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Status of research and development of vaccines for *Streptococcus pyogenes*

Andrew C. Steer[a,b,*], Jonathan R. Carapetis[c], James B. Dale[d], John D. Fraser[e], Michael F. Good[f], Luiza Guilherme[g], Nicole J. Moreland[h], E. Kim Mulholland[i,j], Florian Schodel[k], Pierre R. Smeesters[a,b,l]

[a] *Centre for International Child Health, University of Melbourne, Melbourne, Australia*
[b] *Group A Streptococcal Research Group, Murdoch Children's Research Institute, Melbourne, Australia*
[c] *Telethon Institute for Child Health Research, University of Western Australia, Perth, Australia*
[d] *Medicine, University of Tennessee, Memphis, TN, USA*
[e] *Department of Molecular Medicine and Pathology and Maurice Wilkins Centre, University of Auckland, Auckland, New Zealand*
[f] *Institute for Glycomics, Griffith University, Gold Coast, Australia*
[g] *Heart Institute, School of Medicine, University of São Paulo, São Paulo, Brazil*
[h] *School of Biological Sciences and the Maurice Wilkins Centre, University of Auckland, Auckland, New Zealand*
[i] *Pneumococcal Research Group, Murdoch Children's Research Institute, Melbourne, Australia*
[j] *London School of Hygiene and Tropical Medicine, London, United Kingdom*
[k] *Philimmune LLC, Philadelphia, USA*
[l] *Department of Paediatrics, Université Libre de Bruxelles, Brussels, Belgium*

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ABSTRACT

Streptococcus pyogenes is an important global pathogen, causing considerable morbidity and mortality, especially in low and middle income countries where rheumatic heart disease and invasive infections are common. There is a number of promising vaccine candidates, most notably those based on the M protein, the key virulence factor for the bacterium. Vaccines against *Streptococcus pyogenes* are considered as impeded vaccines because of a number of crucial barriers to development. Considerable effort is needed by key players to bring current vaccine candidates through phase III clinical trials and there is a clear need to develop a roadmap for future development of current and new candidates.

1. Introduction

Streptococcus pyogenes or Group A Streptococcus (GAS) causes a massive disease burden that has been underestimated by global health authorities. A 2005 study estimated that there are >500,000 deaths annually due to the bacteria, mostly occurring in low and middle income countries [1].

The agent is a Gram-positive bacterium with the human as its unique reservoir and an array of virulence factors allowing for a very broad spectrum of clinical expression. The nasopharyngeal mucosa and the skin, the two principal sites of asymptomatic colonization of GAS, represent the primary reservoirs responsible for the maintenance and transmission of GAS to a new host. The ability of GAS to colonize and persist in skin tissue permits transmission through person-to-person contact. GAS can also overcome innate and acquired immune mechanisms present in saliva to remain viable for long periods, enabling transmission from infected persons or asymptomatic carriers via respiratory droplets [2]. In addition, numerous outbreaks caused by food-borne GAS have also been reported [2].

GAS bacteria are able to penetrate normal tissue barriers leading to invasive infection at local (e.g. retropharyngeal abscess or necrotizing fasciitis) as well as distant sites (e.g. septic arthritis). It produces an array of superantigens that can result in streptococcal toxic shock syndrome, which carries a high case fatality rate (>50%). In addition, invasive GAS disease is a frequent cause of sepsis in children and adults and has a high-case fatality rate leading to at least 150,000 deaths annually worldwide, although this figure is almost certainly an underestimate because of sparse data from

* Corresponding author at: Centre for International Child Health, Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Melbourne, Flemington Road, Parkville, Victoria 3052, Australia. Tel.: +61 3 9345 5522; fax: +61 3 9345 6667.
E-mail address: andrew.steer@rch.org.au (A.C. Steer).

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many developing countries [1]. GAS can also cause invasive infection in infants (neonatal sepsis) and the mother (puerperal sepsis); indeed, in the UK, GAS has been reported as the leading single cause of maternal death [3].

The immune response to GAS can be disordered and early symptomatic infection can lead to later, so called post-streptococcal sequelae, including acute rheumatic fever (ARF) that in turn leads to chronic rheumatic heart disease (RHD), as well as post-streptococcal glomerulonephritis (PSGN). ARF/RHD is an uncommon disease today in most resource-rich countries including the United States, but it remains the major cause of acquired heart disease in children, adolescents and young adults in the developing world, responsible for at least 350,000 premature deaths per year [4]. Available data on the prevalence of RHD suggest that there are over 30 million people affected by RHD worldwide. PSGN is thought to contribute to the high rates of end-stage renal failure in GAS endemic regions [5].

GAS pharyngitis and impetigo are responsible for the greatest absolute number of symptomatic GAS infections each year. GAS pharyngitis affects approximately 8–15% of school-aged children per year, whereas GAS impetigo is a very common infection in children, especially in tropical developing countries with a prevalence of >10%, and even over 50% in some settings [6].

GAS remains susceptible to penicillin. In spite of its widespread use there is no evidence to suggest the burden of GAS diseases is decreasing in low and middle-income settings. Serious chronic disease associated with streptococcal disease has decreased in incidence in high income countries in most populations. Antibiotic treatment of pharyngitis is highly effective at preventing ARF [7], however primary prevention programmes are resource intensive especially for low and middle income countries [8,9]. Furthermore, many cases (possibly over 60%) of ARF occur without a history of symptomatic pharyngitis, and it has been hypothesized that GAS skin infection may also contribute to ARF [10]. For those diagnosed with ARF, secondary penicillin prophylaxis is effective in preventing recurrent ARF episodes and consequent worsening of RHD when efficiently delivered [11], but it requires monthly penicillin injections over many years [8].

Serious GAS diseases do appear to be waning in some middle-income countries, probably because of improved living conditions and access to health services. However, GAS diseases continue to exact a toll in terms of mortality, morbidity and economic costs, even in wealthy countries, such as the important contribution of invasive GAS disease to maternal mortality in the United Kingdom [3].

Data are sparse on the economic burden of GAS disease, though a recent study in Fiji found that the cost of RHD mortality over a 5 year period was over US$30 million, close to one third of one percent of total GDP, representing a significant economic burden for the country [12]. An economic evaluation of interventions for ARF/RHD found that a vaccine against GAS would be the most cost-effective intervention for ARF/RHD in ARF-endemic regions at a cost of between US$137–458 per DALY averted, assuming 80% efficacy and 65–95% coverage (compared with 22–33 thousand dollars for treatment of sore throat) [13]. Even wealthy countries are affected – a study of GAS pharyngitis in the US suggested that GAS pharyngitis costs at least US$500 million per annum [14]. Few data are available for impetigo, invasive disease, scarlet fever and PSGN.

Better epidemiologic data are still needed in most developing countries, particularly on the contribution of RHD to premature mortality and long term sequelae [15], and on the rate and mortality of invasive disease, especially in the newborn and the new mother. Data regarding strain diversity of the bacteria are also needed in many low and middle income countries. Even with these limitations, the current data on disease burden make a convincing case for the need for an effective vaccine that could offer a practical strategy for disease control and prevention, especially for ARF and RHD.

2. Overview of current efforts

2.1. Biological feasibility for vaccine development

Although there are no currently licensed GAS vaccines, the biological feasibility for GAS vaccine development is supported by a number of observations including the natural history of GAS infection, available serologic data from natural history studies, animal data from pre-clinical studies, and evidence of protection from challenge in human subjects immunized with purified M proteins.

Pre-school and school-aged children experience repeated episodes of GAS pharyngitis and skin infection until they reach early adulthood when these infections become far less common, indicating that immunity to infection develops with age. One explanation for this observation is that immunity is type-specific – that is, that when a person is infected by a strain of GAS (most commonly referred to as an M-type/*emm*-type) the immune response protects them against subsequent infection due to the homologous type, but not or less completely against heterologous types [16,17]. Over time individuals meet multiple types of GAS and develop immunity against these multiple types. An alternate or complementary explanation is that generation of immunity requires repeated presentation of conserved antigens before a threshold level of protective immunity is achieved. There is no direct evidence for the latter explanation and it is not mutually exclusive with the former.

The paradigm of type-specific immunity is further supported by elegant longitudinal studies that have observed that infection with a single strain of GAS leads to generation of strain specific (M-protein) antibodies that lead to a long period (up to 30 years) of protection against the homologous strains but not against other strains [16].

Pre-clinical (murine) studies of GAS vaccine candidates (predominantly M protein vaccines) have demonstrated protection in challenge studies [18]. Further, subjects vaccinated with purified M proteins from GAS were protected against challenge with virulent homologous strain of GAS [19–21]. These GAS pharyngeal challenge studies, involving a total of 178 healthy adult volunteers in 3 separate studies were successfully used to demonstrate efficacy of prototype M protein vaccines in the 1970s. Vaccine efficacy of up to 89% was demonstrated in these studies [19]. Importantly, GAS challenge was safe, with all participants responding to penicillin therapy without complications or sequelae developing.

2.2. Serotype/strain coverage

A potential barrier to a type-specific M protein-based vaccine is that there are >200 *emm*-types of GAS (the N-terminal part of the M protein has a variable amino acid sequence resulting in antigenic diversity and is the basis for this widely used nucleotide based *emm*-typing scheme). If type-specific antibody protection is the major mechanism by which immunity is generated against GAS then this clearly raises the issue of potential coverage for type-specific vaccines. This was highlighted in an article published in 2009 that identified that the distribution of *emm*-types was quite different in developing compared to developed settings [22]. The study observed that there was a higher diversity of strains in lower to middle income settings versus high income settings, and indicated that the theoretical coverage of a 26-valent (combination of specific strains) vaccine would be favourable in developed settings (>72%) while strain coverage would be much lower in settings were serious GAS disease is more common (e.g. Africa 39% and Pacific 24%). However, recent data from epidemiologic, genomic

and in vitro studies suggest that there may be immunologic "cross-protection" between *emm*-types of GAS that may overcome this issue [23,24]. The hypothesis for this cross-protection is that antibodies generated against individual *emm*-types may actually provide some protection against a selection of heterologous *emm*-types. It is proposed that this cross-protection occurs within 48 *emm*-clusters [23]. There is ongong investigation in this area but it does provide some hope for a broadly effective vaccine based using a type-specific approach. There are fewer epidemiologic data regarding coverage based on the presence or absence of conserved antigens.

2.3. General approaches to vaccine development for this disease for low and middle income country markets

ARF occurs predominantly in school-aged children in low and middle-income countries because that is the age-group in which the triggering infection (GAS pharyngitis) occurs, although in some settings it is hypothesized that earlier skin infection may prime the immune system [25]. Invasive disease occurs in all ages, with an increased incidence in infants and the elderly. PSGN occurs most frequently in pre-school children, reflecting the greatest burden of the triggering infection (GAS impetigo). Therefore, while consensus in the field has not been reached on a target age group for vaccination, an infant or toddler schedule will likely be the most appropriate schedule for most endemic settings, possibly with a school entry booster dose. In non-endemic settings, a school entry schedule that coincides with the schedule for final TDap, IPV, and MMR doses may be appropriate. In areas where GAS is an important cause of maternal and neonatal sepsis, maternal immunization may also be considered.

A successful vaccine could address a huge unmet public health demand, and a vaccine that can prevent ARF (and thus RHD) as well as invasive GAS disease has the potential to save over 500,000 premature deaths per year. In addition, prevention of GAS pharyngitis and impetigo would have an enormous impact on reductions in morbidity through improved quality of life as well as a major economic impact through reduced health care expenditure on these exceedingly common infectious disease problems of childhood.

3. Technical and regulatory assessment

Despite considerable progress, there remain a number of significant barriers to GAS vaccine development and candidates remain in their infancy [26]. There is no clear pathway agreed by multi-disciplinary consensus for a pathway for vaccine licensure, although global efforts are beginning to come together through a rudimentary roadmap for vaccine development [26,27]. GAS vaccines are now considered "impeded vaccines". The major issues include, but are not limited to: safety concerns, an incomplete understanding of immune protection in humans, inadequate epidemiological data, minimal development of vaccines that contain both type-specific *emm* antigens and conserved antigens and limited commercial interest.

A particular issue is development of a correlate of human immune protection [28], reflecting the need for an improved understanding of GAS immunity, including the immune response to GAS skin infection, the role of T-cell immunity and the relative contributions of common conserved antigens in inducing protective immunity. Opsonophagocytic antibodies or bactericidal antibodies are potential correlates, but reliable and reproducible assays to measure these antibodies are lacking [28]. The most frequently used functional assay is the indirect bactericidal test, which is a time-consuming and methodologically challenging test. The establishment of correlates of protection would be much facilitated by the availability of an effective vaccine.

Concerns regarding vaccine safety are based upon a theoretical risk of autoimmune reactions in vaccinees leading to the development of ARF. One small study of a crude M protein vaccine suggested that there may be an increased risk of ARF in vaccine recipients [29]; however, there are a number of concerns about the design of this trial that make it difficult to interpret, and autoimmune reactions have not been observed in the other human GAS vaccine trials involving thousands of study subjects [18].

Better epidemiologic data are also required, for assessing burden of disease to strengthen the case for GAS vaccine development, and also for assessing type diversity and thus vaccine coverage more systematically with high quality, standardized molecular typing studies in more countries, particularly in Africa and Asia.

Combination vaccines may be a viable approach to overcoming "gaps" in *emm* type coverage achieved with multivalent vaccines alone and to potentially broaden the immune response. However, to date there has been minimal progress in combining antigens in a single vaccine [30], and such a move would need to overcome proprietary interests and intellectual property rights. It is unclear exactly why there has been an apparent reluctance of large pharmaceutical companies to invest in clinical development of GAS vaccines. The obstacles listed above, together with the perception of a questionable market for a vaccine in affluent countries, likely combine to create the impression of adverse commercial risk.

Although the ultimate health need is for a vaccine to protect against ARF/RHD and invasive disease, the relatively low incidence of these diseases and the time delay between the initiating event and disease makes these diseases potentially difficult endpoints for phase III efficacy studies because trials would be complicated and require well over 10,000 subjects [31]. However, protective efficacy against pharyngitis is a realistic efficacy endpoint for candidate GAS vaccines in phase III trials and prevention of GAS pharyngitis should enable licensure of efficacious and medically useful vaccines. Importantly, GAS pharyngitis is well established as the triggering event for ARF, and so prevention of pharyngitis can reasonably be assumed to translate to prevention of ARF.

A potential strategy to improve understanding of GAS immunology and also to create a pathway for relatively rapid testing of new GAS vaccine candidates is through the development of human GAS (pharyngeal) challenge studies. Previous studies (in the 1970s) in over 170 volunteers have shown that this approach is feasible [19–21], and proposals are under consideration for funding for a revival of this approach.

4. Status of vaccine research and development activities

GAS vaccines can be broadly divided into M protein-based and non-M protein-based vaccines. The GAS has a broad armamentarium of virulence factors, but it is the M protein that is the major virulence determinant of the organism. The M protein is a coiled-coil protein consisting of 3 domains: an A-repeat/N-terminal domain, which is highly variable and is used for epidemiologic molecular typing (*emm* typing); a B-repeat domain (antibodies against this region are not opsonic and some are cross-reactive with human tissues) and a conserved C-repeat domain. The vaccines that have entered or are nearing clinical investigation are the N-terminal M protein-based multivalent vaccines (26-valent and 30-valent vaccines) and conserved M protein vaccines (the J8 vaccine and the StreptInCor vaccine) [32–35]. There are a variety of other vaccine candidates that are at various stages of discovery and development (Table 1).

Table 1
Development status of current vaccine candidates (*approaching trials).

Candidate name/Identifier	Stage of development			Reference
	Pre-clinical	Phase I	Phase II	
M protein: 6-valent N-terminal	X	X		[36]
M protein: 26-valent N-terminal	X	X	X	[32]
M protein: 30-valent N-terminal	X	*		[33]
M protein: minimal epitope J8	X	X		[34]
M protein: minimal epitope J14/p145	X			[45]
M protein: C-repeat epitope (StreptInCor)	X	*		[38]
M protein: C-repeat epitopes	X			[46]
Three conserved antigens (Combo)	X			[43]
GAS carbohydrate	X			[47]
GAS carbohydrate defective for GlcNAc side-chain	X			[48]
GAS C5a peptidase	X			[49]
Fibronectin-binding protein	X			[50,51]
Streptococcal protective antigen	X			[32]
Serum opacity factor	X			[52]
Streptococcal pyrogenic exotoxin A/B/C	X			[53–55]
Streptococcal pili (T antigen)	X			[56,57]
Serine protease (SpyCEP)	X			[58]
Nine common antigens	X			[42]
Identified but untested antigens: G-related α2-macroglobulin binding (GRAB) protein, metal transporter of streptococcus (MtsA), superoxidase dismutase, lipoproteins				[59,60]

4.1. 26-Valent and 30-valent M protein vaccines

These vaccines consist of fused recombinant peptides from the N-terminal region of M proteins from multiple different *emm* types of GAS [32,33,36]. The original prototype multivalent vaccine was a hexavalent vaccine that was evaluated in a phase I trial and later expanded to a 26-valent vaccine and most recently a 30-valent vaccine. The 26-valent vaccine underwent a phase I/II clinical trial in human adult volunteers and was shown to be safe and immunogenic [32]. Functional opsonic antibodies were induced against all *emm* types of GAS in the vaccine. The 26-valent vaccine was reformulated into a 30-valent vaccine to increase "coverage" of circulating *emm* types in the United States, Canada and Europe as well as developing countries [33]. Epidemiologic surveys suggest that the 26-valent vaccine would provide good coverage of circulating strains of GAS in industrialized countries (over 72%) but poor coverage in many developing countries (as low as 24% in the Pacific region) [22]. In preclinical studies, the 30-valent vaccine has been shown to induce functional opsonic antibodies against all of the *emm* types represented in the vaccine [24]. An intriguing finding of the studies of the 30-valent vaccine is that antibodies produced by the vaccine were shown to cross-opsonize a proportion of non-vaccine *emm* types of GAS [24], implying that cross-protection may mitigate, to a greater or lesser extent, the limited coverage of the 30-valent vaccine in many tropical developing settings where GAS disease is endemic. A phase I clinical evaluation of the 30-valent vaccine in adult volunteers is anticipated in the third quarter of 2015.

4.2. Conserved M protein vaccines

These vaccines contain antigens from the conserved C-repeat portion of the M protein. The StreptInCor vaccine incorporates selected T and B-cell epitopes from the C-repeat region in a synthetic 55 amino acid polypeptide, whereas the J8 and J14 vaccines contain shorter single minimal B cell epitopes from this same region [37,38]. These vaccines have the clear advantage of being comprised of a minimal number of antigens. Extensive studies in mice, particularly of the J8 vaccine candidate, have shown that these antigens produce opsonic antibodies that protect against intraperitoneal challenge when the vaccine is administered parenterally and against intranasal challenge when the vaccine is administered intranasally [34,39]. The J8 vaccine has recently been re-formulated with a CXC chemokine protease which was able to protect mice against both intraperitoneal challenge and also against skin infection in a novel pyoderma mouse model [40]. Limited epidemiological data available for the J8 peptide indicate that its sequence is highly conserved among multiple *emm* types of GAS and across regions [41]. The J8 vaccine entered a phase 1 trial in adult volunteers in 2013 but the results of this trial have not yet been reported. The StreptinCor vaccine has been formulated into GMP StreptInCor plus alum with plans to enter phase I clinical assays in healthy adult volunteers in Brazil in 2016.

4.3. Other vaccines

Cell wall and secreted virulence factors, such as streptococcal C5a peptidase, GAS carbohydrate and streptococcal fibronectin-binding proteins, among others, have been the subject of vaccine research for up to 20 years with some encouraging results, particularly for C5a peptidase, but none of these candidates has entered clinical trials [18]. More recently, a number of promising, apparently conserved, vaccine candidates have been identified using immunization of mice with GAS gene segments [42]. In a large study, immunization of mice with GAS gene segments and challenge studies, identified several known and new antigens, among them three antigens were selected for further development: spy0416 (spyCEP), spy0167 (streptolysin O, SLO) and spy0269, a surface exclusion protein [43]. These three antigens were combined together in a single vaccine (so-called "Combo") and were found to provide broad coverage against multiple GAS strains in mouse models [43]. Combo has not yet entered clinical trials however.

5. Likelihood for financing

GAS vaccine development has been supported in the past by industry (e.g. Merck support of the J8 vaccine and of the 26-valent vaccine, ID Biomedical Corporation support of the 26-valent vaccine, Novartis Vaccines and Diagnostics, now GSK, support of the Combo vaccine and Intercell, now Valneva of a similar vaccine approach). The National Institute of Allergy and Infectious Diseases (NIAID) at the US National Institutes for Health supported the development of potential clinical trial sites in 2005–7 (Fiji, Mali, Nicaragua, and South Africa). The Novartis Vaccines Institute for Global Health (NVGH) included GAS vaccine development in its 2013 work programme.

In 2014 the Australian and New Zealand governments made an initial AU$ 3 million investment into vaccine development in these 2 countries where RHD is a public health priority through an initiative known as CANVAS (Coalition to Advance New Vaccines against group A Streptococcus) [44]. The aim of CANVAS is to evaluate potential GAS vaccine candidates for their potential to protect populations around the world with high rates of GAS diseases, and to support the most promising candidate(s) through phase I

and II clinical trials and, hopefully, to an efficacy study against GAS pharyngitis. The Initiative is also developing a core set of GAS strains that a candidate vaccine should demonstrate protection against, and an independent functional antibody assay that can be used for all GAS vaccines in development. This will coincide with an economic evaluation to make a case for investment in a GAS vaccine, and work to engage industry partners and international agencies in the hope that demonstration of feasibility and safety of a GAS vaccine will encourage further investment to ensure a vaccine is made available.

Moving forward it will be important to gain the interest and support of funders with a track record in supporting vaccine development for organisms that cause a significant burden of disease in low and middle income countries such as the Bill and Melinda Gates Foundation, the Wellcome Trust, NIAID, PATH, GAVI and others.

Conflict of interest: ACS, JRC, JDF, NJM, EKM, FS and PRS are all investigators or advisers to the CANVAS initiative. JBD is the lead developer of the 30 valent M-type specific vaccine. MFG is the lead developer of the J8 vaccine. LG is the lead developer of the Strept-InCor vaccine.

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