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(54) Title: METHODS AND COMPOSITIONS FOR VACCINATING AGAINST *STAPHYLOCOCCUS AUREUS*

(57) Abstract: The invention features a method of vaccinating a mammal against *Staphylococcus aureus* which includes the steps of: a) identifying a mammal at risk for the development of a *Staphylococcus aureus* skin or soft tissue infection; and b) administering to said mammal an immunogenic amount of a vaccine that includes a polypeptide including an isolated agglutinin-like sequence (Als) 3 protein (Als3p), or an immunogenic fragment thereof, in a pharmaceutically acceptable medium.



METHODS AND COMPOSITIONS FOR VACCINATING AGAINST  
*STAPHYLOCOCCUS AUREUS*

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Cross-Reference to Related Application

This application claims benefit of priority to U.S. Provisional Application No. 61/510,896, filed July 22, 2011, which is hereby incorporated by reference.

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Background of the Invention

The invention relates to vaccines against *Staphylococcus aureus*.

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*Staphylococcus aureus* is the leading cause of skin and skin structure infections including cellulitis and furunculosis, and is among the most common causes of bacteremia. Strains of *S. aureus* that exhibit the methicillin-resistant (MRSA) phenotype are predominant causes of healthcare-and community-acquired infections, including invasive disease in immune competent hosts, in immune suppression (e.g. neutropenia, solid-organ or bone marrow transplants), and in inherited immune dysfunctions manifesting recurring cutaneous infection (e.g. Job's Syndrome, Chronic Granulomatous Disease). The significant impact of MRSA on public health is of special concern in light of high rates of mortality associated with invasive *S. aureus* disease even with appropriate antimicrobial therapy (e.g. 15-40% in bacteremia and endocarditis). Increasing rates of life-threatening infections and decreasing susceptibility to antibiotics call for development of an effective vaccine targeting *Staphylococcus aureus*. This invention meets this need.

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Summary of the Invention

The invention, in general, relates to a method of vaccinating a mammal against *Staphylococcus aureus* including the steps of: a) identifying a mammal (e.g., a human or non-human mammal, such as livestock, e.g., a bovine, equine, porcine, or ovine species, or a domestic mammal, e.g., a canine or feline) at risk for the development of a *Staphylococcus aureus* skin or soft tissue infection; and b) administering to said mammal an immunogenic amount of a vaccine including a polypeptide comprising an isolated agglutinin-like sequence (Als) 3 protein (Als3p), or an immunogenic fragment thereof, in a pharmaceutically acceptable medium. Exemplary polypeptides include a *Candida albicans* Als3p (for

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example, an Als3p shown in Figure 1A, e.g., SEQ ID NO: 1 or SEQ ID NO: 2, or an immunogenic fragment thereof). In other embodiments, the polypeptide includes the N-terminal domain of *Candida albicans* Als3p or an immunogenic fragment thereof. The method disclosed herein is especially useful for vaccination against a methicillin-resistant *Staphylococcus aureus* (MRSA) strain of *S. aureus*. The method disclosed herein is also useful for vaccination against other drug-resistant *S. aureus* (e.g., vancomycin resistant, daptomycin-resistant, etc.), or methicillin-sensitive *S. aureus* (MSSA) strains of *S. aureus*. In other embodiments, the polypeptide is conjugated to a carrier such as a keyhole limpet hemocyanin (KLH), CRM197, tetanus toxoid, diphtheria toxoid, enterotoxin B fragments, *N. meningitidis* outer membrane protein complex, or any other carrier protein used in conjugate vaccines in the art. Such carriers also may include a phage, a yeast, a virus, a virosome, or a recombinant virus-like particle. The vaccine, in general, is administered by intramuscular, subcutaneous, intradermal, oral, or sublingual administration, or is administered for inhalation in a microparticulate formulation. If desired, the vaccine is administered as a booster dose. The vaccine optionally may include an immunostimulating adjuvant. In still other embodiments, the method includes administering an antibiotic against *S. aureus* in combination with the vaccine, e.g., wherein the antibiotic is co-formulated or co-administered with the vaccine.

In another aspect, the invention features a method of vaccinating a mammal (e.g., a human or non-human mammal, such as livestock, e.g., a bovine, equine, porcine, or ovine species, or a domestic mammal, e.g., a canine or feline) against *Staphylococcus aureus* including the steps of: a) identifying a mammal at risk for the development of a *Staphylococcus aureus* skin or soft tissue infection; and b) administering to said mammal an effective amount of a vaccine including a polynucleotide (e.g., an isolated polynucleotide) encoding a polypeptide including an Als3p, or an immunogenic fragment thereof, incorporated into a suitable delivery vehicle, which could include single- or double-stranded DNA or RNA, a double-stranded DNA plasmid or a viral vector, in a pharmaceutically acceptable medium, wherein the polynucleotide is expressed *in vivo* and the mammal generates an immune response. The vaccine containing the polynucleotide elicits an immune response in the mammal, e.g., the production of anti-Als3p antibodies that exhibit specificities for Als3p.

In yet another aspect, the invention features an isolated Als3 protein, or an immunogenic fragment thereof, for use in a method of treatment or prevention of a *Staphylococcus aureus* skin or soft tissue infection in a mammal.

In another aspect, the invention features a vaccine including an isolated Als3 protein, or an immunogenic fragment thereof, for use in a method of treatment or prevention of a *Staphylococcus aureus* skin or soft tissue infection in a mammal.

Such Als3p useful for preparing isolated proteins or vaccines include those identified in *Candida albicans*, *Candida krusei*, *Candida tropicalis*, *Candida glabrata* and *Candida parapsilosis*, as well as those Als3p proteins identified in searches of publically available databases.

In still another aspect, the invention features an isolated Als3 protein, wherein the amino acid

sequence of the isolated Als3 protein consists of SEQ ID NO: 2.

In yet another aspect, the invention features a pharmaceutical composition comprising an isolated Als3 protein, wherein the amino acid sequence of the isolated Als3 protein consists of SEQ ID NO: 2, and a pharmaceutically acceptable excipient.

5 In still another aspect, the invention features a vaccine comprising an isolated Als3 protein, wherein the amino acid sequence of the isolated Als3 protein consists of SEQ ID NO: 2. In some embodiments, the protein is conjugated to a carrier such as a keyhole limpet hemocyanin (KLH), CRM197, tetanus toxoid, diphtheria toxoid, enterotoxin B fragments, *N. meningitidis* outer membrane protein complex, or any other carrier protein used in conjugate vaccines in the art. Such carriers also may  
10 include a phage, a yeast, a virus, a virosome, or a recombinant virus-like particle. The vaccine, in general, is administered by intramuscular, subcutaneous, intradermal, oral, or sublingual administration, or is administered for inhalation in a microparticulate formulation. If desired, the vaccine is administered as a booster dose. The vaccine optionally may include an immunostimulating adjuvant. In other embodiments, the vaccine may include a combination of an isolated Als3 protein and one or more other isolated Als  
15 proteins, e.g., derived from a *Candida* strain selected from the group consisting of *Candida albicans*, *Candida krusei*, *Candida tropicalis*, *Candida glabrata* and *Candida parapsilosis*.

In another aspect, the invention features a vaccine including a polynucleotide (e.g., an isolated polynucleotide) encoding a polypeptide including an Als3p, or an immunogenic fragment thereof, incorporated into a suitable delivery vehicle, which could include single- or double-stranded DNA or  
20 RNA, a double-stranded DNA plasmid or a viral vector, in a pharmaceutically acceptable medium. For example, an immunogenic Als3 polynucleotide vaccine, e.g., the nucleic acid sequence of which contains or consists of SEQ ID NO: 3 in part or in its entirety, and which is suitable to be used as a vaccine, may be prepared, e.g., from an Als3 gene or fragment thereof, e.g., a fragment encoding an immunogenic fragment of an Als3p. The vaccine may further include a polynucleotide encoding an immune-stimulant  
25 polypeptide that is co-expressed with the Als3p or immunogenic fragment thereof. Such polynucleotide vaccines may be prepared as injectables, e.g., in physiologically-acceptable liquid solutions or emulsions for polynucleotide administration. The polynucleotide may be associated with liposomes, such as lecithin liposomes or other liposomes known in the art, as a nucleic acid liposome (for example, as described in International Application Pub. No. WO 93/24640) or the polynucleotide may be associated with an  
30 adjuvant. Liposomes including cationic lipids interact spontaneously and rapidly with polyanions, such as DNA and RNA, resulting in liposome/nucleic acid complexes that capture up to 100% of the polynucleotide. In addition, the polycationic complexes fuse with cell membranes, resulting in an intracellular delivery of polynucleotide that bypasses the degradative enzymes of the lysosomal compartment. International Application Pub. No. WO 94/27435 describes compositions for genetic  
35 immunization including cationic lipids and polynucleotides. Agents which assist in the cellular uptake of polynucleotides, such as calcium ions, viral proteins, electroporation and other transfection-facilitating

agents, may advantageously be used. Both liquid as well as lyophilized forms that are to be reconstituted include, preferably buffers, in amounts necessary to suitably adjust the pH of the injected solution.

“*Staphylococcus aureus* skin or soft tissue infection”, “*Staphylococcus aureus* SSTI”, “*Staphylococcus aureus* skin/skin structure infection”, and “*Staphylococcus aureus* SSSI” are used interchangeably herein and refer to a skin or soft tissue infection (e.g. cellulitis, soft tissue abscess, dermonecrosis, myositis, or other infections) resulting from *S. aureus* entering the body at a site where a cut, scrape, bite, or other wound has broken the skin. In some instances, *S. aureus* SSSI is the result of *S. aureus* living on the body, and may occur spontaneously in the absence of a visible site of skin injury or wound. Such infections may affect the layers of the skin or deeper tissues, such as muscle and connective tissue (the interlacing framework of tissue that forms ligaments, tendons, and other supporting structures of the body). Skin abscesses may also occur in areas of the skin where the body has been fighting a *S. aureus* infection. The more important strains of *S. aureus* responsible for skin or soft tissue infections are the antibiotic-resistant *Staphylococcus* known as methicillin-resistant *Staphylococcus aureus* (MRSA); vancomycin-resistant and daptomycin-resistant strains of *S. aureus* may also cause SSSI. MRSA is resistant to commonplace antibiotics. *Staphylococcus aureus* SSSIs may also be caused by methicillin-sensitive *Staphylococcus aureus* (MSSA).

Mammals which are at risk of developing a *S. aureus* skin or soft tissue infection can be treated in a prophylactic mode. Alternatively, mammals may be treated when presenting with symptoms of a *S. aureus* skin or soft tissue infection. Vaccination as described herein will reduce the severity, delay, or prevent the development of symptoms. Mammals are at elevated risk of infection if they are hospitalized or living in an institutionalized community, antibiotic treated, or immunosuppressed including children having HIV/AIDS or other diseases that compromise immune function, individuals having frequent contact with the healthcare system, having a chronic illness such as diabetes, cancer, HIV/AIDS, being very young or very old, frequent use of antibiotics, having an open wound, dermatitis or skin lesions, poor nutrition or poor hygiene. Other mammals at risk include those living in crowded living conditions, military personnel, especially deployed troops, athletes, and prison inmates. Still others at risk of developing a *S. aureus* skin or soft tissue infection are those individuals previously having such infections or individuals scheduled for or having had a surgical or invasive medical procedure.

By “Als3p” is meant a polypeptide that is substantially identical to the amino acid sequence of a sequence shown in Figure 1A, e.g., SEQ ID NO: 1 or SEQ ID NO: 2, or to a Candida ALS3 protein identified in GenBank: XP\_710431.1, XP\_710435.1, AAO72959.1, XP\_712646.1, XP\_712666.1, EAK91173.1, EAK91169.1, AAO72958.1, EAK93494.1, EAK93472.1, O74623.1, AAD02580.1, EAK90704.1, XP\_709985.1. Desirably, a Als3p has at least 70, 75%, 80%, 85%, 90%, 95%, 99%, or even 100% identity to a sequence shown in Figure 1A, e.g., SEQ ID NO: 1 or SEQ ID NO: 2.

By “Als3p fragment” or “fragment of a Als3p” is meant a portion of a Als3p polypeptide containing fewer than 1050, 1025, 1000, 975, 950, or 945 amino acids. In some embodiments, Als3p

fragments are between 300 and 350 or 250 to 500 amino acids in length. In some embodiments, the fragment is fewer than 1050, 1025, 1000, 975, 950, or 945, 940, 937, 936, 935, 934, 933, 932, 931, or 930, 920, 910, 900, 890, 880, 870, 860, 850, 840, 830, 820, 810, 800, 790, 780, 770, 760, 750, 740, 730, 720, 710, 700, 690, 680, 670, 660, 650, 640, 630, 620, 610, 600, 590, 580, 570, 560, 550, 540, 530, 520, 510, 500, 490, 480, 470, 460, 450, 440, 430, 420, 410, 400, 390, 380, 370, 360, 350, 340, 330, 320, 310, 300, 290, 280, 270, 260, 250, 240, 230, 220, 210, 200, 190, 180, 170, 160, 150, 140, 130, 120, 110, 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 15, or 10 amino acids, and, in some instances, is immunogenic.

An exemplary Als3p fragment is SEQ ID NO: 2, as shown in Figure 1A, or fragments thereof. In some instances, Als3p fragments are between 14 and 20 amino acids in length. In general, the fragment may be fewer than, e.g., 325, 320, 310, 300, 290, 280, 270, 260, 250, 240, 230, 220, 210, 200, 190, 180, 170, 160, 150, 140, 130, 120, 110, 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, or 11 amino acids, and desirably, is immunogenic. In some instances, an Als3p fragment is between 14 and 20 amino acids.

In addition, Als3p fragments, for example, may contain one or more conservative amino acid substitutions in a sequence shown in Figure 1A, e.g., SEQ ID NO: 1 or SEQ ID NO: 2. Additional desirable Als3p fragments contain one or more conservative amino acid substitutions in a sequence shown in Figure 1A, e.g., SEQ ID NO: 1 or SEQ ID NO: 2, and/or at least one flanking amino acid (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 flanking amino acids) at the N- and/or C-terminus of a sequence shown in Figure 1A, e.g., SEQ ID NO: 1 or SEQ ID NO: 2. Other preferred Als3p fragments contain seven or more continuous amino acids of a sequence shown in Figure 1A, e.g., SEQ ID NO: 1 or SEQ ID NO: 2.

Non-limiting examples of an Als3p fragment include amino acids 1-40, 10-50, 20-60, 30-70, 40-80, 50-90, 60-100, 70-110, 80-120, 90-130, 100-140, 110-150, 120-160, 130-170, 140-180, 150-190, 160-200, 170-210, 180-220, 190-230, 200-240, 210-250, 220-260, 230-270, 240-280, 250-290, and 260-300, 270-310, 280-320, and 290-331 amino acids of a sequence shown in Figure 1A, e.g., SEQ ID NO: 1 or SEQ ID NO: 2; and these fragments having one or more of the following features: one or more conservative amino acid substitutions (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 conservative amino acid substitutions) in a sequence shown in Figure 1A, e.g., SEQ ID NO: 1 or SEQ ID NO: 2; one or more amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 amino acids) truncated from the N and/or C-terminus of a sequence shown in Figure 1A, e.g., SEQ ID NO: 1 or SEQ ID NO: 2; and at least one flanking amino acid (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 flanking amino acids) at the N- and/or C-terminus of a sequence shown in Figure 1A, e.g., SEQ ID NO: 1 or SEQ ID NO: 2.

By "substantially identical" is meant an amino acid sequence or nucleic acid sequence that exhibits at least 50% identity to a reference sequence. Such a sequence is generally at least, e.g., 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical at the amino acid level or nucleic acid level to a reference sequence. In general, for polypeptides, the length of comparison sequences can be at least five amino acids, e.g., 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200,

250, 300, or more amino acids, up to the entire length of the polypeptide. For nucleic acids, the length of comparison sequences can generally be at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, 250, 300, 400, 500, 600, 700, 800, 900, or more nucleotides, up to the entire length of the nucleic acid molecule. It is understood that for the purposes of determining sequence identity when comparing a DNA  
 5 sequence to an RNA sequence, a thymine nucleotide is equivalent to a uracil nucleotide.

Also contemplated are nucleic acid sequences that encode any of the Als3p polypeptides or fragments thereof recited herein.

As used herein, when a polypeptide or nucleic acid sequence is referred to as having “at least X% sequence identity” to a reference sequence, it is meant that at least X percent of the amino acids or  
 10 nucleotides in the polypeptide or nucleic acid are identical to those of the reference sequence when the sequences are optimally aligned. An optimal alignment of sequences can be determined in various ways that are within the skill in the art, for instance, the Smith Waterman alignment algorithm (Smith et al., J. Mol. Biol. 147:195-7, 1981) and BLAST (Basic Local Alignment Search Tool; Altschul et al., J. Mol. Biol. 215: 403-10, 1990). These and other alignment algorithms are accessible using publicly available  
 15 computer software such as “Best Fit” (Smith and Waterman, Advances in Applied Mathematics, 482-489, 1981) as incorporated into GeneMatcher Plus™ (Schwarz and Dayhof, Atlas of Protein Sequence and Structure, Dayhoff, M.O., Ed pp 353-358, 1979), BLAST, BLAST-2, BLAST-P, BLAST-N, BLAST-X, WU-BLAST-2, ALIGN, ALIGN-2, CLUSTAL, or Megalign (DNASTAR). In addition, those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to  
 20 achieve optimal alignment over the length of the sequences being compared.

By “adjuvant” is meant one or more substances that cause stimulation of the immune system. In this context, an adjuvant is used to enhance an immune response to one or more vaccine antigens or antibodies. An adjuvant may be administered to a subject before, in combination with, or after administration of the vaccine or antibody. Examples of chemical compounds used as adjuvants include,  
 25 but are not limited to, aluminum compounds (e.g., alum, Alhydrogel), oils, block polymers, immune stimulating complexes, vitamins and minerals (e.g., vitamin E, vitamin A, selenium, and vitamin B12), Quil A (saponins), bacterial and fungal cell wall components (e.g., lipopolysaccharides, lipoproteins, and glycoproteins), hormones, cytokines, and co-stimulatory factors.

By “carrier” in the context of a conjugate is meant a moiety or particle, e.g., KLH, CRM197, tetanus toxoid, diphtheria toxoid, enterotoxin B fragments, *N. meningitides* outer membrane protein complex, any other carrier protein, a phage, a yeast, a virus, a virosome, or a recombinant virus-like particle, that is suitable for being linked to or displaying a polypeptide as described herein.  
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By “conjugate” is meant a compound that includes a polypeptide of the invention linked to another moiety or particle, e.g., KLH, CRM197, tetanus toxoid, diphtheria toxoid, enterotoxin B fragments, *N. meningitides* outer membrane protein complex, any other carrier protein, a phage, a yeast, a virus, a virosome, or a recombinant virus-like particle.  
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By “immunogenic” is meant any substance that is capable of inducing an immune response in a subject.

By “immunogenic amount” in the context of a vaccine is meant an amount of the vaccine required to induce an immune response in a subject in a clinically relevant manner. An immunogenic amount of vaccine used to practice the methods of vaccination as described herein varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, prescribers will decide the appropriate amount and dosage regimen.

By “isolated” or “purified” is meant separated from other naturally accompanying components. Typically, a compound (e.g., nucleic acid, polypeptide, antibody, or small molecule) is substantially isolated when it is at least 60%, by weight, free from the proteins and/or naturally occurring organic molecules with which it is naturally associated. The definition also extends, e.g., to a polypeptide or nucleic acid molecule separated from its flanking sequences (e.g., for an amino acid sequence, isolated refers to a sequence that is free from the flanking amino acids with which the sequence is naturally associated in a polypeptide). In some instances, the compound is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, isolated. An isolated compound, e.g., polypeptide, may be obtained by standard techniques, for example, by extraction from a natural source (e.g., purification from a cell infected with *Candida*); by expression of a recombinant nucleic acid encoding an Als3p, an Als3p fragment or variant, or a fusion protein thereof in any standard expression system including but not limited to *E. coli* or *Saccharomyces cerevisiae*; or by chemically synthesizing the polypeptide. Purity can be measured by any appropriate method, e.g., by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

By “linked to” or “conjugated to” in the context of a conjugate is meant a covalent or non-covalent interaction between the polypeptide and the carrier or fusion partner. Non-covalent interactions include, but are not limited to, hydrogen bonding, ionic interactions among charged groups, electrostatic binding, van der Waals interactions, hydrophobic interactions among non-polar groups, lipophobic interactions, and LogP-based attractions.

The terms “peptide,” “polypeptide,” and “protein” are used interchangeably and refer to any chain of two or more natural or unnatural amino acids, regardless of post-translational modification (e.g., glycosylation or phosphorylation), constituting all or part of a naturally-occurring or non-naturally occurring polypeptide or peptide, as is described herein.

The terms “pharmaceutically acceptable carrier” and “pharmaceutically acceptable excipient” are used interchangeably and mean a carrier or excipient that is physiologically acceptable to the treated mammal while retaining the therapeutic properties of the compound with which it is administered. One exemplary pharmaceutically acceptable carrier substance is physiological saline. Other physiologically acceptable carriers and their formulations are known to those skilled in the art and described, for example, in Remington’s Pharmaceutical Sciences, (21<sup>th</sup> edition), ed. A. Gennaro, 2005, Lippincott, Williams &



Wilkins, Philadelphia, PA.

By “pharmaceutical composition” is meant a composition containing a polypeptide, conjugate, vaccine, or antibody of the invention, formulated with a pharmaceutically acceptable excipient, and manufactured or sold with the approval of a governmental regulatory agency as part of a therapeutic regimen for the treatment or prevention of a disease or event in a mammal. Pharmaceutical compositions can be formulated, for example, for intravenous administration (e.g., as a sterile solution free of particulate emboli and in a solvent system suitable for intravenous use), for oral administration (e.g., a tablet, capsule, caplet, gelcap, or syrup), or any other formulation described herein, e.g., in unit dosage form.

By “treating” or “treatment” is meant the medical management of a mammal, e.g., a human or non-human mammal, with the intent to cure, ameliorate, stabilize, reduce the likelihood of, or prevent a disease, pathological condition, disorder, or event, by administering a pharmaceutical composition. This term includes active treatment, that is, treatment directed specifically toward the improvement or associated with the cure of a disease, pathological condition, disorder, or event, and also includes causal treatment, that is, treatment directed toward removal of the cause of the associated disease, pathological condition, disorder, or event. In addition, this term includes palliative treatment, that is, treatment designed for the relief of symptoms rather than the curing of the disease, pathological condition, disorder, or event; symptomatic treatment, that is, treatment directed toward constitutional symptoms of the associated disease, pathological condition, disorder, or event; preventative treatment, that is, treatment directed to minimizing or partially or completely inhibiting the development of the associated disease, pathological condition, disorder, or event, e.g., in a mammal who is not yet ill, but who is susceptible to, or otherwise at risk of, a particular disease, pathological condition, disorder, or event; and supportive treatment, that is, treatment employed to supplement another specific therapy directed toward the improvement of the associated disease, pathological condition, disorder, or event.

By “vaccine,” as used herein, is meant a composition that elicits an immune response in a subject to which it is administered. The mode of administration, dose, and number of administrations can be optimized by those skilled in the art in a known manner.

By “vaccinate” or “vaccinating” as used herein, is meant to treat a mammal by administering a vaccine, e.g., to prevent or ameliorate a disease, pathological condition, disorder, or event.

Other features and advantages of the invention will be apparent from the following Detailed Description, the drawings, and the claims.

#### Brief Description of the Drawings

Fig. 1A is a listing of two Als3p amino acid sequences, SEQ ID NO: 1 and SEQ ID NO: 2.

Fig. 1B is a listing of one Als3 nucleic acid sequence, SEQ ID NO: 3.

Fig. 2 is a set of photographs showing comparative efficacy kinetics of NDV-3 assessed by *in vivo* imaging. The photographs show mice in each of the dosage groups at days 2, 4, and 7 post-infection.

Fig. 3 is a chart showing that NDV-3 restricts MRSA abscess volume in murine SSSI. The chart shows mean volume (cm<sup>3</sup>)/abscesses for the control group and the 3 µg, 10 µg, 30 µg, and 100 µg NDV-3 dosage groups.

Fig. 4 is a pair of photographs showing that NDV-3 restricts MRSA abscess volume in murine SSSI. Left, mouse in control group; right, mouse in 100 µg NDV-3 dosage group.

Fig. 5 is a chart showing that NDV-3 suppresses MRSA proliferation in murine SSSI. The chart shows mean flux/abscess for the control group and the 3 µg, 10 µg, 30 µg, and 100 µg NDV-3 dosage groups.

Fig. 6 is a set of images showing that NDV-3 limits MRSA proliferation and recruits neutrophils. The data shown are from the 100 µg NDV-3 dosage group at day 7 post-infection.

Fig. 7 is a set of images showing that NDV-3 recruits CD3+ T cells and induces IL-17 expression. The data shown are from the 100 µg NDV-3 dosage group at day 7 post-infection.

Fig. 8 is a set of images showing that NDV-3 stimulates IL-22 expression and β-defensin response. The data shown are from the 100 µg NDV-3 dosage group at day 7 post-infection.

Fig. 9 is a chart showing the median flank abscess area of control and vaccinated mice.

Fig. 10 is a chart showing the median flank abscess volume of control and vaccinated mice.

Fig. 11 is a chart showing the mean abscess volume due to MRSA strains in control and vaccinated mice at day 7 post-infection. Asterisks indicate significant reduction as compared to respective control.

Fig. 12 is a composite immunofluorescence image of a MRSA abscess from a NDV-3 (100 µg) vaccinated mouse.

Fig. 13 is a composite immunofluorescence image of a MRSA abscess from a control mouse.

The patent or application file contains drawings (Figures 2, 4, 6-8, 12, and 13) executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

#### Detailed Description

As is described below, agglutinin-like sequence 3 protein (Als3p) allows for vaccination against *S. aureus* in mammals identified as being at risk for development of an *S. aureus* skin or soft tissue infection.

In the following analyses (in particular, the pilot study of Example 1 and the optimized study of Example 2) designed to evaluate the efficacy of an Als3p vaccine against the development of skin or soft tissue infection resulting from *S. aureus* in a murine model of MRSA skin/skin structure infection (SSSI), the organisms and methods are first described.

## **ORGANISMS**

### **MRSA Strains**

- 5           MRSA Xen30 (lux+) Roche-16  
             MRSA LAC-USA300 USA300  
             MRSA MW2 USA400

10           *Staphylococcus aureus* strain Xen30 was used in these in vivo studies. It is derived from the parental strain *S. aureus* MRSA-16 (Roche) and contains a *luxA-E* operon at a single chromosomal integration site. This MRSA strain produces luciferase enzyme and aldehyde substrate, and constitutively emits a bioluminescent signal when metabolically active. Its virulence is equivalent to other MRSA strains in the SSSI murine model used as verified in pilot studies, and all strains tested have otherwise similar phenotypes and growth characteristics. Log-phase cells (BHI; 37°C) were cultured from quantitatively-  
 15           and virulence-validated master cell banks, harvested and suspended in PBS, sonicated and quantified by spectrophotometry to desired CFU.

## **METHODS**

NDV-3 vaccine efficacy was evaluated in a murine SSSI model vs. methicillin-resistant SA (MRSA):  
 20           Xen30 (lux+); LACUSA300; or MW2 (USA400). NDV-3 is a formulation of the recombinant N-terminus of the *Candida* surface protein Als3 protein (Figure 1A; SEQ ID NO:2) and the adjuvant Alhydrogel®, in phosphate-buffered saline, pH 7, e.g., with a 0.5 mL dose containing, e.g., 30-300 µg Als3 protein, and optionally further containing aluminum hydroxide at 1.0 mg Al/mL. Efficacy was compared among NDV-3 regimens administered with alhydrogel adjuvant (IM) on day 0 and boosted on day 21. Controls  
 25           received adjuvant alone. Infection by subcutaneous inoculation of two flanks ( $2 \times 10^7$  CFU) occurred 14 days after boost. Abscess area, volume, and CFU were quantified for multiple days post-challenge. *In vivo* imaging (IVIS) of abscess flux was done in mice infected by Xen30. Serum IgG (ELISA), IFN-γ and IL-17A (ELISpot) responses were quantified in parallel vaccine regimens. Tissue IL-17A, IL-22, mβD-3, CD3+ cell and neutrophil signals were assessed on day 7 post-infection by immunohistochemistry.

30

Vaccine. The NDV-3 vaccination was evaluated across a dose range using an identical regimen of alhydrogel adjuvant. Doses of 3, 10, 30, 100, or 300µg (IM) were studied in parallel. Primary vaccination (day 0) was followed by an identical boost on study day 21. Mice were infected 14 days after boost (study day 35).

35

Murine Model of SSSI. All animal studies were performed per the approved animal use policies of LABioMed at Harbor-UCLA. Balb/C mice (Harlan) were vaccinated as above. A subcutaneous skin / soft tissue abscess model was modified from Ding *et al.* (*J Bacteriol* 2008 190:7123-9) and/or Voyich *et al.* (*J Infect Dis* 2006 194:1761-1770) for these studies. On study day 35, mice were anesthetized, flanks were

shaved and sterilized, and  $2 \times 10^7$  CFU inocula (without beads or matrix) were introduced into the subcutaneous compartment by injection (100  $\mu$ l). A minimum of 20 mice per control or vaccine-regimen groups were used in each study.

- 5    Abscess Quantification. Abscess area / volume were measured in each mouse flank during the study period up to 14 days post-challenge. To do so, mice were anesthetized, and the lesion site length ( $l$ ) and width ( $w$ ) assessed to quantify abscess or dermonecrosis area ( $\text{cm}^2$ ). Abscess volume ( $\text{cm}^3$ ) was calculated per the formula for a spherical ellipsoid: [ $v = (\pi/6) \times l \times w^2$ ].
- 10   Imaging Studies. The Xen30 MRSA strain has a self-contained *lux* operon integrated in its chromosome. The construct encodes the aldehyde substrate and the luciferase enzyme itself; thus, no exogenous luciferin substrate is required (Kadurugamuwa *et al.*, *Infect Immun* 2003 71:882-890). On selected study days, control and vaccinated mice underwent *in vivo* imaging (IVIS) using an IVIS system (Caliper Life Sciences, Inc.). Luminescence signals were captured over a five-minute time period and analyzed using
- 15   the Living Image software as photons / min / abscess.

Quantitative Culture. At pre-selected times post-infection, mice were humanely sacrificed and processed for quantitative culture of abscesses. Each flank was aseptically dissected, the abscess removed and prepared for culture. Abscesses were individually homogenized, and serially diluted in sterile PBS for

20   quantitative culture onto sheep blood agar plates. Cultures were incubated (37°C) for 24 hours, and resulting colonies enumerated.

Immunological Mechanisms. Multiple and complementary approaches were used to assess potential correlates of NDV-3 vaccine efficacy in the murine model of SSSI due to MRSA. These studies focused

25   on strain Xen30, allowing correlation with IVIS data at the 7d endpoint.

A. Antibody Quantification. Serum IgG antibody levels were determined in a 96-well ELISA format over a range dilutions. Values represent geometric mean corrected dilution of triplicate assays comparing immunized vs. control sera.

30

B. Cytokine Quantification. T cell IFN- $\gamma$  and IL-17A responses were determined by ELISpot analysis of splenocytes isolated from immunized vs. control mice, and exposed to the NDV-3 immunogen. The number of spot-forming units (SPUs or SFUs, used interchangeably) was quantified per  $10^6$  cells producing either IFN- $\gamma$  or IL-17A. Cell viability was verified by production of IFN- $\gamma$  following

35   stimulation with phorbol-12-myristate-13-acetate (PMA) and ionomycin per established protocols.

C. Immunohistochemistry. Immunological determinants associated with vaccine efficacy were

assessed in tissues obtained from vaccinated and control animals after 7d of infection by standard methods.

For immunohistochemical studies, in brief 3 µm vertical paraffin embedded sections were de-waxed and rehydrated followed by heat-induced antigen retrieval in target retrieval solution (Dako, Carpinteria, CA).

5 Sections were incubated with dual endogenous blocking buffer (Dako) for 15 min at room temperature to block endogenous peroxidase activity, and non-specific antibody binding was blocked by incubation with 5% normal serum corresponding to the primary antibody. Sections were then incubated overnight at 4°C with a primary antibody targeting a specific antigen of interest (Table 1). Sections were then washed and incubated for 30 min with an appropriate secondary antibody (Table 1), either horseradish peroxidase  
10 (HRP)-conjugated or biotinylated (Santa Cruz Biotechnology, Santa Cruz CA). Immunohistochemical development was then achieved by 30 min development with streptavidin-HRP (Dako) and 3,3'-diaminobenzidine (DAB; Vector Laboratories, Burlingame, CA), and counterstained with hematoxylin. Images were visualized using an Olympus BX43 microscope employing a DP21 digital camera for image capture.

15

D. Immunofluorescence. To evaluate the impact of NDV-3 vaccination on the interrelationships

of immunologic determinants and *S. aureus* in context of infection *in vivo*, immunofluorescence studies

employing confocal microscopy were performed using established methods. In brief, paraffin embedded sections were prepared as above and incubated with immunofluorescence buffer (1% bovine serum

20 albumin and 2% fetal calf serum) for one hour at room temperature. Primary antibodies directed at target antigens of interest (Table 1) were incubated with tissue sections from control or vaccinated mice at 4°C overnight. Next, corresponding secondary antibodies (Table 1) diluted in IFF buffer (2 µg/ml) were incubated for 60 minutes. Sections were then washed in PBS, and mounted using Vectashield H-1500 (Vector Laboratories, Burlingame, CA) to minimize photobleaching. Images were visualized using a  
25 Leica SP2 confocal microscope employing argon (488nm), krypton (568nm) and helium-neon (633nm) lasers and confocal version 2.0 software (Leica Instruments, Germany).

**Table 1.** Antibodies used for immunohistochemical or immunofluorescence studies.**Primary antibodies****Target Antigen**

Ly-6G (granulocytes)  
 5 Ly-6C (monocytes / macrophages)  
 Mouse  $\beta$ -defensin-1 (mBD-1)  
 Mouse  $\beta$ -defensin-3 (mBD-3)  
 Mouse platelet factor 4 (PF-4)  
*Staphylococcus aureus* (mouse)  
 10 *Staphylococcus aureus* (rabbit)  
 Staphylococcal protein A  
 CD3- $\gamma$   
 CD3- $\epsilon$   
 IL-17  
 15 IL-22

**Secondary Antibodies**

Alexa 488-conjugated donkey  $\alpha$ -rabbit  
 Alexa 488-conjugated donkey  $\alpha$ -rat  
 20 Alexa 555-conjugated goat  $\alpha$ -rat  
 Alexa 568-conjugated donkey  $\alpha$ -rabbit  
 Alexa 633-conjugated donkey  $\alpha$ -goat  
 Alexa 647-conjugated donkey  $\alpha$ -rabbit  
 Alexa 633-conjugated streptavidin

25  
Statistical Analyses. Differences in experimental results were compared based on power estimates indicating that 16–20 mice per group yields > 85% power to detect 1 log difference in CFU per gram tissue, or 2 mm abscess area ( $\alpha = 0.05$ ; Mann-Whitney U test. *P* values are defined in Table 2 and Table 3 (below).

**Example 1**

30 In a pilot study, vaccination with NDV-3 reduced SSSI parameters due to MRSA, with equivalent efficacy in limiting abscess area, volume, and CFU for strains Xen30, USA300, and MW2. Murine immune response correlated with NDV-3 dose-related protective efficacy. These results are shown in  
 35 Table 2 and Figure 2. These results indicate the NDV-3 vaccine induced robust B and T cell responses which correspond with protective efficacy against MRSA in the murine model of SSSI.

**Table 2.** NDV-3 Efficacy in MRSA Xen30 SSSI and Immune Response in Murine Models.

Abscess	Control	3 $\mu$ g	10 $\mu$ g	30 $\mu$ g	100 $\mu$ g	300 $\mu$ g
Area 7d	1.88cm <sup>2</sup>	1.47cm <sup>2*</sup>	1.59cm <sup>2*</sup>	0.99cm <sup>2††</sup>	0.77cm <sup>2††</sup>	0.69cm <sup>2††</sup>
Volume 7d	1.29cm <sup>3</sup>	0.95cm <sup>3*</sup>	0.96cm <sup>3**</sup>	0.46cm <sup>3††</sup>	0.34cm <sup>3††</sup>	0.29cm <sup>3††</sup>
Flux 7d	1.92x10 <sup>5</sup>	1.48x10 <sup>5*</sup>	1.81x10 <sup>5</sup>	1.07x10 <sup>5*</sup>	1.65x10 <sup>5*</sup>	9.03x10 <sup>4††</sup>
Median Log CFU 7d IM	7.9 (7.6/8.0) [n=36]	7.8 (7.8/7.8) [n=20]	8.1 (7.8/7.8) [n=20]	8.1 (7.9/7.9) [n=36]	7.9 (7.5/8.0) [n=36]	7.5† (7.4/7.8) [n=36]
Median Log CFU 14d IM	1.70 (1.0/2.8) [n=48]	0.05 <sup>Δ††</sup> (0.05/1.3) [n=39]	ND	ND	0.05 <sup>Δ††</sup> (0.05/1.7) [n=39]	1.48 (0.05/2.6) [n=17]
Median Log CFU 14d SubQ	3.54 (2.6/6.9) [n=20]	3.92 (3.2/5.5) [n=20]	ND	ND	2.26** (1.8/3.6) [n=20]	2.40** (1.4/3.8) [n=20]
Analyte						
IgG	1.0 GCU	44.8 GCU††	ND	97.8 GCU††	81.8 GCU††	ND
IFN- $\gamma$	9.5 SPU	12.8 SPU	ND	21.9 SPU	34.3 SPU*	ND
IL-17	18.9 SPU	132.6 SPU††	ND	62.2 SPU	161.2 SPU**	ND

(25%/75% quartiles); \* $P < 0.5$ ; \*\* $P < 0.1$ ; † $P < 0.05$ ; †† $P < 0.01$ ; GCU, geomean / dilution corrected units; SPU, mean spot forming units / 10<sup>6</sup> splenocytes; <sup>Δ</sup> limit of detection.

## ANALYSIS

The NDV-3 vaccine significantly reduced the abscess area, volume, luminescence signal, and CFU densities in this murine model of MRSA SSSI. NDV-3 efficacy was equivalent for each of the MRSA strains evaluated in this study. Immunological data from mice vaccinated identically to those challenged with infection indicate the NDV-3 vaccine induces robust B and T cell responses which appear to reflect a dose-response relationship. Immunological data from mice vaccinated identically to those challenged with infection indicate the NDV-3 vaccine induces robust B and T cell responses which reflect a dose-response relationship. Collectively these results provide evidence that NDV-3 induces a mixed Th1 / Th17 response that appears to be predominantly associated with protective efficacy. Antibody response may contribute to protective mechanisms of NDV-3. These results indicate that the NDV-3 vaccine is useful as a means to prevent or mitigate MRSA skin infection or abscesses or both in mammals.

### Example 2

A further, optimized analysis was conducted, and results are summarized in Table 3 and Figures 3-8. Like Example 1, this study evaluated the efficacy and immunologic mechanisms of the NDV-3 vaccine in a murine model of skin / skin structure infection due to methicillin-resistant SA (MRSA). Abscess size, MRSA density and CFU were compared over time in NDV-3 immunized and control groups. Serum concentrations of IgG, IFN $\gamma$ , IL-17A, induction of tissue IL-17A, IL-22, and m $\beta$ D-3, and infiltration of CD3+ T cells or neutrophils as mediated by NDV-3 were determined in parallel. NDV-3 immunization achieved protective efficacy against MRSA in terms of abscess area, volume, bacterial density and CFU as compared to adjuvant alone. Protective efficacy of NDV-3 corresponded to increases in serum IgG, serum

and tissue biomarkers of Th1-Th17 polarization, and corresponding neutrophil infiltration and host defense peptide induction in context of abscesses. These data further demonstrated that NDV-3 immunization induces robust B and T cell mechanisms of protective efficacy against MRSA in context of skin and mucosa.

## RESULTS

NDV-3 was efficacious against MRSA as measured by reduced abscess area, volume, and CFU versus adjuvant alone (Table 3). Efficacy as measured by area of dermonecrosis and abscess volume were equivalent for all strains tested. Significant increases in serum IgG, serum and tissue biomarkers of Th1 (INF- $\gamma$ ) and Th17 (IL-17) polarization (Table 3), neutrophil infiltration (Ly6G), IL-22 elaboration, as well as m $\beta$ D-3 induction were correlated with NDV-3 protective efficacy (Figures 3-8).

**Table 3.** NDV-3 efficacy and immune response vs. MRSA Xen30 in murine SSSI.

Abscess	Control	3 $\mu$ g	10 $\mu$ g	100 $\mu$ g
Area d7	1.88cm <sup>2</sup>	1.47cm <sup>2</sup> *	1.59cm <sup>2</sup> *	0.77cm <sup>2</sup> ††
Volume d7	1.29cm <sup>3</sup>	0.95cm <sup>3</sup> *	0.96cm <sup>3</sup> **	0.38cm <sup>3</sup> ††
Flux d7	3.22x10 <sup>5</sup>	1.48x10 <sup>5</sup> *	2.15x10 <sup>5</sup> *	1.06x10 <sup>5</sup> **
Geo Mean	7.50	6.23 †	6.68 †	6.05 †
Log CFU	(8.0/7.4) <sup>a</sup>	(6.4/6.1)	(6.8/6.4)	(6.2/5.6)
d7 IM	[n=54]	[n=20]	[n=20]	[n=20]
Analyte				
IgG	1.0 GCU <sup>b</sup>	44.8 GCU ††	ND	81.8 GCU ††
IFN- $\gamma$	9.5 SFU <sup>c</sup>	12.8 SFU	ND	34.3 SFU *
IL-17	18.9 SFU	132.6 SFU ††	ND	161.2 SFU **

<sup>a</sup> Mean variance

<sup>b</sup> GCU, geomean / dilution corrected units

<sup>c</sup> SPU, mean spot forming units / 10<sup>6</sup> splenocytes

\*  $P < 0.5$ ; \*\* $P < 0.1$ ; † $P < 0.05$ ; †† $P < 0.01$

## CONCLUSION

NDV-3 induces protective efficacy against MRSA in murine SSSI. Immunologic mechanisms of efficacy included robust B and T cell responses consistent with Th1-Th17 paradigms in which neutrophils and host defense peptides are targeted and coordinated in context of infection.

### Example 3

An additional set of experiments was conducted to evaluate the efficacy of the NDV-3 vaccine in a murine model of SSSI due to Xen30 MRSA and comparative strains of MRSA. Experiments were conducted as described in Examples 1 and 2. Median data kinetics of vaccine efficacy versus time is shown in Fig. 9 (median flank abscess area of control and vaccinated mice) and Fig. 10 (mean flank abscess volume of control and vaccinated mice). These data confirm that vaccination with NDV-3



suppresses evolution of the abscess, particularly at dosages greater than 3 µg.

In addition, efficacy of the vaccine was tested against three different MRSA strains: Xen 30, USA300, and MW2. For each MRSA strain, a negative control and a 100 µg dosage group were tested. The mean lesion volume at day 7 post-infection was determined, as shown in Fig. 11. Each strain was the same inoculum ( $2 \times 10^7$ ). MW2 exhibited low virulence in these experiments.

The data demonstrate that regardless of MRSA strain tested, the NDV-3 vaccine has equivalent efficacy (e.g., about 50% reduction) in restricting abscess volume. Thus, NDV-3 efficacy is not MRSA strain-specific.

#### Example 4

In a further set of experiments, composite immunofluorescence images of MRSA abscesses were recorded and analyzed. Fig. 12 is a composite immunofluorescence image of a representative MRSA abscess from a NDV-3 (100 µg) vaccinated mouse, and Fig. 13 is a composite immunofluorescence image of a representative MRSA abscess from a control mouse.

In each of the above images, each component of the image is of the same lesion, magnified approximately 500-fold. As immunofluorescence signal is difficult to resolve at low power, images were recorded for each section of the lesion at higher power, moving from the epidermis of the skin, into the subdermis, and down into the hypodermis. Thus, the components are merged to illustrate a continuous immunofluorescence map of *S. aureus* (blue), neutrophils (red), and CD3+ (T cells) green, throughout a lesion and maintaining magnification sufficient for resolution of immunofluorescence. The image components represent a function of high-power fields positioned to systematically capture equivalent areas in the NDV-3 and control lesions for head-to-head comparison of abscess immunophenotypes.

As Figs. 12 and 13 reveal, in the NDV-3 vaccinated abscess, there are few MRSA organisms (blue), and they are restricted to the epidermis, with infiltration of neutrophils (red) mediated by an influx of CD3+ T cells (green). In contrast, in the control abscess, there are many MRSA organisms, and they are invasive to two distinct regions (epidermis and hypodermis), corresponding with substantially less neutrophil and CD3+ cell infiltration. While the images shown in Figs. 12 and 13 are from individual lesions, they are representative of lesions in vaccinated and control groups overall and are consistent with the quantitative findings described in the preceding Examples.

#### Example 5

The compositions and methods described herein may be used, e.g., to vaccinate a human at risk for the development of a *Staphylococcus aureus* skin or soft tissue infection against *Staphylococcus aureus*. First, a human at risk for the development of an *S. aureus* SSSI is identified. Second, the human is administered an immunogenic amount of a vaccine comprising a polypeptide comprising Als3p, or an immunogenic fragment thereof, in a pharmaceutically acceptable medium. For example, the human is

administered between one and three doses of NDV-3 containing between 3 and 1000 µg of the recombinant N-terminus of the *Candida* surface protein Als3 (SEQ ID NO:2) per dose, with multiple doses occurring at intervals of two weeks to six months.

It is expected that, following administration of the vaccine, the human is at decreased risk for the development of an *S. aureus* SSSI for a period lasting from one month to several years or more.

Likewise, a human who is identified as having an *S. aureus* SSSI may be treated by administration of an immunogenic amount of a pharmaceutical composition comprising a polypeptide comprising Als3p, or an immunogenic fragment thereof, in a pharmaceutically acceptable medium. For example, the human is administered between one and three doses of NDV-3 containing between 3 and 1000 µg of the recombinant N-terminus of the *Candida* surface protein Als3 (SEQ ID NO:2) per dose, with multiple doses occurring at intervals of two weeks to six months.

It is expected that, following administration of the pharmaceutical composition, the *S. aureus* SSSI of the human is decreased in severity.

#### Example 6

The compositions and methods described herein may be used, e.g., to vaccinate a bovine species at risk for the development of a *Staphylococcus aureus* skin or soft tissue infection against *Staphylococcus aureus*. In particular, the bovine species may be at risk of developing bovine mastitis caused by *S. aureus*. First, a bovine species at risk for the development of an *S. aureus* SSSI, e.g., bovine mastitis, is identified. For example, any milk-producing bovine may be considered to be at risk of developing bovine mastitis caused by *S. aureus*. Second, the bovine species is administered an immunogenic amount of a vaccine comprising a polypeptide comprising Als3p, or an immunogenic fragment thereof, in a pharmaceutically acceptable medium. For example, the bovine species is administered between one and three doses of NDV-3 containing between 3 and 1000 µg of the recombinant N-terminus of the *Candida* surface protein Als3 (SEQ ID NO:2) per dose, with multiple doses occurring at intervals of two weeks to six months.

It is expected that, following administration of the vaccine, the bovine species is at decreased risk for the development of an *S. aureus* SSSI, e.g., bovine mastitis.

Likewise, a bovine species identified as having an *S. aureus* SSSI, e.g., bovine mastitis, may be treated by administration of an immunogenic amount of a pharmaceutical composition comprising a polypeptide comprising Als3p, or an immunogenic fragment thereof, in a pharmaceutically acceptable medium. For example, the bovine species is administered between one and three doses of NDV-3 containing between 3 and 1000 µg of the recombinant N-terminus of the *Candida* surface protein Als3 (SEQ ID NO:2) per dose, with multiple doses occurring at intervals of two weeks to six months.

It is expected that, following administration of the pharmaceutical composition, the *S. aureus* SSSI, e.g., bovine mastitis, of the bovine species is decreased in severity.

Other Embodiments

5 All publications and patents cited in this specification are incorporated herein by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

## CLAIMS

1. A method of vaccinating a mammal against *Staphylococcus aureus* comprising the steps of:
  - a) identifying a mammal at risk for the development of a *Staphylococcus aureus* skin or soft tissue infection; and
  - b) administering to said mammal an immunogenic amount of a vaccine comprising a polypeptide comprising an isolated agglutinin-like sequence (Als) 3 protein (Als3p), or an immunogenic fragment thereof, in a pharmaceutically acceptable medium.
2. The method of claim 1, wherein said Als3 protein comprises an Als protein derived from a *Candida* strain selected from the group consisting of *Candida albicans*, *Candida krusei*, *Candida tropicalis*, *Candida glabrata* and *Candida parapsilosis*.
3. The method of claim 1, wherein said polypeptide comprises a *Candida albicans* Als3p.
4. The method of claim 1, wherein said polypeptide comprises the N-terminal domain of *Candida albicans* Als3p or an immunogenic fragment thereof.
5. The method of claim 1, wherein said polypeptide is an Als3p, wherein the amino acid sequence of said Als3p consists of SEQ ID NO: 1 or SEQ ID NO: 2.
6. The method of claim 1, wherein said polypeptide is an immunogenic fragment of an Als3p, wherein the amino acid sequence of said Als3p consists of SEQ ID NO: 1 or SEQ ID NO: 2.
7. The method of claim 1, wherein said *Staphylococcus aureus* is a MRSA strain of *Staphylococcus aureus*.
8. The method of claim 1, wherein said *Staphylococcus aureus* is a MSSA strain of *Staphylococcus aureus*.
9. The method of claim 1, wherein said *Staphylococcus aureus* is a vancomycin-resistant (VRSA) or daptomycin-resistant (DRSA) strain of *Staphylococcus aureus*.
10. The method of claim 1, wherein said polypeptide is conjugated to a carrier.

11. The method of claim 10, wherein said carrier comprises keyhole limpet hemocyanin (KLH), CRM197, tetanus toxoid, diphtheria toxoid, enterotoxin B fragments, or *N. meningitides* outer membrane protein complex.
12. The method of claim 10, wherein said carrier is a phage, a yeast, a virus, a virosome, or a recombinant virus-like particle.
13. The method of claim 1, wherein said vaccine is administered by intramuscular, subcutaneous, intradermal, oral, or sublingual administration, or is administered for inhalation in a microparticulate formulation.
14. The method of claim 1, wherein said administering further comprises administering a booster dose.
15. The method of claim 1, wherein said vaccine comprises an immunostimulating adjuvant.
16. The method of claim 1, further comprising administering an antibiotic.
17. The method of claim 16, wherein said antibiotic is co-formulated or co-administered with said vaccine.
18. The method of claim 1, wherein said vaccine is administered as a pharmaceutical composition.
19. A vaccine comprising an isolated Als3 protein, or an immunogenic fragment thereof, for use in a method of treatment or prevention of a *Staphylococcus aureus* skin or soft tissue infection in a mammal.
20. The vaccine for use according to claim 19, wherein said Als3 protein comprises an Als protein derived from a *Candida* strain selected from the group consisting of *Candida albicans*, *Candida krusei*, *Candida tropicalis*, *Candida glabrata* and *Candida parapsilosis*.
21. The vaccine for use according to claim 19, wherein said polypeptide comprises a *Candida albicans* Als3p.
22. The vaccine for use according to claim 19, wherein said polypeptide comprises the N-terminal domain of *Candida albicans* Als3p or an immunogenic fragment thereof.

23. The vaccine for use according to claim 19, wherein said polypeptide is an Als3p, and wherein the amino acid sequence of said Als3p consists of SEQ ID NO: 1 or SEQ ID NO: 2.
24. The vaccine for use according to claim 19, wherein said polypeptide is an immunogenic fragment of an Als3p, and wherein the amino acid sequence of said Als3p consists of SEQ ID NO: 1 or SEQ ID NO: 2.
25. The vaccine for use according to claim 19, wherein said *Staphylococcus aureus* is a MRSA strain of *Staphylococcus aureus*.
26. The vaccine for use according to claim 19, wherein said *Staphylococcus aureus* is a MSSA strain of *Staphylococcus aureus*.
27. The vaccine for use according to claim 19, wherein said *Staphylococcus aureus* is a vancomycin-resistant (VRSA) or daptomycin-resistant (DRSA) strain of *Staphylococcus aureus*.
28. The vaccine for use according to claim 19, wherein said polypeptide is conjugated to a carrier.
29. The vaccine for use according to claim 28, wherein said carrier comprises keyhole limpet hemocyanin (KLH), CRM197, tetanus toxoid, diphtheria toxoid, enterotoxin B fragments, or *N. meningitidis* outer membrane protein complex.
30. The vaccine for use according to claim 28, wherein said carrier is a phage, a yeast, a virus, a virosome, or a recombinant virus-like particle.
31. The vaccine for use according to claim 19, wherein said vaccine is administered by intramuscular, subcutaneous, intradermal, oral, or sublingual administration, or is administered for inhalation in a microparticulate formulation.
32. The vaccine for use according to claim 19, wherein said administering further comprises administering a booster dose.
33. The vaccine for use according to claim 19, wherein said vaccine comprises an immunostimulating adjuvant.
34. The vaccine for use according to claim 19, which includes further administering an antibiotic.

35. The vaccine for use according to claim 34, wherein said antibiotic is co-formulated or co-administered with said vaccine.

36. The vaccine for use according to claim 19, wherein said vaccine is administered as a pharmaceutical composition.

## FIGURE 1A

SEQ ID NO:1

```

1 MLQQYTLLLI YLSVATAKTI TGVFNSFNLS TWSNAATYNY KGPPTPTWNA VLGWSLDGTS
61 ASPGDTFTLN MPCVEKFTTS QTSVDLTAHG VKYATCQFQA GEEFMTFSTL TCTVSNTLTP
121 SIKALGTVTL PLAFNVGGTG SSVDLEDSKC FTAGTNTVTF NDGGKKISIN VDFERSNVDP
181 KGYLTDSRVI PSLNKVSTLF VAPQCANGYT SGTMGFANTY GDVQIDCSNI HVGITKGLND
241 WNYPVSSSEF SYTKTCSSNG IFITYKNVPA GYRPFVDAI SATDVNSYTL SYANEYTCAG
301 GYWQRAPFTL RWTGYRNSDA GSNIGIVIVAT TRTVTDSTTA VTTLPFDPNR DKTKTIEILK
361 PIPTTTITTS YVGVTTSYST KTAFIGETAT VIVDIPYHTT TTVTSKWTGT ITSTTTHTNP
421 TDSIDTVIVQ VSPNPTVTT TEYWSQSFSAT TTTITGPPGN TDTVLIREPP NHTVTTTEYW
481 SESYTTTSTF TAPPGGTDSDV IIEKPPNPTV TTTEYWSESY TTTSTFTAPP GGTDSVIIKE
541 PPNHTVTTTE YWSQSYTTTT TVTAPPGGTD TVLVREPPNH TVTTTEYWSQ SYTTTTTVIA
601 PPGGTDSDVII REPPNPTVTT TEYWSQSYAT TTTITAPPGE TDTVLIREPP NHTVTTTEYW
661 SQSYATTTTI TAPPGETDTV LIREPPNHTV TTTEYWSQSF ATTTTVTAPP GGTDTVIIRE
721 PPNHTVTTTE YWSQSYATTT TITAPPGETD TVLIREPPNH TVTTTEYWSQ SYATTTTIIA
781 PPGETDTVLI REPPNPTVTT TEYWSQSYTT ATTVTAPPGG TDTVIIYDTM SSSEISSFSR
841 PHYTNHTTLW STTWVIETKT ITETSCEGDK GCSWVSSTR IVTIPNNIET PMVTNTVDST
901 TTESTSQSPS GIFESGVSV ETESSTVTTA QTNPSVPTTE SEVVFTTKGN NENGPYESPS
961 TNVKSSMDEN SEFTTSTAAS TSTDIENTETI ATTGSVEASS PIISSADET TTVTTTAEST
1021 SVIEQPTNNN GGGKAPSATS SPSTTTTANN DSVITGTTST NQSQSQSQYN SDTQQTLSQ
1081 QMTSSLVSLH MLTTFDGSGS VIQHSTWLCG LITLLSLFI

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SEQ ID NO:2

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1 KTITGVFNSF NSLTWSNAAT YNYKGPPTPT WNAVLGWSLD GTSASPGDTF TLNMPCVKFK
61 TTSQTSVDLT AHGVKYATCQ FQAGEEFMTF STLCTVSNT LTPSIKALGT VTLPLAFNVG
121 GTGSSVDLED SKCF TAGTNT VTFNDGGKKI SINVDERSN VDPKGYLTDS RVIPSLNKVS
181 TLFVAPQCAN GYTSGTMGFA NTYGDVQIDC SNIHVGITKG LNDWNPVSS ESFSYTKTCS
241 SNGIFITYKN VPAGYRPFVD AYISATDVNS YTLSYANEYT CAGGYWQRAP FTLRWTGYRN
301 SDAGSNGIVI VATTRTVTDS TTAVTTLPFD PNRDKTKTIE ILKPIPTTTI TTSYVGVTTTS
361 YLTKTAPIGE TATVIVDIPY HTTTTVTSKW TGTITSTTTH TNPTDSIDTV IVQVPL

```



## FIGURE 1B

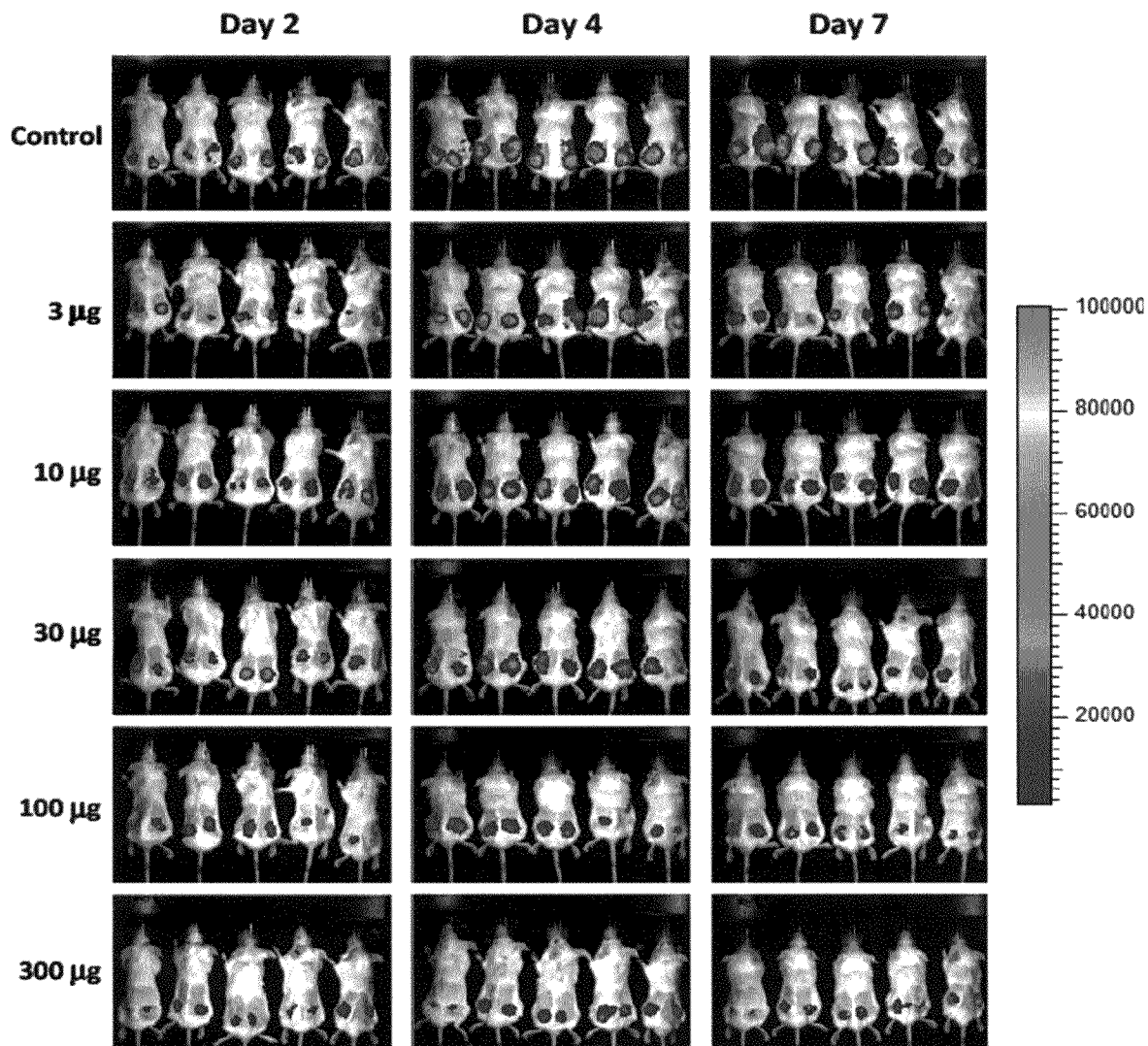
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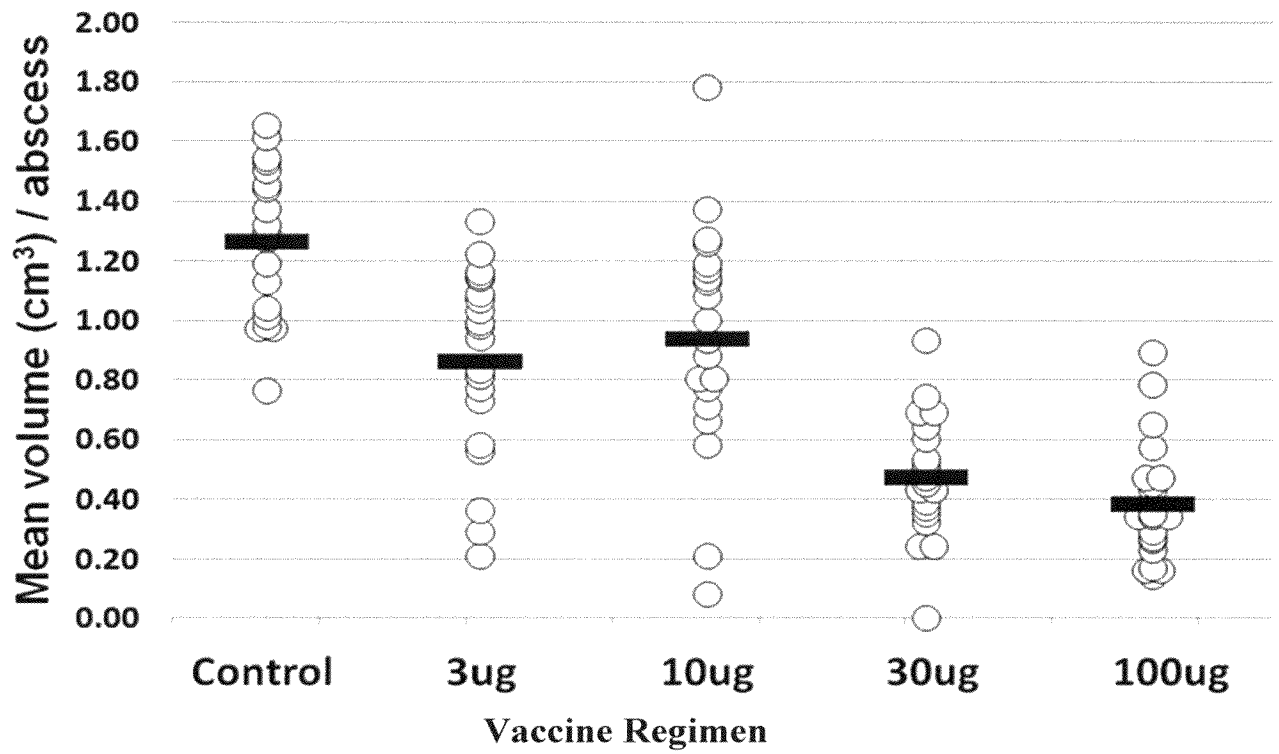
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1      AAGACAATCACTGGTGTGTTTCAACAGTTTT  AATTCATTGACTTGGTCTAATGCTGCTACG
61     TATAATTATAAGGGACCAGGAACCCCAACT  TGGAATGCTGTTTTGGGTGCTTTTAGAT
121    GGTACTAGTGCAAGTCCGGGAGATACATTC  ACATTGAATATGCCATGTGTGTTTAAATTT
181    ACTACTTCTCAAACATCTGTTGATTTGACT  GCTCATGGTGTTAAATATGCTACATGTCAA
241    TTTCAGGCAGGTGAAGAATTTATGACCTTT  TCTACATTAACATGTACTGTGAGCAATACT
301    TTGACTCCATCTATTAAGGCTTTGGGTACT  GTCACTTTACCACTTGCATTCAATGTAGGT
361    GGAAGTGGTTCTTCTGTTGATTTGGAAGAT  TCTAAATGTTTTACTGCTGGTACTAACACA
421    GTTACATTTAATGATGGTGGCAAGAAAATC  TCAATTAATGTTGATTTTGAAAGGTCAAAT
481    GTCGATCCAAAAGGGTACTTAACTGATTCC  AGAGTTATACCAAGTCTCAACAAAGTGTCA
541    ACTCTTTTTGTTGCACCACAATGTGCAAAAT  GGTACACATCTGGTACAATGGGATTTCGCT
601    AACACTTATGGTGATGTTCAAATTGACTGT  TCAAATATTCATGTTGGTATTACAAAAGGA
661    TTGAATGATTGGAATTATCCGGTTTCATCT  GAATCATTTAGTTACACCAAAACTTGTTCA
721    TCTAATGGTATCTTTATCACATATAAAAAAC  GTTCCTGCCGGTTATCGTCCATTTGTTGAC
781    GCTTATATTTCTGCTACAGATGTTAATTCG  TACACCTTGTCGTATGCTAATGAATATACT
841    TGTGCTGGTGGTTATTGGCAACGTGCACCT  TTCACATTAAGATGGACTGGATACAGAAAT
901    AGTGATGCTGGATCTAACGGTATTGTTATT  GTGGCTACTACCAGAACAGTTACAGACAGT
961    ACTACCGCCGTGACCACCTTACCATTTCGAT  CCTAACCGCGACAAAACCTAAGACAATTGAA
1021   ATTTTGAAACCTATTCCAACAACCTACAATC  ACAACATCATATGTTGGTGTGACTACTTCC
1081   TACCTGACCAAAACTGCACCAATTGGGGGAA  ACTGCTACTGTTATTGTTGATATTCCATAT
1141   CACACTACCCTACTGTTACCAGTAAATGG  ACAGGAACAATTACTTCCACCACAACACAT
1201   ACTAATCCAACCTGACTCAATAGACACTGTC  ATTGTACAAGTTCCACTGTGA
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\*

\*stop codon

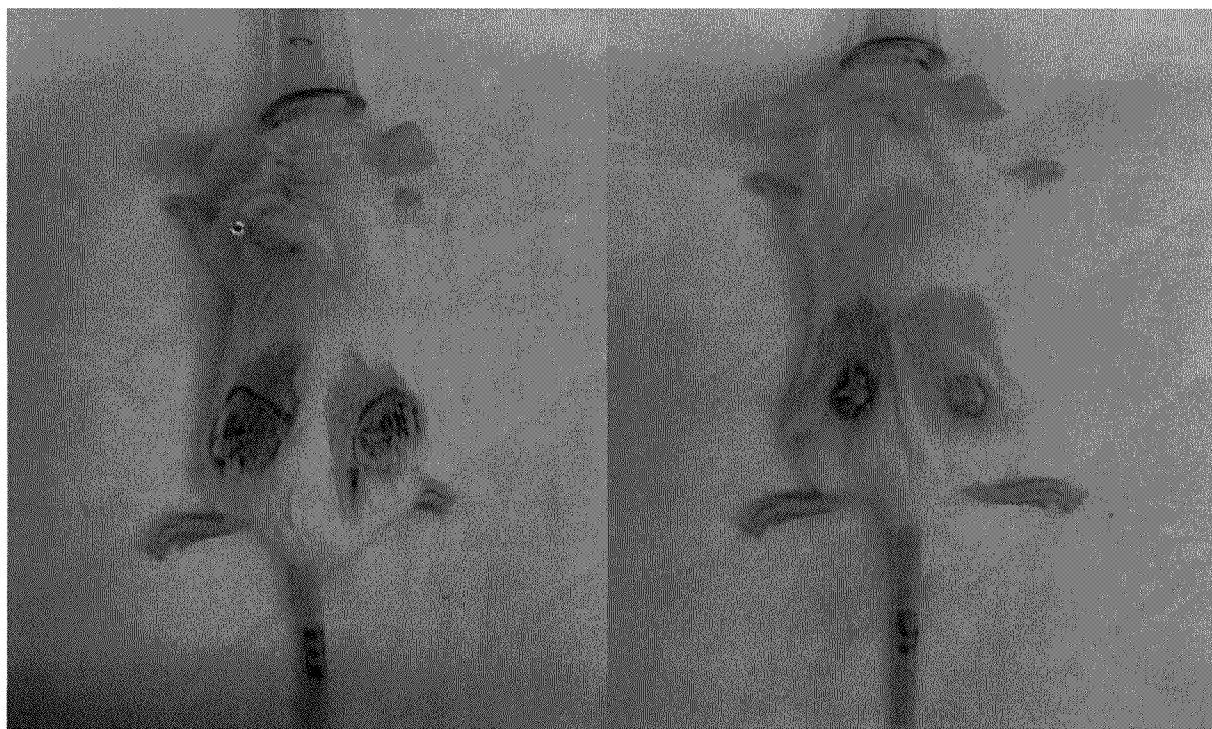
FIGURE 2

Comparative Efficacy Kinetics of NDV-3 Assessed by *in Vivo* Imaging

**FIGURE 3****NDV-3 Restricts MRSA Abscess Volume in Murine SSSI**

**FIGURE 4**

**NDV-3 Restricts MRSA Abscess Volume in Murine SSSI**



**Control**

**100 µg**

**Mean Abscess Volume (cm<sup>3</sup>)**

FIGURE 5

## NDV-3 Suppresses MRSA Proliferation in Murine SSSI

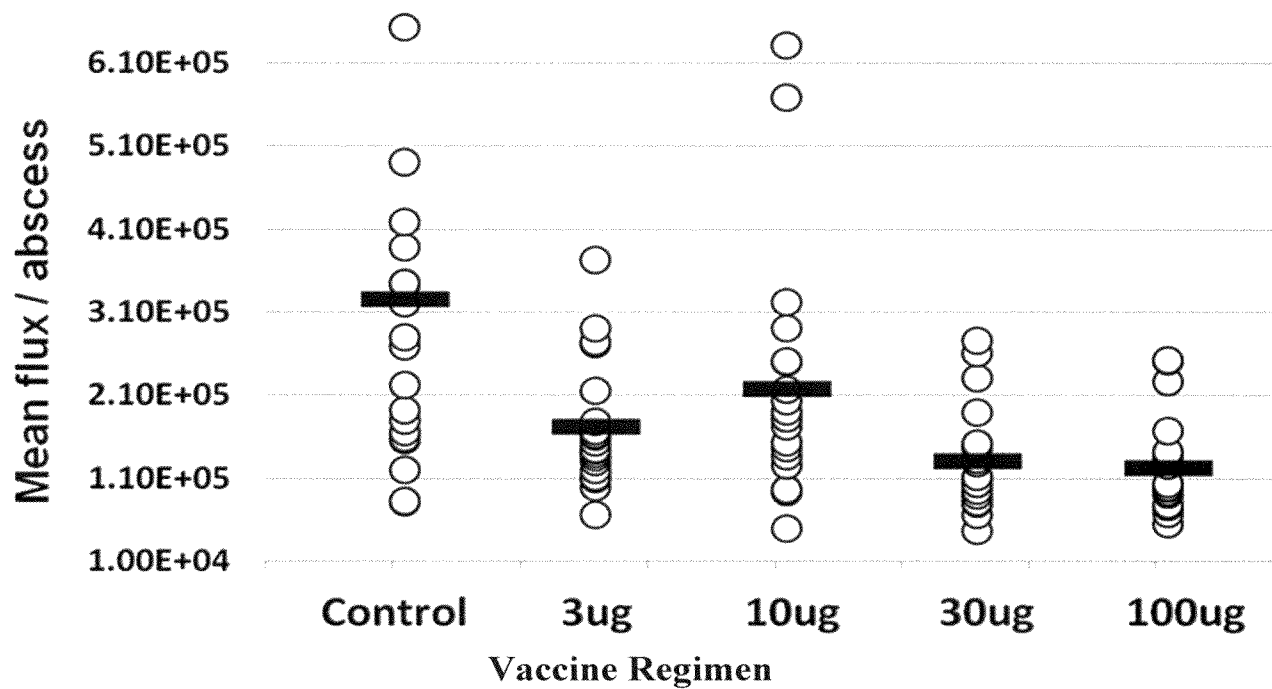
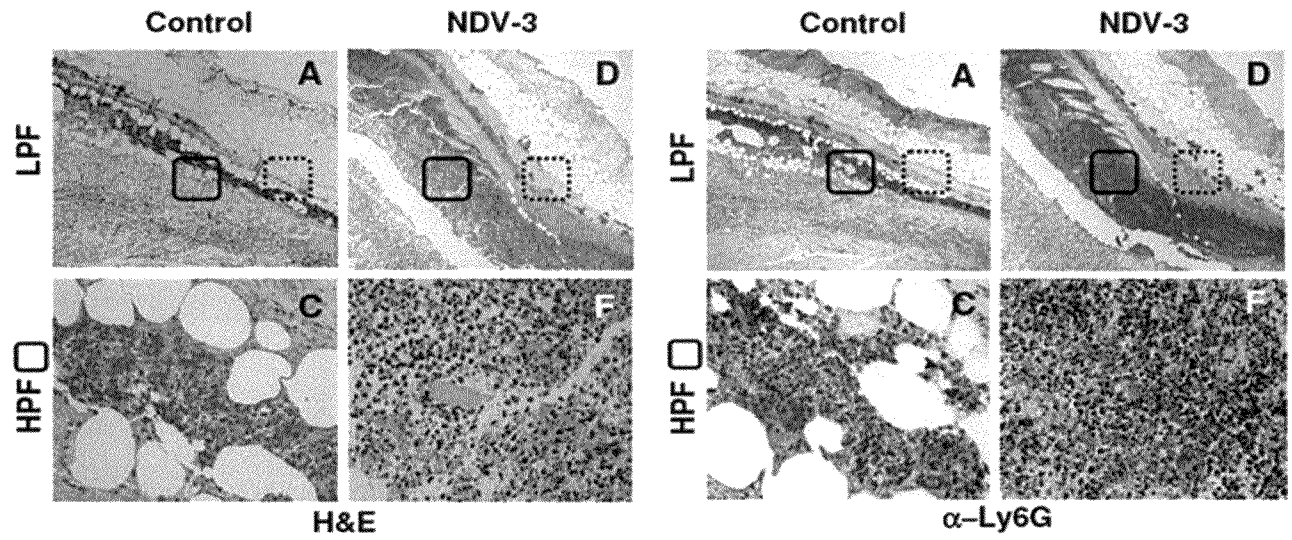
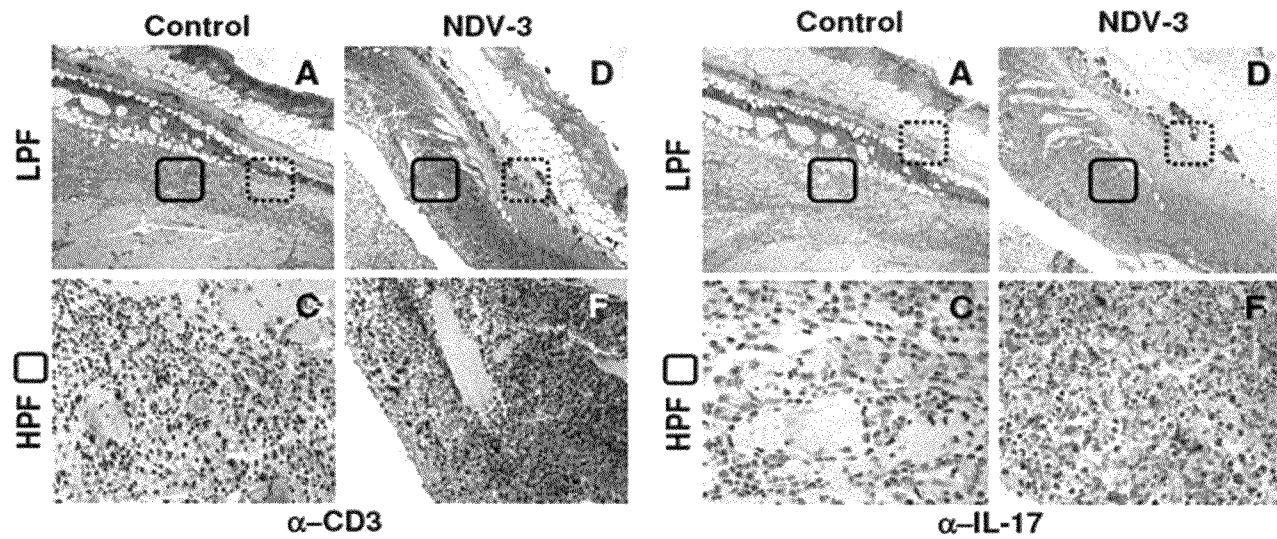


FIGURE 6

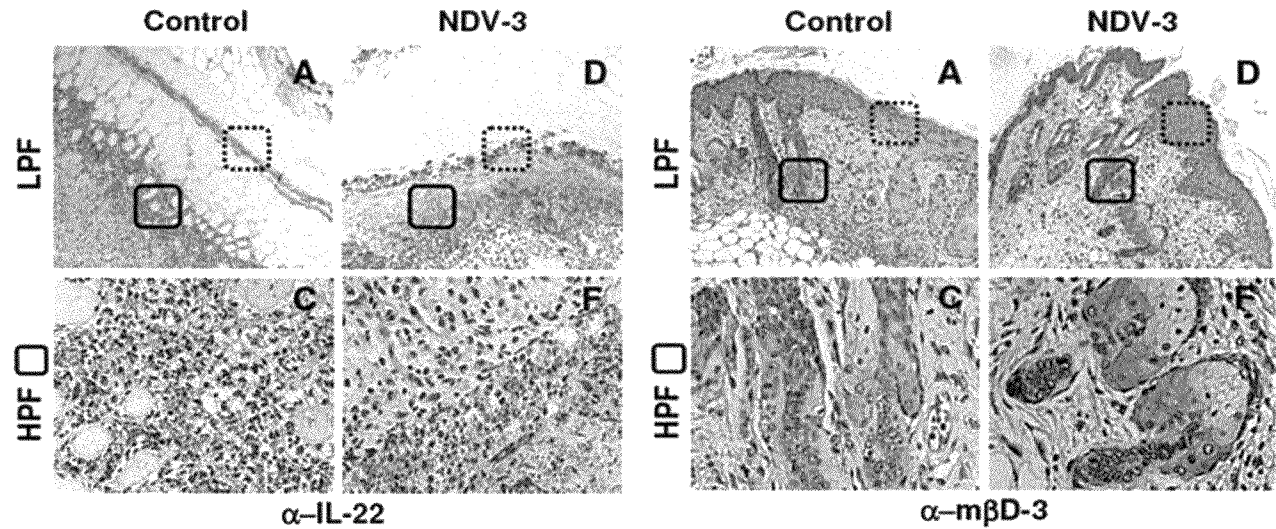
**NDV-3 Limits MRSA Proliferation and Recruits Neutrophils**Data from 100  $\mu$ g dose @ 7d post-infection

**FIGURE 7****NDV-3 Recruits CD3+ T Cells and Induces IL-17 Expression**

Data from 100  $\mu$ g dose @ 7d post-infection

FIGURE 8

NDV-3 Stimulates IL-22 Expression and  $\beta$ -Defensin Response



Data from 100  $\mu$ g dose @ 7d post-infection



FIGURE 9

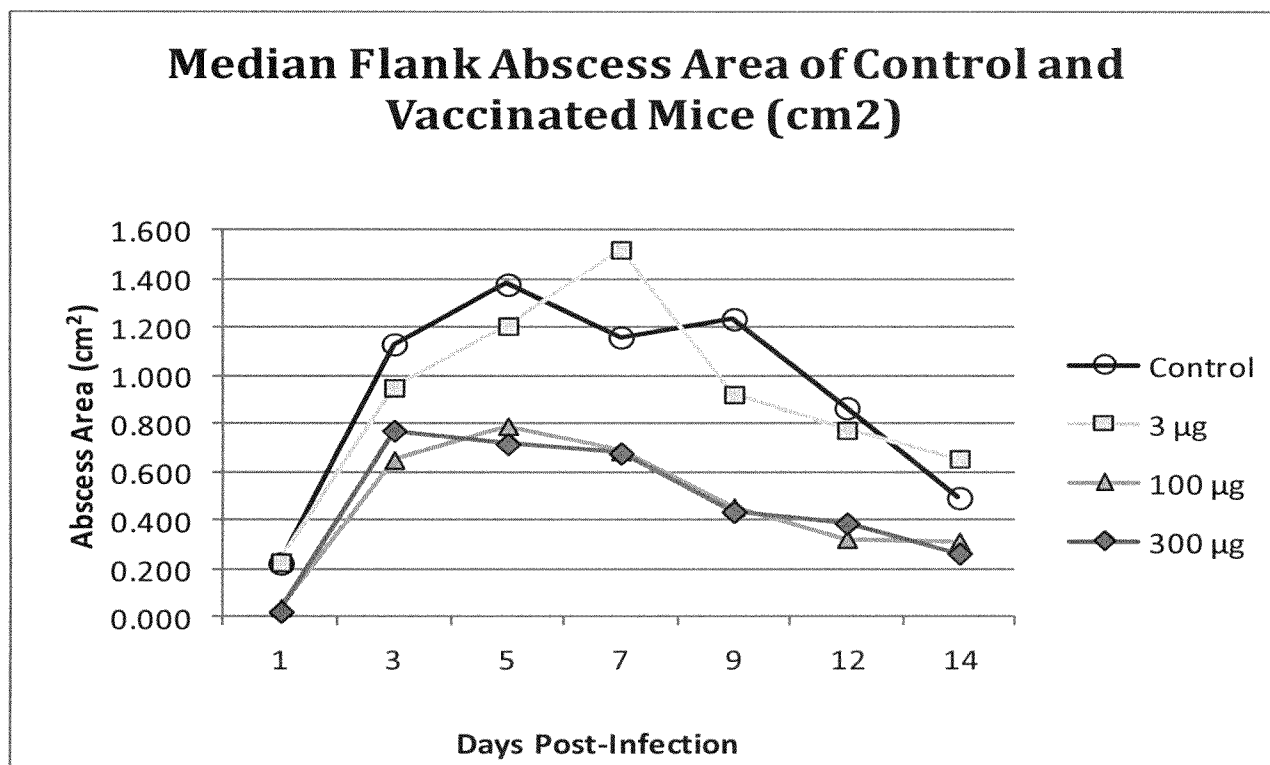


FIGURE 10

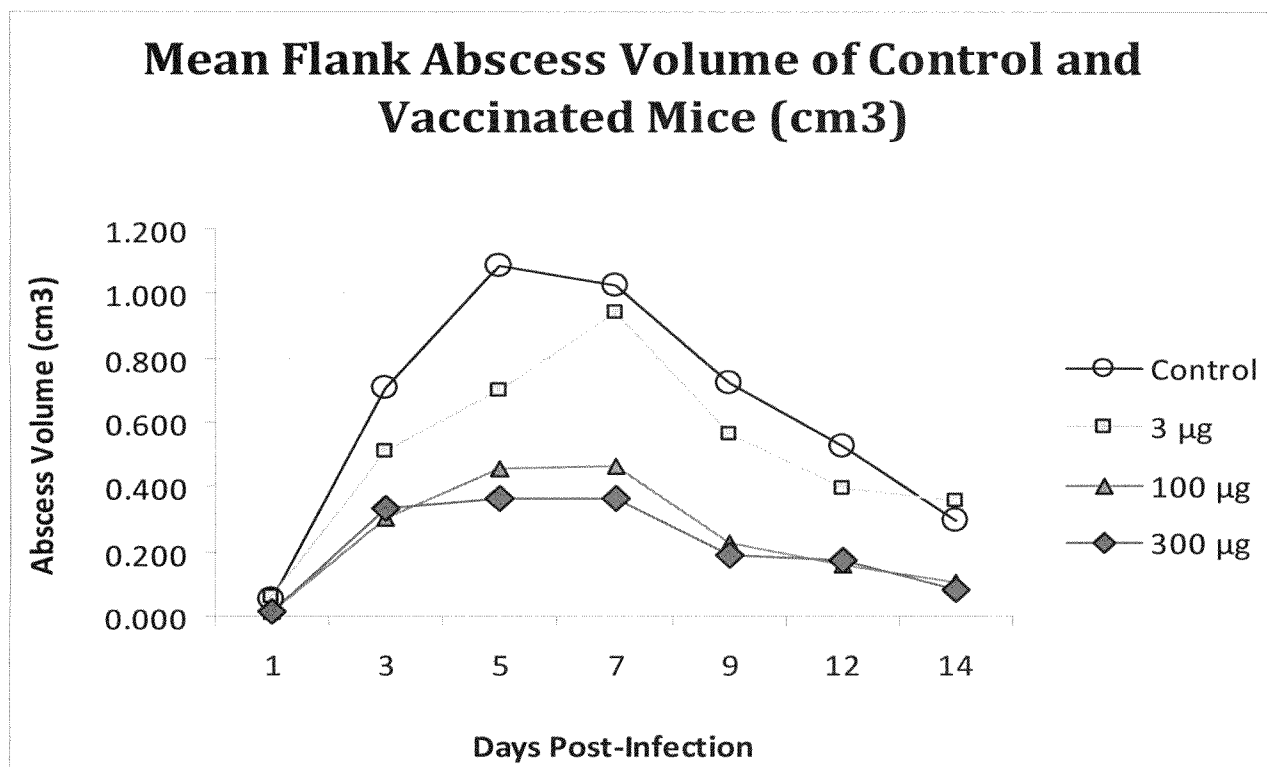
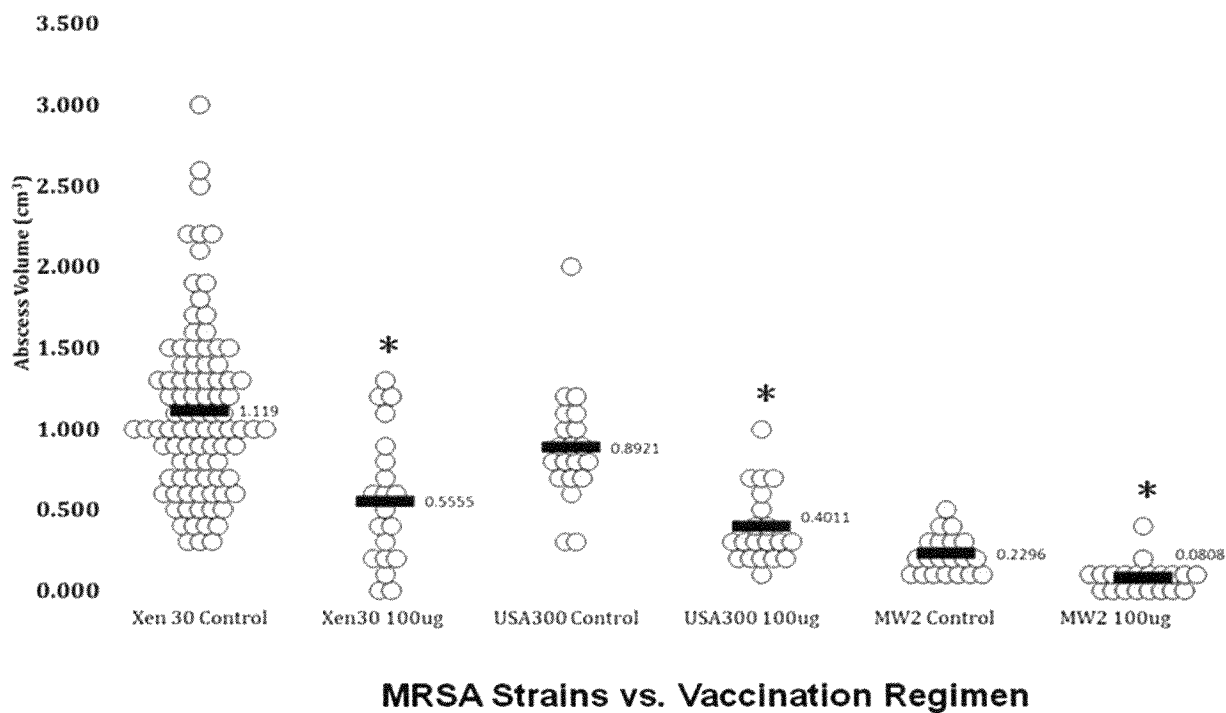


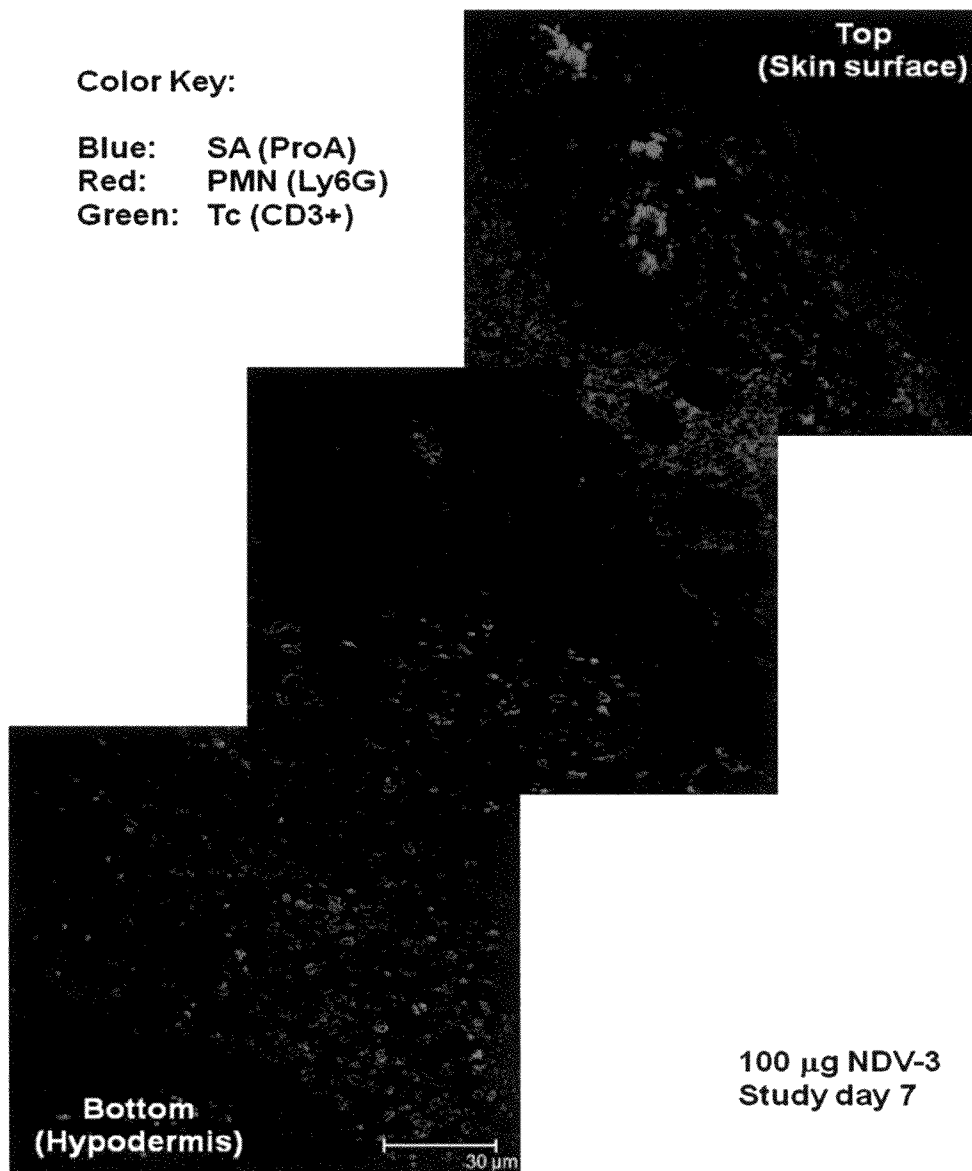
FIGURE 11

## Mean 7d Abscess Volume due to MRSA Strains in Control vs. Vaccinated Mice (cm<sup>3</sup>)



**FIGURE 12****MRSA Abscess from NDV-3 (100 µg) Vaccinated Mouse****Color Key:**

Blue: SA (ProA)  
Red: PMN (Ly6G)  
Green: Tc (CD3+)



# FIGURE 13

## MRSA Abscess from Control Mouse

