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Current status and prospects for development of an HSV vaccine

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Abstract

Herpes simplex virus type 2 (HSV-2) infects 530 million people, is the leading cause of genital ulcer disease, and increases the risk of HIV-1 acquisition. Although several candidate vaccines have been promising in animal models, prophylactic and therapeutic vaccines have not been effective in clinical trials thus far. Negative results from the most recent prophylactic glycoprotein D2 subunit vaccine trial suggest that we must reevaluate our approach to HSV-2 vaccine development. We discuss HSV-2 pathogenesis, immunity, and vaccine efforts to date, as well as the current pipeline of candidate vaccines and design of trials to evaluate new vaccine constructs.

HSV Epidemiology: Global Burden of Disease

Herpes simplex virus type 2 (HSV-2) is an incurable sexually transmitted pathogen that infects over 500 million people worldwide and causes an estimated 23 million new infections annually [1]. In the United States, direct annual medical costs associated with HSV-2 are estimated to be $541 million dollars, making it the third most costly STI after HIV-1 and human papillomavirus (HPV) [2]. HSV-2 seroprevalence ranges from 16% among 14-49 year olds in the United States [3], to >80% in areas of sub-Saharan Africa [4].

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HSV-2 infection rates in heavily exposed populations are nearly 100%, suggesting universal susceptibility [5]. Seroprevalence in women is up to twice as high as men, and increases with age [3, 6]. Although HSV-2 is the leading cause of genital ulcer disease (GUD) worldwide [7, 8], most people are unaware of having the infection [9].

HSV-2 transmission occurs through genital-genital contact during sexual activity. HSV-2 may be transmitted in the absence of signs or symptoms of infection in the infected partner, during episodes of subclinical shedding [10]. In addition, most people who acquire HSV-2 are asymptomatic at the time of acquisition [11]. Transmission of HSV from mother to infant during birth is a serious complication of genital herpes, and can result in long-term neurologic sequelae or mortality [12]. Women who acquire HSV during pregnancy are at the highest risk of transmitting the infection [13]. With an estimated incidence of 4-31/100,000 live births [14, 15], neonatal herpes is too rare to be used as an endpoint in a clinical trial. However, prevention of HSV acquisition during pregnancy is an important goal of developing an effective HSV vaccine.

The greatest public health impact of HSV-2 infection is its role in promulgating the HIV-1 epidemic. Persons with HSV-2 infection are 3-fold more likely to acquire HIV-1 infection [16]; this risk increases up to 8-fold if the exposure occurs soon after acquiring HSV-2 infection [17, 18]. In HIV-1 infected persons, HIV-1 is found in HSV-2 genital ulcers [19], and persons with genital ulcers are at increased risk of transmitting HIV-1 [20]. In regions with high HSV-2 seroprevalence (>80%), 25-50% of HIV-1 infections are attributable to HSV-2 [21]. Mathematical models suggest that even moderately effective prophylactic HSV-2 vaccines would lead to a marked decrease in HIV-1 incidence if given at high coverage [22]. The biologic basis for this predisposition is the persistent mucosal inflammatory response induced by HSV-2. Genital biopsy studies have revealed that HSV-2 ulcers are associated with an infiltrate of CD4+ T-cells bearing the HIV-1 co-receptors CCR5 or CXCR4, which persists during daily antiviral therapy for HSV [23]. Histopathologic studies of foreskins from HIV-1-seronegative men demonstrate that HSV-2 seropositive men have increased concentration of CD4+ and CD8+ T- cells as compared to HSV-2 seronegative men [24]. Similar findings have been found in cervical cytobrush samples from HIV-1 negative, HSV-2 seropositive women [25].

Currently available HSV-2 prevention strategies are inadequate; each reduces the risk of transmission by approximately 50%. Evidence-based methods include use of suppressive antiviral therapy [26], disclosure of serostatus to susceptible partners [27], and consistent condom use [28]. While male circumcision decreases the risk of HSV-2 acquisition by nearly 30% [29], there are conflicting data about the role of circumcision in transmission to women [30, 31]. These partly effective strategies may be useful for management of individual patients, but they are unlikely to be of public health benefit. Indeed, even with availability of suppressive antiherpes viral therapy in the USA, seroprevalence rates are similar to the pre-antiviral era [6].

Changing epidemiology of genital herpes: role of HSV-1

Over the past 2 decades, incident genital herpes in developed countries is increasingly caused by HSV type 1 (HSV-1), especially in persons <25 years of age [32]. This is likely due to declining seroprevalence of HSV-1 in adolescents [6], resulting in the first mucosal exposure to HSV-1 at initiation of sexual activity. As HSV-1 and HSV-2 have similar pathogenesis and host interactions, concepts for effective vaccine development may be relevant to both viruses. Infection with HSV-2 provides partial protection against HSV-1 [15], but the reverse is not true [33]. We need more information about HSV-1 genital infection, the risk of transmission to sex partners and neonates, and interactions between HIV-1 and HSV-1. Vaccines which provide protection against genital HSV-1 infection will be important to reduce genital herpes and its' sequelae.

New insights into HSV pathogenesis: Frequent and dynamic reactivation

During primary infection, HSV infects epithelial cells at skin and mucosa surfaces and is transported along nerve axons to the dorsal root ganglia (DRG), where latency is established [34]. Neuronal cells are not destroyed during initial HSV infection and provide a reservoir for latent virus [35]. During reactivation the virus travels from the ganglia back to the skin and results in detection of virus ("viral shedding") from epithelial surfaces. Viral reactivation is most often asymptomatic, but may be associated with genital symptoms or ulcers. Recent studies have demonstrated that episodes of genital HSV reactivation last a median of 13 hours and are likely rapidly cleared by host responses [36-38]. These may include tissue resident memory (T_{RM}) T cells, discussed below, and suggest that frequent antigen exposure stimulates a chronic immune response in the mucosa.

The Immune Response to HSV-2

Murine HSV models are useful for basic HSV immunology [39], but mimic neither primary nor recurrent human infection. Guinea pigs experience recurrent infection [40], but tools for mechanistic studies are poor, and other models have practical problems or poor evidence for seroconversion [41, 42]. The host and viral determinants of the heterogeneous clinical and virological manifestations of genital HSV-2 in humans are poorly understood. Identification of the components of the host immune system that contain viral reactivation from neurons and promote viral clearance from the mucosa will be essential for development of a successful HSV- 2 vaccine. This information will be gained by detailed immunologic and genetic studies of persons with well-defined HSV-2 severity.

The importance of the innate immune system has been demonstrated by observations that human mutations in a TLR3-centric pathway are associated with severe primary HSV infection [43]. While TLR3 is required for priming CD8+ T-cell responses to HSV infection [44] and can be manipulated by adjuvants [45], data linking variation in TLR3 and recurrent HSV severity in humans are conflicting [46-48]. Variation in other host loci involved in immunity may be associated with HSV severity [49], but the ability manipulate these with vaccines is limited at this time. These findings suggest that adjuvant which promotes innate immune responses may be important for an HSV vaccine.

Antibody-driven vaccines remain of intense interest. The rationale for pursuing neutralizing antibodies is based on the biology of perinatal HSV transmission in the absence vs. presence of pre-existing maternal antibody [15], and animal passive transfer studies [50]. Neutralizing antibody titers correlate with protection to HPV infection, another epithelial STI [51]. The step- wise process of HSV entry, starting with glycoprotein (g)D binding to cell-type specific high affinity receptors and subsequent gB-mediated fusion with mandatory involvement by the gH-gL heterodimer, is becoming clear from structural biology and mutational work [52-55]. Recent advances in human B-cell cloning, high throughput antibody screening, sequencing and expression, and crystallization of complexes of antigens with highly favorable antibodies, as exemplified by HIV-1 and influenza [56, 57] could yield improved HSV immunogen designs.

Evidence is now emerging in both human and murine studies that local T-cells can serve as epithelial sentinels to provide a surveillance function to modulate primary and re-infection episodes. Using *in situ* methods, prolonged residence of HSV-2-specific CD8+ T-cells was documented at the dermo-epidermal junction (DEJ) in humans [58]. These cells have an activated phenotype and a unique expression pattern of homing-related molecules [59]. Elegant murine studies prove prolonged residence of HSV-specific CD8+ T-cells that is spatially limited to sites of previous infection and capable of mediating local protection to exogenous re- scarification, the best model of recurrence in this system [60]. Recently, systemic vaccination with replication-competent, attenuated HSV-2 was followed by a chemoattractant therapy given vaginally in mice [39]. This was found to “pull” vaccine-primed cells to the site of challenge, and to mediate long-lived functional protection [39], providing direct evidence of the importance of CD8 T cells. While vaginal administration of pro-inflammatory chemokines or upstream innate stimuli is challenging in humans, this is an important conceptual advance, establishing the ability to develop tissue resident-memory (T_{RM}) cells without local infection. Mathematical models suggest that small fluctuations in T_{RM} levels could tip the balance between subclinical and clinical reactivation [38]. Therefore, understanding protective T cell responses and stimulating such responses through a vaccine is an ongoing research priority.

At the whole pathogen level, the integrated CD4 and CD8 response in chronically infected persons occupies about 0.1 to 3% of the PBMC compartment [61, 62]. We found no correlation between the magnitude or functionality (IFN-γ, IL-2, TNF-α) of the integrated CD4 responses to whole HSV-2 and shedding or clinical severity in a cross-section survey [61]. Thus, target CD4 levels for preventative vaccines are hard to define, and simply boosting pre-existing CD4 responses may not be rational for immunotherapy. Because HSV-1 and HSV-2 have immune evasive mechanisms and are directly cytotoxic to activated lymphocytes, measuring the size or phenotype of the integrated CD8 response to the whole virus has been challenging. Whether a critical level or phenotype of circulating CD8 responses will correlate with vaccine success is unknown.

Recently developed tools which contain every HSV-1 and HSV-2 open reading frame allow examination of responses at antigen-and epitope-specific levels [62, 63]. Using this unbiased proteomic approach, we found that CD4+ and CD8+ T-cells in HSV-1 infected humans recognize an average of 17 and 22 ORFs, respectively, with a high population prevalence of

both CD8 and CD4 responses to *UL39*, encoding an enzyme, and *UL46*, encoding a tegument protein [62]. These inherently immunogenic proteins are thus potential candidates for a multivalent subunit approach. Responses to individual epitopes and proteins have been correlated with symptom status [64, 65]. A cross-sectional HSV-2 proteome approach in cohorts with clinically defined severity was used to select partial-length HSV-2 ORFs for an adjuvanted, multivalent subunit candidate [66]. These diversity data argue that vaccine candidates using whole viruses are more likely to mimic natural infection with regards to antigenic complexity, albeit whether this is desirable or required is unknown.

Within these poly-specific responses, a pattern of immunodominance is perceptible for both CD8+ and CD4+ T-cell responses. Cells specific for some CD8+ T-cell epitopes are detectable directly *ex vivo* by tetramers or other methods [67], while responder cells specific for most CD8 epitopes are below the limit of detection for most sensitive *ex vivo* method [62]. This implies a steep immunodominance curve, as noted in mice [68]. The dominant epitopes tend to be in tegument and capsid proteins [69]. Dominant CD4 epitope recognition included glycoprotein and regulatory immediate early proteins [70]. Further studies of correlates of immunity using the proteome may identify potentialvaccine candidates.

Predictably, HSV-specific CD8+ and CD4+ T-cells are found at sites of clinically evident recurrent infection [71], because responder cells must physically contact antigen presenting cells (APC). Infiltration of antigen-specific cytotoxic cells correlates with resolution of recurrent genital herpes, and priming or augmenting such cells makes sense for vaccines. The molecular mechanism for homing includes CLA on T-cells and endothelial E-selectin in inflamed tissues [72]. HSV-specific T-cells also localize to the cervix during recurrences in women, to infected ocular tissues, and to the human trigeminal ganglia (for HSV-1) [73-75], suggesting that HSV proteins are expressed at immunologically perceptible sites within the peripheral nervous system throughout the life of the infected host. Mathematical models based on shedding data mirror these findings, and support the view that HSV reactivation is a frequent process with a slow “drip” of virions that are released into the axons [76].

Clinical trials of established HSV vaccine platforms

Prophylactic Vaccines

Several platforms have been tested for prophylactic HSV-2 vaccines; these have been recently reviewed [77]. The most promising and advanced have been recombinant glycoprotein vaccines, with more than 20,000 human volunteers studied in clinical trials. Four envelope glycoproteins elicit neutralizing antibodies to HSV: gD, gB, gH, and gL. The first two are particularly attractive as they bind to high affinity receptors or are involved in membrane fusion, respectively, and are sequence-conserved between strains and relatively conserved between HSV-2 and HSV-1. A recombinant bivalent gB2 and gD2 subunit vaccine formulated with an oil/water emulsion adjuvant was safe and induced strong neutralizing antibody and CD4+ T-cell responses [78, 79] in humans. However, this vaccine did not prevent HSV-2 infection in at-risk members of discordant heterosexual couples or STD clinic enrollees [78]. Two parallel studies showed that a recombinant secreted gD2 subunit vaccine with an adjuvant containing alum and a biologically-derived TLR4 agonist, 3-*O*-deacylated monophosphoryl lipid A (MPL) induced both neutralizing antibody and

CD4+ immune responses [80] in HSV-2 seronegative persons in an HSV-2 discordant sexual relationship. Although the vaccine did not prevent HSV-2 in men or HSV-1 seropositive women, HSV-2 disease was reduced by 70% and HSV-2 infection by 40% in a subgroup analysis of HSV-1 and HSV-2 seronegative women who received vaccine [81]. In a follow-up trial, 8323 sexually active HSV-1/HSV-2 seronegative women in North America received three doses of the gD2 vaccine or control [82]. Unfortunately, the gD2 vaccine failed to prevent HSV-2 infection or disease. However, gD2 vaccine was associated with significant decrease in HSV-1 infection (35% efficacy) and genital disease (58% efficacy). Lower gD2 antibody titers were associated with acquisition of HSV-1 but not HSV-2, suggesting a potential correlate of protection [82]. The magnitude of CD4+ T-cell responses to gD2 was not associated with prevention of HSV infection; CD8+ T-cell responses were not detected. This finding provides proof of concept that an HSV-2 vaccine may also target HSV-1, suggesting potential for cross-reactive immunity [83]. It is unclear why this vaccine showed partial efficacy in HSV-1/2 seronegative women, but not men; differences in the anatomic site at risk of infection (stratum corneum for men, mucosal surfaces, which may contain more vaccine induced immune responses for women), or differences in the immune response to vaccination have been hypothesized to be possible explanations [81].

Therapeutic Vaccines

While an early study of a recombinant gD2 vaccine adjuvanted with alum reduced the rate of virologically confirmed recurrences one year post vaccination [84], later studies of glycoprotein vaccines were not effective [85]. Participants with frequent genital HSV-2 recurrences who received a live, attenuated growth compromised strain of HSV-2 with a deletion in *UL39* (ICP10ΔPK) had decreased self-reported recurrences as compared to placebo [86]. Importantly, this construct was safe, providing proof-of-concept for replication competent vaccine constructs. A replication defective HSV-2 strain with a gH deletion which was able to undergo a single cycle of replication (disabled infectious single cycle, DISC) had similar time to first recurrence, lesion healing rates, and genital shedding rates in HSV-2 seropositive persons with recurrent genital herpes as placebo [87].

Future Clinical Trials: Population, Design & Endpoints

Prophylactic Vaccination

Safe and effective prevention of genital HSV infection is the ultimate goal HSV vaccine research. Because the correlate of protective immunity is unknown, testing the efficacy of prophylactic HSV vaccines requires prospective follow up of persons at risk for genital HSV acquisition. Prior prophylactic vaccine trials have been performed almost exclusively in North America, where the HSV-2 acquisition rate is low. In the per-protocol analysis of the recent gD2 subunit vaccine study, only 1.6% of participants acquired HSV-2 infection, and 1.0% had genital ulcer disease due to HSV-1 or HSV-2, the primary endpoint [82]. In contrast, HSV-2 is rapidly acquired among men and women initiating sexual activity in sub-Saharan Africa, with incidence up to 23 per 100 person years [88]. Prophylactic HSV-2 vaccine studies should be performed in international settings, where the greatest burden of disease exists. Multi-national trials are also important since there may be geographical strain

differences which affect HSV-2 pathogenicity and immunogenicity [89]. It will be important to understand genotypic and phenotypic variation in HSV-2 strains from around the world prior to performing these trials, as these differences may affect vaccine efficacy [89]. Synergy with established networks, such as the HIV Vaccine Trials Network (HVTN), should be explored. Young women are at highest risk for acquiring HSV-2, and serve as an ideal population for prophylactic vaccine trials. Given the sex differences in vaccine efficacy from the gD2 vaccines, it may be important to power trials to stratify vaccine efficacy by sex.

As the efficacy of a vaccine may be different in persons who are HSV-1 seropositive and seronegative, both populations should be evaluated. Importantly, HSV-1 is often acquired early in childhood, especially in resource-limited settings, which may shift the optimal time for vaccination to infancy/early childhood. A vaccine targeting both HSV-1 and HSV-2 could be tested in parallel in HSV-1/HSV-2 seronegative children for prevention of HSV-1 infection.

Acquisition of HSV-1 and HSV-2, measured by seroconversion, should be the primary endpoints for a prophylactic vaccine study. As HSV-2 infection is often subclinical measurement of clinical disease as a primary endpoint is problematic. An important feature of candidate vaccines will be modification of the construct so that an antibody assay can distinguish between vaccinated and infected persons. Secondary endpoints should include frequency of clinically apparent HSV genital disease, and in those who seroconvert, frequency of genital viral shedding. Mathematical modeling suggests that even low efficacy preventative vaccine could impact the HSV-2 epidemic by decreasing shedding and reducing viral transmission [90]. Such a vaccine would have the highest impact in high-prevalence populations [91]; for instance, a vaccine which marginally decreases HSV-2 susceptibility but reduces shedding frequency by 75% could reduce HSV-2 incidence by 30% over a 10 year period [92]. Thus, it is important to study both acquisition, and in those who acquire, frequency of viral shedding.

Therapeutic vaccines

An effective therapeutic HSV-2 vaccine could both improve the clinical course in individual patients, and decrease HSV transmission through reduction in shedding, for a public health benefit. The approach to efficiently evaluate such vaccines relies on evaluation of viral shedding in a cohort of highly adherent persons with clinically apparent genital HSV-2; we have found that this population is highly motivated to participate in daily genital shedding studies [93]. The participants obtain genital swabs for detection of viral shedding before and after vaccination in a one-way crossover study design. These studies are ideal for proof-of-concept, as they can rapidly provide an answer to whether the vaccine has efficacy and can be efficiently performed with fewer than 100 persons [94]. Reduction in viral shedding is the more sensitive primary endpoint for therapeutic vaccine trials, and serves as a useful surrogate endpoint for recurrence rate and transmission likelihood. As initial therapeutic vaccine trials should target persons with symptomatic infection, important secondary endpoints include frequency of genital lesions and prodromal symptoms. These are the clinical endpoints that have been requested in the past by FDA for licensure studies. In

addition, the density of HIV receptor-positive cells in the genital mucosal following therapeutic immunization will need to be evaluated.

The Pipeline—Although prior vaccines that have made it to human clinical trials have almost exclusively targeted glycoproteins, the HSV vaccine pipeline is rich with novel platforms that have shown efficacy in animal models (Table 1). The challenge will be quickly moving these candidate vaccines into human clinical trials.

Whole virus approaches: Replication-competent

There has been concern about safety of replication-competent vaccines due to possibility of recombination with clinical strains or the establishment of latency. However, given the success of the varicella zoster virus (VZV) vaccine, a related alphaherpesvirus, in preventing chicken pox in children and shingles in adults, this is an approach that may reduce the requirement for knowledge of correlates of immunity. Since seroconversion is an appropriate primary outcome in prophylactic vaccine studies, constructs based on whole virus will need to include a serologic marker that identifies the immune response as vaccine- rather than natural infection-specific. Several candidates have yielded promising results in animal models. An HSV-2 ICP0 deletion mutant protected mice from infection, and was more potent than a gD2 subunit approach [95]. HF10 is a highly attenuated naturally occurring HSV-1 mutant that does not express latency associated transcripts and other important viral proteins such as the *UL49.5* product and which prevented genital symptoms, systemic disease, and death after intravaginal HSV-2 challenge in mice [96]. Another attractive replication-competent candidate is an HSV-2 glycoprotein E mutant, which is unable to spread from epithelial cells to neuronal cells [97]. In the guinea pig model, the HSV-2 glycoprotein E mutant has shown potential both as a prophylactic and therapeutic vaccine, although it was unable to completely prevent challenge virus infection or recurrent vaginal shedding [98]. Importantly, infectious glycoprotein E mutant virus was not recovered from dorsal root ganglia or spinal cord in mouse models, although vector DNA was found in the DRG in a minority of animals [98]. AD472, a vaccine strain with deletions in γ*34.5* and several other genes designed to improve genetic stability of the virus also protected guinea pigs, but similar to the glycoprotein E mutant, was not able to prevent wild-type infection [99].

Replication-incompetent—These candidates cannot replicate in normal human cells and therefore, cannot establish latency. This inherent safety advantage may be counterweighed by weaker immunogenicity, possibly requiring higher doses and/or repeated dosing. *dl*5-29 is a double mutant with deletions in *UL5* and *UL29*, two genes which are essential for viral replication [100]. This construct protected against infection and recurrences in the guinea pig model [101]. In both HSV-1 seropositive and HSV-1 seronegative animals, vaccination with *dl*5- 29 resulted in decreased vaginal shedding after challenge compared with gD2 subunit vaccines [102]. Recently described improvements in production and purification of this construct may make it scalable for clinical testing [103]. Another novel replication-incompetent mutant is CJ- gD2, in which both copies the ICP0 gene are replaced by gD2 controlled by HSV-1 ICP4 promoter, resulting in gD2 expression at wild type levels and protection from HSV-2 in the murine model [104].

Other approaches—Other novel platforms include formalin inactivated HSV-2 formulated with MPL/alum adjuvant, which protected guinea pigs from primary infection and recurrent disease when given with a DNA prime [105], and induced neutralizing antibody and HSV-2 specific CD4+ T-cells [106]. In a lentiviral vector delivery system, HSV-1 glycoprotein B expressed in feline immunodeficiency virus vector showed cross-protection against both HSV-1 and HSV-2 vaginal challenge in mice [107]. A plasmid based vaccine which includes gD2, *UL46* and *UL47* formulated with a novel cationic lipid-based adjuvant was effective as a prophylactic and therapeutic vaccine in guinea pigs [108].

Novel routes of delivery are also being evaluated. With increasing evidence for importance of T_{RM} T-cells, there is growing interest in stimulation of genital mucosal immunity through mucosal delivery methods. For instance, intranasal delivery of gB1 packaged in non-ionic surfactant vesicles protected mice from HSV-2 vaginal challenge [109]. Mucosal immunization with gD2 adjuvanted with IC31 [45] or given in a DNA prime followed by a protein boost delivered through liposomal encapsulation [110], both of which stimulate a Th1 response, protected mice from HSV-2 vaginal challenge. Combining the DNA approach with trans-dermal microneedle delivery was found to have a dose-sparing effect in mice; localization of the effector cells is undefined[111]. The “prime-pull” approach in which mice were immunized followed by application of chemokine to genital area is another novel approach that will require further study [39].

There are two ongoing Phase I/II trials of therapeutic vaccines which use novel antigens and adjuvants. One vaccine design consists of 32 35-mer HSV-2 peptides directed against 22 HSV- 2 proteins complexed with human heat shock protein 70 and saponin adjuvant. This vaccine increased detection of HSV-2 specific CD4+ and CD8+ T-cell responses in HSV-2 seropositive persons and was safe in a Phase I trial [112], and is being tested in a Phase II trial for prevention of shedding and lesions (NCT01687595). A subunit vaccine containing secreted gD2, and truncated ICP4, which was identified as a CD8+ T-cell antigen through a high- throughput proteomic screening method, formulated with an adjuvant to stimulate humoral and cellular immunity, showed efficacy against infection and recurrent disease in the guinea pig model [66], and is being tested in a Phase I/II trial as a therapeutic vaccine (NCT01667341).

Conclusions

The field of HSV vaccines is rapidly evolving. Although the results of the prophylactic glycoprotein D2 vaccine were disappointing, the field has been reenergized by improved understanding of the frequency of viral shedding, the importance of the mucosal immune response, availability of novel adjuvants and delivery mechanisms, identification of T cell epitopes via proteomic screening and advancement in replication competent and replication-incompetent candidates. In addition, we have learned from past vaccine studies; we need to depend on objective evidence of seroconversion rather than the variable phenotype of clinical disease in preventative vaccine studies. Similarly, viral shedding should be measured rather than recurrence rate as the most sensitive tool to evaluate both prophylactic and therapeutic vaccines, to provide data on potential impact of vaccination on HSV transmission. Given the increasing incidence of genital HSV-1, we must consider a

vaccination strategy that will provide cross-protection against both HSV-1 and HSV-2, which may ultimately shift the optimal timing of vaccination from adolescence to childhood. Finally, prophylactic vaccines must be tested in populations with high prevalence and incidence of genital HSV-2, as this will provide the benefit of rapid evaluation of candidate vaccine in the populations where it is most desperately needed.

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Highlights

- HSV-2 is a highly prevalent infection that triples the risk of HIV-1 acquisition
- Prophylactic glycoprotein subunit vaccines have failed to decrease HSV-2 acquisition
- Identification of T-cell epitopes and new understanding of mucosal immunity have advanced vaccine development
- Re-evaluation of study population and endpoints for HSV vaccine trials is needed
- The HSV vaccine pipeline is rich with promising novel strategies in preclinical phase

Table 1

HSV Vaccine Pipeline.

Vaccine	Name	Construct	Stage of development
HSV-2 functionally mutated for ICP0 [1]	0ΔNLS	Replication-competent whole virus	Preclinical
HSV-1 functionally mutated for *UL43, UL49.5, UL55, UL56*, and *LAT* expression [2]	HF10	Replication-competent whole virus	Preclinical
HSV2 glycoprotein E deletion mutant [3]	HSV2 gE mutant	Replication-competent whole virus	Preclinical
HSV-2 functionally mutated for *γ34.5, UL43.5, UL55-56 US10, US11, US12* [4]	AD472	Replication-competent whole virus	Preclinical
HSV-2 functionally mutated for *UL5/UL29* [5-7]	ACAM-529	Replication-incompetent whole virus	Preclinical
HSV-2 glycoprotein D dominant negative, functionally mutated for *ICP0/UL9* [8]	CJ2-gD2	Replication-incompetent whole virus	Preclinical
HSV-2 functionally mutated for thymidine kinase (TK) [9]		Replication-competent whole virus, followed by topical vaginal application of CXCL9 and CXCL10	Preclinical
HSV-2 functionally mutated for ICP10 [10, 11]	ICP10APK	Replication-competent whole virus.	No active program, has been in phase I/II, therapeutic
HSV-2 functionally mutated for ICP47, vhs, *γ34.5, US5, UL43* [12]	ImmunoVEX^{HSV2}	Replication-competent whole virus	Phase I
Inactivated HSV-2 in MPL/alum [13, 14]		Formalin-inactivated virus administered after DNA boost	Preclinical
HSV-1 glycoprotein B [15]		Lentiviral vector	Preclinical
Recombinant secreted HSV-1 glycoprotein B [16]		Intranasal immunization with immunogen in non-ionic surfactant vesicles	Preclinical
Recombinant HSV-2 gD [17]		Recombinant gD2 with IC31 adjuvant	Preclinical
Recombinant HSV-2 gD [18]		gD2 DNA prime followed by intranasal protein boost (liposomal encapsulation)	Preclinical
gD2/UL46/UL47 DNA [19]	gD2-Vaxfectin	Plasmid *gD2/UL46/UL47* polyvalent DNA with cationic lipid adjuvant	Phase I therapeutic announced
Modified gD2 DNA [20]		Plasmid gD2 fused to ubiquitin mixed with codon-optimized non-linked gD2	Pre-clinical
32 unique 35-mer HSV-2 peptides [21]	HerpV	Synthetic peptides, multivalent with heat shock protein adjuvant	Phase II, therapeutic
gD2 and ICP4 [22]	GEN-003/MM2	Recombinant bivalent proteins with Iscomatrix adjuvant	Phase 1 b/ II, therapeutic

UL=unique long genes, ICP=infected cell polypeptide. vhs=virion host shutoff protein

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