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SUMMARY

The present disclosure is directed to individual peptide immunogen constructs targeting portions of the Interleukin-31 (IL-31) protein for the treatment and/or prevention of a pruritic
15 condition and/or an allergic condition, such as atopic dermatitis. The present disclosure is also directed to compositions containing the peptide immunogen constructs, methods of making and using the peptide immunogen constructs, and antibodies produced by the peptide immunogen constructs.

In one aspect, the present disclosure refers to an IL-31 peptide immunogen construct can
20 be represented by the formulae: (Th)_m-(A)_n-(IL-31 fragment)-X or (IL-31 fragment)-(A)_n-(Th)_m-X wherein Th is a heterologous T helper epitope; A is a heterologous spacer; (IL-31 fragment) is a B cell epitope having about 15 to about 75 amino acid residues from SEQ ID NO: 1 or SEQ ID NO: 2; X is an α-COOH or α-CONH₂ of an amino acid; m is from 1 to about 4; and n is from 0 to about 10.

25 In another aspect, the present disclosure refers to an IL-31 peptide immunogen construct comprising: a B cell epitope comprising about 15 to about 75 amino acid residues from the full-length IL-31 protein sequence of SEQ ID NO: 1 or SEQ ID NO: 2; a T helper epitope comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 14-42; and an optional heterologous spacer selected from the group consisting of an amino acid, Lys-,
30 Gly-, Lys-Lys-Lys-, (α, ε-N)Lys, and ε-N-Lys-Lys-Lys-Lys (SEQ ID NO: 91), wherein the B cell epitope is covalently linked to the T helper epitope directly or through the optional heterologous spacer.

In yet another aspect, the present disclosure refers to a composition comprising a peptide immunogen construction as disclosed herein.

35 In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising: a. a peptide immunogen construct as disclosed herein; and b. and a pharmaceutically acceptable delivery vehicle and/or adjuvant.

In yet another aspect, the present disclosure refers to an isolated antibody or epitope-binding fragment thereof that specifically binds to the B cell epitope of the IL-31 peptide

5 immunogen construct as disclosed herein.

In yet another aspect, the present disclosure refers to an isolated antibody or epitope-binding fragment thereof that specifically binds to the B cell epitope of the IL-31 peptide immunogen construct as disclosed herein.

10 In yet another aspect, the present disclosure refers to a composition comprising the isolated antibody or epitope-binding fragment thereof as disclosed herein.

The disclosed peptide immunogen constructs contain about 25 or more amino acids. The peptide immunogen constructs contain a B cell epitope from portions of the canine IL-31 protein (GenBank: BAH97742.1). The full-length amino acid sequence of the canine and human IL-31 protein is shown as SEQ ID NO: 1 and SEQ ID NO: 2, respectively, in **Table 1**. The B cell
15 epitope can be linked to a heterologous T helper cell (Th) epitope derived from pathogen proteins through an optional heterologous spacer. The disclosed peptide immunogen constructs stimulate the generation of highly specific antibodies directed against IL-31. The disclosed peptide immunogen constructs can be used as an immunotherapy for animals suffering from a pruritic condition and/or an allergic condition, such as atopic dermatitis.

20 The B cell epitope portion of the peptide immunogen constructs have amino acid sequences from the full-length canine IL-31 protein (SEQ ID NO: 1) or the full-length human IL-31 protein (SEQ ID NO: 2). In some examples, the B cell epitope has a sequence containing any of SEQ ID NOs: 1 to 13 and 93 to 98, as shown in **Table 1**.

The peptide immunogen constructs of the present disclosure can contain a heterologous
25 Th epitope amino acid sequence derived from a pathogenic protein (e.g., SEQ ID NOs: 14 to 42) as shown in **Table 2**. In certain examples, the heterologous Th epitope is derived from natural pathogens, such as Diphtheria Toxin (SEQ ID NO: 18), Plasmodium Falciparum (SEQ ID NO: 19), Cholera Toxin (SEQ ID NO: 21). In other examples, the heterologous Th epitope is an idealized artificial Th epitope derived from Measles Virus Fusion protein (MVF 1 to 5) or
30 Hepatitis B Surface Antigen (HBsAg 1 to 3) in the form of either single sequence or combinatorial sequences (e.g., SEQ ID NOs: 25, 24, and 26).

5 In some examples, the peptide immunogen constructs contain a B cell epitope from IL-31 linked to a heterologous T helper cell (Th) epitope through an optional heterologous spacer. In certain examples, the peptide immunogen constructs contain a B cell antigenic site having the amino acid sequence from IL-31 (e.g., SEQ ID NOs: 1 to 13 and 93 to 98) linked to a heterologous Th epitope derived from a pathogenic protein (e.g., SEQ ID NOs: 14 to 42) through an optional
10 heterologous spacer. In some examples, the optional heterologous spacer is a molecule or chemical structure capable of linking two amino acids and/or peptides together. In certain examples, the spacer is a naturally occurring amino acid, a non-naturally occurring amino acid, or a combination thereof. In specific examples, the peptide immunogen constructs have the amino acid sequence of SEQ ID NOs: 43 to 90 and 99 to 105 shown in **Table 3**.

15 The present disclosure is also directed to compositions containing an IL-31 peptide immunogen construct. In some examples, the disclosed compositions contain more than one IL-31 peptide immunogen constructs. In certain examples, the compositions contain a mixture of IL-31 peptide immunogen constructs (e.g., any combination of SEQ ID NOs: 43-90 and 99 to 105) to cover a broad genetic background in subjects. Compositions containing a mixture of peptide
20 immunogen constructs can lead to a higher percentage in responder rate upon immunization for the treatment of a pruritic condition and/or an allergic condition, such as atopic dermatitis, compared to compositions containing only a single peptide immunogen construct.

The present disclosure is also directed to pharmaceutical compositions for the treatment and/or prevention of a pruritic condition and/or an allergic condition, such as atopic dermatitis. In
25 some examples, the pharmaceutical compositions contain the disclosed peptide immunogen constructs in the form of a stabilized immunostimulatory complex formed through electrostatic associations by mixing a CpG oligomer with a composition containing a peptide immunogen complex. Such stabilized immunostimulatory complexes are able to further enhance the immunogenicity of the peptide immunogen constructs. In some examples, the pharmaceutical
30 compositions contain adjuvants such as mineral salts, including alum gel (ALHYDROGEL), aluminum phosphate (ADJUPHOS), or water-in-oil emulsions including MONTANIDE ISA 50V2, ISA 51, or ISA 720.

The present disclosure is also directed to antibodies directed against the disclosed IL-31 peptide immunogen constructs. In particular, the peptide immunogen constructs of the present
35 disclosure are able to stimulate the generation of highly specific antibodies that are cross-reactive with the IL-31 amino acid sequences (SEQ ID NOs: 1-13) when administered to a subject. The highly specific antibodies produced by the peptide immunogen constructs are cross reactive with recombinant IL-31-containing proteins. The disclosed antibodies bind with high specificity to IL-31 without much, if any, directed to the heterologous Th epitopes employed for immunogenicity

enhancement, which is in sharp contrast to the conventional protein or other biological carriers used for such peptide antigenicity enhancement.

The present disclosure also includes methods for treating and/or preventing a pruritic condition and/or an allergic condition, such as atopic dermatitis, using the disclosed peptide immunogen constructs and/or antibodies directed against the peptide immunogen constructs. In some examples, the methods for treating and/or preventing a pruritic condition and/or an allergic condition, such as atopic dermatitis, include administering to a host a composition containing a disclosed peptide immunogen construct. In certain examples, the compositions utilized in the methods contain a disclosed peptide immunogen construct in the form of a stable immunostimulatory complex with negatively charged oligonucleotides, such as CpG oligomers, through electrostatic association, which complexes are further supplemented, optionally, with mineral salts or oil as adjuvant, for administration to subjects with a pruritic condition and/or an allergic condition, such as atopic dermatitis. The disclosed methods also include dosing regimens, dosage forms, and routes for administering the peptide immunogen constructs to a host at risk for, or with, a pruritic condition and/or an allergic condition, such as atopic dermatitis.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A to 1B – Amino acid sequence of canine IL-31 protein (SEQ ID NO: 1) with structural and functional features highlighted (**Figure 1A**) and sequence alignment and localization of antigenic sites used in the present disclosure relative to the full-length canine IL-31 protein (**Figure 1B**).

Figure 2 – Graphs illustrating the anti-IL-31 polyclonal antibody titers (Log_{10}) with recombinant canine IL-31 protein after immunization of guinea pigs with various formulations containing the IL-31 peptide immunogens in adjuvant ISA50V2. Samples were analyzed at 3, 6, 9, 12, and 15 weeks post injection (wpi).

Figures 3A to 3D – Plasmid map of canine IL-31 with a His-tag at the C-terminus for expression in mammalian cells (Expi 293F) (**Figure 3A**) and a Western blot probed with an anti-His-tag antibody from cell lysate and culture medium (**Figure 3B**). Coomassie blue staining of His-tagged IL-31 protein expression and purification run on a 12% Bis Tris SDS PAGE (**Figure 3C**). Western blot of the SDS-PAGE gel shown in Fig. 3C, probed with an anti-His-tag antibody (**Figure 3D**).

Figures 4A to 4B – schematic illustrating an *in vitro* IL-31 induced signal transduction functional inhibition assay using IL-31 antibodies (**Figure 4A**). An enlarged view of the assay steps is shown