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Abstract: The New Pathways in Syphilis Vaccine Development meeting was held before the start of the STI & HIV 2023 World Congress as a pre-meeting symposium to highlight recent advances in the development of an effective syphilis vaccine and discuss the challenges still faced by investigators. Internationally renowned public health officials, clinical investigators, and basic researchers from academia, government, and community-based organizations met on July 24, 2023, in Chicago, Illinois. Four speakers discussed key research findings in syphilis vaccine development, which included antigen selection, identification of epitopes associated with protective immunity, and delivery platforms, with great emphasis on development of chimeric antigens. Significant progress was also shown on the elucidation of *Treponema pallidum* genomes from virtually all continents to assess the diversity in vaccine candidates of the syphilis spirochete.

BACKGROUND

Syphilis, a sexually and vertically transmitted infection caused by the spirochete *Treponema pallidum* subspecies *pallidum* (*T. pallidum*), remains a global and public health challenge. Despite our understanding of syphilis transmission and the curability of the disease with penicillin or other effective antibiotics, syphilis remains endemic in low- and middle-income countries and has resurged in high-income countries over the past few decades, particularly among men who have sex with men.[1] Syphilis cases have also increased among women in some settings over the past decade, corresponding to an increase in congenital infections.[1] Furthermore, congenital syphilis serves as one of the leading infectious causes of fetal loss or stillbirth in low-income settings.[2] Based on global estimates of maternal and congenital syphilis for 2016, approximately 988,000 pregnant women are infected with active syphilis annually, resulting in an estimated 143,000 cases of early fetal deaths and stillbirths, 61,000 neonatal deaths, 41,000 preterm or low-birth-weight births, and 109,000 infants with clinical congenital syphilis.[3] The high prevalence of syphilis worldwide and the recurring global shortages of benzathine penicillin G underscore the necessity for novel measures to control syphilis transmission.[4] An effective preventative vaccine to complement screening and treatment approaches for syphilis could become a crucial component of the strategy to reduce disease spread and severity, especially in countries where syphilis control measures are suboptimal.

In 2019, the National Institutes of Health awarded 6 Cooperative Research Centers (CRCs) to focus on vaccine development for the bacterial sexually transmitted infections (STIs) including gonorrhea, chlamydia, and syphilis. This meeting summary was prepared to highlight recent advances in syphilis vaccine development presented by the CRCs funded through the University of Connecticut (PIs: Drs. Justin Radolf and Tony Moody) and the University of Washington (PI: Dr. Anna Wald). The meeting was held before the start of the STI & HIV 2023 World Congress on July 24, 2023, in Chicago, Illinois, as a pre-meeting symposium. In attendance were internationally renowned public health officials, clinical investigators and basic scientists from academia, government, and community-based organizations.

EXPERIMENTAL BASIS FOR SYPHILIS VACCINE DEVELOPMENT

Previous attempts to develop a syphilis vaccine have yielded varying levels of success. Using a rabbit model, researchers in the 1950s observed that animals experimentally infected with *T. pallidum* for at least 3 months developed resistance to symptomatic reinfection with the same isolate and partial protection against different isolates.[5] In 1973, Dr. James Miller reported that rabbit immunization with 60 intravenous injections of gamma-irradiated *T. pallidum* cells for 37 weeks, followed by intradermal challenge with the homologous *T. pallidum* strain, provided complete protection against infection for at least 1 year. Challenged rabbits did not develop primary lesions at the challenge sites, and the naive rabbits that received lymph nodes from the challenged immunized rabbits via intratesticular inoculation showed an absence of infection.[6] Although this is not a practical immunization protocol for humans, the study demonstrated that it is possible to elicit a protective response against syphilis. Although the Miller experiment is often cited as the criterion standard for syphilis vaccine experiments, it was pointed out at the meeting that this study has

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never been replicated and subsequent attempts to repeat it with less complex methods failed to confer protection. There is some concern within the field that this study, unless successfully replicated, should not be considered as conclusive evidence of achieving immunity against infectious challenge. A study by Magnuson et al.[7] in the 1950s demonstrated that individuals with untreated latent syphilis lacked clinical and serologic responses after inoculation with the Nichols strain of *T. pallidum* and were presumed to be immune. More recently, Marra et al.[8] and Kenyon et al.[9] independently made the similar observation that patients with previous syphilis had attenuated clinicopathologic findings with subsequent syphilis episodes. These studies highlight variations in the immune response to subsequent *T. pallidum* infection in those with a history of syphilis. Characterizing these responses may inform vaccine development efforts. The syphilis field has been searching for a subunit vaccine capable of conferring a near-complete level of protection in animal experiments. This vaccine ideally would be appealing to industry partners and could be transitioned to clinical trials within a matter of years.

Until very recently, the inability to propagate in vitro the syphilis spirochete in a laboratory setting has significantly hindered the quest for a syphilis vaccine at the technical level. This obstacle was overcome in 2018 when Edmondson et al.[10] revolutionized the syphilis field with a propagation system for this spirochete based on the co-cultivation of *T. pallidum* with SfEp1 rabbit epithelial cells. Despite earlier challenges to cultivate the syphilis spirochete, studies on *T. pallidum* biology have flourished and focused on the antigenic composition of the *T. pallidum* outer membrane and the role of outer membrane proteins (OMPs) in syphilis pathogenesis.[11–13] Given that *T. pallidum* clearance during infection is mediated by phagocytosis of opsonized pathogen cells by activated macrophages, the OMPs of the syphilis spirochete are regarded as primary targets for vaccine development efforts.[13–17]

Advances in genomic and bioinformatics tools have paved the way for reverse and structural vaccinology approaches, which examine the molecular architecture of *T. pallidum* OMPs to guide syphilis vaccine development. In silico experiments and structural modeling have contributed to a better understanding of *T. pallidum* surface-exposed membrane proteins, which make up the current vaccine candidates (Table 1). These include the β-barrel assembly machinery (BAM) complex, the lipopolysaccharide transport (Lpt) complex, 8-stranded β-barrels (OmpW homologs), long-chain-fatty-acid transporters (FadL homologs), the outer membrane components of efflux pump complexes, and the *T. pallidum* repeat proteins (Tpr). For some of these antigens, there still exists a degree of uncertainty regarding their domain architectures and membrane topologies, which eventually will be resolved as more experimental structural and functional data on these proteins accumulate.[17–21]

CHARACTERISTICS REQUIRED FOR AN EFFECTIVE SYPHILIS VACCINE

The presumptive features of an effective syphilis vaccine include the ability to induce opsonic antibodies and strong cellular responses to the vaccinogen, specifically by T-helper 1 response cells.[15,16,23–25] The production of opsonic antibodies and the release of interferon-γ can stimulate macrophages to phagocytose and clear treponemes.[16,26] *T. pallidum* biology also poses a unique challenge for vaccine development. Because of the paucity of surface-exposed antigens, multiple OMP epitopes must be targeted to facilitate pathogen recognition, particularly for antigens whose expression is not constitutive and can be regulated or altered through phase variation at the transcriptional or translational level or through other still unknown mechanisms.[13,22,27–29] An ideal vaccine should target both localized and disseminated infection, attenuate chancre development to reduce transmission in the early stage, and inhibit treponemal dissemination within the body to limit disease progression to the later stages.[26] In addition to the identification of efficacious vaccinogens, significant work is being done to carefully select vaccine adjuvants safe for human use and that can recapitulate the strong activity shown by the ones used in the rabbit model, such as the RIBI (Corixa Corporation, Seattle, WA) or TiterMax (TiterMax USA, Inc., Norcross, GA) adjuvants.

DELIVERY OF *T. pallidum* PROTECTIVE ANTIGENS USING TRANSMEMBRANE β-BARRELS AND VIRUS-LIKE PARTICLES

To increase the efficiency of vaccinogen delivery, chimeric antigens can be created to carry multiple epitopes shown to elicit protective activity. Small de novo designed transmembrane β-barrel proteins (TMBs) can be engineered to display epitopes of *T. pallidum* antigens, serving as a tool in vaccine development worthy of exploration.[30] Dr. Giacani (project and core leader of the University of Washington CRC) presented his team's unpublished work on the design and utilization of small β-barrels to deliver epitopes of protective antigens from *T. pallidum*. Dr. Giacani's group generated TMB constructs with epitopes from three TprC variants shown to be associated with a strong protective effect in a previous vaccination/challenge experiment in rabbits. Using these TMB constructs, they immunized rabbits using a custom RIBI adjuvant developed by PAI Life Sciences, a Seattle-based company. The investigators collected sera samples from the rabbits to assess the development of humoral immunity, performed epitope mapping using scaffolding and antigen-specific peptides, and conducted opsonophagocytosis assays. In addition, splenocytes were collected post-immunization to evaluate interferon-γ production after stimulation with immunogen-based peptides. The animal experiments showed that immunization with these chimeric TMBs induced antibodies against *T. pallidum*–specific extracellular loops (ECLs) that promoted opsonophagocytosis in several instances. Furthermore, it was shown that rabbit splenocytes stimulated with peptides based on the epitopes that were inserted in the small TMBs released interferon-γ, consistent with the induction of a Th1 response.

In addition to TMBs, Dr. Giacani discussed the use of virus-like particles (VLPs) as an alternative delivery platform for *T. pallidum* epitopes. Virus-like particles have gained significant attention in the vaccine field because they can be engineered to express specific surface antigens at high density.[31s] Dr. Giacani's team is exploring the human papillomavirus (HPV) L1 major capsid protein, a major structural protein that can be modified to generate noninfectious VLPs expressing specific antigens on their surface. Currently, their research focuses on engineering the H4 site of the HPV16 L1 protein with epitopes from TprC and TprK.

TRI-ANTIGEN COCKTAIL AND CHIMERIC SYPHILIS VACCINE WITH Tp0751, TprC, AND TprK

Dr. Cameron (project leader of the University of Washington CRC, based at the University of Victoria, BC, Canada) presented ongoing work that focuses on a two-pronged approach to syphilis vaccine design, which targets the localized and disseminated stages of *T. pallidum* infection. In addition to the Tp0751 vascular adhesin that contributes to endothelial cell attachment and *T. pallidum* dissemination, they selected the most conserved regions of the TprC and TprK proteins that have been shown to inhibit chancre formation for their vaccine design.[32s]

Their initial set of protection experiments involved immunizing rabbits with a tri-antigen cocktail consisting of a soluble preparation of Tp0751 (amino acids 24–237) and insoluble preparations of the N-terminal portions of the TprK and TprC proteins

TABLE 1. Potential Vaccine Candidates for *T. pallidum*

Integral Outer Membrane Proteins

Gene (Protein)	Putative Function	References
tp0011 (TprB)	*T. pallidum* repeat protein, putative porin	47s, 49s
tp0117 (TprC)*	*T. pallidum* repeat protein, porin	18, 47s, 48s, 49s, 50s, 51s, 52s
tp0126 (TP0126)	Putative OmpW homolog, porin	17,19,22 47s, 49s, 50s
tp0131 (TprD)	*T. pallidum* repeat protein, porin	17, 47s, 48s, 49s, 50s, 51s, 52s
tp0313 (TprE)	*T. pallidum* repeat protein, putative porin	47s, 49s, 51s, 53s
tp0316 (TprF)	*T. pallidum* repeat protein, putative porin, truncated	49s, 52s, 54s, 55s
tp0317 (TprG)	*T. pallidum* repeat protein, putative porin	49s, 52s, 53s
tp0326 (Tp92)*	β-barrel assembly machinery protein A (BamA) ortholog, outer membrane protein assembly factor	39s, 45s, 47s, 51s, 56s, 57s, 58s, 59s, 60s
tp0479	OprG homolog	17, 51s
tp0515	LPS-assembly outer membrane protein LptD	17, 51s
tp0548	TbuX/FadL-like long-chain fatty acid transport protein	17, 19, 51s
tp0610 (TprH)	*T. pallidum* repeat protein, putative porin	49s
tp0620 (TprI)	*T. pallidum* repeat protein, porin	47s, 49s, 51s, 52s, 57s
tp0621 (TprJ)	*T. pallidum* repeat protein, putative porin	18, 47s, 49s, 51s, 53s, 61s, 62s
tp0698	OprG homolog	17
tp0733	OprG/OmpW-like ion channel involved in transport of small hydrophobic molecules	17,19, 51s
*tp0856**	TodX/FadL-like long-chain fatty acid transporter	17, 19, 40s, 47s, 51s, 57s
*tp0858**	TbuX/FadL-like long-chain fatty acid transporter	17, 19, 40s, 47s, 51s
tp0859	Fad-L like long-chain fatty acid transport protein	17, 21, 47s
tp0865	TbuX/FadL-like long-chain fatty acid transporter	17, 19, 47s, 51s
tp0897 (TprK)*‡	*T. pallidum* repeat protein	18, 51s, 62s, 63s, 64s, 65s, 66s, 67s
tp0966	Outer membrane component for efflux pump complex	17, 19, 51s, 68s
tp0967	Outer membrane component for efflux pump complex	17, 19, 47s, 51s, 68s
tp0968	Outer membrane component for efflux pump complex	17, 19, 47s, 51s, 68s, 69s
tp0969	Outer membrane component for efflux pump complex	17, 19, 47s, 51s, 68s, 69s
tp1031 (TprL)	Putative porin	49s, 51s

Lipoproteins†

Gene (Protein)	Putative Function	References
tp0136 (Tp0136)	Lipoprotein. If exposed to the outer membrane, may bind to fibronectin, microfibronectin, and laminin	18, 70s, 71s, 72s
tp0155	Lipoprotein, fibronectin-binding protein	57s, 58s, 73s
tp0257 (Gpd)	Lipoprotein, glycerophosphodiester phosphodiesterase	74s, 75s, 76s
tp0435 (Tp17)	Lipoprotein, bacterial adhesin	34s, 39s, 77s, 78s, 79s, 80s
tp0483	Lipoprotein, fibronectin-binding protein	57s, 58s, 73s
*tp0751**	Lipoprotein. Vascular adhesin, can bind to laminin and fibrinogen. Exhibits metalloproteinase activity	39s, 81s, 82s

**tp0117* (TprC), *tp0326* (Tp92), *tp0897* (TprK), *tp0856*, *tp0858*, and *tp0751* are some of the current antigens being actively investigated in vaccine development.

†There is an ongoing debate regarding whether some lipoproteins can gain surface exposure and therefore would be possible vaccine candidates.

‡Undergoes phase variation.

using a RIBI-like adjuvant, followed by challenge of the animals with *T. pallidum*.[32s] The TprK and TprC proteins used were solubilized and refolded to restore their native structure as much as possible. Furthermore, each of these recombinant proteins carried regions predicted to code for multiple ECLs in *T. pallidum*. The immunized animals displayed smaller chancres, decreased ulceration, and lower bacterial burdens compared with control unimmunized and challenged rabbits. Naive rabbits inoculated with lymph nodes from immunized rabbits exhibited either delayed or no orchitis, suggesting reduced bacterial dissemination.[32s] These observations were supported by data on cytokine profiles, showing an increase in the production of the proinflammatory cytokines, interferon-γ, and tumor necrosis factor-α, and a decrease in the production of the anti-inflammatory cytokine, interleukin-10 (unpublished).

To move forward with the vaccine development process, Dr. Cameron and her team used the Tp0751 protein as a scaffold and engineered in epitopes from TprC and TprK identified by Dr. Giacani's work, creating a first iteration of a chimeric vaccine construct (unpublished). This chimeric vaccine was soluble and stable, and had endotoxin levels below the Food and Drug Administration threshold (5.0 endotoxin units per kilogram). Immunization with the chimeric vaccine induced high titer antibodies in rabbits and reaffirmed the findings from the protection experiments with the tri-antigen cocktail, which showed attenuated chancre development, reduced bacterial burden at challenge sites, and impaired dissemination to popliteal lymph nodes in immunized animals (unpublished).

TARGETING EXTRACELLULAR LOOPS OF *T. pallidum* OUTER MEMBRANE PROTEINS

The UConn/Duke CRC U19 syphilis vaccine program was founded on an understanding of the molecular architecture of the *T. pallidum* outer membrane derived from years of investigation of this fragile, protein-poor lipid bilayer.[13,17,29, 33s–35s] An important element of this work was the repeated demonstration that the

syphilis spirochete's lipoprotein immunogens reside in the periplasmic compartment where they are inaccessible to circulating antibodies.[13,29, 34s,36s–38s] The starting point for the UConn/Duke vaccine design efforts was a reverse vaccinology approach based on their characterization of *T. pallidum*'s repertoire of OMPs (the *T. pallidum* OMPeome).[13,17] As in other diderm bacteria, OMPs in *T. pallidum* adopt a β-barrel conformation in which ECLs bridge neighboring β-strands; antibodies that promote clearance of spirochetes must target ECLs. To identify potential ECL vaccinogens, the UConn/Duke team first identified ECLs that elicit antibody responses in patients with early syphilis. This was done by screening ECLs displayed on a thioredoxin scaffold from the archaebacterium *Pyrococcus furiosus* (*Pf*Trx)[39s–40s] against patient sera obtained by their CRC's clinical consortium. Dr. Hawley presented results obtained by immunizing animals with scaffolded ECLs of BamA, a central component of the spirochete's outer membrane biogenesis apparatus, and the two fatty acid transporters (FadLs) TP0856 and TP0858.[39s–40s] Immunization with these constructs induced ECL-specific "functional" antibodies that promoted opsonophagocytosis of spirochetes by rabbit peritoneal and murine bone marrow–derived macrophages and that inhibited the growth of *T. pallidum* during in vitro cultivation. Importantly, rabbits immunized with these ECL vaccinogens demonstrated a substantial degree of protection following intradermal challenge with *T. pallidum*. Dr. Hawley concluded by discussing protein engineering efforts to display *T. pallidum* OMP ECLs on VLPs and soluble β-barrels proteins and the team's promising initial experiments in mice immunized with *Pf*Trx ECLs encoded by mRNAs.

GENOMIC EPIDEMIOLOGY OF *T. pallidum*

Over the past few decades, the sequencing and analysis of historical and contemporary *T. pallidum* strains unveiled a significant level of genomic diversity within the genes responsible for encoding OMPs.[41s–46s] These findings may have implications for the design and development of a syphilis vaccine, underscoring the need to obtain high-quality genomes from diverse geographic regions to further assess these genomic differences. Any vaccine developed would need to be effective against circulating strains of *T. pallidum* worldwide. An in-depth exploration of the antigenic targets and their variants within *T. pallidum* OMPs on a global scale will provide valuable insights into the immunogens capable of eliciting opsonic antibodies and help inform future studies of potentially protective mechanisms for vaccine development.[42s] Furthermore, there is a growing need to sequence *T. pallidum* strains in low- and middle-income countries where syphilis remains endemic.[43s] These efforts aim to ensure geographic inclusivity and the development of a broadly protective vaccine.

Dr. Seña (UConn/Duke CRC) presented her team's findings to assess the genomic diversity of *T. pallidum* conducted through a global clinical research consortium to inform vaccine development, consisting of partners in Malawi, Colombia, and China.[46s] The study collected different clinical specimens from a diverse range of participants with early syphilis including heterosexual men and women, pregnant women, men who have sex with men, and HIV-coinfected persons in different regions of the world. Between November 2019 and May 2022, the study screened more than 2800 individuals, enrolling 248 participants. Among those enrolled, 79 (32%) were diagnosed with primary syphilis, 166 (67%) had secondary syphilis, and 2 (1%) had early latent syphilis. In addition, 64 participants (26%) had HIV coinfection. From the collected samples, researchers sequenced and analyzed 133 genomes, which revealed that 80% of the strains belonged to the SS14 lineage. When examining different factors such as age, sex/gender, race/ethnicity, sexual orientation, syphilis stage, and HIV status, the distribution of SS14 lineage and Nichols lineage strains was similar across the groups. Further investigation into the recombination-masked whole-genome phylogenetic analysis including genomes from their study sites, 62 previously published genomes, and 5 reference genomes showed that Nichols-lineage strains formed more distinct subclades and exhibited longer branch lengths, indicating greater genetic divergence within this lineage.[41s] Notably, half of the participants within the Nichols E subclade were living with HIV. Molecular analysis of the 23S ribosomal RNA gene associated with macrolide resistance demonstrated that 14% of strains from Malawi had macrolide resistance mutations compared with 100% of strains from China. Clade-informative variants also were mapped to 3-dimensional models of the *T. pallidum* OMP models to identify lineage and population-associated missense mutations arising in *T. pallidum* OMPs of interest such as the FadLs TP0858 and TP0865.

In attendance at the meeting were also Dr. Alex Greninger (University of Washington CRC Core leader) and Dr. Nicole Lieberman (University of Washington CRC), who described *T. pallidum* genome sequencing work culminating in near-complete genomes from 196 *T. pallidum* strains, including 191 strains sequenced directly from patient samples collected from 8 countries and 6 continents. Their data also revealed that strains from most sites belonged to the SS14 clade. However, 99% (84 of 85) of the samples from Madagascar formed 2 of the 5 distinct Nichols-like subclades. Overall, their data also highlighted the role played by variation in genes encoding putative surface-exposed OMPs in defining separate lineages and provided a critical resource for the design of broadly protective syphilis vaccines targeting surface antigens.[41s]

VACCINE ACCEPTABILITY

Dr. Seña's team also explored syphilis vaccine acceptability through an interview-based qualitative exploratory study among infectious disease and STI clinic patients in North Carolina to assess key stakeholders' perspectives and preferences regarding participation in a syphilis vaccine trial. Eligibility criteria included individuals 18 years or older who identified as one of the following: diagnosed with an STI in the past 12 months, living with HIV, men who have sex with men, or engaged in sex work. Among the 30 participants enrolled, more than half were receptive to the notion of vaccination and would be willing to participate in a trial. However, one-third of those enrolled mentioned that their willingness to participate in a trial would depend on factors such as compensation.

FUTURE STEPS AND CONCLUDING REMARKS

This meeting summary highlights state-of-the-art advances and approaches by leading scientific experts in the syphilis vaccine field. A limitation of this report is the presentation of unpublished data, and therefore, a precise description of the technical procedures that were used by the investigators could not be provided here for deeper critical analysis of the results. Moving forward, there are several areas that require further investigation. Ongoing research has suggested that the vaccine formulation for syphilis must be composed of multiple antigens, whether in the form of an antigen cocktail or chimeric vaccine, to provide complete or nearly complete protection to individuals and geographically diverse populations. Although the chimeric platform evaluated thus far allows for a single vaccine candidate that is stable and easily produced, the protection it offers is only partial. Therefore, further research is needed to boost the level of protection and expand the repertoire of epitopes, potentially including different epitopes from *T. pallidum* repeat proteins and other OMPs. As part of these efforts, it is essential to study the genomic diversity of *T. pallidum* strains from different patient populations, the genetic

variations in OMPs of interest, and their ECLs with respect to antigenicity versus antigenic variability. These studies may generate valuable knowledge and insights to ensure broad protection against *T. pallidum* variants worldwide. Interdisciplinary collaboration between scientists, clinicians, and public health experts, as well as forging innovative partnerships with investigators around the world will be crucial in realizing these goals.

In addition to refining the vaccine candidates, the selection of appropriate delivery vehicles as well as adjuvants also must be considered to ensure the safety and efficacy of the vaccine. Many of the adjuvants being used in preclinical experiments with rabbit models are not suitable for use in humans; thus, vaccine formulations will need to be reevaluated with adjuvants authorized for human use. Once the promising vaccine candidates and adjuvants have been identified in the preclinical setting, transitioning to clinical trials will be the next critical step. Especially considering the fraught history of syphilis research and anti-vaccination sentiment, additional research on the acceptability of syphilis vaccination is also critical. Furthermore, as these vaccines progress into clinical trials, it will be important to gauge the perspectives of policymakers, program implementers, and the vaccine industry. Exploring key parameters that may influence their decisions on vaccine adoption will be crucial. These parameters may include cost-effectiveness, identification of target populations, and the ability to reach these populations, particularly in the context of competing interventions that may be simpler and less expensive.

In conclusion, substantial progress has been made toward development of a syphilis vaccine. Despite the existence of current public health screening and treatment measures, syphilis persists as a pressing global public health concern with devastating consequences for sexually active individuals and maternal/fetal health. The development of a syphilis vaccine to complement traditional prevention and treatment strategies will be vital in the pursuit of syphilis elimination.

REFERENCES

1. Peeling RW, Mabey D, Chen XS, et al. Syphilis. Lancet 2023; 402: 336–346.
2. Lawn JE, Blencowe H, Waiswa P, et al. Stillbirths: Rates, risk factors, and acceleration towards 2030. Lancet 2016; 387:587–603.
3. Newman L, Kamb M, Hawkes S, et al. Global estimates of syphilis in pregnancy and associated adverse outcomes: Analysis of multinational antenatal surveillance data. PLoS Med 2013; 10:e1001396.
4. Nelson R. Syphilis rates soar in the USA amid penicillin shortage. Lancet 2023; 402:515.
5. Turner TB, Hollander DH. Biology of the treponematoses based on studies carried out at the International Treponematosis Laboratory Center of the Johns Hopkins University under the auspices of the World Health Organization. Monogr Ser World Health Organ 1957; 35:3–266.
6. Miller JN. Immunity in experimental syphilis. VI. Successful vaccination of rabbits with *Treponema pallidum*, Nichols strain, attenuated by -irradiation. J Immunol 1973; 110:1206–1215.
7. Magnuson HJ, Thomas EW, Olansky S, et al. Inoculation syphilis in human volunteers. Medicine (Baltimore) 1956; 35:33–82.
8. Marra CM, Maxwell CL, Sahi SK, et al. Previous syphilis alters the course of subsequent episodes of syphilis. Clin Infect Dis 2020; 71: 1243–1247.
9. Kenyon C, Osbak KK, Crucitti T, et al. Syphilis reinfection is associated with an attenuated immune profile in the same individual: A prospective observational cohort study. BMC Infect Dis 2018; 18:479.
10. Edmondson DG, Hu B, Norris SJ. Long-term in vitro culture of the syphilis spirochete *Treponema pallidum* subsp. *pallidum*. mBio 2018; 9:e01153–e01118.
11. Cullen PA, Haake DA, Adler B. Outer membrane proteins of pathogenic spirochetes. FEMS Microbiol Rev 2004; 28:291–318.
12. McKevitt M, Brinkman MB, McLoughlin M, et al. Genome scale identification of *Treponema pallidum* antigens. Infect Immun 2005; 73:4445–4450.
13. Radolf JD, Kumar S. The *Treponema pallidum* outer membrane. Curr Top Microbiol Immunol 2018; 415:1–38.
14. Baker-Zander SA, Lukehart SA. Macrophage-mediated killing of opsonized *Treponema pallidum*. J Infect Dis 1992; 165:69–74.
15. Leader BT, Godornes C, VanVoorhis WC, et al. CD4+ lymphocytes and gamma interferon predominate in local immune responses in early experimental syphilis. Infect Immun 2007; 75:3021–3026.
16. Hawley KL, Cruz AR, Benjamin SJ, et al. IFNγ enhances CD64-potentiated phagocytosis of *Treponema pallidum* opsonized with human syphilitic serum by human macrophages. Front Immunol 2017; 8:1227.
17. Hawley KL, Montezuma-Rusca JM, Delgado KN, et al. Structural modeling of the *Treponema pallidum* outer membrane protein repertoire: A road map for deconvolution of syphilis pathogenesis and development of a syphilis vaccine. J Bacteriol 2021; 203:e0008221.
18. Cox DL, Luthra A, Dunham-Ems S, et al. Surface immunolabeling and consensus computational framework to identify candidate rare outer membrane proteins of *Treponema pallidum*. Infect Immun 2010; 78: 5178–5194.
19. Houston S, Lithgow KV, Osbak KK, et al. Functional insights from proteome-wide structural modeling of *Treponema pallidum* subspecies *pallidum*, the causative agent of syphilis. BMC Struct Biol 2018; 18:7.
20. Lian T, Zhang B, Giacani L, et al. Full-length TprK of *Treponema pallidum* subsp *pallidum* in lipid nanodiscs is a monomeric porin. Enzyme Microb Technol 2022; 153:109897.
21. Parker ML, Houston S, Pětrošová H, et al. The structure of *Treponema pallidum* Tp0751 (Pallilysin) reveals a non-canonical lipocalin fold that mediates adhesion to extracellular matrix components and interactions with host cells. PLoS Pathog 2016; 12:e1005919.
22. Giacani L, Brandt SL, Ke W, et al. Transcription of TP0126, *Treponema pallidum* putative OmpW homolog, is regulated by the length of a homopolymeric guanosine repeat. Infect Immun 2015; 83:2275–2289.
23. Van Voorhis WC, Barrett LK, Koelle DM, et al. Primary and secondary syphilis lesions contain mRNA for Th1 cytokines. J Infect Dis 1996; 173:491–495.
24. Arroll TW, Centurion-Lara A, Lukehart SA, et al. T-cell responses to *Treponema pallidum* subsp. *pallidum* antigens during the course of experimental syphilis infection. Infect Immun 1999; 67:4757–4763.
25. Lukehart SA. Scientific monogamy: Thirty years dancing with the same bug: 2007 Thomas Parran Award Lecture. Sex Transm Dis 2008; 35:2–7.
26. Cameron CE. Syphilis vaccine development: Requirements, challenges, and opportunities. Sex Transm Dis 2018; 45(9S Suppl 1):S17–S19.
27. Giacani L, Molini B, Godornes C, et al. Quantitative analysis of tpr gene expression in *Treponema pallidum* isolates: Differences among isolates and correlation with T-cell responsiveness in experimental syphilis. Infect Immun 2007; 75:104–112.
28. Giacani L, Godornes C, Puray-Chavez M, et al. TP0262 is a modulator of promoter activity of tpr subfamily II genes of *Treponema pallidum* ssp. *pallidum*. Mol Microbiol 2009; 72:1087–1099.
29. Radolf JD, Deka RK, Anand A, et al. *Treponema pallidum*, the syphilis spirochete: Making a living as a stealth pathogen. Nat Rev Microbiol 2016; 14:744–759.
30. Vorobieva AA, White P, Liang B, et al. De novo design of transmembrane β barrels. Science 2021; 371:eabc8182.

For further references, please see "Supplemental References," http://links.lww.com/OLQ/B112.