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Vaccine Design: Emerging Concepts and Renewed Optimism

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Abstract

Arguably, vaccination represents the single most effective medical intervention ever developed. Yet, vaccines have failed to provide any or adequate protection against some of the most significant global diseases. The pathogens responsible for these vaccine-recalcitrant diseases have properties that allow them to evade immune surveillance and misdirect or eliminate the immune response. However, genomic and systems biology tools, novel adjuvants and delivery systems, and refined molecular insight into protective immunity have started to redefine the landscape, and results from recent efficacy trials of HIV and malaria vaccines have instilled hope that another golden age of vaccines may be on the horizon.

Introduction

Traditionally, vaccines have been prepared by isolating an infectious agent, attenuating or inactivating it, and presenting it to the human immune system. This approach has proven extremely efficient against pathogens with relatively low antigen variability such as smallpox, polio, measles, mumps and rubella. However, pathogens with complex immune evasion strategies and the ability to evolve rapidly call for novel and more sophisticated strategies, which have begun to yield new and highly efficacious vaccines (Table 1).

Since the time of Jenner, Koch, and Pasteur, we have attained a detailed molecular understanding of how pathogens interact with the human immune system, permitting molecular identification of particular antigens involved in effective pathogen recognition by our immune system. These antigens can be produced, modified, combined and presented in novel ways to achieve more focused and controlled immune responses. These innovative means of antigen presentation include liposomes [1], virus-derived vectors [2] or even self-amplifying RNA encapsulated in liposomes [3]. Epitope level control over the immune response is now being achieved by grafting epitopes onto protein scaffolds [4]. By sequentially administering diverse immunogens, scientists are currently formulating strategies to elicit certain lineages of protective and potently neutralizing antibodies against HIV [5]. Whole genome sequencing is being used to predict antigens of larger pathogens such as bacteria and protozoa, and to maximize coverage of diverse isolates by enabling vaccination with composite, or mosaic antigens. Systematic approaches to predict protective

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immune responses from transcription and expression profiles of cohorts of genes involved in early immune responses are also being used to guide and accelerate vaccine development.

Collectively, these novel approaches leverage high throughput sequencing and bioinformatics to identify promising antigens, molecular adjuvants to target specific innate cellular receptors and drive desired inflammatory responses, advanced DNA, RNA, and protein delivery systems, and are beginning to exploit detailed molecular insights gained from studying protective immune responses generated in the context of natural infection, and a greater understanding of naïve immune repertoires. This review will discuss the state of the art approaches and technologies being explored to facilitate vaccine development (Table 2).

Reverse vaccinology, systems biology and personalized medicine

The development of next-generation sequencing and proteomic techniques has enabled researchers to mine entire microbial genomes, transcriptomes and proteomes to identify novel candidate immunogens. This reverse vaccinology approach has enjoyed considerable success in the past decade, beginning with *Neisseria meningitides*, and continuing with *Streptococcus pneumonia*, pathogenic *Escherichia coli*, and antibiotic resistant *Staphylococcus aureus* [6, 7]. These and other pathogenic, multi-drug resistant microbial strains pose a major public health threat. The emergence of antibiotic resistance and the slowing development of novel antibiotics may combine to expand the market for vaccines, which is likely to increase the impact of efficient approaches. As described in Figure 1, the reverse vacinology strategy utilizes genome informatics as opposed to traditional biochemical and genetic tools to identify antigen targets with promising characteristics such as surface expression, secretion, and/or high conservation, which can then be empirically tested and screened as candidate immunogens. Similarly, proteomic tools have been utilized to identify surface antigens at high throughput by coupling proteolytic digestion of surface proteins with mass spectrometric protein fragment detection [8].

With decreases in sequencing costs outperforming Moore's law, the number of available genome sequences is rapidly increasing. This development allows for the screening of diverse but related pathogen sequences and identification of shared candidate immunogens, as has been successfully shown for Group B *Streptococci*[9]. It further allows for a comparison of commensal and pathogenic microbe genomes and identification of pathogen-specific candidate immunogens, as has been shown for *E. coli*[10]. With continued decreases in sequencing costs and improved bioinformatic tools, it can be expected that entire gut microbiomes will be screened for new vaccine targets in the future. Candidate immunogens contained in microbiomes that are specific to certain geographic areas could be identified and microbiome sequencing before and after vaccination could reveal immune escape mechanisms on a population scale. Furthermore, larger genomes of eukaryotic pathogens such as parasitic protozoa could be explored, as has been outlined by Goodswen and colleagues[11].

Systems biology approaches, primarily transcriptional profiling of vaccinated subjects, have been implemented with the goal of deciphering the complex network of molecular interactions involved in protective and non-protective immune responses. High-throughput protein arrays and flow cytometry of B and T cell responses to *Chlamydia trachomatis* in infected subjects has likewise yielded partially protective vaccine candidates [12]. Beyond elucidation of the basic biology of immune networks, the chief advantages of these methods lie in their ability to identify possible mechanistic correlates and early predictors of vaccine efficacy [13-16], which could greatly reduce clinical trial costs and facilitate the implementation of adaptive trial designs [17, 18]. Molecules and pathways identified by

systems approaches could be targeted via designed vaccines which could then be iteratively refined by analyzing early predictors of vaccine efficacy (Figure 1) [19].

From a personalized medicine perspective, the future may hold vaccines tailored to specific recipient characteristics, such as age, gender, or genotype. As the immune system develops, its ability to respond to certain types of antigens changes: for example, in infants, thymus-independent responses against microbial polysaccharide antigens are underdeveloped and influenced by maternal antibodies, requiring polysaccharide-based immunogens to be conjugated to protein carriers[20]. Over the course of a life span, the responsiveness of the immune systems to vaccines waxes and then wanes [21], and in elderly populations, higher doses or different adjuvants may be required for optimal protection. From personal genome sequences, either SNP associations, or MHC polymorphisms that affect the presentation of peptides contained in immunogens to T cells, or B cell receptor (BCR) sequences associated with the development of neutralizing antibodies, and hence responsiveness to a given vaccine could be determined. Indeed, successes in personalized vaccination in the setting of cancer immunotherapy demonstrate the potential of such customized treatments[22].

Addressing innate immune cells

The induction of a strong and sustained adaptive immune response against designed subunit vaccine immunogens depends on inflammatory and activating signals provided by innate immune cells such as macrophages, dendritic cells and mast cells. These innate immune cells can be stimulated by adjuvant systems, which provide appropriate danger signals and facilitate antigen delivery for robust immune responses. Traditionally, oil emulsions and highly charged aluminum salts are used, which may destabilize protein immunogens upon adsorption and likely alter the structure of conformational epitopes, as has been shown for model proteins such as lysozyme and albumin[23, 24].

Novel adjuvants may include activating ligands for receptors on innate immune cells such as toll-like receptors (TLRs) [25], C-type Lectin receptors [26], RIG-I-like receptors [27], or moieties to target other cellular receptors such as DEC-205 or FcgRs [28-31]. For example, supplementation of a water in oil emulsion containing multimeric hemagglutinin H5 with the TLR4 ligand glucopyranosyl lipid accelerated priming of protective immune responses, induced antibody class switching and helped mice to recover faster from infection-associated weight loss [32].

Following the success of the human papilloma virus vaccine, which consists of capsid proteins that spontaneously assemble into virus-like particles (VLPs) [33], many recent adjuvant systems have been based on nanoparticles, such as VLPs [34], liposomes [35], phages [36] or synthetic particles [37], which can present antigens in a more native form and orientation than traditional adjuvants (Figure 2). Additional advantages include the ability to spatially cluster antigen in close proximity along with appropriate immune signaling molecules, providing the opportunity to leverage avid interactions and provide co-stimulatory signals. Polylactide-co-glycolide nanoparticles containing different protein antigens were found to induce more robust germinal centers, antibody class switching and high neutralizing antibody titers when supplemented with nanoparticles containing TLR4 and TLR7 ligands [38]. Similar nanoparticles supplemented with the TLR3 ligand polyIC were likewise found to enhance vaccine potency [39]. In both studies, the long persistence of nanoparticles seemed to be required for the enhanced immune response observed. Nanoparticle-based adjuvant systems can also deliver immunogen payloads to cells. For example, cross-linked multilayered liposomes have been shown to stably encapsulate a model vaccine antigen and release it in presence of endolysosomal lipases [40].

Geall and colleagues encapsulated RNA molecules encoding a respiratory syncytial virus (RSV) antigen and alphavirus-derived genes encoding RNA replication machinery in liposomes for delivery. The RNA was autonomously amplified and translated in the cytosol. This approach yielded comparable neutralizing antibody titers and protection against RSV infection as other state of the art particles or electroporated DNA [41]. The potentially improved safety and manufacturing profile of these liposomes over other gene delivery vectors which have demonstrated promising efficacy in some settings, such as virus replicon particles [42], or viral vectors, [43, 44] make them an exciting development.

In general, these and other novel nanoparticle-based adjuvant systems may overcome the limited ability of traditional adjuvants to correctly present the conformational epitopes of complex, oligomeric immunogens. Features of live attenuated viral vaccines such as cell infection and protein synthesis in infected cells may be mimicked through inclusion of genetic polymers. To allow for large-scale vaccinations, it will be important to ensure robust and economical production of nanoparticle-based vaccines, and significant effort has been invested in developing methods for rapid and scalable production of recombinant vaccines, in some cases by using cell-free expression systems[22, 45, 46].

Addressing T cells

Cytotoxic $CD8^+$ T cells (CTLs) can play a critical role in clearing infected cells and controlling pathogen load during chronic infection. However, rapidly evolving pathogens can evade CTL recognition by mutating single residues or swapping expression of variant antigens. Hence, ideal CTL epitopes are located in regions of conserved sequence, where mutation would confer a fitness penalty. The linear nature of CTL epitopes allows bioinformatics tools to be utilized both for optimizing overlap between vaccine and naturally circulating sequences [47, 48], but also for comparing these epitopes for fit into common MHC alleles [49, 50]. These *in silico* methods are readily complemented with experimental immunoproteomic approaches [51, 52].

For example, a composite antigen, termed a mosaic (Figure 3), was assembled to contain conserved T cell epitopes from the HIV-1 gag protein of viral clades B and C. When encoded in an adenoviral vector and delivered to human peripheral blood mononuclear cells, CTL epitopes from both clades were correctly processed and able to induce CTL-mediated cell lysis [53]. Such mosaic antigens can be combined to result in more complete coverage of possible CTL epitopes, as has been shown for mono-, bi- and trivalent HIV-1 Env mosaics administered to rhesus monkeys [54]. The trivalent vaccine was also found to induce a superior neutralizing antibody profile [48]. Mosaic vaccines have also been designed to achieve broader protection against filoviruses such as Ebola and Marburg, as well as chlamydia, and hepatitis C [55-57].

Notably, T cell responses are typically directed against a small number of dominant peptide epitopes among a large number of possible epitopes[58], presumably due to expression, proteolytic processing and MHC presentation biases. To enhance T cell responses, attempts have been made to identify and specifically include these dominant epitopes in DNA vaccines. However, this approach does not take epitope functional constraints or mutant fitness penalties into account. Dominant T cell epitopes may even be pathogenic and elicit T cells that cross-react with related endogenous epitopes, leading to autoimmune reactions[59]. From a vaccine design perspective, such pathogenic epitopes could be identified using bioinformatics tools, and eliminated to reduce the risk of unanticipated side effects.

A general limitation of T cell vaccines is their relatively low immunogenicity, which is related to their delivery to target cells for subsequent intracellular protein processing and

loading on MHC molecules[64]. To improve immunogenicity, better delivery vehicles and means to amplify protein production in vivo may be required. To this end, liposomes containing autonomously amplifying RNA discussed previously may represent a promising development.

Beyond their role in cellular immunity, T cells are critical for mounting a robust humoral immune response. Follicular helper $CD4^+$ T cells (T_{FH}) are essential for the affinity maturation of antibodies as they provide co-stimulatory signals to B cells in germinal centers [60]. Accordingly, vaccines designed to promote germinal center formation and T_{FH} cell expansion may elicit antibodies of higher affinity and sustained titer, providing better long-term protection. Indeed, a number of recent studies have investigated these and other linkages [61-63]. Thus, maximizing the synergy between arms of immunity, and increasing our understanding of the intersection between functionally critical antigens and immune repertoires are strategies that hold a great deal of promise.

Addressing B cells

Antibodies provide the correlate of protection for most vaccine-induced protective immune responses[65]. Difficult vaccine targets including influenza, HIV, and malaria are characterized by their ability to evade the humoral immune response by modifying their surface antigens through mutation, recombination, variant switching, and glycan-masking. Nevertheless, in recent years a number of conserved epitopes have been identified for these pathogens, and some of them are targets for naturally occurring broadly neutralizing antibodies (bnAbs) (Figure 4). Structure-based design [66] has demonstrated promise in a number of settings. Study of antigenic variation, crystal structures, and development of functionally inert mutants of the *N. meningitides* factor H-binding protein has shown positive results[67]. Preparations of toxoids that have been altered recombinantly rather than chemically treated to render them inert represent additional successes of structure-based design[68]. A designed chimeric pili binding protein from Group B *Streptococcus*[69], and recombinant respiratory syncytial virus F protein in its post-fusion conformation[70] have shown excellent profiles in animal models. Currently, even more finely grained methods are being applied to translate knowledge of significant epitopes into candidate vaccines designed to drive the generation of epitope-specific antibodies with broad cross-reactivity and neutralization profiles.

The malaria parasite *P. falciparum* shuffles expression of multiple gene alleles of surface proteins with redundant function but varying sequence as an immune escape mechanism. Promising work is underway in identifying conserved features among these variant antigens to generate vaccine candidates [71, 72]. However, the most exciting results in malaria vaccinology have come from vaccinating with the dominant surface antigen of *P. falciparum* sporozoites, the circumsporozoite protein 1 (CSP-1), which is rarely a target of antibodies raised during natural infection. A fusion protein of CSP-1 and hepatitis-B surface antigen has demonstrated partial protection in infants and children in a clinical phase 3 trial and may become the first approved malaria vaccine (Mosquirix, or RTS,S) [35, 73].

Similar to the mosaic antigens used to induce broad T cell immunity, chimeric protein antigens designed to represent broad pathogen diversity have also been engineered to generate broadly protective antibodies to *Neisseria meningitides* [74]. Notable success has been achieved in pursuit of a universal influenza vaccine using epitope-specific strategies rather than diversity-coverage [75]. Most efforts have revolved around the conserved HA2 stem domain, which has been engineered to stably exist in a pre-fusion conformation [76], spliced into a helix-turn-helix scaffold protein and displayed on a VLP [77], administered as a sequence of variants [78], and used to generate a peptide immunogen [79]. These

candidate vaccines have elicited cross-reactive, HA-specific antibodies, which have in some cases provided protection from infection. Another conserved influenza antigen is the proton channel M2 ectodomain. Song and colleagues demonstrated that M2 embedded in VLPs increased the breath of protection when administered to mice in combination with an inactivated conventional vaccine. Interestingly, no virus-neutralizing M2 antibodies were elicited, but rather non-neutralizing antibodies that may have conferred immune effector functions were thought to be involved in protection [31].

In other viral infections, success has been mixed. The membrane proximal external region (MPER) of HIV envelope glycoprotein gp41 is a target for bnAbs such as 2F5, and efforts have been made to focus the humoral immune response to this particular epitope. The continuous MPER epitope was grafted into five non-related protein scaffolds using Rosetta algorithms [80]. Antibodies elicited by sequential prime-boost administration of the same (homologous) or three different (heterologous) epitope scaffolds containing the shared epitope were found to bind to the original MPER epitope in the same conformational mode as 2F5, but were non-neutralizing. A similar epitope grafting approach was applied to respiratory syncytial virus (RSV). Here, a discontinuous epitope of the RSV F protein was grafted on three-helix bundle protein Z derived from the B domain of *S. aureus* protein A, resulting in the generation of cross-reactive but non-neutralizing antibodies [81]. These studies exemplify progress in directing protective humoral immune responses to defined epitopes, and current limitations in terms of their protective efficacy.

Haynes and colleagues have theorized about the challenges in eliciting not only epitope specific but also functionally protective antibodies in the context of HIV, where a growing number of bnAbs have been isolated but tend to exhibit unusual properties such as long CDRH3s, high mutation rates, polyreactivity, and/or post-translational modifications [5]. Based on the observation that these bnAbs exhibit restricted germline ancestry, the authors suggested the design of vaccine regimens that drive B-cell evolution along certain lineages; assuming that for a given epitope only a limited number of suitable naïve variable gene segments exist to constitute bnAbs. Because weak reactivity was observed between HIV gp120 and these germline ancestors, Jardine and colleagues engineered a gp120 outer domain to sub-micromolar affinity for the putative ancestor while maintaining affinity for matured bnAbs, using a combination of Rosetta-based molecular modeling and yeast surface display-based library screening [82]. This engineered protein was able to stimulate both bnAb and germline antibody-displaying B-cell lines *in vitro*, but it remains to be seen whether bnAbs can be effectively coaxed out of naïve immune repertoires in this way.

While inducing bnAbs has been a cornerstone goal of HIV vaccine development, the recent success of the RV144 vaccine trial [44], in which neither bnAbs, nor potent T cell responses were induced, and protection may have been due to non-neutralizing Abs, presumably through effector mechanisms [83], has both generated a great deal of hope as well as many questions. Follow up analysis has provided evidence that vaccination exerted selective pressure on the viral variants that established infection [84]. Alternative strategies for eliciting protective B cell responses to HIV focus on trimeric spike protein variants that may more closely resemble the native spike on infectious virions, or on the founder viruses responsible for transmission [85]. Kovacs and colleagues have shown that homogeneous trimer preparations elicit more tier 1 virus-neutralizing antibodies than corresponding gp120 monomer [86]. Tong and colleagues suggested a strategy based on protease treatment to remove monomeric and misfolded spike protein leaving homogeneous trimer embedded in VLPs [87]. Toward this end, the lack of a structure of the infectious trimer is still thought to pose a considerable barrier to HIV vaccine development, and the direction of B cell maturation along certain paths towards broad neutralization poses a major challenge to immunologists.

Conclusions

As of January 2013, about 74 vaccines were licensed for immunization and distribution in the US (summarized in Table 1; www.fda.gov). Among these, a majority of 58% represents inactivated or live attenuated, predominantly virus vaccines that are typically produced in fertilized chicken eggs, but also in mammalian cell culture such as Flucelvax™. This group is followed by 35% of licensed vaccines containing microbial toxoids or polysaccharide antigens. Only 7% of all licensed vaccines are composed of recombinant proteins or virus-like particles, although they are comparable in efficacy to vaccines prepared by traditional means, and represent some of the most recent approvals (Table 1). These figures suggest that advanced biotechnology methods are starting to enable development of novel vaccines, and that today's major global disease burdens may be addressable by these means.

More sophisticated vaccine development strategies offer benefits as well as pose challenges (Table 2). Most critically, they offer a path forward where traditional methods have failed. Successes from reverse vaccinology and systems biology approaches, and major efficacy trials in malaria and HIV indicate that these benefits are being realized.

Recombinant subunit vaccines can provide improved safety profiles and may be simpler to manufacture, yet they require more significant upfront investments in research and development. VLPs, liposomes, DNA vaccines, and viral vectors offer means to present antigens in relatively native formats, and these vectors and adjuvant systems can serve both to compensate for the lack of stimulatory co-signals in recombinant subunit vaccines, and to provide specific delivery and immune-modulatory capacities. Vaccines with integrated adjuvant activity may provide excellent co-stimulatory signals while facing fewer regulatory hurdles than next generation traditional adjuvants.

Though instructive, the division of immunity into innate, humoral, and cellular devalues the strong, synergistic connections between arms. An ideal vaccine will likely leverage these arms simultaneously to maximize protection: subunit vaccines can elicit protective responses but depend on suitable adjuvant systems to stimulate the innate immune system, generate T cell help, and encode humoral and cellular memory. As vaccines become more tailored to effectively interact with human innate immune cells and naive B and T cell repertoires, they will become progressively more challenging to evaluate in animal models, but hopefully progressively more effective in preventing major global diseases.

Recent results in major malaria and HIV efficacy trials, and recent approvals of new classes of vaccines have reinstated much hope. The novel approaches described here which leverage high throughput sequencing and bioinformatics to identify promising antigens, molecular adjuvants to target specific innate cell receptors and drive desired inflammatory responses, advanced DNA, RNA, and protein delivery systems, and detailed molecular insights from protective immune responses and naïve immune repertoires hold considerable promise for the future.

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Highlights

- Highly variable and complex pathogens remain challenging vaccine targets.
- Systems biology and reverse vaccinology continue to foster success.
- New ways of presenting conserved, functionally critical epitopes hold promise.
- Adjuvant systems allow for enduring, native antigen presentation.
- An ideal vaccine provides synergy between innate, cellular, and humoral immunity.

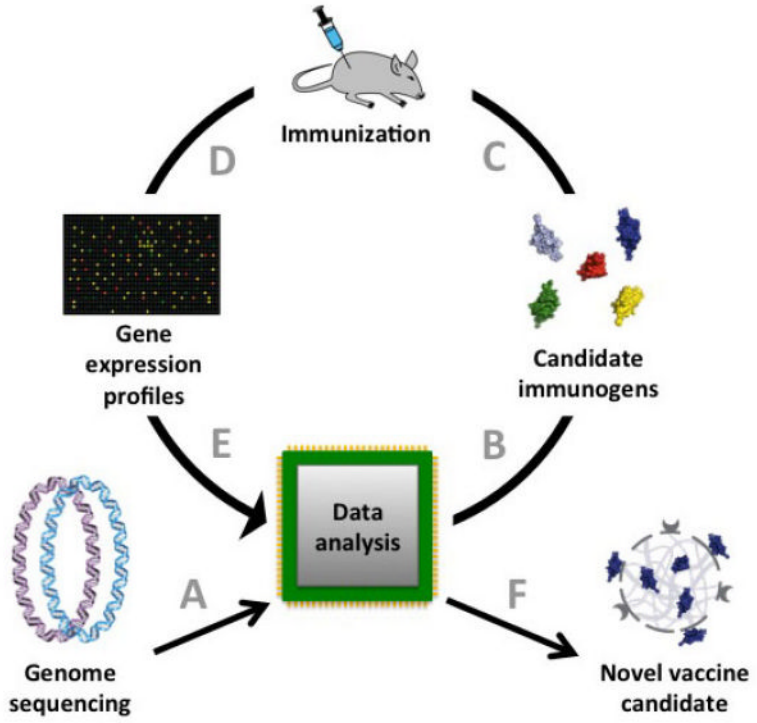


Figure 1. Reverse vaccinology and systems biology approaches in vaccine design

(A) Genomes of pathogens are sequenced and candidate immunogens (cell surface proteins) are predicted using bioinformatic tools. (B, C) Recombinant candidate immunogens are produced, purified and tested in immunogenicity and challenge models. (D) Samples are collected and analyzed for antibody binding properties and gene expression profiles. (E) The data collected is integrated to identify the most promising vaccine candidates and possible correlates of immunogenicity and/or protection. This information can be used to iteratively refine the candidate immunogen. (F) A novel vaccine candidate is designed by combining the selected immunogen(s) with a suitable adjuvant system.

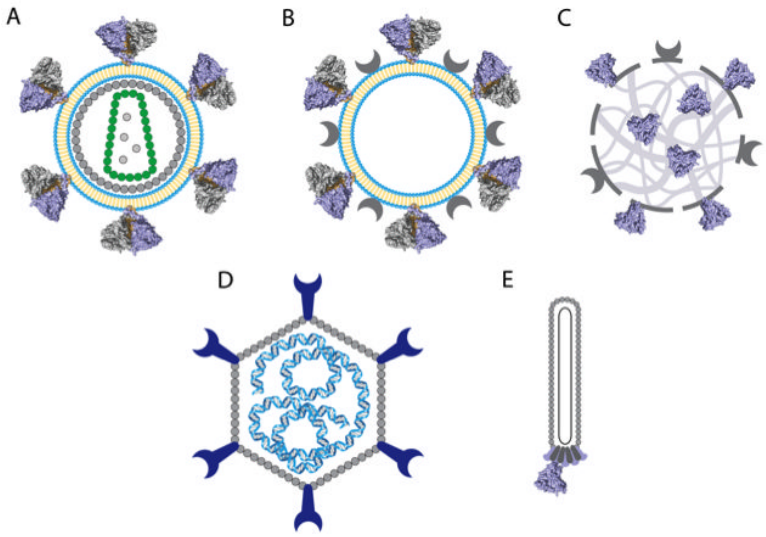


Figure 2. Nanoparticle-based adjuvant systems in vaccine design

Adjuvant systems are antigen delivery vehicles and provide stimulation to the innate immune system. Nanoparticle-based adjuvant systems include: (A) virus-like particles that are identical in structure and composition to virus particles, include capsid and coat proteins but lack genetic material and are hence replication-deficient, (B) liposomes decorated with antigen and optionally stimulatory ligands for innate immune cells, (C) particles composed of synthetic, biodegradable polymers that may be decorated with antigen and stimulatory ligands and are typically loaded with antigen inside of the particle, (D) virus-derived vectors for episomal delivery of genetic material to certain target cells and (E) phage particles that display the antigen as coat protein fusion.

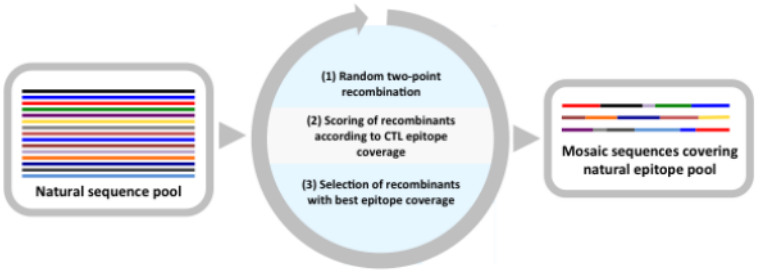


Figure 3. Mosaic CTL immunogen design

From a pool of naturally occurring and diverse pathogen sequences, variants are selected and randomly recombined at two points. The recombinants are scored according to CTL epitope coverage of the sequence pool. Recombinants with best epitope coverage are either recombined with themselves or with new randomly selected variants from the pool. This process is repeated until the resulting mosaic sequences have converged toward maximum possible coverage of CTL epitopes. The valency or number of mosaics is variable, here three.

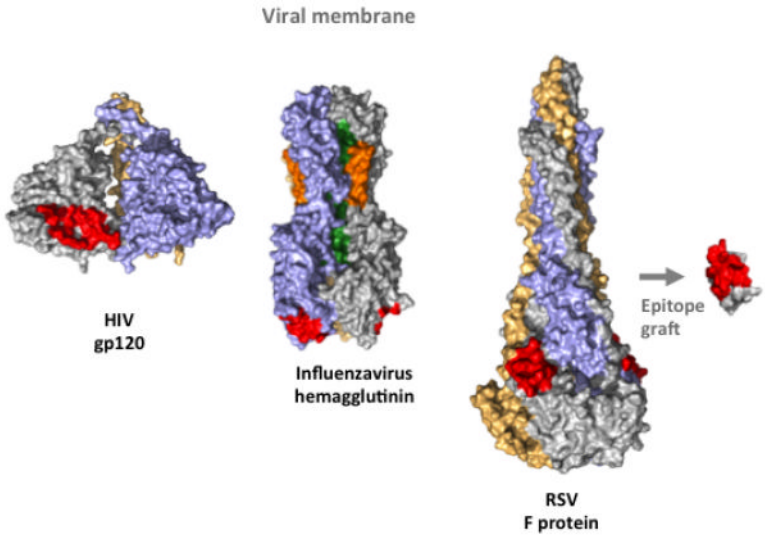
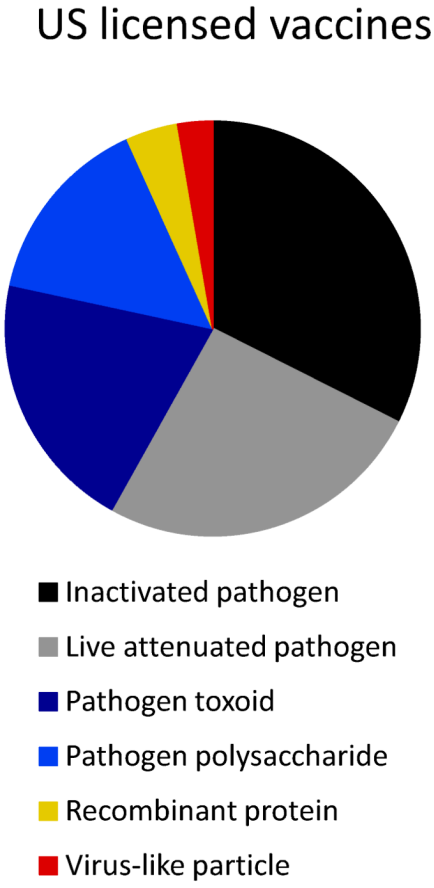


Figure 4. Conserved epitopes for bnAbs and epitope grafting

Relatively conserved epitopes for bnAbs have been identified on large, variable surface antigens. Among those are the CD4 receptor binding site on HIV gp120, the influenzavirus Hemagglutinin receptor binding site and hemagglutinin stem-regions (green and orange), as well as the motavizumab epitope of respiratory syncytial virus F protein. The motavizumab epitope has been grafted on a three-helix bundle scaffold derived from S. aureus protein A. Epitopes are colored in red, green and orange.

Table 1



Class	Target	Example	Licensed	Efficacy
Inactivated pathogen	Hepatitis A	Havrix™	1995	94%
	Influenza	Fluarix™	2005	62%[3)]
	JEV[1)]	Ixiaro™	2009	91%
	Plague virus	Plague Vaccine™	n.a.	n.a.
	Poliovirus	IPOL™	1990	99%
	Rabies virus	RabAvert™	1997	100%
Live attenuated pathogen	Tuberculosis	BCG Live™	1990	50%
	Influenza	FluMist™	2003	87%
	Measles/Mumps/ Rubella	M-M-R II™	1971	100%
	Rotavirus	RotaRix™	2006	87%
	Smallpox	ACAM2000™	2007	95%
	Typhoid	Vivotif Berna™	1989	50-80%
	Varicella	Varivax™	1995	85-90%
	Yellow fever	YF-VAX™	1978	95%
Toxoid	Diphtheria, Tetanus, Pertussis	Tripedia™	2001	95%
Pathogen polysaccharide	*Haemophilus B*	Hiberix™	2009	97%
	Meningococcus	Menveo™	2010	85-100%
	Pneumococcus	Prevnar™	2000	97%
Recombinant protein	Hepatitis B	Comvax™	1996	95%
	Influenza	Flublok™	2013	45%[3)]
VLP	HPV[2)]	Gardasil™	2006	89%

1) Japan. encephalitis virus 2) Human papillomavirus 3) Including unmatched strains

References:

(1) FDA; Complete List of Vaccines Licensed for Immunization and Distribution in the US
(2) http://www.immunizationinfo.org/vaccines

Table 2

Advantages (+) and Disadvantages (−) of different vaccine development methods

Traditional vaccinology	(+) No knowledge of pathogen genome or proteome required
	(+) Offers effective protection against many pathogens
	(+) Well established production and inactivation paths
	(−) Approach has not succeeded for several major, immune evasive pathogens
	(−) Limited to pathogens that can be cultured
	(−) Use of live, attenuated pathogens raise safety concerns
	(−) Inactivation may disrupt critical conformational epitopes
Reverse vaccinology	(+) Offers rapid and systematic discovery of novel vaccine candidates
	(+) Provides for identification of conserved epitopes that ensure broad protection
	(+) Enables the design of non-natural vaccines with enhanced properties
	(−) Ability to identify macromolecules other than proteins is limited
	(−) Requires knowledge of pathogen genomes and proteomes
	(−) Approach may perform best for microbial or other complex pathogens
Structural vaccinology	(+) Offers a path forward for pathogens where traditional methods have failed
	(+) Enables the identification and targeting of critical epitopes
	(+) Enables the design of non-natural vaccines with enhanced properties
	(−) Requires structure and sequence information about critical epitopes
	(−) It may be possible to elicit binding but not neutralizing antibodies, or strong T-cell responses to peptides that are poorly presented during natural infection