Accelerating the development of a group A *Streptococcus* vaccine: an urgent public health need

Group A *Streptococcus* (GAS) infections cause substantial worldwide morbidity and mortality, mostly associated with suppurative complications such as pharyngitis, impetigo, and non-suppurative immune syndromes such as acute rheumatic fever, rheumatic heart disease, and acute post-streptococcal glomerulonephritis. Deaths occur mostly in children, adolescents, and young adults in particular pregnant women in low- and middle-income countries. GAS strains are highly variable, and a GAS vaccine would need to overcome the issue of multiple strains. Several approaches have been used multivalent vaccines using N-terminal polypeptides of different M protein; conserved M protein vaccines with antigens from the conserved C-repete portion of the M protein; incorporation selected T- and B-cell epitopes from the C-repeat region in a synthetic polypeptide or shorter single minimal B-cell epitopes from this same region; and non-M protein approaches utilizing highly conserved motives of streptococcal C5a peptidase, GAS carbohydrate and streptococcal fibronectin-binding proteins. A GAS vaccine represents urgent need for this neglected disease and should therefore deserve the greatest attention of international organizations, donors, and vaccine manufacturers.

**Keywords:** Group A Streptococcus, Vaccines, Rheumatic heart disease, M protein, Low- and middle-income countries

Introduction

The group A *Streptococcus* (GAS) bacterium—is one of the top-10 infectious causes of deaths in the world [1]. According to the Global Burden of Disease 2010 study, this disease affects more than 34 million people, causing >345,000 deaths and 10 million disability-adjusted life years (DALYs) lost per year, almost all in low- and middle-income countries (LMIC) [2,3]. Most cases and deaths occur in children, adolescents, and young adults, depriving developing countries of many young people. In particular GAS, through the progressive cardiac condition known as rheumatic heart disease, causes substantial morbidity and mortality in pregnancy—with consequences for the expectant mother and her child.

GAS infections cause substantial worldwide morbidity and mortality—the combined mortality associated with rheumatic heart disease (RHD) and invasive GAS exceeds all other causes of infectious disease death excluding human immunodeficiency virus, tuberculosis, malaria, and *S. pneumonia* [3,4]. GAS nosology includes suppurative complications (pharyngitis, invasive GAS disease, and impetigo), non-suppurative (immune)
syndromes (acute rheumatic fever [ARF], RHD, and acute post-streptococcal glomerulonephritis), and toxin-mediated diseases such as scarlet fever and streptococcal toxic shock syndrome. A consequence of ARF, RHD is responsible for progressive valvular heart disease (VHD) such as mitral stenosis and mitral regurgitation, and in resource limited settings pregnant women suffer a marked increase in maternal morbidity and unfavorable fetal outcomes, which are related to severity of disease [5]. While RHD has become relatively rare in developed countries, it remains quite common in the developing world where 90% of all heart disorders in women of childbearing age are rheumatic in origin. Although accurate statistics are lacking, the estimated incidence of rheumatic fever in sub-Saharan Africa is approximately 13 cases per 100,000 per year based on clinical screening, while estimations between 21.5 and 30.4 per 1,000 have been reported for example in Cambodia and Mozambique when using echocardiographic screening. Besides its high prevalence, RHD in developing countries is characterized by the occurrence of severe VHD at a younger age than in developed countries [6,7]. ARF, the condition that precedes RHD, is believed to be a form of autoimmunity mediated by the similarity between the GAS coiled M protein and GAS polysaccharides to human myosin and between GAS cell wall and carbohydrates on heart muscle [8]. An alternate explanation is an interaction between M protein and type IV valvular collagen, inducing an immune response to collagen [8-10]. Antibodies found in ARF recognize valvular endothelium structural proteins such as collagen and elastin, and similarly also recognize N-acetylglucosamine and target dopamine receptors in the brain, possibly explaining the choreiform movements found in ARF [10,11].

GAS is a neglected disease of poverty and social injustice [12] that can however be prevented, using one of the cheapest and oldest antibiotics known—penicillin. The urgency for disease control is yet to be recognized at the highest level as it has been largely ignored by international organizations and other key stakeholders. Few affected countries have any coordinated strategy to implement control programs [13]. A GAS vaccine represents and urgent need for this neglected disease and should therefore deserve the greatest attention of international organizations, donors and vaccine manufacturers.

Epidemiology of GAS Infections and Implications for Vaccine Design

GAS strains are categorized by variation in the nucleotide sequence of the emm gene that encodes the variable M protein. Limited data on emm gene sequences are available from LMIC which, as with serotypes, seem to differ from high-income countries [14]. Africa and the Pacific are characterized by a wider diversity of emm types, and many of the common emm types found in industrialized countries are far less common (emm 1, 4, 6, and 12). One explanation provided is the high prevalence of GAS impetigo accompanied by large numbers of circulating GAS of multiple emm types that are readily transmitted and found in some resource-poor settings [15].

Like pneumococcal vaccines, a GAS vaccine would need to overcome the issue of multiple strains. Recent data from both whole M protein sequencing and multivalent vaccines suggest that M protein–based vaccines may evoke cross-protective antibodies that would broaden their potential efficacy and potentially mitigate any concerns about the emergence of new non-vaccine serotypes [16,17]. Some data are available for a vaccine (J8), which contains a common B-cell epitope of M protein whose structure seems highly conserved among GAS strains in a limited number of tropical settings [14]. Another 26-valent M protein-based vaccine, which contains N-terminal M peptides from 26 of the most common serotypes in North America, would likely provide good coverage in high-income countries, particularly United States, Canada, and Europe, but poor coverage in Africa and the Pacific and only intermediate coverage in Asia and the Middle East [18]. These data clearly have significant implications for multivalent M protein vaccines and are the subject of ongoing investigation.

GAS Vaccine Candidates

Several arguments suggest that a GAS vaccine is feasible [19,20]. GAS infection is common in childhood and uncommon in adulthood, suggesting that immunity is acquired through lifetime exposure. Longitudinal data showing the development of antibodies against common emm types in the United States support this hypothesis. Moreover, pre-clinical studies in animals show protection against challenge with GAS. Older studies of a GAS vaccine showed efficacy in a human challenge model against homologous S. pyogenes challenge [21].

Table 1 provides an overview of the vaccine candidates at various pre-clinical and clinical stages. To address strain diversity, several vaccine design approaches have been proposed using M protein– and non-M protein–based vaccine antigens.
M protein–based vaccines

The M protein is a coiled-coil protein consisting of 3 domains: an A-repeat of the N-terminal domain, which is highly variable and 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. A logical approach is the development of vaccines containing multiple serotypes, similar to multivalent pneumococcal polysaccharide or pneumococcal conjugate vaccines [19,22,23].

Twenty six-valent and 30-valent M protein vaccines

Short peptides from the N-terminal region of M proteins from multiple different GAS emm types are fused together in tandem to form larger vaccine-antigen polypeptides [16,24-26]. In humans, the 26-valent vaccine was shown to be safe and immunogenic [24]. Functional opsonic antibodies were induced against all emm types of GAS present in the vaccine. 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) [14]. This 26-valent vaccine was therefore 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 [19]. In preclinical studies, the 30-valent vaccine induced functional opsonic antibodies against all of the emm types represented in the vaccine [26]. Interestingly, the 30-valent vaccine antibodies cross-opsonized a proportion of non-vaccine emm types of GAS [26], suggesting that cross-protection may mitigate, to some 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 has now been initiated.

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 the conjugate version with diphtheria toxoid J8-DT and J14 vaccines contain shorter single minimal B-cell epitopes from this same region [27,28]. These vaccines comprise a minimal number of antigens, which may represent of benefit compared to the use of hypervariable M protein domains. Mouse studies, particularly of the J8-DT 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 [29,30]. In a murine model for infection that closely mimics human skin infection, J8-DT was able to protect against pyoderma and subsequent bacteremia caused by multiple GAS strains. The vaccine was however ineffective against a hypervirulent cluster of virulence responder/sensor mutant GAS strain; this correlated with the strain’s ability to degrade CXC chemokines, thereby preventing neutrophil chemotaxis. By combining J8-DT with an inactive form of the streptococcal CXC protease, *S. pyogenes* cell envelope proteinase, the combination vaccine was highly effective in blocking CXC chemokine degradation and permit opsonic antibodies to kill the bacteria [31]. Limited epidemiological data available for the J8 peptide indicate that its sequence is highly conserved among multiple emm types of GAS and across regions [32]. The J8 vaccine entered a Phase 1 trial in adult volunteers in 2013, but the results are pending. 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.

Non-M protein–based protein vaccines

Since several non-M proteins are highly conserved across
strains, another approach involves the use of conserved non-M protein GAS antigens for vaccine design by reverse vaccinology technology. Cell wall and secreted virulence factors, such as streptococcal C5a peptidase [33], GAS carbohydrate and streptococcal fibronectin-binding proteins are promising candidates although other approaches with GAS gene segments [34] tested in mice identified several known and new antigens including spy0516 (spyCEP), spy0167 (streptolysine O, SLO), and spy0269 (surface exclusion protein) [35]. When combined together, broad coverage of multiple GAS strains was achieved in CD1 mice. None of these approaches has entered yet clinical development.

Vaccine Safety

Nineteen clinical studies of GAS vaccines have been conducted involving thousands of subjects. Candidate vaccines have generally been safe and well tolerated [19]. However, one major concern was the possible induction of ARF through autoimmune mechanisms triggered by vaccine components [36]. Following some initial unfortunate experiences in humans, the U.S. Food and Drug Administration issued a regulation preventing licensure of vaccines containing GAS or its derivatives; a restriction removed in 2006. It is now believed that immunogenic regions of the M protein could be distinguished from those sections believed to be rheumatogenic. Subsequent studies of M protein vaccines that do not include M protein components cross-reacting with human tissues suggest that these vaccines are safe.

Vaccine Development Challenges

Table 2 summarizes the key scientific and strategic challenges to GAS vaccine development. The estimates of the global burden of disease, particularly the morbidity and mortality associated with ARF/RHD, acute post-streptococcal glomerulonephritis and invasive disease have been based on limited data from LMIC [37–40]. Deployment of vaccines into populations at highest risk for ARF/RHD will require additional surveillance and effectiveness studies to provide the data required to support policy decisions and effective implementation. There is also a considerable burden of GAS impetigo in tropical settings. There is a hypothetical link between GAS impetigo and ARF and RHD, although the pathogenetic link remains unknown [41]. Preventing skin infections would be highly desirable for a global GAS vaccine. However, little is known about immune protection against skin infections. Human immune correlates of protection against GAS infection are not clearly defined. The focus for decades has been on the protective role of M protein antibodies in animal models of infection. For most of the potential GAS vaccine antigens, there are few data supporting their role in protection against natural infection in humans. The small animal models currently used to assess potential vaccine efficacy are considered to be of limited predictive value and could lead to the exclusion of potentially efficacious antigens [42]. There are limited criteria for selection of antigens to include in combination vaccines that would optimize vaccine efficacy. Vaccines containing M protein peptides evoke opsonic antibodies that promote bactericidal killing in vitro. C5a peptidase induces antibodies that neutralize the enzyme [43], thus preventing the degradation of this potent chemo-attractant. Adhesins evoke antibodies that block bacterial adherence [44]. Many of the non-M protein common antigens do not have associated functional in vitro assays that could be applied in pre-clinical or clinical studies.

The complex global epidemiology of GAS infections poses a challenge to the development of a single vaccine for the en-

| Table 2. Key scientific and programmatic challenges to GAS vaccine development |
|---------------------------------------------------------------|
| **Scientific challenges**                                       |
| Limited disease burden data associated with acute rheumatic fever and rheumatic heart disease in low- and middle-income countries |
| Prevention of impetigo skin infection                          |
| Human immune correlates of protection against GAS infection not clearly defined |
| Small animal models for assessment of vaccine protection are of limited predictive value |
| Complex global epidemiology of GAS infections and variability of emm types pose a challenge to the development of a single vaccine for the entire world |
| **Strategic challenges**                                       |
| No roadmap developed with vaccine developers, researchers, vaccine manufacturers, global health policy makers and donors |
| Absence of industrial manufacturers and sufficient public/private funding |
| International collaborative effort and leadership gathering key stakeholders urgently needed |
| Strong advocacy effort needed by establishing and maintaining country-level dialogues to facilitate decision-making on GAS vaccine policy |

GAS, group A Streptococcus
tire world [24,25]. Vaccines containing amino-terminal peptides of M proteins may or may not provide sufficient serotype coverage, or durable serotype coverage, in areas of the world where ARF is highly prevalent.

**Urgent Need for a Roadmap**

World Health Organization (WHO) has made the development of GAS vaccines a priority. GAS vaccine development is mentioned in the Global Vaccine Action Plan 2010-2020, framework approved by the World Health Assembly in May 2012 to achieve the Decade of Vaccines vision by delivering universal access to immunization [45]. So far, no detailed plan has been developed with vaccine developers, researchers, vaccine manufacturers, global health policy makers, and donors. The strategic goal to accelerate the development and licensure of an effective and affordable GAS vaccine to prevent ARF and RHD as well as invasive infections in LMIC should receive utmost attention. Unfortunately, this effort seems, at least for the moment, precluded by the absence of industrial manufacturers and sufficient public/private funding. Funding for RHD prevention and GAS vaccines accounts for less than 0.1% of neglected tropical diseases global health funding [46].

The establishment of a roadmap implies an international collaborative effort, leadership, administrative and governance structures, gathering key stakeholders including public health and scientific experts on GAS disease pathogenicity and epidemiology, immunology, pre-clinical and clinical vaccinology, assay development, health policy and cost-effectiveness, vaccine manufacturers, private and public donors to secure sufficient funding for a comprehensive strategy. The WHO is positioned to provide leadership, and a funded product development partnership that is focused on the critical path to GAS vaccine development will be important to the success of this undertaking.

**Conclusion**

GAS infection and its devastating morbidity and mortality remain a ‘hidden’ public health disease borne disproportionately in LMIC that received little attention. The scale and impact of GAS infections and the preliminary evidence that a vaccine may be successful make cogent and compelling case for work on a GAS vaccine. Importantly, engagement of major vaccine manufacturer would facilitate the successful development of this product, and there is experience in developing vaccines for global health needs that have used product development partnerships funded by private philanthropy. To achieve these goals, a strong advocacy effort is needed by establishing and maintaining country-level dialogues to facilitate decision-making on GAS vaccine policy that would benefit to LMIC populations most at risk for GAS infection.

**ORCID**

Jean-Louis Excler  [http://orcid.org/0000-0002-6462-5101](http://orcid.org/0000-0002-6462-5101)

Jerome H. Kim  [http://orcid.org/0000-0003-0461-6438](http://orcid.org/0000-0003-0461-6438)

**References**

1. Carapetis JR, McDonald M, Wilson NJ. Acute rheumatic fever. Lancet 2005;366:155-68.
2. GBD 2013 DALYs and HALE Collaborators, Murray CJ, Barber RM, et al. Global, regional, and national disability-adjusted life years (DALYs) for 306 diseases and injuries and healthy life expectancy (HALE) for 188 countries, 1990-2013: quantifying the epidemiological transition. Lancet 2015;386:2145-91.
3. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2095-128.
4. Carapetis JR, Steer AC, Mulholland EK, Weber M. The global burden of group A streptococcal diseases. Lancet Infect Dis 2005;5:685-94.
5. Hameed A, Karaalp IS, Tummala PP, et al. The effect of valvular heart disease on maternal and fetal outcome of pregnancy. J Am Coll Cardiol 2001;37:893-9.
6. Nanna M, Stergiopoulos K. Pregnancy complicated by valvular heart disease: an update. J Am Heart Assoc 2014;3:e000712.
7. Zuhlke L, Engel ME, Karthikeyan G, et al. Characteristics, complications, and gaps in evidence-based interventions in rheumatic heart disease: the Global Rheumatic Heart Disease Registry (the REMEDY study). Eur Heart J 2015;36:1115-22a.
8. Martin WJ, Steer AC, Smeesters PR, et al. Post-infectious group A streptococcal autoimmune syndromes and the heart. Autoimmun Rev 2015;14:710-25.
9. Kaplan EL. Pathogenesis of acute rheumatic fever and rheumatic heart disease: evasive after half a century of clinical, epidemiological, and laboratory investigation. Heart
2005;91:3-4.
10. Tandon R, Sharma M, Chandrashekhar Y, Kotb M, Yacoub MH, Narula J. Revisiting the pathogenesis of rheumatic fever and carditis. Nat Rev Cardiol 2013;10:171-7.
11. Carapetis JR, Beaton A, Cunningham MW, et al. Acute rheumatic fever and rheumatic heart disease. Nat Rev Dis Primers 2016;2:15084.
12. Brown A, McDonald MI, Calma T. Rheumatic fever and social justice. Med J Aust 2007;186:557-8.
13. Carapetis JR. The stark reality of rheumatic heart disease. Eur Heart J 2015;36:1070-3.
14. Steer AC, Law I, Matatolu L, Beall BW, Carapetis JR. Global emm type distribution of group A streptococci: systematic review and implications for vaccine development. Lancet Infect Dis 2009;9:611-6.
15. Bessen DE, Carapetis JR, Beall B, et al. Contrasting molecular epidemiology of group A streptococci causing tropical and nontropical infections of the skin and throat. J Infect Dis 2000;182:1109-16.
16. Dale JB, Penfound TA, Chiang EY, Walton WJ. New 30-valent M protein-based vaccine evokes cross-opsonic antibodies against non-vaccine serotypes of group A streptococci. Vaccine 2011;29:8175-8.
17. Smeesters PR, Mardulyn P, Vergison A, Leplae R, Van Meleren L. Genetic diversity of group A Streptococcus M protein: implications for typing and vaccine development. Vaccine 2008;26:5835-42.
18. Hu MC, Walls MA, Stroop SD, Reddick MA, Beall B, Dale JB. Immunogenicity of a 26-valent group A streptococcal vaccine. Infect Immun 2002;70:2171-7.
19. Dale JB, Fischetti VA, Carapetis JR, et al. Group A streptococcal vaccines: paving a path for accelerated development. Vaccine 2013;31 Suppl 2:B216-22.
20. Steer AC, Carapetis JR, Dale JB, et al. Status of research and development of vaccines for Streptococcus pyogenes. Vaccine 2016;34:2953-8.
21. Fox EN, Waldman RH, Wittner MK, Mauceri AA, Dorfman A. Protective study with a group A streptococcal M protein vaccine. Infectivity challenge of human volunteers. J Clin Invest 1973;52:1885-92.
22. Kotloff KL. Streptococcus group A vaccines. In: Plotkin SA, Orenstein WA, Offit PA, editors. Vaccines. 6 ed. Philadelphia, PA: Saunders; 2012. p.1169-175.
23. Sheel M, Moreland NJ, Fraser JD, Carapetis J. Development of Group A streptococcal vaccines: an unmet global health need. Expert Rev Vaccines 2016;15:227-38.
24. McNeil SA, Halperin SA, Langley JM, et al. Safety and immunogenicity of 26-valent group A streptococcus vaccine in healthy adult volunteers. Clin Infect Dis 2005;41:1114-22.
25. Kotloff KL, Corretti M, Palmer K, et al. Safety and immunogenicity of a recombinant multivalent group A streptococcal vaccine in healthy adults: phase 1 trial. JAMA 2004;292:709-15.
26. Dale JB, Penfound TA, Tamboura B, et al. Potential coverage of a multivalent M protein-based group A streptococcal vaccine. Vaccine 2013;31:1576-81.
27. Batzloff M, Yan H, Davies M, Hartas J, Good M. Preclinical evaluation of a vaccine based on conserved region of M protein that prevents group A streptococcal infection. Indian J Med Res 2004;119 Suppl:104-7.
28. Guilherme L, Fae KC, Higa F, et al. Towards a vaccine against rheumatic fever. Clin Dev Immunol 2006;13:125-32.
29. Batzloff MR, Hartas J, Zeng W, Jackson DC, Good MF. Intranasal vaccination with a lipopeptide containing a conformationally constrained conserved minimal peptide, a universal T cell epitope, and a self-adjuvanting lipid protects mice from group A streptococcus challenge and reduces throat colonization. J Infect Dis 2006;194:325-30.
30. Batzloff MR, Hayman WA, Davies MR, et al. Protection against group A streptococcus by immunization with J8-diphtheria toxoid: contribution of J8- and diphtheria toxoid-specific antibodies to protection. J Infect Dis 2003;187:1598-608.
31. Pandey M, Langshaw E, Hartas J, Lam A, Batzloff MR, Good MF. A synthetic M protein peptide synergizes with a CXC chemokine protease to induce vaccine-mediated protection against virulent streptococcal pyoderma and bacteremia. J Immunol 2015;194:5915-25.
32. Steer AC, Magor G, Jenney AW, et al. emm and C-repeat region molecular typing of beta-hemolytic Streptococcus in a tropical country: implications for vaccine development. J Clin Microbiol 2009;47:2502-9.
33. Steer AC, Batzloff MR, Mulholland K, Carapetis JR. Group A streptococcal vaccines: facts versus fantasy. Curr Opin Infect Dis 2009;22:544-52.
34. Fritzler A, Senn BM, Minh DB, et al. Novel conserved group A streptococcal proteins identified by the antigenome technology as vaccine candidates for a non-M protein-based vaccine. Infect Immun 2010;78:4051-67.
35. Bensi G, Mora M, Tuscano G, et al. Multi high-throughput approach for highly selective identification of vaccine can-
didates: the Group A Streptococcus case. Mol Cell Proteomics 2012;11:M111.015693.
36. Massell BF, Honikman LH, Amezcuia J. Rheumatic fever following streptococcal vaccination: report of three cases. JAMA 1969;207:1115-9.
37. Marijon E, Ou P, Celermajer DS, et al. Prevalence of rheumatic heart disease detected by echocardiographic screening. N Engl J Med 2007;357:470-6.
38. Paar JA, Berrios NM, Rose JD, et al. Prevalence of rheumatic heart disease in children and young adults in Nicaragua. Am J Cardiol 2010;105:1809-14.
39. Beaton A, Okello E, Lwabi P, Mondo C, McCarter R, Sable C. Echocardiography screening for rheumatic heart disease in Ugandan schoolchildren. Circulation 2012;125:3127-32.
40. Steer AC, Jenney AW, Kado J, et al. Prospective surveillance of streptococcal sore throat in a tropical country. Pediatr Infect Dis J 2009;28:477-82.
41. Parks T, Smeesters PR, Steer AC. Streptococcal skin infection and rheumatic heart disease. Curr Opin Infect Dis 2012;25:145-53.
42. Penfound TA, Chiang EY, Ahmed EA, Dale JB. Protective efficacy of group A streptococcal vaccines containing type-specific and conserved M protein epitopes. Vaccine 2010;28:5017-22.
43. Cleary PP, Prahbu U, Dale JB, Wexler DE, Handley J. Streptococcal C5a peptidase is a highly specific endopeptidase. Infect Immun 1992;60:5219-23.
44. Hasty DL, Ofek I, Courtney HS, Doyle R. Multiple adhesins of streptococci. Infect Immun 1992;60:2147-52.
45. Steer A. Vaccine development for group A Streptococcus [Internet]. Geneva: World Health Organization; 2015 [cited 2016 Mar 1]. Available from: http://who.int/immunization/research/meetings_workshops/pdvc/en.
46. Remenyi B, Carapetis J, Wyber R, Taubert K, Mayosi BM; World Heart Federation. Position statement of the World Heart Federation on the prevention and control of rheumatic heart disease. Nat Rev Cardiol 2013;10:284-92.