The baseline eliciting dose of peanut ranged from 0.015-1 gram in one study[19] with a median eliciting dose of 0.13g. In Vickery et al [24] 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 contamination[26] 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 modeling[28] 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

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.

Schematic timeline

Protocol:

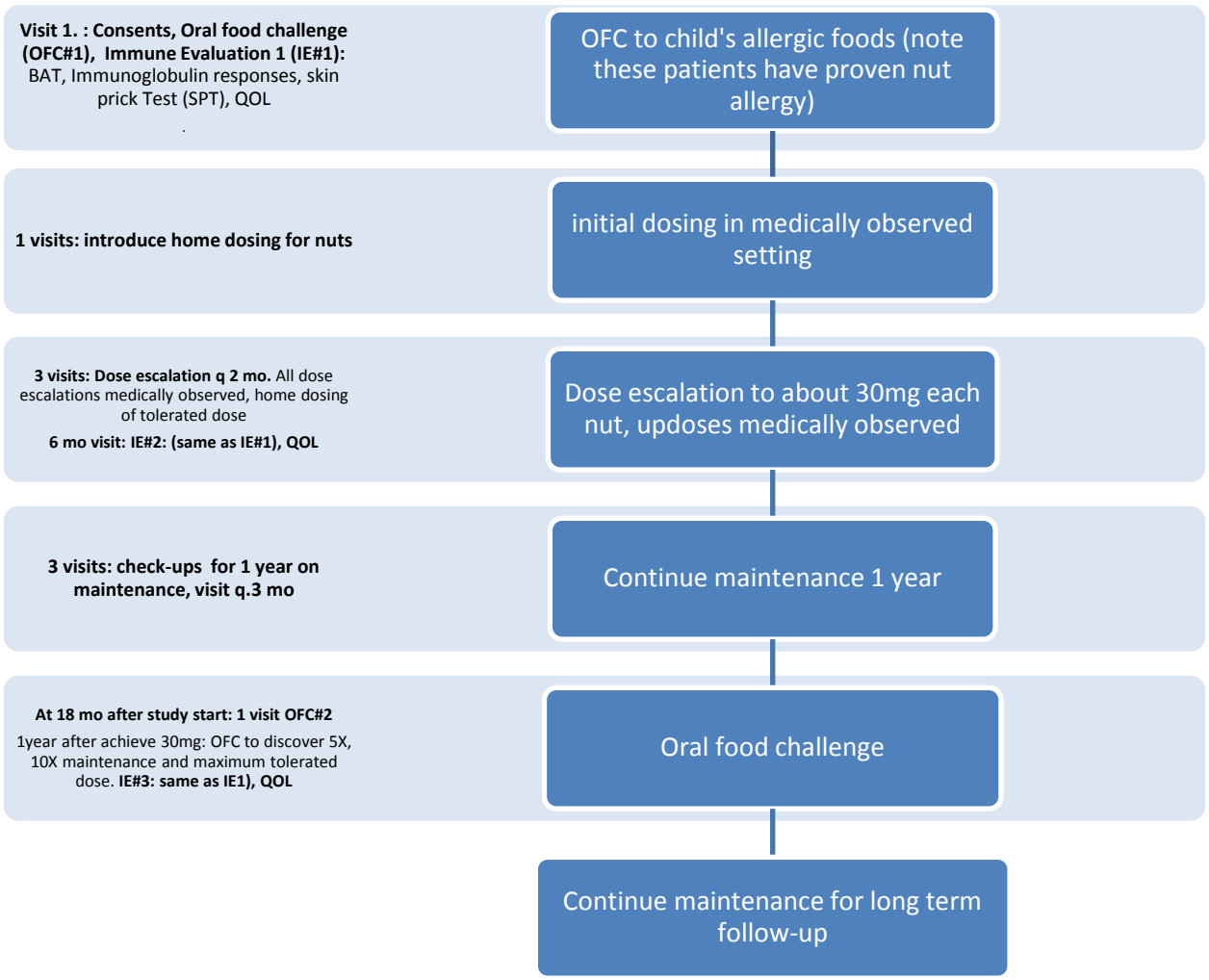

Figure 3: Schematic Timeline

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

- Grade 3 (severe) anaphylaxis on the Brown scale[39] 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.

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 on 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.

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.

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.

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 staturation 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.

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

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.

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.