Clinical Infectious Diseases

VIEWPOINTS ARTICLE

Safety of *Streptococcus pyogenes* vaccines: Anticipating and Overcoming Challenges for Clinical Trials and Post Marketing Monitoring

Edwin J. Asturias[1,2*], Jean-Louis Excler[3*], James Ackland[4], Marco Cavaleri[5], Alma Fulurija[6], Raj Long[7], Mignon McCulloch[8], Shiranee Sriskandan[9], Wellington Sun[10], Liesl Zühlke[11,12], Jerome H. Kim,[3,13] James B Dale[14], Andrew C. Steer[15]**

[1]Colorado School of Public Health, University of Colorado, Aurora CO, USA; edwin.asturias@cuanschutz.edu; [2] University of Colorado School of Medicine, Aurora, CO, USA; [3]International Vaccine Institute (IVI), Seoul, Republic of Korea; Jeanlouis.excler@ivi.int; [4]Global BioSolutions, Melbourne, Australia; jackland@globalbiosolutions.com; [5]European Medicines Agency (EMA), Amsterdam, The Netherlands; Marco.Cavaleri@ema.europa.eu; [6]Telethon Kids Institute (TKI), Perth, Australia; alma.fulurija@telethonkids.org.au; [7]Bill & Melinda Gates Foundation, London, United Kingdom; Raj.Long@gatesfoundation.org; [8]Department of Paediatrics, Red Cross War Memorial Children's Hospital, University of Cape Town, Cape Town, South Africa; mignon.mcculloch@uct.ac.za; [9]Imperial College, London, United Kingdom; s.sriskandan@imperial.ac.uk; [10]Senior Consultant, Vaxcellerant LLC (Formerly US FDA), Silver Spring MD, USA; wellington.sun@vaxcellerant.com; [11]South African Medical Research Council, Francie van Zijl Drive, Parowvallei, Cape Town, South Africa; liesl.zuhlke@uct.ac.za; [12]Division of Paediatric Cardiology, Department of Paediatrics, Institute of Child Health, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa; liesl.zuhlke@uct.ac.za; [13]College of Natural Sciences, Seoul National University, Seoul, Republic of Korea; Jerome.kim@ivi.int; [14]University of *Tennessee* Health Science Center,

*Equal first authors

***Corresponding Author:** Jean-Louis Excler, International Vaccine Institute, SNU Research Park, 1 Gwanak-ro, Gwanak-gu Seoul 08826, Republic of Korea jeanlouis.excler@ivi.int

****Alternate Corresponding Author:** Andrew C Steer**,** Murdoch Children's Research Institute (MCRI), Parkville Victoria, Australia Andrew.Steer@rch.org.au

DOI: 10.1093/cid/ciad311

Memphis TN, USA; bdale@uthsc.edu; [15]Murdoch Children's Research Institute (MCRI), Parkville Victoria, Australia; Andrew.Steer@rch.org.au

Streptococcus. pyogenes (Strep A) infections result in a vastly underestimated burden of acute and chronic disease globally. The Strep A Vaccine Global Consortium (SAVAC) mission is to accelerate the development of safe, effective and affordable *S. pyogenes* vaccines. The safety of vaccine recipients is of paramount importance. A single *S. pyogenes* vaccine clinical trial conducted in the 1960s raised important safety concerns. A SAVAC Safety Working Group was established to review the safety assessment methodology and results of more recent early phase clinical trials and to consider future challenges for vaccine safety assessments across all phases of vaccine development. No clinical or biological safety signals were detected in any of these early phase trials in the modern era. Improvements in vaccine safety assessments need further consideration, particularly for pediatric clinical trials, large-scale efficacy trials, and preparation for post-marketing pharmacovigilance.

Keywords: safety, vaccine, *Streptococcus pyogenes*, acute rheumatic fever, rheumatic heart disease, clinical trial

INTRODUCTION

Infections caused by *Streptococcus pyogenes* ("Strep A"), a human pathogen, afflict more than 800 million people each year and result in an estimated 639,000 deaths - mostly attributable to rheumatic heart disease (RHD) and invasive infections [1]. Clinical manifestations vary from mucosal diseases (pharyngitis, tonsillitis, superficial skin infections) to locally invasive and systemic diseases (bacteremia, meningitis, puerperal sepsis, necrotizing fasciitis, toxic shock syndrome) and immune-related sequelae including acute rheumatic fever (ARF), acute post-streptococcal glomerulonephritis and RHD [1, 2]. While most manifestations of *S. pyogenes* infection can be treated by penicillin, the lack of prevention strategies, particularly in low- and middle-income countries (LMICs), leads infection to progress to severe disease and death. Hence the 71st World Health Assembly asked the World Health Organization (WHO) in May 2018 to prioritize the development of a safe and effective *S. pyogenes* vaccine.

Purpose of the SAVAC Vaccine Safety Working Group

The path to *S. pyogenes* vaccines has been delineated by the WHO Research and Development Technology Roadmap and Preferred Product Characteristics (PPC), with emphasis on vaccine safety considerations and the need to build consensus [3]. The Strep A Vaccine Global Consortium (SAVAC) established in 2019 convened experts from the health and research sectors to facilitate the development of *S. pyogenes* vaccines [4]. The overall objective of the Safety Working Group was to provide, in a non-prescriptive manner, key vaccine safety considerations for vaccine developers, clinicians and regulatory authorities. Four sub working groups were

constituted to address: a) *S. pyogenes* infectious and post-infectious immune-pathogenesis and research into immune markers; b) the current state of knowledge about *S. pyogenes* vaccine safety; c) regulatory perspectives; and d) safety monitoring in phase 1/2 and phase 3 clinical trials and consideration for post marketing pharmacovigilance.

Theoretical concerns about vaccines related to rheumatic fever pathogenesis

The pathogenesis of *S. pyogenes* infection is highly complex and the mechanisms leading to autoimmune diseases such as ARF and RHD remain elusive [5]. It has been proposed that certain *S. pyogenes* antigens are involved in cross-reactivity and pathogenesis of ARF, but the evidence to implicate individual antigens is limited and inconclusive. The primary antigen proposed to generate cross-reactive antibody is the surface M-protein, encoded by the *emm* gene, a highly variable protein with more than 200 sequence variants (*emm*-types) [6]. Some of the monoclonal antibodies generated against a single M-protein variant have been shown to bind human cardiac myosin in heart tissue [7, 8]. Experimental proof that M protein is the ARF antigen is lacking. The absence of adequate models for ARF/RHD represents a major limitation to research in this regard. In addition, there are indications that antibodies to the *S. pyogenes* group A carbohydrate can bind to human heart tissue and may be involved in ARF pathogenesis [8, 9]. Non-M proteins such as hyaluronic capsule and two proteins present in the *S. pyogenes* cell membrane were also identified as cross-reactive antigens [10, 11]. These theoretical concerns have been raised during the development of *S. pyogenes* vaccines based on M protein or group A carbohydrate.

State of Knowledge about *S. Pyogenes* Vaccine Safety

There is a long history of vaccine trials for *S. pyogenes* in humans, dating back over 100 years, and involving over 200,000 participants. One trial, conducted by Massell and colleagues in the US in the 1960s, raised safety concerns, including with US regulators. Over the past two decades, there have been five *S. pyogenes* vaccine clinical trials conducted. Here we review the Massell trial, and the approach and results of safety assessments for the five trials in the modern era.

The Massell trial

The Massell study was conducted between 1965 and 1967 in Boston [12, 13]. The vaccine was a hot-acid extracted M protein of a type 3 *S. pyogenes*, partially purified using ribonuclease and dissolved in thiomersal. Study subjects were 21 healthy siblings of patients with a history of ARF, randomly selected out of a cohort of 106 healthy siblings. There was no control group. The 21 children were given weekly subcutaneous injections of gradually increasing doses of the vaccine, necessary because of reactogenicity, for 18 to 33 weeks.

The vaccination program began in July 1965 with an observation period of 30 months. There were 18 episodes of *S. pyogenes* pharyngitis (none were type 3, **Figure 1**). One child (9-year-old female) developed chorea and a grade 3 pansystolic murmur. This child had a documented

infection with emm18 *S. pyogenes* prior to her ARF illness, a strain type epidemiologically linked to ARF [14]. Another child (11-year-old male) developed fever, right shoulder pain, right knee arthritis and carditis (pansystolic murmur, diastolic murmur, and heart block). A third child (6-year-old male) developed fever and arthritis of both knees. The authors diagnosed definite ARF in the first two children, and probable ARF in the third child. No data were provided on episodes of ARF in the 85 other healthy siblings. The authors compared the number of ARF episodes as a proportion of cases of sore throat (3 out of 18, 17%) with a historical cohort of non-vaccinated children (all siblings of ARF patients) observed over a period of 15 years. In this historical cohort of an unspecified number of children there were 447 episodes of *S. pyogenes* pharyngitis and 5 cases of ARF (1%). The statistical comparison of these two groups had a P-value <0.001. The authors concluded with "*the need for extreme caution in conducting studies with streptococcal vaccine in human subjects"* [13].

Despite the flaws of the study design, this potential safety concern contributed to the US Food and Drug Administration (FDA) regulation enacted in January of 1979. As part of an efficacy review of all Biologicals approved prior to 1972, the FDA convened a "Panel on Bacterial Vaccines and Bacterial Antigens with No U.S. Standard of Potency" to review "mixed bacterial vaccines". Reviewing the Massell study and noting ARF cases following vaccination, the panel concluded that uncontrolled use of *S. pyogenes* antigens in bacterial vaccines with "no U.S. standard of potency" represented unacceptable risks, and the FDA Commissioner codified this conclusion in "21 CFR 610.19 Status of specific products: Group A Streptococcus" [15]. The regulation was revoked from the US Federal Register in December 2005 (**Box 1**) as the FDA believed that its requirement for *S. pyogenes* organisms and derivatives were "both obsolete and a perceived impediment to the development of Group A streptococcus vaccines" [16].

Safety assessment in vaccine clinical trials in the modern era

Since the lift of the FDA ban, there have been five *S. pyogenes* vaccine clinical trials conducted (**Table 1**) using antigens from M protein [17]. To address the historical and hypothetical concern of autoimmune responses elicited by antigens derived from *S. pyogenes*, several specific safety assessments were introduced into clinical protocols (**Table 2).** Standard clinical echocardiographic doppler-flow examinations were performed in all clinical studies to monitor for rheumatic cardiac abnormalities prior to entry and following final vaccination. The echocardiograms were evaluated independently by two cardiologists, and when disagreement occurred, a third independent assessment was obtained. Criteria were used to evaluate pre- and post-vaccination echocardiograms using pre-determined structural and functional changes.

There have been no serious safety signals detected among these five trials (**Supplementary Table 1**)**.** No subject developed clinical, echocardiographic or laboratory evidence of rheumatogenicity or nephritogenicity. No induction of human tissue-reactive antibodies was demonstrated in the first four studies; they were not measured in the fifth.

Regulatory perspectives to inform safety strategies

Further development of *S. pyogenes* vaccines requires safety as a primary outcome to be agreed by developers and relevant national regulatory authorities (NRA). The details of specific safety requirements for licensure may vary due to applicable national laws and product characteristics of the particular investigational vaccine. Currently no specific requirements for a *S. pyogenes* vaccine development are in place by the FDA or other regulatory agencies. General requirements for vaccines for other infectious diseases should be used as a starting point for consideration. However, because of the specific considerations that would be required for *S. pyogenes* vaccines, early, close, and periodic consultation with regulatory agencies throughout the lifecycle of development for *S. pyogenes* vaccines will be necessary (**Box 2**).

Preclinical safety

There is currently no specific regulatory consensus on an adequate preclinical assessment of potential vaccine-induced autoimmunity before the first-in-human study. Harmonization of a core set of preclinical safety data among several NRAs could provide a standardized process of characterization of vaccine-induced immune responses and safety evaluation to accelerate development. Specific studies using modern technologies should be informed by the current understanding of pathogenesis of *S. pyogenes* immune-mediated disorders and validity of animal models as applied to humans, being careful to acknowledge any uncertainty and knowledge gaps. *In vitro* tissue cross-reactivity studies have been used routinely in preclinical safety evaluation of other biologics such as monoclonal antibodies to study on-target and off-target tissue binding [18]. Their role and relevance in *S. pyogenes* vaccine development is uncertain; any tissue cross-reactivity assays to detect cardiac signals would require proof of biological plausibility, validation and standardization. The choice of the most relevant *in vivo* animal model for preclinical safety evaluation should be discussed with the regulator at the earliest opportunity.

Pre-licensure clinical safety

Clinical trials should be first conducted in healthy adults to determine the baseline safety and immunogenicity profile. As children and adolescents bear the major burden of ARF, once safety and immunogenicity have been demonstrated in adults, sequential phase 1/2 trials with proportionate de-escalation in age to the target vaccine population of younger children should be conducted before the full phase 3 demonstration of safety and efficacy in that population. The dose and regimen selected for the pivotal phase 3 placebo-controlled efficacy trial will be based on safety and immunogenicity of phase 2 dose-finding.

While ARF/RHD may occur after *S. pyogenes* infection, they may potentially occur in a vaccine trial as vaccine-induced autoimmune phenomena, with or without *S. pyogenes* infection. In highly endemic areas, background ARF incidence ranges between 8-50 cases per 100,000 population and rarely up to 250 [19]. Thus, even if prevention of ARF/RHD is not a primary

endpoint in the trial, they must be captured as adverse events following immunization (AEFI). While the use of the Jones criteria [20] is sufficient for clinical diagnosis of ARF/RHD, case definitions and ascertainment should be discussed with the regulatory authority prior to initiating clinical trials. A data monitoring committee could monitor in real-time any statistically and clinically significant imbalance of ARF/RHD as a safety signal and apply pause rules where appropriate.

The optimal duration of clinical trials, driven by the need to demonstrate both safety and efficacy for a particular vaccine, is important for discussion with regulatory authorities. Importantly, ARF occurs within 2-4 weeks of *S. pyogenes* pharyngitis, however clinically detectable RHD can have a much longer time to onset, highlighting considerations around duration of safety follow-up and around the role of echocardiography for detecting clinically silent RHD. Although efficacy trials will use symptomatic disease endpoints, careful consideration should be given to evaluation of the vaccine effect on asymptomatic infection during the trial and the effect of asymptomatic infection on the development of ARF/RHD. The trial design should include sound methods to assess the role of *S. pyogenes* carriage (prospectively or retrospectively) in vaccine efficacy given the variable immune responses observed with pharyngeal carriage among children [21, 22].

Post-marketing safety

As has been evident from COVID-19 vaccine implementation, systematic monitoring of vaccine safety is a continuing process, and should be jointly performed with regulatory and public health immunization authorities with a prospectively agreed safety plan. It will be important to develop observational study designs and tools for the evaluation of *S. pyogenes* vaccine safety signals in a larger and more heterogenous population than investigated in pre-approval clinical trials.

A risk management plan needs to be developed [23] when the vaccine is submitted for licensure. Increasingly vaccine manufacturers incorporate a benefit-risk analysis to support policy decision makers [24, 25]. *S. pyogenes* vaccines are likely to be licensed and developed in high-income countries and deployed in LMICs. While LMICs are most likely to benefit from a vaccine when considering invasive disease and RHD, preventing harm in vulnerable populations emphasizes the prominent role of ethics committees in this process.

Strong pharmacovigilance systems in LMICs to detect, analyze, and act on adverse events are essential to the safe scaling of new interventions. A demonstrated favorable track record of safety during larger deployment is essential to maintain trust. Early and proactive engagement with organizations like the WHO, regional public health authorities such as Africa CDC, regional technical advisory groups on immunization, in-country immunization programs, and regulators in LMICs would foster accelerated access of *S. pyogenes* vaccines.

Safety monitoring assessment considerations for future clinical trials

The four phase 1 trials and one phase 2a trial of *S. pyogenes* vaccines conducted so far provide a basis for discussion of safety assessments and monitoring in early phase studies. There have been no phase 2b or phase 3 trials of *S. pyogenes* vaccines in the modern era.

While generic core safety assessments would not fundamentally differ from any other new vaccine for AEFI using the WHO Causality Assessment Algorithm [26], the evaluation of Adverse Events of Special Interest (AESIs) specific to *S. pyogenes* may pose more difficulties, in particular for efficacy studies in children, phase 3 and post-marketing pharmacovigilance studies, underscoring the current gaps in safety assessment methodology. In particular, *S. pyogenes* safety concerns are more "syndromic" in nature and may not be captured and reported into passive reporting systems with discrete diagnoses, but rather a cluster of signs and symptoms. Current approaches to safety surveillance rely on analyses of individual MedDRA adverse event codes. Furthermore, observational studies are performed using predefined discrete case definitions and are most useful for events with clear onset and duration. Finally, safety surveillance infrastructure in LMIC settings, largely using WHO-AEFI causality algorithms, may not be optimal for timely detection of rare safety signals.

Cross-reactive antibody assays

In the vaccine phase 1 trials outlined above, testing for cross-reactive tissue antibodies by immunofluorescence against human cadaver heart, kidney, cartilage, basal ganglia, and cerebral cortex was performed pre-enrollment with a negative test required for subject inclusion. Repeat cross-reactive tissue antibody determinations were performed two weeks after the second and third vaccinations respectively. The methods are laborious and not standardized. Consideration should be given to drawing together an expert group to develop antigen-antibody binding assays using a mixture of suspected human cross-reactive proteins with pre-defined normal ranges.

Clinical assessment

The assessment of ARF events in recent trials of *S. pyogenes* vaccine candidates emphasizes the importance of detailed history and periodic physical exams. Clinical assessment should be general, with focus on cardiac, neurologic, renal, and rheumatologic systems. Because of the non-specific nature of laboratory testing for ARF, it is critical that laboratory results be interpreted in the context of clinical findings.

Safety biomarkers

There are several limitations to the use of biomarkers for safety of *S. pyogenes* vaccines: 1) no well-defined immune markers that could act as a risk surrogate of ARF development; 2) gaps in knowledge of mechanistic correlates of ARF/RHD development to aid biomarker identification;

3) a lack of clear understanding of the biologic time windows for sequelae of *S. pyogenes* infection to inform safety assessment protocols; and 4) the Jones criteria are imperfect as a gold-standard.

Echocardiography

The sensitivity of echocardiography to identify RHD during community surveillance is 3 times greater than that achieved by careful clinical examination alone [27]. However, issues remain with the absence of gold-standard echocardiography criteria for subclinical RHD and for an optimum management strategy for patients with clinically silent, mild valvular abnormalities [28]. A large-scale study conducted in Uganda on secondary antibiotic prophylaxis to prevent progression of latent RHD among children highlighted the feasibility and limitations of screening echocardiograms [29]. These difficulties are particularly relevant for surveillance and detection of safety signals. Anticipation of RHD background rates in different regions could provide vaccine developers, regulators, and public health decision makers with a better interpretation of any safety signal in late phase studies and an assessment of the potential benefits of a protective vaccine. Examples of these anticipatory safety outcomes background rates have been carried out for infant vaccines [30, 31], human papilloma virus vaccines in adolescents [32], influenza H1N1 [33], and COVID-19 vaccines [34]. Therefore, population-based estimates of age-related risk of potential AESI will be essential before phase 3/4 studies are conducted [35, 36].

Consensus is needed to identify and define the major *S. pyogenes* efficacy endpoints that will drive future evaluation and use of *S. pyogenes* vaccines. SAVAC has been developing case definitions of *S. pyogenes* disease endpoints and produced a suite of standardized "best practice" surveillance protocols [37]. In addition, for safety endpoint evaluations, working with available Brighton Collaboration definitions would provide standardized tools for clinical trials and post-marketing surveillance of *S. pyogenes* vaccines.

CONCLUSIONS

The ultimate decision on the use of a vaccine for individual and public health impact rests on the morbidity and mortality that can be prevented or modified, against the safety profile of the vaccine. Whereas the immediate local and systemic reactogenicity of *S. pyogenes* vaccines could be tolerable and transient, there remains concern over the hypothetical induction of adverse post-vaccination immunological responses.

The details of specific safety requirements for licensure may vary with applicable national laws and PPC of a particular investigational vaccine and target population. The field acknowledges there are challenges to safety surveillance and monitoring, as well as interpretation of potential safety signals across the full clinical vaccine development pathway. There is a clear role for the international expert community to contribute to filling these gaps.

Recommendations

The standardization of safety outcome measures for *S. pyogenes* vaccines will be a critical next step for the field. Working groups are needed for the following areas to inform safety assessments: clinical trial design, safety and efficacy endpoint definitions, screening assays for cross-reactivity, role of echocardiography, and biomarker evaluation. Constituting these groups under the umbrella of SAVAC alongside relevant stakeholder engagement will accelerate progress towards vaccine access to curb the impact of this globally significant pathogen.

***Acknowledgements*.** This work was supported by a grant from the Wellcome Trust awarded to the International Vaccine Institute (IVI) (215490/Z/19/Z). The authors thank the members of the Executive Committee of the Strep A Vaccine Global Consortium (https://savac.ivi.int/about) for their constant support and input.

EJA, JLE equally contributed to the design and writing of the manuscript. JA, AF, SS contributed to the immunological considerations; MC, WS to the regulatory considerations; MM, LZ, JD, ACS to the clinical and echocardiography considerations; JHK and ACS and all other authors reviewed and approved the manuscript.

***Support for this work*.** AF reports support for this work from Andrew Steer (ASAVI Collaborator, ASAVIi Co-Director), Jerome Kim (ASAVI Scientific Advisory Board member), Shiranee Sriskandan (ASAVI Scientific Advisory Board member), Jean Louis Excler (ASAVI Collaborator) and Jim Ackland (ASAVI Collaborator). ACS reports support as Co-director of SAVAC. ACS did not receive any direct funding from SAVAC. SAVAC was supported by the Wellcome Trust.

***Competing interests*.** All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest to JLE. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

- AJA is member of the SAVAC Executive Committee
- JLE is member of SAVAC and employee of IVI. He also reports participation on a Data Safety Monitoring Board or Advisory Board for US Military HIV Research Program.
- MM is past President of International Pediatric Transplant Association (IPTA) and Current President of the South African Transplant Society 2022 – 2024. MM also reports IPTA (International Pediatric Transplant Association) Congress travel and accommodation as Past President.

- SS declares membership of the SAVAC Executive committee which is a non-remunerated position
- WS has participated in Advisory Boards for other vaccines not related to Group A Streptococcus. He is an independent consultant with multiple clients developing vaccines, but none developing a Group A Strep vaccine. He owns stocks of ModernaTX Inc.
- LZ is member of the Executive Committee of SAVAC (unpaid). She is funded by the South African Medical Research Council (SAMRC) through its Division of Research Capacity Development under the Mid-Career Scientist Programme from funding received from the South African National Treasury. The content hereof is the sole responsibility of the authors and do not necessarily represent the official views of the SAMRC. LZ also receives support from the National Research Foundation of South Africa (NRFSA), as well as the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement, via the African Research Leader Award (MR/S005242/1). She is board member of World heart Federation (WHF), Non-communicable Diseases Alliance (NCDA) and Food Forward SA. LZ also received funding for contributing to UpToDate.
- JHK is Chair of SAVAC, SK bioscience COVID consultant and Everest unpaid COVID consultant
- JBD is the inventor of certain technologies related to the development of group A streptococcal vaccines. The intellectual property is owned by the University of Tennessee Research Foundation and has not been out-licensed. He is a member of a DSMB for Variation Biotechnologies (VBI) vaccine program. He also reports research funding from NIH/NIAID and Moderna.

- ACS is Co-chair of SAVAC (unpaid), President of the Lancefield Society for Streptococci and Streptococcal Diseases (unpaid). and Co-Director of the Australian Strep A Vaccine Initiative (unpaid) and Co-Chair of the Strep A Global Vaccine Consortium (unpaid). ACS also reports funding from multiple granting agencies for medical research, including Australian government: National Health and Medical Research Council, Medical Research Future Fund, US Government: National Institutes of Health, and other agencies: Viertel Foundation, Macquarie Foundation, Royal Children's Hospital Melbourne Foundaton, Thrasher Foundation; is a member of the DSMB for studies of moxidectin sponsored by Medicines Development for Global Health.
- JA reports consulting fees paid to employer by Australian Strep A Vaccine Initiative (ASAVI) and various companies developing novel vaccines and therapeutic products.
- EJA reports research grants to the University of Colorado from Pfizer Vaccines; consulting fees for serving on Advisory Board for Moderna vaccines; support for attending meetings from SAVAC; participation on a Data Safety Monitoring Board or Advisory Board for Curevac, Inovio, IVI. All other co-authors declare no conflicts of interest.

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FIGURE LEGEND

Figure 1. Pictorial representation of two cases of definite rheumatic fever and one case of probable rheumatic fever after vaccination with a hot-acid extracted M protein of a type 3 S. pyogenes partially purified using ribonuclease and dissolved in thiomersal (NT: not typeable, ARF: acute rheumatic fever; dots represent vaccine dose).

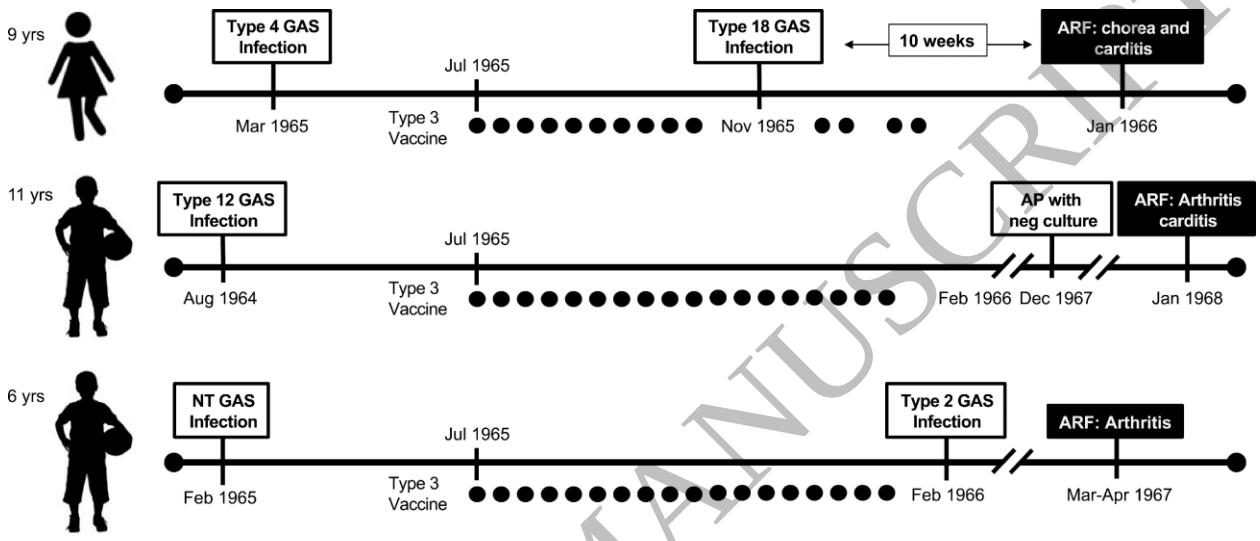

Table 1. Summary of the vaccine product characteristics, dosing regimens, population and designs used in recent S. pyogenes vaccine clinical trials 1990-2020

Trial	Product	Dose Regimen	Control	Population	N	Design	Regulatory Agency
Adult Phase I [38]	Hexavalent Prototype; N-terminal peptides from M1,3,5,6,19 & 24	Successive cohorts received: • 50 µg IM; on days 0, 28 and 56 (N=8) • 100 µg IM; on days 0, 28 and 112 (N=10) • 200 µg IM; on days 0, 28 and 112 (N=10)	None	Healthy adults, ages 18 – 50	29	Open-label, dose-escalation	US FDA
Adult Phase I [39]	StreptAvax 26-valent, N-terminal M peptides	400 µg IM on days 0, 28 and 120	None	Healthy adults, ages 18 – 50	30	Open-label	Health Canada
Adult Phase II [40]	StreptAvax 26-valent	400 µg IM on days 0, 28 and 180*	Hepatitis A vaccine	Healthy adults, ages 18 – 50	90	Randomized double-blind, comparator-controlled (70 StreptAvax, 20 comparator)	Health Canada
Adult Phase I [41, 42]	StreptAnova 30-valent, N-terminal M peptides	600 µg IM on days 0, 28 and 180	Selected licensed vaccines	Healthy adults, ages 18 – 50	36	Randomized double-blind, comparator-controlled (23 StreptAnova, 13 comparator)	Health Canada
Adult Phase I [43]	MJ8VAX (J8-DT) C-terminal 29 aa M peptide	50 µg IM on days 0	Saline	Healthy adults, ages 20 – 44	10	Randomized double-blind, placebo-controlled (8 MJ8VAX, 2 placebo)	QIMR Human Research Ethics Committee

Table 2. Safety assessments performed in recent S. pyogenes vaccine trials

Hexavalent Prototype Multivalent M [38]	26-valent (Phase I) Multivalent M [39]	26-valent (Phase II) Multivalent M [40]	30-valent (Phase I) Multivalent M [41, 42]	J8-DT Conserved C-terminal M peptide conjugate [43]
• 7-day reactogenicity diary • Cardiac exam 14 days after each dose, & 6 and 12 months • Echocardiogram & ECG 14 days after each dose, & 6 and 12 months • Routine clinical labs + troponin, C3, CRP • Human tissue cross-reactive antibodies by IFA 14 days after each dose, & 5 and 12 months AE follow-up x 12 months	• 14-day reactogenicity diary • Cardiac and neuro exam 7 and 14 days after each dose • Echocardiogram & ECG screening and within one month after third dose • Routine clinical labs plus troponin-I, C3, CRP • Human tissue cross-reactive antibodies by IFA within one month after second and third doses • AE follow-up x 12 months	•14-day reactogenicity diary • Cardiac and neuro exam 7 and 14 days after each dose • Echocardiogram & ECG screening and within one month after third dose • Routine clinical labs plus Troponin-I, C3, CRP baseline and if indicated to evaluate clinical findings • Human tissue cross-reactive antibodies by IFA within one month after second and third doses • AE follow-up x 12 months	• 14-day reactogenicity diary • Cardiac, neuro, and joint exam 7 days after dose 1 and 14 days after dose 2 and 3 • Echocardiogram & ECG screening and 30 days after third dose • Routine clinical labs plus C3, CRP baseline and if indicated to evaluate clinical findings • Human tissue cross-reactive antibodies by IFA screening and 14 days after each dose • AE follow-up x 12 months	•7-day reactogenicity diary • Cardiac exam days 28, 180, 266, and 350 • Echocardiogram & ECG screening and days 28, 84, and 350 • Routine clinical labs screening and days 28, 84, 180, 266, and 350 • Serum stored pre-treatment and day 350 for future tissue cross-reactive antibody assays • AE follow-up x 12 months

"We are removing § 610.19 because the existing requirement is obsolete and perceived to be impeding the development of Group A streptococcal vaccines using purified or characterized streptococcal antigens. The regulation is obsolete because it was written to apply to a group of products that are no longer on the market. Therefore, a vaccine to prevent diseases caused by this organism would have a public health benefit. We are taking this action as part of our continuing effort to reduce the burden of unnecessary regulations on industry and to revise outdated regulations without diminishing public health protection."

Box 1. Revocation of the 21 CFR 610.19 Status of specific products: Group A Streptococcus, December 2nd 2005 [16].

1. Unique considerations for *S. pyogenes* vaccines, including the potential for vaccine-induced immune-mediated sequalae
2. Rigorous evaluation of new non-clinical and clinical data for novel vaccines with similarities to *S. pyogenes*
3. Evolution of new technologies used in product development
4. Available and expected testing capacity, including assay development and evaluation of immune and safety responses
5. Evolution of risk-benefit assessment as part of product evaluation and risk management plans
6. Specific pharmacovigilance commitments and phase IV studies
7. Assessment of potential public health impact particularly for a vaccine for which efficacy may be variable according to clinical outcomes (e.g., partial protection against pharyngitis, but higher efficacy against immune-mediated sequelae).

Box 2. Reasons for close and periodic consultation with regulatory agencies throughout the life cycle of vaccine development for *S. pyogenes* vaccines