

Title: Low Dose Multi-Allergen Oral Immunotherapy for Food Allergy

Short Title: Low dose Multi-OIT (LoMO)

PI: Julia Upton

Co-I: Thomas Eiwegger

Amendment: Oct 2018 Changes since last version:

This page: added LoMO abbreviation so that we can keep the samples clear in the lab)

Page 23: Removed that we will treat cashew and pistachio as the same nut. This is because we want to be safe and the literature supporting cashew desensitizing pistachio and vice versa is less developed than walnut/pecan

Page 23 and 31: Modified OFC to harmonize with another collaboration which is already REB approved in Montreal and the PRACTALL guidelines and to ensure that the 10X of baseline tolerated dosing is measurable.

- This lead to slight change to the primary outcome on page 18 (the primary outcome which was used for sample size calculation remains the same)

Page 34: Corrected one error about when labs are drawn on page 34 (we had baseline, 12 mo and 18 mo but all other parts of the document say 0, 6 and 18 mo)

Page 35: clarified that the cells are white blood cells (which includes basophils)

Version 4: Changes after REB review

Clarified primary and secondary outcomes, added pregnancy as an exclusion, added non-fluency as an exclusion, consent process clarified (page 56), separated documents at the end of protocol

Version 3: Changes after Research Quality and Risk Management (RQRM) review: added the make of skin prick test devices and the manufacturer of the test extract and clarified that only clinical equipment in CRC will be used.

Other changes: comments from sci review removed; reduced top age to 16 years to ensure patients have time to complete study before turning 18 y/o, updated that blood sample size is 15ml heparin (page 30), modified dosing of OFC#2 to ensure it can show 5X and 10X maintenance dosing as per objectives, clarified that outcome measure is cumulative dose; noted that Food immunotherapy is being used as an experimental procedure as well as clinically by some practitioners in the risks section (previously mentioned only that is experimental), clarified exclusion is inability to come to hospital for dose escalation (it used to say q2 weeks). Page 34: clarified that we are looking for an increase in threshold of reaction (as was defined in the outcome measures).

Version 2: Changes since Version 1 are in response to scientific review and are outlined in that document

Summary:

Food allergy is a significant problem and is the most important reason for life threatening allergic reactions in childhood. The standard treatment is avoidance and emergency preparedness. Oral immunotherapy (OIT) is being explored for food allergy.

Oral immunotherapy (OIT) is an investigational food allergy treatment where small amounts of the food a child is allergic to is eaten and gradually increased over time with the aim to be able to eat a certain amount of the allergen without experiencing an allergic reaction. While this process works in many children there are concerns about safety, feasibility and drop-outs.

Many OIT trials have targeted approximately 4 grams of food/day. In these trials up to 40% drop-out, or do not complete the protocol. Although some academic associations hold that OIT is not ready for clinical practice, the European Academy (EACCI) has released clinical guidelines and many community allergists are offering this treatment. Community allergists often use slow protocols with low doses. It is important to formally study if such protocols are feasible and safe.

Most OIT trials are single allergen. Up to 30% of children have multiple allergies. Treatments are needed for all children with allergies. Multi-allergen trials have used a biological agent called omalizumab which is expensive and the long term effects are unknown. A lower dose of allergen may show that multi-OIT can be performed without biological drugs in many cases.

At the current time, the main limit to wide implementation in Canada is the extent of resources required to desensitize a patient which requires many regular visits to the hospital for dose escalations to increase the daily allergen dose under medical supervision as well as the preparation of individualized food doses. Private OIT clinics in the USA currently charge between 10k and 27k USD to treat a *single food* allergen at the time. This cost does not include supervision and management of home dosing reactions or time and productivity loss by patients and parents from multiple visits.

The primary aim of this project is to evaluate if low doses of foods can allow for OIT to multiple nuts to be achieved safely and feasibly and be efficacious. We will recruit very well characterized nut allergic patients predominantly from an ongoing, approved study in which their nut allergies are well described in terms of their symptoms and their blood profile. The outcomes will be safety, feasibility (drop-

outs), quality of life, and efficacy (percent eating the prescribed amount of nuts, the amount of nuts eaten without reaction, and immunological markers of OIT effectiveness).

This project is extremely relevant to the practice of OIT in Canada. Approximately 30% of children with allergies have multiple allergies and currently multi-OIT is thought to need a non-approved use of a biological medication (eg. Omalizumab). It may be that low doses can allow multi-OIT without the need for biological medication.

This project integrates two unmet needs of OIT: first, to study the efficacy of low doses which will be important in patients who cannot achieve full dosing of conventional OIT and to be feasible for wide practice, and second, to provide help for children with multiple food allergies. It is crucial to study real-life, feasible ways to give OIT for multiple allergens.

Evidence shows the longer the time on immunotherapy, the greater the gains. If we can have a very agreeable regimen, which may be expected with a very low multi-OIT regimen, then we should see more people stay on the treatment long term and gain the most benefit.

Table of Contents

Title: Low Dose Multi-Allergen Oral Immunotherapy for Food Allergy.....	1
Summary:	2
The Research Problem and Relevant Literature.....	6
Background	6
Food Allergy	6
Food avoidance has risks	6
Food Immunotherapy	6
Oral immunotherapy for foods has difficulties	9
Despite the excitement about OIT, there are challenges with the process that limit its wide clinical applicability.....	9
Allergic reactions on immunotherapy are frequent in the initial months	9
The time and financial commitment from the patient and providers for OIT is long	9
Both OIT and SLIT have significant problems with compliance.....	11
Investigators are actively looking for ways to make OIT have fewer symptoms:	12
Peanut OIT may be on the market soon and real life issues will emerge.....	12
Dosing and Feasibility have been highlighted as Areas needing Research	12
Low-Dose Immunotherapy May Be a Solution to Many of the Challenges of OIT	13
Low Doses take far fewer visits to reach maintenance and so less time for the patient and the health care system	13
Very Low Doses of food are recognized by the body:	13
Only small doses of food are required for protection from contamination of allergens:.....	14
Low dose food exposure has efficacy to increase food allergy thresholds:.....	14
Low Doses would be expected to have fewer allergic reactions:	16
There is interest in continuing to lower the doses used in Immunotherapy	16
Low Dose Summary	16
Summary and Questions remaining.....	16
Hypothesis.....	18
Outcome Measures.....	18
Primary Outcomes:	18
Secondary Outcomes:.....	18
Clinical Outcomes:	19
Immunological Parameters:	19
Exploratory tests:	Error! Bookmark not defined.
Research Design and Methodology	20
Methods.....	20
Schematic timeline	21

Oral Food Challenge	22
Summary of Study Visits	25
Inclusion/Exclusion Criteria	26
Post-Challenge	27
Dose Escalation	28
Formulation of OIT product	28
Oral Food Challenge #2:	31
Assessment of Amount of Nuts Participants Can Eat after OIT:	31
Allergic Reactions in Clinical Research Unit	31
Table 8: Scale for grading reaction severity and treatment (for physician supervised challenges/doses)	32
Laboratory Tests	32
Blood Sampling	32
Component resolved diagnosis.....	32
Basophil activation tests.....	33
IgE, IgG4	33
Multiplex- MicroArray	33
Mass Cytometry	34
Sample size:.....	35
Definition of adverse and serious adverse events	36
Contract with sponsor	37
Future	37
Center Expertise and Feasibility:	38
Budget	40
Safety Information	41
Withdrawal from therapy	41
Home monitoring during desensitization	42
Safety Action Plan: Low Dose Multi-OIT	Error! Bookmark not defined.
Home Diary	Error! Bookmark not defined.
QOL.....	Error! Bookmark not defined.
QOL.....	Error! Bookmark not defined.
QOL.....	Error! Bookmark not defined.
Data Collection Form	Error! Bookmark not defined.
Letter for Emergency Care/Schools	Error! Bookmark not defined.
Ethical Considerations	44
Potential Benefits to Participants and Others	44
Potential Harms to Participants and Others.....	44
Alternative treatments/procedures	45
How we will minimize adverse events.....	45

Phlebotomy:	45
Skin prick testing	45
Oral Challenge to multi-food mix.....	45
Oral Immunotherapy to multi-food:	45
Privacy/Confidentiality	47
Process for Seeking Consent and Assent	47
Blood Sampling Guidelines	48
REFERENCES.....	51

The Research Problem and Relevant Literature

Background

Food Allergy

Food allergy may affect up to 8% of children with nuts being one of the major food allergens.¹ Food allergy is often lifelong, the QOL of people and families with food allergy is reduced,² and treatments are desired. Up to 30% of children have multiple allergies.^{3,4,5} A flexible protocol which can manage single to multiple allergies is ideal for the children.

Food avoidance has risks

The current standard of care for nut allergy is avoidance and emergency preparedness⁶. However, accidental exposures to peanut are common. In a Canadian study of 1941 children with physician confirmed peanut allergy,⁷ accidental exposures to peanut occurred at a rate of 12.4% and were usually managed inappropriately. For children with a moderate to severe reaction less than 30% sought medical attention and only 36.7% received epinephrine.

Food Immunotherapy

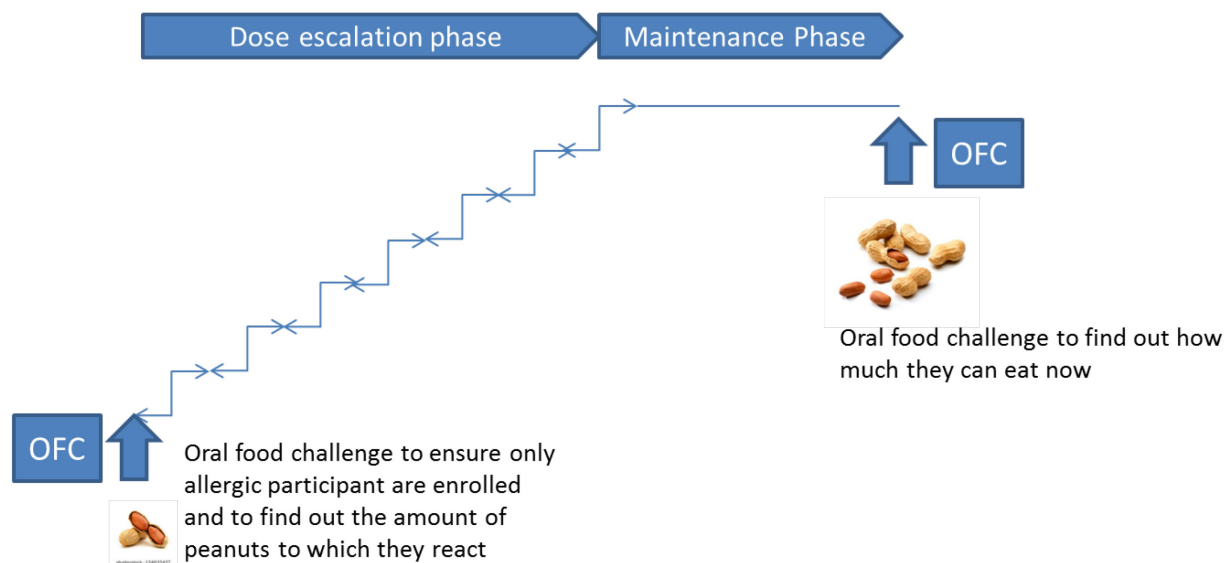


Figure 1. Schematic showing the basic structure of Food OIT using an example of peanuts. Participants are selected to be allergic to peanut and usually undergo a food challenge to ensure they are allergic. Then small doses of peanut are increased over a period of weeks to months until a maintenance dose is reached. Then another oral food challenge is performed to discover how much peanuts they can eat. OFC= oral food challenge.

Food Oral Immunotherapy (OIT) is being explored as a possible treatment for food allergy. Food OIT is a process where a person eats gradually increasing amount of their allergic food up to hundreds or thousands of milligrams. Overtime, this process can increase the amount of allergic food a person can eat without having an allergic reaction and induce immune changes.⁸ The overall process for food oral immunotherapy is shown in Figure 1.

OIT is now considered part of clinical practice by some Allergist Immunologists⁹ and by the European Society of Allergy and Clinical Immunology.¹⁰

A classic picture from the first randomized controlled trial of peanut OIT demonstrates the dramatic change in peanut ingestion which can be achieved with peanut ingestion.¹¹ These participants ingested a median of 280mg peanut per day and the effect was they could eat 5000mg without allergic reaction.

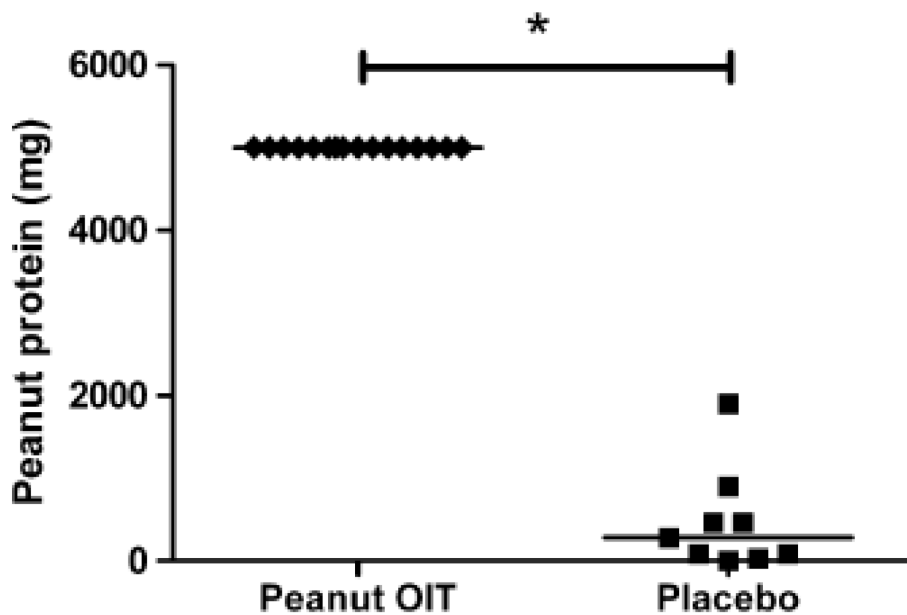


Figure 2. Peanut OIT versus placebo. ¹¹ Peanut OIT allows for a huge increase in the amount of peanut a child can eat without an allergic reaction.

The efficacy of desensitization is not in doubt, as seen in Figure 2, but many questions about how to deliver OIT remain to make it feasible and safe.

Sublingual immunotherapy (SLIT) is where very small doses (typically single digit milligram doses, whereas OIT is usually hundreds to thousands of milligrams) of food are taken under the tongue and this treatment also aims to achieve immune changes and changes in how much a person can eat without an allergic reaction.

Some studies in food immunotherapy aim to evaluate “sustained unresponsiveness,” which is a term meaning that the child or adult can stop eating the food and not react when the allergic food is eaten after a period of weeks or months of avoidance.

Oral immunotherapy for foods has difficulties

Despite the excitement about OIT, there are challenges with the process that limit its wide clinical applicability.

Allergic reactions on immunotherapy are frequent in the initial months

We know that dosing reactions are the most at the beginning of treatment and decrease thereafter and participants in OIT trials continue to have reactions even during maintenance dosing.¹² Investigators often manage these dose escalation reaction by **slowing the dose escalation to stay on a lower dose**.¹³ Table 1 shows the rates of reaction in one peanut OIT study¹² and highlights that the initial escalation days and dose escalation have far more reactions than maintenance.

Treatment	Initial escalation days	Buildup doses	Home doses
Any	71% (20/28)	1.7% (5/301)	0.7% (67/10,184)
Diphenhydramine alone	50% (14/28)	1% (3/301)	0.4% (45/10,184)
Albuterol alone	0%	0%	0.04% (4/10,184)
Diphenhydramine + albuterol	7% (2/28)	0.7% (2/301)	0.2% (18/10,184)
Diphenhydramine + epinephrine	11% (3/28)	0%	0%
Diphenhydramine + albuterol + epinephrine	4% (1/28)	0%	0.02% (2/10,184)

Table 1: The frequency of reactions on initial escalation days versus build up versus home doses.

The time and financial commitment from the patient and providers for OIT is long

At the current time, a limit to wide implementation in Canada is the extent of resources required to desensitize a patient which requires many regular visits to the hospital for dose escalations to increase the daily allergen dose under medical supervision. Private OIT clinics in the USA currently charge between 10k and 27k USD to treat *a single food* allergen at the time (<http://www.oitcenter.com/oit->

[cost.htm](#)). This cost does not include supervision and management of home dosing reactions or time and productivity loss by patients and parents from multiple visits.

Immunotherapy to multiple foods at currently used doses¹⁴ may be difficult to eat in a timely fashion. Most peanut trials have used maintenance doses around 4000mg, as summarized in Trendelenburg et al.¹⁵ A multi-OIT trial¹⁴ targeted 4000mg protein of each food which is about 16 of each nut up to 80 nuts. In this study it took participants *an hour* to eat the daily dose.

OIT may be needed long term for the majority of responders: Long term results are disappointing where people cannot maintain the constant exposure to the food: they become re-sensitized and have signs and symptoms of allergy upon exposure once again.¹⁶

Efficacy improves the longer someone is on immunotherapy. The first double blind placebo controlled study of SLIT in peanut through the coFAR research consortium found an increasing successfully consumed dose the longer the participant was on SLIT. In the SLIT responders the median dose consumed without reaction increased from 3.5mg at baseline to 496 mg at week 44 and then after week 68 the median successfully consumed dose increased even more to 996 mg (compared with Week 44). And these results were obtained on only about 4mg a day of peanut protein SLIT.

In a milk OIT versus SLIT trial, the SLIT had a target of 7mg/day and there were 2 OIT groups of 1g/day and 2g/day. The threshold for reaction increased only 7 fold at 12 weeks versus 64 and 79 fold for the 1gm and 2gm OIT targets respectively. However, at 60 weeks, the threshold was increased from baseline 40X in the SLIT group, 159X in the 1gm group and 54X in the 2gm group.¹⁷ From this milk data we can see that in the long term, the 7mg/day milk SLIT dosing gave basically the same results as 2grams a day of oral dosing and there was no clear dose response evident between the 1gram a day and the 2 grams a day OIT. This study shows that after a long time on immunotherapy, the thresholds increase far above the dose administered.

Sustained unresponsiveness also increases the longer you are on treatment as shown for peanut SLIT¹⁶ and egg OIT.¹⁸

Food immunotherapy dose increases cannot be rushed

One thought to shorten the time for OIT is to give doses more frequently. However, many studies show that “rush” immunotherapy (quick dosing increases) for peanut does not work well. In one study the median baseline positive challenge dose was no different than the maximum dose tolerated after 7 days of rush immunotherapy.¹⁹ Blumschen¹⁹ used a rush oral immunotherapy protocol followed by a long term increase in dosing in which they targeted at least 500mg a day of peanut (125mg peanut protein) with dose escalations q 2-4 weeks and 64% of participants achieved tolerance. The conclusions of this study were that, ***“Rush oral immunotherapy is not successful in most children with peanut allergy. In contrast, a long-term buildup protocol for peanut OIT appears to be safe and effective in reaching clinically relevant protective doses for high-risk patients with peanut allergy.”*** Their data are shown in Table 2.

Patient no.	Maximum tolerated dose before OIT, at baseline DBPCFC (g)	Starting dose of OIT at rush phase (g)	Tolerated dose after the rush phase (g)	Daily tolerated dose after long-term buildup phase (g)*	Maximum tolerated dose after OIT, at final DBPCFC (g)†	Time of peanut exposure during long-term buildup (d)
4	0.13	0.0032	0.16	1.0	0.5	81
10	0.13	0.0032	0.08	0.5	0.25	334
7	0.5	0.012	0.16	0.5	1.0	158
12	0.25	0.006	0.9	1.0	1.0	47
14	0.06	0.0016	0.03	0.5	1.0	314
16	0.13	0.0032	0.14	0.5	1.0	251
21	1.0	0.024	3.0	2.0	2.0	88
23	0.25	0.006	0.4	1.0	4.0	230
25	0.015	0.0004	0.14	0.5	1.0	154
28	0.5	0.012	1.2	1.0	4.0	134
30	1.0	0.024	1.0	1.0	4.0	0
24	0.13	0.0032	0.05	0.5	1.0	389
22	0.25	0.006	0.4	0.5	0.5	476
18	0.06	0.0016	0.04	0.5	0.25	560
26	0.06	0.0016	0.14	0.2	No provocation	458
8	0.13	0.0032	0.16	Dropout	Dropout	Dropout
9	0.13	0.0032	0.024	Dropout‡	Dropout‡	Dropout‡
13	0.13	0.0032	0.12	Dropout	Dropout	Dropout
15	0.03	0.0008	0.04	Dropout‡	Dropout‡	Dropout‡
19	0.5	0.012	1.0	Dropout	Dropout	Dropout
20	0.13	0.0032	0.18	Dropout‡	Dropout‡	Dropout‡
29	0.25	0.006	0.12	Dropout‡	Dropout‡	Dropout‡

*Daily tolerated dose of peanut at maintenance after OIT using the long-term buildup protocol.

†Maximum tolerated dose of peanut at final DBPCFC (after OIT with the long-term buildup protocol and 2 weeks of peanut avoidance).

‡Dropout because of pulmonary adverse events.

Table 2. Summary Table from Blumschen¹⁹ demonstrating that immunotherapy cannot be rushed. There was no difference in the maximum tolerated dose (1st column) versus the amount tolerated after rush immunotherapy (3rd column).

Both OIT and SLIT have significant problems with compliance

The long term follow up study of peanut SLIT showed that more than 50% had discontinued therapy¹⁶ largely due to problems with the daily dosing. In SLIT the dose must be held under the tongue before swallowing so this may contribute to the dropout rate.

For OIT, long term adherence is unclear.² In one of the major studies, 39 were enrolled and 24 completed the protocol and were treated up to 5 years with 4000mg peanut protein (about 20 peanuts) per day. This represents 61.5%. Clearly dropout rates could be improved.

The doses used for SLIT and for OIT cannot be directly compared because of the difference in the route and the prolonged contact with the oral mucosa in SLIT may lead to some of the immunological effects.²⁰ However, this prolonged contact also leads to oropharyngeal symptoms, takes longer to administer and the SLIT trials have high dropout rates. The very small doses used in SLIT have not been used orally.

Investigators are actively looking for ways to make OIT have fewer symptoms:

There is active investigation of OIT with biological therapies such as omalizumab.²¹ There is a cost and a risk to this treatment. It appears to allow for the dose escalation to the typical many hundred gram target doses with fewer symptoms. Certainly this approach has promise although it must be noted that there is currently no evidence that such the many hundred gram target doses are actually required.

Peanut OIT may be on the market soon and real life issues will emerge

It is expected that a highly characterized form of peanut OIT may be on the market within a few years.²² Also, many practitioners are already using OIT clinically.⁹ For those who cannot achieve the target dosing of the Aimmune product (300mg) due to side effects, it will be important to understand if a lower dose is efficacious.

Dosing and Feasibility have been highlighted as Areas needing Research

The most recent international guideline on immunotherapy highlights that there are many unanswered questions about the **dosing** of immunotherapy and how to increase its wide feasibility.²³

Low-Dose Immunotherapy May Be a Solution to Many of the Challenges of OIT

Low Doses take far fewer visits to reach maintenance and so less time for the patient and the health care system

Dose no.	Dose (mg)	Interval (wk)	% Increase
7	6		As above
8	12	2	100
9	25	2	108
10	50	2	100
11	75	2	50
12	100	2	33
13	125	2	25
14	156	2	25
15	195	2	25
16	245	2	25
17	306	2	25
18	383	2	25
19	479	2	25
20	599	2	25
21	749	2	25
22	936	2	25
23	1170	2	25
24	1463	2	25
25	1829	2	25
26	2286	2	25
27	3000	2	31

Table 3. Dosing Schedule of a recent trial showing that traditional dosing can take 20 visits and lower doses take fewer visits.

Table 3 shows the dosing schedule of the recent 300 versus 3000mg peanut OIT trial.²⁴ The first 7 doses were done in one day. Therefore, after the initial dosing day, it took about 10 outpatient visits to get to 300mg maintenance dosing following this schedule, versus about 20 outpatient visits to get to 3000mg. An even lower target would further reduce the number of days required to visit the physician for up dosing. Additionally, from a time perspective, because in this study each visit was approximately q2 weeks, the difference in 10 visits is about 5 months.

Very Low Doses of food are recognized by the body:

The baseline eliciting dose of peanut ranged from 0.015-1 gram in one study¹⁹ with a median eliciting dose of 0.13g. In Vickery et al²⁴ 4/37 children reacted to a cumulative dose of 1mg peanut protein. The peanut threshold eliciting dose for objective symptoms from EuroPrevall has been reported to be 0.2-36mg of peanut protein.²⁵ A recent study showed that 8/381 (2.1%; 95% CI, 0.6%-3.4%) peanut allergic patients had an allergic reaction to 1.5mg of peanut. If a person can have active symptoms to such a low dose, then clearly the immune system is capable of seeing such a low dose and this provides some rationale that a very low dose could be immune modifying.

Only small doses of food are required for protection from contamination of allergens:

Schappi et al reported that some foods had more than 1000mg/kg of peanut contamination²⁶ and Vadas and Pearlman reported that European chocolate bars can have up to 245ppm peanut without any labeling about the presence of peanut.²⁷ This corresponds to 0.245 grams/liter. So an accidental exposure of as much as a half cup of a contaminated food might contain up to about 30mg of peanut. That would be a very large accidental exposure and only deliver 30mg of peanut.

Mathematical modeling²⁸ shows that very few allergic reactions would occur to accidental exposures of foods even with a 30 mg threshold of allergic reaction, even fewer at 100mg and almost all accidental exposure reactions would be expected to be prevented with a threshold above 300mg.

Low dose food exposure has efficacy to increase food allergy thresholds:

Sublingual immunotherapy (SLIT) is where peanut solution is placed under the tongue for a minute or two and then swallowed. The amount used for SLIT is typically in microgram amounts rather than mg. For example, in one study, the high dose target for SLIT was 3696 mcg/day of peanut protein (meaning just under 4mg).²⁹ In this randomized controlled trial of peanut SLIT, responders were defined as being able to consume 10X the amount of peanut after treatment. Peanut SLIT showed efficacy with 70% responders versus 15% responders in the placebo group. This 15% responder rate is not outgrown peanut allergy, it is an increase in threshold as defined. The successfully consumed dose of peanut changed from an average of 3.5mg at week 0 to 496mg at week 44.²⁹ And this result was obtained

consuming fewer than 4mg a day of peanut protein. Additionally, in a long term follow-up study, differences in outcomes using a lower dose of 1386mcg or the high dose of 3696mcg of daily peanut protein were not observed although interpretation is limited due to high dropout rates.¹⁶ It is thought that the immune changes due to SLIT are different than those in OIT because of the under the tongue exposure. However it certainly shows that very low doses can be effective.

For oral immunotherapy, Vickery et al^{30,24} studied 2 doses of immunotherapy and showed efficacy with both. Forty children were enrolled in the study that experienced an allergic reaction to peanut in the previous 6 months or were determined to be very likely to have a reaction because they had a peanut-specific immunoglobulin IgE level above 5 kUA/L. After an oral food challenge confirmed peanut allergy, the children were randomized to either “low-dose” (300 mg/day) or high-dose (3000 mg/day) oral immunotherapy with peanut protein for a minimum of 12 months. The primary end point was sustained unresponsiveness, defined as no allergic reaction to 5 g of peanut ingested 1 month after stopping oral immunotherapy.

The Vickery study is worth exploring further. Of 40 consented subjects, 3 (7.5%) did not qualify. Overall, 29 of 37 (78%) in the intent-to-treat analysis achieved sustained unresponsiveness after 4 weeks of stopping the peanut OIT (300 mg arm, 17 of 20 [85%]; 3000 mg, 12 of 17 [71%], $p=0.43$) over a median of 29 months. Per-protocol, the overall proportion achieving sustained unresponsiveness after 4 weeks of stopping the peanut OIT was 29 of 32 (91%). Peanut-specific IgE levels significantly declined in OIT-treated children, who were 19 times more likely to successfully consume dietary peanut than matched standard-care controls, in whom peanut-specific IgE levels significantly increased (relative risk, 19.42; 95% CI, 8.7-43.7; $P < .001$). There was no difference in the rate of change in the immunology labs between low dose (300mg) and high dose (3000mg) treatment groups. Allergic side effects during OIT were common but all were mild to moderate. Therefore, at both doses tested, OIT had an acceptable safety profile and was highly successful in rapidly suppressing allergic immune responses and achieving safe dietary reintroduction.

Low Doses would be expected to have fewer allergic reactions:

A benefit to SLIT is that the very low doses produce very few systemic allergic reactions.³¹ The reactions are typically local. Using very low doses in OIT may reduce systemic allergic reactions and further balance the benefits with safety.

There is interest in continuing to lower the doses used in Immunotherapy

This proposed study is not the only one to further explore low doses. The Japanese are investigating the efficacy and safety of low-dose OIT (3 mL milk, 1/32 of a whole egg, 2 g boiled udon noodles, or 0.5 g whole peanut (133mg peanut protein) using lower target volumes than what is conventionally used.³²

Low Dose Summary

This section has summarized that overall the amount of peanut a patient tolerates after immunotherapy is typically much higher than the maintenance OIT dose and the lower limit of that dose has not been determined. So far there is no evidence of a dose response for OIT. And in young children only 300mg of peanut a day may have lasting effects which appeared similar to 3000mg. And when applied sublingually, a very small dose can be immune modifying. Thus, a low dose of OIT may also achieve immunological modification, need less visits to achieve a meaningful protection, and cause less significant allergic reactions than traditional dose OIT.

Summary and Questions remaining

We know that immunotherapy to food is efficacious, but this technique is limited by dropout rates, side effects, and it is very labour intensive and costly with multiple visits for providers and family. We know that most accidental exposures are of a very small amount of nut. We know food immunotherapy cannot be rushed. We know that dosing reactions are the most at the beginning of treatment and decrease thereafter and that investigators slow the immunotherapy dose escalation when reactions occur. We know that the longer a participant is on food immunotherapy the better it works.

We know that very, very low doses, such as those used in sublingual immunotherapy (SLIT) have efficacy. However, the maximum dose administrable by this method is limited by the route and the local mouth and tongue symptoms reduce compliance. We know that in a recent study, both 300mg and 3000mg oral immunotherapy maintenance dosing allowed most children to eat 5000mg peanut at the end of treatment, even after stopping the treatment for a month.

There are many remaining questions when it comes to food OIT. It is not known which maintenance dose is required to give meaningful protection. A low target/maintenance dose would mean fewer visits for the participants and may be more feasible. A low dose may work to provide accidental exposure protection without the added risks and discomfort of high doses. This study has the potential to lead to a change the delivery of OIT to an **implementable regimen** with much lower doses which would require **less visits, allow more antigens** without the support of a biologic agent, and most importantly give the patients **less risk of allergic reaction** than avoidance or traditional OIT.

Currently, there is no remuneration for OIT in Ontario. Studies like this are needed to show that OIT can be implementable on a large scale.

Multi OIT:

As described in references 15 and 37 by Begin et al, multiple foods can be desensitized at the same time. When performed without a biological medication (omalizumab), this process took more than a year to updose up to 5 allergens. When performed with omalizumab, the patients could quickly get to target dosing (about 1 month after OIT began), but the large doses took them a very long time to eat.

Hypothesis

Very low maintenance target doses of food will provide meaningful protection from accidental exposure, significant immune changes, and allow for multi-dosing.

Outcome Measures

Primary Outcomes:

Primary Clinical Outcome: Nut allergy Desensitization:

The primary outcome is based on comparing how much nuts the participant can eat without an allergic reaction before the low-dose OIT with how much nuts the participant can eat without an allergic reaction after the low dose OIT (at 18mo). This assessment is based on the result of study oral food challenges (OFC). We will calculate the proportion of subjects who achieve desensitization as determined by tolerating specified challenge doses of peanut protein with no more than mild symptoms. The specified challenge doses are 5X baseline (the basis of the power calculation), 10x baseline, and we will specifically examine 140mg (cumulative), 300 mg (444mg cumulative) because of their importance to accidental protection.

Primary immunological parameter: Change in allergen specific IgG4 change baseline vs 6m vs 18m),

Secondary Outcomes:

Clinical: Nut allergy Desensitization: as a continuous variable amount tolerated at baseline OFC (time 0) versus amount tolerated at OFC at 18mo,

Nut allergy Desensitization: historical rate of passing a repeat OFC after 18mo (no more than 10% of proven nut allergic patients would be expected to pass the OFC after 18 months).

Immunological: Change in IgG4 from the avoidance period (using data from markers of nut tolerance study vs OIT period))

Exploratory tests:

(not powered for these outcomes in this initial cohort)

Clinical Outcomes: Feasibility: Proportion who achieve maintenance doses, proportion who “drop-out” (descriptive) Safety: side-effects (diary), use of epinephrine; (descriptive); Quality of life: Change in quality of life at 18m of parents and children compared to baseline assessment (using validated questionnaires

Immunological Parameters: Change in allergen specific IgE, and components (via microarray) relative (change baseline vs 6m vs 18m), study vs OIT period); Change in basophil sensitivity and activity (change baseline vs 6m vs 18m), SPT reactivity to the individual nut extracts (change baseline vs 6m vs 18m), For those participants previously enrolled in the markers of nut tolerance study (REB# 1000053791), we will analyze their change in allergen specific measures from the time of that study.

We will examine the degree of epitope coverage with microarray techniques during OIT.

High content functional immune profiling via mass cytometry and single cell sorting can reveal specific clinical patterns of OIT response. Systematic assessment of signaling pathways at a single cell level has never been performed during OIT. Even less is known about allergen-driven responses of immunoglobulin receptor bearing cell populations like granulocytes, eosinophils and monocytes. Details of these lab tests are found in Laboratory test section.

Research Design and Methodology

Methods

We propose a prospective cohort study. We will recruit 15 children with multiple nut allergies (at least two allergies to any of peanut and tree nuts).

A multi-nut OIT based on the allergic status (peanut, walnut, hazelnut, almond, cashew, pistachio, pecan, macadamia) with the target of a very low dose of each allergen (about 30mg/day) will be performed. The OIT consists of a 6 months up-dosing period and 1y maintenance therapy. Clinical response will be defined by performing an oral food challenge (OFC) at 18mo. To demonstrate that low doses induce pro-tolerogenic mechanisms an immunological characterization will be conducted at baseline, after the dose escalation (6mo) and after the completion of the study (18mo). We will also use the prior immune evaluation from the nut study to evaluate a period of avoidance versus OIT.

We are proposing no placebo for this study. OIT can be accessed in the community with no regulation or formal tracking. Additionally, a pharmaceutical peanut OIT is expected in the next few years. Additionally, in OIT the effect on the increase in food allergy threshold is quite dramatic and the rate of allergy resolution in the placebo group is very low. The last multi-OIT trial did not have a placebo.¹⁴ Studies are concentrating now on **how to make the OIT process better**, not to prove it works. In Stanford there is a trial of peanut OIT with and without omalizumab, the biological medication which interferes with the allergy pathway.³³ There is no comparator group without peanut OIT.

Schematic timeline

Protocol:

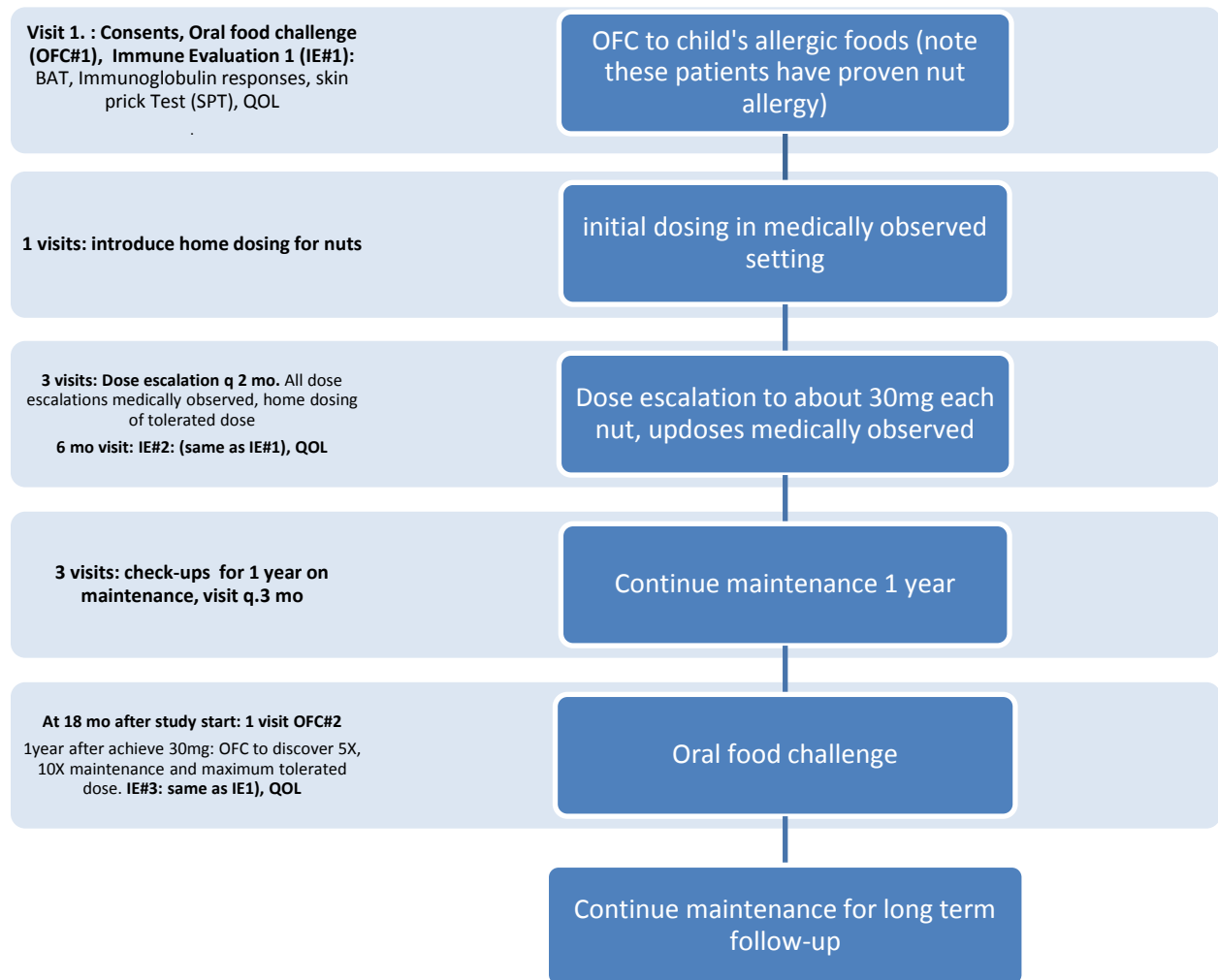


Figure 3: Schematic Timeline

Clinical Equipment:

Skin prick testing will be performed with extracts from Omega and Medipoint steep lancets as used in our Allergy clinic. Equipment from BP, pulse, O2s saturation in the CRC is clinical (Dash 3000 from GE).

Oral Food Challenge #1

Patients will begin with an oral food challenge (OFC#1) to a nut mix that will take place over one day. All challenges and desensitization procedures will be done with commercially available peanut/tree nut proteins. Patients will arrive at the Clinical Research Center (CRC) at SickKids in the morning. The CRC services include equipment and medications required to monitor and treat potential reactions. Patients will be reminded beforehand that they must be in good health (asthma controlled according to GINA guidelines and no intercurrent illness) for the challenge. If they are not in good health, the challenge will be rescheduled. Upon arriving, a physical exam will be performed and vital signs 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, at physician's discretion. Intravenous lines are not routinely required for OFC and the decision to place one is a clinical judgement.³⁴ Situations that may warrant insertion of an intravenous line for OFC are patients with (1) a past history of anaphylaxis or severe emesis (2) patients with severe asthma who are judged to be at higher risk for food-induced anaphylaxis even in the absence of previous anaphylactic reactions, (3) difficult intravenous access, and (4) anticipated need for intravenous medications for resuscitation—for example, glucagon.

The doses will be mixed in a food vehicle tolerated by the participant, such as pudding or fruit puree, that the participant is known to tolerate. Doses will be given by the study nurse every 15-20 minutes, depending on patients' tolerance of the dose (see Table 4 for challenge doses). The study nurse and physician 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 CRC for two hours after the resolution of the last symptoms. Our CRC has experience with OFC procedures from the CHILD study, the Baked milk study, and the Milk OIT studies performed in this setting.

Consideration was given to dosing based on individual thresholds but this has previously been shown to result in more reactions³⁵ than a standardized dosing regime.³⁶

The foods for OIT will be **combined and eaten as one dose** as in prior multi-OIT³⁷. The justification for a nut mix for OFC is these children already have at least one proven nut allergy. A mix will again prove at least one nut allergy, and furthermore find the lowest threshold for reaction, minimize anaphylaxis and be feasible by minimizing labour intensive OFCs. For example, if we know that a child reacted to 20 mg of a nut mix of peanuts and cashews, then we know they reacted to 10mg of cashews or 10 mg of peanuts. If at the end of the study they can tolerate 300mg of peanuts and cashews, we still know that their threshold was markedly increased to at least one nut. They can be further characterized by laboratory parameters and their prior OFC results. It makes sense to ensure the child is clearly allergic to at least one nut in the mix, but not need to check each one individually by OFC. What we really need to know for dosing is the lowest amount of nut they react to and that will be discovered by this procedure. While we cannot 100% prove a patient is allergic to multiple nuts with this procedure, we can prove they are allergic to at least one nut and the evidence for at least one other nut will be from the BAT. Because they have a proven nut allergy, it is worth going through the OIT process. The concept of multi-OIT has already been supported (refs Begin et al). This study will show that far lower amounts of nuts than usually used allow a patient to eat multiple nuts and have a hard outcome of the response of the lowest combined threshold.

We are using small doses which will not be expected to compete nutritionally and this approach provides a strong framework for the future clinical applications.

Given the similarity of allergens between walnut and pecan³⁸ we will include only one of these pairs for participants who report an allergy to these nuts. Table 4 presents the doses used for the initial OFC.

Table 4. OFC#1: Oral Food Challenge to multi-nuts (up to 5)		
Time	Dose (of each nut)	
0	Dose	Cumulative
15	1 mg	1 mg
30	3 mg	4 mg
45	10 mg	14 mg
60	30 mg	44 mg

75	100mg	144mg
90	300mg	444mg

Summary of Study Visits

Table 5 summarizes the Study Visits.

Table 5. Study Visits									
	Study Eligibility and oral food challenge	Initial Desensitization	Dose Escalation (over 6 months), visits q2months			Maintenance (over 1 year), visits q3months			Final Challenge
Visit #	1	2	3	4	5	6	7	8	9
Timeline*			2 months post visit 2	4 months post visit 2	6 months post visit 2	3 months post visit 5	6 months post visit 5	9 months post visit 5	12 months post visit 5
Approx. Cumulative time (weeks)	0	2	10	19	25	38	50	62	75
Oral Food Challenge	X								X
Nut Dose Consumed		X	X	X	X				
Skin Prick Test	X				X				X
Blood Draw	X				X				X
Intravenous Line**	X								X
Physical Exam	X								
Limited physical exam (skin, chest, vital signs)		X	X	X	X	X	X	X	X
QOL Survey	X				X				X
Diary Review			X	X	X	X	X	X	X

*Note that dose escalations are personalized and timeline can be extended if dose escalations take longer, a month has been approximated at 4-5 weeks

**Intravenous line insertion is at the discretion of the study doctor

Inclusion/Exclusion Criteria

Inclusion: Age 6mo-<16years, relevant allergy to 2-5 nuts. Serum IgE >0.35 kU/L (determined by UniCAP within the past 12 months) and/or a SPT to nut >3 mm compared to control, positive OFC to less than 300mg of a nut in the nut mix at baseline.

Exclusion: History of frequent or repeated, severe or life-threatening episodes of anaphylactic shock, use of omalizumab or other non-traditional forms of allergen immunomodulatory therapy (not including corticosteroids) or biologic therapy in the 12 months prior to study entry, history of eosinophilic gastrointestinal disease, uncontrolled asthma as defined by GINA, use of beta-blockers(oral), or angiotensin-converting enzyme inhibitors (ACE), fails to tolerate 4mg of peanut after the first desensitization day Other significant medical conditions that, in the opinion of the investigator, prevent participation in the study

Previous intubation due to allergies or asthma

Symptomatic atopic dermatitis or chronic urticaria which may interfere with ability to evaluate oral immunotherapy and /or requiring daily medication including antihistamines

Patients with problems related to compliance or following study instructions. Inability to come to hospital every for dose escalation.

Pregnancy

Non-fluency in English because participants may need to communicate with us after hours and be able to describe symptoms and concerns and follow instructions to treat anaphylaxis

We will aim to enroll predominantly from the existing nut study (REB#1000053791(as these patients have stated consent to approach for other studies and are very well phenotyped in terms of their reactions to nuts). However participation in the nut study is not essential. Recruitment procedures are outlined under “process for seeking consent and assent.”

Inclusion Criteria from the Nut study REB#1000053791 for reference:

Inclusion criteria

Children between 6 months and 17 years of age attending the SickKids or the Medical University of Vienna allergy clinics or the CARE clinics for routine clinical visits are eligible if they meet one of the following criteria:

Challenge proven peanut and or tree nut allergy.

Peanut or tree nut allergy diagnosis based on an immediate type allergic reaction to peanuts or tree nuts within the last 24 months with a pre-test probability >95%.

Sensitization to peanut or tree nut allergy (confirmed either by SPT or specific IgE testing) that undergo an oral food challenge in the allergy clinics as part of routine clinical care.

Unclear reactivity to peanut or tree nuts and will undergo an oral food challenge in the allergy clinics as part of routine clinical care.

Peanut and tree nut sensitized individuals who regularly consume tree nuts

Exclusion criteria

Infants younger than 6 months of age

Children with an unclear history of allergy to peanuts or tree nuts who will not undergo oral food challenge in the allergy clinics

Sensitization without exposure and a less than 95% pre-test probability who will not undergo oral food challenge.

The patient or the family declined to participate

GINA asthma control (<http://ginasthma.org/2018-pocket-guide-for-asthma-management-and-prevention/>)

All answers must be no to be considered controlled

Daytime symptoms more than twice a week?

Any night waking due to asthma?

Reliever needed more than 2X a week

Any activity limitation due to asthma

Post-Challenge

If a reaction occurred only during the multi-nut challenge, the child will be considered allergic to at least one of the nuts and will be advised to continue avoiding the nuts within the nut mix, with the exception of the nuts consumed for OIT within the study. The child and caregiver will receive a prescription for an epinephrine auto-injector, if they do not already have an epinephrine auto-injector of the appropriate dose. They will receive education regarding anaphylaxis recognition and management, and the caregiver will be requested to demonstrate the ability to recognize anaphylaxis and administer an

epinephrine auto-injector trainer. As developmentally appropriate, the child will also be requested to demonstrate the ability to recognize anaphylaxis and administer an epinephrine auto-injector trainer.

If no reaction occurred during the multi-nut challenge, we will follow-up with a clinical open feeding with an age-appropriate serving of food in its natural form or the least cooked/baked/processed form of food that will be incorporated into the patient's diet at home. If the child can eat nuts with no reaction, the family will be advised that the child should eat at least 4000-8000mg of nuts at least once per week, but that they may include an unlimited amount of nuts in the diet. They will also be advised to keep the child's epinephrine auto-injector available until peanut has been tolerated for at least 6 months, or indefinitely if the child has other allergies that may require emergency treatment with an epinephrine auto-injector. If the child passes the peanut challenge, the results of the challenge will be mailed to the child's primary care physician and allergist after both challenges have been completed and the child's regular consumption of peanut has been established.

Dose Escalation

Table 6. Dose escalation. All dose escalations will be performed under medical supervision. There is in general 3000mg of protein in 1 tablespoon (3 teaspoons) of nut butter. Exact amount will be calculated from foods used.				
Dose Escalation #	Time +/- 2 weeks	Approx. each nut Protein(mg)	Approx. increase % dose	Measured amount of each nut butter In tsp
Introduce multi-OIT	0	2.0 then 1 hour later 4.0mg	n/a	-2.0mg protein weighed at the research center - approx. 4.0 mg is 1/64 diluted ¼ in oil or water, use "drop spoon"
Updose 1	8 weeks	7.81mg	100	1/64 diluted in 1/2 oil or water, use "drop spoon"
Updose 2	16 weeks	15.63mg	100	1/64, use "drop spoon"
Updose 3	24 weeks	31.25 mg	100	1/32 teaspoon "1/32 =Smidgen"
Maintenance	to 84 weeks			

Table 6 shows the doses we will use for dose escalation.

Formulation of OIT product

We will use nut butters because they are stable, they can be diluted, they can be measured, they are readily available, and they will not spill like a powder might. Pure nut butters have approximately 6-7 grams (6000-7000mg) of nut protein in 28 grams of product (1oz, or 30ml, or 2 tablespoons) so they have approximately 3000mg protein in a tablespoon, or 1000mg in a teaspoon. Figure 3 shows a typical nutritional label for nut butter.

The target dosing for each nut butter is approximately 30mg of nut protein. We are aiming for a real life dosing. We will provide spoons which can measure down to 1/64 tsp.

Dosing accuracy will be taught in the research unit on the initial desensitization day with the products they will be using at home. Other OIT studies have used parent dosing including the current milk study at Sickkids (REB 1000050933) which is a multi-center study where we are collaborating with GET FACTS <http://www.getfactsmilk.com>.

The doses in the research unit will be provided to the participant. Home doses will be measured by the participant and added to small amount of pudding or apple sauce or another tolerated food.

If the participant reacts to less than 4mg on the initial challenge day or reacts before the completed dosing on the initial in hospital day, participants will be provided with measured amounts down to 4mg. If they cannot tolerate 4mg they will be excluded from the study.

The dosing regimen is flexible with dose reduction permitted based on investigator discretion. In general dosing will be reduced to the previous tolerated dose if the participant is symptomatic. The investigator can also suggest splitting the dose into two doses administered at least 4 hours apart.



Figure 3: Picture showing typical protein content of a nut butter.

Oral Food Challenge #2:

Assessment of Amount of Nuts Participants Can Eat after OIT:

About 18 months from when the study begins we will perform an oral food challenge to find a change in threshold dosing (the lowest dose which elicits reaction) and to discover the percent of participants who can reach multiples of their baseline as well as approximately 100 mg (140 mg cumulative) and 300 mg (444mg cumulative) without allergic reaction (Table 7). The exit challenge is based on the PRACTALL dosing (Sampson et al JACI 2012) and is also in line with another collaboration on low dose OIT already approved in Montreal lead by Dr. Ben Shoshan.

Note that the first OFC has a top dose of 444mg cumulative of each nut. So if a participant fails at that top dose, he/she tolerated only 144mg cumulative. Therefore, this exit challenge has the ability to discern >10X the entrance dose even if the child reacted to the highest available entrance dose.

The participant will continue to eat their nut mix on an ongoing basis to continue the accidental exposure protection.

Table 7. Oral Food Challenge (OFC#2) to multi-nuts (up to 5) AFTER OIT Participants are all eating 30mg per nut at home already		
Time (minimum time between doses is 15min)	Dose (of each nut, in a mix)	Cumulative
0	10mg	10mg
15	30 mg	40mg
30	100mg	140mg
45	300mg	440mg
60	600mg	1040mg
75	1000mg	2040mg

Allergic Reactions in Clinical Research Unit

If a reaction occurs in OFC#1, during dose escalations, or OFC#2, the symptoms and their time of onset will be recorded on the Data Collection Form, blood pressure, heart rate and respiratory rate will be measured and recorded every 30 minutes and more often as indicated. Epinephrine will be administered by auto-injector according to the anaphylaxis guidelines [5]. We will consider drawing a serum tryptase level, if clinically indicated.

All reactions occurring in the CRC will be graded and treated according to Table 8.

Table 8: Scale for grading reaction severity and treatment (for physician supervised challenges/doses)

SCORE	SYMPTOMS	TREATMENT
Mild	pruritus, urticaria, flushing, rhinoconjunctivitis	Will be treated with antihistamines (Benadryl 1mg/kg) and the regimen will be continued when the patient becomes asymptomatic. The previously tolerated dose will be repeated before resuming the process.
Moderate	angioedema, throat tightness, gastrointestinal complaints (cramping, pain, vomiting, diarrhea) . breathing difficulties (other than wheeze)	Will be treated with antihistamines (Benadryl 1mg/kg) +/- epinephrine (0.01mg/kg) and desensitization would be restarted on the following day at no higher than the previously tolerated dose.
Severe	wheeze, cyanosis, circulatory collapse (sat<92%, hypotension)	Will be treated with the necessary measures (including epinephrine and observed for at least 6 hours) and in the appropriate department, followed by an assessment of whether to interrupt desensitization or reduce the dose on the following day to no more than 1/2 of the dose that caused the reaction.

Laboratory Tests

Blood Sampling

A blood sample for basophil activation testing (15ml in a heparin tube sent immediately to the Eiwegger lab) and for specific IgE testing (4ml serum sample for component specific IgE testing stored at the respective site at -20C) will be drawn by the research nurse or a trained person with phlebotomy privileges. The blood sample will be labelled with the study participant's ID number, date and time when sample is collected and Data Collection Form will be completed.

Component resolved diagnosis

Sensitization to allergen components and positive responses due to cross reactivity among different allergen classes will be assessed via a chip-based approach (MEDALL, Thermofisher, Sweden) using sera of patients from all included patients. (Sirooux: doi:10.1016/j.jaci.2016.05.023)

Basophil activation tests

Basophil activation test (BAT) will be performed according to the manufacture instructions (FlowCAST Buehlmann, Switzerland). In brief whole blood will be exposed to peanut extract or recombinant allergens (Ara h 1, 2, 6, 8) for 15 minutes and CD63 and CD203c expression on basophils (defined as CCR3 positive SSC low population) will be measured. Allergen specific basophil activation will be assessed along 4 log scales of allergen concentration. Basophil activity (%CD63 pos to anti IgE stimulation divided by the maximal stimulation achieve with the allergen), basophil sensitivity concentration of allergen eliciting half-maximal activation of basophils and the percentage of CD63, CD203c positive and the CD203c mean fluorescence intensity basophils at the concentration of 10 and 100 ng will be measured. Moreover, phosphorylation of relevant signaling pathways such as but not exclusively p38, ERK1/2, akt, syk will be assessed either with flow cytometry or mass cytometry.

Analysis and interpretation of the respective allergen tests will be performed in a blinded fashion. Sample size is based on the estimate of a sample size of 12 peanut allergic patients with a power of 0.9 and a 2-sided type one error of 0.05 assuming a BAT non-responder rate of 15%. Based on an estimated response rate of 40% a sample size of 30 patients is required.

IgE, IgG4

During OIT, the induction of allergen-specific IgG4 and IgA has been shown to parallel the initial upregulation, followed by a downregulation of specific IgE. We will measure these responses to the relevant food extracts and food allergens (components) via a nano-particle based multiplex array (ALEX, Microarray Diagnostcs, Vienna, Austria) at baseline, 6mo and 18mo.

Multiplex- MicroArray

Epitope specificity to all reported food allergens of the 15 most common foods will be assessed at a single amino acid resolution (16-mer peptides with a 15AA overlap) using high resolution peptide array

technology (RocheNimbleGene, US). Using this technology will allow to specify the degree of epitope coverage OIT can achieve in different individuals.

Mass Cytometry

Systematic assessment of signaling pathways at a single cell level has never been performed during OIT. Even less is known about allergen-driven responses of immunoglobulin receptor bearing cell populations like granulocytes, eosinophils and monocytes. We will perform Mass Cytometry based, high content functional immune profiling of these cell populations to visualize the multiple layers of modulation via low-dose OIT. A specific panel has been established in the lab of Dr Eiwegger in collaboration with the core unit of flow cytometry at Sickkids (Dr. Cynthia Guidos). It allows whole blood stimulation via food allergens and to assess changes in phosphorylation of eight signaling proteins (ERK, akt, mTOR, s6, syk, tyr, PLC γ , p38). We will use semi- and unsupervised algorithms to identify novel cellular subsets with regards to function. Moreover single cell sorting of basophils and other white blood cells will be performed to perform RNA sequencing to analyze changes of white blood cell characteristics along OIT.

Sample size:

Sample size calculators have been used. <http://clincalc.com/Stats/SampleSize.aspx>

For the primary outcome of efficacy, in this very well-defined cohort we expect a less than 20% will not meet the inclusion criteria by reacting to 4mg and none will be not allergic to their nut mix. Therefore, we will challenge 18 patients to enroll 15. Anticipated efficacy is that the OIT will increase the reaction threshold. At an Alpha 0.05 and Beta 0.2 with a Power 0.8. We anticipate 60% will have at least a 5X increase in threshold, versus no more than 20% historically. At an Alpha 0.05 and Beta 0.2 with a Power 0.8, 9 patients will show this difference (60% vs 20%) with a one sample proportion test. Immunological parameters will be evaluated in repeated-measurement models. Paired t-test will evaluate before and after OIT for IgG4 and other continuous immune markers. With a projected difference of 0.3mgA/L in IgG4 with an alpha of 0.05 and beta 0.8, and standard difference 0.3, we expect to see IgG4 differences with only 8 subjects. This calculation is based on the highly statistically significant changes seen in IgG4 in peanut SLIT with only 17 patients.²⁹ We will target 15 participants to allow for any drop-outs.

Definition of adverse and serious adverse events

Adverse events:

An AE may consist of the following:

- 1) A new event which was not pre-existing at initial study drug administration.
- 2) A pre-existing event which recurs with increased intensity or increased frequency subsequent to study drug administration.
- 3) An event which is present at the time of study drug administration which is exacerbated following initial study drug administration.

Participants will have a diary to record urticaria, wheeze, oropharyngeal symptoms, and administration of any medication including diphenhydramine, albuterol, or epinephrine. We will record all events in the diaries as AE.

Serious adverse event:

A Serious Adverse Event (SAE) is defined by FDA and NCI as any adverse drug event (experience) occurring at any dose that in the opinion of either the investigator or sponsor results in any of the following outcomes:

- 1) Death
- 2) Life-threatening adverse drug experience
- 3) Inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours)
- 4) Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- 5) Congenital anomaly/birth defect
- 6) Important Medical Event (IME) that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon medical judgment, it may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Therefore serious adverse event will be defined as:

- Grade 3 (severe) anaphylaxis on the Brown scale³⁹ AND any of the factors above, OR any event above.

We will report reactions as per SickKids requirements.

Contract with sponsor: N/A

Future

We expect a statistically significant outcome in both the primary clinical and laboratory parameters. This data will support larger trials to have the numbers for feasibility (proportion who achieve maintenance doses, proportion who “drop-out” compared to the literature) and safety (side-effects (diary), use of epinephrine, compare to literature of higher doses) and quality of life in this innovative multi-OIT approach, thus we will measure these parameters in this study.

This multi-OIT protocol is not ideal to compare low dose to traditional dosing due to the volume of food required for traditional multi-OIT. We have worked collaboratively on a protocol already approved at another center directly comparing low doses to the new standard of 300mg nut protein for maintenance.

An extension may be sought to continue to follow these patients for long term outcomes. A future study may explore “sustained responsiveness,” meaning if the participant stops the nuts will they have their nut allergy return. Additionally, we would like to expand this low-dose protocol to other foods.

Overall we plan to incorporate this technique into clinical care if it is successful.

Center Expertise and Feasibility:

Julia Upton has performed oral food challenges to milk in milk allergic children in the Clinical Investigation Unit (CIU), part of the Clinical Research Center, for a previous study on milk allergy in which she was the Primary Investigator. In this study 38 children were screened for baked-milk allergy and 12 children had allergic reactions and 3 of these were given epinephrine.⁴⁰ In addition she has performed double blind placebo controlled challenges for multiple Health Canada approved studies on food immunotherapy as well as a clinical allergist who also performs food challenges in the Allergy clinic. She has published on low dose immunotherapy for food allergy,⁴¹ unusual causes of food allergies^{42 43}, anaphylaxis management^{44,45}, and food allergies as they relate to vaccines.⁴⁶ Her additional clinical trials experience includes blood product trials of IVIG and C1 esterase inhibitor. She is currently enrolled in a master's programme at Harvard in epidemiology which includes teaching on clinical trials. She is the Anaphylaxis and Food Allergy Section Chair for the Canadian Society of Allergy and Immunology.

Thomas Eiwegger is an accomplished clinician and scientist who has over 50 publications on clinical and fundamental allergology, and is a Co-I for CHILd and Site PI for the food component of the CHILd study in Toronto, the work package leader for a multi-national project which focuses on the development on novel and safer alternatives to treat milk and allergies (ALLEVIATE), an associate editor of Allergy (Official journal of EACCI), and PI of multiple trials to develop and optimize human immunological model systems to diagnose food allergy and investigate mechanisms of tolerance development. His lab regularly performs basophil activation test on a research basis (>4 per week). He is the current PI of a CHILd study performing double-blind placebo controlled food challenges in the Clinical Research Unit. He has published in high impact journals on mechanisms of allergy. ^{47 48 49}

At The Hospital for Sick Children we have an ongoing study (PI: Eiwegger, REB # 1000053791) in which the patients nut allergies have been phenotyped clinically by their reaction history and they have extensive molecular phenotyping with basophil activation assay and component antigens. The study is approved to enroll 100 and 50+ are already consented. The benefits to this ongoing study are multiple.

They have a known allergy and we know we have a group of nut allergic patients very keen to have options for studies on OIT.

SickKids also has 4 affiliated community Allergists who estimate they receive 1-2 inquiries about OIT per day. These affiliated allergists provide a large patient referral population. Furthermore, we receive contact information from patients hoping to hear about studies on OIT. We do not think recruitment will be a barrier.

Budget: see separate document,

Budget has been reviewed and approved by Aaron Mulivor

Budget

Overall budget is \$35,000

We have applied for CAAIF funding for the clinical aspects and the BAT, IgG4, IgE, peanut IgE, (\$25,000). Thomas's Start up funds will cover the costs in case of no competitive funding (cost center number 6010100205).

If we do not receive CAAIF funding we will use Thomas Eiwegger startup funds or approach the foundation for Food Allergy Anaphylaxis foundation funds for \$35000. Multi-OIT studies have already been highlighted as a priority for funding within the Food Allergy and Anaphylaxis Programme.

Safety Information

Withdrawal from therapy

Participants will be carefully monitored during the in hospital phase, as well as during the home OIT phase. Instructions for treatment of reactions will be provided. Participants who are enrolled will be informed that in previously published studies, minor allergic reactions are common in these protocols (mild itching, abdominal discomfort such as pain, bloating), but resolve with increased time on therapy.

Participants will be withdrawn if they cannot tolerate the low dose in hospital phase to 4mg of any nut in the nut mix, if they have more than 2 severe reactions during the dose escalation OIT phase, more than 2 severe reactions during the maintenance phase, if they experience a rare or unforeseen complication such as severe flares of eczema or chronic gastrointestinal symptoms (e.g. development of eosinophilic esophagitis). At any time the parents or subjects wish to voluntarily terminate therapy they are welcome to do so.

Home monitoring during desensitization

1. Nut should be given daily based on the dose achieved at the last hospital visit. Doses are only increased at the hospital under observation. The dose of nut should be given at approximately the same time daily, and be given in the presence of an adult. The child must remain under close visual observation (i.e. while maintaining eye contact) for at least 30 minutes after the dose is given.
3. If the child experiences a **mild** reaction (as described in the attached table), nut should be stopped and the child should be observed closely. If symptoms persist for more than 5-10 minutes, antihistamine such as Benadryl® should be given as instructed in the child's safety action plan and the team called prior to giving the next dose.
4. If the child experiences a **moderate** reaction (as described in the attached table) in addition to the actions described for mild reactions, epinephrine autoinjector should be given. An inhaler should be given if the child has difficulties breathing and has used an inhaler previously.
5. If the child experiences a **severe** reaction (as described in the attached table) in addition to the actions described for mild and moderate reactions, a repeated dose of epinephrine autoinjector can be given and emergency services such as 911 should be called immediately.
6. All children and their caregivers will be have two Epinephrine auto-injector along with written emergency plan that will be created by the management team with the parents and children. This plan should address all scenarios of potential use, including home, school, friends, travel, etc. The plan will also include telephone and pager numbers of investigators and nurses for direct consultation 24/7. The copy of the emergency plan and epinephrine autoinjector should be carried with the child at all times, including during visits to the Hospital for Sick Children.
7. Children and their caregivers will follow the written instructions regarding dose of nut based on progression of the protocol.
8. Home diary forms will be provided to record the dose, date, time taken and symptoms.
9. Children and caregivers will be instructed and trained to rank reaction severity and initiate appropriate treatment according to the attached table.
10. If a child has very mild/mild symptoms on 2 consecutive days, a dose will be reduced to the previous tolerated dose. Alternately, the dose can be divided in two, so that the child will take half the dose in the

morning, and half the dose in the evening. This will continue until the next study visit, at which point the child will take the entire dose. The child will then continue at this dose until the next visit.

11. If the child misses more than 5 days of dosing due to illness or non-compliance they will be withdrawn from the protocol.

12. If 1-5 days are missed due to illness or non-compliance, children will continue with the last tolerated dose.

13. Participants will be instructed to remain on a nut-free diet throughout the study and be required to carry an epinephrine auto-injector.

14. Participants will be provided with anticipatory advice about potential causes of reactions and advised to avoid exercise within 2 hours of dosing and to lower the dose with illnesses.

Ethical Considerations

Participants will also be advised that their data will be shared only among the OIT study team.

Potential Benefits to Participants and Others

Participants: Participants in the study may find that they do not have a food allergy during initial screening. If their allergy is confirmed, it is expected that most will be able to increase the amount of food they can eat.

Others: This study will help to understand the immune changes during low dose multi-OIT and to continue the goal of bringing real treatments to patients with food allergy.

Potential Harms to Participants and Others

Skin prick testing to allergens is very safe. In children the risk of generalized reactions is 512 per 100 000 tested children. In a 12 year retrospective review of allergists there was one death reported and this patient had 90 skin prick tests and moderately persistent asthma.⁵⁰

Oral food challenges involve a risk of anaphylaxis. There had been no reported deaths from oral food challenges as accessed by a PubMed search of the literature since 1976³⁴ and then in 2017 there was a death from an OFC to baked milk in Alabama.⁵¹ We will have appropriate emergency medical equipment available for OFC and medical supervision will be used for OFC and for dose increases. Any risk of oral challenges and oral immunotherapy must be compared to the risk of avoidance of the food. Food allergic children avoiding foods have accidental exposures and require epinephrine.

Food immunotherapy is being used as an experimental procedure as well as clinically by some practitioners which can result in anaphylaxis during up-dosing or maintenance. Food immunotherapy may be associated with the development of gastrointestinal symptoms such as eosinophilic esophagitis (EoE).⁵² EoE typically resolves once the food is withdrawn. Risk of OIT must be compared against risk of misdiagnosis of food allergy. This study will prove or exonerate the allergy at baseline.

Alternative treatments/procedures

For nut allergic individuals an alternative is to completely avoid the foods to which they are allergic. Another possible alternative (on a research basis) is to enroll in other immunotherapy studies such as with patches or under the tongue.

How we will minimize adverse events

Phlebotomy: will only be performed by nurses proficient in the procedure.

Skin prick testing will be performed by a physician of the research team who is proficient in the procedure. Skin prick tests have a very low risk of systemic reaction. A physician will remain in attendance and subjects will be monitored for at least 30 minutes after testing and epinephrine will be immediately available.

Oral Challenge to multi-food mix: Oral food challenges are used in many research studies to prove that the child is allergic to nuts. The medically supervised challenge is performed in a graded fashion in the hospital with immediate access to epinephrine and advanced anaphylaxis management. Nursing support will be readily available. The exclusion criteria increases safety by excluding those with risk factors for severe reaction (uncontrolled respiratory disease) and with contraindications to epinephrine or conditions which make epinephrine less effective (B-blockers, cardiac disease, hypertension).

Oral Immunotherapy to multi-food: All dose initiation and dose escalation will be done in the hospital in the research unit with medical attendance and a physician readily available for treatment of allergic

reactions. Full cardiopulmonary equipment will be immediately available. Anaphylaxis kit will be available at bedside.

The patients' caregivers will be instructed **verbally** and **in writing** about the recommendations to be followed after desensitization and how to **treat** possible allergic reactions. They will also be given telephone and pager numbers of investigators for direct consultation 24/7. Parents will be instructed to contact the research team should any symptoms suggestive of an allergic reaction be present. Withdrawal criteria have been set (see Withdrawal from therapy) to remove participants from the protocol if they are having repeated reactions or chronic reactions.

Privacy/Confidentiality

We will follow SickKids policies.

No personally identifiable information will be collected until the patient has successfully undergone pre-screening and signed consents.

Subjects will then receive a unique identifier and code breaking information will be kept separately from data collection forms under double lock.

Data collection forms will collect the minimal required data. They will be kept under double lock.

All research members will complete the privacy course (TCPS2 Tutorial).

The PI is responsible for who has access to the data.

Computers used will be password protected and the information will be kept on the network drive.

Data will be stored from the last publication in accordance with SickKids Guidelines. Paper records will be destroyed by Sickkids confidential waste. Electronic records will be destroyed by informing Information Services.

Process for Seeking Consent and Assent

Patients meeting eligibility will be approached according to local REB guidelines with recruitment until capacity.

Patients thought to be appropriate for the study will be identified by their primary allergist (either at SickKids or in the community) or by prior indication from the parents/participant that they wish to be contacted for studies. They do not have to be existing Sickkids patients. They will be given the choice to contact the research team or for the research team to contact them. A discussion with the research team member by telephone/email/in person or a combination of all three will occur. Through these methods, potential participants will be interviewed for inclusion/exclusion criteria. If they meet criteria for enrollment and want to be further assessed for joining the study they will come to the Clinical Investigation Unit. Consents will be signed in person on the first day. If they meet inclusion/exclusion criteria the study screening will commence.

Four ways of getting into the study:

- 1) Prior consent for contact: potential participant will be called or emailed by the study team because prior consent to hear about research studies is documented.
- 2) Community allergist tells potential participant and asks if they want to hear more about it. Then the potential participant can contact us or we can contact them
- 3) In clinic (previous permission to hear about studies): the patient will be approached by the study team and be informed about the study
- 4) In clinic (has not been asked about prior permission for studies): the potential participant will be approached by someone in the circle of care to be asked would they like to hear about a study on nut allergies

Once the study team can present the study then the potential participant will have the choice to look over the consents for >24hours and then decide if they would like to be in the study. Consents will be signed when they come for first day of the study or by email/in previously scheduled clinic visit. The consent will be taken by team members with permission to take consent. See eREB for further details.

Blood Sampling Guidelines

<http://my.sickkids.ca/research/clinical-research-services/research-ethics/PublishingImages/Pages/Forms-and-Templates/REB%20Blood%20volume%20guidelines%20-%2014Jul2017.docx>

For research of infants, children and adolescents, the REB will allow total blood-drawing of up to 5% of the research participant's total blood volume over an eight week period, on a single occasion or in divided portions.

Blood volume changes with age, thus amount available per kg will be:

	Calculation of 5% Blood Volume by weight
1 month to 10 years	4.0 ml/kg
10-15 years	3.7 ml/kg
Greater than 15 years	3.6 ml/kg

If one volume is used for all participants than the small applicable volume must be used (3.6 ml/kg). (More detailed chart below if further breakdown is required).

Issues to consider:

Vulnerable populations such as premature infants, newborns, cyanotic heart disease, renal disease or inherent anemias must all be considered individually with appropriate research blood volume reduction.

These volumes are based on the assumption that there are not additional clinical blood needs. If there are clinical blood draws within the given time period this amount must be subtracted from what is permitted to be taken for research. For example, a 10 kg 1 year old could have 40 mls of blood removed in an 8 week period. If, however, a 10 ml clinical blood draw will be required at week 2, then only 30 mls will be available for research.

The calculation of blood volume is based on ideal body weight, and should be adjusted for the severely obese or fluid overloaded.

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Chart of changes in blood volume by age:

Age	Mean blood volume per weight	5% of BV=
	(mL/kg)	(mL/kg)
Neonates (*4% of BV)	80	*3.0

Children, 3 months	87	4.4
Children, 6 months	86	4.3
Children, 1 year	80	4.0
Children, 6 years	80	4.0
Children, 10 years	75	3.8
Children, 15 years	71	3.6
Men	71	3.6
Women	70	3.5

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