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Challenges and responses in human vaccine development

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Human vaccine development remains challenging because of the highly sophisticated evasion mechanisms of pathogens for which vaccines are not yet available. Recent years have witnessed both successes and failures of novel vaccine design and the strength of iterative approaches is increasingly appreciated. These combine discovery of novel antigens, adjuvants and vectors in the preclinical stage with computational analyses of clinical data to accelerate vaccine design. Reverse and structural vaccinology have revealed novel antigen candidates and molecular immunology has led to the formulation of promising adjuvants. Gene expression profiles and immune parameters in patients, vaccinees and healthy controls have formed the basis for biosignatures that will provide guidelines for future vaccine design.

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Introduction

Vaccines are generally considered one of the most impressive success stories of medicine [1]. In all documented instances they have reduced the targeted infectious disease remarkably. The success of currently available vaccines is based on their ability to induce antibodies that block or neutralize infectious agents or their products [1]. Antibody-mediated protection is not sufficient in all infectious diseases. The major contagious killers of today for which we do not yet have vaccines are restrained to some extent not only by antibodies but also by cell-mediated immunity. There is currently a strong clinical development pipeline for novel vaccines against infectious diseases. These candidates have entered clinical trials based on evidence for protective efficacy in animal models, or, in the case of malaria and some other infections, in a human challenge model [2]. However, their development has proceeded without knowledge of the correlates of vaccine-induced protection and biomarkers or a biological signature (a custom-made compilation of different biomarkers) that can predict reduced disease incidence. Ideally, clinical trials generate sufficient data to allow definition of a biosignature that can be used to predict vaccine efficacy and safety of novel candidates [3,4] (see Figure 1).

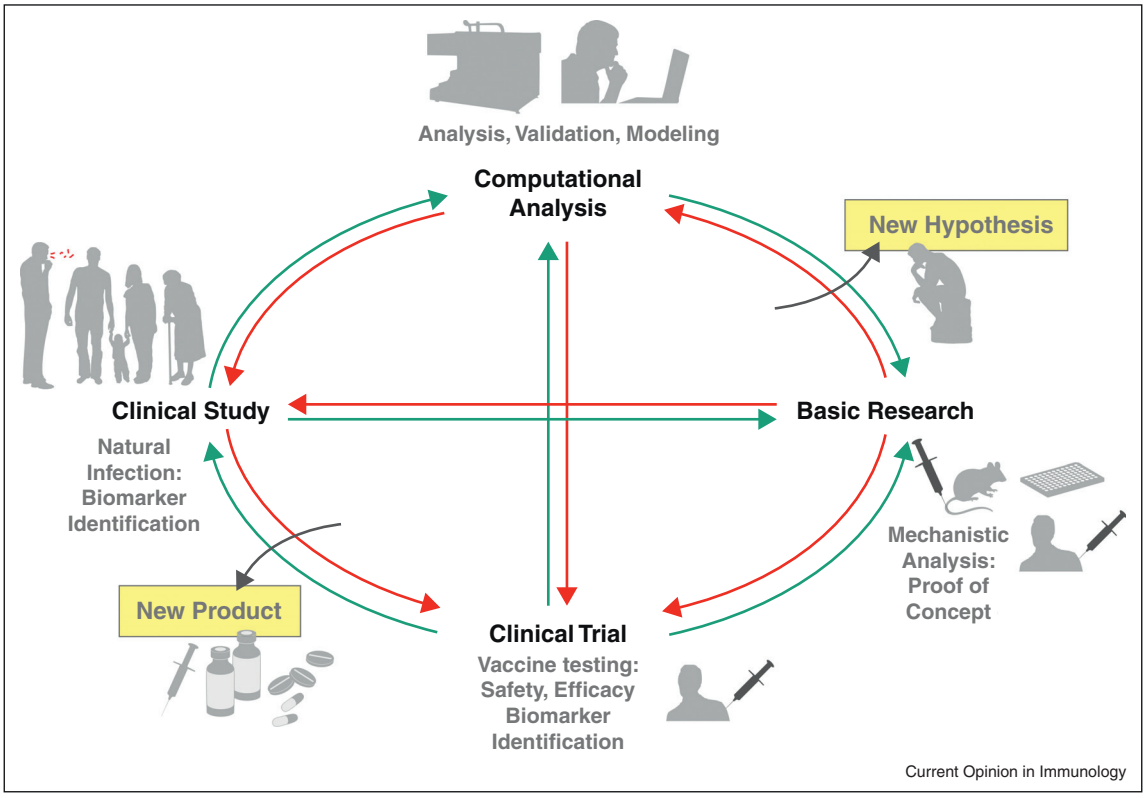
Challenges in human vaccine development

Challenges are faced at all stages of vaccine research and development (R&D) including: shortening the time of discovery of vaccine candidates, production and clinical development; ensuring that vaccines reproduce appropriately native antigens and that the immune response induced is of adequate strength and quality for efficient protection; and predicting at early stages safety and efficacy of vaccine candidates. Novel technologies developed in recent years can diminish these challenges: genomics and proteomics for the vaccine antigen discovery; structural biology to redesign broadly protective antigens; synthetic technologies to accelerate vaccine production; adjuvants and immunopotentiators to tailor the appropriate protective immune responses; systems biology and other computational methods to predict the safety and the efficacy of vaccines. In the following sections, examples of these technologies and their applications will be discussed.

Vaccine target discovery

The potential of vaccine target selection has increased significantly by the sequencing of whole microbial genomes. This approach, named 'reverse vaccinology', started from the MenB genome, for which conventional approaches had only limited success [5], and focused on those proteins predicted to be surface exposed or secreted. Candidate antigens were identified based on their ability to induce bactericidal antibodies, which were known to correlate with protection against disease. This procedure shortened the time of vaccine target discovery from decades to a few years, and allowed the identification of antigens which were completely unknown

Figure 1



'Life cycle' of modern vaccine development comprising iteration of basic research, vaccine trial, clinical study of natural infection and modeling by computational analysis. Vaccine development against HIV/AIDS, malaria and TB starts from basic research providing proof of concept for a novel vaccine candidate. Valid candidates are introduced into clinical trial, which will not only provide information about safety and efficacy but also reveal informative biosignatures. In parallel, clinical studies on naturally infected and diseased individuals can provide information about disease-related biosignatures. Computational analysis and modeling will generate new hypotheses, which can be validated by basic research. This iteration can lead to further vaccine improvement.

before. Three antigens plus outer membrane vesicles were tested in clinical trials in adults, adolescents and young children with satisfactory safety profile, high immunogenicity [6•], and the ability to cover the majority of MenB strains globally [7••]. Results of these studies allowed the European Medicine Agency to approve the first vaccine against MenB in 2012 (commercial name: Bexsero®) [8].

Reverse vaccinology is now applied to a wide variety of other important pathogens [9]. The concomitant use of genomics, bioinformatics, proteomics, and protein arrays can accelerate identification of vaccine targets and the subsequent vaccine development process [10].

The challenge of vaccine preparation

A major component of the MenB vaccine, the factor H binding protein (fHbp), induces strong protective antibodies. This antigen, however, has more than 500 amino acid variants [11] that do not induce cross-protective immunity. The ideal candidate antigen would be able to induce protective antibodies against all the allelic variants of the fHbp. Elucidation of the 3D structure of the antigen revealed protective epitopes in each of the variants. Chimeric molecules were designed to contain the identified epitopes in correct conformation. Many of them elicited broadly protective antibodies and one has been selected for further studies [12••].

Structure-based information is being exploited for rational design of vaccines against viruses. Information about the structure of the F protein of respiratory syncytial virus (RSV) allowed design of a novel F protein in which the protective antigenic site could be stabilized. Immunization of mice and monkeys with the F protein containing the stabilized neutralizing site induced profound levels of protective antibodies [13••]. Similarly, the cryo-electron microscopy reconstruction and structural model of the human immunodeficiency virus (HIV) gp140 trimer complexed with a broadly neutralizing antibody has now provided insights into the trimer assembly, the interactions between gp140 and gp41, and the

interaction with the CD4 binding site and with neutralizing antibodies. This paves the way for the development of structure-based broadly protective vaccines against HIV [14••,15••].

Purified proteins are poorly immunogenic and require the use of adjuvants for the induction of effective responses. Toll-like receptor (TLR) agonists are promising vaccine adjuvants and a TLR-4 agonist has been approved in a vaccine against human papilloma virus [16]. Other adjuvants are emulsions containing a core of biodegradable lipids. MF59 [17] and AS03 [18] have been approved with pandemic and seasonal influenza vaccines (MF59) or with pandemic influenza vaccines (AS03). These adjuvants not only increase antibody titers, they also modify the quality of the antibody response in terms of both breadth of the strains recognized and the repertoire of B-cell epitopes, as exemplified by MF59 with H5N1 and seasonal subunit vaccines [19]. The MF59 adjuvant drives the antibody response towards the HA1 subunit of the hemagglutinin (HA), and in particular to the receptor binding site, and much less to the HA2 moiety, suggesting a role of these phenomena in the enhanced efficacy and effectiveness of the MF59-adjuvanted vaccines in infants and the elderly [20•,21••,22••].

Vaccines against influenza pandemic must become available prior to the pandemic. During the last H1N1 pandemic, mass vaccination was implemented only when transmission already declined [23]. A prompt response to a pandemic has become feasible due to *in vitro* synthesis of genomes [24]. As a prerequisite, sequences of the viruses isolated must be provided as soon as they are available. A combination of enzymatic, cell-free assembly techniques with enzymatic error correction allows rapid and accurate synthesis of genes that are then used to transfect cell lines qualified for vaccine manufacturing, from which viruses can be rescued for vaccine preparation. This process takes as few as five days with the recovery of synthetic viruses antigenically identical to the wild-type viruses. This synthetic process has been successfully used to generate influenza strains including seasonal as well as H1N1, H3N2, H5N1 and H7N9 [25••].

Predictive biosignatures of vaccine safety and efficacy

As discussed below, some recent late stage clinical vaccine trials have unexpectedly shown either a complete [26,27•] or partial [28] lack of efficacy, or raised safety concerns [26]. Biosignatures of safety and efficacy could be used in pre-clinical studies to prioritize available candidates, and in early clinical development to avoid later failure. Parameters may be identified retrospectively [27•], but post hoc analyses may identify misleading chance correlations. An alternative approach is to use vaccines in translational studies to dissect-out mechanisms of reactogenicity and efficacy, and such an approach was taken in an HIV vaccine efficacy trial [29]. This can be applied to human and animal models, in which whole blood or separated cell population gene expression, multiplex cytokine responses, as well as cellular and humoral immune responses are integrated.

Application of biosignatures to predict influenza vaccine efficacy

With influenza vaccines, older adults have suboptimal responses, and using a systems approach Furman *et al.* [30] identified an age-related antibody epitope that predicted the response, possibly by inhibitory memory $CD4^+$ T cells and apoptotic pathways. Observations from limited human samples can be explored in animal models, and apoptosis-deficient mice did exhibit poor serologic responses [30]. Franco *et al.* found a correlation between influenza antibody responses and apoptosis gene expression [31]. In this case T cell-mediated apoptosis of target cells, together with mechanisms involved in antigen presentation, membrane trafficking and intracellular transport were also identified. Tan *et al.* [32] observed that upregulation of genes related to proliferation and immunoglobulin genes segregated high and low vaccine responders. These correlations would have been missed by conventional, single-gene level approaches. Querec *et al.* [33••] also found that whereas molecules in the integrated stress response predicted antiviral CD8 T cell responses to influenza vaccines, an independent signature involving B cells predicted neutralizing antibody responses.

Harmonization of systems vaccinology approaches

A challenge for the systems approach is the diversity of strategies for integrating and analyzing extremely large sets of high-throughput data. Small numbers of samples, repeated or single measures of heterogenous parameters and outcomes, may also lead to contradictory correlations. Harmonization of analytical tools, reporting of data, and experimental conditions is needed [34•]. Liquet *et al.* [35•] combined a multilevel analysis with the usual multivariate approach to analyze data. Whereas the discriminant multilevel analysis identified subsets of related genes, an integrative multilevel analysis revealed clusters of highly correlated genes and cytokines. This highlights that the re-analysis of data from a single trial using different strategies can yield significantly greater information. Different vaccine antigens or adjuvant systems are likely to induce different innate and adaptive responses, making extrapolation from different trials challenging. Obermoser *et al.* [36••] detected significant differences in genes responding to a protein subunit influenza vaccine versus a conjugate polysaccharide pneumococcal vaccine. Differences have also been described between live and non-living vaccines [37•]. Applying 'modules' of responder genes identified in inflammatory diseases, two significant challenges arise

[38]: firstly the selection of related genes to group into a module based on infection or inflammatory states may be misleading for vaccine trials. Different gene expression patterns of inflammation were found in mice and humans caused by trauma or burn injury [39••]. Secondly, gene modules complicate comparison between published results if the modules contain different genes [37•].

Systems vaccinology for biosignatures of reactogenicity

A practical application of a systems approach was the retrospective identification of underlying factors responsible for an increase in febrile reactions associated with a specific trivalent influenza vaccine [40••]. The investigators integrated human, animal, cell line and primary cell culture experiments with gene profiles and cytokine readouts. A combination of the introduction of three entirely new influenza strains, and differences in the manufacturing processes were identified as the probable cause of the unexpected reactogenicity. This opens the possibility to screen vaccine antigen combinations for reactogenicity using an 'omic' approach [34•].

Update on HIV vaccine trials

Improved biomedical strategies are slowly impacting the HIV epidemic, as shown in recent trends of lower infection rates in some at-risk populations [41]. However, an end to the HIV pandemic will likely require widespread immunization with an effective vaccine. After more than two decades of clinical evaluation, no single vaccine regimen has demonstrated sufficient efficacy to advance to licensure [42,43]. Concepts tested in efficacy trials include recombinant bivalent HIV-1 gp120 monomers formulated in alum designed to induce binding and neutralizing antibodies (VAXGEN003 and VAXGEN004 trials) [44,45], recombinant adenovirus (Ad) serotype 5 (Ad5) vectors encoding HIV-1 genes to induce antiviral CD8$^+$ T cells (Step and Phambili trials) [26,46], and prime-boost regimens to induce antibodies and T cells (RV144 and HVTN 505 trials) [47••,48].

The low-level efficacy (31%) demonstrated in the RV144 Thai trial with the canarypox vector and bivalent gp120 in alum prime–boost regimen suggests that a protective vaccine against HIV acquisition may be possible [48]. Enhancing the level and duration of protection seen in the RV144 trial is a major goal in clinical vaccine development, and public–private partnerships to accelerate product development and testing in regions with the highest HIV incidence rates are underway (Table 1). Current plans include a licensure trial in Sub-Saharan Africa using canarypox and subunit envelope protein immunogens that express antigens from HIV-1 clade C, the most common subtype circulating in Sub-Saharan Africa, as well as a series of phase IIb research studies to assess new pox vectors and other immunogens expressing HIV-1 subtype C antigens that may improve upon the RV144 regimen and/or potentially induce protection by alternative mechanisms. The design of these studies will efficiently determine vaccine safety, efficacy and correlates of immunity to better inform which regimens to advance to licensure trials and to guide new vaccine designs.

The HIV vaccine field faced disappointment earlier this year with the failure of the HVTN 505 phase IIb trial [47••], which evaluated a DNA prime-recombinant Ad5 boost HIV vaccine regimen to assess efficacy in reducing either the rate of HIV-1 acquisition or post-infection viremia in 2504 at-risk US men or transgender women who have sex with men. Unlike previous Ad5/HIV vaccine studies, both vaccines in this regimen encoded *env* genes, and induced both strong HIV-specific CD4$^+$ (62%) and CD8$^+$ T cell (64%) response rates and anti-Env IgG (100%) to the vaccine strain envelopes and preferentially to the

Table 1

Accelerating HIV vaccine development building on the RV144 trial outcome and alternative strategies

Strategy	Designs
Post-RV144	Phase III licensure trial in Sub-Saharan Africa evaluating RV144 type regimen (ALVAC + bivalent gp120) using HIV-1 clade C gene inserts and envelope protein. Improved pox virus vectors with prime-boost strategies in phase IIb trials in Sub-Saharan Africa.
T cell immunity	CMV vectors to induce potent and persistent effector memory T cells. Mosaic adenovirus and pox vectors to improve the breadth and depth of T-cell epitopes recognized.
Broad-neutralizing antibodies	Structure-based immunogen designs resembling viral spike and/or distinct neutralizing epitopes. B-cell ontogeny designs to guide specific clonal lineages, circumvent autoimmunity, and select for B-cell repertoires.
Immunoprophylaxis	Mixtures of HIV broadly-reactive mAbs. Genetic immunization with vectors encoding broadly-reactive nAbs.
Improve durability	Alternative adjuvant-protein formulations. Optimized prime-boost regimens, including DNA plasmid as a prime before the protein boost.

Notes: CMV, cytomegalovirus CMV; HIV, human immunodeficiency virus; mAbs, monoclonal antibodies; nAbs, neutralizing antibodies.

gp41 region. Of note, anti-Env V1-V2 IgG response rates were substantially lower than observed in the RV144 trial, and whether this finding is relevant to the lack of efficacy in the HVTN 505 trial remains unclear.

Although no significant difference in infection rates between the vaccine and placebo groups was observed in the HVTN 505 trial, 41 infections occurred in the vaccine and 31 in the placebo group. Taken together with greater infection rates in the Step and Phambili trials testing the Merck Ad5/HIV trivalent vaccine, these data raise concerns about safety and have prompted longitudinal follow up of HVTN 505 subjects, a meta-analysis of infection rates in Ad5-vectored HIV-1 vaccinees, and ongoing experimental studies to understand mechanisms that may contribute to potential enhancement of infection. Because Ad-specific $CD4^+$ T cells induced following natural Ad infections and Ad5 vector immunizations recognize epitopes across multiple Ad serotypes [49•], one hypothesis is that intercurrent Ad infections, commonly detected in mucosal sampling [50], may activate Ad-specific T cells recognizing cross-reactive epitopes and increase the number of HIV-1 target cells in the mucosa. Results from these studies may have implications in future decisions concerning use of Ad5 and Ad vectors of other serotypes as vaccines for HIV and other pathogens in HIV-1-uninfected study participants at risk for HIV-1 infection.

Update on TB vaccine trials

TB vaccine design is in the unique situation that a comparator already exists. As part of the Expanded Program on Immunisation (EPI), the vaccine Bacille Calmette-Guérin (BCG) has been administered more than 4 billion times. It protects against severe extrapulmonary forms of infant TB but not against pulmonary TB in any age group, which represents the vast majority of cases and main source of spreading [51]. Hence, vaccine candidates aimed at replacing BCG need to perform better with respect to efficacy and/or safety than BCG.

The TB vaccine portfolio comprises recombinant live vaccines for prime instead of BCG, as well as recombinant viral vectors expressing *Mycobacterium tuberculosis* (*Mtb*) antigens and adjuvant formulations of *Mtb* antigens for heterologous boost following BCG [51–54]. The most advanced viable recombinant vaccine in clinical trial, VPM1002, is a recombinant BCG (Table 2), which will soon complete a phase IIa trial in infants. Vakzine Projekt Management (VPM) sponsored two phase I trials, which demonstrated safety and immunogenicity of VPM1002 [55]. Adjuvanted protein vaccine candidates against TB have mostly been developed by Statens Serum Institut (SSI) and by GlaxoSmithKline (GSK) (Table 2). One of the first vaccines to enter clinical trial, the H1 vaccine from SSI, comprising a fusion protein of two antigens in the adjuvant Intercell (IC)31, was found to induce strong immune responses as late as 30 weeks after administration [56]. GSK have comprehensively analyzed their protein–adjuvant vaccine candidate M72, by varying the formulation of the vaccine. Although the early formulation M72F protein in adjuvant system (AS)02 proved both safe and immunogenic [52,55,57,58], it was further improved to become the final construct M72 in AS01,

Table 2

News from preventive tuberculosis vaccine candidates in clinical trials

Candidate	Construct	Status of clinical development	Type	Target population
VPM1002	rBCGΔureC::hly	Phase I completed (safe and immunogenic), phase IIa ongoing	Viable rBCG for preexposure	Prime vaccine for infants, to replace BCG
MVA85A	MVA expressing Rv3804 (Ag85A)	Phase IIb completed (safe and immunogenic in target population, no efficacy)	Viral vector for preexposure	Heterologous booster vaccine for BCG-vaccinated infants
H1	Rv1886 (Ag85B) + Rv3875 (ESAT-6) fusion protein in adjuvant IC31 or CAF01	Phase I completed (safe and immunogenic)	Protein adjuvant for preexposure	Heterologous booster vaccine for BCG-vaccinated individuals
MVA85A	MVA expressing Rv3804 (Ag85A)	Phase IIb ongoing (safe and immunogenic in target population)	Viral vector for preexposure	Heterologous booster vaccine for BCG-vaccinated adolescents and adults
Ad5HUAG85A	Human Ad 5 expressing Rv3804 (Ag85A)	Phase I completed (safe and immunogenic)	Viral vector for preexposure	Heterologous booster vaccine for BCG-vaccinated individuals
M72	Rv1196 + Rv0125 fusion protein in adjuvant AS01	Phase IIa completed (safe and immunogenic in target populations)	Protein adjuvant for preexposure and postexposure	Heterologous booster vaccine for BCG-vaccinated individuals

Notes: Ad, adenovirus; Ag, antigen; AS01E, liposomal-based, surface-active saponin plus Toll-like receptor (TLR)-4 ligand; BCG, Bacille Calmette–Guérin; CAF01, liposome-based lipoid MINCLE ligand; IC31, cationic antimicrobial peptide plus Toll-like receptor (TLR)-9 ligand; Mtb, *Mycobacterium tuberculosis*; MVA, modified Vaccinia Ankara; rBCG, recombinant BCG.

which is safe and immunogenic in individuals with or without prior exposure to *Mtb* [59]. Notably, high frequencies of multifunctional T cells were induced by M72 in AS01 [60]. This vaccine qualifies for pre-exposure and post-exposure administration.

Currently, two types of viral vectors are being exploited for TB vaccination: Ad as potent CD8 T cell inducer [61], and modified Vaccinia Ankara (MVA) virus, which preferentially stimulates CD4 T cells [27•] (Table 2). A phase I trial with an Ad5 vector expressing an antigen of *Mtb* not only revealed its safety and immunogenicity, but also assessed effects of preexisting antibodies directed against the vector [61]. Pre-existing antibodies could cause rapid elimination of the Ad5-based vaccine thus reducing its efficacy. Although the study did not support this apprehension, these and other side effects of Ad-based vaccines remain a concern (see also above).

Disappointingly, the phase IIb trial, with the MVA85A vaccine based on MVA-expressing an antigen of *Mtb* [27•], given as a heterologous booster on BCG prime to infants, revealed no evidence of superior protection [27•]. However, the vaccine proved safe and the study, comprising nearly 3000 infants, proved the feasibility of performing large-scale TB vaccine trials in Sub-Saharan Africa [27•]. In parallel, this vaccine is currently undergoing a phase IIb trial in adults after results of a phase IIa trial demonstrated its safety and immunogenicity in individuals independent of their HIV and *Mtb* exposure status [62].

TB biosignatures

Only 10% of those who are infected with *Mtb* develop disease during their lifetime. The vast majority remain healthy but latently infected. Biosignatures that can predict risk of disease would be helpful for stratification of study participants with high TB risk for vaccine trials, leading to reduced size and duration of phase IIb/III clinical trials. Data from vaccine trials, comprising a sufficiently high proportion of protected versus nonprotected individuals, should be harnessed for defining correlates of vaccine-induced protection. This approach, which has been spear-headed in the arena of HIV vaccination [42,43,48,63••], could soon be realized in the area of TB with increasing vaccine candidates entering phase IIb trials.

In parallel, the availability of the BCG vaccine provides an opportunity to analyze vaccine-induced protection. A longitudinal study exploiting canonical immune markers in BCG-vaccinated infants, who developed TB, failed to identify a BCG-induced immune correlate of protection against TB [64•]. Intriguingly, this study questioned the long-held belief that IFN-γ suffices as biomarker of protection.

Ongoing longitudinal studies are comparing blood transcriptome profiles in household contacts of recently diagnosed TB cases, with the aim to identify a biosignature that can predict risk of TB. One ongoing study is analyzing >4000 household contacts of newly diagnosed TB cases at different sites in Africa (http://www.biomarkers-for-tb.net/); the other ongoing study has enrolled >6000 adolescents from a highly endemic area in South Africa [65,66].

Clinical malaria vaccine development

Malaria remains a major public health threat [67]. A malaria vaccine can play an important role in controlling the disease in endemic regions. Hope has been raised by the RTS,S recombinant sporozoite vaccine given in AS01. Many trials have shown its immunogenicity, tolerability, and efficacy in various age groups [68]. Recently, results have been reported from a large multicentric phase III trial carried out at 11 centers in 7 African countries, with more than 15 000 enrolled children. In the first part of the study involving 6000 children (5–17 months old), the efficacy of 3 doses of the RTS,S/AS01 vaccine against clinical malaria during the 14 months after the first dose varied between 50% and 55%, and between 45% and 47% against severe malaria [69•]. In the second part of the study in infants 6–12 weeks of age receiving the vaccine together with the EPI vaccines, the efficacy against clinical malaria and against severe malaria dropped to about 30% or 26%, respectively [70]. Several reasons may account for this drop including: the younger age of these children as compared to those of the first part of the study, the potential interference between RTS,S/AS01 and vaccines of the EPI, the pre-existing immune status towards the hepatitis B vaccine, the possibility that efficacy varied from one site to another.

The possibility that the efficacy of the RTS,S/AS01 vaccine may vary with the intensity of malaria transmission has been evoked by another study conducted in Kenya and Tanzania [71•]. In this smaller study (about 220 5–17 month-old children) the efficacy against clinical malaria was about 30% over a period of 4 years, with 43% during the first year, and 0.4% during the fourth year. The efficacy was higher in children with a lower malaria-exposure index (45%) than in children with a higher malaria exposure index (16%) [71•]. These studies suggest that on the one hand a recombinant subunit vaccine such as RTS,S/AS01 provides some efficacy against clinical and severe malaria, and on the other hand further improvements are necessary to achieve long-term protection by large-scale vaccination in EPI-vaccinated young children in highly endemic areas.

An alternative approach has been reported recently, namely the immunization with non-replicating live attenuated sporozoites given intravenously to healthy volunteers followed by challenge through the bites of infectious

mosquitoes [72••]. All six volunteers receiving the highest dose of sporozoites (1.35×10^5) five times, and 6 out of 9 receiving the same dose four times, did not develop any signs of malaria. Protected individuals had higher titers of anti-sporozoite antibodies and had higher frequencies of sporozoite-specific $CD4^+$ and $CD8^+$ T cells in the peripheral blood, especially $CD8^+$ T cells producing IFN-γ, [72••]. Further studies will be needed to validate these findings in larger sample sizes and to determine the duration of protection. The feasibility of five intravenous injections of live-attenuated sporozoites for mass vaccination campaigns in malaria-endemic areas may be difficult, and strategies to simplify this regimen yet retain its protective response will be required.

Concluding remarks

Although, development of new vaccines still has a long way to go, recent advances in antigen identification, adjuvant development and vector design have brought forward the preclinical vaccine R&D pipeline. These advancements are complemented by recent accomplishments in human vaccinology including rational design of biosignatures, which, by predicting risk of disease as well as vaccine safety and efficacy, can be harnessed for future improvement of novel vaccine candidates. Thus, an iterative combination between wet-lab, *in silico* analyses, and clinical studies represents the best way to accelerate development of vaccines needed for control of major infectious diseases (Figure 1). Even though the first generation of vaccine candidates may not provide satisfactory efficacy, comparative analyses of host responses in protected versus unprotected study participants will reveal information for further improvement of next-generation vaccines. To harness novel information arising from data analysis of ongoing vaccine trials adoptive vaccine protocols would be best suited.

Conflict of interest

SHEK is coinventor of the rBCG preventive tuberculosis vaccine VPM1002. GDG is full-time employee of Novartis Vaccines, Siena, Italy. DJML and MJM declare no conflicts of interest.

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