BAKED MILK ORAL IMMUNOTHERAPY FOR THE TREATMENT OF COW'S MILK ALLERGY

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1. Abstract

Food allergies affect approximately 2-3% of adults and 6-8% of children in the US population.[1-4] Cow's milk allergy (CMA) is the most common food allergy in young children, affecting 2-3% of preschool age children.[5] This allergy is often outgrown, but approximately 20% persists into adolescence and adulthood. A study of our clinic population showed that 19% of CMA resolves by 4 years, 42% by 8 years, 64% by 12 years, and 79% by 16 years.[5] An observational cohort study showed a 50% resolution rate through age 5 years.[6]

Current treatment options for CMA include avoidance and emergency medications for treatment of reactions. Due to the ubiquitous nature of milk, avoidance is particularly difficult leading to frequent and often severe reactions. Furthermore, children with milk allergy have been shown to have lower intake of calcium, zinc, and vitamin B_2[7] and lower weight and BMI.[8] Further, food restrictions have a major impact on quality of life.[9-11] For all of these reasons, effective treatment of food allergy would be highly desirable.

Over the past decade, studies have demonstrated promising results for food oral immunotherapy (OIT), which is a process of providing gradually increasing doses of allergenic proteins with the goal of inducing desensitization. However, OIT studies to date have shown high rates of adverse reactions.[12] In addition, 10-20% of subjects have dropped out of OIT trials due to adverse reactions, most commonly chronic abdominal pain.[12] Treatment approaches that could reduce adverse reactions while maintaining or even improving the development of tolerance are therefore of interest. In that regard, recent studies have shown that extensive heating of milk proteins makes them less allergenic, while still maintaining the antigenic properties needed to induce tolerance. It has been shown that 50-75% of children with CMA tolerate baked milk at full servings, such as a cupcake or muffin, [13] and that children with regular exposure to baked milk will progress to tolerance of unheated milk at a significantly accelerated rate.[14]

While the introduction of baked milk to children with CMA has now become a clinical practice, this is not an option for those patients with more severe milk allergy. These patients can react to even trace amounts of milk in any form and are far less likely to naturally outgrow their CMA. We therefore propose to approach this group of patients in this study by combining our experience with milk OIT with the advantages of utilizing extensively heated milk to make OIT safer. Our hypothesis is that children with severe CMA who are reactive to baked milk can be desensitized to cow's milk protein via baked milk OIT. We propose to explore the safety and efficacy of baked milk OIT in a double-blind, placebocontrolled study in children with persistent CMA, focusing on the more allergic children who are 1) unable to tolerate baked milk at even small servings; 2) very unlikely to naturally outgrow their milk allergy; and 3) more likely to have difficulty tolerating OIT with uncooked milk. If successful, this study could provide a much needed alternative to traditional CMA OIT, providing a safer and more tolerable approach to treatment due to heat-modification of the allergen.

2. *Objectives*

Primary Objective: The primary endpoint of this study is the safety of baked milk OIT, defined as the incidence of adverse events on baked milk OIT compared to adverse events on placebo.

Secondary Objectives include:

- Exploration of the clinical effects of baked milk OIT, defined as tolerating 4 grams of baked milk protein without adverse reactions after 12 and 24 months of treatment
- Exploration of the clinical effects of baked milk OIT, defined as tolerating 2 grams of unheated milk protein without adverse reactions at the end of study year 2

 The change in maximum tolerated dose of baked milk from baseline to end of year 1 and end of year 2

 The exploration of biomarkers and mechanistic correlates of desensitization including milk-specific IgE and IgG4, milk skin prick test responses, T cell responses.

 The exploration of the impact of milk allergy and its treatment on quality of life.

 Differences in clinical response rates based on duration of treatment (1 year versus 2 years)

 The proportion of subjects that achieve desensitization in the placebo crossover group after 1 year of dosing at the year 2 study time point.

 The proportion of subjects who tolerate 8030 mg of unheated milk protein at the end of study year 2

 The incidence of adverse events in the unblinded year of treatment

 Incidence of adverse events after stopping OIT (year 3 and 4)

Impact of cow's milk OIT on goat and sheep milk-specific IgE

 Comparison of maximum tolerated dose of baked milk compared to maximum tolerated dose of unheated milk at the end of year 2

3. Background

Food allergy is a common disease for which there is currently no effective treatment. Food allergies affect 2-3% of adults and up to 8% of children in the US population.[1-4] Cow's milk allergy (CMA) is the most common food allergy in young children, affecting 2-3% of preschool age children.[5] This allergy is often outgrown, but approximately 20% persists into adolescence and adulthood. A study of our clinic population showed that 19% resolve by 4 years, 42% by 8 years, 64% by 12 years, and 79% by 16 years.[5]

Current treatment options for CMA include avoidance and emergency medications for treatment of reactions. Due to the ubiquitous nature of milk, avoidance is particularly difficult and reactions are common and often severe. Gupta et al conducted an electronic survey of US households and found that

among children with CMA, 31% had a history of severe reactions (low blood pressure, anaphylaxis, trouble breathing, or wheezing) and 69% had a history of mild-to-moderate reactions.[2] Furthermore, strict avoidance can have nutritional consequences. Children with CMA have been shown to have lower intake of calcium, zinc, and vitamin B_2[7] and lower weight and BMI.[8] Food restrictions and the fear of accidental food exposures also have a significant impact on quality of life.[9-11]

Allergen immunotherapy is a process of providing gradually increasing doses of allergenic proteins over a period of time with the goal of developing desensitization. For the past century, subcutaneous specific immunotherapy (SCIT) has been effectively used to treat various IgE-mediated hypersensitivity reactions, including allergic rhinitis, asthma, and venom hypersensitivity. The first trial of food immunotherapy conducted in the 1990s with subcutaneous administration of peanut extract had an unacceptably high rate of systemic reactions.[15,16]

Subsequent approaches have therefore focused on different routes of delivery or modification of the allergens to reduce risk but still achieve desensitization. One such approach is referred to as oral immunotherapy (OIT), which involves consuming gradually increasing doses of an allergenic food in some vehicle. Protocols vary widely with regard to type of food and vehicle used.[12] Most current OIT protocols begin with an initial oral food challenge (OFC), followed by an initial dose escalation day. Then there is build-up dosing with dose increases provided under observation weekly or biweekly, with daily home dosing in between until maintenance dosing is achieved. Doses and the duration of maintenance vary greatly between studies. Maintenance dosing is then typically followed by an OFC to assess clinical desensitization.[17]

Several studies have shown effectiveness in desensitization for milk, egg, and peanut.[12,17-24] In 2012, a systematic review and meta-analysis of milk OIT found that 62% of individuals who underwent OIT were able to consume at least 200 mL of cow's milk, compared to 8% of controls.[25] More recently, Wood and Vickery completed a review of OIT for food allergy, which summarized clinical trials for peanut, egg, and milk.[12] Their table summarizing milk OIT studies is listed below. These have all been small studies which have varied in study design, maintenance dose, and duration. All used unheated milk as the OIT allergen. Overall, these studies have shown promising results for achieving desensitization and increasing milk challenge thresholds.

Table 1. Milk OIT Studies[12,26]

Reference	Year	Design	Sample Size	Subject Age	Maintenance dose	Duration	Conclusions
Meglio[20]	2004	Open label	n=21	6-10	200 mL	6 months	72% achieved desensitization to 200 mL of cow's milk daily
Longo[21]	2008	Randomized open label	n=30	5-17	150 mL	10-day rush escalation, 1 year maintenance	36% completely tolerant (150 mL or more) and 54% partially tolerant (5-150 mL)

Skripak[22]	2008	Randomized, placebocontrolled	n=13	6-17	500 mg milk protein	23 weeks	Median milk challenge threshold rose from 40 mg at baseline to 5140 mg after OIT
Nariesty[23]	2009	Open label (Follow up)	n=13	6-16	500 to 4,000 mg milk protein	3-17 months	Ongoing milk intake demonstrated tolerance from 1000 to 16,000 mg (median 7000) with 33% tolerating 16,000 mg on OFC
Pajno[24]	2010	Randomized, placebocontrolled	n=15	4-10	200 mL	18 weeks	67% tolerant to 200 mL cow's milk
Martorell[19]	2011	Randomized, placebocontrolled	n=30	2-3	200 mL	1 year	90% showing complete desensitization
Keet[18]	2012	Randomized, OIT vs SLIT	n=20 for OIT	6-17	1000-2000 mg	60 weeks	70% of patients receiving OIT passed an 8g OFC. Only 40% passed OFC when treatment was discontinued for 6 weeks.
Wood[27]	2015	Omalizumab DBPC, openlabel OIT	n= 57	7-32	3300 mg	24 months	80% desensitized to 10 g OFC, Sustained unresponsiveness in 42% after 8 week

However, OIT studies to date have shown high rates of adverse reactions, ranging from mild, local symptoms, such as oral itching, to severe systemic symptoms, such as anaphylaxis.[12,26] In 2011, Martorell et al conducted a 1 year milk OIT study in 2-3 year old children. While 90% were desensitized, 47% developed moderate reactions (rhinoconjunctivitis, wheezing, vomiting, urticaria) over the course of treatment.[19] In 2012, we examined the safety and efficacy of OIT and SLIT (sublingual immunotherapy) for cow's milk allergy. The study reported reactions in 30% of doses with most confined to oral pruritus, but with more significant reactions with OIT compared to SLIT.[18] In addition, 10-20% of subjects have dropped out of OIT trials due to adverse reactions, most commonly chronic abdominal pain.[12,26]

To mitigate the risks of OIT, there is great interest in developing safer approaches that will still induce desensitization. One approach is to use "modified allergens." The idea behind modified allergens is that it may be possible to alter food proteins in a way that reduces the risk of allergic reactions, while still maintaining the ability to induce desensitization. Extensive heating of milk is attracting increasing interest for the treatment of CMA because it appears to offer the clinical advantages of unheated milk with fewer adverse reactions. In addition, this modified allergen can be created using the simple process of baking in a typical household oven. The current theory is that extensive heating of milk protein induces conformational changes to epitopes responsible for IgE binding. These changes decrease the risk of allergic reactions; however, the T cell epitopes that induce the development of tolerance are largely unchanged.

There are several phenotypes of milk allergy, ranging from patients with persistent disease with a high risk of anaphylaxis to those with more short-lived disease who typically tolerate extensively heated milk. It has been shown that 50% - 75% of children with CMA tolerate baked milk at full servings, such as a cupcake or muffin,[13] and most importantly, that children who have regular exposure to baked milk will develop tolerance to unheated milk at a significantly accelerated rate.[14] Phenotypic characterization is extremely important in the management of milk allergic patients, and usually relies on oral food challenges to different forms of milk, which require significant resources and highly trained staff. To improve diagnostics of milk and other food allergies, the underlying disease mechanisms need to be better understood.

Many of the 25-50% of cow's milk allergic patients who do not tolerate heated milk have a very severe, persistent phenotype. These children have a high risk of reactions due to accidental exposures and a low likelihood of naturally outgrowing their allergy and therefore, would benefit greatly from a safe and effective form of treatment. Our hypothesis is that children with IgE mediated CMA who are reactive to baked milk can be desensitized to cow's milk protein via baked milk OIT, and that this approach will prove far safer than OIT using unheated milk. We propose to explore the efficacy and safety of baked milk OIT in a double-blind, placebo-controlled study in children with persistent CMA, focusing on the more allergic children who are 1) unable to tolerate baked milk even at small servings; 2) unlikely to naturally outgrow their milk allergy; and 3) more likely to have difficulty tolerating OIT with uncooked milk.

This approach has this far been attempted in one small study in Israel with 15 baked milk reactive patients.[28] The primary outcome was defined as attaining a maintenance baked milk dose of 1.3 grams per day without adverse reactions at 1 year of treatment. This study started with a baked milk dose of 25-120 mg. Only 3/14 (21%) patients tolerated the 1.3g goal dose. Eight did not complete the program because of IgE-mediated reactions and 3 did not complete it for other reasons.

While this study had discouraging results, we feel that we have a high chance of success with a substantially revised protocol, most importantly beginning with a far lower starting dose and using a more gradual dose escalation, similar to the protocols we have used with unheated milk. If successful, this study will provide a much needed alternative to traditional milk OIT. We are hopeful that this next generation of OIT will be safer and more tolerable due to modification of the allergen. After becoming desensitized, the need for strict milk avoidance should be eliminated, which would decrease the risk of accidental ingestions and potentially life-threatening reactions and improve overall nutrition and quality of life. We have established a collaboration with the La Jolla Institute for Allergy & Immunology with whom we will seek to define T cell targets from milk allergens, determine the phenotype of antigen specific T cells from donors with persistent cow's milk allergy, and track the longitudinal development of antigen specific T cells during oral immunotherapy. In addition, the immunoglobulin and skin prick studies will help characterize the biomarkers behind desensitization and tolerance.

4. Study Procedures

To effectively address the primary objective of this study, subjects age 3-18 years of age with: 1) a history of cow's milk allergy, 2) a serum cow's milk specific IgE of > 5kU/L and a milk skin prick test with a wheal diameter of wheal 3 mm ≥ negative control and 3) reactivity to baked cow's milk at ≤444 mg of milk protein, will be enrolled, given that all other inclusion and exclusion criteria are satisfied. Subjects will be recruited from the Johns Hopkins Pediatric Allergy Clinic. After obtaining informed consent and assent, participants will undergo an initial screening visit that will include a medical exam and history, quality of life questionnaire, baseline gastrointestinal assessment, blood tests, skin prick testing, and spirometry and/or peak flow meter. During the baseline visit or at the next visit, participants will begin a double-blind placebo-controlled food challenge (DBPCFC) to baked milk /placebo with a maximum cumulative dose of 444 mg milk protein. They will return for the second half of the food challenge within 21 days. Eligible participants will then be randomized to active OIT or placebo and will return for a dose escalation day. Their starting dose for daily home dosing will be determined by the highest tolerated dose on the dose escalation day, with a minimum of 3 mg and a maximum of 25 mg/day. Participants will then return to the Pediatric Clinical Research Unit (PCRU) approximately every 2 weeks for observed dose escalations with a final goal of 2000 mg/day of baked milk protein or placebo. After 1 year, the study will be unblinded and the placebo group will be offered active treatment. The initial active treatment group will continue maintenance therapy for an additional year or, if they did not reach 2000 mg in the first year, they will re-start dose escalation with the goal of reaching 2000 mg, while the placebo group will begin dose escalation from the beginning. Details of the study procedures and visits follow.

Many of the procedures in this study are usual and customary in the care of food allergic children. These include skin prick testing, blood testing for IgE levels, and oral food challenges (OFC). The timing of these procedures is driven by the research protocol. Urine pregnancy testing, blood testing for biomarker and mechanistic studies, and oral immunotherapy are exclusively research related procedures.

1. **OFC**: The initial and year 1 and 2 food challenges will be double-blind placebo-controlled food challenges (DBPCFC) in which both the patient and clinician will be blinded. The DBPCFC will be done in accordance with standard procedures. All oral food challenges will be performed in the PCRU with direct physician supervision, emergency medications immediately available, and in accordance with standard procedures. We will prepare challenge materials using our standard operating procedures (SOP). The initial DBPCFC will be a challenge to baked milk or placebo. Milk will be administered in the form of non-fat milk powder (provided by the University of North Carolina (UNC), manufactured by The Milky Whey, Inc & TMW International), added to a cake and baked at 350°F/180°C (at least) for at least 30 minutes. The first DBPCFC will consist of a maximum cumulative dose of 444 mg of baked milk powder in gradually increasing doses at 15 minute intervals. The doses will be prepared by the research nutrition staff and distributed as listed in table 2 below. Frequent assessments are made for symptoms affecting the skin, gastrointestinal tract, cardiovascular system, and/or respiratory tract. Objective symptoms indicate a positive reaction at which time dosing is terminated and appropriate treatment is administered.

   Table 2. Baseline Food Challenge (Baked Milk) DBPCFC #1:

Dose #	Dose (mg) of milk protein	Cumulative Dose (mg)(mg)
1	1 mg	1 mg
2	3 mg	4 mg
3	10 mg	14 mg
4	30 mg	44mg
5	100 mg	144 mg
6	300 mg	444 mg

At the end of year 1, participants will undergo DBPCFC#2 to up to maximum cumulative dose of 4044 mg of baked milk. The doses will be prepared by the research nutrition staff and distributed as listed in table 3. The subject will return within 21 days for the second half of the challenge.

Table 3. End of Year 1 Food Challenge (Baked Milk) DBPCFC #2:

Dose #	Dose (mg) of milk protein	Cumulative Dose (mg)
1	1 mg	1 mg
2	3 mg	4 mg
3	10 mg	14 mg
4	30 mg	44mg
5	100 mg	144 mg
6	300 mg	444 mg
7	600 mg	1044 mg
8	1000 mg	2044 mg
9	2000 mg	4044 mg

At the end of year 2, participants will undergo DBPCFC #3 to up to maximum cumulative dose of 4044 mg of baked milk. During year 2, all participants will be on active OIT and are required to reach at least 750 mg of baked milk protein at least 8 weeks prior to the end of year 3. Therefore, DBPCFC#3 will start at 444 mg. The doses will be prepared by the research nutrition staff and distributed as listed in table 4.

Table 4. End of Year 2 Food Challenge (Baked Milk) DBPCFC #3:

Dose #	Dose (mg) of milk protein	Cumulative Dose (mg)
1	444 mg	444 mg
2	600 mg	1044 mg
3	1000 mg	2044 mg
4	2000 mg	4044 mg

Subjects who tolerate at least 2000 mg of baked milk the DBPCFC#3 will return within 2 weeks to undergo DBPCFC#4 to up to 8030 mg of unheated milk in the form of non-fat milk powder or placebo in a blinded vehicle. The study nutritionist will create these doses based on the schedule in Table 5.

Table 5. Year 2 Food Challenge (Unheated Milk) DBPCFC #4:

Dose #	Dose (mg) of milk protein	Cumulative Dose (mg)
1	30	30
2	100	130
3	300	430
4	600	1030
5	1000	2030
6	1500	3530
7	2000	5530
8	2500	8030

These challenges will be very helpful to determine the dose of milk that should be safe to incorporate into the diet. All participants will be provided specific guidelines as to the amount of heated and unheated milk that can be introduced into their diets at study completion.

2. **Skin prick tests**: A skin prick test using cow's milk extract will be performed using standard procedures. The patient must be off antihistamines for 5 days for long acting and 3 days for short acting prior to skin testing. A skin test probe (Greer Pick) is pressed through a commercial milk extract (Greer Laboratories) into the epidermis. Histamine (positive) and saline-glycerin (negative) controls are placed to establish that the response is not blocked and to determine if there is dermatographism, respectively. A study physician will be available to monitor for any adverse events.

3. **Blood draws:** Standard procedures will be followed with a maximum of 3 cc/kg in any 8 week period. Laboratory tests will examine milk-specific IgE and IgG4. Laboratory studies will be performed at DACI Laboratory or by La Jolla Institute (LJI) for Allergy & Immunology. LJI will seek to define T cell targets from milk allergens, determine the phenotype of antigen specific T cells from donors with persistent cow's milk allergy, and track the longitudinal development of antigen specific T cells during oral immunotherapy. Cells from the blood will be used for HLA

typing, to allow for the use of HLA-specific experimental assays (e.g., autoantigen-specific cells can be isolated by tetramer reagents) as well as analysis correlation between HLA type and disease or phenotype. Transcriptional profiling will also be done on these samples (e.g. RNA-seq and TCR sequencing).

4. **Spirometry and Peak Flow Rate (PFR)**: Standard procedures will be followed. At the screening visit, spirometry will be attempted in all subjects ≥6 years old. Children < 6 years old will have peak flow rate (PFR) done. If children 6-18 years of age are unable to produce valid spirometry results, the attempt will be documented and PFR will be performed. If children 3-5 years of age are unable to produce valid PFR results, the attempt will be documented. At all subsequent visits, PFR will be performed in all subjects. If children 3-5 years of age are unable to produce valid PFR results, the attempt will be documented.

5. **Urine pregnancy tests:** Urine human chorionic gonadotropin (HCG) testing for pregnancy in females of child bearing age will be conducted prior to administration of the study product at the study visits indicated.

6. **Quality of life questionnaire:** At the baseline visit, completion of study year 1 and 2, and follow-up visits, the subject and/or their parent /guardian will complete a quality of life questionnaire.

7. **Gastrointestinal assessment**: At the baseline visit, all subjects and/or their parent/guardian will complete a baseline gastrointestinal assessment. Follow-up assessments will be completed at least monthly during the build-up period, during each maintenance visit, and at the completion of study year 1 and 2 and during follow-up.

Schedule of visits

All potential participants who express interest on initial contact will receive a copy of the informed consent and be invited for a screening visit. Visits will be as follows:

A. Baseline Visit

This research study will be explained in lay terms to each potential research participant. The potential participant will sign an informed consent before undergoing any screening study procedures. Written informed consent and HIPAA authorization will be obtained from all participants at the beginning of this visit. Study procedures will be stopped and the participant will be deemed ineligible for the study if the participant 1) is unable to complete the procedure being done at any point for any reason or 2) has a result from a study procedure or question that would exclude them from the study.

Historical data will be collected to include demographics (age, race, sex), medical history, dietary history, medications (current and in the past 12 months), and other allergies. A quality of life questionnaire and baseline gastrointestinal assessment will be administered. A physical exam will be conducted along with spirometry or peak flow rate. Spirometry will be attempted in all subjects

≥6 years old. Children < 6 years old will have peak flow rate (PFR) done. If children 6-18 years of age are unable to produce valid spirometry results, the attempt will be documented and PFR will be accepted for the entry criteria if results are ≥ 80% of predicted. Urine pregnancy testing will be conducted where appropriate. Skin prick testing to milk protein extract will be performed and phlebotomy will be conducted. The caregiver will be given a Food Allergy Action Plan. Epinephrine auto-injector teaching will be performed at the screening visit.

In certain participants (e.g. the milk IgE result is known), the baseline visit and first half of the baseline food challenge will be combined.

B. Initial Double Blind Placebo Controlled Oral Food Challenge (OFC):

Within 21 days of the baseline visit, eligible subjects will return for DBPCFC #1 (table 2) to baked milk or placebo. A limited physical exam and peak flow rate will be done. If the exam is normal and PFR ≥80% of predicted, the study participant will undergo an oral food challenge with a cumulative dose of 444 mg of either baked milk powder or Tapioca flour (provided by the University of North Carolina (UNC), manufactured by Ener-G Foods, Inc.) in a blinded manner. The Pediatric nutrition staff will prepare doses by adding milk or Tapioca flour to a cake, which will be baked at 350°F/180°C (at least) for at least 30 minutes. Participants will receive gradually increasing doses at 15 minute intervals as in Table 2 above. Frequent assessments are made for symptoms affecting the skin, gastrointestinal tract, cardiovascular system, and/or respiratory tract. Dosing will be terminated with the onset of symptoms indicating a positive reaction after which appropriate treatment will be administered.

Upon completion of the food challenge, all participants will be observed for at least 2 hours, unless the OFC is unblinded and the participant had a negative challenge to placebo.. For any severe systemic reaction, all subjects will be observed for at least 4 hours or until signs of clinical reactivity subside.

The second half of the DBPCFC will occur within 21 days of the initial food challenge. If the participant does not react with the cumulative dose of 444 mg of baked milk or if they react to placebo, they will not be eligible to enter the treatment phase of the protocol.

At the completion of the DBPCFC, eligible participants will be randomized to active treatment or placebo.

C. Dose Escalation:

Within 4 weeks of the DBPCFC, participants will return for the initial OIT dose escalation. Again, a limited physical exam, peak flow, and urine pregnancy testing (when indicated) will be conducted. If the exam is normal, urine pregnancy test is negative and the PFR is ≥80% of normal, escalation with OIT dosing will be performed. Participants will begin at a dose of 0.1 mg of OIT with dose increases every 15 minutes to a maximum final dose of 25 mg of OIT in one day as listed in table 5.

Table 6: Initial Dose Escalation

Dose #	Milk dose (mg) of milk protein	Interval
1	0.1	Day 1
2	0.2	Day 1
3	0.4	Day 1
4	0.8	Day 1
5	1.5	Day 1
6	3	Day 1
7	6	Day 1
8	12	Day 1
9	25	Day 1

If symptoms, including intolerable localized itching or any systemic reaction, occur, the dose escalation will be stopped and the participant will be sent home on daily doses of the highest dose tolerated (must be at least 3 mg with a maximum of 25 mg). Mild symptoms during escalation can be treated with a single dose of antihistamine with continued milk dosing.

D. Dose Build-Up:

Further dose increases at approximately 50% increments will occur under observation approximately every 2 weeks as listed below in Table 7, with daily home dosing between each observed dose increase. The interval history and dosing log will be reviewed. A gastrointestinal assessment will be done at least once a month. At each visit, alimited physical exam and peak flow testing will be conducted. Urine pregnancy testing will be done every month if indicated. If the participant did not reach 25 mg on the initial dose escalation, they will follow the dose escalation day schedule approximately every 2 weeks until they reach 25mg. At these visits, subjects will demonstrate understanding of preparation and administration of the OIT doses and be observed for reactions for a minimum of 60 minutes. The final dose tolerated will be taken at home for the next 2 weeks (range of 10-21 days). The tolerated protein dose will be dispensed in portion cups. The powder will be packaged into cups by Dr. Wesley Burks' manufacturing facility at the University of North Carolina and shipped to Johns Hopkins Research Pharmacy. Drug dispensing will be performed by Johns Hopkins research pharmacy. The subject's guardian will be given detailed instructions on how to add the powder to a muffin or cupcake for their daily dosing, and bake at 350°F/180°C (at least) for at least 30 minutes.

If the dose is not tolerated, the subject will resume the highest previously tolerated dose until their next visit. The minimum accepted dose for maintenance daily OIT dosing is 750 mg. If the patient does not tolerate three consecutive escalation attempts, but has attained a dose of 750 mg, this dose will become the maintenance daily home dose until the year 1 OFC (DBPCFC #2). If the patient does not tolerate three consecutive escalation attempts and 750 mg has not been obtained, then the study treatment will be discontinued. In addition, OIT will be discontinued for subjects

who do not reach at least 750 mg by study week 44. The dose achieved at study week 44 (which must be ≥750 mg) will be the maintenance dose used in the subsequent 8 weeks.

If build-up was delayed due to the stop in-person visits due to COVID-19, then the patient will be allowed up to an additional 8 weeks in build-up. They would then remain on the maintenance dose for 8 weeks and then complete the end of year OFCs

Between visits, subjects will take the given dose at home daily. It is recommended that the dose be taken at a consistent time (within a 4-hour time period each day) and it is critical to take the dose every day. Doses should be separated by at least 12 hours. They will also be given a daily diary to record the time of administration, adverse events, medications given, and any other symptoms or exposures that may arise.

Subjects may be brought back to the PCRU at any time to observe dosing at the investigator's discretion. If more than 3 consecutive home doses are missed, subjects must return to the PCRU to take their next dose under observation. Subjects should withhold their daily home dose on the build-up day, but should take all other prescribed medications. Blood will be collected at the initial visit, start of maintenance in year 1, at the year 1 and 2 OFCs (DBPCFC #2 and #3), and during study year 3 and 4 follow-up visits. Skin prick testing will occur on the same schedule.

Table 7. Dose Build-up Schedule:

Dose #:	Dose (mg) of milk protein	Interval	Comments:
10	37.5	2 weeks	
11	50	2 weeks	
12	75	2 weeks	
13	125	2 weeks	
14	200	2 weeks	
15	300	2 weeks	
16	500	2 weeks	
17	750	2 weeks	Minimum maintenance dose
18	1000	2 weeks	
19	1500	2 weeks	
20	2000	2 weeks	Goal Maintenance Dose

E. OIT Maintenance:

During the maintenance phase, the subject will continue daily home doses of OIT of 2000 mg (or the minimum tolerated dose ≥750 mg) daily for at least 8 weeks prior to DBPCFC #2. Follow up visits will occur every two months to review the daily diaries and other interval history and to complete the gastrointestinal assessment. A limited physical exam, peak flow, gastrointestinal assessment and urine pregnancy testing (if indicated) will be conducted at

each visit as well. Maintenance visits may occur via phone, video visit, or in-person at the discretion of the PI.

F. Double Blind Placebo Control Food Challenge #2:

At week 52, after at least 8 weeks of OIT maintenance therapy, study participants will undergo DBPCFC #2. Participants will be asked to fill out a gastrointestinal assessment and quality of life questionnaire prior to DBPCFC #2. A limited physical exam, urine pregnancy (if indicated), and peak flow rate will be conducted. Blood will be collected and skin testing will be repeated. If the exam is normal, urine pregnancy test is negative, and PFR ≥80% predicted, the study participants will undergo an oral food challenge with a cumulative dose of 4044 mg of baked milk or placebo as shown above in Table 3. Participants will receive doses every 15 minutes and will be monitored for symptoms of allergic reaction. The second half of the DBPCFC will occur within 21 days.

G. Unblinded Phase:

Unblinding will occur after DBPCFC #2.

1. Build-up Phase Post 1 Year OFC for Placebo-Treated Subjects:

The placebo group will be offered the option of crossing over into active therapy with dose escalation to a maximum of 2000 mg maintenance. They will follow the same protocol as the initial active treatment group. Blood will be collected at the start of maintenance.

2. Build-up Phase Post 1 Year OFC for those in Initial Active Treatment Group

Those in the initial active treatment group who were unable to reach the goal maintenance dose of 2000 mg by study week 44, will resume dose build-up until they reach a maximum of 2000 mg/day if tolerated. They will resume the same initial protocol. If the patient does not tolerate three consecutive escalation attempts, then they will remain at their current dose for the remainder of the study period.

3. Maintenance Phase Post 1 Year OFC:

Those in the active treatment group that tolerated 2000 mg baked milk maintenance will continue maintenance for an additional 52 weeks. Follow up visits will occur every three months to review the daily diaries,other interval history, and the gastrointestinal assessment. A limited physical exam, peak flow, gastrointestinal assessment, and urine pregnancy testing (if indicated) will be conducted at each visit as well. Maintenance visits may occur via phone, video visit, or in-person at the discretion of the PI.

Those who were in the initial placebo group or those in the initial active treatment group who were unable to reach 2000 mg by study week 44 will follow the same initial build-up protocol and requirements mentioned above Their goal maintenance dose for maintenance period #2 will be 2000 mg. If they are unable to reach 2000 mg by week 98, then their maintenance dose will be the achieved dose ≥ 750 mg at week 98. This will allow for at least 8 weeks of maintenance prior to DBPCFC #3. If subjects do not reach at least 750 mg by study week 98, OIT will be discontinued. If build-up was delayed due to the stop in-person visits due to COVID-19, then the patient will be allowed up to an additional 8 weeks in build-up. They would then remain on the maintenance dose for 8 weeks and then complete the end of year OFCs. Follow up visits will occur every two months to review daily diaries and interval history. A limited physical exam, peak flow, gastrointestinal assessment, and urine pregnancy testing (as indicated) will be conducted at each visit as well. Maintenance visits may occur via phone, video visit, or in-person at the discretion of the PI.

4. Study Year 2 OFC to Baked Milk (DBPCFC #3)

At the end of study year 2 (week 106) , study participants will undergo DBPCFC #3 to baked milk with dosing as listed above in Table 4. Participants will be asked to fill out a gastrointestinal assessment and quality of life questionnaire prior to DBPCFC #3. A limited physical exam, urine pregnancy test (if indicated) and peak flow rate will be conducted. Blood will be collected and skin testing will be repeated. If the exam is normal, urine pregnancy test is negative, and PFR ≥80% of normal, the study participants will undergo an oral food challenge with a cumulative dose of 4044 mg of either baked milk or placebo in a blinded manner. Participants will receive doses every 15 minutes and will be monitored for symptoms of allergic reaction. The second half of the DBPCFC will occur within 21 days. If no symptoms occur during the first half of DBPCFC#3, then the second half of the challenge could occur on the same day at the discretion of the principal investigator.

5. Study Year 2 OFC to Unheated Milk (DBPCFC #4)

Within two weeks of the completion of DBPCFC #3, study participants who tolerated at least 2000 mg of baked milk during DBPCFC #3 will undergo DBPCFC#4 with a cumulative of 8030 mg of unheated milk powder or placebo in a blinded manner with dosing as listed in table 5. A limited physical exam, urine pregnancy and peak flow rate will be conducted. If the exam is normal, urine pregnancy test is negative, and PFR ≥80% of normal, the study participants will undergo an oral food challenge to unheated milk powder or placebo. The second half of the DBPCFC will occur within 21 days. If no symptoms occur during the first half of DBPCFC#4, then the second half of the challenge could occur on the same day at the discretion of the principal investigator.

H. Follow-up Period:

1. Subjects who discontinue study treatment before the end of the study will complete an Early Discontinuation Visit 1-2 weeks after their last dose of study treatment. During that visit, they will have a physical exam and urine pregnancy test (if applicable). Daily diaries and interval history will be reviewed. Subjects who complete DBPCFC#2 and then leave the study early will be asked to sign a consent to participate in follow-up (as detailed below in #4) if they have been given instructions to introduce milk at home.

2. For those who had symptoms at <2000 mg of baked milk during DBPCFC#3, they will be considered to have completed the intervention period of the trial. OIT will be stopped. They will be given detailed instructions for the diet to resume at home.

3. For those who tolerated ≥2000 mg of baked milk during DBPCFC#3, they will then complete DBPCFC #4. After the completion of DBPCFC #4, OIT will be stopped and they will be considered to have completed the intervention period of the trial. They will be given detailed instructions for the diet to resume at home.

4. All participants will then have phone follow-up quarterly for 1 year. They will return to clinic after 6, 12, and 24 months. An updated history will be obtained. A limited physical exam will be performed. Milk specific skin prick testing will be performed and blood for laboratory studies will be obtained. Participants will be asked to complete a gastrointestinal symptom and quality of life questionnaire at the follow-up visits.

a. Study duration and number of study visits required of research participants.

The total length of time and number of study visits will vary depending on the subject's response to the immunotherapy and the group to which they are randomized. All eligible subjects will complete a baseline visit, initial DBPCFC (2 visits), and dose escalation day. For those who remain eligible and are enrolled in the study, we anticipate variation in the amount of time required to complete dose escalation. The time in dose escalation will impact the time in maintenance. A subject randomized to the active treatment group who reached a 25 mg starting dose and completed dose escalation visits without any delays would have a minimum of 54 weeks and 20 visits in Study Year 1 and an additional 7 visits and 54 weeks in Study Year 2. For the entire study (including follow-up), a subject would have a minimum of 30 visits over 212 weeks.

During study year 1, a subject who had a lower starting dose would require additional biweekly visits until reaching 25 mg. Furthermore, one who is unable to tolerate a dose escalation would require additional dosing build-up visits. Study Year 1 will last 54 weeks and based on the above factors could require 20-25 visits.

For those in the initial baked milk OIT group, the number of study visits during year 2 will vary based on if a subject is able to achieve the goal maintenance dose of 2000 mg during study year 1. In study year 2, those who started in the active treatment group, but did not reach 2000 mg will have additional biweekly visits in build-up phase #2. They could have a maximum

of 18 weeks (9 visits) in build-up phase #2. Those who did achieve 2000 mg during study year 1 will continue in the maintenance phase with visits every 12 weeks. They will have a minimum of 7 visits during study year 2. All subjects will complete study year 2 at week 108. Those who tolerated ≥2000 mg of baked milk during the DBPCFC #3 will also undergo DBPCFC #4 to unheated milk or placebo, which will occur as a separate visit.

The placebo group should have 20 visits over 54 weeks in study year 1. After crossover to active treatment, their study duration and number of visits required would be the same as described for the initial active treatment group. For the initial placebo group, the total duration of the study (including follow-up) will be 212 weeks and will include a minimum of 40 study visits.

b. Blinding, including justification for blinding or not blinding the trial, if applicable.

The intent of blinding is to maintain maximum objectivity in confirming the diagnosis of food allergy, evaluating any clinical effects of OIT, monitoring adverse reactions and in interpreting results. All 4 sets of food challenges will be double-blind for the same reasons. In addition, there is the possibility of naturally outgrowing a food allergy. Therefore, it is crucial to have a blinded treatment and placebo group in order to evaluate the impact of the baked milk OIT compared to natural history/natural disease progression.

c. Justification of why participants will not receive routine care or will have current therapy stopped.

Standard of care for baked milk allergic individuals in this age group is total avoidance. There is no alternative therapy to treat milk allergy in baked milk allergic children. This treatment offers a potential new therapy for baked milk allergic individuals. Routine care for other allergy concerns will continue in the pediatric allergy clinic.

Participants will be asked to stop antihistamines prior to the screening visit and prior to food challenges. This is necessary to allow for accurate SPT and to avoid masking symptoms during food challenges.

d. Justification for inclusion of a placebo or non-treatment group.

The study will be double-blind, placebo-controlled for the first year. At one year, unblinding will occur and participants in the placebo group will be offered crossover to active therapy. This study design will maximize objectivity when evaluating the clinical effects of OIT, reporting of adverse reactions, and in interpreting study outcomes. In addition, there is the possibility of naturally outgrowing an allergy. Therefore, it is crucial to have a placebo group in order to evaluate the impact of the baked milk OIT compared to natural progression of the disease.

e. Definition of treatment failure or participant removal criteria.

- Severe anaphylaxis secondary to OIT dosing or milk food challenge.
- If the subject is deemed to have severe symptoms and requires aggressive therapy at any time during dose escalation or build up.
- Inability to tolerate at least 3 mg by OIT on the first day of dose escalation.
- Inability to tolerate a dose increase on 3 consecutive attempts during dose build up if one has not reached 750 mg.
- Inability to tolerate 750 mg milk protein 8 weeks prior to DBPCFC #2 or DBPCFC #3 (week 44 and week 98, respectively) (COVID-19 related exceptions deleted above)
- Poor control or persistent activation of asthma or atopic dermatitis
- Severe gastrointestinal symptoms related to treatment which are ongoing and persist for hours beyond dosing.
- For subjects who gastrointestinal symptoms persist for more than 2-3 weeks beyond discontinuation of dosing, a referral to a gastroenterologist will be initiated.
- Circumstances (e.g. concurrent illnesses) requiring missed dosing of > 4 days and inability/lack of desire to repeat desensitization.
- Excessive missed days of home dosing protocol (i.e. >3 consecutive days missed on 3 occasions).
- The principal investigator determined that ongoing treatment is not in the subject's best interest.
- The participant elects to withdraw consent from all future study activities, including followup.
- Pregnancy in study participant.

f. ***Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely*****.**

Those completing the treatment course will be followed as described above; those that withdraw or do not complete the course of treatment will have a follow-up visit within 1-2 weeks after stopping the study. The study termination visit will include: 1) A final physical exam, 2) a pregnancy test for female participants of child bearing age, and 3) a review of the interval history and daily diary.

If subjects complete DBPCFC#2 and then leave the study early, we will ask them to sign a separate consent to participate study in study follow-up if they have been given instructions to introduce milk at home.

All participants who complete treatment will have telephone follow-up quarterly for 1 year and then a clinic visit 6, 12, and 24 months post-study completion. This follow-up visit will include a limited physical exam, dietary history, food reaction history, skin prick tests, blood collection, gastrointestinal symptom questionnaire, and a quality of life questionnaire.

5. *Inclusion/Exclusion Criteria*

Patients who meet *all* of the following criteria are eligible for enrollment as study participants, including participants who:

- Are age 3-18 years, either sex, any ethnicity or race
- Provide signed informed consent by parent or legal guardian and informed assent if applicable
- Have a history of symptomatic reactivity to cow's milk (i.e. eczema, urticarial, upper or lower respiratory symptoms, GI disturbances, rash, oral symptoms)
- Have a skin prick test positive to milk (diameter of wheal 3 mm ≥ negative control) and serum milk-specific IgE level >5 kU/L within the past 6-12 months
- Have a positive reaction to a cumulative dose of ≤444 mg of baked milk protein in the initial qualifying DBPCFC.
- Use an effective method of contraception by females of childbearing potential to prevent pregnancy and agree to continue to practice an acceptable method of contraception for the duration of their participation in the study.
- Have self-injectable epinephrine available at all times

Patients who meet *any* of these criteria are not eligible for enrollment as study participants, including participants who:

- Have a history of severe anaphylaxis resulting in hypotension, neurological compromise, or mechanical ventilation
- Have a history of intubation related to asthma
- Tolerate more than 444 mg of baked milk protein at the initial qualifying DBPCFC.
- Allergy to placebo ingredients OR reacts to any dose of placebo during the qualifying OFC.
- Poor control of atopic dermatitis
- Are unable to tolerate at least 3 mg of baked milk protein on dose escalation day
- Are pregnant or lactating
- Have severe asthma defined by 2007 NHLBI Criteria Steps 5 or 6
- Have severe or poorly controlled asthma defined by with any of the following criteria:
    1. FEV1<80% of predicted
    2. ICS dosing of >500 mcg daily of fluticasone (or equivalent inhaled corticosteroids based on NHLBI dosing chart) or
    3. ≥ 1 hospitalization in the past year for asthma or
    4. > 1 ER visit in the past 6 months for asthma
- Use of steroid medications (oral steroids, such as prednisone or Medrol, steroid injections, such as Kenalog, or IV or oral corticosteroid burst) in the following manners: History of daily oral steroid dosing within 4 weeks prior to baseline visit *or* for > 1 month during the past year *or* >2 burst oral steroid courses in the past 6 months.
- Are unable to discontinue antihistamines for 5 days for long acting and 3 days for short acting prior to skin testing or food challenges

- Are receiving omalizumab, mepolizumab, beta- blocker, ACE inhibitor, angiotensinreceptor blockers, calcium channel blockers, or tricyclic antidepressant therapy
- Have used immunomodulatory therapy (not including corticosteroids) or biologic therapy within the past year
- Have participated in any interventional study for treatment of a food allergy in the past 6 months
- Are on 'build up phase' of environmental allergen immunotherapy. Subjects tolerating maintenance allergen immunotherapy can be enrolled.
- Have a history of eosinophilic esophagitis in the past 3 years
- Have a chronic disease (other than asthma, atopic dermatitis, rhinitis) requiring therapy (e.g., heart disease, diabetes)
- Have used an investigational drug within 90 days or plan to use an investigational drug during the study period
- Severe reaction at initial DBPCFC, defined as:
  - Life-threatening anaphylaxis
  - Requiring overnight hospitalization

6. *Drugs/ Substances/ Devices*

a. The rationale for choosing the drug and dose or for choosing the device to be used.

The study treatment is non-fat cow's milk powder or placebo ingested in a baked form. The non-fat cow's milk powder will be provided by the University of North Carolina (UNC). It is manufactured by The Milky Whey, Inc & TMW International. It will be refrigerated at the site at 2 through 8°C until provided to the participants. Detailed instructions will be provided regarding how the baking should be performed.

The doses chosen are based on prior milk OIT studies, which have all used unheated milk. We anticipate similar efficacy with greater safety using this approach.

b. Justification and safety information if FDA approved drugs will be administered for nonFDA approved indications or if doses or routes of administration or participant populations are changed.

Aside from the skin testing extracts, this study will not use any drugs, but rather foods commonly available in households, prepared using a standard oven. Most milk OIT studies to date have used unheated milk at maintenance doses ranging from 500- 4000 mg. As described in the background section, these have shown high rates of adverse reactions, ranging from mild, local symptoms, such as oral itching, to systemic allergic reactions.

Baking milk has been shown to modify the allergen and make it less allergenic and therefore less likely to induce adverse reactions. It has been shown that 50-75% of children with

CMA tolerate baked milk at full servings, such as a cupcake or muffin, and many are already incorporating these foods into their diet at home.[13] However, the remaining 25-50% of children with CMA are not able to tolerate baked milk at standard doses. Our dosing regimen will start with extremely small doses of baked milk protein and gradual escalation.

We believe that the chemistry and manufacturing of the non-fat milk powder and Tapioca Flour does not present signals of potential human risk, beyond the inherent risk associated with the investigational treatment protocol. This milk powder has been approved by the Food & Drug administration for use in other clinical trials (IND: 77,468) and in our study (IND: 17114).

c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

N/A

7. *Study Statistics*

a. Primary outcome variable.

The primary objective of this trial is to study the safety of baked milk OIT, defined as the incidence of adverse events on baked milk OIT compared to adverse events on placebo.

***b.* Secondary outcome variables.**

The secondary outcome measures are as follows:

- Exploration of the clinical effects of baked milk OIT, defined as tolerating 4 grams of baked milk protein without adverse reactions after 12 and 24 months of treatment
- Exploration of the clinical effects of baked milk OIT, defined as tolerating 2 grams of unheated milk protein without adverse reactions at the end of study year 3 end of year 3

 The exploration of biomarkers and mechanistic correlates of desensitization including milk-specific IgE and IgG4, milk skin prick test responses, T cell responses. The exploration of the impact of milk allergy and its treatment on quality of life. Differences in clinical response rates based on duration of treatment (1 year versus 3 years) 3

 The proportion of subjects that achieve desensitization in the placebo crossover group after 1 year of dosing at the year 2 study time point. The proportion of subjects who tolerate 8030 mg of unheated milk protein at the end of study year 3

 The incidence of adverse events in the unblinded year of treatment Incidence of adverse events after stopping OIT (year 3 and 4) Impact of cow's milk OIT on goat and sheep milk-specific IgE Comparison of maximum tolerated dose of baked milk compared to maximum tolerated dose of unheated milk at the end of year 3

- The change in maximum tolerated dose of baked milk from baseline to end of year 1 and

c. Statistical plan including sample size justification and interim data analysis.

The primary outcome is safety, defined as the incidence of adverse events on baked milk OIT compared to adverse events on placebo.

The main secondary outcome is a clinical response, defined as 1) the proportion tolerating 4 grams of baked milk protein without adverse reactions after 12 and 24 months of treatment and 2) the proportion tolerating 2 grams of unheated milk in the oral food challenge at the end of study year 2. We have proposed a sample size based on 1) data from previous studies and 2) hypothesis testing for test of difference in proportions.

Data from randomized, placebo-controlled unheated milk oral immunotherapy have shown various results. Pajno et al had a maintenance dose of 200 mL and 67% were tolerant to 200 mL of cow's milk after 18 weeks.[24] Martorell et al showed 90% desensitization after 1 year with a maintenance dose of 200 mL.[19] We showed that 70% of patients receiving OIT with a maintenance dose of 1000-2000 mg for 60 weeks passed an 8g OFC.[18]

As mentioned previously, there has only been 1 prior study using baked milk OIT. Goldberg et al had a different primary outcome which was the goal of attaining a maintenance BM dose of 1.3g/day.[28] Only 21% reached that goal but as mentioned above, the study protocol was different than our study protocol.

Based on these prior studies, we would expect that at most 10% of subjects in the placebo arm and at least 60% of subjects in the treatment arm would tolerate 2 grams of unheated milk at the 1 year OFC. To achieve an alpha of 0.05 and a power of 0.8, 14 subjects are needed in each group. 15 participants per group would allow for a 7% drop out rate.

Statistical plan:

We will display summary statistics for each variable to keep track of missing data, and to be alerted to suspicious patterns in the data and outliers. We will initially focus on the primary aim of the study. We will examine adverse event endpoints to estimate 1) the risk associated with OIT and 2) compare the risks of OIT between the risks of adverse reactions in the placebo group. We will tabulate the total number of serious adverse events (SAE) for the active treatment study population and construct a 95% confidence interval for this total number of adverse events. We will also calculate the proportion of subjects who experienced an SAE along with the 95% CI. We will calculate the proportion of doses that was associated with an SAE and construct the 95% CI. We will compare the proportion of subjects who experienced an SAE between treatment and placebo groups and the proportion of doses resulting in an SAE between groups using Fisher's exact test. We will compare the number of adverse events between groups using the Kruskall-Wallis test if the variances between the groups are similar.

In addition to efficacy endpoints, we will tabulate data and calculate the proportion of subjects who achieved a clinical response and construct 95% confidence intervals. We will do this for each time point being evaluated so that we have estimates of clinical response rates after completion of 1 year of OIT, after 2 years of OIT, and after 1 year of placebo.

We will also compare clinical response rates between the active treatment and placebo groups at the year 1 and year 2 OFCs. We will tabulate the clinical response rates by group for each time point and use a t-test for difference in proportions to test for statistical significances in response rates between groups.

We will also compare change in maximum tolerated dose of baked milk from baseline to end of year 1 and end of year 2. We will compare each subject's maximum tolerated dose of baked milk compared to maximum tolerated dose of unheated milk at the end of year 2. We will use a t-test for to test for statistical significances in change in amount tolerated between groups.

Summary descriptive statistics for baseline and demographic characteristics and biomarker and mechanistic studies will be provided for all enrolled participants. Demographic data will include age, race, sex, body weight, and height. Biomarker and mechanistic data will include cow's milk IgE and IgG4, goat and sheep milk IgE, and lymphocyte analysis. These data will be presented in the following manner:

- Continuous data (i.e., age, body weight, and height) will be summarized descriptively by mean, standard deviation, median, and range.
- Categorical data will be presented as enumerations and percentages.

Statistical presentation for baseline and demographic characteristics may be further summarized as appropriate.

d. Early stopping rules.

Study enrollment and treatment will be suspended pending expedited review of all pertinent data by the IRB and the DSMB if the following occur:

- Any death.
- More than one severe anaphylactic reaction related to baked milk immunotherapy dosing at any stage of the protocol.
- More than three subjects require more than one injection of epinephrine during dosing of the baked milk immunotherapy.
- The first 5 subjects cannot reach 3 mg of OIT.

Additionally, the study will be stopped upon enrollment and completion of the defined number of participants that meet the selection criteria.

8. Risks

a. ***Medical risks, listing all procedures, their major and minor risks and expected frequency.***

i. Baked Milk Powder administered via the oral route

The escalation day followed by the build-up phase of immunotherapy is based on previous experience with peanut, egg, wheat, and unheated milk OIT. In the Principal Investigator's experience, the initial doses have been well tolerated. Therefore, the initial dose escalation day was included to shorten the prolonged build-up phase. The likelihood of participants experiencing allergic symptoms will be decreased by the OIT protocol, starting at extremely small amounts with gradual escalation.

Unheated oral immunotherapy has shown a high rate of adverse reactions. In 2011, Martorell et al conducted a 1 year milk OIT study in 2-3 year old children. While 90% were desensitized, 47% developed moderate reactions (rhinoconjunctivitis, wheezing, vomiting, urticaria) over the course of treatment.[19] In 2012, we examined the safety and efficacy of OIT and SLIT (sublingual immunotherapy) for cow's milk allergy. The study reported the reaction rate during the initial OIT escalation (36%), 1000 mg OIT escalation and maintenance (30%), and 2000 mg OIT escalation and maintenance (27%).[18] The majority of symptoms were mild and localized to oral pruritus.[18] During this study, antihistamines were used in 11% of doses during initial OIT escalation, 3.5% of doses in the 1000 mg escalation and maintenance, and 11% of doses in the 2000 mg OIT escalation and maintenance. A β-agonist was required in 1.5%, 2%, and 1.5% of doses in each respective group. Epinephrine was used for 4 OIT doses.[18] In addition, 10-20% of subjects have dropped out of OIT trials due to adverse reactions, most commonly chronic abdominal pain.[12,26]

We would view these risks as worst case scenario, since we expect far fewer adverse reaction with the heat modified milk proteins.

ii. Allergy Skin Testing

There is a risk of minor discomfort and itching and a transient hive. A localized wheal and flare will occur at the site of the histamine control. A similar reaction may occur at the site of milk extract. Fewer than 5 in 10,000 tests results in a systemic reaction (e.g. sneezing, runny nose, itchy eyes, or hives), and, even more rarely, systemic reactions that can be life-threatening.[29-32] There have been no systemic reactions in the principal investigator's experience with over 75,000 skin tests.

iii. Blood Collection

There is a risk of minor discomfort or bruising at the site of the blood draw. Some people may experience lightheadedness. Very rarely, an infection can develop at the site of the venipuncture.

iv. Spirometry and Peak Flow

The minor risk is of slight discomfort associated with forceful expiration and, occasionally, lightheadedness.

v. Oral food challenge

In this study, children will undergo oral food challenges to determine baseline level of reactivity and response to therapy. The risk attendant to an oral food challenge is the same as those that may occur from a food-allergy reaction and are typically short-lived (less than 2 hours). Common symptoms may include urticaria, angioedema, nausea, abdominal discomfort, vomiting and/or diarrhea, rhinorrhea, sneezing and/or mild wheezing. The major risks involved include severe breathing difficulties, and rarely, a drop in blood pressure. A severe outcome is theoretically possible and one death has occurred in a medically supervised oral food challenge. However, to date the primary investigators have performed more than 5000 oral food challenges without a serious life-threatening anaphylactic reaction or need for hospitalization.

We have chosen to only challenge to baked milk at screening and at the end of year 1 in an effort to reduce risk, recognizing that all subjects who react to heated milk would react to unheated milk at the same or lower dose.

vi. Medical Exams: There are no known risks for the physical exam.

vii. Pregnancy Tests: There is a risk of loss of confidentiality of results as positive tests would be provided to the parents in all participants <18 years old.

b. Steps taken to minimize the risks.

i. Baked Milk Powder administered via the oral route

The use of heated milk in this study is specifically designed to minimize the risk of treatment. In addition, as with our studies using unheated milk, the initial doses are extremely small and subsequent dose increases will be gradual. Dose escalation will be done under direct supervision of a physician. Appropriate medications, equipment, and personnel will be available in case of a severe reaction, especially given that the study will be conducted in the PCRU on Bloomberg 9. Upon completion of a food challenge or IDE, all participants will be observed for at least 2 hours, unless the OFC is unblinded and the participant had a negative challenge to placebo. For any severe systemic reaction, all subjects will be observed for at least 4 hours or until signs of clinical reactivity subside. Any subject with ongoing severe systemic symptoms (i.e. persistent vomiting, wheeze, cough, FEV1<80% baseline level, hypotension, or laryngeal edema manifested by difficulty breathing or speaking) will be observed in clinic until symptoms resolve. If they do not resolve, the subject will be hospitalized. In the event of a negative challenge to placebo, participants can be sent home after unblinding.

For the build-up visits, subjects will be observed for at least 1 hour after dosing. For any moderate symptoms, all participants will be observed for at least 2 hours. For any severe systemic reaction, all subjects will be observed for at least 4 hours or until signs of clinical reactivity subside.

All subjects will have injectable epinephrine (available at all times, and clear instructions about its use. The subject will be contacted on the day after dose escalation to monitor for adverse events. A 24-hour physician emergency number will be given to the subject.

ii. Allergy Skin Testing

SPT will be performed by a trained physician or nurse. Appropriate medications will be available at the time of testing (antihistamine, steroids, short-acting beta agonist, and epinephrine). A designated physician will be immediately available to provide medical assistance if necessary. Any participant with an asthma exacerbation at the time of the visit will not undergo skin testing. He/she may return at a later date when his/her asthma is under control. An asthma exacerbation will be defined as wheezing on chest auscultation, currently taking oral corticosteroids, or having FEV1 or PEFR <70% predicted.

iii. Blood Collection

A staff member who is trained to draw blood from children will collect the samples. We will not draw more than 3 cc/kg/month/subject of blood.

iv. Spirometry and Peak Flow

Spirometry and peak flow readings will be performed by trained personnel. **v.**

Oral food challenge

Trained staff will be present to administer study food challenges and a physician will be at the bedside to provide oversight, evaluation, and treatment as needed. The risks of oral food challenges are minimized by the following procedures:

- Children must have a stable baseline examination prior to undergoing the challenge without significant symptoms of flaring atopic dermatitis, exacerbations of rhinorrhea, current urticarial, or other symptoms evaluated during food challenges
- Children must have no wheezing or repetitive cough prior to challenge
- Children must have no current illnesses (e.g. fever) at time of oral challenge
- Medications (epinephrine, intravenous fluids, antihistamines, ranitidine, vasopressors, beta-agonists, and steroids), personnel and equipment (oxygen,

resuscitation equipment) will be immediately available to treat allergic reactions should they occur.

- Children will have discontinued antihistamines and bronchodilators for appropriate periods prior to challenge, thereby allowing investigators to perform challenges only when participants are stable without influence of medications.
- The food will be provided gradually, at 15 minute intervals, beginning with a dose unlikely to trigger a reaction and progress stepwise with escalating doses.
- Vital signs (heart rate, blood pressure, respiratory rate) and physical examination will be undertaken at baseline and at the end of the OFC with a focused examination undertaken prior to each dose
- Challenges will be stopped, and medications administered, in the event of any objective symptoms.

  All participants will be observed for at least 2 hours, unless the OFC is unblinded and the participant had a negative challenge to placebo. For any severe systemic reaction, all subjects will be observed for at least 4 hours or until signs of clinical reactivity subside.

vi. Pregnancy Tests:

Personal identifiers will not be placed on collected data.

c. Plan for reporting unanticipated problems or study deviations.

Reporting will occur as follows:

i. Toxicity will be graded according to the NCI-CTC for application in adverse event reporting. The NCI-CTC has been reviewed specifically for this protocol and is appropriate for this study population. The purpose of using the NCI-CTC system is to provide standard language to describe toxicities, and to facilitate tabulation and analysis of the data and assessment of the clinical significance of treatment-related toxicities. The investigator will try to determine the relationship of toxicity to baked milk immunotherapy as not related, possibly related, or definitely related using standard criteria for clinical trials. All grades of toxicity will be noted. Toxicity grades are assigned by the study site to indicate the severity of adverse experiences and toxicities.

ii. Adverse events, not included in the NCI-CTC listing, should be recorded and graded 0-4 according to the General Grade Definition provided below:

Table 7: General Grade Definitions

Grade 0	Mild	Transient or mild discomforts (< 48 hours), no or minimal medical intervention/therapy required, hospitalization not necessary (non-prescription or single-use prescription therapy may be employed to relieve symptoms, e.g., aspirin for simple headache, acetaminophen for postsurgical pain)
Grade 1	Moderate	Mild to moderate limitation in activity, some assistance may be needed; no or minimal intervention/therapy required, hospitalization possible.
Grade 2	Severe	Marked limitation in activity, some assistance usually required; medical intervention/therapy required hospitalization possible.
Grade 3	Lifethreatening	Extreme limitation in activity, significant assistance required; significant medical/therapy intervention required hospitalization or hospice care probable.
Grade 4	Death	Death

iii. Adverse Event Reporting:

Within the context of this study, blood draws, allergy skin prick tests, dose increases and oral food allergy challenge tests will be observed and recorded. Adverse events (AEs) that are temporally related to these procedures or to home dosing with the study medications (during 3 days following the procedure) will be reportable adverse events. Anticipated local reactions to prick skin tests and oral food challenges reactions occurring during the challenge procedure will not be additionally reported on an adverse event form unless the reaction results in a serious adverse event. In that case it will be reported both on the reaction form and on a serious adverse event form.

The following events would be considered expected events for the procedures being performed:

- Blood draw: nausea and vomiting, local infection, and fainting
- Prick skin test: Pruritus, transient hive, systemic allergic reaction
- Oral food challenges and dosing with study medications: allergic reactions related to the skin (hives, pruritus, eczema flare), the respiratory tract (nasal congestion, rhinorrhea, fits of sneezing, difficulty breathing, asthma), and the GI tract (oropharyngeal pruritus and edema, nausea, vomiting and diarrhea).

All “unanticipated” adverse events related to the experimental procedures will be reported to the DSMB in an expedited manner if they are Grade 2 and above in severity. All “anticipated” adverse events related to the experimental procedures will also be reported in an expedited manner if they are Grade 2 and above in severity. Subject deaths are reportable within 24 hours. The investigator will continue to follow or obtain documentation of the

resolution course of such an event. A copy of the annual report of adverse events will be reported to the IRB.

iv. Data and Safety Monitoring:

A Data Safety Monitoring Board will be convened for overview of this study. The Principal Investigator and Co-Investigators are responsible for collecting and recoding all clinical data. As these results are collected, all toxicities and adverse events will be identified and reported to the PI. Adverse events will be reported as described above. The Principal Investigator will determine the relationship of the event to the study intervention and decide the course of action for the study participant.

Yearly reports will be made to the DSMB and proper Institutional Committees, as required. All adverse events will be kept in a computerized file by numerical identifier.

In the event that the study is stopped because of adverse event(s), it will not be resumed until information regarding the adverse event(s) has been discussed with the DSMB and the DSMB concurs with resumption of the studies.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

The risk of breach of confidentiality will be minimized by using unique identifiers for participants and keeping the record that links the identifier to the participant in a locked file cabinet accessible only to the investigators.

Genomic research and broad sharing of genomic data will have an “opt-in” and “opt-out” area on the consent and will not affect the participants’ eligibility to take part in the study. In addition, personal identifying information about the subject (such as name, phone number, address) will NOT be included in these genomic databases or shared with others, including our collaborator, La Jolla Institute.

e. Financial risks to the participants.

The study does not provide routine care or any medications or treatments for general health or allergic problems (although we will be available for phone consultation regarding possible reactions). All costs associated with study visits, skin testing, spirometry, blood drawing, food challenges, and OIT protein are covered by the investigators. All participants will be required to provide a muffin/cupcake mix in which they can bake the weekly doses of OIT. This will be provided by the family so that flavor/variety can be altered for each child’s preference. All participants will be required to have an epinephrine auto-injector available at

home during immunotherapy treatment. If they do not already have one, they will be responsible for the cost of this medication.

9. Benefits

a. Description of the probable benefits for the participant and for society.

The benefits for the participant include the possibility of a change in sensitivity to milk and decreased allergic reactions following an accidental ingestion of milk. Another possible benefit is the possibility of altering the natural course of the milk allergy.

The potential benefit to the subject and family is that this active protocol may induce tolerance to milk protein in someone that is unlikely to naturally "outgrown" his/her allergy. If tolerance is achieved, this then make impact quality of life.

The results of this study will have important implications for the treatment of cow's milk allergy and food allergy in general. The results would add evidence regarding safety and efficacy of OIT in treating food allergy. By using the baked allergen, we hope to decrease the adverse effects of OIT. The laboratory studies will help us understand the underlying biomarkers surrounding immunotherapy and clinical desensitization. These markers have the potential to serve as diagnostics to indicate progression to tolerance over time and/or to prospectively predict early versus late resolution of milk allergy.

10. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

Subjects will receive $25 per visit and a parking coupon to cover the cost of travel and parking.

11. Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

The study does not provide routine care or any medications or treatments for general health or allergic problems (although we will be available for phone consultation regarding possible reactions). All participants will be required to have an epinephrine auto-injector available at home during immunotherapy treatment. If they do not already have one, they will be responsible for the cost of this medication

All costs associated with study visits, skin testing, spirometry, blood drawing, food challenges, and OIT are covered by the investigator. All participants will be required to provide a muffin/cupcake mix in which they can baked the weekly doses of OIT. This will be provided by the family so that flavor/variety can be altered for each child's preference. The study milk protein or placebo will be provided by the investigator.

Table 8: Study Year 1

	Baseline	DBPCFC #1		Dose Escalation	Build-Up Phase #1											Maintenance Phase #1			DBPCFC #2	
Week	-3	-2	-1*	0	2	4	6	8	10	12	14	16	18	20	22	30	38	46	52	54*
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Informed Consent	X																			
History	X																			
Limited physical exam	X	x	x	x	X	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Vital Signs	X	x	x	x	X	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Symptom/diet information	X			x	X	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
U HCG	X	x		x	X		x		x		x		x		x	x	x	x	x	
Spirometry+	x																			
Peak Flow Rate+	X	x	x	x	X	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
SPT	X															x			x	
Blood draw	X															x			x	
QOL Questionnaire	X																		x	
GI assessment	X			x		x		x		x		x		x	x	x	x	x	x	
DBPCFC #1		x	x																	
DBPCFC #2																			x	x
Randomization			x																	
OIT dose increase				x	X	x	x	x	x	x	x	x	x	x	x					

*Second half of food challenge must occur within 21 days of the first half of the challenge + Spirometry will be attempted in all subjects ≥6 years old. Peak Flow rate will be attempted for children < 6 years old.

Table 9: Study Year 2 -4 schedule: Initial Treatment Group who Achieved 2 grams during Year 1

	Maintenance Phase #2					DBPCFC #3		DBPCFC#4[+]	
Week	56	68	80	92	104	106	108*	110	112*
Visit Number	21	22	23	24	25	26	27	28	29
Informed Consent									
History									
Limited physical exam	x	x	x	x	X	x	x	x	X
Vital Signs	x	x	x	x	X	x	x	x	X
Symptom/diet information	x	x	x	x	X	x	x	x	X
U HCG	x	x	x	x	X	x		x	X
Peak Flow Rate	x	x	x	x	X	x	x	x	X
SPT						x			
Blood draw						x			
QOL Questionnaire						x			
GI assessment	x	x	x	x	x	x		x	
DBPCFC #3						x	x		
DBPCFC #4								x	x
Randomization									
OIT dose increase									

*Second half of food challenge must occur within 21 days of the first half of the challenge [+]
DBPCFC#4 will only be performed if subject tolerates ≥2000 mg of baked milk during DBPCFC#3

Table 10: Study Year 2 -4: Initial Treatment Group who did NOT reach 2000 mg during year 1

	Build-up Phase #2									Maintenance Phase #2				DBPCFC #3		DBPCFC #4+	
Week	56	58	60	62	64	66	68	70	72	80	88	96	104	106	108*	110	112*
Visit Number	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Informed Consent																	
History																	
Limited physical exam	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Vital Signs	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Symptom/diet information	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
U HCG	x		x		x		x		X	x	x	x	x	x	x	x	x
Peak Flow Rate	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
SPT														x			
Blood draw														x			

QOL Questionnaire														x			
GI assessment	X		x		x		x		x	x	x	x	x	x		x	
DBPCFC #3														x	x		
DBPCFC #4																x	x
Randomization																	
OIT dose increase	X	x	x	x	x	x	x	x	x								

*Second half of food challenge must occur within 21 days of the first half of the challenge $^{+}$
DBPCFC#4 will only be performed if subject tolerates ≥2000 mg of baked milk during DBPCFC#3

Table 11: Study Year 2-4: Initial Placebo Group who Crosses Over

	DE day	Build-up Phase #2											Maintenance Phase #2			DBPCFC #3		DBPCFC #4^{+}	
Week	56	58	60	62	64	66	68	70	72	74	76	78	86	94	102	106	108*	110	112
Visit Number	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Informed Consent																			
History	x																		
Limited physical exam	x	X	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Vital Signs	x	X	x	x	x	x	x	x	x	x	x	x	x			x	x	x	x
Symptom/diet information	x	X	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

U HCG	x		x		x		x		x		x		x	x	x	x	x	x	x
Peak Flow Rate	x	x	x	x	x	x	x	x	x	x	x	x	x			x	x	x	x
SPT													x			x			
Blood draw													x			x			
QOL Questionnaire																x			
GI assessment	x		x		x		x		x		x	x	x	x	x	x		x	
DBPCFC #3																x	x		
DBPCFC #4																		x	x
Randomization																			
OIT dose increase	x	x	x	x	x	x	x	x	x	x	x	x							

DE: Dose Escalation

*Second half of food challenge must occur within 21 days of the first half of the challenge

[+] DBPCFC#4 will only be performed if subject tolerates ≥2000 mg of baked milk during DBPCFC#3

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