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(54) **Title**: VACCINES AGAINST HERPES SIMPLEX VIRUS TYPE 2: COMPOSITIONS AND METHODS FOR ELICITING AN IMMUNE RESPONSE

(57) **Abstract**: Herpes Simplex Virus-2 (HSV-2) infection is a major health concern. The present disclosure provides, *inter alia*, certain highly effective vaccines and immunogenic compositions against HSV-2. The antigens can be used therapeutically or prophylactically.

# Vaccines Against Herpes Simplex Virus Type 2: Compositions and Methods for Eliciting an Immune Response

## Related Applications

This application claims the benefit of the filing date of U.S. Provisional Application No. 61/180,784, filed on May 22, 2009, U.S. Provisional Application No. 61/235,628, filed on August 20, 2009, U.S. Provisional Application No. 61/240,587, filed on September 8, 2009, U.S. Provisional Application No. 61/240,626, filed on September 8, 2009, and U.S. Provisional Application No. 61/305,918 filed on February 18, 2010. The entire teachings of the referenced applications are expressly incorporated herein by reference.

## I. Background

Herpes simplex virus type 2 (HSV-2) is the leading cause of genital herpes. HSV-2 is most often transmitted by sexual contact, and infection with the virus typically leads to recurring outbreaks of lesions on the genitals and perianal regions, combined with shedding of virus particles into the genital tract. Viral shedding can also occur in the absence of lesions or other symptoms. HSV-2 also establishes latency in sensory ganglia. HSV-2 infection causes physical discomfort and psychosexual morbidity in affected patients, and introduces additional health risks. In particular, patients infected with HSV-2 are at increased risk for contracting HIV, and pregnant mothers infected with HSV-2 can vertically transmit HSV-2 to their fetuses. In immunocompromised individuals or in neonates, HSV-2 infections can be fatal. Currently, there is no cure for HSV-2 infection.

HSV-2 infection is widespread, with one study estimating that nearly 20% of the population worldwide is infected (Looker et al., 2008, Bulletin of the World Health Organization, October 2008, 86(10)). More women than men are infected, and the prevalence of the disease increases with age. High numbers of adolescents diagnosed with HSV-2 indicate that the prevalence across the population will continue to rise, as HSV-2 infection is lifelong.

Treatment options for HSV-2 symptoms are limited. Antiviral therapy, using compounds such as famciclovir, valaciclovir, or aciclovir, limits the duration of symptoms and, in some cases, speeds healing of lesions and reduces incidence of viral shedding. Antiviral drugs are not curative, however, and do not prevent recurrence of outbreaks or clear the virus completely. In addition, use of antiviral drugs requires patients to recognize symptoms of HSV-2 infection, then obtain a confirmative diagnosis, and ultimately, comply with the antiviral regimen. These requirements may be untenable in regions of the world where antiviral drugs are not readily available. In addition, patients are often unaware that they are infected, either because they do not present symptoms, or because the symptoms of the initial infection subside, suggesting recovery from the disease.

To address the medical and social problems associated with HSV-2, it is highly desirable to develop pharmaceutical compositions to inhibit or counteract infection by HSV-2. An effective composition may be used to elicit an enhanced immune response against HSV-2, thereby preventing initial infection, blocking the ability of the virus to establish latency in sensory ganglia, eliminating recurrence of outbreaks, and/or preventing viral shedding. The immune system is known to mount a defense against HSV-2, as evidenced by recurrent infections which manifest with fewer, less intense symptoms and decreased frequency over time.

While the ultimate goal of an HSV vaccine would be long-lasting protection from viral infection, the suppression of disease symptoms would also provide significant health benefits. One of the current goals for either a prophylactic or therapeutic vaccine is to reduce clinical episodes and viral shedding from primary and latent infections. Three categories of prophylactic vaccines have been tested in clinical trials with disappointing results i) whole virus, ii) protein subunit and iii) gene-based subunit vaccines (Stanberry et al., Clinical Infect. Dis., 30(3):549-566, 2000). In the 1970s a number of killed virus vaccines were explored, none of which were efficacious. More recently an attenuated HSV was found to be poorly immunogenic. Subunit vaccines based on two recombinant glycoproteins have been clinically evaluated in combination with different adjuvant formulations. One developed by Chiron contains truncated forms of both glycoprotein D (gD2) and glycoprotein B (gB2) of HSV-2, purified from transfected Chinese Hamster Ovary

(CHO) cells and formulated in the adjuvant MF59. Another developed by Glaxo-Smithkline (GSK) contains a truncated gD2 formulated with adjuvants alum and 3-O-deacylated monophosphoryl lipid A (MPL). Both vaccines were immunogenic and well tolerated in phase I/II trials. However in phase III analyses, the Chiron vaccine showed no overall efficacy against HSV-2 seroconversion and work was discontinued. The GSK vaccine showed significant efficacy (73-74%) in HSV-1, HSV-2 seranegative women volunteers but no efficacy in men.

While even limited vaccine efficacy would beneficially impact HSV sufferers, these trials are testing only a small number of vaccine possibilities. This is because the vaccine discovery has not been systematic. Pursuance of a whole-virus vaccine assumes that presentation of the pathogen itself to the immune system will generate optimal immunity. Indeed the breadth and duration of immune responses to whole pathogen vaccines historically have been better than subunit vaccines. However, pathogenicity of the vaccine strain must be considered. Subunit vaccines, to date, have been selected for vaccine testing based on their assumed importance in disease pathogenesis and immunogenicity during infection. These approaches have identified one candidate against HSV with limited efficacy in some but no efficacy in other formulations. Thus, new and improved methodologies for herpesvirus vaccine discovery are needed to protect against herpes diseases.

## -II. Summary of the Invention

Infection and transmission of HSV-2 is a major health concern. The present disclosure provides, *inter alia*, certain highly effective vaccines against HSV-2. Such vaccines can be used either therapeutically or prophylactically. The present disclosure also provides specific antigens and methods for using the antigens to elicit an immune response against HSV-2.

In one aspect, the present disclosure describes a vaccine formulation comprising a pharmaceutical-acceptable carrier and at least one polypeptide consisting of SEQ ID NOS: 2, 3, 4 and 5 or an immunogenic fragment thereof, and optionally further comprising SEQ ID NO:1 or an immunogenic fragment thereof.

The vaccine formulation may comprise a first polypeptide consisting of one of the above SEQ ID NOS, and a second polypeptide consisting of another one of the above SEQ ID NOS.

Another aspect of the present invention provides a vaccine formulation comprising a pharmaceutically acceptable carrier, an adjuvant comprising one or more purified fractions of quillaja saponins, and at least one polypeptide comprising any of SEQ ID NOS: 2, 3, 4 and 5 or an immunogenic fragment thereof, and optionally further comprising SEQ ID NO:1 or an immunogenic fragment thereof.

A further aspect of the present invention provides a vaccine formulation comprising a pharmaceutically-acceptable carrier and a polypeptide consisting of SEQ ID NO: 2 or an immunogenic fragment thereof. Residues may be truncated from SEQ ID NO:2. The polypeptide may be glycosylated, or may be unglycosylated.

In still a further aspect, the present invention provides a vaccine formulation comprising a pharmaceutically-acceptable carrier and a polypeptide comprising SEQ ID NO:5, wherein the polypeptide lacks all or at least an 8 contiguous amino acid residue portion of the transmembrane domain spanning residues 340-363. Accordingly, one aspect of the present invention provides a vaccine formulation comprising a pharmaceutically-acceptable carrier and a polypeptide comprising SEQ ID NO:4. The polypeptide may be glycosylated, or may be unglycosylated.

Still another aspect of the present invention provides a vaccine formulation comprising a pharmaceutically-acceptable carrier, a polypeptide comprising SEQ ID NO:5. The polypeptide may be glycosylated, or may be unglycosylated.

Yet another aspect of the present invention provides a vaccine formulation comprising a pharmaceutically-acceptable carrier, a polypeptide comprising SEQ ID NO:3. The polypeptide may be glycosylated, or may be unglycosylated.

In some embodiments, polypeptides in the vaccine formulations that may be conjugated to an immunogenic carrier, for example keyhole limpet hemocyanin. In other embodiments, the vaccine formulations further comprise an adjuvant. The adjuvant may be one or more purified fractions of quillaja saponins.

The invention provides methods or treating a subject suffering from or susceptible to HSV-2 infection by administering an effective amount of a vaccine formulation disclosed herein. In some embodiments, the method inhibits HSV-2 symptoms, for example by reducing the number of herpetic lesions, reducing the number of days a subject experiences herpetic lesions, reducing infection by HSV-2 in an uninfected subject, increasing the IgG titer and/or T cell response to one or more HSV-2 antigens, and/or reducing the number of herpetic lesions at the onset of HSV-2 infection.

In another aspect, the present disclosure describes the results of a high-throughput system for in vitro screening of libraries of efficacious T cells to identify their specific target antigens from the complete proteome of HSV-2. This technology allowed the identification of individual antigens, likely to be effective in vivo, as either a prophylactic or therapeutic composition. In one aspect, herein are provided several critical protective T cell antigens that can be incorporated into protein-based compositions that elicit an immune response.

One aspect of the present invention provides pharmaceutical compositions comprising two or more isolated polypeptides selected from polypeptides having an amino acid sequence of at least one of SEQ ID NOS: 1-38, or an immunogenic fragment thereof.

In another aspect, the invention provides vaccine formulations that include a pharmaceutically-acceptable carrier and a polypeptide comprising at least one of SEQ ID NOS: 1-38, or an immunogenic fragment thereof. In certain embodiments, the polypeptide consists of at least one of SEQ ID NOS: 1-38.

Another aspect of the present invention provides a method of inducing an immune response in a subject, comprising administering to said subject an effective amount of a vaccine formulation or a pharmaceutical composition comprising an effective amount of two or more isolated polypeptides selected from polypeptides having an amino acid sequence of at least one of SEQ ID NOS: 1-38, or an immunogenic fragment thereof.

Yet another aspect of the present invention provides a method of reducing one or more symptoms of HSV-2 infection in a subject, comprising administering to

said subject an effective amount of a vaccine formulation or a pharmaceutical composition comprising two or more isolated polypeptides selected from polypeptides having an amino acid sequence of at least one of SEQ ID NOS: 1-38, or an immunogenic fragment thereof. In some embodiments, the symptoms of HSV-2 infection comprise one or more of lesion formation, pain, irritation, itching, fever, malaise, headache, viral shedding, and prodrome.

A further aspect of the present invention provides a method of inhibiting the onset of HSV-2 infection, comprising administering an effective amount of a vaccine formulation or a composition comprising two or more isolated HSV polypeptides selected from polypeptides having an amino acid sequence of at least one of SEQ ID NOS: 1-38, or an immunogenic fragment thereof.

Applicants disclose another aspect of the present invention, which provides a method of inhibiting development of a latent HSV-2 infection in a subject exposed to HSV-2, comprising administering an effective amount of a vaccine formulation or a composition comprising two or more isolated HSV-2 polypeptides selected from polypeptides having an amino acid sequence of at least one of SEQ ID NOS: 1-38, or an immunogenic fragment thereof.

In a related aspect, the present invention provides a method of reducing viral shedding in a subject infected with HSV-2, comprising administering an effective amount of a vaccine formulation or a composition comprising two or more isolated HSV-2 polypeptides selected from polypeptides having an amino acid sequence of at least one of SEQ ID NOS: 1-38, or an immunogenic fragment thereof.

Further, an aspect of the present invention provides a method of reducing recurrence of outbreaks in a subject infected with HSV-2, comprising administering an effective amount of a vaccine formulation or a composition comprising two or more isolated HSV-2 polypeptides selected from polypeptides having an amino acid sequence of at least one of SEQ ID NOS: 1-38, or an immunogenic fragment thereof.

An additional aspect of the present invention provides a method of producing any of the pharmaceutical compositions described above, comprising expressing said two or more polypeptides; and isolating said two or more polypeptides.

Applicants further disclose an aspect of the present invention which provides a method for diagnosing severity of symptoms in a subjected infected with HSV-2, comprising (i) measuring activation of T cells in response to autologous antigen presenting cells (APC) pulsed with one or more isolated HSV-2 polypeptides selected from polypeptides set forth in SEQ ID NOS: 1-38, or an immunogenic fragment thereof, and (ii) comparing said levels to reference levels obtained from infected subjects experiencing frequent outbreaks; whereby a significant increase in said responses relative to reference levels indicates that said subject has less severe symptoms (e.g., the subject is asymptomatic). A significant increase in response can, for example, comprise a 1.5-fold or greater, 2-fold or greater, 3-fold or greater, 5-fold or greater, 10-fold or greater or even 20-fold or greater increase.

Another aspect of the present invention provides a method for diagnosing severity of symptoms in a subjected infected with HSV-2, comprising (i) measuring activation of T cells from naturally infected or virus-exposed subjects in response to APC presenting one or more isolated HSV-2 polypeptides selected from polypeptides set forth in SEQ ID NOS: 1-38, or an immunogenic fragment thereof, and (ii) comparing said levels to reference levels obtained from infected subjects experiencing frequent outbreaks; whereby a significant decrease in said activation relative to reference levels indicates that said subject has more severe symptoms (e.g., frequent outbreaks).

Another aspect of the present invention provides pharmaceutical compositions comprising an antibody that binds to one or more isolated HSV polypeptides selected from the list consisting of SEQ ID NOS: 1-38, or an immunogenic fragment thereof.

Moreover, a different aspect of the present invention provides a method of identifying immunogenic compositions for HSV-2 by testing two or more polypeptides selected from polypeptides having an amino acid sequence of any one of SEQ ID NOs. 1-38, or an immunogenic fragment thereof, for ability to promote cytokine production in a mammalian T cell, wherein an immunogenic composition is one that elevates levels of a cytokine significantly above the levels of that cytokine produced by a naïve mammalian T cell. A significant increase in cytokine

levels is typically one that is at least 1.5-fold, 2-fold, 3-fold, 5-fold, 10-fold or even 20-fold the level produced by a naïve cell.

Still another aspect of the present invention provides a method of detecting HSV-2 in a sample from a subject, said method comprising (i) contacting said sample with one or more antibodies raised against one or more polypeptides having an amino acid sequence of SEQ ID NOS: 1-38 or an immunogenic fragment thereof, and (ii) detecting said one or more antibodies bound to said one or more HSV-2 polypeptide from the sample.

Finally, one aspect of the present invention provides pharmaceutical compositions comprising two or more isolated polynucleotides, selected from nucleotide SEQ ID NOS: 1-38, or fragments encoding immunogenic peptides thereof.

## III. Brief Description of the Drawings

Figures 1A and B are graphs showing, respectively, $CD4^+$ and $CD8^+$ T cell responses following immunization with gD2 full-length protein, gD2ΔTMR, or gD2 truncated immediately upstream of the transmembrane domain (denoted 306t).

Figures 2A and B are graphs showing, respectively, $CD4^+$ and $CD8^+$ T cell responses following immunization with pooled, overlapping peptides spanning gL2, ICP4.1, or ICP4 fragments encoded by RS1.1, RS1.3.1 and RS1.3.2.

Figure 3A and B are graphs showing, respectively, $CD4^+$ and $CD8^+$ T cell responses following immunization with gD2ΔTMR, or gD2ΔTMR and ICP4.2.

## IV. Detailed Description

This application describes vaccines and immunogenic compositions against HSV-2. Vaccine formulations may include a polypeptide comprising a sequence from Table 1 or an immunogenic fragment thereof, or a combination of at least two polypeptides comprising sequences from Table 1 or immunogenic fragments thereof. In certain embodiments, the polypeptide(s) of the vaccines comprise the

entire sequence of at least one of SEQ ID NOS: 1-26 or consist of the entire sequence of any one of SEQ ID NOS: 1-26. Immunogenic compositions may include a polypeptide comprising a sequence from Table 1 or Table 2 or an immunogenic fragment thereof or a combination of at least two polypeptides comprising sequences from Table 1 or Table 2, or immunogenic fragments thereof. In certain embodiments, the polypeptide(s) of the immunogenic compositions comprise the entire sequence of any one of SEQ ID NOS: 1-38 or consist of the entire sequence of SEQ ID NO: 1-38. The polypeptides in Tables 1 or 2 may be encoded by SEQ ID NOS: 39-46 and 117-134 as indicated and/or by cDNA sequences publically available on http://www.ncbi.nlm.nih.gov/sites/entrez. cDNA and protein sequences may also be obtained from any known strains of HSV-2, including HG52, 333, and Strain G. Accordingly, cDNA sequences may be accessed by gene or protein name from genomic sequence at NC_001798.1, and may be approximately 97% conserved with sequences disclosed at NC_001798.1). As described herein, the polypeptides may be referred to by protein name, by SEQ ID NO, and/or by the name of the gene encoding the protein.

The polypeptides can be prepared in a variety of expression systems. Suitable expression systems include E. coli and Baculovirus-based expression systems (e.g., in insect cells). Polypeptides prepared using E. coli are typically full-length and unglycosylated, although truncated variants can be prepared. In certain embodiments, these truncated variants retain all or part of the signal domain. Polypeptides prepared using a Baculovirus system typically lack the N-terminal signal sequence, but are fully or partially glycosylated.

Table 1. HSV-2 antigens for vaccines or immunogenic compositions

| Protein SEQ ID No. | DNA SEQ ID No. | Gene Name Protein Name | Gene ID No. | GenBank Accession Nos. |
|---|---|---|---|---|
| 1 | 39 | RS1<br>ICP4 | 1869897 | NP_044530.1 |
| 2 | 117 | RS1.2<br>ICP4 internal fragment (ICP4.2) | | RS1.2 corresponds to an internal fragment of the RS1 sequence |

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| Protein SEQ ID No. | DNA SEQ ID No. | Gene Name Protein Name | Gene ID No. | GenBank Accession Nos. |
|---|---|---|---|---|
| 3 | 118 | UL1 gL2 cytoplasmic | 1487292 | NP_044470.1 |
| 4 | 40 | US6ΔTMR gD2 internal deletion (gDΔTMR) | 9629336 | NP_044536.1 US6ΔTMR corresponds to gD2 with a deletion of amino acids 340-363 |
| 5 | | US6 gD2 | | |
| 6 | 41 | RL1 ICP34.5 | 9629329 | NP_044529.1 |
| 7 | 42 | RL2 ICP0 | 109676722 | NP_044528.2 |
| 8 | 121 | RS1.1 ICP4 internal fragments | 1869897 | NP_044530.1 RS1.1 corresponds to residues 1-400 of RS1 |
| 9 | 122 | RS1.3.1 ICP4 internal fragments | 1869897 | NP_044530.1 RS1.3.1 corresponds to residues 750-1024 of RS1 |
| 10 | 123 | RS1.3.2 ICP4 internal fragments | 1869897 | NP_044530.1 RS1.3.2 corresponds to residues 1008-1319 of RS1 |
| 11 | 124 | RS1.3 ICP4 internal fragments | 1869897 | NP_044530.1 RS1.3 corresponds to residues 750-1319_of RS1 |
| 12 | 125 | RS1.4 ICP4 internal fragments | 1869897 | NP_044530.1 RS1.4 corresponds to residues 340-883 of RS1 |
| 13 | 126 | RS1.5 ICP4 internal fragments | 1869897 | NP_044530.1 RS1.5 corresponds to residues 775-1318 of RS1 |
| 14 | 127 | RS1.6 ICP4 internal fragments | 1869897 | NP_044530.1 RS1.6 corresponds to residues 209-1318 of RS1 |
| 15 | 128 | RS1.7 ICP4 internal fragments | 1869897 | NP_044530.1 RS1.7 has a deletion of residues 391-544 of RS1 |
| 16 | 129 | RS1.8 ICP4 internal fragments | 1869897 | NP_044530.1 RS1.8 has a deletion of residues 786-864 of RS1 |
| 17 | | UL2 uracil DNA glycosylase | | |
| 18 | | UL11 myristylated | | |

| Protein SEQ ID No. | DNA SEQ ID No. | Gene Name *Protein Name* | Gene ID No. | GenBank Accession Nos. |
|---|---|---|---|---|
| | | *tegument protein* | | |
| 19 | 119 | UL1 *gL2 secreted* | 1487292 | NP_044470.1 |
| 20 | | UL19 *VP5* | | |
| 21 | 120 | UL19ΔTEV *VP5* | 9629288 | NP_044488.1 UL19ΔTEV is lacking the last 5 amino acids from the C-terminal end of UL19 |
| 22 | | UL36 *ICP1/2* | | |
| 23 | 43 | UL36.3.4.1 *ICP1/2 internal fragments* | 1487322 | NP_044506.1 UL 36.3.4.1 corresponds to residues 1318-2280 of UL36 |
| 24 | 44 | UL36.4.2.5 *ICP1/2 internal fragments* | 1487322 | NP_044506.1 UL 36.4.2.5 corresponds to residues 2253-3122 of UL36 |
| 25 | | UL40 *ribonucleoside reductase* | | |
| 26 | 45 | US12 *ICP47* | 9629343 | NP_044543.1 |

Table 2. Additional HSV-2 antigens for immunogenic compositions

| Protein SEQ ID No. | DNA SEQ ID No. | Gene Name *Protein Name* | Gene ID No. | GenBank Accession Nos. |
|---|---|---|---|---|
| 27 | 134 | UL10 *gM2* | 9629279 | NP_044479.1 |
| 28 | | UL15 *DNA cleavage/packaging protein* | | |
| 29 | | UL26.5 *ICP35* | | |
| 30 | | UL30 *DNA-directed polymerase* | | |
| 31 | | UL5 | | |

| Protein SEQ ID No. | DNA SEQ ID No. | Gene Name *Protein Name* | Gene ID No. | GenBank Accession Nos. |
|---|---|---|---|---|
| | | *DNA helicase/primase complex* | | |
| 32 | | UL8 *DNA helicase/primase complex* | | |
| 33 | | UL15.5 *unknown* | | |
| 34 | | UL32 *cleavage and packaging protein* | | |
| 35 | | UL36.4.2 *ICP1/2 fragment* | | |
| 36 | | UL54 *ICP27* | | |
| 37 | 133 | UL49.5 *Membrane associated virion protein* | 1487337 | NP_044520.1 |
| 38 | 46 | US4 *gG2* | 9629334 | NP_044534.1 |

**A. Immunogenic HSV-2 polypeptides**

Immunogenic polypeptides or polynucleotides as indicated in Table 1 and/or Table 2 may be used in pharmaceutical compositions. The invention provides pharmaceutical compositions containing immunogenic polypeptides or polynucleotides encoding these immunogenic polypeptides together with a pharmaceutical carrier. Antigens from HSV-2 may be identified by screening immune cells from patients infected with HSV-2. Briefly, a library of HSV-2 antigens was expressed by bacteria and mixed with antigen presenting cells (APCs). The APCs, in turn, processed and presented HSV-2-derived polypeptides to lymphocytes that had been isolated from human patients infected with HSV-2. The patients belonged to several populations: (1) exposed to HSV-2 but seronegative for infection, (2) infected with HSV-2 but asymptomatic, (3) infected with HSV-2 and experiencing infrequent outbreaks, (4) infected with HSV-2 and experiencing

frequent outbreaks, (5) naïve and (6) seronegative for HSV-2 (HSV-2$^-$) but seropositive for HSV-1 (HSV-1$^+$). Lymphocyte responses from each population were compared for reactivity to HSV-2-derived polypeptides, and the screen detected antigens that induced reactive lymphocytes with greater frequency in one patient population as compared to the others. Infected but asymptomatic, and exposed but seronegative patients may activate protective immune responses that patients who experience frequent outbreaks do not; in particular, exposed but seronegative patients are presumed to have mounted sterilizing immunity to HSV-2 infection. It is believed that a unique set of polypeptides will activate lymphocytes from these patient populations. Thus, the present invention contemplates compositions of the specific HSV-2 polypeptides that activate the lymphocytes of infected but asymptomatic, or exposed but seronegative patients or a combination of these polypeptides for inhibiting or counteracting infection by HSV-2.

Antigens identified on the basis of their immunogenicity in infected but asymptomatic, or exposed but seronegative patients are similarly expected to be immunogenic in any subject.

In some embodiments, a polypeptide may induce an innate immune response, a humoral immune response, or a cell-mediated immune response. The cell-mediated immune response may involve $T_H1$ cells, and in certain embodiments, the immune response involving $T_H1$ cells is an immune response in which $T_H1$ cells are activated. In some embodiments, an immunogenic polypeptide avoids induction of $T_H2$ cytokines. In some embodiments, the cell-mediated immune response may involve $T_H17$ cells, and in certain embodiments, the immune response involving $T_H17$ cells is an immune response in which $T_H17$ cells are activated.

Polypeptides (or immunogenic fragments thereof) in compositions of the invention may induce T cell responses in multiple individuals, regardless of the HLA haplotype of the individuals. Specifically, epitopes on the polypeptides may induce T cell responses in individuals with one or more of the following HLA supertypes: HLA-A2, -A3, -A24, -A1, -B7, -B8, -B27, -B44, -B58, and B62, and HLA-DQB01, -DQB02, -DQB03, -DQB-04, and -DQB05.

In some embodiments, one or more, e.g. two, three, four, or more polypeptides from Table 1 and/or Table 2 (or immunogenic fragments thereof) are provided in a composition of the invention. In some embodiments, two polypeptides from Table 1 and/or Table 2 are provided in a composition of the invention. In other embodiments, three polypeptides from Table 1 and/or Table 2 are provided in a composition of the invention.

In some embodiments, two, three, four, or more polypeptides from Table 1 and/or Table 2 (or immunogenic fragments thereof) are provided together as a conjugate. In some embodiments, two polypeptides from Table 1 and/or Table 2, or three polypeptides from Table 1 and/or Table 2, are provided as a conjugate. In some embodiments, two, three, four, or more polypeptides from Table 1 and/or Table 2 are covalently bound to each other, e.g., as a fusion protein. In some embodiments, two, three, four, or more polypeptides from Table 1 and/or Table 2 are covalently bound to each other, e.g., as a fusion protein. In some embodiments, two polypeptides from Table 1 and/or Table 2, or three polypeptides from Table 1 and/or Table 2, are covalently bound to each other, e.g. as a fusion protein.

In some embodiments, the compositions comprise two or more polypeptides selected from the group consisting of SEQ ID Nos. 1-38, and may contain or may not contain any other HSV-2 polypeptides.

In certain embodiments, Applicants provide polypeptides that are at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide encoded by a gene in Table 1 and/or Table 2, or a portion of said polypeptide. In certain embodiments, the homologous polypeptide is at least 8, 10, 15, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 350, 400, 450, or 500 amino acids in length. In some embodiments, such as those described immediately above, the polypeptide is no more than 300, 350, 400, 450, or 500 amino acids in length.

An immunogenic composition may also comprise portions of said polypeptides and genes, for example deletion mutants, truncation mutants, oligonucleotides, and peptide fragments. In some embodiments, the portions of said proteins are immunogenic.

The immunogenicity of a portion of a protein or a homolog thereof can be readily determined using the same assays that are used to determine the immunogenicity of the full-length protein. In some embodiments, the portion of the protein has substantially the same immunogenicity as the full-length proteins. In some embodiments, the immunogenicity is no more than 10%, 20%, 30%, 40%, or 50% less than that of the full-length protein. The protein fragments may be, for example, linear, circular, or branched. In some embodiments, a protein or protein fragment comprises one or more non-natural amino acids (e.g. an amino acid other than the 20 typically found in natural proteins). A non-natural amino acid may have an atypical side chain. In addition, peptidomimetics may be used; these may incorporate alterations to the peptide backbone.

Some embodiments of the polypeptide composition described herein include an immunogenic polypeptide that contains a membrane translocating sequence (MTS), to facilitate introduction of the polypeptide into the mammalian cell and subsequent stimulation of the cell-mediated immune response. Exemplary membrane translocating sequences include hydrophobic region in the signal sequence of Kaposi fibroblast growth factor, the MTS of α-synuclein, β-synuclein, or γ-synuclein, the third helix of the Antennapedia homeodomain, SN50, integrin β3 h-region, HIV Tat, pAntp, PR-39, abaecin, apidaecin, Bac5, Bac7, *P. berghei* CS protein, and those MTSs described in US Patents 6,248,558, 6,432,680 and 6,248,558.

In certain embodiments, the immunogenic polypeptide is conjugated (i.e. covalently bound) to another molecule. This may, for example, increase the half-life, solubility, bioavailability, or immunogenicity of the antigen. Molecules that may be conjugated to an immunogenic polypeptide include a carbohydrate, biotin, poly(ethylene glycol) (PEG), polysialic acid, N-propionylated polysialic acid, nucleic acids, polysaccharides, and PLGA. There are many different types of PEG, ranging from molecular weights of below 300 g/mol to over 10,000,000 g/mol. PEG chains can be linear, branched, or with comb or star geometries.

**B. Immunogenic HSV-2 polypeptides and nucleic acids for use in vaccines**

In certain embodiments, one or more, e.g. two, three, four, or more immunogenic fragments or variants thereof are provided in a mixture. For example, a vaccine formulation may comprise any one or more of SEQ ID NOS: 1-26.

In certain embodiments, a vaccine formulation may comprise any one, two, or three of ICP4, ICP4.2, gL2, gD2ΔTMR and gD2 (SEQ ID NOS: 1-5), or immunogenic fragment(s) thereof. In certain embodiments, combinations contain polypeptides or immunogenic fragments from only one of ICP4 (SEQ ID NO 1) and ICP4.2 (SEQ ID NO 2). In other embodiments, combinations contain polypeptides or immunogenic fragments from only one of gD2ΔTMR (SEQ ID NO:4) and gD2 (SEQ ID NO:5).

Exemplary combinations of ICP4, ICP4.2, gL2, gD2ΔTMR and gD2 include:

| **Two antigen combinations** | |
|---|---|
| ICP4<br>SEQ ID NO: 1 | gL2<br>SEQ ID NO: 3 |
| ICP4<br>SEQ ID NO: 1 | gD2ΔTMR<br>SEQ ID NO: 4 |
| ICP4<br>SEQ ID NO: 1 | gD2<br>SEQ ID NO: 5 |
| ICP4.2<br>SEQ ID NO: 2 | gL2<br>SEQ ID NO: 3 |
| ICP4.2<br>SEQ ID NO: 2 | gD2ΔTMR<br>SEQ ID NO: 4 |
| ICP4.2<br>SEQ ID NO: 2 | gD2<br>SEQ ID NO: 5 |
| gL2<br>SEQ ID NO: 3 | gD2ΔTMR<br>SEQ ID NO: 4 |
| gL2<br>SEQ ID NO: 3 | gD2<br>SEQ ID NO: 5 |

| **Three antigen combinations** | | |
|---|---|---|
| ICP4<br>SEQ ID NO: 1 | gL2<br>SEQ ID NO: 3 | gD2ΔTMR<br>SEQ ID NO: 4 |
| ICP4.2<br>SEQ ID NO: 2 | gL2<br>SEQ ID NO: 3 | gD2ΔTMR<br>SEQ ID NO: 4 |
| ICP4<br>SEQ ID NO: 1 | gL2<br>SEQ ID NO: 3 | gD2<br>SEQ ID NO: 5 |
| ICP4.2<br>SEQ ID NO: 2 | gL2<br>SEQ ID NO: 3 | gD2<br>SEQ ID NO: 5 |

The individual antigens and combinations described above can also include additional peptides from or derived from HSV-2, such as polypeptides comprising sequences selected from SEQ ID NO:6-26 or immunogenic fragments thereof.

### *1. ICP4 (SEQ ID NO: 1) encoded by RS1*

RS1 encodes ICP4, a transcriptional transactivator that may interact with and recruit specific components of the general transcription machinery to viral promoters and stabilize their formation for transcription initiation. ICP4 contains distinct domains for transactivation/phosphorylation (approximately spanning acid residues 150-200 of SEQ ID NO:1), DNA binding (approximately spanning residues 380-540 of SEQ ID NO:1), nuclear localization (approximately spanning residues 630-730 of SEQ ID NO:1), and late regulatory transactivation (approximately spanning residues 1220-1319 of SEQ ID NO:1). The DNA and protein sequence of RS1 may be found by searching for RS1 in the publicly available database, Entrez Gene (on the NCBI NIH web site on the World Wide Web, at www.ncbi.nlm.nih.gov/sites/entrez?db=gene), in the Human herpesvirus 2 complete genome.

In some embodiments, vaccines against HSV-2 include a polypeptide containing at least 20 consecutive amino acid residues selected from residues 383-766 of ICP4 (SEQ ID NO: 1), but no more than 1000 amino acids of ICP4 (SEQ ID NO: 1). The polypeptide may also be a variant of the at least 20 residue fragment.

In certain embodiments, the polypeptide includes no more than 950, 900, 850, 800, 750, 700, 650, 600, 550, 500, 450 or even 400 consecutive amino acids from ICP4. Exemplary polypeptides correspond approximately to amino acids residues of full-length ICP4 as follows: 383-766 (RS1.2); 1-400 (RS1.1); 750-1024 (RS1.3.1); 1008-1319 (RS1.3.2); 750-1319 (RS1.3); 280-785 (RS1.4 comprising the full DNA binding region); 680-1319 (RS1.5 comprising the glycosylase/C-terminal region); 208-1319 (RS1.6 which may also comprise a Met residue at the N-term end); 1-380 plus 545-1319 (RS1.7, in which a region spanning approximately residues 381-544 is deleted, removing the DNA binding regions); 1-785 plus 870-1319 (RS1.8, in which a region spanning approximately residues 786-869 is deleted,

removing the nuclear localization domain), or 1-766, 383-1318, 100-750, 400-1300, 250-766, 383-900 of ICP4 (SEQ ID NO. 1) and the like.

**2. ICP4 internal fragment ICP4.2 (SEQ ID NO: 2) encoded by RS1.2**

RS1.2 encodes a 391 amino acid fragment of ICP4, denoted ICP4.2.

In specific embodiments, vaccines against HSV-2 include a polypeptide containing from 50 to all 391 amino acids residues of ICP4.2 (SEQ ID NO: 2), such as from 100 to 391, 200 to 391 or 250 to 350 residues. In particular embodiments, the polypeptide includes all of ICP4.2 (SEQ ID NO: 2) or is ICP4.2 (SEQ ID NO: 2) itself. These polypeptides may, for example, include the full length or fragments of ICP4.2 (SEQ ID NO:2) described herein with amino acids residues 1-382 or 767-1318 of ICP4 (SEQ ID NO. 1) or fragments thereof, which, in certain embodiments, are consecutive with the amino acid residues of ICP4.2 being used. Exemplary fragments that combine the residues of SEQ ID NO:2 with select residues from 1-382 or 767-1318 of SEQ ID NO:1 are described above.

An immunogenic fragment of ICP4.2 comprises at least one immunogenic portion, as measured experimentally or identified by algorithm. Peptides identified by such methods include the following:

GLAHVAAAV (SEQ ID NO:47)
FISGSVARA (SEQ ID NO:48)
QYALITRLL (SEQ ID NO:49)
RYDRAQKGF (SEQ ID NO:50)
GYAMAAGRF (SEQ ID NO:51)
PPHADAPRL (SEQ ID NO:52)
KPAAAAAPL (SEQ ID NO:53)
SEAAVAAV (SEQ ID NO:54)
FGWGLAHV (SEQ ID NO:55)
YALITRLLY (SEQ ID NO:56)
ALPRSPRLL (SEQ ID NO:57)
DLLFQNQSL (SEQ ID NO:58)
ADLLFQNQS (SEQ ID NO:59)

ARNSSSFIS (SEQ ID NO:60)
QACFRISGA (SEQ ID NO:61)
FVRDALVLM (SEQ ID NO:62)
FDGDLAAVP (SEQ ID NO:63)
GLGDSRPGL (SEQ ID NO:64)
WAPELGDAA (SEQ ID NO:65)
ECLAACRGI (SEQ ID NO:66)
RAWLRELRF (SEQ ID NO:67).

Thus, in some aspects, this application provides an immunogenic fragment of ICP4.2. The fragments, in some instances, are close in size to the full-length polypeptide. For example, they may lack at most one, two, three, four, five, ten, or twenty amino acids from one or both termini. In other embodiments, the fragment is 100-391 amino acids in length, or 150-391, or 200-391, or 250-391 amino acids in length. Other exemplary fragments are amino acid residues 1-350, 1-300, 1-250, 1-200, 1-150, 1-100, 1-50, 50-391, 50-350, 50-300, 50-250, 50-200, 50-150, 50-100 , 100-391, 100-350, 100-300, 100-250, 100-200, 100-150, 150-391, 150-350, 150-300, 150-250, 150-200, 200-391, 200-350, 200-300, 200-250, 250-391, 250-350, 250-300, 300-391 and 350-391. The fragments described above or sub-fragments thereof (e.g., fragments of 8-50, 8-30, or 8-20 amino acid residues) preferably have one of the biological activities described below, such as increasing the T cell response by at least 1.5 fold or 2 fold. A fragment may be used as the polypeptide in the vaccines described herein or may be fused to another protein, protein fragment or a polypeptide.

In certain aspects, this application provides immunogenic polypeptides with at least 90%, 95%, 97%, 98%, 99%, or 99.5% identity to ICP4.2 or an immunogenic fragment thereof.

### 3. Glycoprotein L-2 (SEQ ID NO: 3) encoded by UL1

UL1 encodes Glycoprotein L-2 (gL2), a heterodimer glycoprotein that is required for the fusion of viral and cellular membranes and enables the virus to enter

the host cell. The DNA and protein sequence of UL1 may be found by searching in the publicly available database, Entrez Gene (on the NCBI NIH web site on the World Wide Web, at ww.ncbi.nlm.nih.gov/sites/entrez?db=gene), in the Human herpesvirus 2 complete genome.

In some embodiments, vaccines against HSV-2 include a polypeptide containing at least 20 consecutive amino acid residues selected from residues 1-224 of gL2 (SEQ ID NO: 3), but no more than 224 amino acids of gL2 (SEQ ID NO: 3). The polypeptide may also be a variant of the at least 20 residue fragment.

In some embodiments, the polypeptide is at least 85% identical to a fragment of 200-250 amino acids of SEQ ID NO: 3.

In certain embodiments, the polypeptide includes no more than 200 or 100 consecutive amino acids from gL2. Exemplary polypeptides are amino acids residues 1-20, 21-40, 41-60, of 61-80, 81-100, 101-120, 121-140, 141-160, 161-180, 181-200, 201-221 of gL2 (SEQ ID NO. 3) and the like.

In other aspects, this application provides an immunogenic fragment of gL2. An immunogenic fragment of gL2 comprises at least one immunogenic portion, as measured experimentally or identified by algorithm. Peptides identified by such methods include the following:

AYLVNPFLF (SEQ ID NO: 100)
PFLFAAGFL (SEQ ID NO: 101)
TEYVLRSVI (SEQ ID NO: 102)
GSQATEYVL (SEQ ID NO: 103)
RIDGIFLRY (SEQ ID NO: 104)
FLEDLSHSV (SEQ ID NO: 105)
YVLRSVIAK (SEQ ID NO: 106)
YVLRSVIAK (SEQ ID NO: 107)
AYLVNPFLF (SEQ ID NO: 108)
ETTTRRALY (SEQ ID NO: 109)
RIDGIFLRY (SEQ ID NO: 110)
YLVNPFLFA (SEQ ID NO: 111)
FVCLFGLVV (SEQ ID NO: 112)

LYKEIRDAL (SEQ ID NO: 113)
GLDTFLWDR (SEQ ID NO: 114)
RVSPTRGRR (SEQ ID NO: 115)
YVLRSVIAK (SEQ ID NO: 115)
GLDTFLWDR (SEQ ID NO: 116)
DILRVPCMR (SEQ ID NO: 117)
DRHAQRAYL (SEQ ID NO: 118)

**4. Glycoprotein D-2 (SEQ ID NO: 5)encoded by US6 and internally-deleted Glycoprotein D-2 (SEQ ID NO:4) encoded by US6ΔTMR**

US6 encodes envelope glycoprotein D-2 (gD2), an envelope glycoprotein that binds to host cell entry receptors and may trigger fusion of the virus with the host membrane. The gD2 protein has several distinct domains, including a signal domain (amino acid residues 1-25) which is cleaved from the mature protein, and a transmembrane domain (spanning approximately amino acids residues 340-363). The DNA and protein sequence of US6 may be found by searching in the publicly available database, Entrez Gene (on the NCBI NIH web site on the World Wide Web, at www.ncbi.nlm.nih.gov/sites/entrez?db=gene), in the Human herpesvirus 2 complete genome.

In some embodiments, vaccines against HSV-2 include a polypeptide comprising gD2 that is missing all or part of the transmembrane domain (which spans approximately amino acids residues 340-363 inclusive) as well as the signal sequence. In other embodiments, the deleted region may additionally include 5-10 amino acids of the sequence flanking the transmembrane domain. The deleted region may also comprise a portion of the transmembrane domain, for example at least 3 amino acids between residues 340-363. In some embodiments, at least one residue in the transmembrane domain has been modified, deleted or substituted, such that the transmembrane domain is no longer functional. For example, a variant may have its internal deletion begin at amino acid residue 336, 337, 338, 339, 340, 341, 342, 343, 344, 345 or 346 and end at amino acid residue 358, 359, 360, 361, 362, 363, 364, 365, 366, 367 or 368.

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A construct encoding gD2 which is missing amino acid residues 340-363 (the transmembrane domain) is called US6ΔTMR (SEQ ID NO: 40). The corresponding protein is denoted gD2ΔTMR (SEQ ID NO:4). In other embodiments, an immunogenic fragment of gD2 or gD2ΔTMR may comprise a deletion in a portion of the transmembrane domain, and/or may comprise a deletion in the flanking sequence outside of the transmembrane domain.

In other aspects, this application provides an immunogenic fragment of gD2 or gD2ΔTMR. An immunogenic fragment of gD2 or gDΔTMR comprises at least one immunogenic portion, as measured experimentally or identified by algorithm. Peptides identified by such methods include the following:

ALAGSTLAV (SEQ ID NO.68)
LLEDPAGTV (SEQ ID NO.69)
VIGGIAFWV (SEQ ID NO.70)
TVYYAVLER (SEQ ID NO.71)
KYALADPSL (SEQ ID NO.72)
AFETAGTYL (SEQ ID NO.73)
APSNPGLII (SEQ ID NO.74)
IPITVYYAV (SEQ ID NO.75)
APPSHQPLF (SEQ ID NO.76)
FLMHAPAFE (SEQ ID NO.77)
FSAVSEDNL (SEQ ID NO.78)
VYYAVLER (SEQ ID NO.79)
IGMLPRFI (SEQ ID NO.80)
YTECPYNKS (SEQ ID NO.81)
FLMHAPAFE (SEQ ID NO.82)
NLGFLMHAP (SEQ ID NO.83)
VIGGIAFWV (SEQ ID NO.84)
GIAFWVRRR (SEQ ID NO.85)
SEDNLGFLM (SEQ ID NO.86)
RTQPRWSYY (SEQ ID NO.87)
IAFWVRRRA (SEQ ID NO.88)
LVIGGIAFW (SEQ ID NO.89)

FWVRRRAQM (SEQ ID NO.90)

PYTSTLLPP (SEQ ID NO.91)

VGTAALLVV (SEQ ID NO.92)

TAALLVVAV (SEQ ID NO.93)

TSTLLPPEL (SEQ ID NO.94)

GTVSSQIPP (SEQ ID NO.95)

TAGTYLRLV (SEQ ID NO.96)

GVTVDSIGM (SEQ ID NO.97)

AFWVRRRAQ (SEQ ID NO.98)

RVYHIQPSL (SEQ ID NO.99)

Thus, in some aspects, this application provides an immunogenic fragment of gD2 (SEQ ID NO:5) or gDΔTMR (SEQ ID NO: 4). The fragments, in some instances, are close in size to the full-length polypeptide. For example, they may lack at most one, two, three, four, five, ten, or twenty amino acids from one or both termini. In other embodiments, the fragment is 100-393 amino acids in length, or 150-393, or 200-393, or 250-393 amino acids in length. Other exemplary fragments are amino acid residues 1-350, 1-300, 1-250, 1-200, 1-150, 1-100, 1-50, 50-393, 50-350, 50-300, 50-250, 50-200, 50-150, 50-100, 100-393, 100-350, 100-300, 100-250, 100-200, 100-150, 150-393, 150-350, 150-300, 150-250, 150-200, 200-383, 200-350, 200-300, 200-250, 250-393, 250-350, 250-300, 300-393 and 350-393. The fragments described above or sub-fragments thereof (e.g., fragments of 8-50, 8-30, or 8-20 amino acid residues) preferably have one of the biological activities described below, such as increasing the T cell response by at least 1.5 fold or 2 fold. A fragment may be used as the polypeptide in the vaccines described herein or may be fused to another protein, protein fragment or a polypeptide.

In other embodiments, the polypeptide comprises the entire sequence of SEQ ID NO: 4 or SEQ ID NO:5, or consists of the entire sequence of SEQ ID NO: 4 or SEQ ID NO:5. In certain embodiments, an immunogenic fragment of gD2 retains all or part of the signal domain (amino acid residues 1-25) and/or the transmembrane domain (amino acids residues 340-363).

In certain embodiments, polypeptides have less than 20%, 30%, 40%, 50%, 60% or 70% homology with human autoantigens. Examples of such autoantigens include UL6 from HSV-1 and gK or UL53 from HSV-2.

In certain aspects, this application provides immunogenic polypeptides with at least 90%, 95%, 97%, 98%, 99%, or 99.5% identity to gDΔTMR, or an immunogenic fragment thereof.

**C. Additional features of HSV-2 polypeptides**

Typically, the polypeptides present in the vaccine formulations or pharmaceutical compositions described herein are immunogenic, either alone or as a variant, which includes polypeptides fused to another polypeptide or mixed with or complexed to an adjuvant. Variants also include sequences with less than 100% sequence identity, as described herein. In addition, one may use fragments, precursors and analogs that have an appropriate immunogenicity.

These polypeptides may be immunogenic in mammals, for example, mice, guinea pigs, or humans. An immunogenic polypeptide is typically one capable of raising a significant immune response in an assay or in a subject. Alternatively, an immunogenic polypeptide may (i) induce production of antibodies, e.g., neutralizing antibodies, that bind to the polypeptide (ii) induce $T_H1$ immunity, (iii) activate the CD8+ CTL response, for example by increasing CD8+ T cells and/or increasing localization of CD8+ T cells to the site of infection or reinfection, (iv) induce $T_H17$ immunity, and/or (v) activate innate immunity. In some embodiments, an immunogenic polypeptide causes the production of a detectable amount of antibody specific to that antigen.

In certain embodiments, polypeptides have less than 20%, 30%, 40%, 50%, 60% or 70% homology with human autoantigens.

A polypeptide may comprise one or more immunogenic portions and one or more non-immunogenic portions. The immunogenic portions may be identified by various methods, including protein microarrays, ELISPOT/ELISA techniques, and/or specific assays on different deletion mutants (e.g., fragments) of the polypeptide in question. Immunogenic portions may also be identified by computer algorithms. Some such algorithms, like EpiMatrix (produced by EpiVax), use a computational matrix approach. Other computational tools for identifying antigenic epitopes include PEPVAC (Promiscuous EPitope-based VACcine, hosted by Dana Farber Cancer Institute on the world wide web at immunax.dfci.harvard.edu/PEPVAC), MHCPred (which uses a partial least squares approach and is hosted by The Jenner Institute on the world wide web at www.jenner.ac.uk/MHCPred), and Syfpeithi, hosted on the world wide web at www.syfpeithi.de/.

In some embodiments, the vaccine or pharmaceutical composition may comprise fusion proteins and/or fusion DNA constructs. The underlying DNA sequences above may be modified in ways that do not affect the sequence of the protein product. For instance, the DNA sequence may be codon-optimized to improve expression in a host such as *E. coli* or an insect cell line (e.g. using the baculovirus expression system) or mammalian (e.g. Chinese Hamster Ovary) cell line. In particular embodiments, such as when smaller related polypeptides, including those having a molecular weight less than about 5000 daltons, e.g., 1500 to 5000 daltons, are used, modification may be useful in eliciting the desired immune response. For example, the smaller polypeptides can be conjugated to an appropriate immunogenic carrier such as proteins from other pathogenic organisms or viruses (e.g.,tetanus toxoid), large proteins (e.g., keyhole limpet hemocyanin) or the like. Conjugation may be direct or indirect (e.g., via a linker). In other particular embodiments, a fusion protein may comprise a polypeptide disclosed above or an immunogenic fragment or variant thereof and a tag. A tag may be N-terminal or C-terminal. For instance, tags may be added to the nucleic acid or polypeptide to facilitate purification, detection, solubility, or confer other desirable

characteristics on the protein or nucleic acid. For instance, a purification tag may be a peptide, oligopeptide, or polypeptide that may be used in affinity purification. Examples include His, GST, TAP, FLAG, myc, HA, MBP, VSV-G, thioredoxin, V5, avidin, streptavidin, BCCP, Calmodulin, Nus, S tags, lipoprotein D, and β galactosidase. In some embodiments, the fused portion is short. Thus, in some instances, the fusion protein comprises no more than 1, 2, 3, 4, 5, 10, 20, or 50 additional amino acids on one or both termini of a polypeptide described above, such as consecutive amino acids from any of the polypeptides in Table 1.

In some embodiments, tags, secretion signals, or other signal sequences may be added to the C-terminal end and/or to the N-terminal end of the polypeptide. Tags may be used to aid in purification of expresssed polypeptides. Exemplary tags include HHHHHH (SEQ ID NO: 130) and MSYYHHHHHH (SEQ ID NO: 131). Secretion signals may be optimized for use with non-mammalian cells, such as insect cells. An exemplary secretion signal is MKFLVNVALVFMVVYISYIYA (SEQ ID NO: 132).

A detection tag may be used to detect the tag and, consequently, any amino acid sequence fused to it. Detection tags include fluorescent proteins, proteins that bind a fluorescent label, and proteins that bind an electron-dense moeity. Examples of fluorescent proteins include dsRed, mRFP, YFP, GFP, CFP, BFP, and Venus. An example of a protein that binds a fluorescent or electron-dense label is FlAsH.

Another aspect disclosed herein is an antibody preparation generated against a composition of the invention (e.g., a composition comprising one or more or two or more of the polypeptides listed in Table 1). Any of a variety of antibodies are included. Such antibodies include, e.g., polyclonal, monoclonal, recombinant, humanized or partially humanized, single chain, Fab, and fragments thereof, etc. The antibodies can be of any isotype, e.g., IgA, IgG, various IgG isotypes such as $IgG_1$, $IgG_2$, $IgG_{2a}$, $IgG_{2b}$, $IgG_3$, $IgG_4$, etc.; and they can be from any animal species that produces antibodies, including goat, rabbit, mouse, chicken or the like. In some embodiments, Fab molecules are expressed and assembled in a genetically transformed host like *E. coli*. A lambda vector system is available thus to express a

population of Fab's with a potential diversity equal to or exceeding that of subject generating the predecessor antibody. See Huse et al. (1989), Science 246, 1275-81.

## D. Components of vaccines and pharmaceutical compositions

In certain embodiments, the vaccines and pharmaceutical compositions comprise one or more of the polypeptides and nucleic acids described above and one or more of the following: an adjuvant, stabilizer, buffer, surfactant, controlled release component, salt, preservative, and an antibody specific to said antigen.

### *1. Adjuvants*

The vaccine formulations and pharmaceutical compositions described herein may each include an adjuvant. Adjuvants can be broadly separated into two classes, based on their principal mechanisms of action: vaccine delivery systems and immunostimulatory adjuvants (see, *e.g.*, Singh *et al.*, *Curr. HIV Res.* 1:309-20, 2003). Vaccine delivery systems are often particulate formulations, *e.g.*, emulsions, microparticles, immune-stimulating complexes (ISCOMs), which may be, for example, particles and/or matrices, and liposomes. In contrast, immunostimulatory adjuvants are sometimes derived from pathogens and can represent pathogen associated molecular patterns (PAMP), *e.g.*, lipopolysaccharides (LPS), monophosphoryl lipid (MPL), or CpG-containing DNA, which activate cells of the innate immune system.

Alternatively, adjuvants may be classified as organic and inorganic. Inorganic adjuvants include alum salts such as aluminum phosphate, amorphous aluminum hydroxyphosphate sulfate, and aluminum hydroxide, which are commonly used in human vaccines. Organic adjuvants comprise organic molecules including macromolecules. An example of an organic adjuvant is cholera toxin.

Adjuvants may also be classified by the response they induce, and adjuvants can activate more than one type of response. In some embodiments, the adjuvant induces the activation of CD4+ T cells. The adjuvant may induce activation of $T_H1$ cells and/or activation of $T_H17$ cells and/or activation of $T_H2$ cells. Alternately, the adjuvant may induce activation of $T_H1$ cells and/or $T_H17$ cells but not activation of

$T_H2$ cells, or vice versa. In some embodiments, the adjuvant induces activation of CD8+ T cells. In further embodiments, the adjuvant may induce activation of Natural Killer T (NKT) cells. In some embodiments, the adjuvant induces the activation of $T_H1$ cells or $T_H17$ cells or $T_H2$ cells. In other embodiments, the adjuvant induces the activation of B cells. In yet other embodiments, the adjuvant induces the activation of antigen-presenting cells. These categories are not mutually exclusive; in some cases, an adjuvant activates more than one type of cell.

In certain embodiments, an adjuvant is a substance that increases the numbers or activity of antigen presenting cells such as dendritic cells. In certain embodiments, an adjuvant promotes the maturation of antigen presenting cells such as dendritic cells. In some embodiments, the adjuvant is or comprises a saponin. Typically, the saponin is a triterpene glycoside, such as those isolated from the bark of the Quillaja saponaria tree. A saponin extract from a biological source can be further fractionated (e.g., by chromatography) to isolate the portions of the extract with the best adjuvant activity and with acceptable toxicity. Typical fractions of extract from Quillaja saponaria tree used as adjuvants are known as fractions A and C. An exemplary saponin adjuvant is QS-21, which is available from Antigenics. QS-21 is an oligosaccharide-conjugated small molecule. Optionally, QS-21 may be admixed with a lipid such as 3D-MPL or cholesterol.

A particular form of saponins that may be used in vaccine formulations described herein is immunostimulating complexes (ISCOMs). ISCOMs are an art-recognized class of adjuvants, that generally comprise Quillaja saponin fractions and lipids (e.g., cholesterol and phospholipids such as phosphatidyl choline). In certain embodiments, an ISCOM is assembled together with a polypeptide or nucleic acid of interest. However, different saponin fractions may be used in different ratios. In addition, the different saponin fractions may either exist together in the same particles or have substantially only one fraction per particle (such that the indicated ratio of fractions A and C are generated by mixing together particles with the different fractions). In this context, "substantially" refers to less than 20%, 15%, 10%, 5%, 4%, 3%, 2% or even 1%. Such adjuvants may comprise fraction A and fraction C mixed into a ratio of 70-95 A: 30-5 C, such as 70 A : 30 C to 75 A : 25 C, 75 A : 25 C to 80 A : 20 C, 80 A : 20 C to 85 A : 15 C, 85 A : 15 C to 90 A : 10 C, 90

A : 10 C to 95 A : 5 C, or 95 A : 5 C to 99 A : 1 C. ISCOMatrix, produced by CSL, and AbISCO 100 and 300, produced by Isconova, are ISCOM matrices comprising saponin, cholesterol and phospholipid (lipids from cell membranes), which form cage-like structures typically 40-50 nm in diameter. Posintro, produced by Nordic Vaccines, is an ISCOM matrix where the immunogen is bound to the particle by a multitude of different mechanisms, e.g. electrostatic interaction by charge modification, incorporation of chelating groups or direct binding.

In some embodiments, the adjuvant is a TLR ligand. TLRs are proteins that may be found on leukocyte membranes, and recognize foreign antigens (including microbial antigens). An exemplary TLR ligand is IC-31, which is available from Intercell. IC31 comprises an anti-microbial peptide, KLK, and an immunostimulatory oligodeoxynucleotide, ODN1a. IC31 has TLR9 agonist activity. Another example is CpG-containing DNA, and different varieties of CpG-containing DNA are available from Prizer (Coley): VaxImmune is CpG 7909 (a (CpG)-containing oligodeoxy-nucleotide), and Actilon is TLR9 agonist, CpG 10101 (a (CpG)-containing oligodeoxy-nucleotide).

In some embodiments, the adjuvant is a nanoemulsion. One exemplary nanoemulsion adjuvant is Nanostat Vaccine, produced by Nanobio. This nanoemulsion is a high-energy, oil-in-water emulsion. This nanoemulsion typically has a size of 150-400 nanometers, and includes surfactants to provide stability. More information about Nanostat can be found in US Patents 6,015,832, 6,506,803, 6,559,189, 6,635,676, and 7,314,624.

Adjuvants may be covalently bound to antigens (e.g., the polypeptides described above). In some embodiments, the adjuvant may be a protein which induces inflammatory responses through activation of antigen-presenting cells (APCs). In some embodiments, one or more of these proteins can be recombinantly fused with an antigen of choice, such that the resultant fusion molecule promotes dendritic cell maturation, activates dendritic cells to produce cytokines and chemokines, and ultimately, enhances presentation of the antigen to T cells and initiation of T cell responses (see Wu et al., Cancer Res 2005; 65(11), pp 4947-

4954). Other exemplary adjuvants that may be covalently bound to antigens comprise polysaccharides, synthetic peptides, lipopeptides, and nucleic acids.

The adjuvant can be used alone or in combination of two or more kinds. Adjuvants may be directly conjugated to antigens. Adjuvants may also be combined to increase the magnitude of the immune response to the antigen. Typically, the same adjuvant or mixture of adjuvants is present in each dose of a vaccine. Optionally, however, an adjuvant may be administered with the first dose of vaccine and not with subsequent doses (i.e. booster shots). Alternatively, a strong adjuvant may be administered with the first dose of vaccine and a weaker adjuvant or lower dose of the strong adjuvant may be administered with subsequent doses. The adjuvant can be administered before the administration of the antigen, concurrent with the administration of the antigen or after the administration of the antigen to a subject (sometimes within 1, 2, 6, or 12 hours, and sometimes within 1, 2, or 5 days). Certain adjuvants are appropriate for human patients, non-human animals, or both.

### *2. Additional components of vaccines and pharmaceutical compositions*

In addition to the antigens and the adjuvants described above, a vaccine formulation or pharmaceutical composition may include one or more additional components.

In certain embodiments, the vaccine formulation or pharmaceutical composition may include one or more stabilizers such as sugars (such as sucrose, glucose, or fructose), phosphate (such as sodium phosphate dibasic, potassium phosphate monobasic, dibasic potassium phosphate, or monosodium phosphate), glutamate (such as monosodium L-glutamate), gelatin (such as processed gelatin, hydrolyzed gelatin, or porcine gelatin), amino acids (such as arginine, asparagine, histidine, L-histidine, alanine, valine, leucine, isoleucine, serine, threonine, lysine, phenylalanine, tyrosine, and the alkyl esters thereof), inosine, or sodium borate.

In certain embodiments, the vaccine formulation or pharmaceutical composition includes one or more buffers such as a mixture of sodium bicarbonate and ascorbic acid. In some embodiments, the vaccine formulation may be administered in saline, such as phosphate buffered saline (PBS), or distilled water.

In certain embodiments, the vaccine formulation or pharmaceutical composition includes one or more surfactants such as polysorbate 80 (Tween 80), Triton X-100, Polyethylene glycol tert-octylphenyl ether t-Octylphenoxypolyethoxyethanol 4-(1,1,3,3-Tetramethylbutyl)phenyl-polyethylene glycol (TRITON X-100); Polyoxyethylenesorbitan monolaurate Polyethylene glycol sorbitan monolaurate (TWEEN 20); and 4-(1,1,3,3-Tetramethylbutyl)phenol polymer with formaldehyde and oxirane (TYLOXAPOL). A surfactant can be ionic or nonionic.

In certain embodiments, the vaccine formulation or pharmaceutical composition includes one or more salts such as sodium chloride, ammonium chloride, calcium chloride, or potassium chloride.

In certain embodiments, a preservative is included in the vaccine. In other embodiments, no preservative is used. A preservative is most often used in multi-dose vaccine vials, and is less often needed in single-dose vaccine vials. In certain embodiments, the preservative is 2-phenoxyethanol, methyl and propyl parabens, benzyl alcohol, and/or sorbic acid.

In certain embodiments, the vaccine formulation or pharmaceutical composition is a controlled release formulation.

## E. DNA vaccines

In certain aspects, the vaccine comprises one of the nucleic acids disclosed herein. When a nucleic acid vaccine is administered to a patient, the corresponding gene product (such as a desired antigen) is produced in the patient's body. In some embodiments, nucleic acid vaccine vectors that include optimized recombinant polynucleotides can be delivered to a mammal (including humans) to induce a therapeutic or prophylactic immune response. The nucleic acid may be, for example, DNA, RNA, or a synthetic nucleic acid. The nucleic acid may be single stranded or double stranded.

Nucleic acid vaccine vectors (e.g., adenoviruses, liposomes, papillomaviruses, retroviruses, etc.) can be administered directly to the mammal for

transduction of cells *in vivo*. The nucleic acid vaccines can be formulated as pharmaceutical compositions for administration in any suitable manner, including parenteral administration.

In determining the effective amount of the vector to be administered in the treatment or prophylaxis of an infection or other condition, the physician evaluates vector toxicities, progression of the disease, and the production of anti-vector antibodies, if any. Often, the dose equivalent of a naked nucleic acid from a vector is from about 1 μg to 1 mg for a typical 70 kilogram patient, and doses of vectors used to deliver the nucleic acid are calculated to yield an equivalent amount of therapeutic nucleic acid. Administration can be accomplished via single or divided doses. The toxicity and therapeutic efficacy of the nucleic acid vaccine vectors can be determined using standard pharmaceutical procedures in cell cultures or experimental animals.

A nucleic acid vaccine can contain DNA, RNA, a modified nucleic acid, or a combination thereof. In some embodiments, the vaccine comprises one or more cloning or expression vectors; for instance, the vaccine may comprise a plurality of expression vectors each capable of autonomous expression of a nucleotide coding region in a mammalian cell to produce at least one immunogenic polypeptide. An expression vector often includes a eukaryotic promoter sequence, such as the nucleotide sequence of a strong eukaryotic promoter, operably linked to one or more coding regions. The compositions and methods herein may involve the use of any particular eukaryotic promoter, and a wide variety are known; such as a CMV or RSV promoter. The promoter can be, but need not be, heterologous with respect to the host cell. The promoter used may be a constitutive promoter.

A vector useful in the present compositions and methods can be circular or linear, single-stranded or double stranded and can be a plasmid, cosmid, or episome. In a suitable embodiment, each nucleotide coding region is on a separate vector; however, it is to be understood that one or more coding regions can be present on a single vector, and these coding regions can be under the control of a single or multiple promoters.

Numerous plasmids may be used for the production of nucleic acid vaccines. Suitable embodiments of the nucleic acid vaccine employ constructs using the plasmids VR1012 (Vical Inc., San Diego Calif.), pCMVI.UBF3/2 (S. Johnston, University of Texas) or pcDNA3.1 (InVitrogen Corporation, Carlsbad, Calif.) as the vector. In addition, the vector construct can contain immunostimulatory sequences (ISS), such as unmethylated dCpG motifs, that stimulate the animal's immune system. The nucleic acid vaccine can also encode a fusion product containing the immunogenic polypeptide. Plasmid DNA can also be delivered using attenuated bacteria as delivery system, a method that is suitable for DNA vaccines that are administered orally. Bacteria are transformed with an independently replicating plasmid, which becomes released into the host cell cytoplasm following the death of the attenuated bacterium in the host cell.

An alternative approach to delivering the nucleic acid to an animal involves the use of a viral or bacterial vector. Examples of suitable viral vectors include adenovirus, polio virus, pox viruses such as alphaviruses, vaccinia, canary pox, and fowl pox, herpes viruses, including catfish herpes virus, adenovirus-associated vector, and retroviruses. Virus-like vectors include virosomes and virus-like particles. Exemplary bacterial vectors include attenuated forms of Salmonella, Shigella, Edwardsiella ictaluri, Yersinia ruckerii, and Listeria monocytogenes. In some embodiments, the nucleic acid is a vector, such as a plasmid, that is capable of autologous expression of the nucleotide sequence encoding the immunogenic polypeptide.

## F. Use of Vaccines

The vaccines described herein may be used for prophylactic and/or therapeutic treatment of herpes, including HSV-1 and particularly HSV-2. The subject receiving the vaccination may be a male or a female, and may be a child or adult. In some embodiments, the subject being treated is a human. In other embodiments, the subject is a non-human animal.

### *1. Prophylactic use*

In prophylactic embodiments, the HSV-2 vaccine is administered to a subject to induce an immune response that can help protect against the establishment of HSV-2.

In some embodiments, the vaccine compositions of the invention confer protective immunity, allowing a vaccinated individual to exhibit delayed onset of symptoms or reduced severity of symptoms (e.g., reduced number of lesions at the onset of infection), as the result of his/her exposure to the vaccine (e.g., a memory response). In certain embodiments, the reduction in severity of symptoms is at least 25%, 40%, 50%, 60%, 70%, 80% or even 90%. Some vaccinated individuals may display no symptoms upon contact with HSV-2 or even no infection by HSV-2. Protective immunity is typically achieved by one or more of the following mechanisms: mucosal, humoral, or cellular immunity. Mucosal immunity is primarily the result of secretory IgA (sIGA) antibodies on mucosal surfaces of the respiratory, gastrointestinal, and genitourinary tracts. The sIGA antibodies are generated after a series of events mediated by antigen-processing cells, B and T lymphocytes, that result in sIGA production by B lymphocytes on mucosa-lined tissues of the body. Humoral immunity is typically the result of IgG antibodies and IgM antibodies in serum. For example, the IgG titer can be raised by 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 50-fold, or even 100-fold or more following administration of a vaccine formulation described herein. Cellular immunity can be achieved through cytotoxic T lymphocytes or through delayed-type hypersensitivity that involves macrophages and T lymphocytes, as well as other mechanisms involving T cells without a requirement for antibodies. In particular, cellular immunity may be mediated by $T_H1$ cells or $T_H17$ cells. Activation of $T_H1$ cells can be measured by secretion of IFN-γ, relative to the level of IFN-γ released in response to a polypeptide that does not generate an immunologic response. In certain embodiments, the amount of IFN-γ released in 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 50-fold or even 100-fold greater. The primary result of protective immunity is the destruction of HSV-2 viral particles or inhibition of HSV-2's ability to replicate. In some embodiments, the protective immunity conferred by presentation of antigen before exposure to HSV-2 will reduce the likelihood of seroconversion to an HSV-2-positive status.

The duration of protective immunity is preferably as long as possible. In certain embodiments, vaccine formulations produce protective immunity lasting six months, one year, two years, five years, ten years, twenty years or even a lifetime.

***2. Therapeutic use***

In therapeutic applications, the vaccine comprising a polypeptide or nucleic acid of the invention may be administered to a patient suffering from HSV-2, in an amount sufficient to treat the patient. Treating the patient, in this case, may refer to delaying or reducing symptoms of HSV-2 in an infected individual. In some embodiments, treating the patient refers to reducing the duration of lesions, reducing the number of lesions, reducing the duration of symptoms per episode, and/or otherwise reducing the intensity of symptoms per episode. In certain embodiments, the vaccine reduces the duration or severity of mild symptoms; in some embodiments, the vaccine reduces the duration or severity of serious symptoms. In some embodiments, the vaccine reduces viral shedding and therefore the transmissibility of HSV-2 from the vaccinated patient. In certain embodiments, the reductions described above are at least 25%, 30%, 40%, 50%, 60%, 70%, 80% or even 90%. In certain embodiments, the reductions described above include the complete cessation of symptoms, viral shedding and/or future outbreaks (e.g., by blocking the ability of the virus to establish latency in sensory ganglia).

In therapeutic embodiments, the HSV-2 vaccine is administered to an individual post-infection. The HSV-2 vaccine may be administered shortly after infection, e.g. before symptoms manifest, or may be administered during or after manifestation of symptoms. In some embodiments, the HSV-2 may prevent endogenous reactivation of earlier infection. In some embodiments, a postinfection vaccine could be administered to patients in high-risk groups.

The duration of therapeutic effects of a vaccine formulation disclosed herein is preferably as long as possible. In certain embodiments, vaccine formulations produce therapeutic effects lasting one month, two months, three months, six months, one year, two years, five years, ten years, twenty years or even a lifetime.

***3. Assaying vaccination efficacy***

The efficacy of vaccination with the vaccines disclosed herein may be determined in a number of ways.

Vaccine efficacy may be assayed in various model systems. Suitable model systems used to study HSV-2 include a guinea pig model and a mouse model, as described in the examples below. Briefly, the animals are vaccinated and then challenged with HSV-2 or the vaccine is administered to already-infected animals. The response of the animals to the HSV-2 challenge or the vaccine is then compared with control animals, using one of the measures described above. A similar assay could be used for clinical testing of humans. The treatment and prophylactic effects described above represent additional ways of determining efficacy of a vaccine.

In addition, efficacy may be evaluated by *in vitro* immunization of naïve human peripheral blood mononuclear cells (PBMC), where APCs are exposed to the vaccine and then the APCs are co-cultured with naïve T cells from the same donor to evaluate the primary response to immunization in a test tube. An activation of the T-cells by 1.5 fold, 2-fold, 5-fold, 10-fold, 20-fold, 50-fold or 100-fold or more relative to activation of T-cells using APCs not exposed to a vaccine, in certain embodiments, is considered an adequate response.

Vaccine efficacy may further be determined by viral neutralization assays. Briefly, animals are immunized and serum is collected on various days post-immunization. Serial dilutions of serum are pre-incubated with virus during which time antibodies in the serum that are specific for the virus will bind to it. The virus/serum mixture is then added to permissive cells to determine infectivity by a plaque assay. If antibodies in the serum neutralize the virus, there are fewer plaques compared to the control group.

## G. Uses of Pharmaceutical Compositions

### *1. Defense against HSV infection*

The pharmaceutical compositions of the present disclosure are designed to elicit an immune response against HSV-2. Compositions described herein may stimulate an innate immune response, an antibody response or a cell-mediated immune response, or a combination of these responses, in the subject to which it is administered. In some embodiments, the composition stimulates immune cells at

the peripheral site of infection or sensory ganglia, such as neutrophils, macrophages, and NK cells. The composition may stimulate infiltration by macrophages; production of antiviral compounds, such including nitric oxide, TNF-α, interferons (IFN), and interleukin 12 (IL-12) by neutrophils; and/or stimulation of NK cells to produce IFN-γ. IL-2, IFN-α and IFN-β production may also be triggered by the polypeptides of the present composition, and are believed to aid in controlling infection.

In some embodiments, the composition comprises antigens that stimulate production of neutralizing antibodies. Neutralizing antibodies may target the glycoproteins of the viral envelope, which mediate the interaction of virions with host cell and are responsible for attachment, binding, and entry of HSV-2 into cells. Accordingly, an exemplary composition comprises one or more glycoproteins described above or encoded by nucleic acids described above. Immunogenic antigens and/or epitopes as described herein may be administered separately, in series, or in combination with one another.

In some embodiments, the composition elicits a cell-mediated response, which may involve CD4+ T cells, CD8+ T cells and/or production of antiviral cytokines. The composition may trigger IFN-γ secretion, for example through the activation of the innate immune response, and mediate CD8+ T cell clearing of the virus. IFN-γ is also secreted by $T_H1$ cells, ($T_H17$ cells?) $T_C$ cells, dendritic cells, and NK cells, and the composition may trigger IFN-γ secretion by any of these cell types. Such activity of CD8+ T cells may be cytolytic, or, alternately, may be regulated by inhibitor molecules on the surface of the neurons which prevent neuronal killing. CD4+ and/or CD8+ T cells may play a role in maintaining latency of the virus, thus preventing reactivation. In some embodiments, the composition boosts a CD4+ T cell response and/or a CD8+ T cell response that prevents reactivation of the virus from its latent state.

In some embodiments, the composition blocks the ability of HSV to evade the host immune response, or, alternately, boosts immune responses normally evaded by HSV. In some embodiments, the composition inhibits HSV-2 from shifting the immunological balance towards tolerance of HSV antigens. HSV-2 may

mediate tolerance through $T_H2$ cells. First, HSV-2 may induce suppressor T cells, such as CD4+ CD25+ cells and Tr1 cells that secrete IL-10, a $T_H2$ cytokine. $T_H2$ cytokines downregulate costimulatory molecules and inhibit the maturation and function of antigen-presenting dendritic cells. In addition, infection with HSV-2 inhibits the maturation and migration of dendritic cells, which are essential for efficient CTL priming. Notably, $T_H2$ cytokines are produced during recurrence of HSV-2 infection, in contrast to $T_H1$ cytokines, which are produced during recurrence-free episodes. Thus, in certain embodiments, the compositions of the invention repress suppressor T cells and/or induce maturation or migration or both of dendritic cells.

In some embodiments, methods of inducing an immune response against HSV-2 in a mammal comprise administering the compositions described above. The composition may be used to induce an immune response at different time points, such as before exposure to HSV-2, after initial infection with HSV-2, before or after HSV-2 has established latency, before or after HSV-2 shedding occurs, and/or before or after recurrent outbreaks occur. In some embodiments, an immune response against HSV-2 may be induced at one or more of the timepoints above. The composition may induce a $T_H1$ response and/or a $T_H17$ response but not a $T_H2$ response, or may activate the responses at the same time or at different times.

In some embodiments, administration of the composition reduces symptoms associated with initial infection, latency, or recurrent infection with HSV. Such a composition may reduce incidence and/or severity of lesions, sores, pain, irritation, itching, fever, malaise, headache, viral shedding, or prodromes associated with HSV infection or outbreak.

In some embodiments, one or more antibodies to antigens of HSV-2 may be administered to individuals in order to produce passive immunity. Passive immunity results from the transfer of active humoral immunity in the form of ready-made antibodies, from one individual to another. Passive immunization may be used when there is a high risk of infection and insufficient time for the body to develop its own immune response, or to reduce the symptoms of ongoing or immunosuppressive diseases. Adoptive transfer of T cells may provide another

method of eliciting an immune response to HSV-2 antigens in patients. In one embodiment, autologous T cells may be expanded on APCs presenting the antigens derived from the polypeptides described above. Subsequently, the expanded HSV-2-specific T cells are transferred back into the patient from which the T cells were derived.

***2. Diagnostic uses***

This application provides, *inter alia,* a rapid, inexpensive, sensitive, and specific method for detection of HSV-2 in patients. In this respect it should be useful to hospitals and physicians examining and treating patients with or at risk for HSV-2 infection. As used herein, "patient" refers to an individual (such as a human) that either has an HSV-2 infection or has the potential to contract an HSV-2 infection.

In some embodiments, one may use an antibody against one of the polypeptides described herein, such as those of Table 1 and/or Table 2, to detect HSV-2 in an individual. The instant disclosure also provides a method of phenotyping biological samples from patients suspected of having a HSV-2 infection that involves: (a) rendering a biological sample amenable to immunoassay, if necessary; (b) contacting the sample with an appropriate HSV-2-specific antibody or antigen-binding portion thereof under conditions that allow for binding of the antibody or antigen-binding portion to an epitope of HSV-2; and (c) determining if the sample shows the presence of HSV-2 as compared to a control tissue; where if the test tissue shows the presence of HSV-2, the patient is identified as likely having a HSV-2 infection.

Alternatively, one may use the polypeptides described above to detect anti-HSV-2 antibodies in an individual. The instant disclosure also provides a method of phenotyping biological samples from patients suspected of having a HSV-2 infection: (a) rendering a biological sample amenable to an affinity assay such as ELISA, if necessary; (b) contacting the sample with a HSV-2-specific antigen or portion thereof under conditions that allow for binding of the antigen to any host antibodies present in the sample; and (c) determining if the sample shows the presence of HSV-2 as compared to a control tissue; wherein if the test tissue shows

the presence of HSV-2, the patient is identified as likely having a HSV-2 infection. The aforementioned test may be appropriately adjusted to detect other viral infections, for instance by using a homolog (from another viral species) of the proteins described above, such as in Table 1 and/or Table 2.

A number of methods for measuring antibody-antigen binding are known in the art, including ELISA (enzyme-linked immunosorbent assay), Western blotting, competition assay, and spot-blot. The detection step may be, for instance, chemiluminescent, fluorescent, or colorimetric. One suitable method for measuring antibody-protein binding is the Luminex xMAP system, where peptides are conjugated to a dye-containing microsphere. Certain systems, including the xMAP system, are amenable to measuring several different markers in multiplex, and could be used to measure levels of antibodies at once. In some embodiments, other systems are used to assay a plurality of markers in multiplex. For example, profiling may be performed using any of the following systems: antigen microarrays, bead microarrays, nanobarcodes particle technology, arrayed proteins from cDNA expression libraries, protein in situ array, protein arrays of living transformants, universal protein array, lab-on-a-chip microfluidics, and peptides on pins. Another type of clinical assay is a chemiluminescent assay to detect antibody binding. In some such assays, including the VITROS Eci anti-HCV assay, antibodies are bound to a solid-phase support made up of microparticles in liquid suspension, and a surface fluorometer is used to quantify the enzymatic generation of a fluorescent product.

In other embodiments, one may use the polypeptides described above, such as those of Table 1 and/or Table 2, to detect T cells that are specific to HSV-2. The instant disclosure provides a method of phentoyping biological samples from patients suspected of having a HSV-2 infection, involving (a) rendering a biological sample amendable to an assay for activation of T cells, if necessary, (b) contacting the sample with a HSV-2-specific polypeptide or portion thereof under conditions that allow APCs to process the polypeptide, and (c) determining activation of the T cells in response to the HSV-2-specific polypeptide, where an elevated T cell activation relative to an uninfected patient indicates HSV-2 infection. This diagnostic assay is intended to detect the presence of HSV-2-specific T cells in any

patients, including those patients who have been exposed to HSV-2 but have not seroconverted to produce detectable levels of anti-HSV-2 antibodies.

T cell activation may be measured using many proliferation assays, including cytokine-specific ELISA, cell proliferation measured by tritiated thymidine incorporation or membrane intercolating (PKH-67) or cytoplasmic (CFSE) dyes, ELISPOT, flow cytometry, and bead arrays. In addition, one may measure the T cell response in T cell lines or in T cell hybridomas from mice or humans that are specific for the antigens. Readouts for activated T cells include proliferation, cytokine production, or readout of a surrogate enzyme expressed by the hybridoma that is induced when the T cell or T cell hybridoma is activated in response to an antigen. For example, activation of a T cell response may be detected by T cell hybridoma that is engineered to produce β-galactosidase. β-galactosidase may be detected through the use of colorimetric β-galactosidase substrates such as chlorophenyl red β-D galactopyranoside (CPRG).

Infection with HSV-2 may be acute or latent. In some embodiments, if the biological sample shows the presence of HSV-2, one may administer a therapeutically effective amount of the compositions and therapies described herein to the patient. The biological sample may comprise, for example, blood, semen, urine, vaginal fluid, mucus, saliva, feces, urine, cerebrospinal fluid, or a tissue sample. In some embodiments, the biological sample is an organ intended for transplantation. In certain embodiments, before the detection step, the biological sample is subject to culture conditions that promote the growth of HSV-2.

The diagnostic tests herein may be used to detect HSV-2 in a variety of samples, including samples taken from patients and samples obtained from other sources. For example, the diagnostic tests may be used to detect HSV-2 on objects such as medical instruments. In some embodiments, the tests herein may be performed on samples taken from animals such as agricultural animals (cows, pigs, chickens, goats, horses and the like), companion animals (dogs, cats, birds, and the like), or wild animals. In certain embodiments, the tests herein may be performed on samples taken from cell cultures such as cultures of human cells that produce a

therapeutic protein, cultures of bacteria intended to produce a useful biological molecule, or cultures of cells grown for research purposes.

The invention also includes a method of determining the location of a HSV-2 infection in a patient comprising: (a) administering a pharmaceutical composition comprising a labeled HSV-2 antibody or antigen-binding portion thereof to the patient, (b) detecting the label, and (c) determining if the patient has HSV-2 compared to a control. In certain embodiments, the method further comprises, if the patient has an HSV-2 infection, administering a therapeutically effective amount of a composition described herein to the patient. The method may further comprise determining the infected cell types and/or volume of the HSV-2 in the patient. This method may be used to evaluate the spread of HSV-2 in the patient and determine whether a localized therapy is appropriate.

In some embodiments, the polypeptides described herein may be used to make a prognosis of the course of infection. In some embodiments, T cell or antibody responses specific for the polypeptides herein may be detected in a sample taken from a patient. If antibodies or T cells are present at normal levels, it would indicate that the patient has raised an effective immune response against the pathogen. If antibodies or T cells are absent, or present at reduced levels, it would indicate that the patient is failing to raise a sufficient response against the pathogen, and a more aggressive treatment would be recommended. In some embodiments, antibody or T cells present at reduced levels refers to responses that are present at less than 50%, 20%, 10%, 5%, 2%, or 1% the typical level in a patient with a protective immune response. T cell responses may be detected by methods known in the art such as T cell proliferation, ELISPOT or ELISA, and antibodies may be detected by affinity for any of the antigens described herein, using methods known in the art such as ELISA.

In some embodiments, detection of T cells specific for HSV-2 antigens may be used to predict the progress and symptoms of HSV-2 infection in a patient. After infection with HSV-2, some patients remain asymptomatic, although the virus may establish latency. Other patients exhibit symptoms of HSV-2 infection, and may experience recurrent outbreaks. The HSV-2 antigens found in asymptomatic

patients may differ from those antigens found in patients who present symptoms and/or recurrent outbreaks. Accordingly, the detection methods of the present invention may be used to distinguish between subgroups within the population of patients infected with HSV-2. Subgroups may be further divided into patients who experience frequent outbreaks and those who infrequently or never experience outbreaks, or patients who shed high levels of virus and those who shed low levels or do not shed. The categorization of patients, based on the presence and levels of T cell responses to certain HSV-2 antigens but not others, may help health care practitioners to determine appropriate treatment regimens. Similarly, differences in the magnitude of T cell responses and/or differences in the combination and levels of cytokines produced by T cells may also be used to predict the progress and symptoms of HSV-2 infection in a patient. Thus, an infected patient whose complement of HSV-2 antigens to which T cells respond predicts severe symptoms, frequent outbreaks, and/or high levels of viral shedding may require more intensive antiviral therapy and/or a longer course of therapeutic treatment than a patient whose complement of HSV-2 antigens predicts an asymptomatic infection.

It will be understood by one of skill in the art that the methods herein are not limited to detection of HSV-2. Other embodiments include the detection of related viruses including viruses with proteins homologous to the proteins described above, such as those in Table 1 and/or Table 2. Such related viruses include, for example, other members of the *Herpesviridae* family. Depending on the homology, these related viruses may also include viruses that are not members of the *Herpesviridae* family.

***3. Use in groups with increased risk for infection by HSV-2***

Essentially any individual has a certain risk of infection with HSV-2. However, certain sub-populations have an increased risk of infection. In some embodiments, patients receiving the composition for HSV-2 are immunocompromised.

An immunocompromising condition arising from a medical treatment is likely to expose the individual in question to a higher risk of infection. It is possible to treat an infection prophylactically in an individual having the

immunocompromised condition before or during treatments known to generate such a condition. By prophylactically treating with the antigen before or during a treatment known to generate such a condition it is possible to prevent a subsequent infection or to reduce the risk of the individual contracting an infection due to the immunocompromised condition. Should the individual contract an infection, e.g., following a treatment leading to an immunocompromised condition, it is also possible to treat the infection by administering to the individual an antigen composition.

In certain embodiments, the compositions are administered to children or adult patients. In other embodiments, compositions are appropriate for pregnant women who were infected before becoming pregnant, or who became infected during pregnancy, such as to inhibit infection of a fetus or baby. The compositions may also be administered to neonates and infants who became infected in utero or during delivery.

## H. Doses and Routes of Administration

### *1. Dosage amounts and timing*

The amount of antigen in each vaccine dose is selected as an effective amount, which induces an prophylactic or therapeutic response, as described above, in either a single dose or over multiple doses. Preferably, the dose is without significant adverse side effects in typical vaccinees. Such amount will vary depending upon which specific antigen is employed. Generally, it is expected that a dose will comprise 1-1000 μg of protein, in some instances 2-100 μg, for instance 4-40 μg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of antibody titers, T cell activation levels, and other responses in subjects. In some embodiments, the appropriate amount of antigen to be delivered will depend on the age, weight, and health (e.g., immunocompromised status) of a subject. When present, typically an adjuvant will be present in amounts from 1 μg – 250 μg per dose, for example 50-150 μg, 75-125μg or 100 μg.

In some embodiments, only one dose of the vaccine is administered to achieve the results described above. In other embodiments, following an initial vaccination, subjects receive one or more boost vaccinations, for a total of two, three, four or five vaccinations. Advantageously, the number is three or fewer. A boost vaccination may be administered, for example, about 1 month, 2 months, 4 months, 6 months, or 12 months after the initial vaccination, such that one vaccination regimen involves administration at 0, 0.5-2 and 4-8 months. It may be advantageous to administer split doses of vaccines which may be administered by the same or different routes.

The pharmaceutical compositions described herein may take on a variety of dosage forms. In certain embodiments, the composition is provided in solid or powdered (e.g., lyophilized) form; it also may be provided in solution form. In certain embodiments, a dosage form is provided as a dose of lyophilized composition and at least one separate sterile container of diluent.

In some embodiments, the antigen is delivered to a patient at an amount of 1μmol per dose. In some embodiments, the antigen is delivered at a dose ranging from 10 nmol to 100 nmol per dose. The appropriate amount of antigen to be delivered may be determined by one of skill in the art. In some embodiments, the appropriate amount of antigen to be delivered will depend on the age, weight, and health (e.g., immunocompromised status) of a subject.

Pharmaceutical compositions disclosed herein are (in some embodiments) administered in amounts sufficient to elicit production of antibodies as part of an immunogenic response. In some embodiments, the composition may be formulated to contain 5 mcg/0.5 mL or an amount ranging from 10 mcg/1mL to 200mcg/1 mL of an antigen. In other embodiments, the composition may comprise a combination of antigens. The plurality of antigens may each be the same concentration, or may be different concentrations.

In some embodiments, the composition will be administered in a dose escalation manner, such that successive administrations of the composition contain a higher concentration of composition than previous administrations. In some embodiments, the composition will be administered in a manner such that successive

administrations of the composition contain a lower concentration of composition than previous administrations.

In therapeutic applications, compositions are administered to a patient suffering from a disease in an amount sufficient to cure or at least partially arrest the disease and its complications.

Therapeutic applications of a composition described herein include reducing transmissibility, slowing disease progression, reducing viral shedding, or eliminating recurrent infections in patients that have been infected with HSV-2, such as by 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20% or 10% of the levels at which they would occur in individuals who are not treated with the composition. The composition may also reduce the quantity of HSV-2 shed by infected individuals, inhibit the expression of proteins required for reactivation of HSV-2 from the latent stage in infected patients, and/or inhibit replication of HSV-2 in neurons of infected patients, such as by 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, or 10% of the levels at which they would occur in individuals not treated with the composition.

In prophylactic embodiments, compositions are administered to a human or other mammal to induce an immune response that can inhibit the establishment of an infectious disease or other condition. In some embodiments, a composition may partially block the virus from establishing latency or reduce the efficiency with which latency is established.

In some embodiments, only one dose (administration) of the composition is given. In other embodiments, the composition is administered in multiple doses. In various embodiments, the composition is administered once, twice, three times, or more than three times. The number of doses administered to a subject is dependent upon the antigen, the extent of the disease or the expected exposure to the disease, and the response of a subject to the composition.

In some embodiments, the compositions are administered in combination with antimicrobial molecules. Antimicrobial molecules may include antiviral molecules. Many antiviral molecules are currently known in the art, and target one or more stage of the viral life cycle, including viral attachment to host cells, release of viral genes and/or enzymes into the host cell, replication of viral components

using host-cell machinery, assembly of viral components into complete viral particles, and release of viral particles to infect new hosts.

***2. Routes of administration***

The vaccine formulations and pharmaceutical compositions herein can be delivered by administration to an individual, typically by systemic administration (e.g., intravenous, intraperitoneal, intramuscular, intradermal, subcutaneous, transdermal, subdermal, intracranial, intranasal, mucosal, anal, vaginal, oral, sublingual, buccal route or they can be inhaled) or they can be administered by topical application.

In some embodiments, the composition may be administered directly to the likely sites of infection. In female patients, the composition may be applied topically to mucosal membranes, or delivery vaginally or rectally using devices and methods known in the art. The vaginal and rectal routes of delivery permits extended, continuous or pulsed delivery and administration of composition dosages, and may be administered either before or after exposure to HSV, depending on the use of a prophylactic or therapeutic composition. In male patients, the composition may be applied topically to the skin or mucosal membranes, or delivered rectally. In both patient populations, the composition may also be targeted to the sensory ganglia.

An HSV-2 vaccine or pharmaceutical composition is often administered via the intramuscular route. Typically, in this route, the vaccine is injected into an accessible area of muscle tissue. Intramuscular injections are, in some embodiments, given in the deltoid, vastus lateralis, ventrogluteal or dorsogluteal muscles. The injection is typically given at an approximately 90° angle to the surface of the skin, so the vaccine penetrates the muscle.

An HSV-2 vaccine may also be administered subcutaneously. The injection is typically given at a 45° angle to the surface of the skin, so the vaccine is administered to the subcutis and not the muscle.

In some embodiments, the HSV-2 vaccine is administered intradermally. Intradermal administration is similar to subcutaneous administration, but the injection is not as deep and the target skin layer is the dermis. The injection is

typically given at a 10-15° angle to the surface of the skin, so the vaccine is delivered just beneath the epidermis.

### *3. Formulations*

The vaccine formulation may be suitable for administration to a human patient, and vaccine preparation may conform to USFDA guidelines. In some embodiments, the vaccine formulation is suitable for administration to a non-human animal. In some embodiments, the vaccine is substantially free of either endotoxins or exotoxins. Endotoxins include pyrogens, such as lipopolysaccharide (LPS) molecules. The vaccine may also be substantially free of inactive protein fragments. In some embodiments, the vaccine has lower levels of pyrogens than industrial water, tap water, or distilled water. Other vaccine components may be purified using methods known in the art, such as ion-exchange chromatography, ultrafiltration, or distillation. In other embodiments, the pyrogens may be inactivated or destroyed prior to administration to a patient. Raw materials for vaccines, such as water, buffers, salts and other chemicals may also be screened and depyrogenated. All materials in the vaccine may be sterile, and each lot of the vaccine may be tested for sterility. Thus, in certain embodiments the endotoxin levels in the vaccine fall below the levels set by the USFDA, for example 0.2 endotoxin (EU)/kg of product for an intrathecal injectable composition; 5 EU/kg of product for a non-intrathecal injectable composition, and 0.25-0.5 EU/mL for sterile water.

In some embodiments, the vaccine comprising a polypeptide contains less than 5%, 2%, 1%, 0.5%, 0.2%, 0.1% of other, undesired unpolypeptides, relative to the amount of desired polypeptides. In some embodiments, the vaccine contains less than 5%, less than 2%, less than 1%, less than 0.5%, less than 0.2%, or less than 0.1% DNA and/or RNA.

It is preferred that the vaccine has low or no toxicity, within a reasonable risk-benefit ratio.

The formulations suitable for introduction of the pharmaceutical composition vary according to route of administration. Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous,

intramuscular, intradermal, intraperitoneal, intranasal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials.

Injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. Cells transduced by the packaged nucleic acid can also be administered intravenously or parenterally.

Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the polypeptides or packaged nucleic acids suspended in diluents, such as water, saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as liquids, solids, granules or gelatin; (c) suspensions in an appropriate liquid; and (d) suitable emulsions. Tablet forms can include one or more of lactose, sucrose, mannitol, sorbitol, calcium phosphates, corn starch, potato starch, tragacanth, microcrystalline cellulose, acacia, gelatin, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, stearic acid, and other excipients, colorants, fillers, binders, diluents, buffering agents, moistening agents, preservatives, flavoring agents, dyes, disintegrating agents, and pharmaceutically compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin or sucrose and acacia emulsions, gels, and the like containing, in addition to the active ingredient, carriers known in the art. The pharmaceutical compositions can be encapsulated, e.g., in liposomes, or in a formulation that provides for slow release of the active ingredient.

The antigens, alone or in combination with other suitable components, can be made into aerosol formulations (e.g., they can be "nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

Suitable formulations for vaginal or rectal administration include, for example, suppositories, which consist of the polypeptides or packaged nucleic acids with a suppository base. Suitable suppository bases include natural or synthetic triglycerides or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the polypeptides or packaged nucleic acids with a base, including, for example, liquid triglycerides, polyethylene glycols, and paraffin hydrocarbons. The formulation may be suitable for administration to a human patient, and the preparation may conform to US FDA guidelines. In some embodiments, the formulation is suitable for administration to a non-human animal. In some embodiments, the composition is substantially free of either endotoxins or exotoxins. Endotoxins may include pyrogens, such as lipopolysaccharide (LPS) molecules. The composition may also be substantially free of inactive protein fragments which may cause a fever or other side effects. In some embodiments, the composition contains less than 1%, less than 0.1%, less than 0.01%, less than 0.001%, or less than 0.0001% of endotoxins, exotoxins, and/or inactive protein fragments. In some embodiments, the composition has lower levels of pyrogens than industrial water, tap water, or distilled water. Other components may be purified using methods known in the art, such as ion-exchange chromatography, ultrafiltration, or distillation. In other embodiments, the pyrogens may be inactivated or destroyed prior to administration to a patient. Raw materials for compositions, such as water, buffers, salts and other chemicals may also be screened and depyrogenated. All materials in the composition may be sterile, and each lot of the composition may be tested for sterility. Thus, in certain embodiments the endotoxin levels in the composition fall below the levels set by the USFDA: 0.2 endotoxin (EU)/kg of product for an intrathecal injectable composition; 5 EU/kg of product for a non-intrathecal injectable composition, and 0.25-0.5 EU/mL for sterile water.

In certain embodiments, the preparation comprises less than 50%, 20%, 10%, or 5% (by dry weight) contaminating protein. In certain embodiments, the desired molecule is present in the substantial absence of other biological macromolecules, such as other proteins (particularly other proteins which may substantially mask, diminish, confuse or alter the characteristics of the component proteins either as

purified preparations or in their function in the subject reconstituted mixture). In certain embodiments, at least 80%, 90%, 95%, 99%, or 99.8% (by dry weight) of biological macromolecules of the same type present (but water, buffers, and other small molecules, especially molecules having a molecular weight of less than 5000, can be present).

It is preferred that the composition has low or no toxicity, within a reasonable risk-benefit ratio. In certain embodiments, the composition comprises ingredients at concentrations that are less than $LD_{50}$ measurements for the animal being treated with the composition. $LD_{50}$ measurements may be obtained in mice or other experimental model systems, and extrapolated to humans and other animals. Methods for estimating the $LD_{50}$ of compounds in humans and other animals are well-known in the art. A composition, and any component within it, might have an $LD_{50}$ value in rats of greater than 100 g/kg, greater than 50g/kg, greater than 20 g/kg, greater than 10 g/kg, greater than 5 g/kg, greater than 2 g/kg, greater than 1 g/kg, greater than 500 mg/kg, greater than 200 mg/kg, greater than 100 mg/kg, greater than 50 mg/kg, greater than 20 mg/kg, or greater than 10 mg/kg. In some embodiments, the therapeutic index of the composition (measured as the toxic dose for 50% of the population ($TD_{50}$) divided by the minimum effective dose for 50% of the population ($ED_{50}$)), is greater than 1, greater than 10, or greater than 100.

## I. Preparation and Storage of Vaccines Formulations and Immunogenic Compositions

The HSV-2 vaccines described herein may be produced using a variety of techniques. For example, a polypeptide may be produced using recombinant DNA technology in a suitable host cell. A suitable host cell may be bacterial, yeast, mammalian, or other type of cell. The host cell may be modified to express an exogenous copy of one of the relevant polypeptide genes. Typically, the gene is operably linked to appropriate regulatory sequences such as a strong promoter and a polyadenylation sequence. In some embodiments, the promoter is inducible or repressible. Other regulatory sequences may provide for secretion or excretion of the polypeptide of interest or retention of the polypeptide of interest in the cytoplasm or

in the membrane, depending on how one wishes to purify the polypeptide. The gene may be present on an extrachromosomal plasmid, or may be integrated into the host genome. One of skill in the art will recognize that it is not necessary to use a nucleic acid 100% identical to the naturally-occurring sequence. Rather, some alterations to these sequences are tolerated and may be desirable. For instance, the nucleic acid may be altered to take advantage of the degeneracy of the genetic code such that the encoded polypeptide remains the same. In some embodiments, the gene is codon-optimized to improve expression in a particular host. The nucleic acid may be produced, for example, by PCR or by chemical synthesis.

Once a recombinant cell line has been produced, a polypeptide may be isolated from it. The isolation may be accomplished, for example, by affinity purification techniques or by physical separation techniques (e.g., a size column).

In a further aspect of the present disclosure, there is provided a method of manufacture comprising mixing one or more polypeptides or an immonogenic fragment or variant thereof with a carrier and/or an adjuvant. In some embodiments, the adjuvant is one that stimulates a $T_H1$ cell response.

In some embodiments, antigens for inclusion in compositions of the invention may be produced in cell culture. One method comprises providing one or more mammalian expression vectors and cloning nucleotides encoding two or more polypeptides selected from polypeptides having an amino acid sequence of any one of SEQ ID NOS: 1-38, then expressing and isolating the polypeptides.

The immunogenic polypeptides described herein, and nucleic acid compositions that express the polypeptides, can be packaged in packs, dispenser devices, and kits for administering nucleic acid compositions to a mammal. For example, packs or dispenser devices that contain one or more unit dosage forms are provided. Typically, instructions for administration of the compounds will be provided with the packaging, along with a suitable indication on the label that the compound is suitable for treatment of an indicated condition, such as those disclosed herein.

**V. Examples**

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**Example 1. Identification of HSV-2 antigens.**

A library of HSV-2 antigens (from HSV-2 Strain G, Lot # 7C0013, from Advanced Biotechnologies Inc, Maryland) was prepared and screened with peripheral blood mononuclear cells (PBMC) from human donors. Briefly, a library of HSV antigens was expressed by bacteria and mixed with antigen presenting cells (APCs). The APCs, in turn, presented HSV-derived peptides to lymphocytes that had been isolated from human patients infected with HSV-2. The patients belonged to several populations, as described below. Lymphocyte responses from each population were compared for reactivity to each expressed protein, and the screen detected antigens that induced reactive lymphocytes with greater frequency in one patient population as compared to the others. Infected but asymptomatic, and exposed but seronegative patients may activate protective immune responses that patients who experience frequent outbreaks do not; in particular, exposed but seronegative patients are presumed to have mounted sterilizing immunity to HSV-2 infection. It is believed that a unique set of polypeptides will activate lymphocytes from these patient populations.

The release of IFN-γ from $CD4^+$ T cells and $CD8^+$ T cells from each population was measured by ELISA following exposure to candidate antigens. Antigens were selected on the basis of the fold increase of IFN-γ released, relative to the level of IFN-γ released by frequent recurrers who experience more than four outbreaks per year, as well as the frequency of responders in the infected but asymptomatic, or exposed but seronegative populations, compared to frequent and less-frequent recurrers.

**A. Identification of antigens encoded by UL10, UL19, UL40, US4, US6, RS1 (RS1.1, RS1.2, RS1.3), UL 36 (UL36.3, UL36.4, UL36.5), UL32, and RL2**

Lymphocytes were isolated from patients belonging to several populations: infected but asymptomatic (n=40), exposed but seronegative (n=40), frequent recurrers who experience 4 or more outbreaks per year (n=43), less-frequent recurrers who experience less than 4 outbreaks per year (n=19), naïve (n=10), and $HSV\text{-}2^-/HSV\text{-}1^+$ (n=10). Table 3 shows the frequency analysis for thirteen HSV-2 antigens encoded by UL10, UL19, UL40, US4, US6, RS1 (RS1.1, RS1.2, RS1.3),

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UL36 (UL36.3, UL 36.4, UL36.5), UL32, and RL2 in the exposed patient cohort compared to the recurrer cohorts (frequent and less-frequent recurrers combined).

Table 3. Frequency analysis for antigens encoded by UL10, UL19, UL40, US4, US6, RS1 (RS1.1, RS1.2, RS1.3), UL36 (UL36.3, UL36.4, UL36.5), UL32 and RL2

| **HSV-2 Gene** | **Protein Name** | **Frequency Analysis (HSV-1/HSV-2 seronegative)** | |
|---|---|---|---|
| | | **% response from exposed donors** | **fold increase over recurrer response** |
| **UL10** | gM | 23% | 1.4 |
| **UL19** | VP5 | - | - |
| **UL40** | ribonucleotide reductase | 36% | 3.0 |
| **Us4** | gG | 24% | 1.6 |
| **Us6** | gD | 27% | 1.9 |
| **RS1**<br>**RS1.1**<br>**RS1.2**<br>**RS1.3** | ICP4 | <br>54%<br>46%<br>23% | <br>3.0<br>2.3<br>1.2 |
| **UL36**<br>**UL36.3**<br>**UL36.4**<br>**UL36.5** | Major tegument protein | <br>46%<br>46%<br>31% | <br>2.3<br>4.2<br>1.9 |
| **UL32** | DNA cleavage & packaging proteiin | - | - |
| **RL2** | ICP0 | 45% | 1.6 |

**B. Identification of antigens encoded by UL1, UL49.5, and UL54**

Lymphocytes were isolated from patients belonging to several populations: infected but asymptomatic (n=40), exposed but seronegative (n=40), frequent recurrers who experience 4 or more outbreaks per year (n=43), less-frequent recurrers who experience less than 4 outbreaks per year (n=19), naïive (n=10), and HSV-$2^-$/HSV-$1^+$ (n=10).

Table 4 shows the frequency analysis for three HSV-2 antigens encoded by UL1, UL49.5 and UL54, in the exposed patient cohort compared to the recurrer cohorts (frequent and less-frequent recurrers combined).

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Table 4. Frequency analysis for antigens encoded by UL1, UL49.5, and UL54

| HSV-2 Gene | Protein Name | Frequency Analysis (HSV-1/HSV-2 seronegative) | |
|---|---|---|---|
| | | % response from exposed donors | fold increase over recurrer response |
| **UL1** | gL2 | 64% | 2.7 |
| **UL49.5** | (virion p) | 37% | 2.1 |
| **UL54** | ICP27 | 22% | 5.8 |

### C. Identification of antigens encoded by RL1, UL2, and UL11

Lymphocytes were isolated from patients belonging to several populations: infected but asymptomatic (n=40), exposed but seronegative (n=40), frequent recurrers who experience 4 or more outbreaks per year (n=43), less-frequent recurrers who experience less than 4 outbreaks per year (n=19), naïive (n=10), and HSV-$2^-$/HSV-$1^+$ (n=10).

Table 5 shows the frequency analysis for three HSV-2 antigens encoded by RL1, UL2, and UL11 in the exposed patient cohort compared to the recurrer cohorts (frequent and less-frequent recurrers combined).

Table 5. Frequency analysis for HSV-2 antigens encoded by RL1, UL2, and UL11

| HSV-2 Gene | Protein Name | Frequency Analysis (HSV-1/HSV-2 seronegative) | |
|---|---|---|---|
| | | % response from exposed donors | fold increase over recurrer response |
| **RL1** | ICP34.5 | 45% | 1.3 |
| **UL2** | DNA glycosylase | 23% | 1.4 |
| **UL11** | tegument protein | 21% | <1.0 |

## Example 2. In vivo data

### A. [Protocol A] Guinea pig therapeutic vaccination protocol

Female Hartley guinea pigs were challenged intravaginally with HSV-2 strain MS at $5x10^5$ pfu to establish a genital tract infection. Animals were monitored for infection by vaginal swab on day 1 post-infection, and acute disease between days 3 and 14 post-infection. On day 14, after resolution of primary disease, the animals were randomized into groups of 12 and immunized subcutaneously with

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antigen (HSV-2 polypeptide at 15 μg dose) plus adjuvant (50 μg dose of an ISCOM matrix with a 91:9 mixture of Quillaja saponin fractions A and C). Each group received a total of 3 vaccinations, on days 14, 21, and 34 post-infection. Genital swabs were collected during the vaccination period to monitor viral shedding, and daily observations were recorded. Symptoms were scored on a scale from 0 to 4 based upon severity, 0 = no symptoms; 1= redness or swelling; 2 = a few small vesicles; 3 = several large vesicles; 4 = several large vesicles with maceration. In addition, animals with lesions intermediate in severity between the above scores were given a score of 0.5, 1.5, 2.5, or 3.5.

**1. Results of therapeutic vaccination studies with ICP4.2, gD2ΔTMR, and gD2**

The results of the studies are presented below in Tables 6-10. The IgG titer was determined at day 41 post-infection and 7 days after third immunization using an average of 4 out of the 12 animals in each group. The mean recurrent lesion scores and mean lesion days were each determined from day 15 to day 63 post-infection. The lesion scores represent total lesions for each group from day 15 to 60 and then a mean was calculated. Mean lesion days represent the mean number of days post-infection that immunized or non-immunized animals had herpetic lesions present. Vaginal-swab samples were collected from all animals for 12 days between days 20-59 post-infection and stored at -80°C until assayed for virus shedding titers by quantitative real-time PCR.

Table 6. Results of therapeutic vaccination studies with ICP4.2 (SEQ ID NO: 2): lesions

| **Groups N=12** | **Dose** | **gD2 IgG Titer** | **Mean Recurrent Lesion Score** | **% Reduction** | **Mean Lesion Days** | **% Reduction** |
|---|---|---|---|---|---|---|
| Phosphate-Buffered Saline | - | 1:263 | 8.1 | - | 9.0 | - |
| adjuvant only | 50 μg x 3 | 1:331 | 7.1 | 14 | 8.5 | 6 |

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| | | | | | | |
|---|---|---|---|---|---|---|
| ICP4.2 + adjuvant | 15 µg x 3 | 1:1079 | 4.3 | 47 | 5.1 | 44 |

Table 7. Results of therapeutic vaccination studies with ICP4.2 (SEQ ID NO: 2): viral shedding

| **Groups** | **No. of animals with no detectable viral shedding/total** | **Mean number of days viral shedding detected ±SEM** | **% Reduction** | **P value*** |
|---|---|---|---|---|
| Phosphate-Buffered Saline | 0/11 | 4.5 ± 0.8 | - | - |
| Adjuvant only | 0/12 | 4.4 ± 0.7 | 2 | 0.971 |
| ICP4.2 + adjuvant | 5/11 | 1.5 ± 0.5 | 67 | 0.004 |

Table 8. Results of therapeutic vaccination studies with gD2ΔTMR (SEQ ID NO:4): lesions

| **Groups** | **Mean Recurrent Lesion Score** | **% Reduction** | **Mean Lesion Days** | **% Reduction** |
|---|---|---|---|---|
| Adjuvant only | 8.7 | - | 11.7 | - |
| gD2ΔTMR + adjuvant | 5.7 | 34 | 8.6 | 26 |

Table 9. Results of therapeutic vaccination studies with gD2 (SEQ ID NO: 5): lesions

| **Groups** | **Dose** | **gD2 IgG** | **Mean Recurrent** | **%** | **Mean Lesion** | **%** |
|---|---|---|---|---|---|---|

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| N=12 | | Titer | Lesion Score | Reduction | Days | Reduction |
|---|---|---|---|---|---|---|
| Phosphate-Buffered Saline | - | 1:263 | 8.1 | - | 9.0 | - |
| Adjuvant only | 50 µg x 3 | 1:331 | 7.1 | 14 | 8.5 | 6 |
| gD2 + adjuvant | 15 µg x 3 | >1:6400 | 4.0 | 51 (p=0.04) | 5.0 | 45 |

Table 10. Results of therapeutic vaccination studies with gD2 (SEQ ID NO: 5): viral shedding

| Groups | No. of animals with no detectable viral shedding/total | Mean number of days viral shedding detected ±SEM | % Reduction | P value* |
|---|---|---|---|---|
| Phosphate-Buffered Saline | 0/11 | 4.5 ± 0.8 | - | - |
| Adjuvant only | 0/12 | 4.4 ± 0.7 | 2 | 0.971 |
| gD2 + adjuvant | 4/12 | 2.4 ± 0.6 | 47 | 0.047 |

**B. [Protocol B] Murine prophylactic vaccination protocol**

Female C57BL/6 mice from 6 to 8 weeks of age were immunized subcutaneously with antigen (HSV-2 polypeptide) plus adjuvant (12 µg dose of an ISCOM matrix with a 82:18 mixture of Quillaja saponin fractions A and C) on day 0 and day 9. On day 11, estrous cycles were synchronized with depo provera and then the mice were challenged on day 16 via intravaginal deposition of 10 times the $LD_{50}$ of HSV-2 strain 333 while under anaesthesia. All animals were monitored for morbidity (clinical score) and mortality, and body weights and vaginal swabs were

collected between days 17 and 28 post-infection. Clinical scores were recorded using the following scale: 0 = no symptoms, 1 = vaginal erythema, 2 = vaginal erythema and edema, 3 = vaginal herpetic lesions, 4 = unilateral paralysis or severe genital ulceration, and 5 = bilateral paralysis or death.

5

**1. Results of murine prophylactic vaccination studies with ICP4.2, VP5, gD2ΔTMR and gD2ΔTMR and ICP4.2**

In the experimental group, mice were immunized subcutaneously with either 5 μg or 10 μg of antigen plus adjuvant (12 μg dose of an ISCOM matrix with a 82:18 mixture of Quillaja saponin fractions A and C) on day 0 and day 9. Control animals received phosphate buffered saline (PBS) only, or adjuvant only.

Mice receiving PBS only or adjuvant only all died by day 9 post-challenge (no survivors). In contrast, mice receiving antigen largely survived to day 9, and 20-75% survived to day 12 post-challenge. The severity of disease symptoms (genital and neurological disease) were also scored at either day 9 or 10 post-challenge. Mice immunized with ICP4.2, VP5, gD2ΔTMR, or gD2ΔTMR and ICP4.2 with ISCOM adjuvant showed a significant decrease in disease symptoms compared to the PBS only or adjuvant only groups.

Table 11. Results of murine prophylactic vaccination studies

| **Groups** | **Mean Disease Score Day 10** | **% Reduction** | **P value*** | **% Survival Day 12** |
|---|---|---|---|---|
| PBS only/adjuvant only | 5.00/4.81 | - | -- | 0% |
| ICP4.2 | 3.6 | 28 | -- | 2.0% |

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| | | | | |
|---|---|---|---|---|
| VP5 + adjuvant | 3.13 | 35 | 0.146 | 3.8% |
| gD2ΔTMR + adjuvant | 1.44 | 70 | 0.023 | 7.5% |
| gD2ΔTMR + ICP4.2 + adjuvant | 0.75 | 84 | 0.020 | 8.8% |

*Student's t test

**C. [Protocol C] Guinea pig prophylactic vaccination protocol**

Female Hartley guinea pigs from 250-350 grams (weight) were immunized subcutaneously with 15 μg of antigen plus adjuvant (50 μg dose of an ISCOM matrix with a 91:9 mixture of Quillaja saponin fractions A and C) on day 0 and day 14-21. Sera were collected by toenail clip 2-3 weeks after the boost and then the guinea pigs were challenged via intravaginal deposition of 5 x $10^5$ PFU of HSV-2 strain MS. Vaginal-swab samples were collected from all animals on days 30 and 32 and stored at -80°C until assayed for virus titers by quantitative real-time PCR. Guinea pigs were evaluated daily (day 1-14), and primary genital skin disease was quantified using a lesion severity score scale from 1-4. Numerical scores were assigned to specific disease signs as follows: 0, no disease; 1, redness or swelling; 2, a few small vesicles; 3, several large vesicles; 4, several large vesicles with maceration. At the end of the study, the guinea pigs were euthanized , and the dorsal root ganglia (DRG) were harvested, stored at -80°C until they were processed for quantitative real-time PCR analysis.

Table 12. Results of guinea pig prophylactic vaccination studies with gD2ΔTMR and VP5

| Groups | Viral titer, PFU/ml Day 2 | Total mean acute lesion score | % Reduction | Copies HSV-2 DNA/ 1 µg DRG DNA | % Reduction |
|---|---|---|---|---|---|
| Adjuvant only | $2.3 \times 10^6$ | 22.6 | - | 959 | - |
| gD2ΔTMR + Adjuvant | $1.7 \times 10^6$ | 7.7 | 66% | 274 | 71% |
| VP5 + adjuvant | $5.9 \times 10^5$ | 18.2 | 17% | 283 | 70% |

**D. [Protocol D] Immunogenicity assay I (standard)**

Mice were immunized subcutaneously in the scruff of the neck with a 100 µl injection of 5 µg antigen plus adjuvant (12 µg dose of an ISCOM matrix with a 82:18 mixture of Quillaja saponin fractions A and C) in saline. The mice received one or two injections, 7 days apart. Analysis of the immunogenicity of the injection occurred 7 days after the final injection.

The immunogenicity assay was an *ex vivo* IFN-γ ELISPOT. $CD4^+$ and $CD8^+$ T cells were enriched from the spleen and analyzed separately. For the ELISPOT assay, membrane plates were prepared by coating them overnight with capture antibody and subsequently blocked by supplemented medium for a minimum of 2 hours at 37 °C. The mice were euthanized and their spleens harvested. The T cells were then prepared by sorting the splenocytes for $CD4^+$ and $CD8^+$ T cells using magnetic beads. The blocking solution was washed out from ELISPOT plates and the T cells were plated out onto the blocked plates. The plates were returned to the incubator to allow the T cells to settle. APCs were prepared by pulsing naïive T-depleted splenocytes with antigen for 2 hours at 37°C. For $CD4^+$ ELISPOTs, APCs were pulsed with whole protein. For $CD8^+$ ELISPOTs, APCs were pulsed with *E. coli* expressing protein plus cLLO. A medium control was APCs incubated for 2 hours at 37 °C with no additional antigen. The pulsed APCs were irradiated, washed

and adjusted to 2 x $10^6$ cells/ml. The APCs were added to appropriate wells of plates containing T cells. Then phorbol myristate acetate (PMA) and ionomycin were added to control wells as a positive control. The plates were allowed to incubate for 18 hours at 37°C under 5% $CO_2$. The plates were then developed using a secondary biotinylated antibody, horseradish peroxidase (HRP) and 3-amino-9-ethylcarbazole (AEC) substrate.

***1. Results of immunogenicity assay I with ICP4.2***

The immunogenicity assay I showed a robust immunogenic response for both the one and two injection regimens with ICP4.2. For the one injection regimen, the number of IFN-γ spots per 200,000 T cells were 8 and 101 for $CD4^+$ and $CD8^+$ cells, respectively. For the two injection regimen, there were 50 and 70 spots, respectively. In contrast, less than 15 spots were observed for media or adjuvant alone in either $CD4^+$ or $CD8^+$ cells.

***2. Results of immunogenicity assay I with gD2ΔTMR and gD2***

Results of immunogenicity assay I are shown in Figure 1A and B. Robust $CD4^+$ and $CD8^+$ T cell responses were obtained for both full-length gD2 and for gD2ΔTMR. In contrast, gD2 antigen truncated immediately upstream of the transmembrane domain (denoted 306t in Figure 1) showed significantly reduced responses.

**E. [Protocol E] Immunogenicity assay II (rapid)**

Recombinant *E. coli* from Genocea's proprietary library of HSV-2 orfeome were induced to express gL2 or fragments of ICP4 protein (ICP4.2, and polypeptides encoded by RS1.1, RS1.3.1 and RS 1.3.2). The protein was retained within bacterial cells. The bacteria were then fixed with PFA, washed extensively with PBS and stored at -80C until used for immunization.

Three mice per group were immunized with 1x$10^8$ bacteria in PBS per mouse by intraperitoneal injection. Mice received 1-2 additional boosters at 1 week intervals. Seven days after last boost, sera were collected and analyzed in an HSV-2 neutralization assay. Five-fold serial dilutions were prepared for plasma or serum samples in a 96-well round-bottom plate, followed by the addition of 50 PFUs HSV-2 (strain 333) to each well. The plates were covered and incubated at 37°C for 1

hour. 200µl of virus-serum dilution was transferred in duplicate to Vero cells grown in a 48-well tissue culture plate and incubated for 1 hour at 37°C. 300µl of DMEM containing 2% FBS was then added to each well and the plates were incubated for 48 hours at 37°C. To visualize virus plaques the plates were stained with crystal violet.

Table 13. Results of HSV-2 neutralization assay with gL2, ICP4.2, and polypeptides encoded by RS1.1, RS1.3.1 and RS1.3.2

| Immunogen | HSV-2 Neutralization IgG Titer* |
|---|---|
| E coli//gL2 | 1:50 |
| Ecoli//RS1.1 | <1:20 |
| Ecoli//ICP4.2 | <1:20 |
| E.coli/RS1.3.1 | 1:100 |
| E.coli//RS1.3.2 | <1:20 |
| Positive control (DL11 Mab) | 1:2500 |
| Negative control (Naïve mouse serum) | <1:20 |

* Serum dilution that inhibits 50% of virus control

**F. [Protocol F] Immunogenicity assay III (overlapping peptide pools)**

Mice were immunized with 2 µg/mouse of pooled, overlapping peptides (OLP) spanning the entire sequence of gL2, ICP4, and ICP4 fragments encoded by RS1.3.1 and RS1.3.2. OLPs were formulated in TiterMax adjuvant (Alexis Biochemical) in a total volume of 100 µl per mouse where adjuvant represented 1/3 of the subcutaneous dose. Mice were immunized on day 0, boosted on day 6 and spleens and blood were collected on day 11. Single cell suspensions were prepared from spleens and erythrocytes were lysed. The splenocyte suspensions were then divided into halves. The first half was separated into antigen presenting cells, $CD4^+$ and $CD8^+$ T cells; 200,000 T cells were seeded per well of IFN-gamma ELISPOT plate and stimulated with 100, 000 APCs and OLP pool corresponding to immunization, irrelevant peptide, positive and negative control. Cells were incubated in plates overnight after which the plates were developed and spots per well were counted. The second half of each splenocyte suspension was run as

unseparated splenocytes (400,000/well), pulsed with peptides, and assayed as described above.

Results are shown in Figure 2A and B as magnitude of response per immunization group.

**G. [Protocol G] Vaccination with at least two antigens**

***Example 1. Immunogenicity of gD2ΔTMR and ICP4 or ICP4.2 in C57BL/6 mice***

Purified protein was mixed with adjuvant and immunized into naïve mice to evaluate the ability to make $CD4^+$ and $CD8^+$ T cell responses to the protein antigens. Briefly, antigen alone (gD2ΔTMR (5μg)) or combinations of antigens (gD2ΔTMR and ICP4.2 (10μg)) were mixed with adjuvant (12μg dose of an ISCOM matrix with a 82:18 mixture of Quillaja saponin fractions A and C) and administered subcutaneously to mice, twice, 9 days apart. Seven days after the second immunization, mice were euthanized and spleens were harvested for *ex vivo* IFNγ ELISPOT assays. $CD4^+$ and $CD8^+$ T cells were sorted out of the splenocyte population using antibody-coated magnetic beads and then co-cultured on IFNγ-specific antibody-coated membranes in 96-well plates with naïve splenocytes that were pulsed with specific or non-specific antigens (as described) and irradiated with an x-ray irradiator. After 18 hours of incubation, captured IFNγ was detected with a biotinylated secondary IFNγ-specific antibody and visualized with horseradish peroxidase and 3-amino-9-ethylcarbazole substrate. Data are reported as the number of IFN-γ spot forming units per $2x10^5$ T cells ± standard deviation of three mice per group. Figure 3 shows the number of IFN-γ spot forming units per $2x10^5$ $CD4^+$ or $CD8^+$ T cells ± standard deviation of three mice per group. As seen in Figures 3A and B, the number of IFN-γ spot forming units per $CD4^+$ T cells or $CD8^+$ T cells is increased in mice immunized with gD2ΔTMR antigen combined with ICP4.2 compared to gD2ΔTMR antigen alone.

***Example 2. Combinations of gD2 and ICP4.2 plus adjuvant immunization reduced disease symptoms and mortality in mice.***

The ability to trigger protective immunity after immunization with the ICP4.2 protein in combination with gD2 plus adjuvant was evaluated in a lethal HSV-2 challenge mouse model. Briefly, eight C57BL/6 mice per group were immunized with either gD2 (2μg) or ICP4.2 (10μg) plus adjuvant individually or with both antigens mixed together plus adjuvant. Formulations were administered subcutaneously in the scruff of the neck twice, 9 days apart. Estrus cycles were synchronized with depo provera 5 days prior to virus infection, and animals were challenged intravaginally 7 days after the second immunization with 20 times the $LD_{50}$ of HSV-2 strain 333. Disease symptoms were scored post-infection, and survival monitored. Disease severity scores were as follows: 0= no symptoms, 1= redness, 2= redness and swelling, 3= herpetic lesions, 4=severe ulceration or unilateral paralysis, and 5= bilateral paralysis or death.

Table 14. Effect of HSV-2 proteins gD2 and ICP4.2 on disease symptoms, viral replication and mortality

| **Antigen (+ adjuvant) N=8** | **Mean disease score Day 7** | **Reduction in disease score** | **P value**** | **Reduction in virus titer** | **% Survival Day 11** |
|---|---|---|---|---|---|
| PBS | 3.5 ± 0.3 | --- | --- | --- | 0% |
| gD2* (2ug) | 2.5 ± 0.2 | 29% | 0.016 | 0% | 25% |
| ICP4.2 (10ug) | 1.7 ± 0.4 | 51% | 0.005 | 0% | 13% |
| gD2 (2ug) + ICP4.2 (10ug) | 1.3 ± 0.3 | 63% | 0.0004 | 20% | 50% |

*EC; **Student's t-test

***Example 3. Combinations of gD2ΔTMR and ICP4.2 plus adjuvant immunization reduced disease symptoms and mortality in mice.***

Mice immunized with a combination of gD2ΔTMR and ICP4.2 antigens showed a lower mean disease score at ten days after virus challenge compared to animals receiving the individual antigen with adjuvant.

Table 15. Effect of HSV-2 proteins gD2ΔTMR and ICP4.2 on disease symptoms and survival rate in mice

| Groups | Mean Disease Score Day 10 | % Reduction | P value* | % Survival Day 12 |
|---|---|---|---|---|
| Adjuvant only | 4.81 | - | - - | 00% |
| gD2ΔTMR + adjuvant | 1.44 | 70 | 0.023 | 75% |
| gD2ΔTMR + ICP4.2 + adjuvant | 0.75 | 84 | 0.020 | 88% |

***Example 4. Combination of gD2 and ICP4.2 plus adjuvant immunization reduces severity of recurrent lesions when administered therapeutically to HSV-2 infected guinea pigs***

The ability to affect HSV-2 reactivation in infected guinea pigs after therapeutic immunization with antigens plus adjuvant was evaluated. Briefly, guinea pigs were infected intravaginally with 5 x $10^5$ pfu of HSV-2 strain MS, monitored for primary disease for 14 days, and then randomized into immunization groups (N=15). Animals were immunized three times subcutaneously on day 14, 21, and 35 post-infection with antigen (15μg) plus adjuvant (50μg) or adjuvant alone, or vehicle control and scored daily for local disease severity. The scoring system was as follows: 0= no symptoms, 1= redness, 2=single lesions, 3= large or fused lesions, 4=severe ulceration or unilateral paralysis, and 5= bilateral paralysis or death.

Table 16 shows the data as the mean recurrent lesion score for each week after the guinea pigs recovered from their acute disease. The guinea pigs treated with a combination of gD2 and ICP4.2 antigens showed a reduction in the mean lesion score at 7 (day 42) and 14 (day 49) days after their last immunization compared to animals receiving the individual antigens with adjuvant.

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Table 16. Effect of HSV-2 proteins gD2 and ICP4.2 vaccine on recurrent genital skin disease

| Mean Recurrent Lesion Score Post HSV-2 Infection | | | | | |
|---|---|---|---|---|---|
| Antigen + Adjuvant | Day 15-21 | Day 22-28 | Day 29-35 | Day 36-42 | Day 43-49 |
| PBS | 2.00 ± 0.45 | 1.17 ± 0.35 | 1.50 ± 0.50 | 0.87 ± 0.28 | 1.33 ± 0.33 |
| gD2 | 1.00 ± 0.30 | 0.67 ± 0.24 | 0.80 ± 0.19 | 0.83 ± 0.26 | 0.77 ± 0.28 |
| ICP4.2 | 1.97 ± 0.38 | 1.07 ± 0.29 | 1.03 ± 0.33 | 0.53 ± 0.16 | 0.83 ± 0.29 |
| gD2 & ICP4.2 | 1.43 ± 0.32 | 0.80 ± 0.27 | 1.07 ± 0.33 | 0.43 ± 0.19 | 0.70 ± 0.27 |

Throughout this specification and the claims, unless the context requires otherwise, the word "comprise" and its variations, such as "comprises" and "comprising," will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgement or any form of suggestion that such art forms part of the common general knowledge in Australia. Further, the reference to any prior art in this specification is not, and should not be taken as, an acknowledgement or any form of suggestion that such art would be understood, ascertained or regarded as relevant by the skilled person in Australia.

Sequences

SEQ ID NO: 1 = ICP4, full-length

SAEQRKKKKTTTTTQGRGAEVAMADEDGGRLRAAAETTGGPGSPDPADGPPPTPNPDR
RPAARPGFGWHGGPEENEDEADDAAADADADEAAPASGEAVDEPAADGVVSPRQLALLASMVDEAVRT
IPSPPPERDGAQEEAARSPSPPRTPSMRADYGEENDDDDDDDDDDRDAGRWVRGPETTSAVRGAYPD
PMASLSPRPPAPRRHHHHHHHRRRRAPRRRSAASDSSKSGSSSSASSASSSASSSSSASASSSDDDDD
DDAARAPASAADHAAGGTLGADDEEAGVPARAPGAAPRPSPPRAEPAPARTPAATAGRLERRRARAAV
AGRDATGRFTAGRPRRVELDADAASGAFYARYRDGYVSGEPWPGAGPPPPGRVLYGGLGDSRPGLWGA
PEAEEARARFEASGAPAPVWAPELGDAAQQYALITRLLYTPDAEAMGWLQNPRVAPGDVALDQACFRI
SGAARNSSSFISGSVARAVPHLGYAMAAGRFGWGLAHVAAAVAMSRRYDRAQKGFLLTSLRRAYAPLL
ARENAALTGARTPDDGGDANRHDGDDARGKPAAAAAPLPSAAASPADERAVPAGYGAAGVLAALGRLS
AAPASAPAGADDDDDDDGAGGGGGGRRAEAGRVAVECLAACRGILEALAEGFDGDLAAVPGLAGARPA
APPRPGPAGAAAPPHADAPRLRAWLRELRFVRDALVLMRLRGDLRVAGGSEAAVAAVRAVSLVAGALG
PALPRSPRLLSSAAAAAADLLFQNQSLRPLLADTVAAADSLAAPASAPREARKRKSPAPARAPPGGAP
RPPKKSRADAPRPAAAPPAGAAPPAPPTPPPRPPRPAALTRRPAEGPDPQGGWRRQPPGPSHTPAPSA
AALEAYCAPRAVAELTDHPLFPAPWRPALMFDPRALASLAARCAAPPPGGAPAAFGPLRASGPLRRAA
AWMRQVPDPEDVRVVILYSPLPGEDLAAGRAGGGPPPEWSAERGGLSCLLAALGNRLCGPATAAWAGN
WTGAPDVSALGAQGVLLLSTRDLAFAGAVEFLGLLAGACDRRLIVVNAVRAADWPADGPVVSRQHAYL
ACEVLPAVQCAVRWPAARDLRRTVLASGRVFGPGVFARVEAAHARLYPDAPPLRLCRGANVRYRVRTR
FGPDTLVPMSPREYRRAVLPALDGRAAASGAGDAMAPGAPDFCEDEAHSHRACARWGLGAPLRPVYVA
LGRDAVRGGPAELRGPRREFCARALLEPDGDAPPLVLRDDADAGPPPQIRWASAAGRAGTVLAAAGGG
VEVVGTAAGLATPPRREPVDMDAELEDDDDGLFGE

SEQ ID NO: 2 = ICP4 internal fragment

MVLYGGLGDSRPGLWGAPEAEEARARFEASGAPAPVWAPELGDAAQQYALITRLLYTP
DAEAMGWLQNPRVAPGDVALDQACFRISGAARNSSSFISGSVARAVPHLGYAMAAGRFGWGLAHVAAA
VAMSRRYDRAQKGFLLTSLRRAYAPLLARENAALTGARTPDDGGDANRRDGDDARGKPAAAAAPLPSA
AASPADERAVPAGYGAAGVLAALGRLSAAPASAPAGADDDDDDDDGAGGGGGGGGGGGGRRAEAGRVA
VECLAACRGILEALAEGFDGDLAAVPGLAGARPAAPPRPGPAGAAAPPHADAPRLRAWLRELRFVRDA
LVLMRLRGDLRVAGGSEAAVAAVRAVSLVAGALGPALPRSPRLLSSAAAAAADLLFQNQSL

SEQ ID NO: 3 = gL2 cytoplasmic

MGFVCLFGLVVMGAWGAWGGSQATEYVLRSVIAKEVGDILRVPCMRTPADDVSWRYEA
PSVIDYARIDGIFLRYHCPGLDTFLWDRHAQRAYLVNPFLFAAGFLEDLSHSVFPADTQETTTRRALY

KEIRDALGSRKQAVSHAPVRAGCVNFDYSRTRRCVGRRDLRPANTTSTWEPPVSSDDEASSQSKPLAT
QPPVLALSNAPPRRVSPTRGRRRHTRLRRN

SEQ ID NO: 4 = gD2 internal deletion

NRWKYALADPSLKMADPNRFRGKNLPVLDQLTDPPGVKRVYHIQPSL
EDPFQPPSIPITVYYAVLERACRSVLLHAPSEAPQIVRGASDEARKHTYNLTIAWYRMGDNCAIPITV
MEYTECPYNKSLGVCPIRTQPRWSYYDSFSAVSEDNLGFLMHAPAFETAGTYLRLVKINDWTEITQFI
LEHRARASCKYALPLRIPPAACLTSKAYQQGVTVDSIGMLPRFIPENQRTVALYSLKIAGWHGPKPPY
TSTLLPPELSDTTNATQPELVPEDPEDSALLEDPAGTVSSQIPPNWHIPSIQDVAPHHAPAAPSNPRR
RAQMAPKRLRLPHIRDDDAPPSHQPLFY

SEQ ID NO: 5 = predicted gD2 sequence

MGRLTSGVGTAALLVVAVGLRVVCAKYALADPSLKMADPNRFRGKNLPVLDQLTDPPGVKRVYHIQPS
LEDPFQPPSIPITVYYAVLERACRSVLLHAPSEAPQIVRGASDEARKHTYNLTIAWYRMGDNCAIPIT
VMEYTECPYNKSLGVCPIRTQPRWSYYDSFSAVSEDNLGFLMHAPAFETAGTYLRLVKINDWTEITQF
ILEHRARASCKYALPLRIPPAACLTSKAYQQGVTVDSIGMLPRFIPENQRTVALYSLKIAGWHGPKPP
YTSTLLPPELSDTTNATQPELVPEDPEDSALLEDPAGTVSSQIPPNWHIPSIQDVAPHHAPAAPSNPG
LIIGALAGSTLAVLVIGGIAFWVRRRAQMAPKRLRLPHIRDDDAPPSHQPLFY

SEQ ID NO: 6 = ICP34.5

MSRRRGPRRRGPRRRPRPGAPAVPRPGAPAVPRPGALPTADSQMVPAYDSGTAVESAPAASSLLRRWL
LVPQADDSDDADYAGNDDAEWANSPPSEGGGKAPEAPHAAPAAACPPPPPRKERGPQRPLPPHLALRL
RTTTEYLARLSLRRRRPPASPPADAPRGKVCFSPRVQVRHLVAWETAARLARRGSWARERADRDRFRR
RVAAAEAVIGPCLEPEARARARARARAHEDGGPAEEEEAAAAARGSSAAAGPGRRAV

SEQ ID NO: 7 = ICP0

MEPRPGTSSRADPGPERPPRQTPGTQPAAPHAWGMLNDMQWLASSDSEEETEVGISDD
DLHRDSTSEAGSTDTEMFEAGLMDAATPPARPPAERQGSPTPADAQGSCGGGPVGEEEAEAGGGGDVC
AVCTDEIAPPLRCQSFPCLHPFCIPCMKTWIPLRNTCPLCNTPVAYLIVGVTASGSFSTIPIVNDPRT
RVEAEAAVRAGTAVDFIWTGNPRTAPRSLSLGGHTVRALSPTPPWPGTDDEDDDLADVDYVPPAPRRA
PRRGGGGAGATRGTSQPAATRPAPPGAPRSSSSGGAPLRAGVGSGSGGGPAVAAVVPRVASLPPAAGG
GRAQARRVGEDAAAAEGRTPPARQPRAAQEPPIVISDSPPPSPRRPAGPGPLSFVSSSSAQVSSGPGG
GGLPQSSGRAARPRAAVAPRVRSPPRAAAAPVVSASADAAGPAPPAVPVDAHRAPRSRMTQAQTDTQA
QSLGRAGATDARGSGGPGAEGGPGVPRGTNTPGAAPHAAEGAAARPRKRRGSDSGPAASSSASSSAAP

RSPLAPQGVGAKRAAPRRAPDSDSGDRGHGPLAPASAGAAPPSASPSSQAAVAAASSSSASSSSASSS
SASSSSASSSSASSSSASSSSASSSAGGAGGSVASASGAGERRETSLGPRAAAPRGPRKCARKTRHAE
GGPEPGARDPAPGLTRYLPIAGVSSVVALAPYVNKTVTGDCLPVLDMETGHIGAYVVLVDQTGNVADL
LRAAAPAWSRRTLLPEHARNCVRPPDYPTPPASEWNSLWMTPVGNMLFDQGTLVGALDFHGLRSRHPW
SREQGAPAPAGDAPAGHGE

SEQ ID NO: 8 = ICP4 internal fragments (RS1.1, #1-400)

msaeqrkkkkttttttqgrgaevamadedggrlraaaettggpgspdpadgpppptpn
pdrrpaarpgfgwhggpeenedeaddaaadadadeaapasgeavdepaadgvvspr
qlallasmvdeavrtipsppperdgaqeeaarspspprtpsmradygeenddddddd
dddddrdagrwvrgpettsavrgaypdpmaslsprppaprrhhhhhhhrrrraprr
rsaasdssksgssssassasssassssasasssddddddaarapasaadhaagg
tlgaddeeagvparapgaaprpsppraepapartpaatagrlerrraraavagrda
tgrftagrprrveldadaasgafyaryrdgyvsgepwpgagppppgrvlygglgds
rpglwgap

SEQ ID NO: 9 = ICP4 internal fragments (RS1.3.1, #750-1024)

ssaaaaaadllfqnqslrplladtvaaadslaapasaprearkrkspaparappgg
aprppkksradaprpaaappagaappapptpppprpprpaaltrrpaegpdpqggwr
rqppgpshtpapsaaaleaycapravaeltdhplfpapwrpalmfdpralaslaar
caapppggapaafgplrasgplrraaawmrqvpdpedvrvvilysplpgedlaagr
agggpppewsaergglscllaalgnrlcgpataawagnwtgapdvsalgaq

SEQ ID NO: 10 = ICP4 internal fragments (RS1.3.2, #1008-1319)

wagnwtgapdvsalgaqgvlllstrdlafagaveflgllagacdrrlivvnavraa
dwpadgpvvsrqhaylacevlpavqcavrwpaardlrrtvlasgrvfgpgvfarve
aaharlypdapplrlcrganvryrvrtrfgpdtlvpmspreyrravlpaldgraaa
sgagdamapgapdfcedeahshracarwglgaplrpvyvalgrdavrggpaelrgp
rrefcarallepdgdapplvlrddadagpppqirwasaagragtvlaaagggvevv
gtaaglatpprrepvdmdaeledddglfge

SEQ ID NO: 11 = ICP4 internal fragments (RS1.3, #750-1319)

Ssaaaaaadllfqnqslrplladtvaaadslaapasaprearkrkspaparappgg
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rqppgpshtpapsaaaleaycapravaeltdhplfpapwrpalmfdpralaslaar
caapppggapaafgplrasgplrraaawmrqvpdpedvrvvilysplpgedlaagr
agggpppewsaergglscllaalgnrlcgpataawagnwtgapdvsalgaqgvlll
strdlafagaveflgllagacdrrlivvnavraadwpadgpvvsrqhaylacevlp
avqcavrwpaardlrrtvlasgrvfgpgvfarveaaharlypdapplrlcrganvr
yrvrtrfgpdtlvpmspreyrravlpaldgraaasgagdamapgapdfcedeahsh
racarwglgaplrpvyvalgrdavrggpaelrgprrefcarallepdgdapplvlr
ddadagpppqirwasaagragtvlaaagggvevvgtaaglatpprrepvdmdaele
ddddglfge

SEQ ID NO: 12 = ICP4 internal fragments (RS1.4, #340-883)

tagrprrveldadaasgafyaryrdgyvsgepwpgagppppgrvlygglgdsrpgl
wgapeaeeararfeasgapapvwapelgdaaqqyalitrllytpdaeamgwlqnpr
vapgdvaldqacfrisgaarnsssfisgsvaravphlgyamaagrfgwglahvaaa
vamsrrydraqkgflltslrrayapllarenaaltgartpddggdanrhdgddarg
kpaaaaaplpsaaaspaderavpagygaagvlaalgrlsaapasapagaddddddd
gaggggggrraeagrvaveclaacrgilealaegfdgdlaavpglagarpaapprp
gpagaaapphadaprlrawlrelrfvrdalvlmrlrgdlrvaggseaavaavravs
lvagalgpalprsprllssaaaaaadllfqnqslrplladtvaaadslaapasapr
earkrkspaparappggaprppkksradaprpaaappagaappapptppprpprpa
altrrpaegpdpqggwrrqppgpshtpapsaaaleayca

SEQ ID NO: 13 = ICP4 internal fragments (RS1.5, #775-1318)

aaadslaapasaprearkrkspaparappggaprppkksradaprpaaappagaap
papptppprpprpaaltrrpaegpdpqggwrrqppgpshtpapsaaaleaycapra
vaeltdhplfpapwrpalmfdpralaslaarcaapppggapaafgplrasgplrra
aawmrqvpdpedvrvvilysplpgedlaagragggpppewsaergglscllaalgn
rlcgpataawagnwtgapdvsalgaqgvlllstrdlafagaveflgllagacdrrl
ivvnavraadwpadgpvvsrqhaylacevlpavqcavrwpaardlrrtvlasgrvf

gpgvfarveaaharlypdapplrlcrganvryrvrtrfgpdtlvpmspreyrravl
paldgraaasgagdamapgapdfcedeahshracarwglgaplrpvyvalgrdavr
ggpaelrgprrefcarallepdgdapplvlrddadagpppqirwasaagragtvla
aagggvevvgtaaglatpprrepvdmdaeledddglfge

SEQ ID NO: 14 = ICP4 internal fragments (RS1.6, #209-1318)

hrrrraprrrsaasdssksgssssassasssassssasasssddddddd
aarapasaadhaaggtlgaddeeagvparapgaaprpsppraepapartpaatagr
lerrraraavagrdatgrftagrprrveldadaasgafyaryrdgyvsgepwpgag
ppppgrvlygglgdsrpglwgapeaeeararfeasgapapvwapelgdaaqqyali
trllytpdaeamgwlqnprvapgdvaldqacfrisgaarnsssfisgsvaravphl
gyamaagrfgwglahvaaavamsrrydraqkgflltslrrayapllarenaaltga
rtpddggdanrhdgddargkpaaaaaplpsaaaspaderavpagygaagvlaalgr
lsaapasapagaddddddddgaggggggrraeagrvaveclaacrgilealaegfdg
dlaavpglagarpaapprpgpagaaapphadaprlrawlrelrfvrdalvlmrlrg
dlrvaggseaavaavravslvagalgpalprsprllssaaaaaadllfqnqslrpl
ladtvaaadslaapasaprearkrkspaparappggaprppkksradaprpaaapp
agaappapptpppprpprpaaltrrpaegpdpqggwrrqppgpshtpapsaaaleay
capravaeltdhplfpapwrpalmfdpralaslaarcaapppggapaafgplrasg
plrraaawmrqvpdpedvrvvilysplpgedlaagragggpppewsaergglscll
aalgnrlcgpataawagnwtgapdvsalgaqgvlllstrdlafagaveflglaga
cdrrlivvnavraadwpadgpvvsrqhaylacevlpavqcavrwpaardlrrtvla
sgrvfgpgvfarveaaharlypdapplrlcrganvryrvrtrfgpdtlvpmsprey
rravlpaldgraaasgagdamapgapdfcedeahshracarwglgaplrpvyvalg
rdavrggpaelrgprrefcarallepdgdapplvlrddadagpppqirwasaagra
gtvlaaagggvevvgtaaglatpprrepvdmdaeledddglfge

SEQ ID NO: 15 = ICP4 internal fragments (RS1.7, deletion of 391-544)

msaeqrkkkkttttqgrgaevamadedggrlraaaettggpgspdpadgppptpn
pdrrpaarpgfgwhggpeenedeaddaaadadadeaapasgeavdepaadgvvspr
qlallasmvdeavrtipsppperdgaqeeaarspspprtpsmradygeenddddd

ddddrdagrwvrgpettsavrgaypdpmaslsprppaprrhhhhhhhrrrraprr
rsaasdssksgssssassasssassssasasssddddddaarapasaadhaagg
tlgaddeeagvparapgaaprpsppraepapartpaatagrlerrraraavagrda
tgrftagrprrveldadaasgafyaryrdgyvsgepwpgagppppgrvlygglgar
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adtvaaadslaapasaprearkrkspaparappggaprppkksradaprpaaappa
gaappapptpppprpprpaaltrrpaegpdpqggwrrqppgpshtpapsaaaleayc
apravaeltdhplfpapwrpalmfdpralaslaarcaapppggapaafgplrasgp
lrraaawmrqvpdpedvrvvilysplpgedlaagragggpppewsaergglsclla
algnrlcgpataawagnwtgapdvsalgaqgvlllstrdlafagaveflgllagac
drrlivvnavraadwpadgpvvsrqhaylacevlpavqcavrwpaardlrrtvlas
grvfgpgvfarveaaharlypdapplrlcrganvryrvrtrfgpdtlvpmspreyr
ravlpaldgraaasgagdamapgapdfcedeahshracarwglgaplrpvyvalgr
davrggpaelrgprrefcarallepdgdapplvlrddadagpppqirwasaagrag
tvlaaagggvevvgtaaglatpprrepvdmdaeledddglfge

SEQ ID NO: 16 = ICP4 internal fragments (RS1.8, deletion of 786-864)

msaeqrkkkkttttqgrgaevamadedggrlraaaettggpgspdpadgpppt pnpdrrpaarpgfg
whggpeenedeaddaaadadadeaapasgeavdepaadgvvsprqlallasmvdeavrtipsppperd
gaqeeaarspspprtpsmradygeenddddddddddrdagrwvrgpettsavrgaypdpmaslsprp
paprrhhhhhhhrrrraprrrsaasdssksgssssassasssassssasasssddddddaarapas
aadhaaggtlgaddeeagvparapgaaprpsppraepapartpaatagrlerrraraavagrdatgrf
tagrprrveldadaasgafyaryrdgyvsgepwpgagppppgrvlygglgdsrpglwgapeaeearar
feasgapapvwapelgdaaqqyalitrllytpdaeamgwlqnprvapgdvaldqacfrisgaarnsss
fisgsvaravphlgyamaagrfgwglahvaaavamsrrydraqkgflltslrrayapllarenaaltg
artpddggdanrhdgddargkpaaaaaplpsaaaspaderavpagygaagvlaalgrlsaapasapag
addddddgagggggrraeagrvaveclaacrgilealaegfdgdlaavpglagarpaapprpgpag
aaapphadaprlrawlrelrfvrdalvlmrlrgdlrvaggseaavaavravslvagalgpalprsprl
lssaaaaaadllfqnqslrplladtvaaadslaapastpapsaaaleaycapravaeltdhplfpapw
rpalmfdpralaslaarcaapppggapaafgplrasgplrraaawmrqvpdpedvrvvilysplpged
laagragggpppewsaergglscllaalgnrlcgpataawagnwtgapdvsalgaqgvlllstrdlaf

agaveflgllagacdrrlivvnavraadwpadgpvvsrqhaylacevlpavqcavrwpaardlrrtvl
asgrvfgpgvfarveaaharlypdapplrlcrganvryrvrtrfgpdtlvpmspreyrravlpaldgr
aaasgagdamapgapdfcedeahshracarwglgaplrpvyvalgrdavrggpaelrgprrefcaral
lepdgdapplvlrddadagpppqirwasaagragtvlaaagggvevvgtaaglatpprrepvdmdael
eddddglfge

SEQ ID NO: 17 = predicted sequence for uracil DNA glycosylase (encoded by UL2)

MFSASTTPEQPLGLSGDATPPLPTSVPLDWAAFRRAFLIDDAWRPLLEPELANPLTARLLAEYDRRCQ
TEEVLPPREDVFSWTRYCTPDDVRVVIIGQDPYHHPGQAHGLAFSVRADVPVPPSLRNVLAAVKNCYP
DARMSGRGCLEKWARDGVLLLNTTLTVKRGAAASHSKLGWDRFVGGVVQRLAARRPGLVFMLWGAHAQ
NAIRPDPRQHYVLKFSHPSPLSKVPFGTCQHFLAANRYLETRDIMPIDWSV

SEQ ID NO: 18 = predicted sequence for tegument protein encoded by UL11

MGLAFSGARPCCCRHNVITTDGGEVVSLTAHEFDVVDIESEEEGNFYVPPDVRVVTRAPGPQYRRASD
PPSRHTRRRDPDVARPPATLTPPLSDSE

SEQ ID NO: 19 = gL2 secreted

NRWGFVCLFGLVVMGAWGAWGGSQATEYVLRSVIAKEVGDILRVPCM
RTPADDVSWRYEAPSVIDYARIDGIFLRYHCPGLDTFLWDRHAQRAYLVNPFLFAAGFLEDLSHSVFP
ADTQETTTRRALYKEIRDALGSRKQAVSHAPVRAGCVNFDYSRTRRCVGRRDLRPANTTSTWEPPVSS
DDEASSQSKPLATQPPVLALSNAPPRRVSPTRGRRRHTRLRRN

SEQ ID NO: 20 = predicted sequence for VP5 encoded by UL19

DYDIPTTENLYFQGMAAPARDPPGYRYAAAMVPTGSILSTIEVASHRRLFDFFARVRS
DENSLYDVEFDALLGSYCNTLSLVRFLELGLSVACVCTKFPELAYMNEGRVQFEVHQPLIARDGPHPV
EQPVHNYMTKVIDRRALNAAFSLATEAIALLTGEALDGTGISLHRQLRAIQQLARNVQAVLGAFERGT
ADQMLHVLLEKAPPLALLLPMQRYLDNGRLATRVARATLVAELKRSFCDTSFFLGKAGHRREAIEAWL
VDLTTATQPSVAVPRLTHADTRGRPVDGVLVTTAAIKQRLLQSFLKVEDTEADVPVTYGEMVLNGANL
VTALVMGKAVRSLDDVGRHLLEMQEEQLEANRETLDELESAPQTTRVRADLVAIGDRLVFLEALEKRI
YAATNVPYPLVGAMDLTFVLPLGLFNPAMERFAAHAGDLVPAPGHPEPRAFPPRQLFFWGKDHQVLRL
SMENAVGTVCHPSLMNIDAAVGGVNHDPVEAANPYGAYVAAPAGPGADMQQRFLNAWRQRLAHGRVRW
VAECQMTAEQFMQPDNANLALELHPAFDFFAGVADVELPGGEVPPAGPGAIQATWRVVNGNLPLALCP
VAFRDARGLELGVGRHAMAPATIAAVRGAFEDRSYPAVFYLLQAAIHGSEHVFCALARLVTQCITSYW

NNTRCAAFVNDYSLVSYIVTYLGGDLPEECMAVYRDLVAHVEALAQLVDDFTLPGPELGGQAQAELNH
LMRDPALLPPLVWDCDGLMRHAALDRHRDCRIDAGEHEPVYAAACNVATADFNRNDGRLLHNTQARAA
DAADDRPHRPADWTVHHKIYYYVLVPAFSRGRCCTAGVRFDRVYATLQNMVVPEIAPGEECPSDPVTD
PAHPLHPANLVANTVNAMFHNGRVVVDGPAMLTLQVLAHNMAERTTALLCSAAPDAGANTASTANMRI
FDGALHAGVLLMAPQHLDHTIQNGEYFYVLPVHALFAGADHVANAPNFPPALRDLARHVPLVPPALGA
NYFSSIRQPVVQHARESAAGENALTYALMAGYFKMSPVALYHQLKTGLHPGFGFTVVRQDRFVTENVL
FSERASEAYFLGQLQVARHETGGGVSFTLTQPRGNVDLGVGYTAVAATATVRNPVTDMGNLPQNFYLG
RGAPPLLDNAAAVYLRNAVVAGNRLGPAQPLPVFGCAQVPRRAGMDHGQDAVCEFIATPVATDINYFR
RPCNPRGRAAGGVYAGDKEGDVIALMYDHGQSDPARPFAATANPWASQRFSYGDLLYNGAYHLNGASP
VLSPCFKFFTAADITAKHRCLERLIVETGSAVSTATAASDVQFKRPPGCRELVEDPCGLFQEAYPITC
ASDPALLRSARDGEAHARETHFTQYLIYDASPLKGLSL

SEQ ID NO: 21 = VP5 encoded by UL19ΔTEV

MAAPARDPPGYRYAAAMVPTGSILSTIEVASHRRLFDFFARVRSDENSLYDVEFDALL
GSYCNTLSLVRFLELGLSVACVCTKFPELAYMNEGRVQFEVHQPLIARDGPHPVEQPVHNYMTKVIDR
RALNAAFSLATEAIALLTGEALDGTGISLHRQLRAIQQLARNVQAVLGAFERGTADQMLHVLLEKAPP
LALLLPMQRYLDNGRLATRVARATLVAELKRSFCDTSFFLGKAGHRREAIEAWLVDLTTATQPSVAVP
RLTHADTRGRPVDGVLVTTAAIKQRLLQSFLKVEDTEADVPVTYGEMVLNGANLVTALVMGKAVRSLD
DVGRHLLEMQEEQLEANRETLDELESAPQTTRVRADLVAIGDRLVFLEALEKRIYAATNVPYPLVGAM
DLTFVLPLGLFNPAMERFAAHAGDLVPAPGHPEPRAFPPRQLFFWGKDHQVLRLSMENAVGTVCHPSL
MNIDAAVGGVNHDPVEAANPYGAYVAAPAGPGADMQQRFLNAWRQRLAHGRVRWVAECQMTAEQFMQP
DNANLALELHPAFDFFAGVADVELPGGEVPPAGPGAIQATWRVVNGNLPLALCPVAFRDARGLELGVG
RHAMAPATIAAVRGAFEDRSYPAVFYLLQAAIHGSEHVFCALARLVTQCITSYWNNTRCAAFVNDYSL
VSYIVTYLGGDLPEECMAVYRDLVAHVEALAQLVDDFTLPGPELGGQAQAELNHLMRDPALLPPLVWD
CDGLMRHAALDRHRDCRIDAGEHEPVYAAACNVATADFNRNDGRLLHNTQARAADAADDRPHRPADWT
VHHKIYYYVLVPAFSRGRCCTAGVRFDRVYATLQNMVVPEIAPGEECPSDPVTDPAHPLHPANLVANT
VNAMFHNGRVVVDGPAMLTLQVLAHNMAERTTALLCSAAPDAGANTASTANMRIFDGALHAGVLLMAP
QHLDHTIQNGEYFYVLPVHALFAGADHVANAPNFPPALRDLARHVPLVPPALGANYFSSIRQPVVQHA
RESAAGENALTYALMAGYFKMSPVALYHQLKTGLHPGFGFTVVRQDRFVTENVLFSERASEAYFLGQL
QVARHETGGGVSFTLTQPRGNVDLGVGYTAVAATATVRNPVTDMGNLPQNFYLGRGAPPLLDNAAAVY
LRNAVVAGNRLGPAQPLPVFGCAQVPRRAGMDHGQDAVCEFIATPVATDINYFRRPCNPRGRAAGGVY
AGDKEGDVIALMYDHGQSDPARPFAATANPWASQRFSYGDLLYNGAYHLNGASPVLSPCFKFFTAADI
TAKHRCLERLIVETGSAVSTATAASDVQFKRPPGCRELVEDPCGLFQEAYPITCASDPALLRSARDGE
AHARETHFTQYLIYDASPLKGLSL

SEQ ID NO: 22 = predicted sequence for ICP1/2 encoded by UL36

MIPAALPHPTMKRQGDRDIVVTGVRNQFATDLEPGGSVSCMRSSLSFLSLLFDVGPRDVLSAEAIEGC
LVEGGEWTRAAAGSGPPRMCSIIELPNFLEYPAARGGLRCVFSRVYGEVGFFGEPTAGLLETQCPAHT
FFAGPWAMRPLSYTLLTIGPLGMGLYRDGDTAYLFDPHGLPAGTPAFIAKVRAGDVYPYLTYYAHDRP
KVRWAGAMVFFVPSGPGAVAPADLTAAALHLYGASETYLQDEPFVERRVAITHPLRGEIGGLGALFVG
VVPRGDGEGSGPVVPALPAPTHVQTPGADRPPEAPRGASGPPDTPQAGHPNRPPDDVWAAALEGTPPA
KPSAPDAAASGPPHAAPPPQTPAGDAAEEAEDLRVLEVGAVPVGRHRARYSTGLPKRRRPTWTPPSSV
EDLTSGERPAPKAPPAKAKKKSAPKKKAPVAAEVPASSPTPIAATVPPAPDTPPQSGQGGGDDGPASP
SSPSVLETLGARRPPEPPGADLAQLFEVHPNVAATAVRLAARDAALAREVAACSQLTINALRSPYPAH
PGLLELCVIFFFERVLAFLIENGARTHTQAGVAGPAAALLDFTLRMLPRKTAVGDFLASTRMSLADVA
AHRPLIQHVLDENSQIGRLALAKLVLVARDVIRETDAFYGDLADLDLQLRAAPPANLYARLGEWLLER
SRAHPNTLFAPATPTHPEPLLHRIQALAQFARGEEMRVEAEAREMREALDALARGVDSVSQRAGPLTV
MPVPAAPGAGGRAPCPPALGPEAIQARLEDVRIQARRAIESAVKEYFHRGAVYSAKALQASDSHDCRF
HVASAAVVPMVQLLESLPAFDQHTRDVAQRAALPPPPPLATSPQAILLRDLLQRGQPLDAPEDLAAWL
SVLTDAATQGLIERKPLEELARSIHGINDQQARRSSGLAELQRFDALDAALAQQLDSDAAFVPATGPA
PYVDGGGLSPEATRMAEDALRQARAMEAAKMTAELAPEARSRLRERAHALEAMLNDARERAKVAHDAR
EKFLHKLQGVLRPLPDFVGLKACPAVLATLRASLPAGWTDLADAVRGPPPEVTAALRADLWGLLGQYR
EALEHPTPDTATALAGLHPAFVVVLKTLFADAPETPVLVQFFSDHAPTIAKAVSNAINAGSAAVATAS
PAATVDAAVRAHGALADAVSALGAAARDPASPLSFLAVLADSAAGYVKATRLALEARGAIDELTTLGS
AAADLVVQARRACAQPEGDHAALIDAAARATTAARESLAGHEAGFGGLLHAEGTAGDHSPSGRALQEL
GKVIGATRRRADELEAAVADLTAKMAAQRARGSSERWAAGVEAALDRVENRAEFDVVELRRLQALAGT
HGYNPRDFRKRAEQALAANAEAVTLALDTAFAFNPYTPENQRHPMLPPLAAIHRLGWSAAFHAAAETY
ADMFRVDAEPLARLLRIAEGLLEMAQAGDGFIDYHEAVGRLADDMTSVPGLRRYVPFFQHGYADYVEL
RDRLDAIRADVHRALGGVPLDLAAAAEQISAARNDPEATAELVRTGVTLPCPSEDALVACAAALERVD
QSPVKNTAYAEYVAFVTRQDTAETKDAVVRAKQQRAEATERVMAGLREALAARERRAQIEAEGLANLK
TMLKVVAVPATVAKTLDQARSVAEIADQVEVLLDQTEKTRELDVPAVIWLEHAQRTFETHPLSAARGD
GPGPLARHAGRLGALFDTRRRVDALRRSLEEAEAEWDEVWGRFGRVRGGAWKSPEGFRAMHEQLRALQ
DTTNTVSGLRAQPAYERLSARYQGVLGAKGAERAEAVEELGARVTKHTALCARLRDEVVRRVPWEMNF
DALGGLLAEFDAAAADLAPWAVEEFRGARELIQYRMGLYSAYARAGGQTGAGAESAPAPLLVDLRALD
ARARASSSPEGHEVDPQLLRRRGEAYLRAGGDPGPLVLREAVSALDLPFATSFLAPDGTPLQYALCFP
AVTDKLGALLMRPEAACVRPPLPTDVLESAPTVTAMYVLTVVNRLQLALSDAQAANFQLFGRFVRHRQ
ATWGASMDAAAELYVALVATTLTREFGCRWAQLGWASGAAAPRPPPGPRGSQRHCVAFNENDVLVALV
AGVPEHIYNFWRLDLVRQHEYMHLTLERAFEDAAESMLFVQRLTPHPDARIRVLPTFLDGGPPTRGLL
FGTRLADWRRGKLSETDPLAPWRSALELGTQRRDVPALGKLSPAQALAAVSVLGRMCLPSAALAALWT
CMFPDDYTEYDSFDALLAARLESGQTLGPAGGREASLPEAPHALYRPTGQHVAVLAAATHRTPAARVT
AMDLVLAAVLLGAPVVVALRNTTAFSRESELELCLTLFDSRPGGPDAALRDVVSSDIETWAVGLLHTD
LNPIENACLAAQLPRLSALIAERPLADGPPCLVLVDISMTPVAVLWEAPEPPGPPDVRFVGSEATEEL
PFVATAGDVLAASAADADPFFARAILGRPFDASLLTGELFPGHPVYQRPLADEAGPSAPTAARDPRDL
AGGDGGSGPEDPAAPPARQADPGVLAPTLLTDATTGEPVPPRMWAWIHGLEELASDDAGGPTPNPAPA
LLPPPATDQSVPTSQYAPRPIGPAATARETRPSVPPQQNTGRVPVAPRDDPRPSPPTPSPPADAALPP

PAFSGSAAAFSAAVPRVRRSRRTRAKSRAPRASAPPEGWRPPALPAPVAPVAASARPPDQPPTPESAP
PAWVSALPLPPGPASARGAFPAPTLAPIPPPPAEGAVVPGGDRRRGRRQTTAGPSPTPPRGPAAGPPR
RLTRPAVASLSASLNSLPSPRDPADHAAAVSAAAAAVPPSPGLAPPTSAVQTSPPPLAPGPVAPSEPL
CGWVVPGGPVARRPPPQSPATKPAARTRIRARSVPQPPLPQPPLPQPPLPQPPLPQPPLPQPPLPQPP
LPQPPLPQPPLPQPPLPQPPLPPVTRTLTPQSRDSVPTPESPTHTNTHLPVSAVTSWASSLALHVDSA
PPPASLLQTLHISSDDEHSDADSLRFSDSDDTEALDPLPPEPHLPPADEPPGPLAADHLQSPHSQFGP
LPVQANAVLSRRYVRSTGRSALAVLIRACRRIQQQLQRTRRALFQRSNAVLTSLHHVRMLLG

SEQ ID NO: 23 = ICP1/2 internal fragments encoded by UL36.3.4.1

AAQRARGSSERWAAGVEAALDRVENRAEFDVVELRRLQALAGTHGYNPRDFRKRAEQA
LAANAEAVTLALDTAFAFNPYTPENQRHPMLPPLAAIHRLGWSAAFHAAAETYADMFRVDAEPLARLL
RIAEGLLEMAQAGDGFIDYHEAVGRLADDMTSVPGLRRYVPFFQHGYADYVELRDRLDAIRADVHRAL
GGVPLDLAAAAEQISAARNDPEATAELVRTGVTLPCPSEDALVACAAALERVDQSPVKNTAYAEYVAF
VTRQDTAETKDAVVRAKQQRAEATERVMAGLREALAARERRAQIEAEGLANLKTMLKVVAVPATVAKT
LDQARSVAEIADQVEVLLDQTEKTRELDVPAVIWLEHAQRTFETHPLSAARGDGPGPLARHAGRLGAL
FDTRRRVDALRRSLEEAEAEWDEVWGRFGRVRGGAWKSPEGFRAMHEQLRALQDTTNTVSGLRAQPAY
ERLSARYQGVLGAKGAERAEAVEELGARVTKHTALCARLRDEVVRRVPWEMNFDALGGLLAEFDAAAA
DLAPWAVEEFRGARELIQYRMGLYSAYARAGGQTGAGAESAPAPLLVDLRALDARARASSSPEGHEVD
PQLLRRRGEAYLRAGGDPGPLVLREAVSALDLPFATSFLAPDGTPLQYALCFPAVTDKLGALLMRPEA
ACVRPPLPTDVLESAPTVTAMYVLTVVNRLQLALSDAQAANFQLFGRFVRHRQATWGASMDAAAELYV
ALVATTLTREFGCRWAQLGWASGAAAPRPPPGPRGSQRHCVAFNENDVLVALVAGVPEHIYNFWRLDL
VRQHEYMHLTLERAFEDAAESMLFVQRLTPHPDARIRVLPTFLDGGPPTRGLLFGTRLADWRRGKLSE
TDPLAPWRSALELGTQRRDVPALGKLSPAQALAAVSVLGRMCLPSAALAALWTCMFPDDYTEYDSFDA
LLAARLESGQTLGPAGGREASL

SEQ ID NO: 24 = ICP1/2 internal fragments encoded by UL36.4.2.5

EYDSFDALLAARLESGQTLGPAGGREASLPEAPHALYRPTGQHVAVLAAATHRTPAAR
VTAMDLVLAAVLLGAPVVVALRNTTAFSRESELELCLTLFDSRPGGPDAALRDVVSSDIETWAVGLLH
TDLNPIENACLAAQLPRLSALIAERPLADGPPCLVLVDISMTPVAVLWEAPEPPGPPDVRFVGSEATE
ELPFVATAGDVLAASAADADPFFARAILGRPFDASLLTGELFPGHPVYQRPLADEAGPSAPTAARDPR
DLAGGDGGSGPEDPAAPPARQADPGVLAPTLLTDATTGEPVPPRMWAWIHGLEELASDDAGGPTPNPA
PALLPPPATDQSVPTSQYAPRPIGPAATARETRPSVPPQQNTGRVPVAPRDDPRPSPPTPSPPADAAL
PPPAFSGSAAAFSAAVPRVRRSRRTRAKSRAPRASAPPEGWRPPALPAPVAPVAASARPPDQPPTPES
APPAWVSALPLPPGPASARGAFPAPTLAPIPPPPAEGAVVPGGDRRRGRRQTTAGPSPTPPRGPAAGP
PRRLTRPAVASLSASLNSLPSPRDPADHAAAVSAAAAAVPPSPGLAPPTSAVQTSPPPLAPGPVAPSE
PLCGWVVPGGPVARRPPPQSPATKPAARTRIRARSVPQPPLPQPPLPQPPLPQPPLPQPPLPQPPLPQ
PPLPQPPLPQPPLPQPPLPQPPLPPVTRTLTPQSRDSVPTPESPTHTNTHLPVSAVTSWASSLALHVD

SAPPPASLLQTLHISSDDEHSDADSLRFSDSDDTEALDPLPPEPHLPPADEPPGPLAADHLQSPHSQF
GPLPVQANAVLSRRYVRSTGRSALAVLIRACRRIQQQLQRTRRALFQRSNAVLTSLHHVRMLLG

SEQ ID NO: 25 = predicted sequence for reductase encoded by UL40

MDPAVSPASTDPLDTHASGAGAAPIPVCPTPERYFYTSQCPDINHLRSLSILNRWLET
ELVFVGDEEDVSKLSEGELGFYRFLFAFLSAADDLVTENLGGLSGLFEQKDILHYYVEQECIEVVHSR
VYNIIQLVLFHNNDQARRAYVARTINHPAIRVKVDWLEARVRECDSIPEKFILMILIEGVFFAASFAA
IAYLRTNNLLRVTCQSNDLISRDEAVHTTASCYIYNNYLGGHAKPEAARVYRLFREAVDIEIGFIRSQ
APTDSSILSPGALAAIENYVRFSADRLLGLIHMQPLYSAPAPDASFPLSLMSTDKHTNFFECRSTSYA
GAVVNDL

SEQ ID NO: 26 = ICP47 encoded by US12

MSWALKTTDMFLDSSRCTHRTYGDVCAEIHKREREDREAARTAVTDPELPLLCPPDVRSDPASRNPTQ
QTRGCARSNERQDRVLAP

SEQ ID NO: 27 = gM2 encoded by UL10

MGRRAPRGSPEAAPGADVAPGARAAWWVWCVQVATFIVSAICVVGLLVLASVFRDRFPCLYAPATSYA
KANATVEVRGGVAVPLRLDTQSLLATYAITSTLLLAAAVYAAVGAVTSRYERALDAARRLAAARMAMP
HATLIAGNVCAWLLQITVLLLAHRISQLAHLIYVLHFACLVYLAAHFCTRGVLSGTYLRQVHGLIDPA
PTHHRIVGPVRAVMTNALLLGTLLCTAAAAVSLNTIAALNFNFSAPSMLICLTTLFALLVVSLLLVVE
GVLCHYVRVLVGPHLGAIAATGIVGLACEHYHTGGYYVVEQQWPGAQTGVRVALALVAAFALAMAVLR
CTRAYLYHRRHHTKFFVRMRDTRHRAHSALRRVRSSMRGSRRGGPPGDPGYAETPYASVSHHAEIDRY
GDSDGDPIYDEVAPDHEAELYARVQRPGPVPDAEPIYDTVEGYAPRSAGEPVYSTVRRW

SEQ ID NO: 28 = predicted sequence for cleavage/package protein encoded by UL15

MFGQQLASDVQQYLERLEKQRQQKVGVDEASAGLTLGGDALRVPFLDFATATPKRHQTVVPGVGTLHD
CCEHSPLFSAVARRLLFNSLVPAQLRGRDFGGDHTAKLEFLAPELVRAVARLRFRECAPEDAVPQRNA
YYSVLNTFQALHRSEAFRQLVHFVRDFAQLLKTSFRASSLAETTGPPKKRAKVDVATHGQTYGTLELF
QKMILMHATYFLAAVLLGDHAEQVNTFLRLVFEIPLFSDTAVRHFRQRATVFLVPRRHGKTWFLVPLI
ALSLASFRGIKIGYTAHIRKATEPVFDEIDACLRGWFGSSRVDHVKGETISFSFPDGSRSTIVFASSH
NTNGIRGQDFNLLFVDEANFIRPDAVQTIMGFLNQANCKIIFVSSTNTGKASTSFLYNLRGAADELLN
VVTYICDDHMPRVVTHTNATACSCYILNKPVFITMDGAVRRTADLFLPDSFMQEIIGGQARETGDDRP
VLTKSAGERFLLYRPSTTTNSGLMAPELYVYVDPAFTANTRASGTGIAVVGRYRDDFIIFALEHFFLR

ALTGSAPADIARCVVHSLAQVLALHPGAFRSVRVAVEGNSSQDSAVAIATHVHTEMHRILASAGANGP
GPELLFYHCEPPGGAVLYPFFLLNKQKTPAFEYFIKKFNSGGVMASQELVSVTVRLQTDPVEYLSEQL
NNLIETVSPNTDVRMYSGKRNGAADDLMVAVIMAIYLAAPTGIPPAFFPITRTS

SEQ ID NO: 29 = predicted sequence for ICP35 encoded by UL26.5

MNPVSASGAPAPPPPGDGSYLWIPASHYNQLVTGQSAPRHPPLTACGLPAAGTVAYGHPGAGPSPHYP
PPPAHPYPGMLFAGPSPLEAQIAALVGAIAADRQAGGLPAAAGDHGIRGSAKRRRHEVEQPEYDCGRD
EPDRDFPYYPGEARPEPRPVDSRRAARQASGPHETITALVGAVTSLQQELAHMRARTHAPYGPYPPVG
PYHHPHADTETPAQPPRYPAKAVYLPPPHIAPPGPPLSGAVPPPSYPPVAVTPGPAPPLHQPSPAHAH
PPPPPPGPTPPPAASLPQPEAPGAEAGALVNASSAAHVNVDTARAADLFVSQMMGSR

SEQ ID NO: 30 = predicted sequence for polymerase encoded by UL30

MFCAAGGPASPGGKPAARAASGFFAPHNPRGATQTAPPPCRRQNFYNPHLAQTGTQPKALGPAQRHTY
YSECDEFRFIAPRSLDEDAPAEQRTGVHDGRLRRAPKVYCGGDERDVLRVGPEGFWPRRLRLWGGADH
APEGFDPTVTVFHVYDILEHVEHAYSMRAAQLHERFMDAITPAGTVITLLGLTPEGHRVAVHVYGTRQ
YFYMNKAEVDRHLQCRAPRDLCERLAAALRESPGASFRGISADHFEAEVVERADVYYYETRPTLYYRV
FVRSGRALAYLCDNFCPAIRKYEGGVDATTRFILDNPGFVTFGWYRLKPGRGNAPAQPRPPTAFGTSS
DVEFNCTADNLAVEGAMCDLPAYKLMCFDIECKAGGEDELAFPVAERPEDLVIQISCLLYDLSTTALE
HILLFSLGSCDLPESHLSDLASRGLPAPVVLEFDSEFEMLLAFMTFVKQYGPEFVTGYNIINFDWPFV
LTKLTEIYKVPLDGYGRMNGRGVFRVWDIGQSHFQKRSKIKVNGMVNIDMYGIITDKVKLSSYKLNAV
AEAVLKDKKKDLSYRDIPAYYASGPAQRGVIGEYCVQDSLLVGQLFFKFLPHLELSAVARLAGINITR
TIYDGQQIRVFTCLLRLAGQKGFILPDTQGRFRGLDKEAPKRPAVPRGEGERPGDGNGDEDKDDDEDG
DEDGDEREEVARETGGRHVGYQGARVLDPTSGFHVDPVVVFDFASLYPSIIQAHNLCFSTLSLRPEAV
AHLEADRDYLEIEVGGRRLFFVKAHVRESLLSILLRDWLAMRKQIRSRIPQSTPEEAVLLDKQQAAIK
VVCNSVYGFTGVQHGLLPCLHVAATVTTIGREMLLATRAYVHARWAEFDQLLADFPEAAGMRAPGPYS
MRIIYGDTDSIFVLCRGLTAAGLVAMGDKMASHISRALFLPPIKLECEKTFTKLLLIAKKKYIGVICG
GKMLIKGVDLVRKNNCAFINRTSRALVDLLFYDDTVSGAAAALAERPAEEWLARPLPEGLQAFGAVLV
DAHRRITDPERDIQDFVLTAELSRHPRAYTNKRLAHLTVYYKLMARRAQVPSIKDRIPYVIVAQTREV
EETVARLAALRELDAAAPGDEPAPPAALPSPAKRPRETPSHADPPGGASKPRKLLVSELAEDPGYAIA
RGVPLNTDYYFSHLLGAACVTFKALFGNNAKITESLLKRFIPETWHPPDDVAARLRAAGFGPAGAGAT
AEETRRMLHRAFDTLA

SEQ ID NO: 31 = predicted sequence for helicase/primase complex encoded by UL5

MAASGGEGSRDVRAPGPPPQQPGARPAVRFRDEAFLNFTSMHGVQPIIARIRELSQQQLDVTQVPRLQ
WFRDVAALEVPTGLPLREFPFAAYLITGNAGSGKSTCVQTLNEVLDCVVTGATRIAAQNMYVKLSGAF
LSRPINTIFHEFGFRGNHVQAQLGQHPYTLASSPASLEDLQRRDLTYYWEVILDITKRALAAHGGEDA
RNEFHALTALEQTLGLGQGALTRLASVTHGALPAFTRSNIIVIDEAGLLGRHLLTTVVYCWWMINALY
HTPQYAGRLRPVLVCVGSPTQTASLESTFEHQKLRCSVRQSENVLTYLICNRTLREYTRLSHSWAIFI
NNKRCVEHEFGNLMKVLEYGLPITEEHMQFVDRFVVPESYITNPANLPGWTRLFSSHKEVSAYMAKLH
AYLKVTREGEFVVFTLPVLTFVSVKEFDEYRRLTQQPTLTMEKWITANASRITNYSQSQDQDAGHVRC
EVHSKQQLVVARNDITYVLNSQVAVTARLRKMVFGFDGTFRTFEAVLRDDSFVKTQGETSVEFAYRFL
SRLMFGGLIHFYNFLQRPGLDATQRTLAYGRLGELTAELLSLRRDAAGASATRAADTSDRSPGERAFN
FKHLGPRDGGPDDFPDDDLDVIFAGLDEQQLDVFYCHYALEEPETTAAVHAQFGLLKRAFLGRYLILR
ELFGEVFESAPFSTYVDNVIFRGCELLTGSPRGGLMSVALQTDNYTLMGYTYTRVFAFAEELRRRHAT
AGVAEFLEESPLPYIVLRDQHGFMSVVNTNISEFVESIDSTELAMAINADYGISSKLAMTITRSQGLS
LDKVAICFTPGNLRLNSAYVAMSRTTSSEFLHMNLNPLRERHERDDVISEHILSALRDPNVVIVY

SEQ ID NO: 32 = predicted sequence for helicase/primase complex encoded by UL8

MEAPGIVWVEESVSAITLYAVWLPPRTRDCLHALLYLVCRDAAGEARARFAEVSVGSSDLQDFYGSPD
VSAPGAVAAARAATAPAASPLEPLGDPTLWRALYACVLAALERQTGRWALFVPLRLGWDPQTGLVVRV
ERASWGPPAAPRAALLDVEAKVDVDPLALSARVAEHPGARLAWARLAAIRDSPQCASSASLAVTITTR
TARFAREYTTLAFPPTRKEGAFADLVEVCEVGLRPRGHPQRVTARVLLPRGYDYFVSAGDGFSAPALV
ALFRQWHTTVHAAPGALAPVFAFLGPGFEVRGGPVQYFAVLGFPGWPTFTVPAAAAAESARDLVRGAA
ATHAACLGAWPAVGARVVLPPRAWPAVASEAAGRLLPAFREAVARWHPTATTIQLLDPPAAVGPVWTA
RFCFSGLQAQLLAALAGLGEAGLPEARGRAGLERLDALVAAAPSEPWARAVLERLVPDACDACPALRQ
LLGGVMAAVCLQIEQTASSVKFAVCGGTGAAFWGLFNVDPGDADAAHGAIQDARRALEASVRAVLSAN
GIRPRLAPSLAPEGVYTHVVTWSQTGAWFWNSRDDTDFLQGFPLRGAAYAAAAEVMRDALRRILRRPA
AGPPEEAVCAARGVMEDACDRFVLDAFGRRLDAEYWSVLTPPGEADDPLPQTAFRGGALLDAEQYWRR
VVRVCPGGGESVGVPVDLYPRPLVLPPVDCAHHLREILREIQLVFTGVLEGVWGEGGSFVYPFDEKIR
FLFP

SEQ ID NO: 33 = predicted sequence for unknown protein encoded by UL15.5

MDGAVRRTADLFLPDSFMQEIIGGQARETGDDRPVLTKSAGERFLLYRPSTTTNSGLMAPELYVYVDP
AFTANTRASGTGIAVVGRYRDDFIIFALEHFFLRALTGSAPADIARCVVHSLAQVLALHPGAFRSVRV
AVEGNSSQDSAVAIATHVHTEMHRILASAGANGPGPELLFYHCEPPGGAVLYPFFLLNKQKTPAFEYF
IKKFNSGGVMASQELVSVTVRLQTDPVEYLSEQLNNLIETVSPNTDVRMYSGKRNGAADDLMVAVIMA
IYLAAPTGIPPAFFPITRTS

SEQ ID NO: 34 = predicted sequence for cleavage and packaging protein encoded by UL32

MATSAPGVPSSAAVREESPGSSWKEGAFERPYVAFDPDLLALNEALCAELLAACHVVGVPPASALDED
VESDVAPAPPRPRGAAREASGGRGPGSARGPPADPTAEGLLDTGPFAAASVDTFALDRPCLVCRTIEL
YKQAYRLSPQWVADYAFLCAKCLGAPHCAASIFVAAFEFVYVMDHHFLRTKKATLVGSFARFALTIND
IHRHFFLHCCFRTDGGVPGRHAQKQPRPTPSPGAAKVQYSNYSFLAQSATRALIGTLASGGDDGAGAG
AGGGSGTQPSLTTALMNWKDCARLLDCTEGKRGGGDSCCTRAAARNGEFEAAAGALAQGGEPETWAYA
DLILLLLAGTPAVWESGPRLRAAADARRAAVSESWEAHRGARMRDAAPRFAQFAEPQPQPDLDLGPLM
ATVLKHGRGRGRTGGECLLCNLLLVRAYWLAMRRLRASVVRYSENNTSLFDCIVPVVDQLEADPEAQP
GDGGRFVSLLRAAGPEAIFKHMFCDPMCAITEMEVDPWVLFGHPRADHRDELQLHKAKLACGNEFEGR
VCIALRALIYTFKTYQVFVPKPTALATFVREAGALLRRHSISLLSLEHTLCTYV

SEQ ID NO: 35 = predicted sequence for ICP1/2 fragment enoded by UL36.4.2

MEYDSFDALLAARLESGQTLGPAGGREASLPEAPHALYRPTGQHVAVLAAATHRTPAARVTAMDLVLA
AVLLGAPVVVALRNTTAFSRESELELCLTLFDSRPGGPDAALRDVVSSDIETWAVGLLHTDLNPIENA
CLAAQLPRLSALIAERPLADGPPCLVLVDISMTPVAVLWEAPEPPGPPDVRFVGSEATEELPFVATAG
DVLAASAADADPFFARAILGRPFDASLLTGELFPGHPVYQRPLADEAGPSAPTAARDPRDLAGGDGGS
GPEDPAAPPARQADPGVLAPTLLTDATTGEPVPPRMWAWIHGLEELASDDAGGPT

SEQ ID NO: 36 = predicted sequence for ICP27 encoded by UL54

MATDIDMLIDLGLDLSDSELEEDALERDEEGRRDDPESDSSGECSSSDEDMEDPCGDGGAEAIDAAIP
KGPPARPEDAGTPEASTPRPAARRGADDPPPATTGVWSRLGTRRSASPREPHGGKVARIQPPSTKAPH
PRGGRRGRRRGRGRYGPGGADSTPKPRRRVSRNAHNQGGRHPASARTDGPGATHGEARRGGEQLDVSG
GPRPRGTRQAPPPLMALSLTPPHADGRAPVPERKAPSADTIDPAVRAVLRSISERAAVERISESFGRS
ALVMQDPFGGMPFPAANSPWAPVLATQAGGFDAETRRVSWETLVAHGPSLYRTFAANPRAASTAKAMR
DCVLRQENLIEALASADETLAWCKMCIHHNLPLRPQDPIIGTAAAVLENLATRLRPFLQCYLKARGLC
GLDDLCSRRRLSDIKDIASFVLVILARLANRVERGVSEIDYTTVGVGAGETMHFYIPGACMAGLIEIL
DTHRQECSSRVCELTASHTIAPLYVHGKYFYCNSLF

SEQ ID NO: 37 = virion protein encoded by UL49.5

MTGKPARLGRWVVLLFVALVAGVPGEPPNAAGARGVIGDAQCRGDSAGVVSVPGVLVP
FYLGMTSMGVCMIAHVYQICQRALAAGSA

SEQ ID NO: 38 = gG2 encoded by US4

NRWGSGVPGPINPPNSDVVFPGGSPVAQYCYAYPRLDDPGPLGSADA
GRQDLPRRVVRHEPLGRSFLTGGLVLLAPPVRGFGAPNATYAARVTYYRLTRACRQPILLRQYGGCRG
GEPPSPKTCGSYTYTYQGGGPPTRYALVNASLLVPIWDRAAETFEYQIELGGELHVGLLWVEVGGEGP
GPTAPPQAARAEGGPCVPPVPAGRPWRSVPPVWYSAPNPGFRGLRFRERCLPPQTPAAPSDLPRVAFA
PQSLLVGITGRTFIRMARPTEDVGVLPPHWAPGALDDGPYAPFPPRPRFRR

SEQ ID NO: 39 = nucleotide sequence for RS1 (ICP4), full-length

ATGTCGTACTACCATCACCATCACCATCACAGTGCCGAACAGCGTAAAAAGAAAAAAACCACCACCAC
GACCCAAGGACGTGGAGCTGAAGTTGCTATGGCGGATGAGGATGGAGGCCGCTTGAGAGCTGCTGCTG
AGACTACTGGAGGACCTGGATCACCGGACCCTGCCGATGGACCCCCCCCCTACACCAAACCCCGATCGT
AGACCGGCTGCTAGACCTGGATTCGGATGGCATGGAGGACCCGAGGAAAACGAGGACGAGGCGGACGA
CGCCGCTGCCGACGCCGACGCCGATGAGGCTGCCCCTGCTTCTGGAGAGGCGGTAGACGAACCTGCTG
CCGATGGAGTTGTTAGCCCTAGGCAATTGGCTTTGTTGGCGAGCATGGTAGACGAGGCTGTGAGAACA
ATCCCTTCCCCTCCCCCTGAACGTGATGGAGCACAAGAGGAGGCGGCTAGGAGTCCCTCACCACCCCG
TACACCTTCTATGAGAGCGGATTACGGCGAGGAAAACGACGACGACGACGATGATGATGACGACGATG
ATCGTGATGCCGGACGCTGGGTTAGGGGACCTGAAACCACTTCTGCTGTCCGTGGAGCATACCCCGAT
CCTATGGCGAGTTTGAGCCCTAGACCACCTGCCCCGAGGAGACACCACCACCACCACCATCATAGGCG
TAGACGTGCTCCTAGACGTCGTTCTGCCGCTAGTGACTCTTCCAAATCTGGCTCTTCTTCATCTGCCT
CTTCCGCTTCATCTTCGGCCTCATCGTCCTCTTCGGCATCCGCTTCGAGTAGTGATGATGATGATGAC
GACGACGCTGCTAGAGCCCCCGCTTCTGCTGCCGACCACGCTGCTGGCGGAACTTTGGGAGCCGACGA
CGAGGAGGCGGGAGTTCCTGCTCGTGCCCCGGGAGCTGCTCCGAGGCCTTCTCCACCCCGTGCTGAAC
CTGCTCCGGCTAGAACACCGGCCGCTACTGCTGGTAGACTGGAGCGTAGACGTGCCCGTGCTGCTGTG
GCTGGTAGAGATGCTACTGGCCGCTTCACTGCTGGCCGTCCTAGACGTGTTGAACTGGACGCCGATGC
TGCTTCTGGTGCTTTCTACGCCCGTTACCGTGATGGTTACGTGTCTGGTGAACCTTGGCCTGGCGCTG
GTCCACCTCCGCCCGGACGTGTACTCTACGGTGGATTGGGCGATTCTCGCCCTGGTCTGTGGGGCGCT
CCGGAGGCTGAGGAGGCTAGAGCCCGTTTCGAGGCTTCTGGTGCCCCTGCTCCTGTTTGGGCTCCTGA
ATTGGGCGACGCTGCTCAACAATACGCCCTCATCACACGCTTGCTGTACACTCCCGACGCCGAGGCTA
TGGGATGGCTCCAAAACCCTAGAGTTGCCCCTGGTGATGTTGCTCTGGATCAGGCTTGTTTCCGTATC
TCCGGCGCTGCTCGTAACTCTTCTTCGTTCATCTCCGGTTCTGTGGCTAGAGCTGTGCCTCACTTGGG
ATACGCCATGGCCGCTGGACGTTTCGGCTGGGGACTGGCTCATGTTGCTGCCGCTGTAGCAATGTCTA
GACGCTACGACCGTGCTCAAAAAGGATTCTTGCTCACGTCACTGAGGCGTGCTTACGCCCCTTTGTTG
GCCCGTGAAAACGCTGCCCTCACTGGCGCCCGTACCCCCGATGACGGTGGCGACGCCAACCGCCACGA
TGGTGATGATGCTAGAGGCAAACCCGCTGCCGCTGCTGCTCCTTTGCCCTCTGCCGCCGCTTCCCCTG
CCGATGAACGTGCTGTTCCTGCCGGTTACGGTGCCGCTGGTGTGTTGGCTGCTTTGGGACGCTTGAGT
GCTGCCCCGGCTAGTGCCCCCGCTGGTGCCGATGACGATGACGATGACGATGGTGCTGGCGGAGGCGG
TGGCGGTAGACGTGCTGAGGCTGGACGTGTTGCTGTTGAATGCCTGGCTGCCTGTAGAGGAATCTTGG
AGGCTCTGGCCGAGGGATTCGACGGAGACTTGGCGGCTGTACCGGGACTGGCGGGAGCGAGGCCTGCC
GCTCCACCTCGCCCCGGTCCTGCTGGTGCTGCCGCTCCTCCTCATGCCGACGCTCCTAGACTCCGTGC

TTGGCTCCGTGAACTCCGTTTCGTTCGTGACGCTTTGGTTCTGATGAGACTGAGAGGCGACTTGAGAG
TGGCTGGAGGATCCGAGGCTGCTGTTGCTGCTGTCCGTGCTGTTTCTTTGGTTGCTGGTGCTTTGGGC
CCTGCTTTGCCGAGATCTCCCCGTTTGTTGTCGAGTGCCGCCGCTGCTGCCGCCGATTTGTTGTTCCA
AAACCAATCCCTCCGCCCTCTGCTCGCCGACACTGTTGCCGCTGCCGATTCTCTGGCTGCTCCGGCTT
CTGCCCCACGTGAAGCTCGTAAACGTAAATCACCCGCTCCGGCTCGTGCTCCCCCTGGTGGCGCCCCT
AGACCCCCTAAAAAATCCCGTGCCGATGCCCCTAGACCTGCTGCTGCTCCCCCCGCTGGTGCTGCTCC
CCCCGCTCCCCCTACTCCCCCCCCCACGCCCACCTCGTCCCGCTGCCCTCACACGCCGTCCTGCTGAGG
GACCCGATCCACAAGGCGGCTGGCGTAGACAACCTCCTGGCCCATCCCATACACCGGCACCATCTGCC
GCTGCTTTGGAGGCTTACTGTGCTCCTCGTGCTGTGGCTGAACTCACCGATCATCCGCTGTTCCCTGC
TCCCTGGCGTCCCGCCCTCATGTTCGATCCTAGAGCTTTGGCTTCCTTGGCCGCTCGTTGTGCTGCCC
CTCCCCCTGGCGGTGCTCCGGCTGCTTTCGGTCCTCTCCGTGCCTCTGGTCCACTCCGCCGTGCCGCT
GCCTGGATGAGACAAGTTCCCGACCCTGAGGATGTTAGAGTTGTGATCTTGTACTCGCCCTTGCCTGG
CGAGGATTTGGCCGCTGGTAGAGCTGGCGGTGGCCCCCCTCCTGAATGGTCTGCTGAACGTGGTGGTT
TGTCTTGCTTGTTGGCCGCCCTGGGAAACCGTCTGTGTGGTCCTGCTACTGCTGCTTGGGCTGGAAAC
TGGACTGGCGCTCCCGATGTTTCTGCTCTCGGTGCTCAAGGAGTTTTGCTGCTCTCTACTCGTGACTT
GGCATTCGCTGGAGCTGTTGAATTCCTGGGACTCTTGGCTGGCGCTTGTGATAGGAGACTCATCGTCG
TAAACGCTGTGAGAGCTGCCGATTGGCCTGCCGATGGTCCTGTTGTGTCTCGTCAACACGCTTACTTG
GCTTGTGAAGTGTTGCCCGCTGTCCAATGTGCTGTTCGCTGGCCTGCTGCTCGTGATCTGAGGCGTAC
TGTTCTGGCTAGTGGTCGTGTTTTCGGACCTGGTGTTTTCGCTCGTGTCGAAGCTGCTCACGCTAGAC
TGTACCCCGATGCCCCACCCCTCCGTTTGTGTCGTGGAGCAAACGTTCGCTACCGTGTCCGTACTCGT
TTCGGACCCGATACTCTGGTTCCAATGTCCCCTCGTGAATACCGTCGTGCTGTTCTGCCTGCCCTCGA
TGGACGTGCTGCCGCTTCTGGCGCTGGTGACGCTATGGCTCCTGGCGCTCCGGACTTCTGTGAGGATG
AGGCTCACTCACATCGTGCCTGTGCCCGCTGGGGACTGGGCGCTCCATTGAGGCCTGTATACGTGGCA
CTGGGCCGTGATGCTGTTAGAGGCGGACCCGCTGAATTGAGAGGCCCTCGTCGTGAATTCTGTGCTAG
GGCTCTGCTCGAACCCGATGGAGATGCTCCTCCTTTGGTACTCCGTGACGACGCCGATGCTGGTCCTC
CCCCACAAATTCGCTGGGCTAGTGCTGCTGGACGTGCTGGTACTGTATTGGCTGCTGCTGGCGGTGGC
GTTGAAGTTGTTGGTACTGCCGCTGGACTCGCTACACCTCCCCGCCGTGAACCTGTAGACATGGATGC
TGAACTCGAGGATGATGACGACGGATTGTTCGGAGAGTAATAG

SEQ ID NO: 40 = US6ΔTMR

ATGAAGTTCCTCGTGAACGTGGCCCTGGTGTTCATGGTGGTGTACATCAGCTACATCTACGCCAACCG
TTGGAAGTACGCTCTGGCTGACCCATCCCTGAAGATGGCTGACCCCAACCGTTTCCGTGGCAAGAACC
TGCCCGTGCTGGACCAGCTGACCGACCCCCCCTGGCGTGAAGCGTGTGTACCACATCCAGCCATCCCTC
GAAGACCCCTTCCAGCCCCCCTCCATCCCCATCACCGTGTACTACGCTGTGCTGGAACGCGCTTGCCG
TTCCGTGCTGCTGCACGCTCCTTCCGAGGCTCCCCAGATCGTGCGTGGTGCTTCCGACGAGGCTCGCA
AGCACACCTACAACCTGACTATCGCTTGGTACAGGATGGGTGACAACTGCGCTATCCCTATCACCGTC
ATGGAATACACCGAGTGCCCCTACAACAAGTCCCTGGGCGTGTGCCCTATCCGTACCCAGCCCCGTTG
GTCCTACTACGACTCCTTCAGCGCTGTGTCCGAGGACAACCTGGGTTTCCTGATGCACGCTCCCGCTT

TCGAGACTGCTGGCACCTACCTGCGTCTGGTCAAGATCAACGACTGGACCGAGATCACCCAGTTCATC
CTGGAACACCGTGCTCGTGCTTCGTGCAAGTACGCCCTGCCCCTGCGTATCCCTCCTGCTGCTTGCCT
GACCTCCAAGGCTTACCAGCAGGGCGTGACCGTGGACTCCATCGGCATGCTGCCCCGTTTCATCCCCG
AGAACCAGCGTACCGTGGCTCTGTACTCTCTGAAGATCGCTGGCTGGCACGGTCCTAAGCCCCCCTAC
ACCTCCACTCTGCTGCCCCCTGAGCTGTCCGACACCACCAACGCTACTCAGCCCGAGTTGGTGCCTGA
GGACCCCGAGGACTCCGCTCTGTTGGAGGACCCCGCTGGAACCGTGTCCTCCCAGATCCCCCCCAACT
GGCACATCCCTTCCATCCAGGACGTGGCCCCTCACCACGCTCCAGCTGCTCCCTCCAACCCCCGTCGT
CGTGCTCAGATGGCTCCCAAGCGTCTGCGTCTGCCCCACATCCGTGACGACGACGCTCCTCCATCCCA
CCAGCCCCTGTTCTACCACCACCACCATCACCACTAATAA

SEQ ID NO: 41 = nucleotide sequence for RL1 (ICP34.5)

ATGTCTCGTCGTCGTGGTCCTCGTCGTCGTGGTCCTCGTCGTCGTCCGCGTCCGGGTGCGCCGGCGGT
ACCACGCCCGGGTGCGCCGGCAGTGCCGCGTCCAGGCGCACTGCCTACCGCGGACTCTCAAATGGTGC
CGGCGTATGATTCTGGTACTGCCGTCGAATCTGCTCCGGCAGCGAGCTCCCTGCTGCGTCGTTGGCTG
CTGGTCCCTCAGGCGGACGATTCCGATGACGCAGACTACGCGGGCAACGACGACGCGGAGTGGGCTAA
CAGCCCGCCAAGCGAGGGTGGTGGCAAAGCGCCGGAGGCTCCGCACGCAGCGCCTGCCGCAGCGTGCC
CGCCTCCGCCTCCTCGTAAAGAACGTGGCCCTCAACGTCCTCTGCCGCCGCACCTGGCTCTGCGTCTG
CGTACTACCACTGAGTACCTGGCGCGTCTGTCTCTGCGTCGTCGCCGTCCGCCGGCTAGCCCGCCGGC
CGATGCACCGCGTGGCAAAGTGTGCTTCTCTCCACGTGTTCAAGTTCGTCACCTGGTGGCTTGGGAAA
CGGCTGCCCGTCTGGCTCGCCGTGGCAGCTGGGCACGTGAGCGCGCAGACCGTGACCGCTTCCGTCGC
CGTGTGGCGGCTGCTGAAGCCGTTATCGGCCCGTGCCTGGAACCTGAGGCTCGCGCTCGCGCGCGTGC
GCGCGCTCGTGCCCACGAAGATGGCGGTCCAGCAGAGGAAGAAGAGGCAGCTGCAGCAGCGCGCGGTA
GCTCCGCGGCTGCGGGTCCAGGTCGTCGTGCCGTA

SEQ ID NO: 42 = nucleotide seqeunce for RL2 (ICP0)

ATGTCGTACTACCATCACCATCACCATCACATGGAGCCACGTCCTGGTACTTCTTCTCGCGCTGATCC
TGGTCCTGAACGTCCGCCACGCCAGACTCCGGGCACCCAGCCGGCCGCCCCTCACGCTTGGGGCATGC
TGAACGATATGCAGTGGCTGGCGTCCTCTGATTCCGAAGAGGAGACTGAGGTTGGTATCAGCGATGAT
GATCTGCACCGCGACTCTACCAGCGAAGCAGGTTCCACTGACACCGAAATGTTTGAAGCGGGCCTGAT
GGATGCCGCGACCCCGCCGGCTCGTCCGCCGGCTGAACGTCAGGGTAGCCCTACGCCTGCGGATGCGC
AAGGCTCTTGTGGTGGTGGTCCAGTAGGCGAAGAGGAGGCTGAGGCCGGTGGCGGCGGTGATGTGTGT
GCGGTTTGTACCGATGAAATCGCACCGCCGCTGCGTTGTCAGTCTTTCCCGTGCCTGCACCCGTTTTG
CATTCCGTGCATGAAAACCTGGATCCCGCTGCGCAACACTTGCCCGCTGTGCAACACTCCGGTTGCTT
ATCTGATCGTTGGTGTAACCGCATCTGGTTCCTTTTCTACCATCCCGATTGTCAACGACCCACGTACG
CGTGTTGAGGCGGAGGCGGCTGTACGTGCGGGCACCGCGGTGGACTTTATCTGGACCGGTAACCCGCG
CACCGCGCCACGCTCCCTGTCTCTGGGTGGCCATACCGTTCGTGCTCTGAGCCCGACCCCACCTTGGC
CAGGCACCGATGACGAAGACGACGATCTGGCTGACGTTGACTATGTTCCGCCGGCACCGCGTCGCGCA

CCACGCCGTGGTGGCGGTGGCGCCGGTGCGACGCGCGGTACCTCCCAGCCGGCAGCAACTCGCCCAGC
ACCGCCGGGTGCCCCGCGTTCTAGCAGCTCCGGTGGCGCACCGCTGCGTGCTGGCGTGGGTTCTGGTT
CCGGTGGTGGTCCGGCCGTGGCGGCTGTCGTCCCGCGTGTGGCTTCTCTGCCACCGGCAGCTGGTGGC
GGTCGTGCTCAAGCTCGTCGTGTCGGCGAGGACGCAGCGGCTGCTGAGGGCCGTACTCCACCGGCCCG
TCAACCGCGCGCAGCACAGGAACCGCCGATCGTGATCTCCGATTCCCCGCCACCGAGCCCGCGTCGCC
CGGCGGGTCCGGGTCCGCTGTCTTTTGTATCCTCCAGCTCTGCTCAGGTAAGCAGCGGTCCTGGCGGT
GGCGGCCTGCCACAGTCCTCTGGTCGTGCTGCTCGTCCTCGTGCGGCGGTTGCTCCTCGTGTACGTTC
TCCGCCACGCGCTGCTGCCGCGCCGGTCGTTTCTGCCTCTGCTGACGCGGCAGGTCCGGCTCCGCCTG
CAGTTCCGGTTGATGCACACCGTGCACCGCGCTCTCGTATGACCCAGGCGCAGACTGATACCCAGGCA
CAATCCCTGGGTCGCGCGGGTGCGACTGACGCTCGTGGTAGCGGTGGTCCGGGCGCTGAAGGTGGCCC
GGGTGTTCCACGCGGTACTAACACTCCGGGCGCTGCGCCACACGCGGCTGAAGGTGCGGCTGCACGTC
CGCGTAAACGTCGTGGTTCCGACAGCGGTCCGGCTGCAAGCAGCAGCGCGAGCTCTTCCGCTGCGCCT
CGCAGCCCGCTGGCGCCGCAGGGTGTTGGCGCCAAGCGTGCTGCTCCGCGTCGTGCACCGGACTCCGA
TTCTGGCGACCGCGGTCACGGCCCGCTGGCCCCTGCTAGCGCAGGCGCTGCGCCGCCATCCGCCAGCC
CGTCTTCTCAGGCAGCTGTGGCTGCGGCGTCCTCTTCTTCCGCTAGCAGCTCTTCCGCCTCTTCTAGC
AGCGCGTCCTCTAGCAGCGCATCTTCCTCTTCTGCTTCTTCTTCTAGCGCTTCTAGCTCTTCCGCGTC
CTCTTCCGCTGGCGGTGCAGGCGGCTCTGTTGCTTCCGCCAGCGGCGCAGGTGAGCGTCGTGAAACGA
GCCTGGGCCCACGTGCTGCTGCACCGCGTGGCCCGCGTAAGTGTGCGCGCAAGACCCGCCACGCTGAA
GGCGGTCCGGAGCCGGGTGCGCGTGATCCGGCTCCGGGTCTGACCCGTTACCTGCCGATTGCGGGTGT
GTCCTCCGTTGTGGCACTGGCGCCGTATGTGAACAAAACTGTCACGGGCGATTGCCTGCCTGTTCTGG
ACATGGAAACCGGTCATATCGGCGCTTACGTCGTTCTGGTTGACCAAACCGGCAACGTGGCGGATCTG
CTGCGTGCGGCCGCTCCGGCTTGGTCCCGTCGTACCCTGCTGCCGGAACATGCTCGCAACTGTGTACG
CCCACCGGATTACCCAACCCCGCCGGCCTCCGAGTGGAACTCCCTGTGGATGACCCCGGTTGGTAACA
TGCTGTTCGACCAGGGCACGCTGGTTGGTGCTCTGGACTTTCACGGCCTGCGCTCCCGTCACCCGTGG
TCCCGTGAGCAAGGCGCTCCGGCCCCTGCGGGCGATGCCCCGGCTGGCCACGGCGAGAGTACTAGAGG
ATCATAA

SEQ ID NO: 43 = nucleotide sequence for UL36.3.4.1

ATGTCGTACTACCATCACCATCACCATCACGCCGCTCAACGTGCTAGGGGATCCTCTGAACGCTGGGC
TGCTGGTGTCGAGGCTGCTTTGGATAGAGTGGAGAACCGTGCCGAATTCGATGTTGTCGAGCTGAGGA
GACTCCAAGCTTTGGCTGGTACTCACGGCTACAACCCTCGTGATTTCCGTAAACGTGCCGAACAGGCT
TTGGCGGCAAACGCTGAGGCCGTAACATTGGCTCTGGACACTGCCTTCGCTTTCAACCCATACACGCC
CGAAAACCAACGTCATCCTATGCTCCCACCTCTCGCTGCTATTCACCGCCTGGGATGGAGCGCTGCTT
TCCATGCTGCTGCTGAAACTTACGCCGACATGTTCCGTGTCGATGCCGAACCACTGGCTAGACTGCTC
CGTATCGCTGAGGGACTGCTGGAGATGGCTCAAGCTGGCGACGGATTCATCGATTACCATGAGGCTGT
CGGTAGACTGGCCGATGATATGACTTCTGTGCCCGGATTGAGGCGCTACGTTCCTTTCTTCCAACATG
GCTACGCCGATTACGTGGAACTGAGAGATCGCCTGGATGCTATTAGGGCCGACGTCCATAGAGCACTC
GGTGGTGTTCCGCTGGATTTGGCGGCTGCTGCCGAACAAATTTCCGCTGCTCGTAACGATCCTGAGGC

TACTGCTGAATTGGTCCGTACTGGTGTAACATTGCCTTGCCCTAGTGAGGACGCTCTCGTGGCTTGTG
CTGCTGCCCTGGAGAGAGTCGATCAATCTCCCGTGAAAAACACGGCTTACGCCGAATACGTTGCCTTC
GTGACCCGTCAAGACACTGCTGAGACTAAAGACGCTGTGGTCCGTGCTAAACAACAACGTGCTGAGGC
CACTGAACGTGTTATGGCTGGCCTGAGAGAGGCTCTGGCTGCTAGAGAACGTCGTGCTCAAATTGAGG
CTGAGGGATTGGCAAACCTGAAAACCATGCTCAAAGTCGTGGCTGTACCCGCTACTGTTGCTAAAACT
CTCGACCAGGCTCGTAGTGTTGCCGAAATTGCCGATCAAGTCGAAGTGTTGCTGGATCAAACCGAAAA
AACTCGTGAACTGGATGTGCCTGCTGTGATCTGGCTCGAACACGCCCAAAGAACATTCGAGACACACC
CTTTGTCTGCCGCTCGTGGTGATGGTCCTGGACCCTTGGCTCGTCATGCTGGCCGCCTCGGTGCCCTC
TTCGATACTCGTCGTAGAGTAGACGCCTTGAGGAGATCCCTGGAGGAGGCTGAGGCTGAATGGGACGA
AGTTTGGGGACGCTTCGGTAGAGTGAGGGGCGGAGCGTGGAAATCTCCGGAGGGATTCCGTGCAATGC
ATGAGCAACTGAGGGCCCTCCAAGACACAACAAACACCGTGTCTGGCCTGAGGGCTCAACCTGCTTAC
GAACGCTTGTCTGCTCGCTACCAAGGAGTACTCGGAGCGAAAGGCGCTGAGAGAGCTGAGGCTGTTGA
GGAACTCGGTGCTCGTGTCACTAAACACACCGCTCTGTGTGCTAGGCTGAGAGATGAGGTCGTCCGTA
GAGTGCCTTGGGAAATGAACTTCGATGCTCTGGGAGGATTGTTGGCTGAGTTCGATGCCGCTGCTGCC
GATTTGGCACCTTGGGCTGTAGAGGAATTCCGTGGTGCTAGAGAACTCATTCAATACCGTATGGGCCT
GTACTCTGCCTACGCTAGAGCTGGAGGACAAACTGGTGCTGGAGCTGAATCTGCTCCTGCTCCTTTGC
TCGTGGATCTGAGGGCTTTGGATGCTCGTGCTCGTGCTTCTTCTTCCCCTGAGGGACATGAAGTGGAC
CCACAACTGCTGAGGAGGCGTGGAGAGGCTTACTTGAGAGCTGGCGGCGACCCTGGACCTCTCGTGCT
CCGTGAAGCTGTTTCTGCTTTGGACCTGCCATTCGCCACATCTTTCTTGGCCCCCGATGGAACTCCCC
TCCAATACGCTTTGTGCTTCCCTGCCGTAACGGACAAACTCGGAGCTTTGCTCATGAGGCCCGAGGCC
GCTTGTGTTAGACCTCCTTTGCCTACCGATGTGCTGGAATCTGCCCCAACTGTGACTGCCATGTACGT
ACTCACTGTGGTCAACCGCCTCCAACTGGCATTGAGTGATGCTCAAGCGGCAAACTTCCAACTGTTCG
GTCGTTTCGTTCGTCATAGGCAGGCAACCTGGGGAGCGTCAATGGATGCCGCCGCTGAATTGTACGTT
GCCCTGGTGGCTACAACTCTCACACGTGAATTCGGTTGTCGCTGGGCACAATTGGGATGGGCTAGTGG
AGCTGCTGCTCCTAGACCCCCACCTGGACCCCGTGGCTCACAACGTCACTGTGTGGCATTCAACGAGA
ACGATGTCCTCGTCGCTTTGGTTGCCGGTGTTCCCGAACACATCTACAACTTCTGGCGCCTGGACTTG
GTCCGTCAACACGAGTACATGCACCTCACACTGGAGCGTGCCTTCGAGGATGCTGCCGAGTCTATGCT
CTTCGTTCAACGCCTCACTCCACATCCCGACGCTCGTATTAGAGTTCTGCCGACCTTCTTGGATGGTG
GTCCTCCTACACGTGGTCTGTTGTTCGGAACCCGCTTGGCGGACTGGCGTCGTGGTAAACTGTCTGAA
ACCGACCCATTGGCCCCATGGAGATCTGCTTTGGAACTCGGAACCCAACGTCGTGACGTGCCTGCTTT
GGGAAAACTGTCCCCTGCTCAAGCTTTGGCCGCTGTGTCGGTACTGGGCCGTATGTGCTTGCCCTCGG
CTGCCTTGGCTGCTTTGTGGACCTGTATGTTCCCCGACGACTACACTGAATACGACTCATTCGACGCC
CTCTTGGCGGCTCGCCTGGAATCGGGACAAACATTGGGACCTGCTGGCGGTAGAGAGGCTTCATTGTA
ATAG

SEQ ID NO: 44 = nucleotide sequence for UL36.4.2.5

ATGTCGTACTACCATCACCATCACCATCACGAATACGACTCCTTCGACGCTTTGTTGGCTGCTAGACT
GGAATCTGGTCAAACCTTGGGACCCGCTGGCGGTAGAGAGGCTTCTTTGCCCGAGGCTCCTCATGCTT

TGTACCGTCCAACCGGACAACATGTTGCTGTGTTGGCGGCTGCTACTCATAGAACCCCTGCTGCTCGT
GTTACTGCTATGGACCTGGTCTTGGCGGCCGTTTTGCTGGGCGCTCCTGTGGTGGTCGCTCTGAGAAA
CACTACTGCCTTCTCCCGTGAATCCGAATTGGAACTGTGCCTCACCCTGTTCGATTCTCGTCCCGGCG
GACCGGATGCTGCCCTGAGAGATGTGGTATCCTCCGACATTGAAACCTGGGCTGTGGGCTTGCTCCAC
ACCGATTTGAACCCTATTGAGAACGCTTGCTTGGCGGCTCAACTGCCACGCTTGTCTGCCCTCATTGC
TGAACGTCCTTTGGCCGATGGACCCCCTTGTTTGGTGTTGGTGGACATTTCGATGACACCTGTCGCTG
TTTTGTGGGAGGCCCCTGAACCACCTGGCCCTCCCGATGTTCGTTTCGTCGGTAGCGAGGCCACTGAG
GAATTGCCTTTCGTGGCTACTGCTGGTGATGTTTTGGCGGCGAGTGCTGCCGATGCCGATCCTTTCTT
CGCTCGTGCTATCCTGGGCCGTCCTTTCGATGCTTCTCTGCTCACTGGTGAACTGTTCCCTGGTCACC
CCGTTTACCAACGTCCCCTGGCGGATGAGGCTGGTCCTTCTGCTCCTACTGCCGCTCGTGATCCTAGA
GATCTGGCTGGAGGCGACGGTGGATCCGGACCTGAGGATCCCGCTGCTCCACCTGCTAGACAGGCCGA
TCCTGGTGTTTTGGCTCCTACTCTGCTCACCGATGCTACTACTGGCGAACCTGTGCCACCCCGTATGT
GGGCTTGGATTCATGGACTGGAGGAACTGGCTTCCGATGATGCCGGCGGTCCTACCCCAAACCCTGCC
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GCTCCCCCGGCTTGGGTTTCCGCTCTGCCATTGCCACCCGGACCTGCTAGTGCTCGTGGTGCTTTCCC
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CCTCGTCGCCTCACTAGGCCTGCTGTTGCTAGTCTGTCCGCTTCTTTGAACTCTCTGCCTTCCCCCCG
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GCCACCTGTTACTCGTACACTCACTCCCCAATCTCGTGACTCTGTGCCTACACCTGAGTCTCCAACTC
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TGCCACCTGCCGATGAACCCCCCGGACCTCTGGCTGCCGACCATCTCCAATCACCTCACTCACAATTC
GGTCCTTTGCCCGTTCAAGCGAACGCTGTTCTGTCTCGTCGTTACGTGAGATCAACTGGCCGTTCTGC
CTTGGCTGTGCTCATTAGAGCTTGTCGCCGTATCCAACAACAACTCCAGCGTACTAGGAGAGCACTCT
TCCAACGCTCAAACGCCGTGCTCACATCACTCCACCATGTCCGTATGCTCTTGGGATAATAG

SEQ ID NO: 45 = nucleotide sequence for US12 (ICP47)

ATGTCTTGGGCTCTGAAAACCACCGACATGTTCCTGGACTCTTCTCGTTGCACCCACCGTACCTACGG
TGACGTTTGCGCTGAAATCCACAAACGTGAACGTGAAGACCGTGAAGCTGCTCGTACCGCTGTTACCG
ACCCGGAACTGCCGCTGCTGTGCCCGCCGGACGTTCGTTCTGACCCGGCTTCTCGTAACCCGACCCAG
CAGACCCGTGGTTGCGCTCGTTCTAACGAACGTCAGGACCGTGTTCTGGCTCCGTGA

SEQ ID NO: 46 = nucleotide sequence for US4

ATGAAGTTCCTCGTGAACGTGGCCCTGGTGTTCATGGTGGTGTACATCAGCTACATCTACGCTAACCG
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CCGTGGCTCAGTACTGCTACGCTTACCCCCGTCTGGACGACCCTGGTCCCCTGGGTTCTGCTGACGCT
GGTCGTCAGGACCTGCCCCGTCGTGTCGTGCGTCACGAGCCCCTGGGTCGTAGCTTCCTGACCGGTGG
CCTGGTGCTGTTGGCTCCCCCTGTGCGCGGTTTCGGTGCTCCCAACGCTACCTACGCTGCTCGTGTGA
CCTACTACCGTCTGACCCGTGCTTGCCGTCAGCCCATCCTGCTGCGTCAGTACGGTGGTTGCCGTGGT
GGAGAGCCCCCATCCCCCAAGACCTGCGGTTCTTACACCTACACCTACCAGGGTGGTGGTCCCCCTAC
CCGTTACGCTCTGGTCAACGCTTCCCTGCTGGTGCCCATCTGGGACCGTGCTGCTGAGACTTTCGAGT
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SEQ ID NO: 117 = RS1.2

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CGCGCCCCTGCTGGCGCGCGAGAACGCGGCGCTGACCGGGGCGCGGACCCCCGACGACGGCGGCGACG
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SEQ ID NO: 118 = UL1(cytoplasmic),gL full length

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TGGGGGACATACTAAGAGTGCCTTGCATGCGGACCCCCGCGGACGATGTTTCTTGGCGCTACGAGGCC
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GTTTTTGTGGGATAGGCACGCCCAGAGGGCGTATCTTGTTAACCCCTTTCTCTTTGCGGCGGGATTTT
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CAGCCGCCCGTCCTCGCCCTTTCGAACGCCCCCCCACGGCGGGTCTCCCCGACGCGAGGTCGGCGCCG
GCATACTCGCCTCCGACGCAACTGA

SEQ ID NO: 119 = UL1(Secreted),gL full length (preferred Ag)

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CGGAATATTTCTTCGCTATCACTGCCCGGGGTTGGACACGTTTTTGTGGGATAGGCACGCCCAGAGGG
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SEQ ID NO: 120 UL19 delta TEV VP5 full length

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SEQ ID NO: 121 = RS1.1

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SEQ ID NO: 122 = RS1.3.1

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SEQ ID NO: 123 = RS1.3.2

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SEQ ID NO: 124 = RS1.3

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SEQ ID NO: 125 = RS1.4

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SEQ ID NO: 128 = RS1.7

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SEQ ID NO: 129 = RS1.8

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SEQ ID NO: 130 = His tag

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SEQ ID NO: 131 = Tag

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SEQ ID NO: 132 = Secretion Signal

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SEQ ID NO: 133 = UL49.5

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2010249330 14 Oct 2015

Claims

1. A vaccine formulation comprising a pharmaceutically-acceptable carrier and a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or comprising the amino acid sequence of SEQ ID NO:2 lacking 1-20 amino acids from the N-terminus, C-terminus, or both, provided that the polypeptide does not consist of the amino acid sequence of SEQ ID NO:1.

2. The vaccine formulation of claim 1, further comprising a gD2 polypeptide.

3. The vaccine formulation of claim 1, further comprising a gD2 polypeptide lacking a transmembrane domain and lacking a cytoplasmic domain.

4. The vaccine formulation of claim 1, further comprising a gD2 polypeptide lacking a transmembrane domain.

5. The vaccine formulation of claim 1, further comprising a second polypeptide consisting of the amino acid sequence of SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5.

6. A vaccine formulation comprising a pharmaceutically-acceptable carrier and a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 2, provided that the polypeptide does not consist of the amino acid sequence of SEQ ID NO:1.

7. A vaccine formulation comprising a pharmaceutically acceptable carrier and a polypeptide comprising the amino acid sequence of SEQ ID NO:2 conjugated to an immunogenic carrier, a signal sequence, or a peptide of no more than 20 amino acids at the N-terminus or C-terminus of the polypeptide, provided that the polypeptide does not consist of the amino acid sequence of SEQ ID NO:1.

8. The vaccine formulation of claim 1, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:2 lacking 1-10 amino acid residues from the N-terminus, C-terminus, or both.

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9. The vaccine formulation of any of claims 1, 6, 7, or 8, further comprising a second polypeptide comprising the amino acid sequence of SEQ ID NO:5 lacking all or at least 8 contiguous amino acid residues of residues 340-363 of SEQ ID NO:5, or comprising the amino acid sequence of SEQ ID NO:5 lacking all or at least 8 contiguous amino acid residues of residues 340-363 of SEQ ID NO:5 and lacking 1-25 amino acids from the N-terminus, C-terminus, or both.

10. The vaccine formulation of any of claims 1, 6, 7, or 8, further comprising a second polypeptide comprising the amino acid sequence of SEQ ID NO:4 or the amino acid sequence of SEQ ID NO:4 lacking 1-20 amino acids from the N-terminus, C-terminus, or both.

11. The vaccine formulation of any of claims 1, 6, 7, or 8, further comprising a second polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:4.

12. The vaccine formulation of any of claims 1, 6, 7, or 8, further comprising a second polypeptide comprising the amino acid sequence of SEQ ID NO:5 or the amino acid sequence of SEQ ID NO:5 lacking 1-25 amino acids from the N-terminus, C-terminus, or both.

13. The vaccine formulation of any of claims 1, 6, 7, or 8, further comprising a second polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:5.

14. The vaccine formulation of any of claims 1, 6, 7, or 8, further comprising a second polypeptide comprising the amino acid sequence of SEQ ID NO:3 or the amino acid sequence of SEQ ID NO:3 lacking 1-25 amino acid residues from the N-terminus, C-terminus, or both.

15. The vaccine formulation of any of claims 1-14, further comprising an adjuvant.

16. The vaccine formulation of claim 15, wherein the adjuvant is one or more purified fractions of quillaja saponins.

17. The vaccine formulation of claim 16, wherein the adjuvant comprises saponin fraction A and saponin fraction C.

18. The vaccine formulation of claim 17, wherein the adjuvant comprises cholesterol, phosphatidyl choline, saponin fraction A and saponin fraction C.

19. The vaccine formulation of claim 18, wherein the adjuvant is in the form of particles.

20. The vaccine formulation of claim 19, wherein particles comprising saponin fraction A are free of saponin fraction C and particles comprising saponin fraction C are free of saponin fraction A.

21. The vaccine formulation of any of claims 15-20, wherein the vaccine formulation comprises 5-200 μg of each polypeptide and 5-200 μg of the adjuvant.

22. The vaccine formulation of any of claims 1-21, wherein the vaccine formulation inhibits HSV-2 symptoms.

23. The vaccine formulation of claim 22, wherein the vaccine formulation reduces the number of herpetic lesions.

24. The vaccine formulation of claim 22, wherein the vaccine formulation reduces the number of days a subject experiences herpetic lesions.

25. The vaccine formulation of any of claims 1-21, wherein the vaccine formulation inhibits infection by HSV-2 in an uninfected subject.

26. The vaccine formulation of claim 25, wherein the vaccine formulation increases the IgG titer to one or more HSV-2 antigens.

27. The vaccine formulation of claim 25, wherein the vaccine formulation increases the T cell response to one or more HSV-2 antigens.

28. The vaccine formulation of claim 25, wherein the vaccine formulation reduces the number of herpetic lesions at the onset of HSV-2 infection.

29. The vaccine formulation of any of claims 1-21, wherein the vaccine formulation inhibits HSV-2 symptoms or inhibits infection by HSV-2 in three or fewer doses.

30. A pharmaceutical composition for treating a subject suffering from or susceptible to HSV-2 infection, comprising an effective amount of a vaccine formulation according to claims 1-21.

31. The pharmaceutical composition of claim 30, wherein the pharmaceutical composition inhibits HSV-2 symptoms.

32. The pharmaceutical composition of claim 31, wherein the pharmaceutical composition reduces the number of herpetic lesions.

33. The pharmaceutical composition of claim 32, wherein the pharmaceutical composition reduces the number of days a subject experiences herpetic lesions.

34. The pharmaceutical composition of claim 30, wherein the pharmaceutical composition inhibits infection by HSV-2 in an uninfected subject.

35. The pharmaceutical composition of claim 34, wherein the pharmaceutical composition increases the IgG titer to one or more HSV-2 antigens.

36. The pharmaceutical composition of claim 34, wherein the pharmaceutical composition increases the T cell response to one or more HSV-2 antigens.

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37. The pharmaceutical composition of claim 34, wherein the pharmaceutical composition reduces the number of herpetic lesions at the onset of HSV-2 infection.

38. The pharmaceutical composition of claim 30, wherein the pharmaceutical composition treats a subject within a three dose regimen.

39. The pharmaceutical composition of claim 30, wherein the subject is a human.

40. A pharmaceutical composition comprising a first polypeptide having an amino acid sequence that is at least 90% identical to SEQ ID NO:2 and one or more additional polypeptides selected from polypeptides having an amino acid sequence that is at least 90% identical to SEQ ID NOS: 4 or 5 or 90% identical to any one of SEQ ID NOS: 1, 3, and 6-38.

41. A pharmaceutical composition for inducing an immune response in a subject comprising an effective amount of a first polypeptide having an amino acid sequence that is at least 90% identical to SEQ ID NO:2, and one or more additional polypeptides selected from polypeptides having an amino acid sequence that is at least 90% identical to SEQ ID NOS: 4 or 5 or 90% identical to any one of SEQ ID NOS: 1, 3, and 6-38.

42. The pharmaceutical composition of claim 41, characterized in that said pharmaceutical composition is administered two, three, four, or five times.

43. The pharmaceutical composition of claim 41, characterized in that said pharmaceutical composition is administered before exposure to herpes simplex virus-2 (HSV-2).

44. The pharmaceutical composition of claim 41, characterized in that said pharmaceutical composition is administered after exposure to HSV-2.

45. A pharmaceutical composition for reducing one or more symptoms of HSV-2 infection in a subject, comprising a first polypeptide having an amino acid sequence that is at least 90%

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identical to SEQ ID NO:2, and one or more additional polypeptides selected from polypeptides having an amino acid sequence that is at least 90% identical to SEQ ID NOS: 4 or 5 or 90% identical to any one of SEQ ID NOS: 1, 3, and 6-38.

46. The pharmaceutical composition of claim 45, wherein the symptoms of HSV-2 infection comprise one or more of lesion formation, pain, irritation, itching, fever, malaise, headache, viral shedding, and prodrome.

47. A pharmaceutical composition for inhibiting onset of HSV-2 infection, inhibiting development of a latent HSV-2 infection in a subject, reducing viral shedding in a subject infected with HSV-2, and/or reducing recurrence of outbreaks in a subject infected with HSV-2, comprising a first polypeptide having an amino acid sequence that is at least 90% identical to SEQ ID NO:2, and one or more additional HSV-2 polypeptides selected from polypeptides having an amino acid sequence that is at least 90% identical to SEQ ID NOS: 4 or 5 or 90% identical to any one of SEQ ID NOS: 1, 3, and 6-38.

Fig. 1A

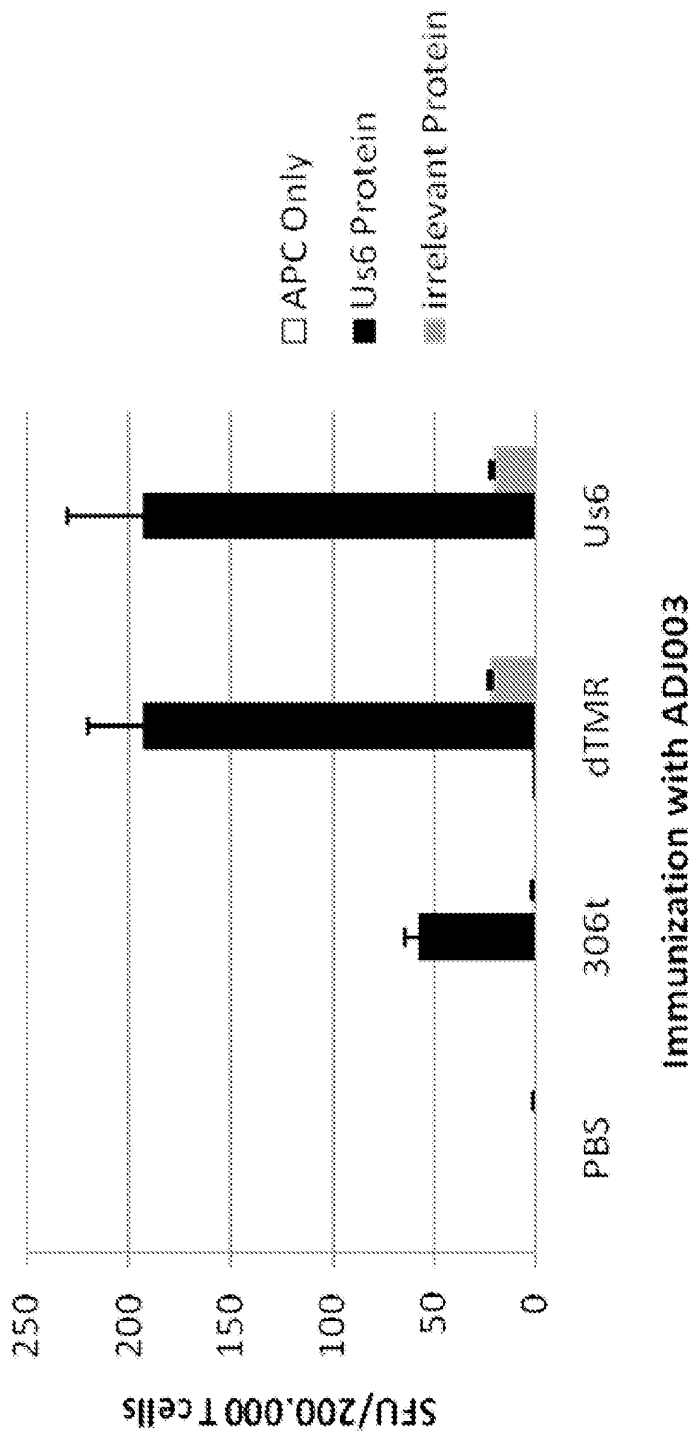
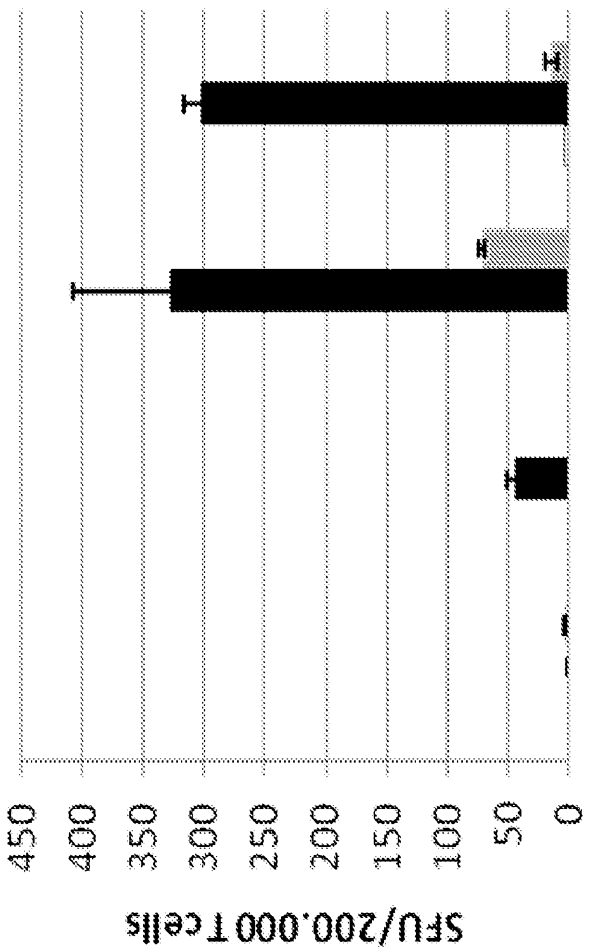

Responses of CD4 T cells to different forms of Us6
APC Only
Us6 Protein
irrelevant Protein
SFU/200,000 T cells
250
200
150
100
50
0
PBS
306t
dTMR
Us6
Immunization with ADJ003

Fig. 1B

Responses of CD8 T cells to different forms of Us6




All proteins were expressed in E. coli

Results are interferon-gamma spot forming units per 200,000 T cells (CD4+ or CD8+) seeded per well of Elipot plate

**306t**: Us6 protein without transmembrane (TMR) or cytoplasmic region

**dTMR**: Us6 protein without transmembrane (TMR) region

**Us6**: full-length Us6 protein

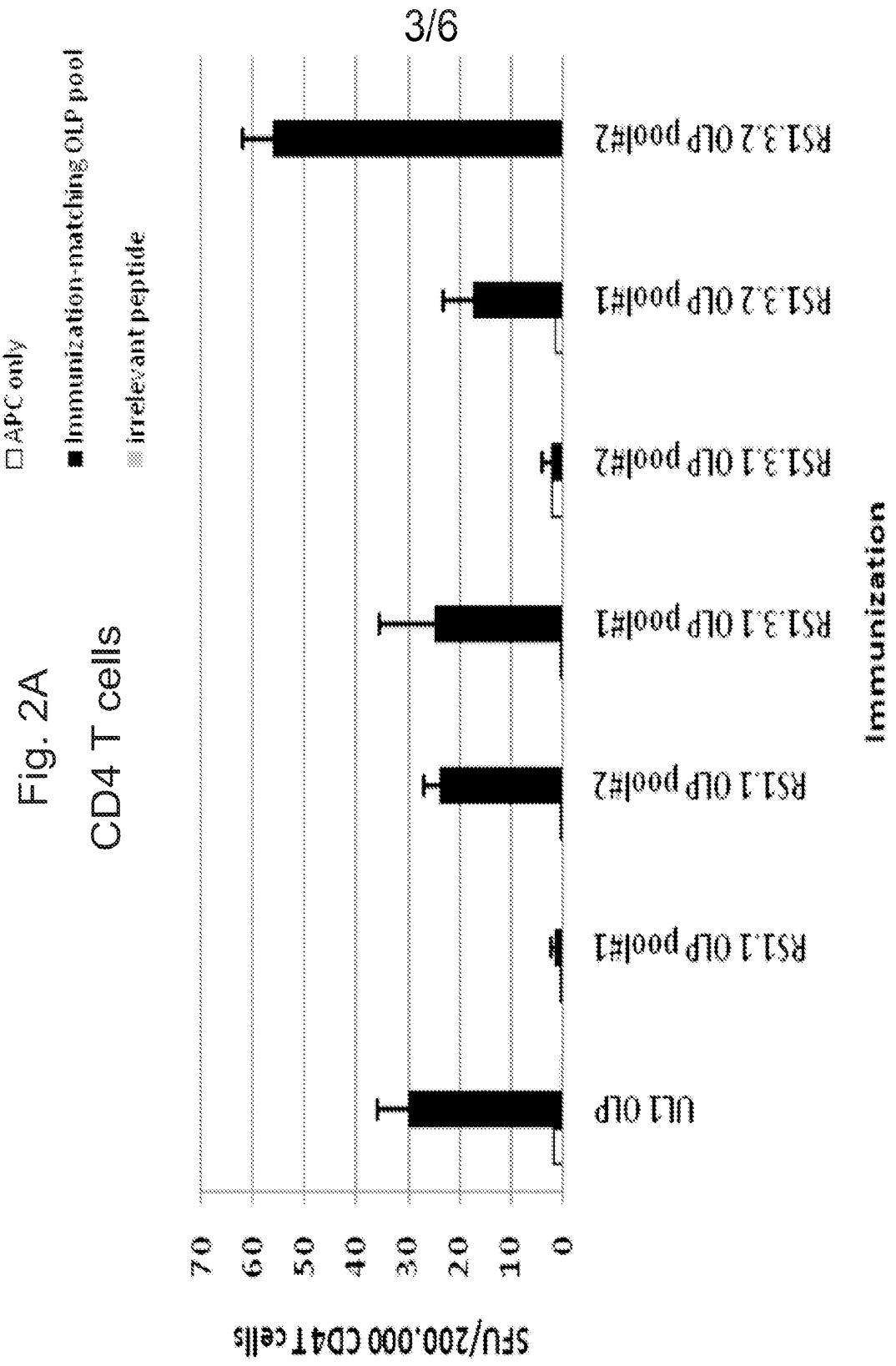



Fig. 2A
CD4 T cells

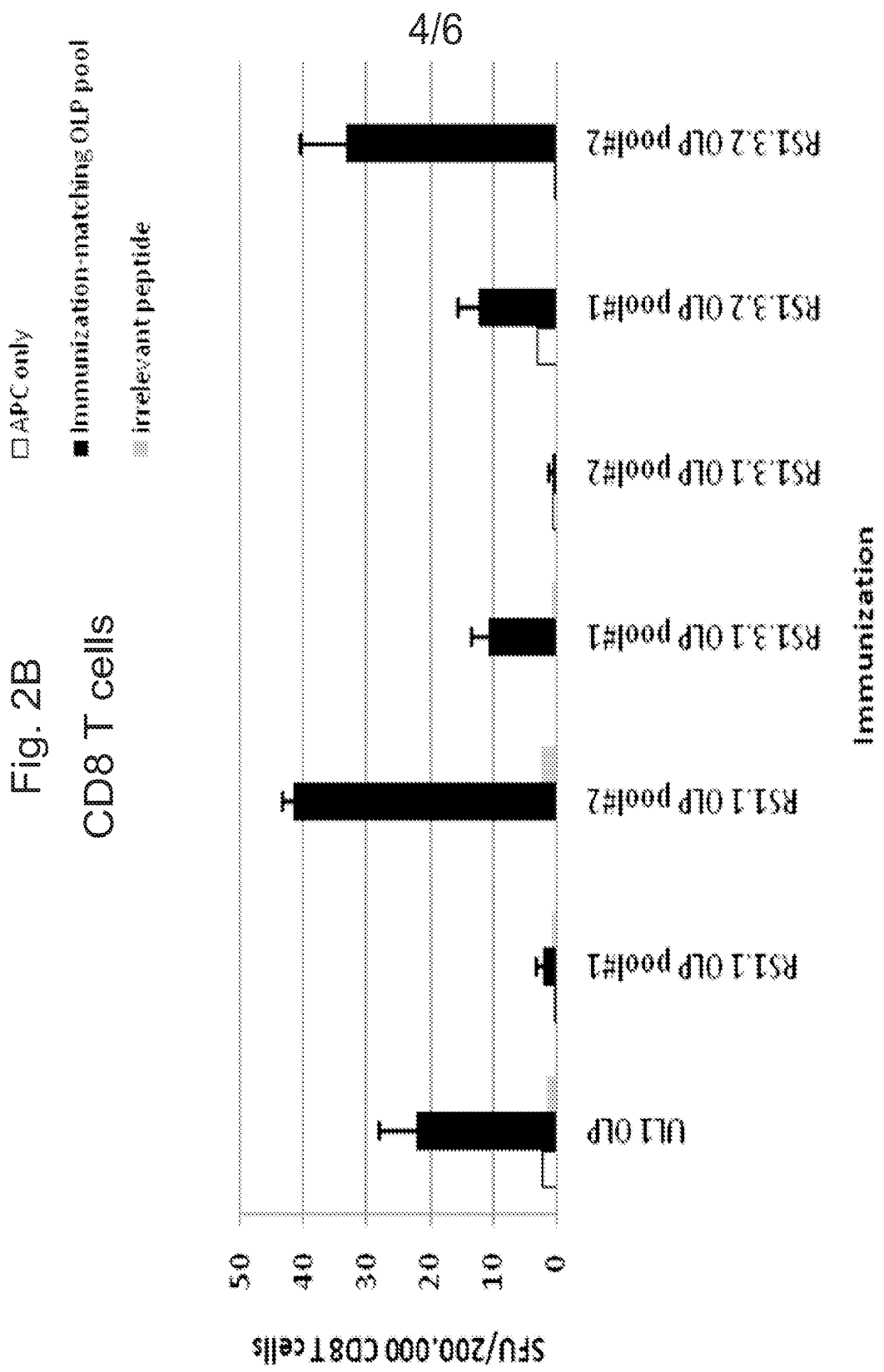
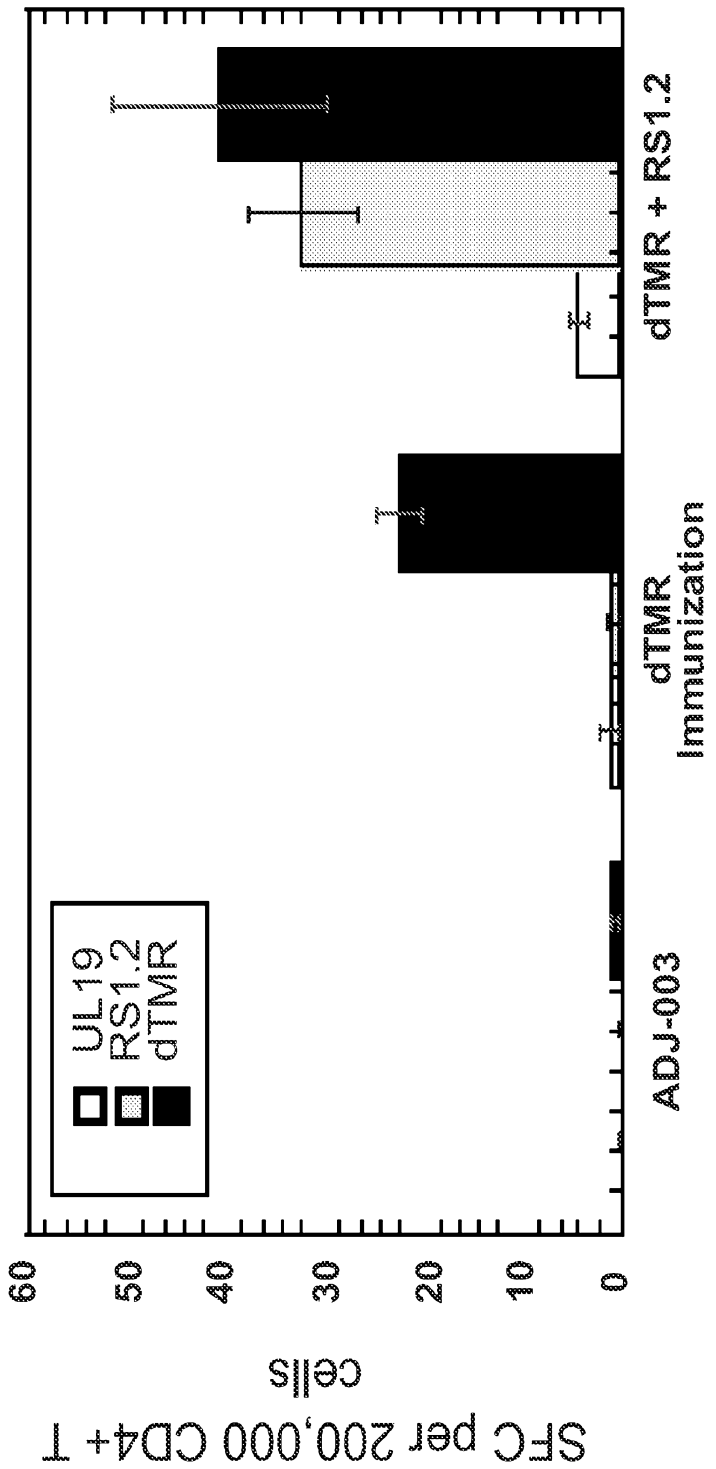



Fig. 2B

CD8 T cells

Fig. 3A


SFC per 200,000 CD4+ T cells
60
50
40
30
20
10
0
UL19
RS1.2
dTMR
ADJ-003
dTMR Immunization
dTMR + RS1.2

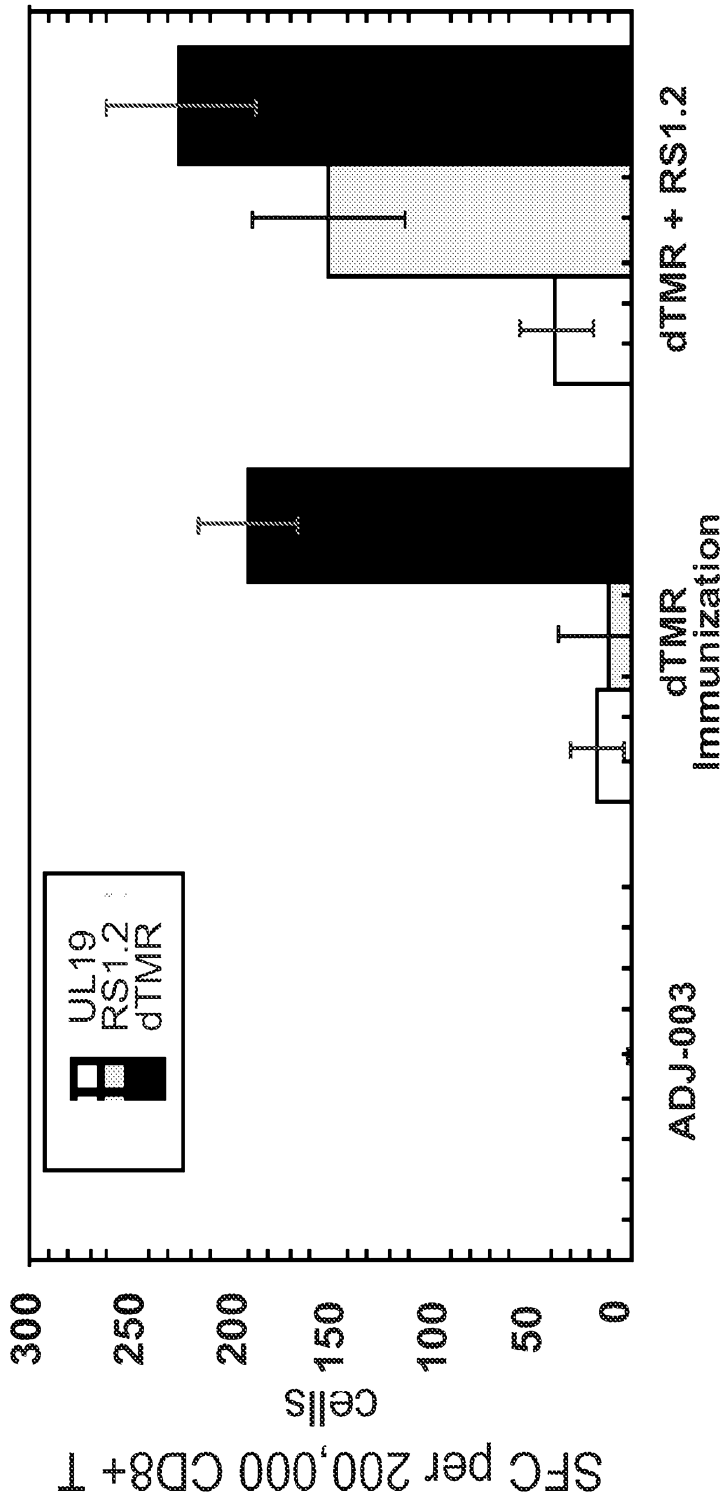

SFC per 200,000 CD8+ T cells
300
250
200
150
100
50
0
UL19
RS1.2
dTMR
ADJ-003
dTMR Immunization
dTMR + RS1.2

Fig. 3B