IMPROVES : Investigating Modified Protocols of Oral immunotherapy to validate Efficacy and Safety

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1. Background, knowledge gaps and Impact of the research: (*our publications marked* *)

1.1 Food allergy is a common problem with substantial societal, nutritional and economic impact.

Almost 3,500,000 Canadians are reported to have food allergy, a life threatening condition associated with substantial social and economic burden.[1-5]* Studies suggest a worrisome increase in food allergy prevalence in the last decade mainly in North America.*[6,7] Up to 20% of households in Canada have at least one member with a food allergy while another 30% consider food allergies when purchasing or preparing foods (e.g., their school age child attends a school where certain allergenic foods are prohibited). [4,6,8]* Further, food-induced anaphylaxis, the most severe manifestation of food allergy, has almost doubled in the last decade among Canadian children presenting to the emergency department (ED).[2,9]* It is estimated that the annual cost (direct & indirect) for Canadians with food allergies is 2.9 billion dollars.[10-12]

The most common food allergy affecting Canadian children and the major cause of anaphylaxis is peanut,[2,4,13]* while milk allergy is associated with the highest annual risk for accidental allergic reactions (up to 40%).[14,15] Hence, managing these food allergies appropriately is of crucial importance. Until recently the only management for food allergy was avoidance.[16]* However, elimination of peanut and milk in children's diets has substantial nutritional and health implications. Peanuts are one of the most widely consumed legumes globally due to their nutritional value, taste, and affordability.[17] Studies have shown that consumption of dairy products are beneficial for building muscle and bone, lowering blood pressure and low-density lipoprotein cholesterol, and preventing tooth decay, diabetes, cancer, and obesity.[18] Indeed an expanding body of research suggests that food allergy, mainly milk allergy, and elimination diets can negatively affect childhood growth and nutrition.[19,20] For egg, the perfect balance and diversity in nutrients along with its high digestibility and its affordable price has highlighted egg as a basic food for humans. Further, given the need for strict avoidance, high risk of accidental reactions,[21-24]* paying meticulous attention to food labelling[25,26]* and perpetual vigilance for potential undeclared sources of contamination, it is unsurprising that individuals with food allergies and their families have higher levels of anxiety and a lower quality of life than those with other chronic conditions such as diabetes mellitus and inflammatory bowel disease.[27] We must develop management strategies that replace avoidance of these important sources of nutrients, and that are highly accessible, in order to improve the health of Canadians with food allergies.

1.2 The shift of paradigm in food allergy management and the use of oral immunotherapy

While only 10 years ago management relied solely on avoidance of the offending allergen and rescue treatment for accidental reactions with epinephrine injection,[16]* it is now agreed that almost 80% of children[28-30]* can be desensitized and protected from accidental reactions through gradual and medically supervised introduction of the allergic food, via oral immunotherapy (OIT).[30,31]* OIT has a

marked effect on immunological and clinical markers and allows for many fold increases in the threshold of food which elicits an allergic reaction.[21,30]* This increase in tolerance protects children from reactions after accidental exposure to their food allergen and in some cases allows normal introduction into their diet.[30] Accordingly, protocols for OIT for the main food allergens have been recently incorporated in clinical practice and their clinical benefits have been acknowledged in European and Canadian official guidelines.[30,32]

Adaption of OIT as a management for food allergy has been slow for two main reasons. Firstly, the most widely used OIT protocols create more allergic reactions than they avoid.[29,33]* Secondly, in the USA, the FDA treats peanut OIT as a drug because it is treating a disease (peanut allergy). The clinical efficacy of OIT for food allergy is further attested by the FDA approval (https://www.wsj.com/articles/fda-approves-first-drug-for-peanut-allergy-11580510666) of the very first pharmaceutical peanut allergy treatment, Palforzia(https://www.fda.gov/media/134838/download), encapsulated peanut protein manufactured from defatted peanut flour. However, the prescription of Palfozia requires both the patient and physician to agree to a risk mitigation strategy, deterring potential users. Further, while thousands of children have undergone OIT treatment with regular, store bought foods and this approach is endorsed by the Canadian[30] and European[32] Allergy associations, it requires intense medical and nursing management which limits its accessibility.

Hence, while the clinical benefits of OIT inducing desensitization are promising, safer protocols using regular, store-bought foods are required.

2. Overarching Objective: Using a randomized trial design, the investigators aim to develop, through our **IMPROVES** study, new OIT protocols that will promote a safer, effective strategy for OIT. This proposal will focus on peanut, milk and egg allergy given their frequency and effect on the health of Canadian children.

3. Hypothesis: We hypothesize the proportion of anaphylactic reactions among all therapeutic doses will be at least five times lower among participants managed through our two modified protocols when compared to the standard protocol in use today. We also anticipate that immune biomarkers will change similarly over time in the three OIT protocols assessed indicating similar efficacies between the three protocols. Finally, we expect that accidental reactions due to inadvertent exposure after the maintenance dose is attained will occur in less than 5 % of our patients over a three-year period.

4. Experimental aims: We will test our hypothesis using the following three Aims:
Aim 1. Develop and evaluate modified protocols that will improve the safety of OIT.
Aim 2: Determine the long-term adherence and protective effect of OIT for all protocols used.
Aim 3: Explore changes in immune biomarkers over the course of desensitization.

5. Research plan/Methodological approach/Study design

AIM1: DEVELOP AND EVALUATE MODIFIED PROTOCOLS THAT WILL IMPROVE THE SAFETY OF

Background and Rationale: Gaps related to current oral immunotherapy protocols
Would lower target doses be as efficacious while safer?

The threshold doses provoking reactions to peanut, the most common food allergy in Canadian children, range from 15mg to 1000mg,[34,35] and it is reported that eliciting doses for milk proteins are as low as 0.1 mg for sensitized individuals.[36] Alarmingly, almost a third of products from Western Europe and two thirds of products from eastern Europe without precautionary labeling contain detectable levels of peanut up to 245 mg per liter.[37] It was reported that products marked as milk free can contain up to 15 mg of milk protein[31]. Hence it is crucial that desensitization protocols cover threshold doses of at least 300mg of food protein, a dose that is equivalent approximately to 1.5 peanuts and was reported as a target safe dose by our group and by others[30,31]. Interestingly, studies reveal that OIT using doses that are 10 times lower still offer protection against this target dose.[31*] A recent small sample study reveals that children undergoing OIT using peanut powder up to 133 mg/day tolerated a challenge of 300 mg with a good safety profile.[38] **Preliminary data from our group on the use of low dose protocols clearly indicate the feasibility and safety of these protocols in a small group of 14 children with peanut allergy who were randomized to either high dose of maintenance (300mg) or low dose (30mg) . Our preliminary findings reveal that children desensitized to 30 mg versus 300mg tolerated similar amount of peanut at the exit challenge (5522.5mg versus 5795.2mg on average respectively) and that the rate of anaphylaxis was three times higher in the higher dose group (45 versus 15 cases of anaphylaxis). Further, there were six severe anaphylactic reactions in the high dose group (defined as the development of hypoxia or hypotension) and no severe reactions in the low dose group.**

Similarly for milk, it was reported that ***low doses*** (e.g. 20 ml of milk)[39,40] ***were as effective as high doses*** (100ml)[40-42] and, that the risk of severe symptoms in the maintenance phase was five times lower in the low-dose group.[39,43] Using lower doses (e.g. 10 times lower than currently used protocols) if still effective, is clearly of advantage given the better safety profile.[29,30]

Would OIT using processed forms be as effective while safer?

Studies suggest that a large subset of children who react to uncooked foods, mainly milk, can tolerate cooked forms of these foods.[44,45] Further, immunologic changes induced by a diet containing well-cooked milk are reported to be similar to changes that have been observed during OIT.[45] Similarly, there is ample evidence that boiled peanut versus raw and roasted have decreased the risk of allergic reactions to peanut.[46] It was also reported in two small sample studies that desensitization to fresh milk can be achieved with gradual exposure to well-cooked milk in baked goods in almost half of the cases.[47,48] However, the safety and efficacy of this approach compared to current protocols using uncooked milk was not yet been established. Such a comparison is crucial in order to determine the best strategy to desensitize children. In addition, the peanut snack used for the LEAP study was a processed form consisting of corn puff/stick containing approximately 50% peanut protein (Bamba; Osem Food Industries, Shoham, Israel), and was reported to be an effective OIT strategy.[49] Given the practicality and safety of OIT using processed/cooked forms of food allergens, it is of particular interest to compare protocols incorporating initially processed/cooked forms and then transitioning to unprocessed/uncooked forms of peanut/milk to currently used protocols.

Primary objective: Compare the rate of anaphylaxis in different OIT protocols.
Secondary objective: Compare the efficacy of protocols A to C

Study Procedures: (Figure 1): Three OIT protocols (A, B, C) will be assessed each for milk, egg or peanut. Each protocol will use a group of 20 males and 20 females, between 2 to 40 years, with physician diagnosed food allergy. Participants will be recruited from the allergy clinic at the Montreal Children's Hospital and at the collaborating site The Hospital for Sick Kids in Toronto. We will base our proposed desensitization protocol on previous studies by our group on milk, egg and peanut

desensitization protocols that have been active in the last year at the McGill University Health Centre[29,31]* and at the Hospital for Sick Children[29,31]* and on current existing guidelines.[30]

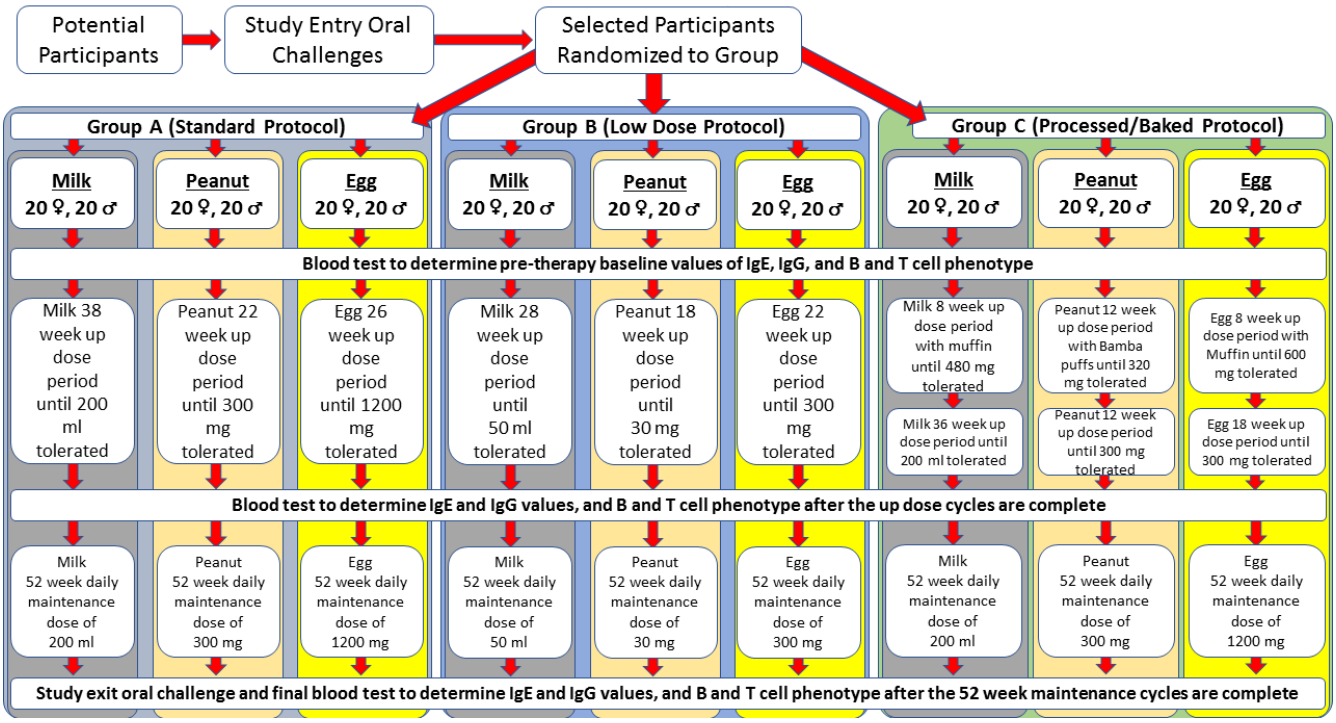

Figure 1. Flow chart for study procedures: Protocols A to C for milk and peanut

Inclusion criteria:

- Patients between 2 and 40 who satisfy all the following criteria will be included:
- A history suggestive of immediate allergy to the food. A convincing clinical history of an IgE mediated reaction to a specific food will be defined as a minimum of 2 mild signs/symptoms or 1 moderate or 1 severe sign/symptom that was likely IgE mediated and occurred within 120 minutes after ingestion or contact. [29,50]*
- The presence of at least one of the following confirmatory tests:
  - Positive SPT to the culprit food allergen (weal diameter 3 mm larger than that of the normal saline control). The allergens used will be commercial extracts of the foods (Omega Labs, Toronto, Ontario).
  - Detection of serum specific IgE (>0.35 kU/L) to the culprit food or any of its proteins, measured by fluorescence enzyme immunoassay (Phadia, CAP System, Uppsala, Sweden).
  - Informed consent form signed by the parents or legal guardian.

Exclusion criteria:

- Patients who have uncontrolled respiratory disease (asthma, cystic fibrosis, etc.)
- Patients who present with intercurrent disease active at the time of starting desensitization.
- Non IgE mediated or non-immunological adverse reactions to milk or peanuts.
- Malignant or immunopathological diseases and/or severe primary or secondary immune deficiencies.
- Patients receiving oral immunosuppressor therapy.
- Patients receiving β-blockers (including topical formulations), or who receive daily doses of NSAIDs, aspirin or ACE inhibitors for cardiac issues.

- Associated diseases contraindicating the use of epinephrine: cardiovascular disease, severe hypertension or hypotension.
- Patients diagnosed with eosinophilic gastrointestinal disorders, including patients with a history of antacid use for reflux related to food impaction or with a history of esophageal spasm.
- Patients already tolerating processed/cooked forms of the food (e.g.,baked goods with milk).

Asthmatic patients will be eligible to participate in the study. These patients will have pulmonary function testing at the beginning of the study. Their asthma must be very well controlled: they must be symptom-free, and must have been on a stable dose of asthma medication for at least the previous three months. Potential subjects who do not meet these criteria at screening will will have their asthma control assessed by one of the study physicians. Their screening visit will be rescheduled for three months later. At this visit, they will have a second pulmonary function test. If the study physician decides that the subject's asthma is now well controlled, they will be able to proceed with the screening visit.

Once these criteria are assessed qualifying participants take the following steps: A qualifying oral food challenge, a two-day rush period to ensure they are able to tolerate the minimum allergen intake requirements (for peanut and milk desensitization only), an up-dosing period (also referred to as build-up phase) of 21 to 44 weeks depending upon the protocol, a maintenance period of 52 weeks followed by a final exit oral food challenge.

Qualifying Oral Food Challenge (OFC)

Studies exemplify the need for oral food challenges prior to OIT to establish the food allergy given high false positive rates for skin tests and IgE levels alone.[30,51,52] Further, conducting food challenges at study entry also allow us to determine the tolerated cumulative dose of the culprit food and allow us to evaluate the effect of OIT on this threshold. Patients will begin with a double blinded, placebo-controlled oral food challenge (DBPCFC) to the culprit food that will take place over two days, with patients receiving doses of placebo one day and the food protein the other. For peanut, the protein will be in the form of crushed peanut, powdered egg white for egg protein for milk the protein will be in the form of fresh cow's milk. Patients, study nurses and evaluating physicians will be blinded, but not the study coordinator. On challenge days, patients will arrive at the Center for Innovative Medicine (CIM) in the morning. The CIM services include equipment and medications required to monitor and treat potential reactions. The study nurses that assist in the desensitization protocol have been trained to work in an intensive care unit. Patients will be reminded beforehand that they must be in good health for the challenge and must not have taken anti-histamines in the last seven days. If they are not in good health, the challenge will be rescheduled. Upon arriving, a physical exam will be performed and vital signs taken. A complete allergic history will be taken. Skin prick test to peanut/tree nut will be performed to establish baseline values in both. An IV catheter will be inserted in the patient's arm. In the case of egg and peanut/tree nut, the doses will be mixed in a food vehicle tolerated by the participant, such as pudding, fruit puree or crumbled crackers. On the placebo days, a substance that the participant is known to tolerate, such as wheat flour, will be used. Doses will be given by the study nurse every 15-30 minutes, depending on patients' tolerance of the dose (see Appendix B for challenge doses). If participants tolerate the last dose, they will be given a dose of food that contains the allergen in question – peanut butter in the case of a peanut challenge, or a softly coked omelet for egg allergy. For the milk challenges, to maintain the blind as well as making the doses more palatable, the doses will be mixed with chocolate syrup. The placebo doses of the milk challenges will be a milk substitute

that the participant is known to tolerate, such as soy, oat, rice or almond milk. The study nurse will be with the participant for the duration of the challenge and as soon as the participant demonstrates objective signs of an allergic reaction, the challenge will be stopped and the reaction treated. Participants will be kept under observation in the CIM for a minimum of two hours after a dose of epinephrine and/or one hour after the resolution of the last allergic symptoms.

Participants will then be randomly assigned in a 1:1:1 fashion to one of 3 study groups (A, B, C) using a process of stratified randomization (according to sex) using opaque envelops. Participants tolerating a minimum cumulative dose of 154.4 ml of milk, or the 300 mg dose of peanut/egg will be considered screen fails and **NOT** be included in the study.

Two-Day Rush Desensitization Period

Milk: The two-day rush desensitization will begin with a first single dose of milk diluted 1/1000 and will advance in 10 increments over 2 days until the participant can tolerate 2.5 ml 1:10 diluted milk on day 2. Patients will be instructed on how to make the diluted milk doses by a member of the study team. They will then continue this dosage daily at home for 2 weeks (all Doses for Rush Desensitization are in Appendix B).

Peanut: The two-day rush desensitization period will begin with 0.5 mg of peanut protein. After the initial dose, subjects will receive approximately doubling doses every 30 minutes until a dose of 5 mg. Those who tolerate 5 mg, will be asked to return the next day for one dose of 3mg. They will continue this dosage daily at home for 2 weeks.

For these allergens, if a participant develops allergic symptoms during the Rush desensitization phase, the procedure will be stopped. The participant will begin their home dosing with the last tolerated dose during the Rush Desensitization that caused no allergic symptoms.

Egg: No Rush Desensitization will take place for egg desensitization. Rather, participants will begin their desensitization dosing with the last dose of egg powder that was tolerated during the challenge (for example, if the participant tolerated the third dose of egg powder during the challenge, but reacted to the fourth dose, desensitization will begin with the amount of egg powder present in the third dose). Participants will come to the CIM and take this dose under nursing supervision. If the dose is well tolerated, they will begin taking this dose at home.

Up Dosing period

Two weeks after the two-day rush desensitization, participants will begin the build-up phase. Participants will come to the clinic every two weeks to receive their first new dose at each up-dose level. Subsequent home doses of milk are measured by the participant or their parent/caregiver using a syringe. The study staff will train them in the use of the syringe, and supply all syringes required.

Home doses of peanut and egg will be provided in individual pre-weighed containers.

Group A (the standard protocol currently used in our centers and by other groups[21,29,30]*):

Milk: Beginning with a dose of 2.5 ml (1:10 dilution) subsequent doses are incrementally increased every two weeks until the maintenance dose of **200 ml** is tolerated. This phase will take approximately 38 weeks.

Peanut: Beginning with a dose of 3 mg subsequent doses are incrementally increased every two weeks until the maintenance dose of **300 mg** is tolerated. This phase will take approximately 22 weeks.

Egg : Beginning with the dose established during the Screening Challenge, subsequent doses are incrementally increased every two weeks until the maintenance dose of **1200mg** egg protein (~1/5 of an egg) is tolerated. This phase will take approximately 24 weeks.

Group B:

Milk: Beginning with a dose of dose of 2.5 ml (1:10 dilution) subsequent doses are incrementally increased every two weeks until the maintenance dose of **50 ml** is tolerated. This phase will take approximately 28 weeks.

Peanut: Beginning with a dose of 3 mg subsequent doses are incrementally increased every two weeks until the maintenance dose of **30 mg** is tolerated. This phase will take approximately 18 weeks.

Egg: Beginning with the dose established during the Screening Challenge, subsequent doses are incrementally increased every two weeks until the maintenance dose of **300mg** egg protein (~1/20 of an egg) is tolerated. This phase will take approximately 22 weeks.

Group C:

Participants in group C will start the up-dose phase with a processed form of the allergen.

Milk: Participants randomized to Group C will be given a recipe (Appendix D) for baked goods and will use a portion of a cupcake containing 50 mg protein respectively (e.g., 1/16 of a muffin). Because allergenic protein levels are based on weights of ingredients, participants will be instructed that the recipe is to be strictly followed. This start dose has been chosen as it is reported to be well tolerated in studies using well-cooked forms of milk.[48] Participants will take these portions daily and will double the portion every two weeks until 800mg protein equivalent in baked goods is reached ~ 1 muffin). Once participants have reached this dose, they will transition to 40mg of milk protein (equivalent to a dose of 1 ml of pure milk). They will double this dose every 2 weeks until 4 ml are reached. At this point the subject will continue from week 7 according to protocol A (Figure 1). This phase will take approximately 44 weeks.

Peanut: will start with 10 mg of peanut puff (one **Bamba**, a peanut-butter-flavored **snack**, one peanut puff = 80 mg protein). The dose will be doubled every 2 weeks and once 4 puffs are reached (320 mg processed protein) and then we will transition to 80 mg crushed standard peanut protein. The protocol will continue from week 7 according to protocol A. Total duration of the up-dosing phase is 24 weeks.

Egg Participants randomized to Group C will be given a recipe (Appendix D) and will use a portion of a muffin 75 mg protein (e.g. 1/8 of a muffin). Because allergenic protein levels are based on weights of ingredients, participants will be instructed that the recipe is to be strictly followed. Participants will take this portion daily and will double the portion every two weeks until 600mg protein equivalent in baked goods is reached (~ 1 muffin). Once participants have reached this dose, they will transition to 50 mg of egg protein and will continue according to protocol A. This phase will take approximately 26 weeks.

In addition, participants will continue to consume the tolerated baked/processed forms at least 3 times a week after the transition.

For all groups, a slow regimen will be used in those patients who will experience repeated moderate reactions or any severe reaction to a given dose; that regimen will consist of reducing the dose to the

previously tolerated dose for 1-2 weeks followed by an increase of the dose under supervision, when at least 3-4 days without symptoms.[29]*

Maintenance dose period

Once subjects have reached their group's maximum dose, they will take that dose daily for 52 weeks. During this period, subjects and families will be asked to complete daily home diaries to document that daily doses were taken, as well as to report accidental ingestions, problems with dose administration, or related symptoms. Follow-up visits in addition to or in conjunction with the biweekly visits for dose escalation will be planned at 3, 6, 9, 12 months from enrollment. Each visit will involve a recent medical history and physical examination. A complete nursing assessment of the symptoms and reactions at home, as well as the management of the co-factors and adherence to the study will be assessed at enrollment and at follow-up visits.

The patients' parents will be instructed verbally and in writing about the recommendations to be followed during desensitization and how to treat possible allergic reactions. They will also be given a direct telephone line to members of the study staff for consultation. Patients will be instructed not to perform physical exercise for 2 h before or after eating and not to take non-steroidal anti-inflammatory drugs for 3 h before or after ingestion. No special recommendations will be given for viral infections.

Group A (Standard protocol): will receive a daily maintenance dose of 200 ml milk, 300 mg peanut or 1200 mg egg for 52 weeks.

Group B (low dose protocol): will receive a daily maintenance dose of 50 ml milk, 30 mg peanut or 300 mg egg or 52 weeks.

Group C (baked /processed protocol): will receive a daily maintenance dose of 200 ml milk, or 300 mg peanut, or 1200mg egg for 52 weeks.

Study Exit oral challenge

A single oral challenge will be conducted immediately at the end of the 52-week maintenance period. The challenge will be conducted in a similar fashion to the entry challenge (IV inserted, physical exam, etc). Doses are specified in Appendix B.

Documentation of intake doses adverse events: Subjects and families will be asked to complete daily home diaries to document that doses were taken, as well as to report accidental ingestions, problems with dose administration, or adverse symptoms. The patients' parents will be instructed verbally and in writing about the recommendations to be followed during desensitization and how to treat possible allergic reactions. They will also be given a direct telephone line to members of the study staff for consultation. In addition patients will be able to contact our team through a dedicated email and text messages.

Classification of adverse reactions: Reactions during the desensitization protocol will be classified according to the categories proposed by Perry et al.[53] Mild reactions are defined when symptoms are limited to the oral mucosa or the skin; severe reactions include cardiovascular or respiratory symptoms or involvement of any four systems; all other reactions will classified as moderate, although the investigators will consider isolated abdominal discomfort as mild when it lasted for 30 min or less.[29]*

Anaphylaxis will be defined when reactions involve at least 2 organ systems or hypotension develops.[54] A modified grading system described by Muraro et al. will be used to classify anaphylaxis severity to mild, moderate and severe.[55]

Definition of successful desensitization: Successful OIT will be defined when the patient will be able to tolerate 200ml (8000mg protein of milk) , one egg (6000mg egg protein) 300 mg of peanut protein(~ one peanut). Partial desensitization will be defined when a dose greater than 100ml milk/ 1000mg egg / 100 mg of the peanut protein is tolerated. Patients who do not tolerate 100ml milk / 1000mg egg protein / 100 mg of peanut protein at the exit challenge will be considered as study failure. Participants in Group B-C who have allergic reactions during the exit challenge will be offered protocol A if they wish to continue.

Successful OIT will be defined when the patient will be able to tolerate 200ml (8000mg) of milk protein or 300mg of peanut protein. Partial desensitization will be defined when a dose greater than 100ml/100mg respectively of the culprit protein is tolerated. Patients who do not tolerate 100ml of the milk or 100mg peanut protein at the exit challenge will be considered as study failure. Participants in Group B-C who have allergic reactions during the exit challenge will be offered protocol A if they wish to continue.

Discontinuation of study treatment: Discontinuation of study treatment for a patient occurs when the study treatment is stopped earlier than the protocol planned duration. This can be initiated by the participant or by the investigator at any time. Participants can withdraw their consent to participate at any time for any reason. Participants who do withdraw will be asked to attend a last visit at the study site in order to review their allergic status. Discontinuation can also be initiated by the investigator if they believe that continuation would negatively impact the risk/benefit of study participation, such as pregnancy or pregnancy planning.

Attrition and failure: Studies by our group and by others report that almost 10% of participants in protocol A will withdraw from the study mainly due to anaphylactic reactions occurring during the buildup phase, while 20% will complete the program but fail the oral food challenge at the end of the therapy.[29]*[30].

Statistical Considerations: We will base our sample size calculations on our primary objective, i.e., the number of anaphylactic reactions among participants in the test protocols - B, C versus the non-modified protocol A. Given that preliminary data by our group (unpublished) and previous published studies by others suggest that modified protocols reduce by the risk of severe side effects compared to the high dose non modified protocol (protocol A)[43] by at least 5-fold, and given previous research by our group reveals that 80% of participants will report anaphylactic reactions, a sample size of 40 participants per group (a total of 120) will provide > 90% statistical power at a significance level of 0.05 using chi-squared tests, with Bonferroni correction accounting for multiple comparison to show increased safety. This sample size will also provide adequate statistical power for exploratory analyses in our secondary objectives.

Descriptive statistics, including means and standard deviations for continuous data, and proportions for categorical data, will be computed for all study variables. multiple logistic regression models will be used to assess factors associated with increased risk of anaphylaxis (i.e. baseline demographic characteristics; gender; sex, age, presence of co-morbidities such as asthma or eczema).

For the secondary objective(Compare the efficacy of protocols A to C) , we will be able to determine an efficacy of 70% with a 95% confidence interval of 53.3% to 82.9%.

AIM 2: DETERMINE THE LONG-TERM ADHERENCE AND PROTECTIVE EFFECTS OF OIT

Background and rational:*Do patients adhere to OIT protocols after maintenance achieved?*

Only two studies have so far attempted to determine long term adherence to OIT.[56,57] The first study followed for 6 months 145 children reaching peanut OIT maintenance. The researchers reported that adherence to treatment was significantly higher in patients consuming 1200 mg (96.1%) versus those consuming 3000 mg (72.2%), (P =.001).[56] The second study followed 101 children going through egg OIT for one year and revealed that in children with daily consumption the risk of allergic events decreased five folds from build-up phase to maintenance (from 5.3 on average per patient to 1.3 on average) and that this risk was higher in those consuming egg every two days ([0.76 ± 1.85 (0-7) with daily consumption vs 2.1 ± 3.49 (0-7) when consumed every two days ($P < 0.05$) respectively].[57] No study so far assessed long term adherence to milk OIT. It is crucial to assess longer follow up periods using different protocols in order to determine which protocol is more likely to be followed in the long term and to determine the annual risk of allergic reactions. Such a comparison between protocols will contribute to determine which protocol will have a higher long term social and health impact.

What is the risk of accidental reactions after achieving OIT maintenance?

It is estimated that up to 50% of children with food allergy will experience an accidental reaction annually[14] despite continuous vigilance[22,58]* and that these reactions may be fatal.[59,60] Although several studies reported on high rate of allergic reactions during the build-up phase of OIT,[29,30]* no studies so far assessed long term (years) effect of OIT on the incidence of allergic reactions after maintenance is attained.

Objectives: 1.To determine long term (4 years) adherence to OIT 2. To assess the annual risk of allergic reactions <u>over a 4 year period.</u>

<u>Study Procedures:</u> Long term compliance with OIT and the risk of future reactions will be assessed through a follow up questionnaire that will be administered annually to patients after the exit challenge for 4 years (please see Appendix D for questionnaires). Patients will be queried on the continuous consumption (how often do they consume the maintenance dose per week), the occurrence of allergic reactions (the suspected culprit, the clinical presentation defined as mild, moderate, severe or anaphylaxis as previously defined in this proposal and based on previous publications by our group [29]*) and their management.

<u>Statistical Considerations:</u> The proportion of patients adhering to the OIT protocol and factors associated with lack of compliance will be assessed with a cox model and Kaplan-Meier curve. For each time interval, cumulative compliance probability will be calculated as the number of subjects compliant with the protocol divided by the number of patients at risk. Similarly, the incidence of patients experiencing allergic reactions over the 4 years follow up will be calculated.

<u>AIM 3: EXPLORE CHANGES IN IMMUNE BIOMARKERS OVER THE COURSE OF DESENSITIZATION</u>

<u>Background and rationale: Are there significant long-term immunologic changes that would predict sustained desensitization?</u>

The symptoms of food allergy are initiated by the presence of IgE antibodies against foods. In individuals without food allergies, the level of IgE antibodies is low, and in fact although individuals make IgG against foods, this is of unknown significance.[61] The exception to this is IgG4, which we and others have shown slowly increases as OIT progresses, and correlates with improvement in clinical performance.[62] At the same time, production of IgE against the food used in OIT actually increases and does not decrease significantly until well after maintenance is initiated, 6-9 months after starting therapy. The balance between increasing IgG4 despite maintaining IgE has led to the concept that IgG4 plays the role of a "blocking antibody" to counteract the adverse functions of IgE. It is not known if the lower dose OIT protocols will induce the same incremental changes in IgG4 within the same time

frames, or if there will be delays in the diminution of IgE.[63] Based on our preliminary work with milk we expect at least a 50% increase in the values of specific IgG4, and a 50% decrease in milk component specific IgE over one year (Figure 2 A and B respectively).

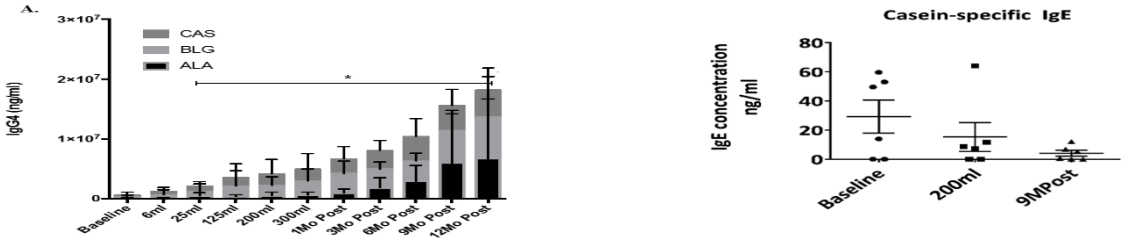

Figure 2: A. IgG4 increases in 10 patients undergoing Milk OIT using the standard protocol (A:200 ml maintenance dose) and in **B.** temporal changes in specific IgE in 8 patients undergoing Milk OIT using the standard protocol over 1 year (CAS: Casein, BLG: betalactoglobulin, ALA-alphalactalbumin)

What influences the production of IgG4? B-lymphocytes are cells specialized for producing antibodies, and play a crucial role in the pathogenesis of food allergy by producing IgE. In addition to this already important role, B-cells likely play other crucial roles in the ability tolerate food proteins. While B-cells can produce cytokines such as IL-4 and IL-13, which contribute to allergic inflammation in lungs and sinuses [64] and increase IgE production. However, B-cells can play an active role as regulatory cells in various infectious and inflammatory processes. B-regulatory(Breg) cells produce regulatory cytokines, such as IL-10 and TGF-β. **The production of IgG4 is highly influenced by the presence of IL-10**.[65] B-cells make both IgE which causes allergies and IgG4 (which regulates allergies). We have shown in preliminary data that OIT can we understanding the contribution of Breg to food desensitization is an important facet of clarifying the immunological network that is in play in this disease. Thus, we will examine the total numbers of Breg (designated by the surface markers CD19 and CD38 as well as the intracellular cytokines IL-10) and determine changes in these cells in children undergoing different OIT protocols.

What are the changes regulatory T (Treg) and regulatory B cells in different OIT protocols?

Another key cell that is crucial for inducing tolerance in allergy is the regulatory T-cell.Regulatory T cells (Treg) expressing the FOXP3 transcription factor are a subset of $CD4^+$ T cells that play a key role in the induction and maintenance of tolerance to a plethora of self and non-self-antigens in murine models and humans. Importantly, $FOXP3^+$ Treg cells are implicated in the control of allergen-specific effector T (Teff) cells, and as such, serve as a key cellular mechanism for the induction and maintenance of oral tolerance to food.A potential suppressive role of Treg in food allergies was exemplified both in animal models [66,67] and in human studies. At least two studies showed increased Treg cells in peripheral blood in children who spontaneously outgrew milk allergy[68,69]. We have shown that antigen-specific Treg attenuate airway inflammation in a mouse model of asthma.[70,71]*, in part due to production of regulatory cytokines like IL-10 and TGF-β. It is not known if increases in Treg during the natural outgrowth of peanut/milk allergy are mirrored by similar increased in Treg following in active oral immunotherapy. In a pilot study in millk-allergic children, we reported that the systemic frequency of milk allergen (casein)-specific Treg cells correlated with successful milk OIT, while a similar study showed no correlation in similar settings when evaluating polyclonal Treg cells. Similarly for peanut[72] it was reported that oral immunotherapy results in increased antigen-induced Treg cell function.[72,73] Thus, we will investigate the effect of immunotherapy to peanut and milk on the circulating frequency and function of allergen-specific Teff and Treg cells.

We will track Breg from peripheral blood mononuclear cells taken from children at the indicated time points; we predict that as IgG4 increases, so will the percentage of CD19+IL-10+ positive cells in their

blood, as detected by flow cytometry. We also predict that the Breg population will be stable over the 3 years of follow up, leading to maintenance of IgG4 and tolerance to milk or peanut.

We will carefully monitor and quantify antigen-specific, $CD4^+$ T cell subsets in blood cells by multi-parametric flow cytometry. To this end, we will identify antigen-activated Treg and Teff directly *ex vivo* through the differential expression of CD137 (Treg) and CD154 (Teff) in conjunction with reference Treg signature markers CD25, FOXP3 and Helios, as we have previously published. We will establish a correlation between cycling (intranuclear Ki67 expression) and function (cytokine expression by intracellular staining: allergic [IL-4] and non-allergic [IFNg]), activated Teff cells in various groups and correlate these responses with the frequency of antigen-specific Treg cells. We will confirm the function of these allergen-specific CD4+ T cell subsets following in vitro re-stimulation with designated allergens and quantitation of suppressive or inflammatory functions, as we have shown.[74,75]* We will also determine if children who have undergone OIT through protocols A-C have decreased T-eff responses to food in different OIT protocols, and determine the cytokines that are produced by the Treg. It is yet known if changes in Treg cells will occur to the same extent in protocols based on low doses or baked /processed forms.

Objective: To assess the change in immune biomarkers over the course of OIT.

Study Procedures: We will aim to assess biomarkers including Skin Prick Tests (SPT) at study entry, after the up-dose period is complete, and after the 52 week maintenance cycle is complete and then annually through the entire follow up period in 12 consecutive participants for each group A to C. Milk and Peanut component specific IgE and IgG4 will be measured according to our published ELISA protocols for milk[29]* and recent protocols for peanut (Levy, Cohen et al submitted). At these visits, 30 mls of blood will be collected from participants. We will also assess biomarkers including tryptase levels and specific IgE levels at study entry one week and one month post challenge to assess the temporal accuracy of this biomarkers. As negative controls we will assess specific IgE and skin tests for a food allergen tolerated by the patient (typically wheat. Soy will be used as the negative control allergen in the case of a participant with wheat allergy).

Statistical Considerations: Descriptive statistics, including means and standard deviations for continuous data, and proportions for categorical data, will be computed for all study variables. The concentrations of specific IgE and IgG ,Breg and Treg at the different study time-points will be compared with their baseline values and between treatment groups using a linear mixed-effects regression model.

6. Knowledge Translation (KT) plan

Our integrated KT plans target patients and their families, caregivers and policy makers. We will involve all members of our transdisciplinary team. This collaborative and participatory approach focuses on impactful and meaningful solutions. To this end, we will continue to use social media (e.g. Facebook, Twitter) to distribute our findings effectively to all Canadians[76,77]* Going forward, we will also hold physicians and patient-focused webinars, and when the situation permits, in-person Café Scientifique-style presentations, where families can raise questions and expect meaningful answers. We will also engage with national and local patient advocacy organizations (e.g, Food Allergy Canada, Allergies Quebec), with whom we already enjoy strong collaborations, and, who participate in all stages of our study design. We highlight that, consistent with CIHR's mission of knowledge mobilization, knowledge users including clinicians, research members of the CSACI and patient representatives (Food Allergy Canada) have been active partners at all stages of preparing this

proposal. Hence, the findings from our studies will be distributed to care givers and patients and contribute to reincorporate allergenic foods in infancy and childhood, ultimately avoiding nutritional deficiencies,[78] morbidity and fatality.[78,79]

7. Potential challenges and mitigation strategies

The OIT protocols require substantial commitment from patients and family which may lead to high rate of attrition. However, in our experience patients and families with food allergy are highly motivated and follow diligently the protocol. In fact, in our last 10 years of experience with OIT, less than 15% withdrew from OIT mainly due to adverse effects. Further, we have currently more 500 families on the waiting list for OIT. Hence, we are confident we will be able to address our study aims.

8. Equity diversity and inclusion

At study entry data are collected on age, sex, and allergen. Studies by our group and by others do not suggest that these factors affect the efficacy of OIT or increase the risk of allergic reactions. However, as discussed above (Statistical Considerations section, aim 1) we will evaluate these factors through comparing uni- and multivariate logistic regressions.

9. Significance and Implications

Safer approaches of OIT are required to be able to allow patients to access the therapeutic effects of OIT. Some approaches of interest include using biological therapies to protect against anaphylaxis[80] and certainly adjuvant approaches may be needed in some very highly allergic individuals. However, these strategies are often associated with high costs and increased risk of adverse events after weaning from adjuvant therapies.[81] We maintain that there remain significant opportunities to improve safety of OIT with natural food through protocol modification as elaborated in our proposal.

The proposed research program will be the first to strategically examine the effectiveness of different OIT protocols and will contribute to future safe and effective protocols that will be incorporated in clinical practice. The study will also be one of the first to examine the safety and efficacy of oral immunotherapy in adults. **Improved practices and incorporation of safe protocols will save enormous amounts of money in both direct and indirect costs to the system and reductions in emergency room visits, will contribute to better health and will save lives**. **Further, improved protocols will also have important nutritional impact that includes a ready source of protein, minerals and vitamins, which are crucial components for growth and health. Most importantly, given the safety concerns related to current protocols, developing safe and effective protocol using regular, store bought foods will allow use of OIT outside of the hospital setting and hence increase OIT accessibility to all Canadians.**

10. Expertise, Experience & Resources

The McGill University Health center is a multi-disciplinary research institute that is an integral part of the Montreal Children's Hospital facilitating the collaboration between clinicians, epidemiologists and basic science researchers. The nominated principal applicant, Dr. Moshe Ben-Shoshan will oversee all aspects of the proposed research and will dedicate 15 hours per week (h/w) for the IMPROVES study. Dr. Ben-Shoshan is an epidemiologist and allergist at the Montreal Children's Hospital and has extensive experience managing cohorts/registries, and OIT trials[29,31*] whose findings have been published in more than 100 publications in major allergy and paediatrics journals. Dr. McCusker (Montreal Children's Hospital), Dr Mazer (Montreal Children's Hospital) (8h /W each) bring expertise in clinical immunology to the research team and will assist in patient recruitment and in supervising the

OIT procedures and laboratory analysis of IgE, and IgG4. Dr Xun Zhang, a biostatistician in McGill university is a collaborator in this initiative (1h /W). He has assisted in the development of this protocol and will supervise, together with Dr Ben-Shoshan, the statistical analysis. The collaborator, Dr Piccirillo who is a leader in the field of Regulatory T-cells, and has collaborated with our group previously, will co-supervise the analysis of these antibodies (5h /W).[74,75,82-84] Dr Julia Upton (Sick Kid's Hospital, Toronto, 10h/w) is an external applicant and will recruit patients from Sick kids Hospital. Mr Duncan Lejtenyi who has been an integral part of our team in numerous studies, will serve as research coordinator and data entry. We will continue and work with our medical nurse Mrs Liane Beaudette who has been part of the OIT team over the last 5 years and gained tremendous skills and experience in managing individuals with OIT. In addition, as in previous studies our team will include an MSc student in experimental medicine who will base their thesis on the results on this analysis. The students will dedicate at least 8 hours a week to help supervise data collection, data entry and analysis.

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APPENDICES

Appendix A : Disease burden and gaps.

Table 1. Primary and secondary prevention measures for food allergies[a]

Trigger	Primary prevention	Route of desensitization	Reference number	Secondary preventi
Foods		PO, SL	(14;68-70)	Avoidance of allerge
	Tree nut	PO	(11-13)	education of allergic inc
	Peanut	PO	(27)	their care-givers on imp
	Tree nut	SL	(29)	avoidance, improved lab
	Peach	SL	(71)	prepackaged foods for a
				wearing of Medic-Alert
				specific food allergy

[a] only the most common foods , drugs and insect desensitization approaches are mentioned.

PO, *Per os*; SL, *Sublingual*; SC,

Table 2.Environmental factors associated with food allergy.

Factor	study	Type of study	Effect
Season	Vassallo MF	Case control	Children younger than 5 years born in fall or winter had a 53% higher odds of food allergy compared with controls.
Drugs	Palli-Scholl	Case control	The relative risk to develop food-specific IgE after anti-acid therapy was 10.5 (95% CI,1.44,76.48).
Microbial exposure	Gourbeyre	Review of case control and cohort studies	No clear conclusion regarding probiotic beneficial effects on the prevention or treatment of allergy .

Food consumption (quantity and timing)	Poole JA	Cohort	After adjusting for breastfeeding duration, introduction of rice cereal, family history of allergy, and history of food allergy before 6 months of age, age at initial exposure to cereal grains continued to be strongly associated with wheat allergy (≥7 months: adjusted OR: 3.8; 95% CI, 1.18,12.28)
	Du Toit	Case control	After adjustment for atopy, other food allergies, age, and sex, the RR for peanut allergy in the UK vs Israel is 5.8 (95% CI, 2.8,11.8), and largest and most significant difference in aning between the UK and Israel was observed in the age of introductio of peanut (P < .0001). By 9 months of age, 69% of Israelis were eating peanut compared with only 10% of UK infants.
	Katz	Cohort	The OR was 19.3 (95% CI, 6.0,62.1) for development of IgE mediated CMA among infants with exposure to cow protein at th age of 15 days or more (P < .001) vs those introduced to cow protein before 15 days.
	Joseph	Cohort	Early feeding reduced the risk of peanut sensitization among children with a parental history [adjusted OR, 0.2 (95% CI, 0.1,0.7); P = .007]. The relationship also became significant forwhen a cutoff for IgE of ≥0.70 IU/mL was used [adjustedOR, 0.5 (95% CI, 0.3,0.9)].
	Koplin	Case control	Introduction of cooked at age 4 to 6 months, vs later exposure reduced the risk of allergy [OR, 0.2 (95% CI, 0.06-0.71)].
	Des	Case control	The reported consumption of peanuts during pregnancy and

	Roches		breastfeeding was higher in the case group (those who developed peanut allergy and associated with an increased risk of peanut allergy in offspring [OR, 4.22 (95%CI, 1.57,11.30) and OR, 2.28 (95% CI, 1.31,3.97) for pregnancy and breastfeeding, respectively].
	Sicherer	Case control	Multivariate analysis including clinical, laboratory, and demographic variables showed frequent peanut consumption during pregnancy {OR, 2.9(95% CI, 1.7,4.9)] to be associated with peanut IgE ≥5 kUA/L.
Food processing	Chung	Laboratory analysis	After curing and roasting, mature peanuts exhibited approximately 20% higher levels of advanced glycation end adducts and higher IgE binding vs immature peanuts.
	Yadzir	Laboratory analysis	Extracts from raw shrimp bound higher IgE than extracts from boiled shrimp, but the purified boiled tropomysoin (the main shrimp allergen) demonstrates higher IgE binding vs raw shrimp.
	Samson	Laboratory analysis	Thermal processing can lead to the formation of new antigenic structures.
Vitamin D	Milner	Cohort	Early vitamin D use (within the first 6 months of life) was associated with a higher risk for food allergies in the exclusively formula-fed population [OR,1.63(95% CI,1.21,2.20)]. Vitamin use at 3 years of age was associated with increased risk for food allergies but not asthma in both breastfed [OR,1.62(95% CI,1.19,2.21)]and exclusively formula-fed infants [OR, 1.39(95%

			CI,1.03,1.88)].
	Cramago	Ecologic study	Strong north-south gradient for the prescription of EpiPens in the United States, with the highest rates found in New England. [adjusted β for New England vs the rest of the US, 4.07 (95%CI, 2.77,5.36)]
	Mulins et al	Ecologic study	Using multivariate analysis , EpiPen prescription rates the were higher in southern latitudes (less sunlight) compared with northern regions [β, −19.22(95% CI, −26.71 , −11.73)].
	Mulins et al	Ecologic study	Southern latitudes were associated with higher hypoallergenic formulae prescription rates [beta, -147.98(95% CI,-281.83 , -14.14)].

OR, odds ratio;RR, Relative Risk; CI,confidence interval ;CMA, Cow's Allergy

Appendix B. Procedures

I. Food Challenges

1. Screening Double-Blind, Placebo Controlled Oral Food Challenges (DBPCFC)

a) Peanut

Double-blind, placebo-controlled food challenge (BPCFC) up to 100 mg (444 mg cumulative) done at screening

Dose (n:0)	Dose (mg peanut protein)	Cumulative (mg peanut protein)
1	1	1
2	3	4
3	10	14
4	30	44
5	100	144
6	300	444

For placebo day, a substance that the participant is known to tolerate

b) Milk

Double-blind, placebo-controlled food challenge (BPCFC) up to 154.4 ml done at screening

Dose (n:0)	Dose (l milk	Cumulative dose in ml
1	0.1	0.1
2	0.3	0.4
3	1	1.4
4	3	4.4
5	5	9.4
6	10	19.4
7	30	49.4
8	45	94.4
9	60	154.4

c) Egg

Double-blind, placebo-controlled food challenge (BPCFC) up to 845.1 ml done at screening

Dose (n:0)	Egg protein/placebo (mg)	Cumulative egg dose (mg)

1	**0.2 mg placebo**	**0**
2	**0.2 mg egg**	**0.2**
3	**0.4 placebo**	**0.2**
4	**0.4 mg egg**	**0.6**
5	**1.5 placebo**	**0.6**
6	**1.5 egg**	**2.1**
7	**6 egg**	**8.1**
8	**12 egg**	**20.1**
9	**25 egg**	**45.1**
10	**50 egg**	**95.1**
11	**100 egg**	**195.1**
12	**150 egg**	**345.1**
13	**200 egg**	**545.1**
14	**300 egg**	**845.1**

2. Exit Challenges

a. Peanut exit challenge

Dose	Dose (mg peanut protein)	Cumulative (peanut protein)
1	3	3
2	10	13
3	30	43
4	100	143
5	300	443
6	600	1043
7	1000	2043

b.Milk exit challenge

Dose	**Dose ml milk**	**Cumulative dose ml milk**
1	**200 ml**	**200 ml**
2	**100 ml**	**300 ml**

c. Egg exit challenge

Dose	Egg protein mg dose	Cumulative dose
1	**100**	**100**
2	**300**	**400**
3	**600**	**1000**
4	**1000**	**2000**
5	**2000**	**4000**
6	**2000**	**6000**

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Scale for Grading Reaction Severity(non anaphylactic reactions)

Score	Symptom	Action
Mild	Pruritus, Urticaria, Flushing, Rhinoconjunctivitis	Observe May give Antihistamine (e.g. Rupatidine or Reactin as prescribed **Call Research Team** Research team will evaluate if dose adjustment is needed and if next dose will be given at home or in hospital.
Moderate	Angioedema, Throat tightness, Gastrointestinal complaints (cramping, . pain,vomiting,diarrhea) Respiratory symptoms (Cough, Mucous production)	Give epinephrine IM as per protocol Give Antihistamine (e.g. Reactin or Rupatidine as prescribed Seek urgent care (hospital emergency room) Call Research team To give next adjusted dose in hospital research unit (CIM)
Severe	Wheeze, Respiratory Distress Hypoxia, Cyanosis, Hypotension Circulatory collapse (Shock)	Give epinephrine IM as per protocol Give Antihistamine (e.g. Reactin or Rupatidine as prescribed Call 911 Seek urgent care (transfer to hospital emergency room) Call Research team; if the symptoms are not improving within 10 minutes of the first dose,

		instructions will be given from the team regarding use of a second dose of epinephrine.

Anaphylaxis is defined as involvement of **2 organ systems** and/or hypotension in response to a potential allergen and anaphylaxis.[81]

Anaphylaxis severity is classified according to a modified grading system published by Muraro. [82]

a. **Mild anaphylaxis:** characterized by the presence of skin and subcutaneous tissues symptoms (urticaria, erythema, and angioedema) as well as oral pruritus, nausea (i.e. gastrointestinal involvement and cutaneous) or nasal congestion, sneezing, rhinorrhea or throat tightness (i.e. respiratory involvement).

b. **Moderate anaphylaxis**: characterized by the presence of any of the previous symptoms as well as crampy abdominal pain, diarrhea or recurrent vomiting, hoarseness, "barky" cough, difficulty swallowing, dyspnea, moderate wheezing, and or "light headedness."

c. **Severe anaphylaxis**: characterized by cyanosis, hypoxia (saturation<92%), respiratory arrest, hypotension, dysrhythmia, confusion or loss of consciousness.

II. Rush Desensitization

Day 1 and 2 common for group A, B, C

- **Verify no wheezing, flare of atopic disease, or other intercurrent illness**
- **Update history and con meds**

- **Vital signs and PE**
- **PEFR**
- **Dose escalation at 20 minute intervals**
- **Post-dose vital signs**
- **Vital signs q30min x3 post last dose**
- **Monitoring for AEs (see**

1 Peanut /tree nut: groups A/B/C

Day 1	Day 1	Day 2	Day 2
Dose (mg peanut protein)	Dose (mg crushed peanut)	Dose(mg peanut protein)	Dose (mg crushed peanut)
0.5	2.0	3	12
1.0	4.0		
1.5	6.0		
2.5	10.0		
3.0	12		
3.5	14		
4.0	16		
4.5	18		
5.0	20		

2 Milk: Groups A/B/C

Time Point	Milk Dilution/dose	Volume (ml)
Day 1	1/1000	1
	1/1000	2
	1/1000	4
	1/1000	8
	1/100	1.6
Day 2	1/100	1.6
	1/100	3.2
	1/100	6.4
	1/100	12
	1/10 to continiue	2.5

3 Egg: No Rush Desensitization will be done for egg desensitization – for participants in Groups A and B, the highest tolerated dose established during the Screening Challenge will be used to determine the first dose for the buildup phase.

III. Escalation Phase

1. Peanut Group A

Time Point	Dose peanut protein (mg)	Dose crushed peanut (mg)
Week 2	**3**	**12**
Week 4	**6**	**24**
Week 6	**12**	**48**
Week 8	**20**	**80**
Week 10	**40**	**160**
Week 12	**80**	**320**
Week 14	**120**	**480**
Week 16	**160**	**640**
Week 18	**200**	**800**
Week 20	**240**	**960**
Week 22	**300**	**1200**

Peanut/ tree nut: Build up phase for-group B

Time Point	Dose peanut protein (mg)	Dose crushed peanut (mg)
Week 2	**3**	**12**
Week 4	**6**	**24**
Week 6	**12**	**48**
Week 8	**20**	**80**
Week 10	**30**	**120**
Week 12	**30**	**120**
Week 14	**30**	**120**
Week 16	**30**	**120**
Week 18	**30**	**120**

Peanut/ tree nut: Build up phase for processed -group C

Time Point	Dose peanut protein (mg)	Dose crushed peanut (mg)
Week 2	**10mg ***	**1/8 puff**

Week 4	**20mg ***	**1/4**
Week 6	**40mg***	**1/2**
Week 8	**80mg***	**1**
Week 10	**160mg***	**2puffs**
Week 12	**320mg***	**4 puffs**
Week 14	**80****	**320**
Week 16	**120****	**480**
Week 18	**160****	**640**
Week 20	**200****	**800**
Week 22	**240****	**960**
Week 24	**300****	**1200**

***Processed form " Bamba" puffs for peanut**
****** crushed form of the peanut/ tree nut**

Milk :Build up phase -group A
1ml= 35mg milk protein
Desensitization protocol

Time Point	Dose	Volume (ml)	Milk protein (mg)
Week 2	**1/10**	**5**	**3.5**
Week 4	**Undiluted**	**1**	**35**
Week 6	**Undiluted**	**2**	**70**
Week 8	**Undiluted**	**4**	**140**
Week 10	**Undiluted**	**6**	**210**
Week 12	**Undiluted**	**8**	**280**
Week 14	**Undiluted**	**10**	**350**
Week 16	**Undiluted**	**12**	**420**
Week 18	**Undiluted**	**15**	**525**
Week 20	**Undiluted**	**20**	**700**
Week 22	**Undiluted**	**25**	**875**
Week 24	**Undiluted**	**30**	**1050**
Week 26	**Undiluted**	**40**	**1400**

Week 28	**Undiluted**	**50**	**1750**
Week 30	**Undiluted**	**50**	**1750**
Week 32	**Undiluted**	**50**	**1750**
Week 34	**Undiluted**	**50**	**1750**
Week 36	**Undiluted**	**150**	**5250**
Week 38	**Undiluted**	**200**	**7000**

Milk: Build up phase -group B

Desensitization protocol

Time Point	Dose	Volume (ml)	Milk protein (mg)
Week 2	**1/10**	**5**	**3.5**
Week 4	**Undiluted**	**1**	**35**
Week 6	**Undiluted**	**2**	**70**
Week 8	**Undiluted**	**4**	**140**
Week 10	**Undiluted**	**6**	**210**
Week 12	**Undiluted**	**8**	**280**
Week 14	**Undiluted**	**10**	**350**
Week 16	**Undiluted**	**12**	**420**
Week 18	**Undiluted**	**15**	**525**
Week 20	**Undiluted**	**20**	**700**
Week 22	**Undiluted**	**25**	**875**
Week 24	**Undiluted**	**30**	**1050**
Week 26	**Undiluted**	**40**	**1400**
Week 28	**Undiluted**	**50**	**1750**

Milk : Build up phase for processed -group C

Time Point	Dose	Volume /form	Milk protein (mg)

Week 2	**1/16 muffin**	**muffin**	**50mg**
Week 4	**1/8 muffin**	**muffin**	**100mg**
Week 6	**1/4 muffin**	**muffin**	**200mg**
Week 8	**1/2 muffin**	**muffin**	**400mg**
Week 10	**1 muffin**	**muffin**	**800mg**
Week 12	**Undiluted**	**1**	**35mg**
Week 14	**Undiluted**	**2**	**70mg**
Week 16	**Undiluted**	**4**	**140mg**
Week 18	**Undiluted**	**6**	**210mg**
Week 20	**Undiluted**	**8**	**280mg**
Week 22	**Undiluted**	**10**	**350mg**
Week 24	**Undiluted**	**12**	**420mg**
Week 26	**Undiluted**	**15**	**525mg**
Week 28	**Undiluted**	**20**	**700mg**
Week 30	**Undiluted**	**25**	**875mg**
Week 32	**Undiluted**	**30**	**1050mg**
Week 34	**Undiluted**	**40**	**1400mg**
Week 36	**Undiluted**	**50**	**1750mg**
Week 38	**Undiluted**	**50**	**1750mg**
Week 40	**Undiluted**	**50**	**1750mg**
Week 42	**Undiluted**	**50**	**1750mg**
Week 44	**Undiluted**	**150**	**1750mg**
Week 46	**Undiluted**	**200**	**1750mg**

1ml= 35mg milk protein

Egg :Build up phase -group A

Note: the highest tolerated dose established during the Screening Challenge will be used to determine the first dose for the buildup phase

Time Point	Dose egg powder (mg)	Dose egg protein (mg)
Week 2	0.2	0.2
Week 4	0.4	0.4
Week 6	1.5	1.5
Week 8	6	6
Week 10	12	12
Week 12	25	25
Week 14	50	50
Week 16	100	100
Week 18	150	150
Week 20	200	200
Week 22	300	300
Week 24	600	600
Week 26	1200	1200

Egg :Build up phase -group B

Note: the highest tolerated dose established during the Screening Challenge will be used to determine the first dose for the buildup phase

Time Point	Dose egg powder (mg)	Dose egg protein (mg)
Week 2	0.2	0.2
Week 4	0.4	0.4
Week 6	1.5	1.5
Week 8	6	6
Week 10	12	12
Week 12	25	25
Week 14	50	50

Week 16	**100**	**100**
Week 18	**150**	**150**
Week 20	**200**	**200**
Week 22	**300**	**300**

Egg :Build up phase -group C

All participants randomized to this group will begin with 1/8 muffin, irrespective of amount of egg powder tolerated at Screening Challenge.

Time Point	Dose muffin/egg powder (mg)	Dose egg protein (mg)
Week 2	**1/8 muffin**	**75**
Week 4	**¼ muffin**	**150**
Week 6	**½ muffin**	**300**
Week 8	**1 muffin**	**600**
Week 10	**50 (mg powder)**	**50**
Week 12	**75 (mg powder)**	**75**
Week 14	**100 (mg powder)**	**100**
Week 16	**150 (mg powder)**	**150**
Week 18	**200 (mg powder)**	**200**
Week 20	**250 (mg powder)**	**250**
Week 22	**300 (mg powder)**	**300**
Week 24	**600 (mg powder)**	**600**
Week 26	**1200 (mg powder)**	**1200**

Appendix C. Recipes

1. Milk baked

Muffin Recipe for Baked Milk

For 10 muffins (each muffin is 800 mg milk protein 1/16 muffin = 50mg)

- Follow this recipe carefully and use all the batter.
- Following baking time and temperature carefully.
- Please bring at least four (4) regular size cupcakes with you.
- Do not use jumbo or mini muffin/cupcake pans.

Ingredients:

200 of 2% Natrel Lactose-Free milk (~8000mg milk protein)
1 tablespoon of vegetable oil
1 large egg*
*If egg allergic: 1⁄4 cup of applesauce
1 teaspoon vanilla extract
1 1⁄4 cups white flour
1⁄2 cup sugar
2 teaspoon baking powder
1⁄4 teaspoon salt

Directions:

1. Preheat oven to 350 degrees F
2. Line muffin/cupcake pan(s) with standard size paper liners
3. Mix liquid ingredients: milk, vegetable oil, vanilla extract, egg or applesauce
4. In separate bowl, mix the dry ingredients: flour, sugar, salt, and baking powder
5. Add the liquid ingredients to the dry ingredients and mix thoroughly.
6. Pour mixture into pan dividing equally, scraping bowl so to use ALL the batter.

Bake for 30-35 minutes (not less).

2. Egg baked

Muffin Recipe for Baked egg - 10 muffins (each with 600mg protein of egg – will start with 1/8 =75mg)

• Follow this recipe carefully and use all the batter.
• Following baking time and temperature carefully.
• Please bring at least four (4) regular size cupcakes with you.
• Do not use jumbo or mini muffin/cupcake pans.

Ingredients:
1 cup of 2% Natrel Lactose Free milk*
'if allergic to milk can replace milk rice milk/ soy milk/ coconut milk
1 tablespoon of vegetable oil
1 large egg (6000mg protein)
1 teaspoon vanilla extract
1 1⁄4 cups white flour
1⁄2 cup sugar
2 teaspoon baking powder
1⁄4 teaspoon salt

Directions:
1. Preheat oven to 350 degrees F
2. Line muffin/cupcake pan(s) with paper liners
3. Mix liquid ingredients: milk, vegetable oil, vanilla extract, egg or applesauce
4. In separate bowl, mix the dry ingredients: flour, sugar, salt, and baking powder
5. Add the liquid ingredients to the dry ingredients and mix thoroughly.
6. Pour mixture into pan dividing equally, scraping bowl so to use ALL the batter.
Bake for 30-35 minutes (not less).