *T. pallidum* proteins, thus ensuring limited off-target immune responses. One such endogenous scaffold that has shown promise at providing partial protection against *T. pallidum* dissemination within the body[25,30] and is amendable to incorporation of heterologous epitopes into flexible loop regions is the *T. pallidum* protein Tp0751.[38,40] The ability to graft functionally and/or immunologically relevant *T. pallidum* protein epitopes onto a soluble and stable *T. pallidum*-derived scaffold will address many of the constraints associated with subunit vaccines. Overall, the innovative and flexible design of contemporary *T. pallidum* protein scaffolds, paired with the abundance of existing infrastructure for subunit vaccine production, position this vaccine platform as a leading

formulation for developing an efficacious and cost-effective syphilis vaccine.[24,29,38,40,41,45]

Virus-like particle vaccines

Virus-like particles (VLPs) are a vaccine technology used in several commercially available vaccines, including vaccines against human papilloma virus (Cervarix®, Gardasil® and Gardasil9®), hepatitis B virus (Sci-B-Vac™), and malaria (Mosquirix™).[56] For this technology custom epitopes are presented on viral capsid proteins that self-assemble into an ultrastructure that resembles or mimics that of a virus.[57] The immune system recognizes repeated viral patterns, making VLPs innately immunogenic, thereby bypassing the requirement for an adjuvant.[58,59] These repetitive viral structures allow for greatly increased epitope presentation which enables B-cell crosslinking, and thus may require fewer doses to achieve desired immunity thresholds.[57,59] VLPs can be enveloped, or non-enveloped, and range from 1 to 3 protein capsid layers. VLPs naturally accommodate the presentation of multiple epitopes, which meets the need for a syphilis vaccine to target multiple *T. pallidum* proteins.

Drawbacks to VLP production include that it is significantly more expensive than traditional recombinant protein production, epitopes that can be presented on VLPs have a size restriction, and epitopes may misfold during presentation or interfere with VLP assembly.[59] VLPs also require further processing and purification steps, often involving specialized equipment and techniques such as density gradients and chromatography for particle assembly and purification. Additional analysis techniques are therefore also necessary to ensure product quality and purity.[60] The need for specialized equipment, skilled personnel, and the increased complexity of the product all contribute to higher production costs, hinder scalability, and reduce the number of existing production facilities that can produce VLPs. Stability, which has been documented to be a concern for VLP vaccines,[58,59] would need to be tested to ensure that storage and shelf-life are practical for delivery and use in resource-limited settings, and are compatible with cold-chain restrictions. Given these technical and economic constraints, a syphilis-based VLP vaccine may pose feasibility concerns for production and use in LMIC settings.

mRNA vaccines

mRNA vaccines consist of a core mRNA strand that encodes one or more vaccine antigens, which are then intracellularly translated into protein(s) in vaccinated individuals. mRNA vaccines can also be self-replicating, where mRNA strands include an RNA-dependent RNA polymerase which replicate the mRNA strand, thereby lowering the mRNA required per dose.[61] Naked mRNA requires packaging in a delivery vehicle, such as a lipid nanoparticle, to cross cell membranes. mRNA vaccines typically require an adjuvant to elicit specific immune responses; however, mRNA vaccines may also self-adjuvant based on the characteristics of mRNA or of delivery components such as the lipid nanoparticle.[62] Since all mRNA products share the same physical and chemical properties, manufacturing only differs by the specific nucleotide sequences.[63] This feature allows production to be versatile and responsive to current vaccine needs, and the manufacturing process to be standardized to enable production of multiple different mRNA products by a single manufacturing facility.[63] Further benefits of mRNA vaccines include fast production time (i.e. completed within hours) and cell-free production, thus avoiding impurities derived from production organisms.[64]

Although recent advances have been made in mRNA vaccine technology, manufacturing challenges for mRNA vaccines persist. These challenges include the high costs and shortages of essential mRNA production reagents, particularly enzymes.[64] Additionally, mRNA purification and downstream processing, such as encapsulation in a lipid nanoparticle, are costly and difficult to scale.[64] mRNA vaccines also rely on strict cold- or ultra-cold-chain temperatures.[64] The future development of continuous manufacturing processes, and innovation in mRNA structure, encapsulation, and delivery, will increase accessibility and affordability of this technology.[64,65]

With the flexibility of mRNA manufacturing facilities and COVID 19-related investment in global infrastructure, mRNA vaccine manufacturers are able to produce vaccines on-demand, a potential benefit for a disease such as syphilis that may experience fluctuations in the demand for a vaccine.[63,64] Manufacturing costs will also decrease as the technology further matures, and current limitations and cost-barriers are overcome.[64] Therefore, mRNA vaccine platforms show promise for syphilis vaccine development, addressing both economic and antigen design constraints. Similar to other vaccine formulations, research on syphilis vaccines utilizing mRNA technology must evaluate stability, immunogenicity, and continued efficacy after long-term storage. The duration of protection from mRNA vaccines is not well-established, and likely varies depending on antigen properties.[61] Hence, investigations into syphilis mRNA vaccines should include evaluation of protection duration, antibody titers, and other relevant metrics during pre-clinical testing. Like other vaccine platforms, aligning research and development with product feasibility for use in LMIC settings enhances the likelihood of bringing a successful vaccine to market.

Outer membrane vesicle vaccines

Outer membrane vesicle (OMV) vaccines are an emerging platform for the development of vaccines against infectious pathogens.[66] OMVs are released from bacteria, and thus retain both the lipid and protein structures from the bacterial membrane, but are non-replicative and noninfectious.[66] Since OMVs are derived from bacterial membranes, they can naturally elicit an immune response and do not require adjuvants.[66,67] However, OMVs also contain toxic membrane components such as LPS and pathogen associated molecular patterns (PAMPs), often requiring the removal of these components to ensure vaccine safety.[66,68] Further, OMVs need to be purified from any cytoplasmic bacterial proteins released during cell lysis.[66] The success of the OMV vaccines against *Neisseria meningitidis* serogroup B[69] positions OMV vaccines as an attractive platform for the development of vaccines against infectious pathogens.

OMV vaccines can be produced by harvesting OMVs released from bacteria, termed natural OMV (nOMV), though this technique typically results in low yields. To address low nOMV yields, OMV vaccines can be produced using detergent extraction (dOMV) or by genetically modified bacteria that release increased levels of OMVs, termed mutant-derived OMV (mdOMV) or GMMA (Generalized Modules for Membrane Antigens).[66] OMV vaccine manufacturing generally involves growth of bacterial strains, harvesting or induction of OMVs, and purification of OMVs using affinity purification, size exclusion chromatography, hydrostatic filtration dialysis, or differential centrifugation.[67] As with other vaccine platforms, temperature sensitivity, cold chain limitations, and long-term potency are concerns for OMV vaccines. However, recent assessments demonstrate that OMV vaccines show promising stability and potency following long-term storage, with comparable or superior stability relative to other vaccine platforms.[70] Other recent advances in OMV production include the development of continuous *N. meningitidis* OMV vaccine production, which is estimated to increase OMV yields 9-fold and significantly reduce production costs.[71] Collectively these technology advances position OMV vaccines as an attractive platform for syphilis vaccine design.

Electron microscopy images suggest that *T. pallidum* may naturally produce OMVs though membrane blebbing.[72] However, detection and isolation of *T. pallidum* OMVs is challenging due to low yields and technical limitations associated with *T. pallidum* growth. An alternative approach is to develop genetically engineered strains of *N. meningitidis* or other OMV production organisms to heterologously express *T. pallidum* vaccine candidates. An OMV vaccine production approach could address requirements associated with a syphilis vaccine, including the need to incorporate multiple *T. pallidum* proteins, accommodate the hydrophobic or amphipathic nature of many *T. pallidum* OMP vaccine candidates, and present *T. pallidum* proteins in a conformation that mimics the natural folding state of the proteins.[39] Overall, the success of recent OMV vaccines,[66,67,69] improved continuous OMV vaccine production[71] and OMV stability and long-term potency,[70] and decreased OMV vaccine manufacturing costs identify this platform as a leading candidate for a syphilis vaccine.

Conclusion

Syphilis is a growing global public health threat despite increased surveillance and treatment initiatives, which emphasizes the need for a syphilis vaccine to complement public health approaches to disease prevention. To ensure successful development of a syphilis vaccine, researchers and other stakeholders must align pre-clinical vaccine design with economic and technical feasibility, with prioritization of syphilis vaccine design that is compatible with delivery in LMIC settings. Key factors influencing feasibility include cost-effective production that achieves cGMP standards, and compatibility with the logistical requirements of vaccine delivery and cold-chain limitations in LMICs. By keeping these considerations in mind, researchers can develop products that have reasonable manufacturing costs and reduce the risk of product failure at early clinical stages due to manufacturing difficulties. Here we outline the optimal product performance characteristics of a syphilis vaccine, and assess the benefits, limitations, and costs for subunit, VLP, mRNA, and OMV vaccine platforms. Although the most effective platform for syphilis vaccine development is currently unknown, by proactively addressing the benefits and constraints associated with each platform during the design phase, researchers can maximize the likelihood of developing an effective vaccine that is feasible to produce and distribute on a global scale. This proactive approach is critical in the effort to eliminate syphilis and to foster equitable healthcare access globally.

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Sean Waugh is a PhD candidate studying with Dr. Caroline Cameron at the University of Victoria (UVic) in Victoria, B.C., Canada. UVic is located on the unceded territories of the Lək^{w}əŋən (Songhees and Esquimalt) Peoples, on whose traditional lands the university stands, and where the Lək^{w}əŋən and W̱SÁNEĆ Peoples' historical relationships with the land continue to this day. Sean's research focuses on characterizing host-pathogen interactions, the host molecular response to Treponema pallidum, and using these discoveries to inform syphilis vaccine development.

Dr. Caroline Cameron is a Professor in the Department of Biochemistry and Microbiology at the University of Victoria and an Affiliate Professor in the Division of Infectious Diseases (Department of Medicine) at the University of Washington. Dr. Cameron also serves as President of the Canadian branch of the International Union against Sexually Transmitted Infections and President of the International Society for Sexually Transmitted Diseases Research Foundation. Dr. Cameron's research program, which focuses on diagnostic and vaccine development for infectious and congenital syphilis, has been recognized with an NIH MERIT Award and a CIHR Canada Research Chair.

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SW: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing.

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