2. SCIENTIFIC ABSTRACT

Allertein Therapeutics, LLC (Allertein) is developing EMP-123 for the treatment of peanut allergy. EMP-123 consists of three heat/phenol-killed, E. coli-encapsulated, recombinant modified peanut proteins, Ara h 1, Ara h 2 and Ara h 3, formulated in a 1:1:3:1.4 ratio in phosphate buffered saline (PBS), 10% glycerol, 0.5% phenol, and 0.5% hydroxypropyl methylcellulose (HPMC) for rectal delivery. This product is designed to act as a vaccine, or immunotherapy, in which peanut allergic patients are gradually vaccinated against progressively larger doses of the drug product to reduce or eliminate sensitivity to peanut allergens and to prevent potentially life-threatening or fatal reactions in peanut allergic patients who are inadvertently exposed to small quantities of peanut proteins.

Peanut allergy is common, with studies from the United States (US), the United Kingdom (UK), and France estimating a prevalence of 0.8% in children and an overall prevalence of 0.5-1% in the general population (Sicherer et al. 2003; Emmett et al. 1999; Kanny et al. 2001). There is evidence that the prevalence of peanut allergy in the US is rising based on a follow-up study that showed the prevalence of peanut allergy had doubled in children over the 5 year interval from 1997–2002 (Sicherer et al. 2003). In addition, an earlier report from the Isle of Wight showed a two-fold increase in reported peanut allergy, a three-fold increase in peanut sensitization, and an overall estimate of peanut allergy of 1.5% (Grundy et al. 2002). Further complicating matters is the fact that consumption of peanut in Western countries is rising, which may be due to its use as a source of protein in health foods, the popularity of vegetarian diets, and increased use of prepared foods.

Currently, the standard of care for peanut allergy is dietary avoidance and emergency treatment for accidental ingestions. However, strict avoidance diets can be complicated due to difficulty in interpreting labels (Joshi et al. 2002) and by the presence of undeclared or hidden allergens in commercially prepared foods (Altschul et al. 2001; Vierk et al. 2002). Accidental ingestions are unfortunately common, with up to 50% of food-allergic patients having an allergic reaction over a two-year period (Sicherer et al. 1998). Allergic reactions to peanut can be severe and life threatening; and peanut and/or tree nut allergies account for the vast majority of fatal food-induced anaphylaxis (Sampson et al. 2005). This combination of strict avoidance diets, the high incidence of accidental exposures, and the risk of severe or even fatal reactions with accidental exposures adds a tremendous burden and stress on patients and their families. Further complicating matters is the fact that only about 20% of children will outgrow peanut allergy (Skolnick et al. 2001), meaning that the majority of people with peanut allergy will have it for the rest of their lives. If the rising prevalence and increased consumption of peanut in Western countries is coupled with the facts that only approximately 1 in 5 will outgrow their allergy, that allergic reactions have the potential to be severe or even fatal (Bock et al. 2001), and that accidental exposures are common, developing an effective treatment for peanut allergy becomes even more imperative.
Allertein, in conjunction with the National Institute of Allergy and Infectious Diseases (NIAID), Division of Allergy, Immunology, and Transplantation (DAIT), and the Consortium for Food Allergy Research (CoFAR) proposes to conduct a phase I clinical study of EMP-123, titled "A Phase 1 Safety Study of Heat/Phenol-Killed, E. coli-Encapsulated, Recombinant Modified Peanut Proteins Ara h 1, Ara h 2, and Ara h 3 (EMP-123) in Normal Volunteers Followed by Subjects Allergic to Peanuts" (Protocol No. APA-001). The primary objectives of this study are to 1) determine in a small cohort of normal volunteers whether weekly rectal administration of escalating doses of EMP-123 is associated with any symptoms, and 2) to determine in a small cohort of subjects with peanut allergy whether weekly rectal administration of escalating doses of EMP-123 is associated with more than mild allergic symptoms. The secondary objectives of this study are to 1) determine the rate of serious adverse events and adverse events reported, and 2) determine the rate of desensitization, as determined by oral food challenge (OFC) in peanut allergic subjects on EMP-123. The tertiary objectives of this study are to conduct and evaluate a set of immune-focused mechanistic studies in the peanut allergic subjects.

The purpose of this submission is to provide the protocol for the above referenced clinical trial to NIH OBA and RAC for review. EMP-123 is intended for therapeutic purposes, and under the strictest interpretation, recombinant DNA will be transferred into human participants in the process. However, it should be noted that EMP-123 is not intended as gene therapy. In gene therapy, the objective is to deliver deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) into an individual's cells with the intent of replacing or repairing a defective gene that is responsible for disease. For this purpose, the nucleic acids must be taken up by the cell, transported to the nucleus, and integrated into the genome. The delivery/uptake of the nucleic acids is typically facilitated by a viral vector or by physical means, and specific sequences are utilized to enhance the likelihood of integration.

EMP-123 is not designed to deliver nucleic acids into human cells, nor is it designed to favor integration into the human genome. The objective of EMP-123 therapy is to deliver modified peanut allergens (i.e., proteins) encapsulated in heat/phenol killed E. coli to the rectal mucosa where they can suppress/elicit immune responses that will reduce or eliminate sensitivity to peanut allergens. The modified peanut allergens are expressed from recombinant DNA within E. coli during the manufacturing process, and the E. coli are subsequently killed as the last step in manufacturing. The modified peanut allergens encapsulated in dead E. coli are formulated and packaged for rectal administration to humans with a syringe and flexible rectal adaptor. The DNA present in the final EMP-123 product is essentially carryover from the process used to manufacture the encapsulated allergens. The likelihood that DNA from lysed EMP-123 E. coli will be taken up by human cells and integrated into the genome is minimal. Further, in the event of such an occurrence, the cells of the gastrointestinal (GI) tract epithelium have a relatively short lifespan, and such an event would be short lived.

A bacterial infection within a subject administered EMP-123 or horizontal transmission of a bacterial infection between a subject administered EMP-123 and other individuals (e.g., relatives
or investigational site staff) is unlikely, as the *E. coli* in the EMP-123 product are heat/phenol-killed prior to administration. Further, the *E. coli* strain used to manufacture EMP-123 [i.e., BLR(DE3), a derivative of BL21] has been shown to lack well recognized pathogenic mechanisms, and is considered nonpathogenic (Kuhnert et al. 1997; Chart et al. 2000). Vertical transmission (i.e., transmission of bacterial infection directly from mother to baby during the period immediately before and after birth) is unlikely for the same reasons listed above. Regardless, pregnant or lactating females will not be allowed to participate in the proposed clinical trial, and sexually active patients will be required to use an effective method of contraception throughout the study.

The background of the research and development of EMP-123 can be described in four main areas corresponding to the characteristics of EMP-123 that define it as a lead compound:

1) selection of the three peanut proteins Ara h 1, Ara h 2, and Ara h 3 as the antigens,
2) modification of the peanut proteins to remove sequential immunoglobulin E (IgE) binding epitopes,
3) encapsulation of the modified peanut proteins in heat/phenol-killed *E. coli*, and
4) rectal administration of EMP-123. The rationale for each of these areas is described below.

**Selection of the Three Peanut Proteins Ara h 1, Ara h 2, and Ara h 3 as the Antigens:** In order for efficacy to be achieved, EMP-123 should protect against exposure to the main peanut proteins responsible for anaphylactic reactions. The three major peanut proteins that are responsible for peanut allergy are Ara h 1, Ara h 2, and Ara h 3. Several labs in the US and Europe have conducted studies using serum IgE from peanut allergic patients and protein extracts from peanuts to identify the protein components of peanuts that elicit allergic reactions. In general, each of these studies involved the identification of IgE binding proteins by immunoblot analysis to establish the size of the protein and the prevalence of recognition in the peanut allergic population.

Ara h 1 and Ara h 2 were the first peanut allergens to be identified by this process in the US (Burks et al. 1991; Burks et al. 1992). Ara h 1 and Ara h 2 were initially identified as major allergens because they were recognized by IgE from >90% of a peanut allergic population. Subsequent multi-center studies have established these two proteins as major peanut allergens in larger peanut allergic populations. Ara h 3, a third peanut allergen, was initially identified by serum IgE from peanut allergic individuals that had cross-reacting soy IgE antibodies adsorbed using soy protein (Eigenmann et al. 1996). A multi-center study established that IgE from about 45-65% of the peanut allergic population recognized this allergen (Rabjohn et al. 1999; Shreffler et al. 2002). Studies using a peanut allergic population in Europe have confirmed Ara h 1, Ara h 2, and Ara h 3 as the main allergens from peanut (Kleber-Janke et al. 1999). Of the four new peanut protein allergens identified in these studies, three (Ara h 4, Ara h 6, and Ara h 7) were isoforms of these already identified proteins. The fourth, Ara h 5, is a profilin that is recognized by about 13% of the peanut allergic population. In summary, Ara h 1, Ara h 2, and Ara h 3 were selected as the antigens for EMP-123 as they represent the three major peanut proteins that are responsible for peanut allergy.
Modification of the Peanut Proteins to Disrupt Sequential IgE Binding Epitopes: The investigators hypothesized that disruption of the sequential IgE binding epitopes on wild type peanut proteins by point mutations would reduce the ability of these proteins to produce allergic responses following administration of the proteins during immunotherapy by reducing the response of mast cells. This provided the rationale for the research initiative to identify and disrupt sequential IgE binding epitopes on Ara h 1, Ara h 2, and Ara h 3. The coding regions of Ara h 1, Ara h 2, and Ara h 3 peanut proteins were modified relative to the wild type proteins by single amino acid substitutions (i.e., point mutations) designed to interfere with known sequential IgE binding epitopes (Burks et al. 1997; Stanley et al. 1997; Rabjohn et al. 1999).

Briefly, Ara h 1, Ara h 2, and Ara h 3 proteins were isolated and purified, and the DNA encoding these proteins cloned. Sequential IgE-binding epitopes and their critical amino acids were then mapped for each wild type recombinant protein using serum IgE from a peanut allergic population. The wild type recombinant Ara h 1, Ara h 2, and Ara h 3 constructs were then modified by single amino acid substitutions to disrupt the mapped IgE epitopes. In summary, the rationale for modifying the peanut proteins to disrupt sequential IgE binding epitopes was to potentially reduce the ability of these proteins to produce allergic responses following administration of the proteins during immunotherapy by reducing the response of mast cells. However, regardless of the scientific rationale for using modified peanut proteins, and the safety profile demonstrated in animal models, it has not been proven that EMP-123 has reduced IgE binding, or has an “improved” safety profile over wild type proteins because of reduced IgE binding.

Encapsulation of the Modified Peanut Proteins in Heat/Phenol-Killed E. coli: One therapeutic strategy for suppressing antigen-specific Th2 responses is to specifically activate antigen-specific Th1 T-cells. Experimentally, the activation of antigen-presenting cell (APCs) and the induction of Th1 immunity have been accomplished by the use of adjuvants, many of which contain fragmented bacteria. More recently, immunization with defined adjuvants has proven extremely effective in the elicitation of long-term Th1 responses. Most notably recombinant bacterial vaccine vectors have been used to elicit or boost Th1 immune responses to protozoan (Matsumoto et al. 1998), viral (Shen et al. 1995), or protein antigens (Shen et al. 1998). In these cases, a gene from a viral, protozoan, or protein antigen (i.e., a protein) is introduced and expressed in a bacterial vector (i.e., resulting in cellular encapsulated antigen). These engineered bacteria provide the APC activating component necessary for the induction of Th1 immunity, as well as the antigen to which the Th1 cells response is desired. E. coli is a well-characterized, gram-negative bacterium that induces potent Th1 immunity. Unique to gram-negative bacteria is the expression of lipopolysaccharide that also activates APCs. Hence the rationale for encapsulating the modified peanut proteins in E. coli. Heat/phenol-killed E. coli are used to avoid the obvious risk of exposing patients to live bacteria. Encapsulation of the modified peanut proteins has yet another potential advantage. The delivery of modified peanut proteins encapsulated within an intact cellular delivery system should reduce the potential for
allergic reactions upon administration of the proteins by hiding the proteins from mast cells (i.e., the antigens are not available to trigger mast cell degranulation while encapsulated).

**Rectal Administration of EMP-123:** There are potential advantages to using rectal administration for EMP-123. The lower colon and rectal vault are colonized with millions of bacterial organisms that the local mucosal immune system “tolerates”. Therefore, it is anticipated that rectal administration of heat/phenol-killed *E. coli* will pose minimal risk. It is also hypothesized that the mucosal immune response of the lower colon may favor tolerance induction. Furthermore, given that absorption of peanut antigen following accidental peanut ingestion occurs via the GI tract, it is also desirable to choose a route of administration that might specifically alter the gut immune response to the peanut antigens. In support of the hypothesis that the route of administration can be used as a means of targeting the immune response, clinical studies of a whole cell biologic cholera vaccine demonstrated that rectal administration of the study drug was efficacious in modulating rectal immune responses (Kozlowski et al. 1997; Kozlowski et al. 2002). Studies in a murine model of peanut anaphylaxis demonstrated that rectal administration of EMP-123 induced desensitization (described further below).

Alleretein is currently conducting two nonclinical studies of EMP-123 in support of the proposed clinical trial: 1) an efficacy study in a murine model of peanut anaphylaxis that also serves as a safety study of the anaphylactic potential of EMP-123 in peanut sensitized animals, and 2) a 16-week repeated dose toxicity study of rectally administered EMP-123 in dogs, that includes cardiovascular safety parameters. Both studies utilized EMP-123 that is comparable to that which will be used in the proposed clinical study. These studies are summarized briefly below.

**In Vivo Desensitization Study of Rectally Administered EMP-123 in Murine Model of Peanut Anaphylaxis (Study No. PNSK9):** The objective of this study was to evaluate the *in vivo* desensitization efficacy of rectally administered EMP-123 in peanut-sensitized female C3H/HeJ mice and to evaluate the safety of EMP-123 by assessing its anaphylactic potential in peanut-sensitized animals. Four groups of mice (10/group) were used for this study. All but one group (naïve control, receiving no treatments of any type) were first sensitized with peanut plus cholera toxin over a period of 8 weeks. Two weeks after the completion of sensitization, at Study Week 10, all but the naïve mice began a desensitization treatment regimen consisting of either vehicle control, vector control, or 0.23 mg/mouse EMP-123 rectally administered once a week for three consecutive weeks. At Study Weeks 14, 18, and 22 the mice were challenged orally with freshly ground whole peanut. Mice were observed for symptoms of anaphylaxis after each desensitization treatment (Weeks 10, 11, and 12). After each challenge, the mice were evaluated for anaphylactic symptoms, body temperature, and plasma histamine levels. Serum levels of peanut-specific IgE, IgG1 and IgG2a were measured throughout the course of the study. Following the final oral peanut challenge, the spleens were removed, spleen cells isolated, and the resulting spleen cell cultures assayed for cytokines [interleukin-4 (IL-4), IL-5, and interferon-gamma (IFN-γ)] and T-cell proliferation.
Mice treated with EMP-123 showed no symptoms of anaphylaxis during the EMP-123 treatment period, indicating that EMP-123 itself lacks anaphylactic potential. Therefore, EMP-123 appears to be safe in peanut sensitized mice. Further, rectal administration of 0.23 mg/mouse EMP-123 once a week for three consecutive weeks in peanut sensitized mice resulted in desensitization of some but not all of the mice to oral food challenge, as demonstrated by a reduction in the anaphylactic scores and a reduced effect on body temperature. A similar effect was noted in the animals that received the vector control but to a lesser extent. The peanut-specific IgE, IgG1 and IgG2a assessments, and cytokine and T-cell proliferation assessments are underway.

16-Week Repeated Dose Toxicity Study of Rectally Administered EMP-123 in Beagle Dogs (Study No. 12110.01.02): The objective of this study was to assess the toxicity of rectally administered EMP-123 when given once weekly for 16 weeks to beagle dogs. Three groups of dogs (7/sex/group) were rectally administered either vehicle control, low dose EMP-123 (3.7 mg, approximately equivalent to the maximum anticipated clinical dose of the modified peanut proteins, unadjusted for body weight) and high dose EMP-123 (35.1 mg, equivalent to 10 times the maximum anticipated clinical dose of the modified peanut proteins, unadjusted for body weight) once a week for 16 weeks, followed by a 2-week recovery period. The study included a full battery of clinical observations, clinical pathology, gross pathology, and histopathological assessments, as well as cardiovascular and peanut-specific IgE and IgG assessments. The in-life portion of this study is complete and there were no adverse findings or ECG abnormalities during the in-life phase that were attributed to the EMP-123. Necropsy, histopathology, and peanut-specific IgE and IgG assessments are underway.