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Minireview

New developments and concepts related to biomarker application to vaccines

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Summary

This minireview will provide a perspective on new developments and concepts related to biomarker applications for vaccines. In the context of preventive vaccines, biomarkers have the potential to predict adverse events in select subjects due to differences in genetic make-up/underlying medical conditions or to predict effectiveness (good versus poor response). When expanding them to therapeutic vaccines, their utility in identification of patients most likely to respond favourably (or avoid potentially negative effects of treatment) becomes self-explanatory. Despite the progress made so far on dissection of various pathways of biological significance in humans, there is still plenty to unravel about the mysteries related to the quantitative and qualitative aspects of the human host response. This review will provide a focused overview of new concepts and developments in the field of vaccine biomarkers including (i) vaccine-dependent signatures predicting subject response and safety, (ii) predicting therapeutic vaccine efficacy in chronic diseases, (iii) exploring the genetic make-up of the host that may modulate subject-specific adverse events or affect the quality of immune responses, and (iv) the topic of volunteer stratification as a result of biomarker screening (e.g. for therapeutic vaccines but also potentially for preventive vaccines) or as a reflection of an effort to compare select groups (e.g. vaccinated subjects versus patients recovering from infection) to enable the discovery of clinically relevant biomarkers for preventive vaccines.

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Introduction

Existing model for bridging preclinical studies to clinical trials

The standard approach to drug development involves research studies in animals prior to testing the most efficacious and safest candidates in a stepwise approach in human volunteers and then patients. Clearly, challenges remain when attempting to translate data from animal studies into human studies but at least they serve as a starting point for hypothesis testing. For small molecules and biologics, efforts have been undertaken in the pharmaceutical industry to bridge this gap between preclinical data and human clinical trials by studying biomarkers related to an improved understanding of human disease mechanisms. This approach has been labelled 'clinical sciences', 'translational sciences' or 'translational medicine' and has found homes in both academic centres and industry. However, the application of an approach tailored to small molecules and biologics needs to be adapted, when targeting vaccines, since the majority of them will be preventive vaccines administered to otherwise healthy individuals. Except for the specific case of therapeutic vaccines, there is not a clinical phenotype in a patient due to an active disease mechanism that can be targeted for biomarker studies when developing a preventive vaccine.

Adapting translational studies to the challenges facing vaccines

Since vaccines are immunogens, vaccine biomarker efforts tend to focus on basic immune responses (antibodies, T cells) post-vaccination but it is becoming clear that the significance of the measured signals is difficult to ascertain given the heterogeneity of the human immune response. Multiple variables can complicate the application of a standard biomarker approach based on immune

Table 1. Distinguishing potential markers.

Term	Definition	Reference
Biomarker	A characteristic that is objectively measured and evaluated an indicator of normal biological processes, pathogenic processes or pharmacological response to a therapeutic intervention	Biomarkers Definition Working Group (2001)
Surrogate marker	A laboratory measurement or physical sign that is used in therapeutic trials as a substitute for a clinically meaningful end-point that is a direct measure of how a patient feels, functions or survives and is expected to predict the effect of therapy	Temple (1999)
Correlate of protection	A laboratory parameter that has been shown, from adequate and well-controlled trials, to be associated with protection from clinical disease	FDA Guidance for Industry (1997)

components, which include differences in the following: quality of the immune response at various ages, approaches when targeting vaccines that prevent disease versus those that treat disease, and the genetic make-up of the subject (and their environment) in modulating the type of immune response. Recent efforts have begun to benchmark the results of these studies against natural infection or against vaccines that are highly immunogenic and efficacious in a majority of the population. Such comparisons provide a pragmatic approach to deciphering the relevance of data generated before, during and after exposure, and set a standard on which to base experimental biomarkers – either they should mimic the host response triggered by infection as closely as possible or they should mimic the response typical for a licensed vaccine proven to be highly immunogenic and efficacious. In both cases, the goals are to reliably predict negative and positive outcomes (e.g. safety versus adverse events; protection versus disease). Such an approach has been labelled 'systems biology' which requires filtering data with biological significance from many diverse sources and hierarchical levels and assimilating this information in a manner that is not readily apparent on examination of the individual components in isolation (Zak and Aderem, 2009). As touched upon in this review, the establishment of biomarkers related to vaccine efficacy and safety will hinge in the short term on the ability of systems biology to make sense of the immune response in relation to vaccination and in the mid-term on the ability to unravel the mysteries of innate and adaptive immunity that will be critical for advancing personalized medicine.

The main goal of current early stage vaccine trials is to demonstrate safety and immunogenicity. In later stage trials, efficacy in preventing disease can be measured and provides the opportunity to develop a biomarker that can serve as a 'correlate of protection'. Examples of correlates of protection include those for hepatitis B (10 mIU ml^{-1} of anti-hepatitis B surface antigen antibodies) and tetanus (0.1 IU ml^{-1} of anti-tetanus toxin antibodies) (Newell *et al*., 1971; Jack *et al*., 1999; Centers for Disease Control and Prevention, 2002). Table 1 distinguishes between the various markers (e.g. biomarker, surrogate marker and correlate of protection). With these definitions in mind, it is hoped that the reader will better appreciate the obstacles and successes related to discovering and applying the broad spectrum of biomarkers to vaccine clinical development.

Signatures for vaccine response and safety

It was almost a decade ago that gene expression signatures were being applied to predict prognosis in cancer (Van 't Veer *et al*., 2002) and seem to suggest that a biomarker technology facilitating clinical decision making and opening the door to patient-specific personalized medicine was around the corner. For the successful application of genomic signatures to vaccine development, however, the complexity of the innate and adaptive immune responses will need to be dissected and these responses to vaccines and natural infection will need to be mapped carefully integrating well-defined expression signatures. It is with this better understanding of the heterogeneity of the adaptive immune response that correlates of protective immunity can be defined, as comprehensively discussed in a perspective paper (Haining and Wherry, 2010) on the concept that gene expression signatures can serve as surrogates for biological phenotypes of cell populations within the immune system. In addition, early signalling of the innate immune system (within minutes to hours after vaccination) has the potential to yield valuable information about the eventual performance of the vaccine, particularly for those vaccines containing adjuvants designed to specifically stimulate the innate immune system. Furthermore, genomic signatures have the potential to correlate with important clinical phenotypes such as immunological protection and may enable the discovery of true correlates of protective immunity enabling the generation of broadly protective vaccines against diseases such as influenza virus, malaria, human immunodeficiency virus, hepatitis C virus, malaria and tuberculosis (TB). In the following sections, the potential for biomarkers will be explored using the examples of yellow fever and human papilloma virus (HPV).

Yellow fever

A fascinating study in the field of vaccinology (Querec *et al*., 2009) has provided encouragement for the use of gene signatures as a biomarker and explored the gene expression profiles in PBMC obtained from 15 subjects receiving the yellow fever vaccine (YFV-17D). What is important to note about this elegant study is that it targeted one of the most effective vaccines ever made that has been administered to more than 600 million people and is capable of inducing a broad spectrum of immune responses with a single injection. Utilizing a systems biology approach, investigators were able to decipher a pattern of genes correlating with the magnitude of the YFV-induced CD8 T-cell response that was distinct from the signature correlating with antibody titre and both signatures were validated in an independent trial ($n = 10$ subjects) using microarray analysis at a similar time point. These signatures predicted response rate with 80–90% accuracy and predicted, in as little as a few days after vaccination, the subsequent development of protective levels of antibody (surrogate of protection). This application of systems biology could be expanded to include various groups of people (e.g. responders versus non-responders) to explore potential age-, gender- and genetic-dependent effects on vaccine effectiveness or to study the role that different types of vaccines (non-adjuvanted versus adjuvanted, live versus subunit or effective versus non-effective) may play in these populations. Such an approach related to predicting vaccine efficacy could be extended to vaccine safety and may set the framework for comparing novel vaccines to benchmark vaccines known for their safety track record.

Human papilloma virus (HPV)

Another study explored the gene expression patterns in PBMC from 17 subjects receiving the HPV-16 L1 virus-like particles (versus four placebo recipients) before vaccination and 1 month after the second immunization to explore potential early predictors of long-term efficacy and decipher the innate and adaptive immune responses (Garcia-Pineres *et al*., 2009). Although antibodies are thought to be responsible for HPV vaccine-mediated protection, the gene signature demonstrated vaccine-induced stimulation of both the cellular and innate arms of immunity illustrating how biomarker efforts may help to unravel the complexities, roles and relationships among the various arms of the immune system to vaccination. Correlations with neutralizing antibody titres were found for three genes in particular (cyclin D2, galectin and interleukin-1 receptor antagonist) that may be predictive for prolonged antibody responses. While these finding are interesting, they should be interpreted with caution for their clinical relevance as subjects in this study were immunized with a monovalent, non-adjuvanted HPV-16 L1VLP, in contrast to the bi- and tetra-valent licensed vaccines currently in use. Furthermore, the predictive value related to gene signatures on immunogenicity and long-term outcome need to be validated in larger, independent clinical studies.

It is currently expected that a single vaccine should protect against a particular infectious disease in all people, with few exceptions (e.g. the need for an adjuvanted influenza vaccine for the elderly). However, it is likely that certain individuals will be predisposed to respond differently to a vaccine. One well-established example is the relatively poor responsiveness to the hepatitis B vaccine in people with certain HLA haplotypes (Milich and Leroux-Roels, 2003) or polymorphisms in cytokine-related genes (Chen *et al*., 2011). Hence, as technologies develop, it may become possible to tailor vaccines to particular populations, based on their genetic make-up. Both of the above mentioned studies highlight the promise that gene expression signatures may hold for preventive vaccines in predicting responses and eventually safety provided that such signatures are extensively validated. The next section will provide a contrast with therapeutic vaccines for which similar challenge exists related to immune biomarker discovery coupled with identifying biomarkers targeting the disease process itself (e.g. cancer).

Biomarkers for therapeutic vaccines against chronic diseases

The World Health Organization (WHO) defines chronic diseases as ‘diseases of long duration and generally slow progression. Chronic diseases, such as heart disease, stroke, cancer, chronic respiratory diseases, and diabetes are by far the leading cause of mortality in the world, representing 60% of all deaths’ (WHO, 2011). In the USA, seven out of 10 deaths annually are from the top three chronic diseases indicated previously, which account for more than 50% of deaths (Kung *et al*., 2008), and arthritis is the most common cause of disability (Centers for Disease Control and Prevention, 2006). Given the increased activity of vaccine efforts in cancer, this will also likely be a focus of biomarker developments in chronic disease. Despite the induction of specific T cells against tumour antigens, active immunization therapies targeting various cancers have had a low rate of clinical response, due in part to the advanced stage of disease and immunocompromised status of the patients treated. In addition, often there is a lack of understanding of the important antigens to target with a vaccine strategy. Thus, to increase the chances of success, it will be important to understand the individual’s tumour microenvironment and overall ‘receptiveness’ to treatment in order to have a

high-quality patient population and set the stage for the identification of biological correlates predictive of anti-tumour responses. In the following sections, the potential for biomarkers will be explored using the examples of melanoma, prostate cancer and breast cancer.

Melanoma

The first example illustrates the efforts related to candidate biomarkers for melanoma vaccines (recently reviewed by Gajewski *et al*., 2010). Gene expression profiling of pre-treatment tumour biopsies identified a cluster of transcripts predicting outcome to treatment in melanoma metastases when categorized into 'inflamed' and 'non-inflamed' subsets. The 'non-inflamed' tumours are characterized by high expression of vascular markers as well as macrophages and fibroblasts and low levels of innate inflammation with poor chemokine production and scarcity of lymphocytes (suggesting that poor effector cell trafficking is responsible for tumour escape). The 'inflamed' phenotype is dominated by innate immune signals, chemokines necessary for T-cell recruitment and variable presence of T cells but also contains important immune suppressive mechanisms (e.g. increased expression of indoleamine-2,3-dioxygenase, PD-L1, FoxP3 and decreased/absent expression of co-stimulatory ligands B7-1 and B7-2) which through dominant effects of negative regulation may afford tumour escape. These biomarkers, when combined with preclinical experiments on tumour escape mechanisms, could guide therapeutic strategies tailored to the particular tumour microenvironment. A personalized therapy approach could be envisioned with patients having 'non-inflamed' tumours being administered systemic vaccination and local application of inflammatory signals to promote T-cell recruitment while those with the 'inflamed' tumours additionally receiving blockade of inhibitory pathways mediated by PD-1.

Prostate cancer

Prostate cancer is another field in which biomarkers have been exploited in the development of therapeutic interventions. A recent review (Detchokul and Frauman, 2011) provides an overview of recent clinical trials targeting biomarkers in advanced prostate cancer and includes 19 new therapeutic agents summarized by the biomarker being targeted and therapeutic approaches. The biomarkers being targeted by vaccines include the following: (i) granulocyte-macrophage colony-stimulating factor (GM-CSF), which is based on prolonging specific prostate cancer immunity using genetically modified prostate cancer cells expressing GM-CSF via dendritic cell differentiation and proliferation (GVAX vaccine), (ii) the combination of prostate-specific antigen, prostate-specific membrane antigen, prostate stem cell antigen and six-transmembrane epithelial antigen of the prostate delivered via mRNA to direct anti-tumour activity (CV9103 vaccine), and (iii) interleukin-2 (IL-2) and interferon-γ (IFN-γ) to facilitate specific anti-tumour responses by enhancing antigen-presenting cells and T-cell responses to tumour antigens using allogeneic prostate cancer cell lines with recombinant human IL-2 and IFN-γ (IL-2-IFNγ-secreting tumour vaccine).

Breast cancer

Breast cancer has also been an active field of vaccine development and illustrates how a biomarker (HER2/neu expression level) correlates with response to the E75 peptide vaccine (Benavides *et al*., 2009) in disease-free, node-positive and high-risk, node-negative breast cancer patients. HER2/neu FISH expression (low-expresser versus overexpresser) and immunohistochemistry status revealed a more robust immune response to the vaccine in low-expressing patients who also had decreased mortality. The intriguing aspect of this classic biomarker approach (receptor status) was how expression status of this receptor could have diverse effects on the efficacy of a monoclonal antibody approach (trastuzumab) versus a vaccine approach. Trastuzumab has been shown to be less effective in low-expressers of HER2/neu (and thus indicated for overexpressers) while the E75 peptide vaccine has a greater response rate in patients with low expression of this biomarker suggesting a role for immunologic tolerance related to HER2/neu overexpression. While this section and the previous illustrate how a normal subject's response to vaccination or a patient's response to disease, respectively, can be exploited to develop biomarkers predicting efficacy, the next section will explore how to capitalize on the host's genetic make-up for predicting vaccine safety and response.

Genetic make-up of host as predictor of safety and response

Subject-dependent safety

The application of bench side research using serum obtained from select subjects experiencing adverse events during vaccine clinical trials may be a way of identifying predictive biomarkers in this age of personalized medicine. One study illustrating the potential of such an effort focused on the prediction of fever risk after the smallpox vaccine based on genetic predisposition (Stanley *et al*., 2007). This call-back study performed genotyping and sequencing of DNA obtained from whole blood of 346 subjects who received the smallpox vaccine (Dryvax). Certain haplotypes in the interleukin-1 (IL-1)

gene complex and in interleukin-18 (IL-18) were predictive of fever after vaccination while a haplotype in the interleukin-4 gene was associated with reduced development of fever. It would be interesting to explore, if validated, whether these biomarkers could potentially identify individuals at risk of fever after receipt of other live virus vaccines.

Another study applied a systems biology approach to identify biomarkers associated with adverse events following smallpox vaccination (Reif *et al*., 2009). The investigators used high-dimension genetic and proteomic data to shed light on the mechanisms underlying the development of adverse events in subjects following smallpox vaccination. The vaccination was considered successful in all of the patients in the study (all developed clinically observable pustules at the immunization site) and thus the adverse events reported were attributed to components of the immune system interacting in a manner which promoted excessive or prolonged immune stimulation. Sixty-one subjects were examined on five visits in the first month post vaccination for adverse events with collection of blood for cytokine measurements before vaccination and during the 5- to 7-day post-vaccination evaluation period. Systemic adverse events being considered were fever, generalized rash and lymphadenopathy. Besides the 108 proteomic variables being assessed (serum cytokines), genetic data were also gathered to examine 1442 single-nucleotide polymorphisms to model adverse event risk. Utilizing the Random Forest method and combining information from previous studies on adverse events related to smallpox vaccination, the investigators developed a step-wise decision tree based on three proteomic variables (intracellular adhesion molecule 1 [CD54], interleukin-10 and colony-stimulating factor 3), as well as a genetic polymorphism in the interleukin-4 cytokine gene which, altogether, correctly classified 89% of individuals. The elegance of such an approach is that it visualizes adverse events as a complex interaction among multiple factors including genetics (the genetic data included SNPs in and around genes having various immunological functions) and proteomics (cytokine arrays designed to capture variations in important systemic mediators).

Clearly, such types of assessments or models need to be evaluated for their reproducibility so that targets for screening a wider population can be reliably identified. One could envision more informative prospective studies that collect different cell types, sera, DNA and RNA at various points leading up to the time of immunization (to establish normal variance from baseline) and shortly afterwards (e.g. 1, 6 and 12 h post vaccine) that might allow a better correlation of adverse events with changes in the subjects' immune system in response to immunization. One needs to keep in mind, also, that genetic analyses may not allow more than to estimate different degrees of risk for reactions among a population (unlikely to identify those with no risk or near 100% risk).

Subject-dependent vaccine responses

Regarding the role of host genetics and the vaccine response, one could envision similar genotyping and sequencing efforts on DNA obtained from whole blood of responders and non-responders to highly efficacious vaccines (e.g. yellow fever) to see whether there was a genotype associated with a good versus poor immune response. While this search for a genetic profile that would predict the quantity or quality of an immune response against a specific vaccine or family of vaccines or even all vaccines seems unrealistic today, this may be a hypothesis that can be tested with the advent of new technologies. A recent publication in the field of epigenetics (Feinberg *et al*., 2010) illustrated how non-sequence-based modification in DNA methylation of the epigenome may affect normal phenotypes and predisposition to disease. Using comprehensive array-based relative methylation analysis, they discovered variably methylated regions of which half were stable over an average of 11 years and defined a personalized epigenomic signature. In their study, four of these variably methylated regions showed covariation with body mass index at separate study visits and were located near genes implicated in regulating body weight or diabetes. With greater insight into the human immune response, such approaches may identify similar personalized epigenomic signatures that capture individuals likely to respond or not respond to vaccines based on signatures pertaining to subtle defects in the innate and adaptive immune systems.

Role for biomarkers during clinical development

Implication for therapeutic vaccines

Biomarkers have a role in influencing the selection or stratification of subjects or patients being considered for clinical trials and, in the case of therapeutic vaccines, are particularly important since they may enable enrolment of patients with early disease. In Alzheimer's disease, a potential biomarker (Aβ40) has been discovered that may facilitate earlier diagnosis of Alzheimer's disease (Gao *et al*., 2010). The Aβ peptides (1–40) and (1–42) are cleavage products of the amyloid precursor protein which aggregate and form insoluble plaques in the brain of patients afflicted with Alzheimer's disease. The presence of the Aβ40 oligomer in patients with early stages of disease would make them attractive candidates for inclusion in vaccine studies thus affording patients and vaccine interventions the best chance for success prior to the

formation of amyloid plaques and the occurrence of irreversible neuronal damage.

Implication for preventive vaccines

One could also potentially stratify volunteers in preventive vaccine trials in order to discover biomarkers relevant for disease prophylaxis. For example, volunteers could be stratified into those that were willing to receive a licensed vaccine versus those who did not want to be vaccinated to gain a better understanding of differences in the immune response to vaccination versus natural infection in those not being vaccinated. In addition, in controlled situations human volunteers have been challenged with live virus (e.g. influenza, norovirus) to study the protective effects of vaccine versus placebo. Such approaches could enable the discovery of 'bioprocess markers' which is a term being coined in this minireview to indicate the immune response signature seen in patients that become infected. Such studies could be informative in that they may provide proteomic (e.g. cytokine) and genetic (e.g. haplotype) data points from subjects promptly recovering from infection (in contrast to those progressing to chronic or severe disease) with the goal of developing a similar vaccine profile. The following discussions illustrate how biomarkers discovered in previous studies on infected patients may be informative for vaccine clinical studies.

'Bioprocess' markers in TB

A recent paper (Berry *et al*., 2010) illustrates how a transcriptional signature in human TB could be exploited as a 'bioprocess marker' for stratifying subjects in clinical trials. Their studies focused on the problem related to the lack of a licensed test to detect latent TB which carries a 10% lifetime risk of developing active TB. They identified a whole-blood 393 transcript signature (dominated by an interferon-inducible signature containing increased abundance of plasma cell transcripts) specific for active TB that reverted to healthy control signatures post-treatment and was also specific for a subset of subjects with latent TB. One could envision the use of such patient-related biomarker information to facilitate targeted preventive vaccine therapy in patients with latent TB. In this regard, two recent papers have shown promising preclinical results for vaccines targeting latency antigens in *Mycobacterium tuberculosis* (Bertholet *et al*., 2010; Aagaard *et al*., 2011).

'Bioprocess' markers in invasive candidiasis

Along the lines of learning from infection, an investigation used serological proteome analysis as a global profiling technique to assess reactivity of antibodies from 45 patients with invasive candidiasis (IC) to the whole soluble *Candida* proteome to discover a prognostic signature (Pitarch *et al*., 2011). The investigators developed an IC prognosis score using a five-IgG antibody-reactivity signature obtained with supervised discriminant analysis which was able to discriminate IC patients at high risk for death from those at low risk within 2 months. These were associated with good prognosis and protective patterns (Met6p, Hsp90p and Pgk1p – putative virulence factors and anti-apoptosis mediators) or poor prognosis and risk patterns (Ssb1p and Gap1p/Tdh3p – proapoptotic mediators). The information related to the protective and non-protective patterns could be informative for guiding the desired type of profile to be elicited by vaccines against IC.

'Bioprocess' markers in viral challenge studies

Another spin on this 'infectious' approach is the development of biomarkers in the setting of 'challenge' studies. In a study (Statnikov *et al*., 2010) re-analysing the gene expression profile reported in a prior publication (Zaas *et al*., 2009), the authors developed a molecular signature that allowed for an accurate differentiation (0.85 AUC) between uninfected subjects prior to immunization ($n = 56$) compared with subjects remaining asymptomatic after challenge with rhinovirus, respiratory syncytial virus or influenza A ($n = 30$). Using an improved data-analytical protocol, they developed a compact molecular signature distinguishing these two groups which was comprised of genes involved in the host immune response (e.g. eukaryotic translation initiation factor 2, zinc finger protein 91, RNA-binding motif protein 3 and CD24 molecule). This findings may shed light on understanding the molecular factors that enable some subjects to combat infection more effectively (e.g. remain asymptomatic after exposure), as well as provide targets enabling the development of more effective vaccines.

Lessons to be learned from convalescent plasma used therapeutically

Finally, there should be something said about the value of convalescent plasma used therapeutically in infected patients and the potential for de-convoluting what makes convalescent plasma effective and to model vaccine responses on this 'bioprocess'. A group of investigators in Hong Kong, China published results indicating how convalescent plasma treatment reduced mortality in patients with severe pandemic influenza (H1N1) 2009 virus infection (Hung *et al*., 2011). Of the 93 patients with severe H1N1 requiring intensive care recruited for this study, 20 patients received plasma treatment obtained by apheresis in patients recovering from H1N1 2009 infection (neutralizing antibody titre of > 1:160). The mortality was lower in

patients receiving convalescent plasma (20%) compared with those who refused plasma therapy (54%). Plasma therapy was associated with significantly lower day 3, 5 and 7 viral load and lower interleukin-6, interleukin-10 and tumour necrosis factor levels. While the aetiology of the improved outcome afforded by plasma therapy remains unclear, the investigators proposed that neutralizing and non-neutralizing antibody in the plasma could facilitate virus entry into Fc-receptor-bearing antigen-presenting cells such as macrophages and B lymphocytes. These host cells would not permit growth of the influenza virus and remain functionally intact and be able to increase viral antigen processing to augment T lymphocyte-mediated adaptive immune responses. These data and hypotheses illustrate how patient stratification to various treatment modalities may enable us to uncover bio (process) markers of relevance to vaccine development.

Concluding remarks

The above discussions highlight the advances in molecular biology and immunology for discovering biomarkers relevant to vaccines, and two large NIH-funded initiatives are underway for vaccine biomarkers including an extramural effort called Human Immune Phenotyping Consortium (http://www.hhs.gov/news/press/2010pres/08/20100811a.html) and an intramural effort called Center for Human Immunology and Inflammation (http://www.nhlbi.nih.gov/resources/chi/). Studies such as these and others in the future will need to provide greater insight into the quantity and quality of antibodies needed for protection against disease and carriage. The availability of adequate biomarkers will facilitate selection of individuals or patients who would most benefit from vaccination thereby enabling the design of adequately powered trials with a reduced number of enrolled volunteers and considerably more modest overall costs. Until then, vaccine effectiveness will need to be determined through large phase III trials with clinical outcomes rather than cost-effective biomarkers. The hopes offered by these preliminary studies will become a reality with a multidisciplinary approach to biomarker development. This will necessitate the involvement of scientists dissecting immunological pathways, physicians assessing human safety and efficacy, and trial managers designing human studies containing the relevant target population, an adequately powered sample size, and the inclusion of nested exploratory studies utilizing an appropriate number of samples obtained at immunologically relevant time points. However, a recent perspective (Koscielny, 2010) has methodically illustrated why biomarker signatures (e.g. gene array) have failed to fulfil their promise in the clinic – emphasizing the critical need for adequate validations in independent clinical trials to prevent overestimation of a signature's performance. A key message from this perspective included the fact that knowledge of how to read the messages in the genome is elusive and that without a breakthrough in the way data are analysed, there is a risk of collecting data sufficient to explain everything but unable to predict anything.

What also needs to be capitalized on is the wealth of information in other fields such as biotherapeutics (e.g. monoclonal antibody treatment for diseases) which may be of relevance to vaccines (Flower, 2009). Ironically for vaccines, biotherapeutics is a field where immunogenicity is highly undesirable as it can lead to reduced efficacy of the treatment, can manifest as allergic reactions (swelling, skin eruption, fever, anaphylaxis), or lead to potentially life-threatening symptoms when antibody mediated neutralization of the therapeutic protein cross-reacts with an endogenous protein of vital function. For this reason, a field of de-immunization has been developed to reduce the immunogenicity of biotherapeutic agents and targets the immunogenicity conferred by T-cell epitopes and that conferred by antibody-mediated epitopes. The experiences and biomarkers deemed to be negative for this field may be paradoxically sought after as desirable for improving the immunogenicity in response to vaccination. With the average cost of discovering and developing a vaccine approaching US $800 million and typically requiring more than a decade to reach licensure (Serdobova and Kieny, 2006; Global Malaria Action Plan, 2008), it is clear that biomarkers have the potential to play an increasingly important role in accelerating both the developmental time frame and likelihood of success of future vaccines.

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