

**PEPTIDE IMMUNOGENS TARGETING CALCITONIN GENE-RELATED PEPTIDE
(CGRP) AND FORMULATIONS THEREOF FOR PREVENTION AND TREATMENT OF
MIGRAINE**

5 The present application is a PCT International Application that claims the benefit of U.S. Provisional Application Serial No. 62/787,102, filed December 31, 2018, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

10 This disclosure relates to peptide immunogen constructs targeting Calcitonin Gene-Related Peptide (CGRP) and formulations thereof for prevention and treatment of migraine.

BACKGROUND OF THE INVENTION

15 Migraine is a common medical condition that affects as many as 37 million people in the US. It is considered a systemic illness, not just a headache. Recent research has demonstrated that changes may begin to occur in the brain as early as 24 hours before migraine symptoms begin. Migraine symptoms vary depending on the affected individual, but can include a severe throbbing headache, often on only one side of the head, nausea, vomiting, light sensitivity (photophobic), sound sensitivity (phonophobic), or combinations of these symptoms. These symptoms can
20 persist even after the headache pain goes away.

 There are a variety of migraine subtypes with symptoms that include weakness, numbness, visual changes or loss, vertigo, and difficulty speaking (some patients may appear as if they are having a stroke). The disability resulting from this chronic condition is tremendous, causing missed days of work and loss of ability to join family activities.

25 It is sometimes possible for people to use an “abortive” medication, which, when taken early, can arrest the migraine process. For many patients, a preventive medication can decrease both the frequency and the severity of the migraines. However, many of the medications that are used to prevent or treat migraines were developed primarily for other conditions: seizures, depression, high blood pressure, and muscle spasms, for example.

30 Researchers have been working for decades to develop a “targeted” preventive therapy specifically for migraine. Calcitonin gene-related peptide (CGRP) is a molecule that is synthesized in both peripheral and central neurons. It has been implicated in different pain processes, including migraine, and functions as a vasodilator. Abortive treatments for migraines focus on stopping CGRP from being activated at the start of a migraine. Small-molecule CGRP antagonist drugs
35 have been shown to decrease migraine pain based on certain measures, but these antagonists can

have serious side effects including liver toxicity.

Monoclonal antibodies that target the CGRP molecule have an inhibitory effect on the pain process and can be used as an abortive treatment. Monoclonal antibodies against CGRP can have a long half-life, which means that they can be administered less frequently than typical migraine medications that are taken daily (with the exception of botulinum toxin, which is injected every 90 days). Monoclonal antibodies for migraines can be injected under the skin monthly, and have thus far demonstrated a statistically significant decrease in days of migraine. Several different drug companies are developing these new antibodies for FDA approval.

Although such monoclonal anti-CGRP or anti-CGRP receptor antibodies may prove efficacious in immunotherapy of migraine, they are expensive and must be administered monthly to maintain sufficient suppression of serum and body fluid CGRP levels and the clinical benefits derived therefrom. Cost effective immunotherapeutic treatment targeting CGRP molecule through vaccination approach that is safe and well tolerated remains an exciting new intervention and development for migraine therapies.

There are a number of disadvantages and deficiencies associated with the classical peptide/hapten-carrier protein immunogen preparation method. For example, the preparation methods can involve complicated chemical coupling procedures, they use expensive pharmaceutical grade KLH or toxoid protein as the T helper cell carrier, most of the antibodies elicited by the protein immunogens are directed against the carrier protein and not the target B cell epitope(s), etc.

In view of the economic and practical disadvantages and limitations with monoclonal therapy and classical peptide/hapten-carrier protein preparations, there is clearly an unmet need to develop an efficacious immunotherapeutic composition capable of eliciting highly specific immune responses against the functional site(s) on CGRP, that can be easily administered to patients, that is able to be manufactured under stringent good manufacturing practices (GMP), and that is cost effective for worldwide application to treat patients suffering from migraine.

Two review articles that cite to additional supporting documents can be found for statements made in the above background section are hereby incorporated by reference in their entireties. The first article contains an updated review on CGRP and CGRP receptors (website: en.wikipedia.org/wiki/Calcitonin_gene-related_peptide), and the second article addresses the biology of CGRP signaling, key clinical evidence for the role of CGRP in migraine headache including the efficacy of CGRP-targeted treatment, the role of CGRP in the trigemino vascular system, and new insight into the central role of the trigeminal ganglion in the pathophysiology of migraine (Edvinsson, et al., 2018).

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SUMMARY OF THE INVENTION

The present disclosure is directed to portions of the Calcitonin Gene-Related Peptide (CGRP) that can be used as B cell epitopes. The present disclosure is also directed to peptide immunogen constructs containing B cell epitopes from CGRP, compositions containing the peptide immunogen constructs, methods of making and using the peptide immunogen constructs, and antibodies produced by the peptide immunogen constructs.

One aspect of the present disclosure is directed to portions of CGRP from different organisms as B cell epitopes in peptide immunogen constructs, as well as formulations thereof, that can be used for the prevention and/or treatment of migraine. The disclosed CGRP peptide immunogen constructs (SEQ ID NOs: 116-127 and 130-180) have 30 or more total amino acids and contain a functional B cell epitope peptide having about 7 to about 30 amino acids (SEQ ID NOs: 4-13, 15-19, and 20-24 of **Table 1**) derived from CGRP from human, marmoset, or rat/mouse (i.e., SEQ ID NOs: 1-3, respectively). The functional B cell epitope peptide can be linked through an optional heterologous spacer to a heterologous T helper cell (Th) epitope peptide derived from pathogen proteins (e.g., SEQ ID NOs: 74-115), to form the disclosed peptide immunogen construct.

The disclosed CGRP peptide immunogen constructs can contain a CGRP B cell epitope peptide having about 7 to about 30 amino acids. The B cell epitope peptide can be derived from the CGRP receptor binding region (e.g., SEQ ID NOs: 5-9 and 15-22, shown in **Table 1**) located at the C-terminal and central regions of the CGRP molecule. The B cell epitope peptide can also
5 be derived from the CGRP receptor activation site around a cyclic C2-C7 loop (e.g. SEQ ID NOs: 4, 10-13 and 23-24, shown in **Table 1**) located at the N-terminal and central regions of the CGRP molecule. The designed CGRP B cell epitope peptide can be linked to a heterologous Th epitope derived from a pathogenic protein (e.g., SEQ ID NOs: 74-115 of **Table 2**) at either the N- or C-terminus of the CGRP peptide. The B cell and Th epitopes act together to stimulate the generation
10 of highly specific antibodies cross-reactive with full-length CGRP (SEQ ID NO:1-3) of various species.

In certain embodiments, the heterologous Th epitopes employed to enhance the CGRP B cell epitope peptide are derived from natural pathogens EBV BPLF1 (SEQ ID NO: 112), EBV CP (SEQ ID NO: 109), Clostridium Tetani (SEQ ID NOs: 74, 77, 104, 106-108), Cholera Toxin (SEQ
15 ID NO: 81), and Schistosoma mansoni (SEQ ID NO: 80), as well as those idealized artificial Th epitopes derived from Measles Virus Fusion protein (MVF 1 to 5) and Hepatitis B Surface Antigen (HBsAg 1 to 3) in the form of either single sequence or combinatorial sequences (e.g. SEQ ID NOs: 75, 82-99).

The disclosed CGRP peptide immunogen constructs, containing both designed B cell- and
20 Th epitope peptides, act together to stimulate the generation of highly specific antibodies directed against CGRP functional sites, including the CGRP receptor binding region located at the C-terminal of the CGRP molecule or the cyclic C2-C7 loop involved in receptor activation, offer therapeutic immune responses to patients predisposed to, or suffering from, migraine.

Another aspect of the present disclosure is directed to peptide compositions containing a
25 CGRP peptide immunogen construct. In some embodiments, the compositions contain one peptide immunogen construct. In other embodiments, peptide compositions comprising a mixture of CGRP peptide immunogen constructs. In certain embodiments, the mixture of CGRP peptide immunogen constructs have heterologous Th epitopes derived from different pathogens that can be used to allow coverage of as broad a genetic background in patients leading to a higher
30 percentage in responder rate upon immunization for the prevention and/or treatment of migraine.

Synergistic enhancement in CGRP immunogen constructs can be observed in the peptide compositions of this disclosure. The antibody response derived from the administration of such compositions containing CGRP peptide immunogen constructs was mostly (>90%) focused on the desired cross-reactivity against the CGRP functional site(s) or receptor binding region peptides
35 (SEQ ID NOs: 4-13 and 15-24) without much, if any, directed to the heterologous Th epitopes

employed for immunogenicity enhancement. This is in sharp contrast to standard methods that use a conventional carrier protein, such as KLH, toxoid, or other biological carriers used for such peptide antigenicity enhancement

5 The present disclosure is also directed to pharmaceutical compositions and formulations for the prevention and/or treatment of migraine. In some embodiments, pharmaceutical compositions comprising a stabilized immunostimulatory complex, which is formed by mixing a CpG oligomer with a peptide composition containing a mixture of CGRP peptide immunogen constructs through electrostatic association, to further enhance the CGRP peptide immunogenicity towards the desired cross-reactivity with the full-length CGRP (e.g., SEQ ID NOs:1-3).

10 In other embodiments, pharmaceutical compositions comprising a peptide composition of a mixture of CGRP peptide immunogen constructs in contact with mineral salts including Alum gel (ALHYDROGEL) or Aluminum phosphate (ADJU-PHOS) to form a suspension formulation, or with MONTANIDE™ ISA 51 or 720 as adjuvant to form water-in-oil emulsions, that can be used for the prevention and/or treatment of migraine.

15 Furthermore, the present disclosure also provides a method for the low cost manufacture and quality control of CGRP peptide immunogen constructs and formulations thereof, capable of preventing and/or treating migraine in animals.

The present disclosure is also directed to antibodies directed against the disclosed CGRP peptide immunogen constructs. In particular, the CGRP peptide immunogen constructs of the
20 present disclosure are able to stimulate the generation of highly specific functional antibodies that are cross-reactive with the full-length CGRP molecule. The disclosed antibodies bind with high specificity to CGRP without much, if any, directed to the heterologous Th epitopes employed for immunogenicity enhancement, which is in sharp contrast to antibodies produced using conventional proteins or other biological carriers used for such peptide immunogenicity
25 enhancement. Thus, the disclosed CGRP peptide immunogen constructs are capable of breaking the immune tolerance against self-CGRP, with a high responder rate, compared to other peptide or protein immunogens.

In some embodiments, the disclosed antibodies are directed against and specifically bind to the CGRP receptor binding sites on the C-terminal portion of the CGRP molecule (e.g., SEQ
30 ID NOs: 5-9 and 15-22) when the peptide immunogen constructs are administered to a subject. The highly specific antibodies elicited by these CGRP peptide immunogen constructs can inhibit CGRP and CGRP receptor binding and the downstream activation event in the rise of cellular cAMP caused by the region around the cyclic C2-C7 loop of the CGRP, leading to effective prevention and/or treatment of migraine.

35 In other embodiments, the disclosed antibodies are directed against either the N-terminal

or central regions of CGRP around the cyclic C2-C7 loop responsible for the downstream cell activation event or against the C-terminal and central regions of the CGRP receptor binding site, when the peptide immunogen constructs of current invention are administered to a subject (e.g., SEQ ID NOs: 4, 10-13, and 23-24). The highly specific antibodies elicited by the CGRP peptide immunogen constructs can inhibit (1) CGRP and CGRP receptor binding and (2) the downstream activation event caused by the region around the cyclic C2-C7 loop of the CGRP, resulting in the suppression of the rise of cellular cAMP, thus leading to effective treatment of patients suffering from migraine.

Based on their unique characteristics and properties, the disclosed antibodies elicited by the CGRP peptide immunogen constructs are capable of providing a prophylactic immunotherapeutic approach to treating patients suffering from migraine.

In a further aspect, the present invention provides human monoclonal antibodies against CGRP induced by patients receiving compositions containing CGRP peptide immunogen constructs of this disclosure. An efficient method to make human monoclonal antibodies from B cells isolated from the blood of a human patient is described by Traggiai, E., et al, 2004, which is incorporated by reference.

The present disclosure is also directed to methods of making the disclosed CGRP peptide immunogen constructs, compositions, and antibodies. The disclosed methods provide for the low cost manufacture and quality control of CGRP peptide immunogen constructs and compositions containing the constructs, which can be used in methods for treating patients suffering from migraine.

The present disclosure also includes methods for preventing and/or treating subjects predisposed to, or suffering from, migraine using the disclosed CGRP peptide immunogen constructs and/or antibodies directed against the CGRP peptide immunogen constructs. The methods for preventing and/or treating migraine in a subject include administering to the subject a composition containing a disclosed CGRP peptide immunogen construct. In certain embodiments, the compositions utilized in the methods contain a disclosed CGRP peptide immunogen construct in the form of a stable immunostimulatory complex with negatively charged oligonucleotides, such as CpG oligomers, through electrostatic association, which can be further supplemented with an adjuvant, for administration to patients suffering from migraine.

The disclosed methods also include dosing regimens, dosage forms, and routes for administering the CGRP peptide immunogen constructs to prevent and/or treat migraine in a subject.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 presents alignment of CGRP sequences from multispecies including human (SEQ ID NO: 1), marmoset (SEQ ID NO:2), mouse (SEQ ID NO: 3), rat (SEQ ID NO: 3), and many others including horse, chicken, pig, sheep, cow, dog, opossum, Gecko, Frog, Pufferfish, Flounder, Goldfish, Salmon, Medalea, Zebrafish. This Figure is adapted from Watkins, et al., 2013.

Figure 2 depicts pathways from discovery to commercialization of high precision CGRP designer peptide immunogen constructs and formulations thereof for the treatment of migraine.

Figure 3 depicts results from immunogenicity studies of CGRP peptide immunogen constructs (SEQ ID NOs: 116-118, 124-127, 128, 129, 131, 133, 135, 137, 119-122, 139, 123, 130) in guinea pigs with CGRP B cell epitope peptides derived from the N-terminal, Central, and C-terminal regions of the CGRP molecule.

Figure 4 depicts neutralization activities, expressed as IC_{50} of intracellular cAMP production in CGRP activated cell culture in the presence of purified antibodies from guinea pig immune sera (collected from 6 wpi bleeds) of representative CGRP peptide immunogen constructs (SEQ ID NOs: 116-118, 124-127, 128, 129, 131, 133, 135, 137, 119-123, 130, 139, and 150).

Figure 5 illustrates the results from immunogenicity studies in Balb/C mice of representative CGRP peptide immunogen constructs (SEQ ID NOs: 123, 131, 141, 151) in both ADJUPHOS and ISA51 formulations with corresponding antibodies purified from guinea pig immune sera of 7 wpi, 10 wpi and 13 wpi bleeds, for their reactivities and cross-reactivities with full-length human CGRP molecule.

Figure 6 illustrates the inhibitory effect of Capsaicin-induced dermal blood flow measured in Balb/C mice that had been vaccinated at 0, 3 and 6 wpi with CGRP peptide immunogen constructs (SEQ ID NOs: 123, 131, 141) formulated in either ADJUPHOS or ISA emulsion formulations as indicated, when compared to the control articles of adjuvant alone or saline.

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure is directed to portions of the Calcitonin Gene-Related Peptide (CGRP) that can be used as B cell epitopes. The present disclosure is also directed to peptide immunogen constructs containing B cell epitopes from CGRP, compositions containing the peptide immunogen constructs, methods of making and using the peptide immunogen constructs, and antibodies produced by the peptide immunogen constructs.

One aspect of the present disclosure is directed to portions of CGRP from different organisms as B cell epitopes in peptide immunogen constructs, as well as formulations thereof,

that can be used for the prevention and/or treatment of migraine. The disclosed CGRP peptide immunogen constructs (SEQ ID NOs: 116-127 and 130-180) have 30 or more total amino acids and contain a functional B cell epitope peptide having about 7 to about 30 amino acids (SEQ ID NOs: 4-13, 15-19, and 20-24 of **Table 1**) derived from CGRP from human, marmoset, or rat/mouse (i.e., SEQ ID NOs: 1-3, respectively). The functional B cell epitope peptide can be linked through an optional heterologous spacer to a heterologous T helper cell (Th) epitope peptide derived from pathogen proteins (e.g., SEQ ID NOs: 74-115), to form the disclosed peptide immunogen construct.

The disclosed CGRP peptide immunogen constructs can contain a CGRP B cell epitope peptide having about 7 to about 30 amino acids. The B cell epitope peptide can be derived from the CGRP receptor binding region (e.g., SEQ ID NOs: 5-9 and 15-22, shown in **Table 1**) located at the C-terminal and central regions of the CGRP molecule. The B cell epitope peptide can also be derived from the CGRP receptor activation site around a cyclic C2-C7 loop (e.g. SEQ ID NOs: 4, 10-13, and 20-22, shown in **Table 1**) located at the N-terminal and central regions of the CGRP molecule. The designed CGRP B cell epitope peptide can be linked to a heterologous Th epitope derived from a pathogenic protein (e.g., SEQ ID NOs: 74-115 of **Table 2**) at either the N- or C-terminus of the CGRP peptide. The B cell and Th epitopes act together to stimulate the generation of highly specific antibodies cross-reactive with full-length CGRP (SEQ ID NO:1-3) of various species.

In certain embodiments, the heterologous Th epitopes employed to enhance the CGRP B cell epitope peptide are derived from natural pathogens EBV BPLF1 (SEQ ID NO: 112), EBV CP (SEQ ID NO: 109), Clostridium Tetani (SEQ ID NOs: 74, 77, 104, 106-108), Cholera Toxin (SEQ ID NO: 81), and Schistosoma mansoni (SEQ ID NO: 80), as well as those idealized artificial Th epitopes derived from Measles Virus Fusion protein (MVF 1 to 5) and Hepatitis B Surface Antigen (HBsAg 1 to 3) in the form of either single sequence or combinatorial sequences (e.g. SEQ ID NOs: 75, 82-99).

The disclosed CGRP peptide immunogen constructs, containing both designed B cell- and Th epitope peptides, act together to stimulate the generation of highly specific antibodies directed against CGRP functional sites, including the CGRP receptor binding region located at the C-terminal of the CGRP molecule or the cyclic C2-C7 loop involved in receptor activation, offer therapeutic immune responses to patients predisposed to, or suffering from, migraine.

Another aspect of the present disclosure is directed to peptide compositions containing a CGRP peptide immunogen construct. In some embodiments, the compositions contain one peptide immunogen construct. In other embodiments, peptide compositions comprising a mixture of CGRP peptide immunogen constructs. In certain embodiments, the mixture of CGRP peptide

immunogen constructs have heterologous Th epitopes derived from different pathogens that can be used to allow coverage of as broad a genetic background in patients leading to a higher percentage in responder rate upon immunization for the prevention and/or treatment of migraine.

Synergistic enhancement in CGRP immunogen constructs can be observed in the peptide compositions of this disclosure. The antibody response derived from the administration of such compositions containing CGRP peptide immunogen constructs was mostly (>90%) focused on the desired cross-reactivity against the CGRP functional site(s) or receptor binding region peptides (SEQ ID NOs: 4-13, 15-19, and 20-24) without much, if any, directed to the heterologous Th epitopes employed for immunogenicity enhancement. This is in sharp contrast to standard methods that use a conventional carrier protein, such as KLH, toxoid, or other biological carriers used for such peptide antigenicity enhancement

The present disclosure is also directed to pharmaceutical compositions and formulations for the prevention and/or treatment of migraine. In some embodiments, pharmaceutical compositions comprising a stabilized immunostimulatory complex, which is formed by mixing a CpG oligomer with a peptide composition containing a mixture of CGRP peptide immunogen constructs through electrostatic association, to further enhance the CGRP peptide immunogenicity towards the desired cross-reactivity with the full-length CGRP (e.g., SEQ ID NOs: 1-3).

In other embodiments, pharmaceutical compositions comprising a peptide composition of a mixture of CGRP peptide immunogen constructs in contact with mineral salts including Alum gel (ALHYDROGEL) or Aluminum phosphate (ADJU-PHOS) to form a suspension formulation, or with MONTANIDE™ ISA 51 or 720 as adjuvant to form water-in-oil emulsions, that can be used for the prevention and/or treatment of migraine.

Furthermore, the present disclosure also provides a method for the low cost manufacture and quality control of CGRP peptide immunogen constructs and formulations thereof, capable of preventing and/or treating migraine in animals.

The present disclosure is also directed to antibodies directed against the disclosed CGRP peptide immunogen constructs. In particular, the CGRP peptide immunogen constructs of the present disclosure are able to stimulate the generation of highly specific functional antibodies that are cross-reactive with the full-length CGRP molecule. The disclosed antibodies bind with high specificity to CGRP without much, if any, directed to the heterologous Th epitopes employed for immunogenicity enhancement, which is in sharp contrast to antibodies produced using conventional proteins or other biological carriers used for such peptide immunogenicity enhancement. Thus, the disclosed CGRP peptide immunogen constructs are capable of breaking the immune tolerance against self-CGRP, with a high responder rate, compared to other peptide or protein immunogens.

In some embodiments, the disclosed antibodies are directed against and specifically bind to the CGRP receptor binding sites on the C-terminal portion of the CGRP molecule (e.g., SEQ ID NOs: 5-9 and 15-22) when the peptide immunogen constructs are administered to a subject. The highly specific antibodies elicited by these CGRP peptide immunogen constructs can inhibit
5 CGRP and CGRP receptor binding and the downstream activation event in the rise of cellular cAMP caused by the region around the cyclic C2-C7 loop of the CGRP, leading to effective prevention and/or treatment of migraine.

In other embodiments, the disclosed antibodies are directed against either the N-terminal or central regions of CGRP around the cyclic C2-C7 loop responsible for the downstream cell
10 activation event or against the C-terminal and central regions of the CGRP receptor binding site, when the peptide immunogen constructs of current invention are administered to a subject (e.g., SEQ ID NOs: 4, 10-13, and 23-24). The highly specific antibodies elicited by the CGRP peptide immunogen constructs can inhibit (1) CGRP and CGRP receptor binding and (2) the downstream activation event caused by the region around the cyclic C2-C7 loop of the CGRP, resulting in the
15 suppression of the rise of cellular cAMP, thus leading to effective treatment of patients suffering from migraine.

Based on their unique characteristics and properties, the disclosed antibodies elicited by the CGRP peptide immunogen constructs are capable of providing a prophylactic immunotherapeutic approach to treating patients suffering from migraine.

20 In a further aspect, the present invention provides human monoclonal antibodies against CGRP induced by patients receiving compositions containing CGRP peptide immunogen constructs of this disclosure. An efficient method to make human monoclonal antibodies from B cells isolated from the blood of a human patient is described by Traggiai, E., et al, 2004, which is incorporated by reference.

25 The present disclosure is also directed to methods of making the disclosed CGRP peptide immunogen constructs, compositions, and antibodies. The disclosed methods provide for the low cost manufacture and quality control of CGRP peptide immunogen constructs and compositions containing the constructs, which can be used in methods for treating patients suffering from migraine.

30 The present disclosure also includes methods for preventing and/or treating subjects predisposed to, or suffering from, migraine using the disclosed CGRP peptide immunogen constructs and/or antibodies directed against the CGRP peptide immunogen constructs. The methods for preventing and/or treating migraine in a subject include administering to the subject a composition containing a disclosed CGRP peptide immunogen construct. In certain
35 embodiments, the compositions utilized in the methods contain a disclosed CGRP peptide

immunogen construct in the form of a stable immunostimulatory complex with negatively charged oligonucleotides, such as CpG oligomers, through electrostatic association, which can be further supplemented with an adjuvant, for administration to patients suffering from migraine.

5 The disclosed methods also include dosing regimens, dosage forms, and routes for administering the CGRP peptide immunogen constructs to prevent and/or treat migraine in a subject.

General

10 The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All references or portions of references cited in this application are expressly incorporated by reference herein in their entirety for any purpose.

Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The singular terms “a,” “an,” and “the” include plural referents unless context clearly indicates otherwise. Similarly, the word “or” is intended to include “and” unless the context clearly indicates otherwise. Hence, the phrase “comprising A or B” means including A, or B, or A and B. 15 It is further to be understood that all amino acid sizes, and all molecular weight or molecular mass values, given for polypeptides are approximate, and are provided for description. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the disclosed method, suitable methods and materials are described below. All 20 publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

CGRP peptide immunogen construct

25 The present disclosure provides peptide immunogen constructs containing a B cell epitope peptide with an amino acid sequence from CGRP (SEQ ID NOs: 1-3) or fragments thereof. The CGRP peptide immunogen constructs can contain a CGRP B epitope peptide having about 7 to about 30 amino acids. The B cell epitope peptides can be from (1) the CGRP receptor binding region (e.g., SEQ ID NOs: 5-9 and 15-22, shown in **Table 1**) located at the C-terminal/central 30 region of the CGRP molecule; or (2) from the CGRP receptor activation site around a cyclic C2-C7 loop (e.g. SEQ ID NOs: 4, 10-13 and 23-24, shown in **Table 1**) located at the N-terminal/central region of the CGRP molecule. The B cell epitope can be covalently linked to a heterologous T helper cell (Th) epitope derived from a pathogen protein (e.g., SEQ ID NOs: 74-115, shown in **Table 2**) directly or through an optional heterologous spacer. These constructs,

containing both designed B cell- and Th cell epitopes act together to stimulate the generation of highly specific antibodies that are cross-reactive with full-length CGRP (SEQ ID NO:1-3) of various species.

The phrase “CGRP peptide immunogen construct” or “peptide immunogen construct”, as used herein, refers to a peptide with more than about 30 amino acids containing (a) a B cell epitope having about more than about 7 contiguous amino acid residues from the full-length CGRP (SEQ ID NOs: 1-3); (b) a heterologous Th epitope; and (c) an optional heterologous spacer.

In certain embodiments, the CGRP peptide immunogen construct can be represented by the formulae:

$$\begin{array}{c}
 (\text{Th})_m-(\text{A})_n-(\text{CGRP functional B epitope peptide})-\text{X} \\
 \text{or} \\
 (\text{CGRP functional B epitope peptide})-(\text{A})_n-(\text{Th})_m-\text{X} \\
 \text{or} \\
 (\text{Th})_m-(\text{A})_n-(\text{CGRP functional B epitope peptide})-(\text{A})_n-(\text{Th})_m-\text{X}
 \end{array}$$

wherein

Th is a heterologous T helper epitope;

A is a heterologous spacer;

(CGRP functional B epitope peptide) is a B cell epitope peptide having from 7 to 30 amino acid residues from CGRP that are involved in either receptor binding or receptor activation;

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.

The CGRP peptide immunogen constructs of the present disclosure were designed and selected based on a number of rationales, including:

- i. the CGRP B cell epitope peptide is non-immunogenic on its own to avoid autologous T cell activation;
- ii. the CGRP B cell epitope peptide can be rendered immunogenic by using a protein carrier or a potent T helper epitope(s);
- iii. when the CGRP B cell epitope peptide rendered immunogenic and administered to a host, the peptide immunogen construct:
 - a. elicits high titer antibodies preferentially directed against the CGRP B cell epitope(s) and not the protein carrier or T helper epitope(s);
 - b. breaks immune tolerance in the immunized host and generates highly specific antibodies having cross-reactivity with the CGRP (SEQ ID NOs: 1-3);
 - c. generates highly specific antibodies capable of inhibiting CGRP and CGRP receptor

binding and the associated downstream events such as rise in intracellular cAMP production; and

- d. generates highly specific antibodies capable reduction in vivo of capsaicin triggered dermal blood flow.

5 The disclosed CGRP peptide immunogen constructs and formulations thereof can effectively function as a pharmaceutical composition to prevent and/or treat subjects predisposed to, or suffering from, migraine.

The various components of the disclosed CGRP peptide immunogen construct are described in further detail below.

10 **a. B cell epitope peptide from CGRP**

The present disclosure is directed to a novel peptide composition for the generation of high titer antibodies with specificity for the Calcitonin Gene-Related Peptide (CGRP) protein of multi-species (e.g., SEQ ID NOs: 1-3). The site-specificity of the peptide immunogen constructs minimizes the generation of antibodies that are directed to irrelevant sites on other regions of CGRP or irrelevant sites on carrier proteins, thus providing a high safety factor.

15 The term “CGRP”, as used herein, refers to the 37-amino acid neuropeptide α -CGRP belonging to the calcitonin (CT) family of peptides. Human CGRP is derived from UniProtKB: P06881-1 and has the amino acid sequence of SEQ ID NO: 1. Marmoset (*Callithrix jacchus*) CGRP is derived from GenBank Accession No.: AAL35592.1 and has the amino acid sequence of SEQ ID NO:2. Rat (*Rattus norvegicus*) CGRP is derived from UniProtKB: P01256 and mouse (*Mus musculus*) CGRP is derived from UniProtKB: Q99JA0 and both rat and mouse CGRP have the amino acid sequence of SEQ ID NO: 3. The amino acid sequences of CGRP used in the present disclosure are shown in **Table 1**.

25 In humans, CGRP is derived from the gene encoding calcitonin, is formed from the alternative splicing of the calcitonin/CGRP gene located on chromosome 11. In humans, CGRP has two isoforms: α -CGRP and β -CGRP. The α -isoform differs from the β -isoform in the amino acids located at positions 3, 22 and 25. On a molecular level within smooth muscle cells, CGRP could bind to its receptor via its C-terminal region and then activate the receptor by using its loop region. The cyclic C2-C7 loop with a disulfide bridge has a basic role in receptor activation and correlates closely with a rise in intracellular cAMP. In the mammalian plasma, the half-life of CGRP is approximately 10 minutes. In the human trigeminal ganglia, CGRP-reactive neurons account for up to 50% of all neurons (Tajti, et al., 1999).

30 CGRP is widely expressed in the central and peripheral nervous system. It is primarily associated with small unmyelinated sensory neurons in close proximity of blood vessels. CGRP

is a potent vasodilator and local administration of CGRP causes transient increases in blood flow. CGRP has also been associated with pain transmission, pain modulation, and neurogenic inflammation. CGRP can be released from sensory neurons via activation of the transient receptor potential cation channel V1 using capsaicin. Laser Doppler Imaging (LDI) has been used to detect
5 the resulting changes in dermal blood flow caused by CGRP.

CGRP is also linked to inflammatory pain as demonstrated by attenuated responses in CGRP knock-out mice in a number of pain models. This role in pain perception is congruent with the expression of CGRP in sensory neurons.

One aspect of the present disclosure is to prevent and/or treat CGRP-migraine headaches
10 with an active immunotherapy that targets CGRP to exert long-term CGRP blockade and clinical efficacy. Thus, the present disclosure is directed to peptide immunogen constructs targeting portions of the full-length CGRP protein (SEQ ID NO: 1-3) and formulations thereof for prevention and treatment of migraine.

The B cell epitope portion of the CGRP peptide immunogen construct can contain between
15 about 7 to about 30 amino acids from any portion of the full-length CGRP protein represented by SEQ ID NOs: 1-3. In certain embodiments, the B cell epitope peptide, screened and selected based on design rationales, contains an amino acid sequence of SEQ ID NOs: 4-13, and 15-24 as shown in **Table 1**.

In some embodiments, the B cell epitope peptide is from the CGRP receptor binding region
20 located at the C-terminal/central region of the CGRP molecule R11-F37 (SEQ ID NO: 9), or fragments thereof (e.g. SEQ ID NOs: 5-8 and 15-22). In other embodiments, the B cell epitope peptide is from the CGRP receptor activation region around the cyclic C2-C7 loop such as A1-N25 (SEQ ID NO: 13) or fragments thereof (e.g. SEQ ID NOs: 4, 10-12, and 23-24).

The CGRP B cell epitope peptide of the present disclosure also includes immunologically
25 functional analogues or homologues of the CGRP. Functional immunological analogues or homologues of CGRP B cell epitope peptide include variants that retain substantially the same immunogenicity as the original peptide. Immunologically functional analogues can have a conservative substitution in an amino acid position; a change in overall charge; a covalent attachment to another moiety; or amino acid additions, insertions, or deletions; and/or any
30 combination thereof (e.g. CGRP peptides of SEQ ID NOs: 9 vs 25).

Antibodies generated from peptide immunogen constructs containing these B cell epitope
from CGRP are highly specific and cross-reactive with the full-length CGRP of various species (e.g., SEQ ID NOs: 1-3). Based on their unique characteristics and properties, the disclosed antibodies elicited by the CGRP peptide immunogen constructs are capable of providing a
35 prophylactic immunotherapeutic approach to preventing and/or treating migraine.

b. Heterologous T helper cell epitopes (Th epitopes)

The present disclosure provides peptide immunogen constructs containing a B cell epitope from CGRP covalently linked to a heterologous T helper cell (Th) epitope directly or through an optional heterologous spacer.

5 The heterologous Th epitope in the CGRP peptide immunogen construct enhances the immunogenicity of the CGRP fragment, which facilitates the production of specific high titer antibodies directed against the optimized target CGRP B cell epitope peptide screened and selected based on design rationales.

10 The term “heterologous”, as used herein, refers to an amino acid sequence that is derived from an amino acid sequence that is not part of, or homologous with, the wild-type sequence of CGRP. Thus, a heterologous Th epitope is a Th epitope derived from an amino acid sequence that is not naturally found in CGRP (i.e., the Th epitope is not autologous to CGRP). Since the Th epitope is heterologous to CGRP, the natural amino acid sequence of CGRP is not extended in either the N-terminal or C-terminal directions when the heterologous Th epitope is covalently
15 linked to the CGRP B epitope peptide.

 The heterologous Th epitope of the present disclosure can be any Th epitope that does not have an amino acid sequence naturally found in CGRP. The Th epitope can also have promiscuous binding motifs to MHC class II molecules of multiple species. In certain embodiments, the Th epitope comprises multiple promiscuous MHC class II binding motifs to allow maximal activation
20 of T helper cells leading to initiation and regulation of immune responses. The Th epitope is preferably immunosilent on its own, i.e. little, if any, of the antibodies generated by the CGRP peptide immunogen constructs will be directed towards the Th epitope, thus allowing a very focused immune response directed to the targeted B cell epitope peptide of the CGRP molecule.

 Th epitopes of the present disclosure include, but are not limited to, amino acid sequences
25 derived from foreign pathogens, as exemplified in **Table 2** (SEQ ID NOs: 74-115). Further, Th epitopes include idealized artificial Th epitopes and combinatorial idealized artificial Th epitopes (e.g., SEQ ID NOs: 75 and 82-99). The heterologous Th epitope peptides presented as a combinatorial sequence (e.g., SEQ ID NOs: 85, 91, 94, and 97), contain a mixture of amino acid residues represented at specific positions within the peptide framework based on the variable
30 residues of homologues for that particular peptide. An assembly of combinatorial peptides can be synthesized in one process by adding a mixture of the designated protected amino acids, instead of one particular amino acid, at a specified position during the synthesis process. Such combinatorial heterologous Th epitope peptides assemblies can allow broad Th epitope coverage for animals having a diverse genetic background. Representative combinatorial sequences of
35 heterologous Th epitope peptides include SEQ ID NOs: 85, 91, 94, and 97 which are shown in

Table 2. Th epitope peptides of the present invention provide broad reactivity and immunogenicity to animals and patients from genetically diverse populations.

c. Heterologous Spacer

The disclosed CGRP peptide immunogen constructs optionally contain a heterologous spacer that covalently links the CGRP B cell epitope peptide to the heterologous T helper cell (Th) epitope.

As discussed above, the term “heterologous”, refers to an amino acid sequence that is derived from an amino acid sequence that is not part of, or homologous with, the natural type sequence of CGRP. Thus, the natural amino acid sequence of CGRP is not extended in either the N-terminal or C-terminal directions when the heterologous spacer is covalently linked to the CGRP B cell epitope peptide because the spacer is heterologous to the CGRP sequence.

The spacer is any molecule or chemical structure capable of linking two amino acids and/or peptides together. The spacer can vary in length or polarity depending on the application. The spacer attachment can be through an amide- or carboxyl- linkage but other functionalities are possible as well. The spacer can include a chemical compound, a naturally occurring amino acid, or a non-naturally occurring amino acid.

The spacer can provide structural features to the CGRP peptide immunogen construct. Structurally, the spacer provides a physical separation of the Th epitope from the B cell epitope of the CGRP fragment. The physical separation by the spacer can disrupt any artificial secondary structures created by joining the Th epitope to the B cell epitope. Additionally, the physical separation of the epitopes by the spacer can eliminate interference between the Th cell and/or B cell responses. Furthermore, the spacer can be designed to create or modify a secondary structure of the peptide immunogen construct. For example, a spacer can be designed to act as a flexible hinge to enhance the separation of the Th epitope and B cell epitope. A flexible hinge spacer can also permit more efficient interactions between the presented peptide immunogen and the appropriate Th cells and B cells to enhance the immune responses to the Th epitope and B cell epitope. Examples of sequences encoding flexible hinges are found in the immunoglobulin heavy chain hinge region, which are often proline rich. One particularly useful flexible hinge that can be used as a spacer is provided by the sequence Pro-Pro-Xaa-Pro-Xaa-Pro (SEQ ID NO: 71), where Xaa is any amino acid, and preferably aspartic acid.

The spacer can also provide functional features to the CGRP peptide immunogen construct. For example, the spacer can be designed to change the overall charge of the CGRP peptide immunogen construct, which can affect the solubility of the peptide immunogen construct. Additionally, changing the overall charge of the CGRP peptide immunogen construct can affect

the ability of the peptide immunogen construct to associate with other compounds and reagents. As discussed in further detail below, the CGRP peptide immunogen construct can be formed into a stable immunostimulatory complex with a highly charged oligonucleotide, such as CpG oligomers, through electrostatic association. The overall charge of the CGRP peptide immunogen construct is important for the formation of these stable immunostimulatory complexes.

Chemical compounds that can be used as a spacer include, but are not limited to, (2-aminoethoxy) acetic acid (AEA), 5-aminovaleric acid (AVA), 6-aminocaproic acid (Ahx), 8-amino-3,6-dioxaoctanoic acid (AEEA, mini-PEG1), 12-amino-4,7,10-trioxadodecanoic acid (mini-PEG2), 15-amino-4,7,10,13-tetraoxapenta-decanoic acid (mini-PEG3), trioxatridecan-succinamic acid (Ttds), 12-amino-dodecanoic acid, Fmoc-5-amino-3-oxapentanoic acid (O1Pen), and the like.

Naturally-occurring amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine.

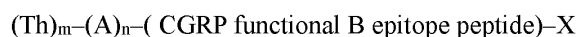
Non-naturally occurring amino acids include, but are not limited to, ϵ -N Lysine, β -alanine, ornithine, norleucine, norvaline, hydroxyproline, thyroxine, γ -amino butyric acid, homoserine, citrulline, aminobenzoic acid, 6-aminocaproic acid (Aca; 6-Aminohexanoic acid), hydroxyproline, mercaptopropionic acid (MPA), 3-nitro-tyrosine, pyroglutamic acid, and the like.

The spacer in the CGRP peptide immunogen construct can be covalently linked at either N- or C- terminal end of the Th epitope and the CGRP B cell epitope peptide. In some embodiments, the spacer is covalently linked to the C-terminal end of the Th epitope and to the N-terminal end of the CGRP B cell epitope peptide. In other embodiments, the spacer is covalently linked to the C-terminal end of the CGRP B cell epitope peptide and to the N-terminal end of the Th epitope. In certain embodiments, more than one spacer can be used, for example, when more than one Th epitope is present in the CGRP peptide immunogen construct. When more than one spacer is used, each spacer can be the same as each other or different. Additionally, when more than one Th epitope is present in the CGRP peptide immunogen construct, the Th epitopes can be separated with a spacer, which can be the same as, or different from, the spacer used to separate the Th epitope from the CGRP B cell epitope peptide. There is no limitation in the arrangement of the spacer in relation to the Th epitope or the CGRP B cell epitope peptide.

In certain embodiments, the heterologous spacer is a naturally occurring amino acid or a non-naturally occurring amino acid. In other embodiments, the spacer contains more than one naturally occurring or non-naturally occurring amino acid. In specific embodiments, the spacer is Lys-, Gly-, Lys-Lys-Lys-, (α , ϵ -N)Lys, ϵ -N-Lys-Lys-Lys-Lys (SEQ ID NO: 72), or Lys-Lys-Lys- ϵ -N-Lys (SEQ ID NO: 73).

d. Specific embodiments of the CGRP peptide immunogen constructs

In certain embodiments, the CGRP peptide immunogen constructs can be represented by the following formulae:

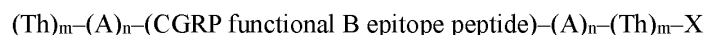


5

or



or



wherein

10

Th is a heterologous T helper epitope;

A is a heterologous spacer;

(CGRP functional B epitope peptide) is a B cell epitope peptide having from 7 to 30 amino acid residues from CGRP that are involved in either receptor binding or receptor activation;

X is an α -COOH or α -CONH₂ of an amino acid;

15

m is from 1 to about 4; and

n is from 0 to about 10.

The B cell epitope peptide can contain between about 7 to about 30 amino acids from any portion of the full-length CGRP protein represented by SEQ ID NOs: 1-3. In some embodiments, the B cell epitope has an amino acid sequence selected from any of SEQ ID NOs: 4-13 and 15-22, shown in **Table 1**. In certain embodiments, the B cell epitope peptide is from the CGRP receptor binding region located at the C-terminal/central region of the CGRP molecule R11-F37 (SEQ ID NO: 9), or fragments thereof (e.g. SEQ ID NOs: 5-8 and 15-22). In other embodiments, the B cell epitope peptide is from the CGRP receptor activation region around the cyclic C2-C7 loop such as A1-N25 (SEQ ID NO: 13) or fragments thereof (e.g. SEQ ID NOs: 4, 10-12, and 23-24).

25

The heterologous Th epitope in the CGRP peptide immunogen construct has an amino acid sequence selected from any of SEQ ID NOs: 74-115, and combinations thereof, shown in **Table 2**. In some embodiments, the CGRP peptide immunogen construct contains more than one Th epitope.

30

The optional heterologous spacer is selected from any of Lys-, Gly-, Lys-Lys-Lys-, (α , ϵ -N)Lys, Pro-Pro-Xaa-Pro-Xaa-Pro (SEQ ID NO: 71), ϵ -N-Lys-Lys-Lys-Lys (SEQ ID NO: 72), Lys-Lys-Lys- ϵ -N-Lys (SEQ ID NO: 73), and any combination thereof, where Xaa is any amino acid, but preferably aspartic acid. In specific embodiments, the heterologous spacer is ϵ -N-Lys-Lys-Lys-Lys (SEQ ID NO: 72) or Lys-Lys-Lys- ϵ -N-Lys (SEQ ID NO: 73).

35

In certain embodiments, the CGRP peptide immunogen construct has an amino acid

sequence selected from any of SEQ ID NOs: 116-127 and 130-180 as shown in **Table 3**.

The CGRP peptide immunogen constructs comprising Th epitopes are produced simultaneously in a single solid-phase peptide synthesis in tandem with the CGRP fragment. Th epitopes also include immunological analogues of Th epitopes. Immunological Th analogues
5 include immune-enhancing analogs, cross-reactive analogues and segments of any of these Th epitopes that are sufficient to enhance or stimulate an immune response to the CGRP B cell epitope peptide.

The Th epitope in the CGRP peptide immunogen construct can be covalently linked at either N- or C- terminal end of the CGRP B cell epitope peptide. In some embodiments, the Th
10 epitope is covalently linked to the N-terminal end of the CGRP B cell epitope peptide. In other embodiments, the Th epitope is covalently linked to the C-terminal end of the CGRP B cell epitope peptide. In certain embodiments, more than one Th epitope is covalently linked to the CGRP B cell epitope peptide. When more than one Th epitope is linked to the CGRP B cell epitope peptide, each Th epitope can have the same amino acid sequence or different amino acid sequences. In
15 addition, when more than one Th epitope is linked to the CGRP B cell epitope peptide, the Th epitopes can be arranged in any order. For example, the Th epitopes can be consecutively linked to the N-terminal end of the CGRP B cell epitope peptide, or consecutively linked to the C-terminal end of the CGRP B cell epitope peptide, or a Th epitope can be covalently linked to the N-terminal end of the CGRP B cell epitope peptide while a separate Th epitope is covalently
20 linked to the C-terminal end of the CGRPB cell epitope peptide. There is no limitation in the arrangement of the Th epitopes in relation to the CGRP B cell epitope peptide.

In some embodiments, the Th epitope is covalently linked to the CGRP B cell epitope peptide directly. In other embodiments, the Th epitope is covalently linked to the CGRP fragment through a heterologous spacer.

25 **e. Variants, homologues, and functional analogues**

Variants and analogs of the above immunogenic peptide constructs that induce and/or cross-react with antibodies to the preferred CGRP B cell epitope peptides can also be used. Analogs, including allelic, species, and induced variants, typically differ from naturally occurring peptides at one, two, or a few positions, often by virtue of conservative substitutions. Analogs
30 typically exhibit at least 80 or 90% sequence identity with natural peptides. Some analogs also include unnatural amino acids or modifications of N- or C-terminal amino acids at one, two, or a few positions.

Variants that are functional analogues can have a conservative substitution in an amino acid position; a change in overall charge; a covalent attachment to another moiety; or amino acid

additions, insertions, or deletions; and/or any combination thereof.

Conservative substitutions are when one amino acid residue is substituted for another amino acid residue with similar chemical properties. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine; the polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; the positively charged (basic) amino acids include arginine, lysine and histidine; and the negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

In a particular embodiment, the functional analogue has at least 50% identity to the original amino acid sequence. In another embodiment, the functional analogue has at least 80% identity to the original amino acid sequence. In yet another embodiment, the functional analogue has at least 85% identity to the original amino acid sequence. In still another embodiment, the functional analogue has at least 90% identity to the original amino acid sequence.

Functional immunological analogues of the Th epitope peptides are also effective and included as part of the present invention. Functional immunological Th analogues can include conservative substitutions, additions, deletions and insertions of from one to about five amino acid residues in the Th epitope which do not essentially modify the Th-stimulating function of the Th epitope. The conservative substitutions, additions, and insertions can be accomplished with natural or non-natural amino acids, as described above for the CGRP B cell epitope peptide. **Table 2** identifies another variation of a functional analogue for Th epitope peptide. In particular, SEQ ID NOs: 75 and 82 of MvF1 and MvF2 Th are functional analogues of SEQ ID NOs: 94 and 98 of MvF4 and MvF5 in that they differ in the amino acid frame by the deletion (SEQ ID NOs: 75 and 82) or the inclusion (SEQ ID NOs: 94 and 98) of two amino acids each at the N- and C-termini. The differences between these two series of analogous sequences would not affect the function of the Th epitopes contained within these sequences. Therefore, functional immunological Th analogues include several versions of the Th epitope derived from Measles Virus Fusion protein MvF1-4 Ths (SEQ ID NOs: 75, 82, 85, and 94) and from Hepatitis Surface protein HBsAg 1-3 Ths (SEQ ID NOs: 91, 97, and 99).

Compositions

The present disclosure also provides compositions comprising the disclosed CGRP immunogen peptide constructs.

a. Peptide compositions

Compositions containing the disclosed CGRP peptide immunogen constructs can be in liquid or solid/lyophilized form. Liquid compositions can include water, buffers, solvents, salts, and/or any other acceptable reagent that does not alter the structural or functional properties of the

CGRP peptide immunogen constructs. Peptide compositions can contain one or more of the disclosed CGRP peptide immunogen constructs.

b. Pharmaceutical compositions

The present disclosure is also directed to pharmaceutical compositions containing the disclosed CGRP peptide immunogen constructs.

Pharmaceutical compositions can contain carriers and/or other additives in a pharmaceutically acceptable delivery system. Accordingly, pharmaceutical compositions can contain a pharmaceutically effective amount of an CGRP peptide immunogen construct together with pharmaceutically-acceptable carrier, adjuvant, and/or other excipients such as diluents, additives, stabilizing agents, preservatives, solubilizing agents, buffers, and the like.

Pharmaceutical compositions can contain one or more adjuvant that act(s) to accelerate, prolong, or enhance the immune response to the CGRP peptide immunogen constructs without having any specific antigenic effect itself. Adjuvants used in the pharmaceutical composition can include oils, oil emulsions, aluminum salts, calcium salts, immune stimulating complexes, bacterial and viral derivatives, virosomes, carbohydrates, cytokines, polymeric microparticles. In certain embodiments, the adjuvant can be selected from alum (potassium aluminum phosphate), aluminum phosphate (e.g. ADJU-PHOS®), aluminum hydroxide (e.g. ALHYDROGEL®), calcium phosphate, incomplete Freund's adjuvant (IFA), Freund's complete adjuvant, MF59, adjuvant 65, Lipovant, ISCOM, liposyn, saponin, squalene, L121, EmulsIL-6n®, monophosphoryl lipid A (MPL), Quil A, QS21, MONTANIDE® ISA 35, ISA 50V, ISA 50V2, ISA 51, ISA 206, ISA 720, liposomes, phospholipids, peptidoglycan, lipopolysaccharides (LPS), ASO1, ASO2, ASO3, ASO4, AF03, lipophilic phospholipid (lipid A), gamma inulin, algamulin, glucans, dextrans, glucomannans, galactomannans, levans, xylans, dimethyldioctadecylammonium bromide (DDA), as well as the other adjuvants and emulsifiers.

In some embodiments, the pharmaceutical composition contains MONTANIDE™ ISA 51 (an oil adjuvant composition comprised of vegetable oil and mannide oleate for production of water-in-oil emulsions), TWEEN® 80 (also known as: Polysorbate 80 or Polyoxyethylene (20) sorbitan monooleate), a CpG oligonucleotide, and/or any combination thereof. In other embodiments, the pharmaceutical composition is a water-in-oil-in-water (i.e. w/o/w) emulsion with EmulsIL-6n or EmulsIL-6n D as the adjuvant.

Pharmaceutical compositions can also include pharmaceutically acceptable additives or excipients. For example, pharmaceutical compositions can contain antioxidants, binders, buffers, bulking agents, carriers, chelating agents, coloring agents, diluents, disintegrants, emulsifying agents, fillers, gelling agents, pH buffering agents, preservatives, solubilizing agents, stabilizers,

and the like.

Pharmaceutical compositions can be formulated as immediate release or for sustained release formulations. Additionally the pharmaceutical compositions can be formulated for induction of systemic, or localized mucosal, immunity through immunogen entrapment and co-administration with microparticles. Such delivery systems are readily determined by one of
5 ordinary skill in the art.

Pharmaceutical compositions can be prepared as injectables, either as liquid solutions or suspensions. Liquid vehicles containing the CGRP peptide immunogen construct can also be prepared prior to injection. The pharmaceutical composition can be administered by any suitable
10 mode of application, for example, i.d., i.v., i.p., i.m., intranasally, orally, subcutaneously, etc. and in any suitable delivery device. In certain embodiments, the pharmaceutical composition is formulated for intravenous, subcutaneous, intradermal, or intramuscular administration. Pharmaceutical compositions suitable for other modes of administration can also be prepared, including oral and intranasal applications.

Pharmaceutical compositions can also be formulated in a suitable dosage unit form. In some embodiments, the pharmaceutical composition contains from about 0.1 µg to about 1 mg of the CGRP peptide immunogen construct per kg body weight. Effective doses of the pharmaceutical compositions vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an
20 animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the patient is a human but nonhuman mammals including transgenic mammals can also be treated. When delivered in multiple doses, the pharmaceutical compositions may be conveniently divided into an appropriate amount per dosage unit form. The administered dosage will depend on the age, weight and general health of the subject as is well known in the therapeutic arts.
25

In some embodiments, the pharmaceutical composition contains more than one CGRP peptide immunogen construct. A pharmaceutical composition containing a mixture of more than one CGRP peptide immunogen construct to allow for synergistic enhancement of the immunoefficacy of the constructs. Pharmaceutical compositions containing more than one CGRP
30 peptide immunogen construct can be more effective in a larger genetic population due to a broad MHC class II coverage thus provide an improved immune response to the CGRP peptide immunogen constructs.

In some embodiments, the pharmaceutical composition contains an CGRP peptide immunogen construct selected from SEQ ID NOs: 120-127 and 130-180 (**Table 3**), as well as
35 homologues, analogues and/or combinations thereof.

In certain embodiments, CGRP peptide immunogen constructs (SEQ ID NOs: 161-163) with heterologous Th epitopes derived from MVF and HBsAg in a combinatorial form (SEQ ID NOs: 85, 91, 94, and 97) were mixed in an equimolar ratio for use in a formulation to allow for maximal coverage of a host population having a diverse genetic background.

5 Furthermore, the antibody response elicited by CGRP peptide immunogen construct (e.g. UBITH®1 with SEQ ID NO: 108) was mostly (>90%) focused on the desired cross-reactivity against the B epitope peptide of CGRP without much, if any, directed to the heterologous Th epitopes employed for immunogenicity enhancement (Example 6, **Table 10**). This is in sharp contrast to the conventional protein such as KLH or other biological protein carriers used for such
10 CGRP peptide immunogenicity enhancement.

In other embodiments, pharmaceutical compositions comprising a peptide composition of for example a mixture of the CGRP peptide immunogen constructs in contact with mineral salts including Alum gel (ALHYDROGEL) or Aluminum phosphate (ADJUPHOS) as adjuvant to form a suspension formulation was used for administration to hosts.

15 Pharmaceutical compositions containing an CGRP peptide immunogen construct can be used to elicit an immune response and produce antibodies in a host upon administration.

c. Immunostimulatory complexes

The present disclosure is also directed to pharmaceutical compositions containing an CGRP peptide immunogen construct in the form of an immunostimulatory complex with a CpG
20 oligonucleotide. Such immunostimulatory complexes are specifically adapted to act as an adjuvant and as a peptide immunogen stabilizer. The immunostimulatory complexes are in the form of a particulate, which can efficiently present the CGRP peptide immunogen to the cells of the immune system to produce an immune response. The immunostimulatory complexes may be formulated as a suspension for parenteral administration. The immunostimulatory complexes may
25 also be formulated in the form of water in oil (w/o) emulsions, as a suspension in combination with a mineral salt or with an in-situ gelling polymer for the efficient delivery of the CGRP peptide immunogen construct to the cells of the immune system of a host following parenteral administration.

The stabilized immunostimulatory complex can be formed by complexing an CGRP
30 peptide immunogen construct with an anionic molecule, oligonucleotide, polynucleotide, or combinations thereof via electrostatic association. The stabilized immunostimulatory complex may be incorporated into a pharmaceutical composition as an immunogen delivery system.

In certain embodiments, the CGRP peptide immunogen construct is designed to contain a cationic portion that is positively charged at a pH in the range of 5.0 to 8.0. The net charge on the

cationic portion of the CGRP peptide immunogen construct, or mixture of constructs, is calculated by assigning a +1 charge for each lysine (K), arginine (R) or histidine (H), a -1 charge for each aspartic acid (D) or glutamic acid (E) and a charge of 0 for the other amino acid within the sequence. The charges are summed within the cationic portion of the CGRP peptide immunogen construct and expressed as the net average charge. A suitable peptide immunogen has a cationic portion with a net average positive charge of +1. Preferably, the peptide immunogen has a net positive charge in the range that is larger than +2. In some embodiments, the cationic portion of the CGRP peptide immunogen construct is the heterologous spacer. In certain embodiments, the cationic portion of the CGRP peptide immunogen construct has a charge of +4 when the spacer sequence is (α , ϵ -N)Lys, (α , ϵ -N)-Lys-Lys-Lys-Lys (SEQ ID NO: 72), or Lys-Lys-Lys- ϵ -N-Lys (SEQ ID NO: 73).

An "anionic molecule" as described herein refers to any molecule that is negatively charged at a pH in the range of 5.0-8.0. In certain embodiments, the anionic molecule is an oligomer or polymer. The net negative charge on the oligomer or polymer is calculated by assigning a -1 charge for each phosphodiester or phosphorothioate group in the oligomer. A suitable anionic oligonucleotide is a single-stranded DNA molecule with 8 to 64 nucleotide bases, with the number of repeats of the CpG motif in the range of 1 to 10. Preferably, the CpG immunostimulatory single-stranded DNA molecules contain 18-48 nucleotide bases, with the number of repeats of CpG motif in the range of 3 to 8.

More preferably the anionic oligonucleotide is represented by the formula: 5' X¹CGX² 3' wherein C and G are unmethylated; and X¹ is selected from the group consisting of A (adenine), G (guanine) and T (thymine); and X² is C (cytosine) or T (thymine). Or, the anionic oligonucleotide is represented by the formula: 5' (X³)₂CG(X⁴)₂ 3' wherein C and G are unmethylated; and X³ is selected from the group consisting of A, T or G; and X⁴ is C or T. In specific embodiments, the CpG oligonucleotide has the sequence of CpG1: 5' TCg TCg TTT TgT CgT TTT gTC gTT TTg TCg TT 3' (fully phosphorothioated) (SEQ ID NO: 182), CpG2: 5' Phosphate TCg TCg TTT TgT CgT TTT gTC gTT 3' (fully phosphorothioated) (SEQ ID NO: 183), or CpG3 5' TCg TCg TTT TgT CgT TTT gTC gTT 3' (fully phosphorothioated) (SEQ ID NO: 184).

The resulting immunostimulatory complex is in the form of particles with a size typically in the range from 1-50 microns and is a function of many factors including the relative charge stoichiometry and molecular weight of the interacting species. The particulated immunostimulatory complex has the advantage of providing adjuvantation and upregulation of specific immune responses in vivo. Additionally, the stabilized immunostimulatory complex is suitable for preparing pharmaceutical compositions by various processes including water-in-oil

emulsions, mineral salt suspensions and polymeric gels.

The present disclosure is also directed to pharmaceutical compositions, including formulations, for the prevention and/or treatment of migraine. In some embodiments, pharmaceutical compositions comprising a stabilized immunostimulatory complex, which is formed through mixing a CpG oligomer with a peptide composition containing a mixture of the CGRP peptide immunogen constructs (e.g., SEQ ID NOs: 120-127 and 130-180) through electrostatic association, to further enhance the immunogenicity of the CGRP peptide immunogen constructs and elicit antibodies that are cross-reactive with the CGRP proteins of SEQ ID NOs: 1-3 that are directed at the CGRP receptor binding or receptor activation region (Example 6).

In yet other embodiments, pharmaceutical compositions contain a mixture of the CGRP peptide immunogen constructs (e.g., any combination of SEQ ID NOs: 120-127 and 130-180) in the form of a stabilized immunostimulatory complex with CpG oligomers that are, optionally, mixed with mineral salts, including Alum gel (ALHYDROGEL) or Aluminum phosphate (ADJUPHOS) as an adjuvant with high safety factor, to form a suspension formulation for administration to hosts.

Antibodies

The present disclosure also provides antibodies elicited by the CGRP peptide immunogen constructs.

The present disclosure provides CGRP peptide immunogen constructs and formulations thereof, cost effective in manufacturing, optimal in their design that are capable of eliciting high titer antibodies targeting the CGRP receptor binding or receptor activation region of the CGRP molecule (SEQ ID NOs: 4-13 and 15-24) that is capable of breaking the immune tolerance against self-protein CGRP with a high responder rate in immunized hosts. The antibodies generated by the CGRP peptide immunogen constructs have high affinity towards the CGRP receptor binding or activation region.

In some embodiments, CGRP peptide immunogen constructs for eliciting antibodies comprise a hybrid of a CGRP peptide targeting the CGRP receptor binding or receptor activation region of the CGRP molecule (SEQ ID NOs: 4-13 and 15-24) linked to a heterologous Th epitope derived from pathogenic proteins such as Measles Virus Fusion (MVF) protein and others (SEQ ID NOs: 74-115) through an optional spacer. The B epitope and Th epitope peptide of the CGRP peptide immunogen constructs act together to stimulate the generation of highly specific antibodies cross-reactive with the CGRP receptor binding or activation region of the CGRP protein (SEQ ID NO: 1-3).

Traditional methods for immunopotentiating a peptide, such as through chemical coupling

to a carrier protein, for example, Keyhole Limpet Hemocyanin (KLH) or other carrier proteins such as Diphtheria toxoid (DT) and Tetanus Toxoid (TT) proteins, typically result in the generation of a large amount of antibodies directed against the carrier protein. Thus, a major deficiency of such peptide-carrier protein compositions is that most (>90%) of antibodies generated by the immunogen are the non-functional antibodies directed against the carrier protein KLH, DT or TT, which can lead to epitopic suppression.

Unlike the traditional method for immunopotentiating a peptide, the antibodies generated by the disclosed CGRP peptide immunogen constructs (e.g. SEQ ID NOs: 116-127 and 130-180) bind with highly specificity to the CGRP B epitope peptide (SEQ ID NO:4-13 and 15-24) with little, if any, antibodies directed against the heterologous Th epitope (e.g., SEQ ID NOs: 74-115) or optional heterologous spacer.

Methods

The present disclosure is also directed to methods for making and using the CGRP peptide immunogen constructs, compositions, and pharmaceutical compositions.

a. Methods for manufacturing the CGRP peptide immunogen construct

The CGRP peptide immunogen constructs of this disclosure can be made by chemical synthesis methods well known to the ordinarily skilled artisan (see, e.g., Fields, et al., 1992). The CGRP peptide immunogen constructs can be synthesized using the automated Merrifield techniques of solid phase synthesis with the α -NH₂ protected by either t-Boc or F-moc chemistry using side chain protected amino acids on, for example, an Applied Biosystems Peptide Synthesizer Model 430A or 431. Preparation of CGRP peptide immunogen constructs comprising combinatorial library peptides for Th epitopes can be accomplished by providing a mixture of alternative amino acids for coupling at a given variable position.

After complete assembly of the desired CGRP peptide immunogen construct, the resin can be treated according to standard procedures to cleave the peptide from the resin and the functional groups on the amino acid side chains can be deblocked. The free peptide can be purified by HPLC and characterized biochemically, for example, by amino acid analysis or by sequencing. Purification and characterization methods for peptides are well known to one of ordinary skill in the art.

The quality of peptides produced by this chemical process can be controlled and defined and, as a result, reproducibility of CGRP peptide immunogen constructs, immunogenicity, and yield can be assured. Detailed description of the manufacturing of the CGRP peptide immunogen construct through solid phase peptide synthesis is shown in Example 1.

The range in structural variability that allows for retention of an intended immunological

activity has been found to be far more accommodating than the range in structural variability allowed for retention of a specific drug activity by a small molecule drug or the desired activities and undesired toxicities found in large molecules that are co-produced with biologically-derived drugs.

5 Thus, peptide analogues, either intentionally designed or inevitably produced by errors of the synthetic process as a mixture of deletion sequence byproducts that have chromatographic and immunologic properties similar to the intended peptide, are frequently as effective as a purified preparation of the desired peptide. Designed analogues and unintended analogue mixtures are effective as long as a discerning QC procedure is developed to monitor both the manufacturing
10 process and the product evaluation process so as to guarantee the reproducibility and efficacy of the final product employing these peptides.

 The CGRP peptide immunogen constructs can also be made using recombinant DNA technology including nucleic acid molecules, vectors, and/or host cells. As such, nucleic acid molecules encoding the CGRP peptide immunogen construct and immunologically functional
15 analogues thereof are also encompassed by the present disclosure as part of the present invention. Similarly, vectors, including expression vectors, comprising nucleic acid molecules as well as host cells containing the vectors are also encompassed by the present disclosure as part of the present invention.

 Various exemplary embodiments also encompass methods of producing the CGRP peptide
20 immunogen construct and immunologically functional analogues thereof. For example, methods can include a step of incubating a host cell containing an expression vector containing a nucleic acid molecule encoding an CGRP peptide immunogen construct and/or immunologically functional analogue thereof under such conditions where the peptide and/or analogue is expressed. The longer synthetic peptide immunogens can be synthesized by well-known recombinant DNA
25 techniques. Such techniques are provided in well-known standard manuals with detailed protocols. To construct a gene encoding a peptide of this invention, the amino acid sequence is reverse translated to obtain a nucleic acid sequence encoding the amino acid sequence, preferably with codons that are optimum for the organism in which the gene is to be expressed. Next, a synthetic gene is made typically by synthesizing oligonucleotides which encode the peptide and
30 any regulatory elements, if necessary. The synthetic gene is inserted in a suitable cloning vector and transfected into a host cell. The peptide is then expressed under suitable conditions appropriate for the selected expression system and host. The peptide is purified and characterized by standard methods.

b. Methods for the manufacturing of immunostimulatory complexes

Various exemplary embodiments also encompass methods of producing the Immunostimulatory complexes comprising CGRP peptide immunogen constructs and CpG oligodeoxynucleotide (ODN) molecule. Stabilized immunostimulatory complexes (ISC) are derived from a cationic portion of the CGRP peptide immunogen construct and a polyanionic CpG ODN molecule. The self-assembling system is driven by electrostatic neutralization of charge. Stoichiometry of the molar charge ratio of cationic portion of the CGRP peptide immunogen construct to anionic oligomer determines extent of association. The non-covalent electrostatic association of CGRP peptide immunogen construct and CpG ODN is a completely reproducible process. The peptide/CpG ODN immunostimulatory complex aggregates, which facilitate presentation to the “professional” antigen presenting cells (APC) of the immune system thus further enhancing the immunogenicity of the complexes. These complexes are easily characterized for quality control during manufacturing. The peptide/CpG ISC are well tolerated in vivo. This novel particulate system comprising CpG ODN and CGRP peptide immunogen constructs was designed to take advantage of the generalized B cell mitogenicity associated with CpG ODN use, yet promote balanced Th-1/Th-2 type responses.

The CpG ODN in the disclosed pharmaceutical compositions is 100% bound to immunogen in a process mediated by electrostatic neutralization of opposing charge, resulting in the formation of micron-sized particulates. The particulate form allows for a significantly reduced dosage of CpG from the conventional use of CpG adjuvants, less potential for adverse innate immune responses, and facilitates alternative immunogen processing pathways including antigen presenting cells (APC). Consequently, such formulations are novel conceptually and offer potential advantages by promoting the stimulation of immune responses by alternative mechanisms.

c. Methods for the manufacturing of pharmaceutical compositions

Various exemplary embodiments also encompass pharmaceutical compositions containing CGRP peptide immunogen constructs. In certain embodiments, the pharmaceutical compositions employ water in oil emulsions and in suspension with mineral salts.

In order for a pharmaceutical composition to be used by a large population, safety becomes another important factor for consideration. Despite there has been use of water-in-oil emulsions in many clinical trials, Alum remains the major adjuvant for use in formulations due to its safety. Alum or its mineral salts Aluminum phosphate (ADJUPHOS) are, therefore, frequently used as adjuvants in preparation for clinical applications.

Other adjuvants and immunostimulating agents include 3 De-O-acylated monophosphoryl

lipid A (MPL) or 3-DMP, polymeric or monomeric amino acids, such as polyglutamic acid or polylysine. Such adjuvants can be used with or without other specific immunostimulating agents, such as muramyl peptides (e.g., N-acetylmuramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2' dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), N-acetylglucosaminyl-N-acetylmuramyl-L-Al-D-isoglu-L-Ala-dipalmitoxy propylamide (DTP-DPP) Theramide™), or other bacterial cell wall components. Oil-in-water emulsions include MF59 (see WO 90/14837 to Van Nest et al., which is hereby incorporated by reference in its entirety), containing 5% Squalene, 0.5% TWEEN 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE) formulated into submicron particles using a microfluidizer; SAF, containing 10% Squalene, 0.4% TWEEN 80, 5% pluronic-blocked polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion; and the Ribi™ adjuvant system (RAS) (Ribi ImmunoChem, Hamilton, Mont.) containing 2% squalene, 0.2% TWEEN 80, and one or more bacterial cell wall components selected from the group consisting of monophosphoryllipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL+CWS (Detox™). Other adjuvants include Complete Freund's Adjuvant (CFA), Incomplete Freund's Adjuvant (IFA), and cytokines, such as interleukins (IL-1, IL-2, and IL-12), macrophage colony stimulating factor (M-CSF), and tumor necrosis factor (TNF- α).

The choice of an adjuvant depends on the stability of the immunogenic formulation containing the adjuvant, the route of administration, the dosing schedule, the efficacy of the adjuvant for the species being immunized, and, in humans, a pharmaceutically acceptable adjuvant is one that has been approved or is approvable for human administration by pertinent regulatory bodies. For example, alum, MPL or Incomplete Freund's adjuvant (Chang, et al., 1998), which is hereby incorporated by reference in its entirety) alone or optionally all combinations thereof are suitable for human administration.

The compositions can include pharmaceutically-acceptable, non-toxic carriers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological phosphate-buffered saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may also include other carriers, adjuvants, or nontoxic, nontherapeutic, non-immunogenic stabilizers, and the like.

Pharmaceutical compositions can also include large, slowly metabolized macromolecules, such as proteins, polysaccharides like chitosan, polylactic acids, polyglycolic acids and

copolymers (e.g., latex functionalized sepharose, agarose, cellulose, and the like), polymeric amino acids, amino acid copolymers, and lipid aggregates (e.g., oil droplets or liposomes). Additionally, these carriers can function as immunostimulating agents (i.e., adjuvants).

The pharmaceutical compositions of the present invention can further include a suitable delivery vehicle. Suitable delivery vehicles include, but are not limited to viruses, bacteria, biodegradable microspheres, microparticles, nanoparticles, liposomes, collagen minipellets, and cochleates.

d. Methods of using pharmaceutical compositions

The present disclosure also includes methods of using pharmaceutical compositions containing CGRP peptide immunogen constructs.

In certain embodiments, the pharmaceutical compositions containing CGRP peptide immunogen constructs can be used for the treatment of migraine.

In some embodiments, the methods comprise administering a pharmaceutical composition comprising a pharmacologically effective amount of an CGRP peptide immunogen construct to a host in need thereof. In certain embodiments, the methods comprise administering a pharmaceutical composition comprising a pharmacologically effective amount of an CGRP peptide immunogen construct to a warm-blooded animal (e.g., humans, Cynomolgus macaques, mice) to elicit highly specific antibodies cross-reactive with the human CGRP protein (SEQ ID NO: 1), or CGRP proteins from other species (e.g., SEQ ID NOs: 2 and 3).

In certain embodiments, the pharmaceutical compositions containing CGRP peptide immunogen constructs can be used to treat migraine as shown in in vivo capsaicin induced dorsal blood flow model.

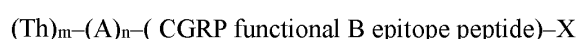
e. In vitro functional assays and in vivo proof of concept studies

Antibodies elicited in immunized hosts by the CGRP peptide immunogen constructs can be used in in vitro functional assays. These functional assays include, but are not limited to:

- (1) in vitro binding to CGRP protein (SEQ ID NOs: 1-3);
- (2) inhibition in vitro of CGRP binding to its receptor;
- (3) inhibition in vitro of intracellular cAMP elevation;
- (4) inhibition in vivo of capsaicin induced dorsal blood flow model in mice.

Specific Embodiments

(1) A CGRP peptide immunogen construct having about 30 or more amino acids, represented by the formulae:



or

(CGRP functional B epitope peptide)-(A)_n-(Th)_m-X

or

(Th)_m-(A)_n-(CGRP functional B epitope peptide)-(A)_n-(Th)_m-X

5 wherein

Th is a heterologous T helper epitope;

A is a heterologous spacer;

(CGRP functional B epitope peptide) is a B cell epitope peptide having from 7 to about 30 amino acid residues derived from CGRP receptor binding or activation region with SEQ ID NOs:

10 4-13, 15-24) of CGRP (SEQ ID NOs: 1-3) as shown in **Table 1**;

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.

(2) The CGRP peptide immunogen construct according to (1), wherein the CGRP receptor
15 binding or activation region is selected from the group consisting of SEQ ID NOs: 4-13 and 15-24.

(3) The CGRP peptide immunogen construct according to any of (1) or (2), wherein the Th epitope is selected from the group consisting of SEQ ID NOs: 74-115.

(4) The CGRP peptide immunogen construct according to (1), wherein the peptide
20 immunogen construct is selected from the group consisting of SEQ ID NOs: 120-127 and 130-180.

(5) An CGRP peptide immunogen construct comprising:

- a. a B cell epitope comprising from about 7 to about 30 amino acid residues from the CGRP sequence of SEQ ID NOs: 1 to 3;
- 25 b. a T helper epitope comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 74-115, and any combination thereof; and
- c. an optional heterologous spacer selected from the group consisting of an amino acid, Lys-, Gly-, Lys-Lys-Lys-, (α , ϵ -N)Lys, ϵ -N-Lys-Lys-Lys-Lys (SEQ ID NO: 72), Lys-Lys-Lys- ϵ -N-Lys (SEQ ID NO: 73), and Pro-Pro-Xaa-Pro-Xaa-Pro (SEQ ID NO: 71), and any
30 combination thereof,

wherein the B cell epitope is covalently linked to the T helper epitope directly or through the optional heterologous spacer.

(6) The CGRP peptide immunogen construct of (5), wherein the B cell epitope is selected from the group consisting of SEQ ID NOs: 4-13 and 15-24.

35 (7) The CGRP peptide immunogen construct of (5), wherein the T helper epitope is selected

from the group consisting of SEQ ID NOs: 78, 77, 80-82, 85, 91, 94, 97, 98-99, 106-109, 112, and any combination thereof.

- (8) The CGRP peptide immunogen construct of (5), wherein the optional heterologous spacer is (α , ϵ -N)Lys, ϵ -N-Lys-Lys-Lys-Lys (SEQ ID NO: 72), Lys-Lys-Lys- ϵ -N-Lys (SEQ ID NO: 73),
5 or Pro-Pro-Xaa-Pro-Xaa-Pro (SEQ ID NO: 71), where Xaa is any amino acid.
- (9) The CGRP peptide immunogen construct of (5), wherein the T helper epitope is covalently linked to the amino terminus of the B cell epitope.
- (10) The CGRP peptide immunogen construct of (5), wherein the T helper epitope is covalently linked to the amino terminus of the B cell epitope through the optional heterologous spacer.
- 10 (11) A composition comprising an CGRP peptide immunogen construct according to (1).
- (12) A pharmaceutical composition comprising:
 - a. a peptide immunogen construct according to (1); and
 - b. a pharmaceutically acceptable delivery vehicle and/or adjuvant.
- (13) The pharmaceutical composition of (12), wherein
 - 15 a. the CGRP functional B epitope peptide is selected from the group consisting of SEQ ID NOs: 4-13 and 15-24;
 - b. the Th epitope is selected from the group consisting of SEQ ID NOs: 74-115; and
 - c. the heterologous spacer is selected from the group consisting of an amino acid, Lys-, Gly-, Lys-Lys-Lys-, (α , ϵ -N)Lys, ϵ -N-Lys-Lys-Lys-Lys (SEQ ID NO: 72), Lys-Lys-Lys- ϵ -N-Lys
20 (SEQ ID NO: 73), and Pro-Pro-Xaa-Pro-Xaa-Pro (SEQ ID NO: 71), and any combination thereof; and

wherein the CGRP peptide immunogen construct is mixed with an CpG oligodeoxynucleotide (ODN) to form a stabilized immunostimulatory complex.
- (14) The pharmaceutical composition of (12), wherein
 - 25 a. the CGRP peptide immunogen construct is selected from the group consisting of SEQ ID NOs: 120-127 and 130-180; and

wherein the CGRP peptide immunogen construct is mixed with an CpG oligodeoxynucleotide (ODN) to form a stabilized immunostimulatory complex.
- (15) A method for generating antibodies against CGRP in an animal comprising administering
30 the pharmaceutical composition according to (12) to the animal.
- (16) An isolated antibody or epitope-binding fragment thereof that specifically binds to the CGRP receptor binding or activation region of SEQ ID NOs: 4-13 and 15-24.
- (17) The isolated antibody or epitope-binding fragment thereof according to (16) bound to the CGRP peptide immunogen construct.
- 35 (18) A composition comprising the isolated antibody or epitope-binding fragment thereof

according to (16).

(19) A method of preventing and/or treating migraine in an animal comprising administering the pharmaceutical composition of (12) to the animal.

EXAMPLE 1

5 SYNTHESIS OF CGRP RELATED PEPTIDES AND PREPARATION OF FORMULATIONS THEREOF

a. Synthesis of CGRP related peptides

Methods for synthesizing CGRP related peptides that were included in the development effort of CGRP peptide immunogen constructs are described. The peptides were synthesized in
10 small-scale amounts that are useful for serological assays, laboratory pilot and field studies, as well as large-scale (kilogram) amounts, which are useful for industrial/commercial production of pharmaceutical compositions. A large repertoire of CGRP related antigenic peptides having sequences with lengths from approximately 10 to 70 amino acids were designed for epitope mapping and for the screening and selection of the most optimal peptide immunogen constructs
15 for use in an efficacious CGRP targeted therapeutic vaccine.

Representative full-length CGRP of human, mouse, rat and macaque species (SEQ ID NOs: 1-3), CGRP peptide fragments, and 10-mer peptide employed for epitope mapping in various serological assays are listed in **Table 1** (SEQ ID NOs: 1-70).

Selected CGRP B cell epitope peptides were made into CGRP peptide immunogen
20 constructs by synthetically linking to a carefully designed helper T cell (Th) epitope peptide which was derived from pathogen proteins including Measles Virus Fusion protein (MVF), Hepatitis B Surface Antigen protein (HBsAg), peptide influenza, Clostridium tetani, and Epstein-Barr virus (EBV) identified in **Table 2** (SEQ ID NOs: 74-115). The Th epitope peptides were used either in a single sequence (SEQ ID NOs: 74-84, 86-90, 92-93, 95-96, 98-115) or a combinatorial library
25 (SEQ ID NOs: 85, 91, 94, and 97) to enhance the immunogenicity of their respective CGRP peptide immunogen constructs.

Representative CGRP peptide immunogen constructs selected from hundreds of peptide constructs are identified in **Table 3** (SEQ ID NOs: 116-180). All peptides used for immunogenicity studies or related serological tests for detection and/or measurement of anti-CGRP antibodies were
30 synthesized on a small scale using F-moc chemistry by peptide synthesizers of Applied BioSystems Models 430A, 431 and/or 433. Each peptide was produced by an independent synthesis on a solid-phase support, with F-moc protection at the N-terminus and side chain protecting groups of trifunctional amino acids. Completed peptides were cleaved from the solid

support and side chain protecting groups were removed by 90% Trifluoroacetic acid (TFA). Synthetic peptide preparations were evaluated by Matrix-Assisted Laser Desorption/Ionization-Time-Of-Flight (MALDI-TOF) Mass Spectrometry to ensure correct amino acid content. Each synthetic peptide was also evaluated by Reverse Phase HPLC (RP-HPLC) to confirm the synthesis profile and concentration of the preparation. Despite rigorous control of the synthesis process (including stepwise monitoring the coupling efficiency), peptide analogues were also produced due to unintended events during elongation cycles, including amino acid insertion, deletion, substitution, and premature termination. Thus, synthesized preparations typically included multiple peptide analogues along with the targeted peptide.

Despite the inclusion of such unintended peptide analogues, the resulting synthesized peptide preparations were nevertheless suitable for use in immunological applications including immunodiagnosis (as antibody capture antigens) and pharmaceutical compositions (as peptide immunogens). Typically, such peptide analogues, either intentionally designed or generated through synthetic process as a mixture of byproducts, are frequently as effective as a purified preparation of the desired peptide, as long as a discerning QC procedure is developed to monitor both the manufacturing process and the product evaluation process to guarantee the reproducibility and efficacy of the final product employing these peptides. Large scale peptide syntheses in the multi-hundred to kilo gram quantities were conducted on a customized automated peptide synthesizer UBI2003 or the like at 15 mmole to 150 mmole scale.

For active ingredients used in the final pharmaceutical composition for clinical trials, CGRP related peptide immunogen constructs were purified by preparative RP-HPLC under a shallow elution gradient and characterized by MALDI-TOF mass spectrometry, amino acid analysis and RP-HPLC for purity and identity.

b. Preparation of compositions containing CGRP peptide immunogen constructs

Formulations employing water in oil emulsions and in suspension with mineral salts were prepared. In order for a pharmaceutical composition designed to be used by a large population, safety becomes another important factor for consideration. Despite the fact that water-in-oil emulsions have been used in humans as pharmaceutical compositions in many clinical trials, Alum remains the major adjuvant for use in pharmaceutical composition due to its safety. Alum or its mineral salts ADJUPHOS (Aluminum phosphate) are therefore frequently used as adjuvants in preparation for clinical applications.

Briefly, the formulations specified in each of the study groups described below generally contained all types of CGRP designer peptide immunogen constructs. Over 200 designer CGRP peptide immunogen constructs were carefully evaluated in guinea pigs for their relative

immunogenicity with the corresponding CGRP peptide representative of the immunogen's B epitope peptides. Epitope mapping and serological cross-reactivities were analyzed amongst the varying homologous peptides by ELISA assays using plates coated with peptides selected from the list with SEQ ID NOs: 1-70.

- 5 The CGRP peptide immunogen constructs at varying amounts were prepared in a water-in-oil emulsion with Seppic MONTANIDE™ ISA 51 as the approved oil for human use, or mixed with mineral salts ADJUPHOS (Aluminum phosphate) or ALHYDROGEL (Alum) as specified. Compositions were typically prepared by dissolving the CGRP peptide immunogen constructs in water at about 20 to 2000 µg/mL and formulated with MONTANIDE™ ISA 51 into water-in-oil
10 emulsions (1:1 in volume) or with mineral salts ADJUPHOS or ALHYDROGEL (Alum) (1:1 in volume). The compositions were kept at room temperature for about 30 min and mixed by vortex for about 10 to 15 seconds prior to immunization. Animals were immunized with 2 to 3 doses of a specific composition, which were administered at time 0 (prime) and 3 week post initial immunization (wpi) (boost), optionally 5 or 6 wpi for a second boost, by intramuscular route. Sera
15 from the immunized animals were then tested with selected B epitope peptide(s) to evaluate the immunogenicity of the various CGRP peptide immunogen constructs present in the formulation and for the corresponding sera's cross-reactivity with CGRP proteins. Those CGRP peptide immunogen constructs with potent immunogenicity found in the initial screening in guinea pigs were further tested in in vitro assays for their corresponding sera's functional properties. The
20 selected candidate CGRP peptide immunogen constructs were then prepared in water-in-oil emulsion, mineral salts, and alum-based formulations for dosing regimens over a specified period as dictated by the immunizations protocols.

- Only the most promising CGRP peptide immunogen constructs were further assessed extensively prior to being incorporated into final formulations for immunogenicity, duration,
25 toxicity and efficacy studies in GLP guided preclinical studies in preparation for submission of an Investigational New Drug application followed by clinical trials in patients suffering from migraine.

The following examples serve to illustrate the present invention and are not to be used to limit the scope of the invention.

30

EXAMPLE 2

SEROLOGICAL ASSAYS AND REAGENTS

Serological assays and reagents for evaluating functional immunogenicity of the CGRP peptide immunogen constructs and formulations thereof are described in details below.

a. CGRP or CGRP B epitope peptide based ELISA tests for immunogenicity and antibody specificity analysis

ELISA assays for evaluating immune serum samples described in the following Examples were developed and described below. The wells of 96-well plates were coated individually for 1 hour at 37°C with 100 µL of CGRP or CGRP B epitope peptides etc. (SEQ ID NOs: 1 to 70), at 2 µg/mL (unless noted otherwise), in 10mM NaHCO₃ buffer, pH 9.5 (unless noted otherwise).

The CGRP or CGRP B epitope peptide-coated wells were incubated with 250 µL of 3% by weight gelatin in PBS at 37°C for 1 hour to block non-specific protein binding sites, followed by three washes with PBS containing 0.05% by volume TWEEN® 20 and dried. Sera to be analyzed were diluted 1:20 (unless noted otherwise) with PBS containing 20% by volume normal goat serum, 1% by weight gelatin and 0.05% by volume TWEEN® 20. One hundred microliters (100 µL) of the diluted specimens (e.g., serum, plasma) were added to each of the wells and allowed to react for 60 minutes at 37°C. The wells were then washed six times with 0.05% by volume TWEEN® 20 in PBS in order to remove unbound antibodies. Horseradish peroxidase (HRP)-conjugated species (e.g., guinea pig or rat) specific goat polyclonal anti-IgG antibody or Protein A/G were used as a labeled tracer to bind with the antibody/peptide antigen complex formed in positive wells. One hundred microliters of the HRP-labeled detection reagent, at a pre-titrated optimal dilution and in 1% by volume normal goat serum with 0.05% by volume TWEEN® 20 in PBS, was added to each well and incubated at 37°C for another 30 minutes. The wells were washed six times with 0.05% by volume TWEEN® 20 in PBS to remove unbound antibody and reacted with 100 µL of the substrate mixture containing 0.04% by weight 3', 3', 5', 5'-Tetramethylbenzidine (TMB) and 0.12% by volume hydrogen peroxide in sodium citrate buffer for another 15 minutes. This substrate mixture was used to detect the peroxidase label by forming a colored product. Reactions were stopped by the addition of 100 µL of 1.0M H₂SO₄ and absorbance at 450 nm (A₄₅₀) determined. For the determination of antibody titers of the vaccinated animals that received the various peptide vaccine formulations, a 10-fold serial dilutions of sera from 1:100 to 1:10,000 or a 4-fold serial dilutions of sera from 1:100 to 1: 4.19 x 10⁸ were tested, and the titer of a tested serum, expressed as Log₁₀, was calculated by linear regression analysis of the A₄₅₀ with the cutoff A₄₅₀ set at 0.5.

b. Assessment of antibody reactivity towards Th peptide by Th peptide based ELISA tests

The wells of 96-well ELISA plates were coated individually for 1 hour at 37°C with 100 µL of Th peptide at 2 µg/mL (unless noted otherwise), in 10mM NaHCO₃ buffer, pH 9.5 (unless noted otherwise) in similar ELISA method and performed as described above. For the determination of antibody titers of the vaccinated animals that received the various CGRP peptide

vaccine formulations, 10-fold serial dilutions of sera from 1:100 to 1:10,000 were tested, and the titer of a tested serum, expressed as Log₁₀, was calculated by linear regression analysis of the A₄₅₀ with the cutoff A₄₅₀ set at 0.5.

c. Fine specificity analyses of a target CGRP B cell epitope peptide determined by epitope mapping through B cell epitope cluster 10-mer peptide-based ELISA tests

5 Fine specificity analyses of anti-CGRP antibodies from hosts immunized with CGRP peptide immunogen constructs were determined by epitope mapping using B cell epitope cluster 10mer peptide-based ELISA tests. Briefly, the wells of 96-well plates were coated with individual CGRP or related 10-mer peptides (SEQ ID NOs: 26-70) at 0.5 µg per 0.1mL per well and then
10 100 µL serum samples (1:100 dilution in PBS) were incubated in 10-mer plate wells in duplicate following the steps of the antibody ELISA method described above. The target B cell epitope related fine specificity analyses of anti-CGRP antibodies from immunized hosts were tested with corresponding CGRP peptide, or with non-relevant control peptide for specificity confirmation.

d. Immunogenicity Evaluation

15 Preimmune and immune serum samples from animal or human subjects were collected according to experimental vaccination protocols and heated at 56°C for 30 minutes to inactivate serum complement factors. Following the administration of the vaccine formulations, blood samples were obtained according to protocols and their immunogenicity against specific target site(s) were evaluated by corresponding CGRP B cell epitope peptide-based ELISA tests. Serially
20 diluted sera were tested and positive titers were expressed as Log₁₀ of the reciprocal dilution. Immunogenicity of a particular vaccine formulation is assessed for its ability to elicit high titer antibody response directed against the desired epitope specificity within the target antigen and high cross-reactivities with CGRP proteins, while maintaining a low to negligible antibody reactivity towards the “Helper T cell epitopes” employed to provide enhancement of the desired
25 B cell responses.

EXAMPLE 3

ASSESSMENT OF FUNCTIONAL PROPERTIES OF ANTIBODIES ELICITED BY THE CGRP PEPTIDE IMMUNOGEN CONSTRUCTS AND FORMULATIONS THEREOF IN AN IN VITRO ASSAY FOR INTRACELLULAR cAMP PRODUCTION

30 Immune sera or purified anti-CGRP antibodies in immunized vaccines were further tested for their ability to suppress the CGRP -induced intracellular AMP production.

a. Antibody purification

All antibody purification procedures were followed according to manual of antibody purification kit (Thermo fisher, Cat no. 89953). The respective concentrations of IgG purification for each of the groups were carefully calibrated for use in in vitro assay.

5 b. Cell Preparation and Maintenance

Cell line L6 (ATCC® CRL-1458™) was purchased from ATCC. The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. Fetal bovine serum was added to a final concentration of 10% to make a complete growth medium. Atmosphere: air, 95%; carbon dioxide (CO₂), 5% Temperature: 37°C.

10 c. Treatment of CGRP with/without IgGs

Anti-CGRP IgGs were further screened for neutralization activity in vitro using cell based cAMP activation assay. All procedures were done in 384 well round bottom low volume plate (Mediomics, LLC Cat. No. 163301). Five micro liters of rat α -CGRP (final concentration 10 nM) in the presence of an anti-CGRP IgG (final concentration 1-20 μ g/ml), was incubated at room temperature for 30 minutes. Then five microliters of 5000 rat L6 muscle cells in 1XKRB-IBMS buffer were added. The plate was incubated at room temperature for 30 minutes.

d. Cell based CGRP neutralization test (cAMP level detection)

After the incubation, cAMP activation was performed using Mediomics Bridge-It cAMP all in one Fluorescence Assay (Mediomics, Cat. No. 122938/122939) following manufacture's instruction. 10 μ l of the cAMP all in one assay solution was added to each of the wells and mixed by pipetting up and down to lyse the cells and start the cAMP assay. The plate was covered to avoid evaporation and exposure to light. The plate was incubated at room temperature for 30 minutes with fluorescence intensity (excitation 485nm, emission 540nm) read by a fluorescence reader (SpectraMax i3x Multi-Mode Microplate Reader). Data are recorded in percentage. 0% represents L6 cell alone and 100% stands for CGRP treated L6 cell.

EXAMPLE 4**ANIMALS USED IN SAFETY, IMMUNOGENICITY, TOXICITY AND EFFICACY STUDIES****a. Guinea Pigs:**

30 Immunogenicity studies were conducted in mature, naïve, adult male and female Duncan-Hartley guinea pigs (300-350 g/BW). The experiments utilized at least 3 Guinea pigs per group.

Protocols involving Duncan-Hartley guinea pigs (8–12 weeks of age; Covance Research Laboratories, Denver, PA, USA) were performed under approved IACUC applications at a contracted animal facility under UBI sponsorship.

b. Cynomolgus macaques:

- 5 Immunogenicity and repeated dose toxicity studies in adult male and female monkeys (Macaca fascicularis, approximately 3-4 years of age; Joynn Laboratories, Suzhou, China) were conducted under approved IACUC applications at a contracted animal facility under UBI sponsorship.

c. Mice:

- 10 Female Balb/C mice (n = 6/group) were dosed for intramuscular injection (IM) with 40 µg /0.1ml/dose test vaccine or 0.1ml/dose control article in one injection site (quadricep femoral muscle on hind limb), 5 shots at 0, 3, 6, 9, 12 wpi prior to capsaicin challenge. Animals were housed at UBI Asia Laboratory Animal Facility and acclimatized for 1 week under constant temperature (22°C), humidity (72%), 12-h light/12-h dark cycle. Mice had free access to chow
15 and water. All protocols followed the Principles of Laboratory Animal Care. Blood collection was carried out as indicated in the protocol. Antibody titers were tested for anti-CGRP (mouse) by ELISA assay.

EXAMPLE 5

**VACCINE FORMULATIONS FOR IMMUNOGENICITY ASSESSMENT OF CGRP
20 PEPTIDE CONSTRUCTS IN GUINEA PIGS**

Pharmaceutical compositions and vaccine formulations used in each experiment are described in greater detail as shown below.

- Briefly, the formulations specified in each of the study groups generally contained all types of designer CGRP peptide immunogen constructs with a segment of the CGRP B cell epitope peptide linked via different type of spacers (e.g., εLys (εK) or lysine-lysine-lysine (KKK) to
25 enhance the peptide construct's solubility) and promiscuous helper T cell epitopes including two sets of artificial T helper epitopes derived from Measles virus fusion protein and Hepatitis B surface antigen. The CGRP B epitope peptides are linked at the N- or C- terminus of the designer peptide constructs. Hundreds of designer CGRP peptide immunogen constructs were initially
30 evaluated in guinea pigs for their relative immunogenicity with the corresponding CGRPB cell epitope peptides. The CGRP peptide immunogen constructs were either prepared under varying amounts in a water-in-oil emulsion with Seppic MONTANIDE ISA 51 as the approved oil for

human vaccine use, or with mineral salts (ADJUPHOS) or ALHYDROGEL (Alum) as a suspension, as specified. Vaccine formulations were usually prepared by dissolving the CGRP peptide constructs in water at about 20 to 800 µg/mL and formulated either with MONTANIDE ISA 51 into water-in-oil emulsions (1:1 in volume) or with mineral salts (ADJUPHOS) or ALHYDROGEL (Alum) (1:1 in volume). The vaccine formulations were kept at room temperature for about 30 min and mixed by vortex for about 10 to 15 seconds prior to immunization.

Some animals were immunized with 2 to 5 doses of a specific vaccine formulation, which were administered at time 0 (prime) and 3 weeks post initial immunization (wpi) (boost), optionally 5 or 6 wpi for a second boost, by intramuscular route. These immunized animals were then evaluated for the immunogenicity of the corresponding CGRP peptide immunogen constructs used in the respective vaccine formulations for their cross-reactivity with the corresponding CGRP B epitope peptides or full-length CGRP. Those CGRP peptide immunogen constructs with potent immunogenicity in the initial screening in guinea pigs were further tested in both water-in-oil emulsion, mineral salts, and alum-based formulations in macaques for dosing regimens over a specified period as dictated by the immunization protocols.

Only the most promising CGRP peptide immunogen construct candidates were further assessed extensively to evaluate for their ability to breakout immune tolerance in mice using corresponding mouse CGRP peptide immunogen constructs. The CGRP peptide immunogen constructs with best immunogenicity in mice, which elicited anti-CGRP antibody titers against endogenous CGRP; especially for their capability of suppressing Capsaicin induced dermal blood flow in mice model. The optimized CGRP peptide immunogen constructs were incorporated into final vaccine formulations for GLP guided immunogenicity, duration, toxicity and proof of efficacy studies in preparation for submission of an Investigational New Drug application and clinical trials in patients with migraine.

EXAMPLE 6

DESIGN RATIONALE, SCREENING, IDENTIFICATION, ASSESSMENT OF FUNCTIONAL PROPERTIES AND OPTIMIZATION OF MULTI-COMPONENT VACCINE FORMULATIONS INCORPORATING CGRP PEPTIDE IMMUNOGEN CONSTRUCTS FOR TREATMENT OF MIGRAINE

Based on scientific information provided by **Figures 1 and 2**, as well as information disclosed in Fig. 5 of Russel, et al., 2014 and Figs. 2(a)-2(c) of Edvinsson, et al., 2018, CGRP is selected as the target molecule for design and as the content of this instant invention. **Figure 1**

presents alignment of CGRP sequences from human (SEQ ID NO: 1), marmoset (SEQ ID NO: 2), mouse/rat (SEQ ID NO: 3) and many other species; Fig. 5 of Russel, et al., 2014 depicts the physiology and pathophysiology associated with CGRP; and Figs. 2(a)-2(c) of Edvinsson, et al., 2018 depicts why CGRP could be a target for new migraine therapies. **Figure 2** depicts the pathways from discovery to commercialization of high precision designer synthetic peptide based vaccines. A general summary of the steps is described in **Figure 2** with a flow chart identifying the development process from discovery to commercialization (industrialization) of an CGRP vaccine formulation. Detailed evaluation and analyses of each of the steps, with pleasant and unpleasant surprises, had led to a myriad of experiments in the past which would ultimately result in commercialization of a safe and efficacious CGRP vaccine formulation.

a. Design History

Each peptide immunogen construct or immunotherapeutic product requires its own design focus and approach based on its specific disease mechanism and the target protein(s) required for intervention. For treatment of migraine, CGRP was selected as the target molecule based on the scientific information available to us as outlined in **Figure 1** and in Fig. 5 of Russel, et al., 2014 and Figs. 2(a)-2(c) of Edvinsson, et al., 2018. The pathways from discovery to commercialization as shown in **Figure 2** typically require one or more decades to accomplish. Identification of the CGRP B cell epitope peptides correlating to the functional site(s) for intervention are key to the immunogen construct design. Consecutive pilot immunogenicity studies in guinea pigs incorporating various T helper support (carrier proteins or suitable T helper peptides) in various formulations were conducted and subsequently evaluated for the functional properties of the elicited purified antibodies or the vaccine formulations employing specific CGRP peptide immunogen constructs in specific in vitro functional assays or proof of concept in vivo studies in selected animal models. Upon extensive serological validation, candidate CGRP B epitope peptide immunogen constructs were then further tested in non-human primates to further validate the immunogenicity and direction of the CGRP peptide immunogen design. Selected CGRP peptide immunogen constructs are then prepared in varying mixtures to evaluate the subtle differences in functional properties related to the respective interactions amongst peptide constructs when used in combinations. Upon additional evaluation, the final peptide constructs, peptide compositions and formulations thereof, along with the respective physical parameters of the formulations are established leading to the final product development process.

The amino acid sequences of the CGRP peptide immunogen constructs were selected based on a number of design rationales. Several of these rationales include employing a CGRP B epitope peptide sequence that:

(i) is devoid of an autologous T helper epitope within CGRP to prevent autologous T cell activation which could lead to inflammation of the brain resulting in meningococcal encephalitis as previously reported in clinical trials using AN1792 vaccine targeting A β ₁₋₄₂ for treatment of Alzheimer's Disease;

5 (ii) is non-immunogenic on its own, since it is a self-molecule;

(iii) can be rendered immunogenic by a protein carrier or a potent T helper epitope(s) upon administration to a host:

(iv) elicits high titer antibodies directed against the CGRP peptide sequence (B cell epitope) and not against the protein carrier or potent T helper epitope(s);

10 (v) elicits high titer antibodies that would suppress the induction of intracellular cAMP rise due to CGRP and CGRP receptor interaction and cellular activation; and

(vi) such vaccine formulations, when administered in an animal model, e.g. BALB/C mice, would suppress the dorsal blood flow induced by Capsaicin, as a proof of concept validation for the treatment of migraine.

15 **b. Design and validation of CGRP peptide immunogen constructs for pharmaceutical compositions with potential to treat patients suffering from migraine.**

In order to generate the most potent peptide constructs for incorporation into the pharmaceutical compositions, a repertoire of human CGRP B cell epitope peptides (e.g. SEQ ID NOs: 4-24) and promiscuous T helper epitopes derived from various pathogens or artificially T
20 helper epitopes (e.g. SEQ ID NOs: 74-115) were further designed and made into for example representative CGRP peptide immunogen constructs (e.g., SEQ ID NOs: 116-180) for immunogenicity studies initially in guinea pigs.

i) Selection of CGRP B cell epitope peptide sequences from the receptor binding or receptor activation region for design

25 The CGRP receptor binding region located at the central/C-terminus of CGRP and the receptor activation region from N-terminus C2-C7 loop/central region of CGRP were selected for CGRP B epitope design and then further made into peptide immunogen constructs to elicit immune sera in guinea pigs initially for immunogenicity by ELISA on CGRP B epitope peptide coated plates and subsequently for in vitro functional assay assessment.

30 Upon binding of CGRP to CGRP receptor, CGRP receptor transmits the activation signals intracellularly leading to intracellular rise of cAMP level amongst other cellular events. The ability of purified antibodies from guinea pig immune sera against specific CGRP peptide immunogen constructs to neutralize the functional properties of CGRP is assessed for IC₅₀ to inhibit 50% of cAMP rise when compared to the control in the absence of antibodies as shown in **Figure 6** and

the tables displayed within **Figure 3**.

These CGRP peptide immunogen constructs were formulated initially with ISA 51 and CpG for prime immunization in guinea pigs at 400µg/1mL and boosts (3, 6 and 9 wpi) at 100µg/0.25mL for immunogenicity studies. To test the immunogenicity in guinea pigs, ELISA assay was used with guinea pig immune sera from various (wpi) bleeds, diluted at a 10-fold serial dilution from 1: 100 to 1:10000. ELISA plates were coated with corresponding mouse/rat CGRP B epitope peptide and full-length CGRP peptide at 0.5 µg peptide per well. The titer of a tested serum, expressed as Log₁₀, was calculated by linear regression analysis of the A_{450nm} with the cutoff A₄₅₀ set at 0.5 as shown in **Figure 3** with detailed titers for representative B epitope derived CGRP peptide immunogen constructs as shown in **Tables 4, 5 and 6**. Despite that the designed short CGRP peptides frequently are non-immunogenic due to their lack of endogenous Th epitopes, addition of foreign Th epitopes could enhance the immunogenicity of the specific CGRP peptide immunogen constructs. Detailed analyses of the reactivity/specificity pattern of various constructs from **Tables 4, 5, and 6** along with **Figure 3**, immunogenicity imparted by certain residues within the CGRP molecule can be assessed which would facilitate further design of the optimal peptide immunogen constructs.

ii) Devoid of an autologous T helper epitope within selected CGRP B epitope to prevent autologous T cell activation

As shown in **Table 7**, representative CGRP B cell epitopes such as those with SEQ ID Nos: 5, 6 and 15, when administered in potent vaccine formulations used for counterpart peptide immunogen constructs, they themselves did not elicit any antibodies to CGRP thus are devoid of the undesirable endogenous Ths epitope within the selected CGRP B cell epitopes.

iii) Focused antibody response elicited by CGRP peptide immunogen constructs is targeted at the CGRP B cell epitope only

It is well known that all carrier proteins (e.g. Keyhole Limpet Hemocyanin (KLH), Diphtheria toxoid (DT) and Tetanus Toxoid (TT) proteins) used to potentiate an immune response directed against the targeted B cell epitope peptide, by chemical conjugation of such B cell epitope peptide to the respective carrier protein, will elicit more than 90% of the antibodies directed against the potentiating carrier protein with less than 10% of the antibodies directed against the targeted B cell epitope in immunized hosts.

It is therefore of interest to assess the specificity of the CGRP peptide immunogen constructs of the present invention. Two representative CGRP peptide immunogen constructs (SEQ ID NOs: 142 and 142 from **Table 8**), with B cell epitopes of varying lengths from CGRP 20-37 and 22-37 that are linked through a spacer sequence to the heterologous T cell epitope

UBITH®1 (SEQ ID NO: 98), were prepared for immunogenicity assessment. The UBITH®1 (T helper peptide used for B epitope immunopotentiality) was coated to the plates and the guinea pig immune sera were employed to test for cross-reactivities with the UBITH®1 peptide used for immunopotentiality. In contrast to the high immunogenicity of these constructs towards the corresponding targeted CGRP B cell epitope peptides as illustrated by the high titers of antibodies (>5 Log₁₀) generated towards the CGRP B epitope(s) upon even a single shot, while as most, if not all, of the immune sera were found non-reactive to the UBITH®1 peptide as shown in **Table 8**.

In summary, simple immunogen design incorporating target CGRP B cell epitope peptide linked to carefully selected T helper epitope allows the generation of a focused immune response targeted only to the corresponding CGRP B cell epitope peptide. For pharmaceutical composition design, the more specific the immune response it generates, the higher safety profile it provides for the composition. The CGRP peptide immunogen constructs of this instant invention is thus highly specific yet highly potent against its B cell target.

iv) Fine epitope mapping with immune sera directed against selected CGRP peptide immunogen constructs

In a fine epitope mapping study as shown in **Table 9** to localize the antibody binding site(s) to specific residues within the target B epitope region, 45 overlapping 10-mer peptides (SEQ ID NOs: 26-70) were synthesized that cover from amino acid -9 to amino acid 45 sequence covering the full-length region of CGRP along with the precursor sequences before and after the processed CGRP molecule. These 10-mer peptides were individually coated onto 96-well microtiter plate wells as solid-phase immune-absorbents. The pooled guinea pig antisera were added at a 1:100 dilution in specimen diluent buffer to the plate wells coated with 10-mer peptide at 2.0 µg/mL followed by incubation for one hour at 37° C. After washing the plate wells with wash buffer, the horseradish peroxidase-conjugated rProtein A/G was added and incubated for 30 min. After washing with PBS again, the substrate was added to the wells for measurement of absorbance at 450nm by ELISA plate reader, when the samples were analyzed in duplicate. The binding of CGRP peptide immunogen elicited immune sera to the corresponding CGRP B cell epitope peptide coated wells represent the maximal antibody binding signal.

The fine epitope mapping results as shown in **Table 9** revealed that the pooled guinea pig sera from CGRP peptide immunogen constructs of SEQ ID NOs: 122, 123, 127, 129, 130, 132, 137, and 139 comprising CGRP B epitope peptides from both the receptor binding region from AAs 11 to 37 and the receptor activation region around the C2-C7 loop from AAs 1-25, 11-25, 8-18, 11-35, 11-37, 15-37 and 18-37 induced high titer antibodies mainly against a cluster of 10mer

peptides from amino acid 1-37 (SEQ ID NO: 1) with high cross-reactivities to peptides mainly with amino acids of varying patterns from 1-13 for B epitope 1-25; 15-26 for B epitope 11-25; none major for B epitope 8-18; 22-33 and 26-36 for B epitope 11-35; 23-33 and 28-37 for B epitope 11-37; 23-33 and 28-37 for B epitope 18-37; 22-33 and 26-36 for B epitope 11-35; 17-26, 20-30 and 28-37 for B epitope 15-37. It is of interest to find that despite the same B epitope was employed for the design of peptide immunogen constructs SEQ ID NOs: 123 and 130 with only spacer difference with SEQ ID NO: 123 being with an extra KKK spacer when compared to SEQ ID NO: 130, extra reactivities towards AAs 20-30 and AAs 26-36 were found with the construct SEQ ID NO: 130 which had a shorter spacer without the KKK residues.

In summary, the designed synthetic CGRP peptide immunogen constructs induced robust immune responses in guinea pigs generating polyclonal antibodies targeted at distinct clusters of 10mer peptides within CGRP, which have close proximity to both the CGRP receptor binding region near by the respective C-terminus and the receptor activation region nearby the C2-C7 loop, allowing for important medical interventions. Epitope mapping along with functional assay assessment would allow identification of the most optimal peptide immunogen constructs for use in vaccine formulations.

EXAMPLE 7

ASSESSMENT OF FUNCTIONAL PROPERTIES OF ANTIBODIES ELICITED BY THE CGRP PEPTIDE IMMUNOGEN CONSTRUCTS AND FORMULATIONS THEREOF IN AN EX- VIVO MODE

After demonstration of the high immunogenicity and cross-reactivities of the antibodies purified from immune sera of guinea pigs immunized with carefully selected candidate CGRP immunogen constructs as shown in **Tables 4, 5, 6, 7, 8 and 9**, the following studies were designed to assess whether representative purified IgGs from these immune sera collected at 6 wpi from each animal could suppress intracellular rise of cAMP due to activation by the C2-C7 loop within the CGRP upon binding of CGRP to its receptor.

At a molecular level within smooth muscle cells, CGRP could bind to its receptor via its C-terminal region and then activate the receptor by using its loop region (reference). The cyclic C2-C7 loop with a disulfide bridge has a basic role in receptor activation which correlates closely with a rise in intracellular cAMP. Various anti-CGRP IgGs were used to characterize their potential anti-CGRP influence in a neutralization assay. Specifically, the effect was assessed by functional pharmacology using the alterations in intracellular cAMP levels. This in vitro functional assessment is particularly important to assess the anti-CGRP effect of those guinea pig immune

sera directed against CGRP peptide immunogen constructs of the current invention with assay procedures detailed in EXAMPLE 4.

Suppression of intracellular cAMP rise in CGRP activated phosphorylation by anti-CGRP antibodies

5 Immune sera from 6 wpi bleeds of each animal were collected with antibodies purified as described in EXAMPLE 4. Twenty-one CGRP peptide immunogen constructs were tested in guinea pigs for their respective immunogenicity as demonstrated in EXAMPLE 6. The purified antibodies were grouped into three categories based on their respective target B cell epitope peptides employed in the peptide immunogen constructs. They are those directed at B cell epitope peptides from the N-terminal region, Central region and those from the C-terminal region respectively. Data are recorded in percentage of cAMP detected within the CGRP treated L6 cell. Zero % represents L6 cell alone and 100% stands for CGRP treated L6 cell. As shown in **Figure 4**, along with the accompanying tables, there are constructs incorporating CGRP B epitope peptides from N-terminal, Central, or C-terminal region that showed IC_{50} ($\mu\text{g/mL}$) for cAMP level from 0.60 to >20. For practical purpose, we use IC_{50} at less than $10\mu\text{g/mL}$ as significant in antibody mediated suppression of cAMP production. Representative constructs that showing effective functional immunogenicity are ranked (with IC_{50} $\mu\text{g/mL}$ from low to high) as SEQ ID NOs: 130 > 127 > 150 > 125 > 137 > 123 > 121 > 119 > 124 > 131 > 126 > 133 > 120 > 129 > 135 > 116 ~ 117 ~ 118 ~ 128 ~ 139 and we can delineate the optimal design for CGRP peptide immunogen constructs with high precision up to a few residues within the CGRP 37mer structure.

In summary, CGRP peptide immunogen constructs as shown above in their relative ranking for respective functional properties are of value for use in subsequent CGRP vaccine formulations to demonstrate functional efficacy.

EXAMPLE 8

ASSESSMENT OF REPRESENTATIVE MOUSE CGRP PEPTIDE IMMUNOGEN CONSTRUCT IN A PREVENTIVE MODE ON THE CAPSAICIN INDUCED DERMAL BLOOD FLOW MODEL IN BALB/C MICE AS A PROOF OF CONCEPT CGRP VACCINE STUDY

a. Study rationales.

30 Calcitonin gene-related peptide (CGRP) is a 37 amino acid peptide that is widely expressed in the central and peripheral nervous system. It is primarily associated with small unmyelinated sensory neurons, which are in close proximity of blood vessels. CGRP is a potent

vasodilator and local administration of CGRP causes transient increases in blood flow. CGRP has also been associated with pain transmission, pain modulation, and neurogenic inflammation. CGRP can be released from sensory neurons via activation of the transient receptor potential cation channel using capsaicin.

5 Laser Doppler Imaging (LDI) has been used to detect the resulting changes in dermal blood flow which has been shown to be predominantly caused by CGRP. CGRP is also linked to inflammatory pain as demonstrated by attenuated responses in CGRP knock-out mice in a number of pain models. This role in pain perception is congruent with the expression of CGRP in sensory neurons.

10 In the mammalian plasma, the half-life of CGRP is ~ 10 min. In the human trigeminal ganglia, CGRP-immunoreactive neurons account for up to 50% of all neurons (Tajti, et al., 1999). By targeting the CGRP to treat the migraine headaches with anti-CGRP vaccination approach can fulfill an unmet medical needs to treat migraine in a prophylactic mode cost effectively over a long duration with the potential for long-term CGRP blockade.

15 This study is designed to test in a Balb/C mice model the murine CGRP peptide immunogen constructs of representative counterparts of human CGRP peptide immunogen constructs counterpart identified in EXAMPLE 7 for their ability in reduction of Capsaicin induced dermal blood flow as a proof of concept study to illustrate the efficacy of the anti CGRP vaccine treatment for migraine.

20 **b. Study design.**

i) Representative Test Articles:

CGRP peptide immunogen construct vaccine formulations used in this study are listed below:

- Group 1: UBITH®1-human α CGRP₁₁₋₃₇ (SEQ ID NO: 123) with ISA51 and CpG3
 - 25 Group 2: UBITH®3-human α CGRP₁₁₋₃₇ (SEQ ID NO: 141) with ISA51 and CpG3
 - Group 3: UBITH®3-rat/mouse α CGRP₁₁₋₃₇ (SEQ ID NO: 151) with ISA51 and CpG3
 - Group 4: UBITH®1-human α CGRP₁₁₋₃₇ (SEQ ID NO: 123) with ADJUPHOS and CpG3
 - Group 5: UBITH®1-human α CGRP (SEQ ID NO: 131) with ISA51 and CpG3
- These articles along with group information were listed in **Table 2**.

30 **ii) Control Articles:**

- Group 6: ISA51 and CpG3
- Group 7: ADJUPHOS and CpG3
- Group 8: Saline

iii) Groups and Dosage:

Grouping is executed as following:

iv) Individual identification:

Tested animals were identified by picric acid marker.

v) Group identification:

Cages were properly labeled for identification, including the Study Title, IACUC No., Route of Administration, Observation Period, Cage No., Quantity/cage, Species, Strain, Gender, In House Date, In House Age, Animal ID No., Keeper and Deputy

vi) Administration Route and Injection Site:

Female Balb/C mice (n = 6/group) were dosed intramuscularly (IM) with 40 µg /0.1ml/dose for test vaccine article or 0.1ml/dose for control article in one injection site (quadricep femoral muscle on hind limb), 5 shots at 0, 3, 6, 9, 12 wpi prior to capsaicin challenge.

vii) Treatment Schedule:

A total of 5 injections of control or test articles were administered during the course of treatment at 0, 3, 6, 9 and 12 wpi.

viii) Bio-sample Collection and preparation:

Blood were clotted in tube at room temperature, undisturbed for a minimum of 30 to a maximum of 60 minutes to separate serum from blood clot. Blood clot was removed by centrifugation at 1,000 x g for 10 minutes using a refrigerated centrifuge. Serum was transferred immediately into sterile 1.5 mL Eppendorf polypropylene tubes. All samples were maintained on wet ice during handling. Unused serum samples were stored frozen at -80°C.

ix) Laser Doppler Imaging (LDI):

On the day of the experiment, the mouse lower backs were shaved and the mice were placed on a heating pad under the LDI instrument. Anesthesia was induced with 25mg/kg Zoletil and the animal was stabilized under anesthesia for approximately 20 min prior to scanning. The scan series began with two baseline scans after which

2 µL of capsaicin solution (50 mg capsaicin in a solution of 83.4µL EtOH, 55.6µL Tween 20, 27.8µL purified H₂O in a 3:2:1 ratio) was applied to each of the two O-rings which were placed on the lower back of the animal. Scanning was continued with a scan every 2.5 min for an additional 10 min. Data were analyzed using moorVMS-LDF software for regions of interest. Excel worksheets were used for averaging the signal from the regions of interest at each time

point. Data are reported as percent change from the baseline.

x) Immunological Analysis of Antibody Titers:

Samples of serum or CSF were collected with anti- CGRP antibody titers measured by ELISA kits prepared as shown in EXAMPLE 2 using full-length human CGRP for antigen coating.

- 5 Serum samples were serially diluted 3-fold with a starting dilution of 1:1000. The antibody ELISA titers, expressed in Log₁₀, were determined using an automated plate reader at absorbance, A₄₅₀ nm.

c. Study Results.

i) Injection site reaction:

- 10 Slight and temporal swelling at the injection site was occasionally observed as a result of immunization procedure.

ii) Antibody Titers of the Immune Sera from Immunized Mice:

- 15 Representative ELISA results showed that two peptide immunogen constructs from mice counterparts (e.g. SEQ ID NO: 151) of CGRP peptide immunogen constructs (SEQ ID NOs: 132 and 141) not only induced high immunogenicity titers against their respective B epitope peptides (SEQ ID NOs: 15 and 9), antibodies from these two immune sera were also found to have moderate cross-reactivity against their homologous full-length human CGRP as shown in **Figure 5**. The study indicated that the two representative CGRP peptide immunogens are able to induce specific antibodies with cross-reactivity against human CGRP B epitope peptide and its mouse
20 counterpart peptide. The mouse/rat CGRP B epitope peptide immunogen construct counterparts employed UBITH®3 (a combinatorial Th peptide library that is more potent than the UBITH®1) as the T helper peptide to enhance further the immunogenicity of the selected constructs in mice (e.g. SEQ ID NO: 132, 134, 136, 138, 140, 141, and 151) and linker (e.g. SEQ ID NO: 72) in this POC animal study.

25 **iii) LDI result:**

- The Capsaicin model for dermal blood flow measurement is an in vivo pharmacodynamic model in both animals as well as humans. It is non-invasive, technically uncomplicated and gives a rapid and objective endpoint. This model can be repeatedly tested with measurements being adequately reproducible. This model therefore offers an ideal assessment for clinical evaluation
30 of CGRP blocking therapeutics. Like all other biomarker models, this model also has its limitations. The Capsaicin model remains a simulation of a naturally occurring pathophysiological process of a desired study. The effect of a drug or vaccine induced antibodies on Capsaicin-

induced dermal blood flow could offer trends of anti-CGRP activity that are indicative for their efficacy in inhibiting peripheral dermal blood flow.

As shown in **Figure 6**, when compared to the control articles of saline or various adjuvant alone formulations, the CGRP peptide immunogen constructs with SEQ ID NOs: 123, 131, 141, and 151 in both ADJUPHOS and ISA51 formulations containing CpG in the formation of peptide/CpG complexes, elicited significant specific titers (Log_{10} from 4 to 6) of anti CGRP antibodies in the vaccinated mice after receiving three immunizations. The animals showed respective suppression of the Capsaicin-induced dermal microvascular blood flow (**Figure 6**) with the measurements taken at 8 wpi, 12 wpi and 14 wpi. The duration of such inhibition lasted 14 weeks for the period measured. Despite differences found in the titers of the selected antibody preparations in the immunogenicity study, or their corresponding IC_{50} s in an in vitro assay measurement for the respective antibodies' cAMP inhibition potencies, the three representative CGRP peptide immunogen constructs (e.g. SEQ ID NOs: 123, 131, and 141) and formulations thereof, shared similar capability in the reduction of Capsaicin-induced dermal blood flow in these vaccinated mice, indicative of the efficacy in using these CGRP peptide immunogen constructs for the treatment of migraine.

Table 1
Amino Acid Sequences of α -CGRP and Fragments Thereof Employed in Serological Assays

Amino Acid positions within α -CGRP	SEQ ID NO:	Sequence
Human α -CGRP 1-37	1	ACDTA TCVTH RLAGL LSRSG GVVKN NEVPT NVGSK AF
Marmoset α -CGRP 1-37	2	ACDTA TCVTH RLAGL LSRSG GMVKN NEVPT NVGSE AF
Rat/Mouse α -CGRP 1-37	3	SCNTA TCVTH RLAGL LSRSG GVVKD NEVPT NVGSE AF
α -CGRP 1-10	4	ACDTA TCVTH
α -CGRP 31-37	5	NVGSK AF
α -CGRP 28-37	6	VPTNV GSKAF
α -CGRP 25-37	7	NNFVP TNVGS KAF
α -CGRP 18-37	8	RSGGV VKNNF VPTNV GSKAF
α -CGRP 11-37	9	RLAGL LSRSG GVVKN NEVPT NVGSK AF
α -CGRP 1-15	10	ACDTA TCVTH RLAGL
α -CGRP 1-18	11	ACDTA TCVTH RLAGL LSR
α -CGRP 1-20	12	ACDTA TCVTH RLAGL LSRSG
α -CGRP 1-25	13	ACDTA TCVTH RLAGL LSRSG GVVKN
α -CGRP 8-18	14	VTHRL AGLLS R
α -CGRP 11-25	15	RLAGL LSRSG GVVKN
α -CGRP 11-30	16	RLAGL LSRSG GVVKN NEVPT
α -CGRP 11-33	17	RLAGL LSRSG GVVKN NEVPT NVG
α -CGRP 11-35	18	RLAGL LSRSG GVVKN NEVPT NVGSK
α -CGRP 15-37	19	LLSRS GGVVK NNFVP TNVGS KAF
α -CGRP 20-37	20	GGVVK NNFVP TNVGS KAF
α -CGRP 22-37	21	VVKNN FVPTN VSKA F
α -CGRP 11-18	22	RLAGL LSR
α -CGRP 5-18	23	ATCVT HRLAG LLSR
α -CGRP 4-18	24	TATCV THRLA GLL
Rat α -CGRP 11-37	25	RLAGL LSRSG GVVKD NEVPT NVGSE AF
α -CGRP -9 - 1	26	GSRII AQKRA
α -CGRP -8 - 2	27	SRIIA QKRAC
α -CGRP -7 - 3	28	RIIAQ KRACD
α -CGRP -6 - 4	29	IIAQK RACDT
α -CGRP -5 - 5	30	IAQKR ACDTA
α -CGRP -4 - 6	31	AQKRA CDTAT
α -CGRP -3 - 7	32	QKRAC DTATC
α -CGRP -2 - 8	33	KRACD TATCV
α -CGRP -1 - 9	34	RACDT ATCVT
α -CGRP 1 - 10	35	ACDTA TCVTH
α -CGRP 2 - 11	36	CDTAT CVTHR
α -CGRP 3 - 12	37	DTATC VTHRL
α -CGRP 4 - 13	38	TATCV THRLA
α -CGRP 5 - 14	39	ATCVT HRLAG
α -CGRP 6 - 15	40	TCVTH RLAGL
α -CGRP 7 - 16	41	CVTHR LAGLL
α -CGRP 8 - 17	42	VTHRL AGLLS
α -CGRP 9 - 18	43	THRLA GLLSR

Amino Acid positions within α -CGRP	SEQ ID NO:	Sequence
α -CGRP 10 – 19	44	HRLAG LLSRS
α -CGRP 11 – 20	45	RLAGL LSRSG
α -CGRP 12 – 21	46	LAGLL SRSGG
α -CGRP 13 – 22	47	AGLLS RSGGV
α -CGRP 14 – 23	48	GLLSR SGGVV
α -CGRP 15 – 24	49	LLSRS GGVVK
α -CGRP 16 – 25	50	LSRSG GVVKN
α -CGRP 17 – 26	51	SRSGG VVKNN
α -CGRP 18 – 27	52	RSGGV VKNNF
α -CGRP 19 – 28	53	SGGVV KNNFV
α -CGRP 20 – 29	54	GGVVK NNFVP
α -CGRP 21 – 30	55	GVVKN NFVPT
α -CGRP 22 – 31	56	VVKNN FVPTN
α -CGRP 23 – 32	57	VKNNF VPTNV
α -CGRP 24 – 33	58	KNNFV PTNVG
α -CGRP 25 – 34	59	NNFVP TNVGS
α -CGRP 26 – 35	60	NFVPT NVGSK
α -CGRP 27 – 36	61	FVPTN VGSKA
α -CGRP 28 – 37	62	VPTNV GSKAF
α -CGRP 29 – 38	63	PTNVG SKAFG
α -CGRP 30 – 39	64	TNVGS KAFGR
α -CGRP 31 – 40	65	NVGSK AFGRR
α -CGRP 32 – 41	66	VGSKA FGRRR
α -CGRP 33 – 42	67	GSKAF GRRRR
α -CGRP 34 – 43	68	SKAFG RRRRD
α -CGRP 35 – 44	69	KAFGR RRRDL
α -CGRP 36 – 45	70	AFGRR RRD _{LQ}
Flexible Hinge Spacer	71	PPXPXP
Spacer1	72	ϵ K-KKK
Spacer2	73	KKK- ϵ K

Table 2
Amino Acid Sequences of Pathogen Protein Derived Th Epitopes Including Idealized Artificial Th Epitopes for Employment in the Design of α -CGRP Peptide Immunogen Constructs

Description	SEQ ID NO:	Sequence
Clostridium tetani1 Th	74	KKQYIKANSKFIGITEL
MvF1 Th	75	LSEIKGVIVHRLEGV
Bordetella pertussis Th	76	GAYARCPNGTRALTVAELRGNAEL
Clostridium tetani2 Th	77	WVRDIIDDFTNESQKT
Diphtheria Th	78	DSETADNLEKTVAALSILPGHGC
Plasmodium falciparum Th	79	DHEKKHAKMEKASSVFNVNS
Schistosoma mansoni Th	80	KWFKTNAPNGVDEKHRH
Cholera Toxin Th	81	ALNIWDRFDVFCTLGATTGYLKGNS
MvF2 Th	82	ISEIKGVIVHKIEGI
KKKMvF3 Th	83	KKKISISEIKGVIVHKIEGILF
	84	T RT TR T
	85	KKKISIXEIXXVIVXXIEXILF
HBsAg1 Th	86	KKKLFLLTLLTPQSLD
	87	RRRIKII RII I L IR
	88	VRVV VV V I V
	89	F FF FF F V F
	90	F
	91	XXXXXXXXTXXXTXPSXX
	92	ISISEIKGVIVHKIETILF
MvF4 Th (UBITh®3)	93	T RT TR
	94	ISIXEIXXVIVXXIETILF
	95	KKKIITITRIITIPQSLD
HBsAg2 Th	96	FLL L ITTI
	97	KKKXXXXTRIXTIXXXD
MvF5 Th (UBITh®1)	98	ISITEIKGVIVHRIETILF
HBsAg3 Th (UBITh®2)	99	KKKIITITRIITIITID
Influenza MP1_1 Th	100	FVFTLTVPSEER
Influenza MP1_2 Th	101	SGPLKAEIAQRLEDV
Influenza NSP1 Th	102	DRLRRDQKS
EBV BHRF1 Th	103	AGLTLSLLVICSYLFISRG
Clostridium tetani TT1 Th	104	QYIKANSKFIGITEL
EBV EBNA-1 Th	105	PGPLRESIVCYFMVFLQTHI
Clostridium tetani TT2 Th	106	FNNFTVSFVLRVPKVSASHLE
Clostridium tetani TT3 Th	107	KFIKRYTPNNEIDSF
Clostridium tetani TT4 Th	108	VSIDKFRIFCKALNPK
EBV CP Th	109	VPGLYSPCRAFFNKEELL
HCMV IE1 Th	110	DKREMMMACIKELH
EBV GP340 Th	111	TGHGARTSTEPTDY
EBV BPLF1 Th	112	KELKRQYEKKLRQ
EBV EBNA-2 Th	113	TVFYNIIPMPL
HBsAg4 Th (UBITh®4)	114	FLLTTRILITIPQSLD
Yersinia Invasin Th (Inv)	115	ALNIWDRFDVFCTLGATTGYLKGNS

Table 3
Amino Acid Sequences of α -CGRP Peptide Immunogen Constructs

Peptide Description	SEQ ID NO:	Sequence
UBIth1- ϵ K-KKK- α -CGRP 1-10	116	UBIth1- ϵ K-KKK-ACDTATCVTH
α -CGRP 1-10-KKK- ϵ K-UBIth1	117	ACDTATCVTH-KKK- ϵ K-UBIth1
UBIth1- ϵ K-KKK- α -CGRP 1-10-KKK- ϵ K-UBIth1	118	UBIth1- ϵ K-KKK-ACDTATCVTH-kkk- ϵ K-UBIth1
UBIth1- ϵ K-KKK- α -CGRP 31-37	119	UBIth1- ϵ K-KKK-NVGSKAF
UBIth1- ϵ K-KKK- α -CGRP 28-37	120	UBIth1- ϵ K-KKK-VPTNVGSKAF
UBIth1- ϵ K-KKK- α -CGRP 25-37	121	UBIth1- ϵ K-KKK-NNFVPTNVGSKAF
UBIth1- ϵ K-KKK- α -CGRP 18-37	122	UBIth1- ϵ K-KKK-RSGGVVKNNFVPTNVGSKAF
UBIth1- ϵ K-KKK- α -CGRP 11-37	123	UBIth1- ϵ K-KKK-RLAGLLSRGGVVKNNFVPTNVGSKAF
α -CGRP 1-15-KKK- ϵ K-UBIth1	124	ACDTATCVTHRLAGL-KKK- ϵ K-UBIth1
α -CGRP 1-18-KKK- ϵ K-UBIth1	125	ACDTATCVTHRLAGLLSR-KKK- ϵ K-UBIth1
α -CGRP 1-20-KKK- ϵ K-UBIth1	126	ACDTATCVTHRLAGLLSRSG-KKK- ϵ K-UBIth1
α -CGRP 1-25-KKK- ϵ K-UBIth1	127	ACDTATCVTHRLAGLLSRGGVVKNN-KKK- ϵ K-UBIth1
α -CGRP 8-18-KKK- ϵ K-UBIth1	128	VTHRLAGLLSR-KKK- ϵ K-UBIth1
UBIth1- ϵ K-KKK- α -CGRP 8-18	129	UBIth1- ϵ K-KKK-VTHRLAGLLSR
UBIth1- ϵ K- α -CGRP 11-37	130	UBIth1- ϵ K-RLAGLLSRGGVVKNNFVPTNVGSKAF
UBIth1- ϵ K-KKK- α -CGRP 11-25	131	UBIth1- ϵ K-KKK-RLAGLLSRGGVVKNN
UBIth3- ϵ K-KKK- α -CGRP 11-25	132	UBIth3- ϵ K-KKK-RLAGLLSRGGVVKNN
UBIth1- ϵ K-KKK- α -CGRP 11-30	133	UBIth1- ϵ K-KKK-RLAGLLSRGGVVKNNFVPT
UBIth3- ϵ K-KKK- α -CGRP 11-30	134	UBIth3- ϵ K-KKK-RLAGLLSRGGVVKNNFVPT
UBIth1- ϵ K-KKK- α -CGRP 11-33	135	UBIth1- ϵ K-KKK-RLAGLLSRGGVVKNNFVPTNVG
UBIth3- ϵ K-KKK- α -CGRP 11-33	136	UBIth3- ϵ K-KKK-RLAGLLSRGGVVKNNFVPTNVG
UBIth1- ϵ K-KKK- α -CGRP 11-35	137	UBIth1- ϵ K-KKK-RLAGLLSRGGVVKNNFVPTNVGSK
UBIth3- ϵ K-KKK- α -CGRP 11-35	138	UBIth3- ϵ K-KKK-RLAGLLSRGGVVKNNFVPTNVGSK
UBIth1- ϵ K-KKK- α -CGRP 15-37	139	UBIth1- ϵ K-KKK-LLSRGGVVKNNFVPTNVGSKAF
UBIth3- ϵ K-KKK- α -CGRP 15-37	140	UBIth3- ϵ K-KKK-LLSRGGVVKNNFVPTNVGSKAF
UBIth3- ϵ K-KKK- α -CGRP 11-37	141	UBIth3- ϵ K-KKK-RLAGLLSRGGVVKNNFVPTNVGSKAF
UBIth1- ϵ K-KKK- α -CGRP 20-37	142	UBIth1- ϵ K-KKK-GGVVKNNFVPTNVGSKAF
UBIth1- ϵ K-KKK- α -CGRP 22-37	143	UBIth1- ϵ K-KKK-VVKNNFVPTNVGSKAF
α -CGRP 11-18-KKK- ϵ K-UBIth1	144	RLAGLLSR-KKK- ϵ K-UBIth1
α -CGRP 5-18-KKK- ϵ K-UBIth1	145	ATCVTHRLAGLLSR-KKK- ϵ K-UBIth1
α -CGRP 4-16-KKK- ϵ K-UBIth1	146	TATCVTHRLAGLL-KKK- ϵ K-UBIth1
UBIth3- ϵ K-KKK- α -CGRP 28-37	147	UBIth3- ϵ K-KKK-VPTNVGSKAF
α -CGRP 1-20-KKK- ϵ K-UBIth3	148	ACDTATCVTHRLAGLLSRSG-KKK- ϵ K-UBIth3
UBIth3- ϵ K-KKK- α -CGRP 1-20	149	UBIth3- ϵ K-KKK-ACDTATCVTHRLAGLLSRSG
UBIth1- ϵ K-KKK-Rat α -CGRP 11-37	150	UBIth1- ϵ K-KKK-RLAGLLSRGGVVKDNFVPTNVGSEAF
UBIth3- ϵ K-KKK-Rat α -CGRP 11-37	151	UBIth3- ϵ K-KKK-RLAGLLSRGGVVKDNFVPTNVGSEAF

Peptide Description	SEQ ID NO:	Sequence
Clostridium tetani1 Th-εK-KKK-α-CGRP 11-25	152	KKQYIKANSKFIGITEL-εK-KKK-RLAGLLSRSGGVVKN
MvF1 Th-εK-KKK-α-CGRP 11-25	153	LSEIKGVIVHRLEGV-εK-KKK-RLAGLLSRSGGVVKN
Bordetella pertussis Th-εK-KKK-α-CGRP 11-25	154	GAYARCPNGTRALTVAELRGNAEL-εK-KKK-RLAGLLSRSGGVVKN
Clostridium tetani2 Th-εK-KKK-α-CGRP 11-25	155	WVRDIIIDFTNESSQKT-εK-KKK-RLAGLLSRSGGVVKN
Diphtheria Th-εK-KKK-α-CGRP 11-25	156	DSETADNLEKTVAALSILPGHGC-εK-KKK-RLAGLLSRSGGVVKN
Plasmodium falciparum Th-εK-KKK-α-CGRP 11-25	157	DHEKKHAKMEKASSVFNVVNS-εK-KKK-RLAGLLSRSGGVVKN
Schistosoma mansoni Th-εK-KKK-α-CGRP 11-25	158	KWFKTNAPNGVDEKHRH-εK-KKK-RLAGLLSRSGGVVKN
Cholera Toxin Th-εK-KKK-α-CGRP 11-25	159	ALNIWDRFDVFCTLGATTGYLKGNS-εK-KKK-RLAGLLSRSGGVVKN
MvF2 Th-εK-KKK-α-CGRP 11-25	160	ISEIKGVIVHKIEGI-εK-KKK-RLAGLLSRSGGVVKN
KKKMvF3 Th-εK-KKK-α-CGRP 11-25	161	KKKISISEIKGVIVHKIEGILF-εK-KKK-RLAGLLSRSGGVVKN T RT TR T
HBsAg1 Th-εK-KKK-α-CGRP 11-25	162	KKKLFLLTKLLTLPQSLD-εK-KKK-RLAGLLSRSGGVVKN RRRIKII RII I L IR VRVV VV V I V F FF FF F V F F
HBsAg2 Th-εK-KKK-α-CGRP 11-25	163	KKKIITITRIITIPQSLD-εK-KKK-RLAGLLSRSGGVVKN FFLL L ITTI
HBsAg3 Th-εK-KKK-α-CGRP 11-25	164	KKKIITITRIITIIITID-εK-KKK-RLAGLLSRSGGVVKN
Influenza MP1_1 Th-εK-KKK-α-CGRP 11-25	165	FVFTLTVPSE-εK-KKK-RLAGLLSRSGGVVKN
Influenza MP1_2 Th-εK-KKK-α-CGRP 11-25	166	SGPLKAEIAQRLEDV-εK-KKK-RLAGLLSRSGGVVKN
Influenza NSP1 Th-εK-KKK-α-CGRP 11-25	167	DRLRRDQKS-εK-KKK-RLAGLLSRSGGVVKN
EBV BHRF1 Th-εK-KKK-α-CGRP 11-25	168	AGLTSLLVICSYLFIISRG-εK-KKK-RLAGLLSRSGGVVKNF
Clostridium tetani TT1 Th-εK-KKK-α-CGRP 11-25	169	QYIKANSKFIGITEL-εK-KKK-RLAGLLSRSGGVVKN
EBV EBNA-1 Th-εK-KKK-α-CGRP 11-25	170	PGPLRESIVCYFMVFLQTHI-εK-KKK-RLAGLLSRSGGVVKN
Clostridium tetani TT2 Th-εK-KKK-α-CGRP 11-25	171	FNNFTVSEFWLRVPKVSASHLE-εK-KKK-RLAGLLSRSGGVVKN
Clostridium tetani TT3 Th-εK-KKK-α-CGRP 11-25	172	KFIKRYTPNNEIDSF-εK-KKK-RLAGLLSRSGGVVKN
Clostridium tetani TT4 Th-εK-KKK-α-CGRP 11-25	173	VSIDKFRIFCKALNPK-εK-KKK-RLAGLLSRSGGVVKN
EBV CP Th-εK-KKK-α-CGRP 11-25	174	VPGLYSPCAFFNKEELL-εK-KKK-RLAGLLSRSGGVVKN
HCMV IE1 Th-εK-KKK-α-CGRP 11-25	175	DKREMMMACIKELH-εK-KKK-RLAGLLSRSGGVVKN
EBV GP340 Th-εK-KKK-α-CGRP 11-25	176	TGHGARTSTEPTDY-εK-KKK-RLAGLLSRSGGVVKN
EBV BPLF1 Th-εK-KKK-α-CGRP 11-25	177	KELKRQYEKKLRQ-εK-KKK-RLAGLLSRSGGVVKN
EBV EBNA-2 Th-εK-KKK-α-CGRP 11-25	178	TVFYNIIPMPL-εK-KKK-RLAGLLSRSGGVVKN
HBsAg4 Th-εK-KKK-α-CGRP 11-25	179	FFLLTRILTIIPQSLD-εK-KKK-RLAGLLSRSGGVVKN
Yersinia Invasin Th-εK-KKK-α-CGRP 11-25	180	TAKSKKFPSYTATYQF-εK-KKK-RLAGLLSRSGGVVKN

* Peptides are cyclized by cysteine disulfide bonds with the cysteines underlined.

Table 4
Immunogenicity Assessment in Guinea Pigs of α -CGRP Peptide Immunogen Constructs

Grp #	Peptide immunogen description	SEQ ID NO:	Animal No	α -CGRP ₁₋₁₀ (SEQ ID NO: 4) ELISA Log ₁₀ Titer						α -CGRP ₁₁₋₃₇ (SEQ ID NO: 9) ELISA Log ₁₀ Titer					
				0 w	3 w	6 w	9 w	12 w	15 w	0 w	3 w	6 w	9 w	12 w	15 w
1	UBITH1- ϵ K-KKK- α CGRP (1-10)	116	6096	0.14	4.62	5.20	5.10	5.14	5.09	0.17	0.00	1.12	1.62	/	0.00
			6097	0.12	4.48	4.93	4.90	5.07	5.00	0.16	0.00	2.08	2.69	3.49	3.17
			6098	0.10	4.42	5.10	5.05	5.18	5.24	0.14	0.00	0.11	0.00	2.38	2.44
			Avg.	0.12	4.51	5.08	5.02	5.13	5.11	0.16	0.00	1.10	1.44	2.93	1.87
2	α CGRP (1-10)-KKK- ϵ K-UBITH1	117	6099	0.11	1.24	3.29	3.21	3.66	3.67	0.12	0.00	0.00	0.00	0.00	0.00
			6100	0.12	2.03	3.82	4.73	4.81	5.00	0.13	0.00	0.91	0.00	1.74	3.50
			6101	0.10	0.00	2.95	2.93	4.33	4.58	0.14	0.00	0.00	0.00	0.57	3.41
			Avg.	0.11	1.09	3.35	3.62	4.27	4.42	0.13	0.00	0.30	0.00	0.77	2.30
3	UBITH1- ϵ K-KKK- α CGRP (1-10)-KKK- ϵ K-UBITH1	118	6102	0.11	1.61	4.26	4.62	4.85	4.73	0.14	0.00	2.40	0.00	0.74	2.03
			6103	0.11	0.52	3.36	3.58	4.32	4.75	0.15	0.00	0.00	0.00	1.85	3.60
			6104	0.12	2.46	3.05	3.98	4.67	4.57	0.26	0.00	0.69	0.00	1.04	2.89
			Avg.	0.11	1.53	3.56	4.06	4.61	4.68	0.18	0.00	1.03	0.00	1.21	2.84
4	UBITH1- ϵ K-KKK- α CGRP (31-37)	119	6105	0.11	0.00	0.00	0.00	0.00	2.08	0.16	5.71	13.93	9.39	9.70	7.72
			6106	0.09	0.00	0.00	0.00	0.00	2.86	0.14	12.57	10.43	7.12	7.30	7.29
			6107	0.11	0.00	0.00	0.00	0.00	2.57	0.14	7.08	>10	>10	>10	>10
			Avg.	0.10	0.00	0.00	0.00	0.00	2.50	0.15	8.45	12.18	8.25	8.50	7.50
5	UBITH1- ϵ K-KKK- α CGRP (28-37)	120	6108	0.11	0.00	0.00	0.00	0.00	0.00	0.14	6.63	>10	7.71	8.03	7.43
			6109	0.08	0.00	0.00	0.00	0.00	0.00	0.15	10.08	11.22	7.95	7.37	6.90
			6110	0.10	0.00	0.00	0.00	0.00	0.00	0.15	6.64	8.90	6.97	7.63	/
			Avg.	0.10	0.00	0.00	0.00	0.00	0.00	0.15	7.78	10.06	7.54	7.68	7.17
6	UBITH1- ϵ K-KKK- α CGRP (25-37)	121	6111	0.10	0.00	0.00	0.00	0.00	0.00	0.17	11.80	10.07	6.64	7.00	7.20
			6112	0.12	0.00	0.00	0.00	0.00	0.00	0.15	9.21	8.52	6.91	6.67	10.53
			6113	0.09	0.00	0.00	0.00	0.00	0.00	0.13	9.49	11.26	8.01	8.58	7.30
			Avg.	0.10	0.00	0.00	0.00	0.00	0.00	0.15	10.17	9.95	7.19	7.42	8.34
7	UBITH1- ϵ K-KKK- α CGRP (18-37)	122	6114	0.08	0.00	0.00	0.00	0.00	0.00	0.12	9.17	7.85	6.02	6.07	5.90
			6115	0.10	0.00	0.00	0.00	0.00	0.00	0.13	10.20	8.40	5.96	6.66	7.51
			6116	0.12	0.00	0.00	0.00	0.00	1.19	0.14	10.79	7.43	6.32	6.69	6.07
			Avg.	0.10	0.00	0.00	0.00	0.00	0.40	0.13	10.05	7.89	6.10	6.47	6.49
8	UBITH1- ϵ K-KKK- α CGRP (11-37)	123	6117	0.08	0.00	0.00	0.00	0.00	0.71	0.12	12.78	12.30	9.52	8.81	8.41
			6118	0.10	0.00	0.00	0.00	0.00	1.16	0.12	10.81	13.87	9.13	7.83	6.65
			6119	0.11	0.00	0.00	0.00	0.00	0.00	0.15	12.14	15.51	10.15	9.56	7.12
			Avg.	0.09	0.00	0.00	0.00	0.00	0.63	0.13	11.91	13.89	9.60	8.73	7.39

Table 5
Immunogenicity Assessment in Guinea Pigs of α -CGRP Peptide Immunogen Constructs

Group #	Peptide immunogen description	SEQ ID NO:	Animal No	α -CGRP ₁₋₃₇ (SEQ ID NO: 9) ELISA Log ₁₀ Titer					α -CGRP ₁₋₂₅ (SEQ ID NO: 13) ELISA Log ₁₀ Titer					α -CGRP ₃₋₁₂ (SEQ ID NO: 14) ELISA Log ₁₀ Titer				
				0 w	3 w	6 w	9 w	12 w	0 w	3 w	6 w	9 w	12 w	0 w	3 w	6 w	9 w	12 w
1	α CGRP (1-15)-KKK- eK-UBIth1	124	6721	0.127	3.109	3.854	3.140	3.122	0.095	3.399	5.164	5.054	5.051	0.089	0.000	0.000	0.000	1.362
			6722	0.234	0.000	1.963	0.000	2.252	0.079	3.540	5.384	5.142	5.099	0.075	0.000	1.170	0.000	0.000
			6723	0.118	2.963	4.086	3.158	2.968	0.073	3.135	5.336	5.163	5.109	0.078	0.000	1.016	0.000	0.000
2	α CGRP (1-18)-KKK- eK-UBIth1	125	6724	0.129	3.462	4.977	4.696	3.696	0.076	4.012	5.165	5.103	5.019	0.073	0.000	2.465	0.000	0.780
			6725	6.691	2.938	5.524	5.178	5.330	0.085	3.015	5.077	4.697	4.961	0.062	0.000	3.292	3.150	3.176
			6726	0.079	2.297	5.023	4.757	4.913	0.085	3.226	5.347	5.078	5.079	0.075	0.000	2.858	2.575	2.992
3	α CGRP (1-20)-KKK- eK-UBIth1	126	6727	0.075	2.957	5.098	4.921	5.000	0.080	3.255	5.335	5.111	5.118	0.077	0.000	2.957	2.429	3.065
			6728	0.077	3.441	5.284	5.043	4.937	0.082	3.825	5.537	5.397	5.528	0.076	0.000	2.957	2.273	2.602
			6729	0.094	2.833	5.021	4.732	4.602	0.093	2.978	5.094	4.926	4.774	0.077	0.000	2.227	1.345	0.000
4	α CGRP (1-25)-KKK- eK-UBIth1	127	6730	0.072	4.959	5.922	5.157	5.034	0.069	4.190	5.630	5.151	5.040	0.067	0.000	0.771	0.000	0.000
			6731	0.104	4.752	5.413	5.235	5.086	0.075	4.560	5.348	5.446	5.159	0.077	0.000	3.026	3.788	2.326
			6732	0.111	4.605	5.873	5.134	5.071	0.081	3.900	5.752	5.278	5.173	0.078	0.000	2.473	1.667	0.672
5	α CGRP (8-18)-KKK- eK-UBIth1	128	6733	0.100	3.194	4.766	4.628	4.732	0.080	3.282	4.932	4.745	4.789	0.080	0.000	2.495	2.678	3.148
			6734	0.082	2.161	4.211	4.298	4.769	0.079	2.902	4.568	4.537	4.687	0.075	0.000	0.265	2.589	3.118
			6735	0.071	3.154	4.695	4.678	4.663	0.076	3.940	4.862	4.745	4.698	0.078	0.000	2.978	3.079	3.094
6	UBIth1-eK-KKK- α CGRP (8-18)	129	6736	0.126	4.674	5.250	5.012	4.940	0.086	4.682	5.324	5.095	4.934	0.080	2.269	4.540	4.462	4.348
			6737	0.079	4.623	5.149	4.755	4.593	0.095	4.492	5.164	4.815	4.597	0.068	3.558	5.178	4.927	4.820
			6738	0.106	4.574	5.552	5.181	5.040	0.084	4.847	5.082	5.531	5.220	0.062	1.824	4.486	4.552	4.549
7	UBIth1-eK- α CGRP (11-37)	130	6739	0.117	10.661	>10	8.478	7.453	0.087	0.000	1.433	2.655	3.040	0.070	0.000	0.000	0.000	0.000
			6740	0.124	>10	>10	>10	>10	0.089	2.661	4.645	4.659	4.632	0.077	0.000	0.000	0.000	0.000
			6741	0.095	>10	>10	>10	>10	0.103	2.931	4.808	4.672	4.788	0.083	0.000	0.000	0.000	0.000

Table 6
Immunogenicity Assessment in Guinea Pigs of α -CGRP Peptide Immunogen Constructs

Group #	Peptide immunogen description	SEQ ID NO:	Animal No	α -CGRP ₁₁₋₃₅ (SEQ ID NO: 18) ELISA Log ₁₀ Titer					α -CGRP ₁₅₋₃₇ (SEQ ID NO: 19) ELISA Log ₁₀ Titer				
				0 w	3 w	6 w	9 w	12 w	0 w	3 w	6 w	9 w	12 w
1	UBITh1- ϵ K-KKK- α CGRP (11-25)	131	6823	0.089	5.136	>10	5.271	5.020	0.079	4.948	>10	5.826	5.122
			6824	0.094	6.042	>10	5.395	5.056	0.083	5.341	>10	7.013	5.191
			6825	0.086	5.942	7.707	5.255	4.994	0.076	5.029	6.660	5.262	4.949
2	UBITh1- ϵ K-KKK- α CGRP (11-30)	133	6826	0.088	5.397	5.668	5.051	4.879	0.095	5.169	5.634	5.231	5.025
			6827	0.074	5.202	5.633	5.057	4.893	0.108	4.907	5.409	5.150	5.106
			6828	0.073	5.425	5.729	5.091	4.917	0.079	5.194	5.405	5.267	5.106
3	UBITh1- ϵ K-KKK- α CGRP (11-33)	135	6829	0.093	5.599	5.366	5.112	5.096	0.075	5.277	5.298	5.162	5.087
			6830	0.082	6.234	>10	5.577	5.222	0.072	5.730	7.937	7.026	5.487
			6831	0.075	5.458	5.963	5.172	5.051	0.061	5.225	5.453	5.380	5.180
4	UBITh1- ϵ K-KKK- α CGRP (11-35)	137	6832	0.08	10.05	8.83	7.364	5.819	0.082	8.101	6.224	5.453	5.036
			6833	0.075	10.06	>10	7.443	6.106	0.067	7.523	7.232	5.896	5.313
			6834	0.08	7.833	>10	8.988	5.759	0.086	5.424	5.567	5.780	5.221
5	UBITh1- ϵ K-kkk- α CGRP (15-37)	139	6835	0.112	5.075	4.914	4.859	4.727	0.096	>10	10.351	>10	6.721
			6836	0.073	4.943	4.983	5.290	5.022	0.099	5.428	6.345	10.811	5.828
			6837	0.093	5.041	4.832	4.903	4.878	0.073	>10	9.509	6.990	5.625

Table 7
Cross-Reactivities of Immune Sera with Full-Length CGRP

CGRP peptide immunogen	SEQ ID NO:	Animal ID	α -CGRP ₁₋₃₇ (SEQ ID NO: 1) ELISA Log ₁₀ Titer		
			6 wpi	9 wpi	12 wpi
UBITH1- ϵ K-KKK- α CGRP (1-10)	116	6096	5.41	5.16	5.02
		6097	5.29	5.40	5.65
		6098	5.47	5.67	5.63
α CGRP (1-10)-KKK- ϵ K-UBITH1	117	6099	5.39	5.39	5.61
		6100	5.61	7.11	7.43
		6101	5.41	5.33	5.66
UBITH1- ϵ K-KKK- α CGRP (1-10)-KKK- ϵ K-UBITH1	118	6102	8.21	>10	>10
		6103	5.44	5.71	7.25
		6104	5.71	6.34	7.92
UBITH1- ϵ K-KKK- α CGRP (31-37)	119	6105	>10	>10	>10
		6106	>10	>10	10.50
		6107	>10	>10	>10
UBITH1- ϵ K-KKK- α CGRP (28-37)	120	6108	>10	>10	>10
		6109	>10	>10	>10
		6110	>10	10.84	11.63
UBITH1- ϵ K-KKK- α CGRP (25-37)	121	6111	>10	11.89	11.48
		6112	>10	8.97	7.12
		6113	>10	>10	>10
UBITH1- ϵ K-KKK- α CGRP (18-37)	122	6114	>10	9.88	>10
		6115	>10	9.67	>10
		6116	>10	11.23	10.42
UBITH1- ϵ K-KKK- α CGRP (11-37)	123	6117	>10	>10	10.97
		6118	>10	9.48	7.09
		6119	>10	>10	>10
α CGRP (1-15)-KKK- ϵ K-UBITH1	124	6721	5.39	5.39	5.61
		6722	5.61	7.11	7.43
		6723	5.41	5.33	5.66
α CGRP (1-18)-KKK- ϵ K-UBITH1	125	6724	8.21	>10	>10
		6725	5.44	5.71	7.25
		6726	5.71	6.34	7.92
CGRP peptide immunogen	SEQ ID NO:	Animal ID	α -CGRP ₁₋₃₇ (SEQ ID NO: 1) ELISA Log ₁₀ Titer		
			6 wpi	9 wpi	12 wpi
α CGRP (1-20)-KKK- ϵ K-UBITH1	126	6727	>10	>10	>10
		6728	>10	>10	10.50
		6729	>10	>10	>10
α CGRP (1-25)-KKK- ϵ K-UBITH1	127	6730	>10	>10	>10
		6731	>10	>10	>10
		6732	>10	10.84	11.63
α CGRP (8-18)-KKK- ϵ K-UBITH1	128	6733	>10	11.89	11.48
		6734	>10	8.97	7.12
		6735	>10	>10	>10
UBITH1- ϵ K-KKK- α CGRP (8-18)	129	6736	>10	9.88	>10
		6737	>10	9.67	>10
		6738	>10	11.23	10.42
UBITH1- ϵ K-KKK- α CGRP (11-25)	131	6823	6.400	5.414	5.239
		6824	6.648	5.507	5.241
		6825	6.305	5.116	5.038
UBITH1- ϵ K-KKK- α CGRP (11-30)	133	6826	5.411	5.138	5.056
		6827	5.357	5.078	4.994
		6828	5.679	5.149	5.165
UBITH1- ϵ K-KKK- α CGRP (11-33)	135	6829	5.153	5.147	5.348
		6830	8.112	5.540	6.021
		6831	5.487	5.301	5.673
UBITH1- ϵ K-KKK- α CGRP (11-35)	137	6832	6.008	5.347	5.386
		6833	8.078	5.649	5.482
		6834	5.603	5.582	6.109
UBITH1- ϵ K-KKK- α CGRP (15-37)	139	6835	>10	>10	>10
		6836	7.322	7.323	5.937
		6837	>10	8.384	9.439

Table 8
Lack of Endogenous Th Epitopes within the Selected CGRP B Epitope Sequences

Peptide description	SEQ ID NO:	Animal ID	α -CGRP ₁₋₂₅ (SEQ ID NO: 13) ELISA Log ₁₀ Titer			α -CGRP ₁₁₋₃₇ (SEQ ID NO: 9) ELISA Log ₁₀ Titer		
			0 w	3 w	6 w	0 w	3 w	6 w
α -CGRP (31-37)	5	7174	0.073	0.000	0.000	0.067	0.000	0.000
		7175	0.073	0.000	0.000	0.066	0.000	0.000
		7176	0.079	0.000	0.000	0.064	0.000	0.000
α -CGRP (28-37)	6	7177	0.075	0.000	0.000	0.059	0.000	0.349
		7178	0.075	0.000	0.000	0.079	0.000	0.000
		7179	0.070	0.000	0.000	0.067	0.000	0.000
α -CGRP (11-25)	15	7195	0.067	0.000	0.000	0.054	0.000	0.000
		7196	0.087	0.000	0.000	0.061	0.000	0.000
		7197	0.096	0.000	0.000	0.085	0.000	0.000

Table 9
Immunogenicity Assessment in Guinea Pigs against the Th Epitope Portion of the selected CGRP Peptide Immunogen Constructs

Peptide immunogen description	SEQ ID NO:	Animal ID	α -CGRP ₂₀₋₃₇ (SEQ ID NO: 20) ELISA Log ₁₀ Titer			UBI _{Th} 1 (SEQ ID NO: 98) ELISA Log ₁₀ titer		
			0 wpi	3wpi	6wpi	0 wpi	3wpi	6wpi
UBI _{Th} 1- ϵ K-KKK- α CGRP (20-37)	142	7282	0.092	5.511	5.323	0.127	0.000	0.000
		7283	0.092	5.405	5.085	0.106	0.000	0.000
		728	0.090	5.991	5.058	0.111	0.000	0.000
UBI _{Th} 1- ϵ K-KKK- α CGRP (22-37)	143	7285	0.104	5.823	5.811	0.114	0.000	0.000
		7286	0.090	5.766	5.686	0.121	0.000	0.000
		7287	0.095	6.087	5.151	0.107	0.000	0.000

Table 10
Mapping of α -CGRP Binding B epitopes with Immune Sera from α -CGRP Peptide Immunogen Constructs

10 mer peptide design for epitope mapping from -9 to 46 of α -CGRP	SEQ ID NO	Sequence	Arb ^{Unit} ELISA of Immune Sera from α -CGRP Peptide Immunogen Constructs									
			SEQ ID NO									
			123	122	127	129	130	132	137	139	137	139
GSRTIAQKACDTATCVTHRLAGLLSRSGGVKNEVPINVGSKATCFRRRLQA	181		CGRP 11-37	CGRP 18-37	CGRP 1-25	CGRP 8-18	CGRP 11-37	CGRP 11-25	CGRP 11-35	CGRP 15-37		
GSRTIAQKSA	26	-9 - 1	0.575	0.245	0.190	0.099	0.153	0.116	0.109	0.126		
SRRTIAQKDAC	27	-8 - 2	0.564	0.203	0.140	0.102	0.141	0.110	0.110	0.124		
RIITIAQKACD	28	-7 - 3	0.476	0.191	0.136	0.102	0.147	0.110	0.101	0.134		
ITIAQKACDT	29	-6 - 4	0.489	0.203	0.129	0.091	0.136	0.113	0.094	0.103		
IAQKACDIA	30	-5 - 5	0.472	0.186	0.142	0.097	0.120	0.103	0.099	0.125		
AQKACDTAT	31	-4 - 6	0.448	0.249	0.112	0.093	0.108	0.093	0.087	0.089		
QKACDTATC	32	-3 - 7	0.691	1.372	0.258	0.094	0.141	0.090	0.094	0.148		
KKACDTATCV	33	-2 - 8	0.680	1.071	2.378	0.180	0.129	0.102	0.094	0.116		
PACDTATCVT	34	-1 - 9	0.436	0.265	2.286	0.263	0.169	0.123	0.118	0.139		
ACDTATCVTH	35	1 - 10	0.493	0.194	3.076	0.340	0.126	0.104	0.098	0.110		
CDTATCVTHR	36	2 - 11	0.472	0.183	3.965	1.117	0.126	0.105	0.099	0.121		
DATCVTHRL	37	3 - 12	0.494	0.189	3.875	0.105	0.131	0.104	0.097	0.116		
TATCVTHRLA	38	4 - 13	0.515	0.194	3.723	0.111	0.138	0.108	0.101	0.114		
ATCVTHRLAG	39	5 - 14	0.514	0.199	0.636	0.109	0.136	0.113	0.108	0.107		
TCVTHRLAGL	40	6 - 15	0.884	0.324	0.522	0.214	0.235	0.836	0.322	0.471		
CVTHRLAGLL	41	7 - 16	0.950	0.524	0.708	0.733	0.489	0.479	0.477	0.426		
VTHRLAGLLS	42	8 - 17	0.502	0.185	0.228	1.076	0.149	0.108	0.099	0.104		
THRLAGLLSR	43	9 - 18	0.588	0.209	0.300	0.249	0.166	0.166	0.161	0.117		
HLAGLLSR	44	10 - 19	0.552	0.203	0.297	0.171	0.137	0.134	0.130	0.101		
RLAGLLSRSG	45	11 - 20	0.473	0.196	0.213	0.119	0.127	0.114	0.110	0.104		
LAGLLSRSGG	46	12 - 21	0.462	0.210	0.228	0.109	0.122	0.100	0.089	0.108		
AGLLSRSGGV	47	13 - 22	0.423	0.175	0.216	0.094	0.159	0.103	0.089	0.096		
GLLSRSGGVV	48	14 - 23	0.399	0.179	0.928	0.115	0.565	0.268	0.108	0.100		
LLSRSGGVVK	49	15 - 24	0.666	0.301	3.642	0.109	1.336	3.120	0.510	0.102		
LSRSGGVVFN	50	16 - 25	0.425	0.584	3.850	0.185	0.734	4.000	0.268	0.851		
SRSGGVVFNH	51	17 - 26	0.426	0.848	3.797	0.145	0.112	3.282	0.310	2.235		

10 mer peptide design for epitope mapping from -8 to 46 of α-CGRP	SEQ ID NO	Sequence	A _{560nm} ELISA of Immune Sera from α-CGRP Peptide Immunogen Constructs															
			SEQ ID NO															
			123 CGRP 11-37	122 CGRP 18-37	127 CGRP 1-25	129 CGRP 8-18	130 CGRP 11-37	132 CGRP 11-25	137 CGRP 11-35	139 CGRP 15-37								
GSRILAQKACDIATCVTHRLAGLLSRSGGVVKNNEVFETNVGSKARGRRRDLQA	181																	
	52	ESGGVKNNEF	0.594	0.537	1.972	0.130	0.203	0.295	0.176	0.661								
	53	SGGVKNNEV	0.423	0.261	0.192	0.101	0.158	0.100	0.100	0.178								
	54	GGVKNNEVP	0.708	0.572	0.201	0.092	1.249	0.090	0.252	2.268								
	55	GVVKNNEVFT	0.863	0.491	0.205	0.123	1.105	0.108	0.283	1.697								
	56	VKNNEVPIN	0.719	0.418	0.201	0.105	0.321	0.259	1.274	0.437								
	57	VKNNEVPINV	1.768	2.259	0.207	0.118	1.031	0.111	4.000	0.325								
	58	KNNEVFETVGS	1.265	3.381	0.210	0.102	0.878	0.103	4.000	0.777								
	59	MNEVFETVGS	0.425	0.194	0.208	0.104	0.141	0.106	0.271	0.124								
	60	NEVFETVGSK	0.705	0.542	0.205	0.106	1.697	0.106	4.000	0.248								
FVETNVGSKA	61	FVETNVGSKA	0.535	0.407	0.232	0.115	1.255	0.120	3.238	0.189								
	62	VETNVGSKAF	3.730	3.796	0.222	0.103	4.000	0.106	0.964	4.000								
	63	ETNVGSKAFG	3.537	2.160	0.132	0.090	2.709	0.095	0.478	1.299								
	64	ETNVGSKAFGR	0.921	0.344	0.138	0.091	0.367	0.088	0.386	0.263								
	65	NVGSKAFGR	0.660	0.223	0.142	0.096	0.155	0.097	0.083	0.125								
	66	VGSKAFGRER	0.697	0.243	0.173	0.106	0.149	0.114	0.091	0.119								
	67	GSKAFGRERK	0.570	0.229	0.164	0.105	0.134	0.120	0.096	0.114								
	68	SKAFGRERKD	0.619	0.237	0.152	0.100	0.158	0.135	0.099	0.126								
	69	KAFGRERKDL	0.699	0.252	0.176	0.113	0.210	0.121	0.121	0.134								
	70	AFGRERKDLQ	0.606	0.233	0.177	0.129	0.163	0.139	0.120	0.137								
RLAGLLSRSGGVVKNNEVFETNVGSKAF	8	11 - 37	3.759	3.814	2.080	0.348	3.708	1.693	2.274	3.788								
	12	1 - 25	0.564	0.150	3.061	0.445	0.279	4.000	0.582	0.149								
	1	1 - 37	3.774	3.862	3.441	0.495	3.885	1.914	2.944	3.813								

CLAIMS

1. A CGRP peptide immunogen construct having about 30 or more amino acids, represented by the formulae:

$$(\text{Th})_m-(\text{A})_n-(\text{CGRP functional B epitope peptide})-\text{X}$$

or

$$(\text{CGRP functional B epitope peptide})-(\text{A})_n-(\text{Th})_m-\text{X}$$

or

$$(\text{Th})_m-(\text{A})_n-(\text{CGRP functional B epitope peptide})-(\text{A})_n-(\text{Th})_m-\text{X}$$

wherein

Th is a heterologous T helper epitope;

A is a heterologous spacer;

(CGRP functional B epitope peptide) is a B cell epitope peptide having from 7 to about 30 amino acid residues derived from CGRP receptor binding or activation region with SEQ ID NOs: 4-13 and 15-24) of CGRP (SEQ ID NOs: 1-3);

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.

2. The CGRP peptide immunogen construct according to claim 1, wherein the CGRP receptor binding or activation region is selected from the group consisting of SEQ ID NOs: 4-13 and 15-24.
3. The CGRP peptide immunogen construct according to any of claims 1 or 2, wherein the Th epitope is selected from the group consisting of SEQ ID NOs: 74-115.
4. The CGRP peptide immunogen construct according to claim 1, wherein the peptide immunogen construct is selected from the group consisting of SEQ ID NOs: 120-127 and 130-180.
5. An CGRP peptide immunogen construct comprising:
- a B cell epitope comprising from about 7 to about 30 amino acid residues from the CGRP sequence of SEQ ID NOs: 1 to 3;
 - a T helper epitope comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 74-115, and any combination thereof; and
 - an optional heterologous spacer selected from the group consisting of an amino acid, Lys-, Gly-, Lys-Lys-Lys-, (α , ϵ -N)Lys, ϵ -N-Lys-Lys-Lys-Lys (SEQ ID NO: 72), Lys-Lys-Lys- ϵ -

N-Lys (SEQ ID NO: 73), and Pro-Pro-Xaa-Pro-Xaa-Pro (SEQ ID NO: 71), and any combination thereof,

wherein the B cell epitope is covalently linked to the T helper epitope directly or through the optional heterologous spacer.

6. The CGRP peptide immunogen construct of claim 5, wherein the B cell epitope is selected from the group consisting of SEQ ID NOs: 4-13 and 15-24.
7. The CGRP peptide immunogen construct of claim 5, wherein the T helper epitope is selected from the group consisting of SEQ ID NOs: 68, 71, 74-82, 87, 89-92, 95, and any combination thereof.
8. The CGRP peptide immunogen construct of claim 5, wherein the optional heterologous spacer is (α , ϵ -N)Lys, ϵ -N-Lys-Lys-Lys-Lys (SEQ ID NO: 72), Lys-Lys-Lys- ϵ -N-Lys (SEQ ID NO: 73), or Pro-Pro-Xaa-Pro-Xaa-Pro (SEQ ID NO: 71), where Xaa is any amino acid.
9. The CGRP peptide immunogen construct of claim 5, wherein the T helper epitope is covalently linked to the amino terminus of the B cell epitope.
10. The CGRP peptide immunogen construct of claim 5, wherein the T helper epitope is covalently linked to the amino terminus of the B cell epitope through the optional heterologous spacer.
11. A composition comprising an CGRP peptide immunogen construct according to claim 1.
12. A pharmaceutical composition comprising:
 - a. a peptide immunogen construct according to claim 1; and
 - b. a pharmaceutically acceptable delivery vehicle and/or adjuvant.
13. The pharmaceutical composition of claim 12, wherein
 - a. the CGRP functional B epitope peptide is selected from the group consisting of SEQ ID NOs: 4-13 and 15-24;
 - b. the Th epitope is selected from the group consisting of SEQ ID NOs: 74-115; and
 - c. the heterologous spacer is selected from the group consisting of an amino acid, Lys-, Gly-, Lys-Lys-Lys-, (α , ϵ -N)Lys, ϵ -N-Lys-Lys-Lys-Lys (SEQ ID NO: 72), Lys-Lys-Lys- ϵ -N-Lys (SEQ ID NO: 73), and Pro-Pro-Xaa-Pro-Xaa-Pro (SEQ ID NO: 71), and any combination thereof; and

wherein the CGRP peptide immunogen construct is mixed with an CpG oligodeoxynucleotide (ODN) to form a stabilized immunostimulatory complex.
14. The pharmaceutical composition of claim 12, wherein

- a. the CGRP peptide immunogen construct is selected from the group consisting of SEQ ID NOs: 120-127 and 130-180; and
wherein the CGRP peptide immunogen construct is mixed with an CpG oligodeoxynucleotide (ODN) to form a stabilized immunostimulatory complex.
- 15. A method for generating antibodies against CGRP in an animal comprising administering the pharmaceutical composition according to claim 12 to the animal.
- 16. An isolated antibody or epitope-binding fragment thereof that specifically binds to the CGRP receptor binding or activation region of SEQ ID NOs: 4-13 and 15-24.
- 17. The isolated antibody or epitope-binding fragment thereof according to claim 16 bound to the CGRP peptide immunogen construct.
- 18. A composition comprising the isolated antibody or epitope-binding fragment thereof according to claim 16.
- 19. A method of preventing and/or treating migraine in an animal comprising administering the pharmaceutical composition of claim 12 to the animal.