Interventional study with investigational medicinal product (IMP)

Clinical Study Protocol

Immunological analysis of lymph node tissue after intralymphatic immunotherapy: A prospective case control study

Study Type:	Interventional study with investigational medicinal product (IMP) for intralymphatic allergen immunotherapy.
Study Categorisation:	Clinical trial, Risk category B.
Study Registration:	Registry on SNCTP via BASEC (Nr:…)
Study Identifier:	ILIT-FNA-2023
Principle Investigator	Professor Peter Schmid-Grendelmeier, MD, Allergy Unit, Department of Dermatology, University Hospital Zurich The Circle 59, 8058 Zurich-Flughafen, Switzerland Mail : peter.schmid@usz.ch Tel. +41 44 255 30 78
Sponsor-:	Professor Pål Johansen, PhD, Department of Dermatology University Hospital Zurich Rämistrasse 100 8091 Zurich, Switzerland Mail: pal.johansen@usz.ch Tel. +41 44 255 8616
Investigational Product:	Grass pollen extract (Polvac™) produced by Allergy Therapeutics
Protocol Version and Date:	Version 2, 06/06/2023

Signature Page

Study number	BASEC Number 2023-00821
Study Title	Immunological analysis of lymph node tissue after intralymphatic immunotherapy: A prospective case control study

The Principle-Investigator, the sponsor and the trial statistician have approved the protocol version 2 (dated.2023), and confirm hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki, the ICH-GCP guidelines and the local legally applicable requirements.

The Principle Investigator and the sponsor generally oversees the whole project and at all sections of the project (hospital and laboratories). Especially, but not exclusively, it is assures that

- approval is obtained from ethical review board and from Swissmedic,
- that all involved investigator or project partners are informed and educated,
- that the project is financially secured,
- that the investigational product is provided,
- that responsible and qualified medical investigators are available,
- that biological samples obtained from patients are process by qualified personnel as planned,
- that all documentation is obtained, filed and stored as planned,
- that contracts are made when required (e.g. monitoring) and
- that reporting is made to authorities.

We have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines and the local legally applicable requirements.

Principle Investigator

Professor Peter Schmid-Grendelmeier, MD

Department of Dermatology, University Hospital Zurich

Place/Date	Signature

Sponsor

Professor Pål Johansen, PhD

Department of Dermatology, University Hospital Zurich

Place/Date	Signature

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STUDY SYNOPSIS

Principle investigator	Professor Peter Schmid Grendelmeier, MD
Sponsor	Professor Pål Johansen, PhD
Study Title:	Immunological analysis of lymph node tissue after intralymphatic immunotherapy: A prospective case control study
Study ID:	ILIT-FNA-2023
Version/Date:	Version 2, 30.05.2023
Trial registration:	Registry on SNCTP via BASEC (Nr: 2023 - 00821)
Study category and Rationale	Interventional study with investigational medicinal product (IMP) for intralymphatic allergen immunotherapy. Clinical trial, Risk category B.
Clinical Phase:	Not applicable
Background and Rationale:	IgE-mediated allergy can be treated by subcutaneous allergen-specific immunotherapy (SCIT) (1-3). However, SCIT is not used by the majority of allergic individuals, mainly due to the long duration of the therapy and the risk of allergic reactions associated with allergen administration (4-6). Intralymphatic immunotherapy (ILIT), the administration of the allergens directly into a lymph node, could reduce treatment time, number of injections and adverse events. ILIT could raise patient compliance and improve allergy management. Clinical trials with only three monthly intralymphatic injections have shown comparable efficacy and safety to that of three years of SCIT (7). While efficacy of ILIT is becoming increasingly recognized, the mechanism by which ILIT acts is not known. The advantages of ILIT are typically explained by a more potent and efficient delivery of allergen to the lymphatic system. However since a different tissue is targeted than when using SCIT or SLIT, it may be assumed that other cells are directly involved or providing bystander effects. Moreover, while the existing ILIT trials have investigated long term clinical effects as well long term antibody and T-cell responses, early immunological events have not been investigated so far. Therefore, the current study aims to analyse and characterize local and systemic immunological changes upon a single ILIT injection in allergy patients receiving AIT as standard of care. For this, we will collect lymph node tissue and blood within one month of a single ILIT injection. One group of patients will receive a SCIT injection as a reference to ILIT. Cells and serum will be analysed by standard diagnostic methods as well as modern immunological methods. The study may highlight the immune properties of ILIT and potentially further propagate ILIT as a valid alternative to SCIT.
Objective(s):	The study seeks primarily to determine hallmarks in immunological responses (analysis of inflammatory cell and proteins) in lymph nodes and in peripheral blood after allergen ILIT and SCIT.
Outcome(s):	1) Fine needle aspiration (FNA) material of lymph node tissue at baseline and 2 or 6 or 24 hours after allergen ILIT or SCIT will be evaluated for changes in cell populations and cell functions. 2) Immunological changes (analysis of cell populations and functions ex vivo and in vitro) in peripheral blood at baseline, after 2 or 6 or 24 hours, after 7 days and after 28 days of allergen ILIT or SCIT.

Study design:	This is a prospective, comparative, clinical study. We will recruit 30 grass-pollen allergic patients undergoing pre-seasonal short-term SCIT scheme with Polvac™ Grass+Rye at the USZ Allergy Unit. 4 weeks after last SCIT injection and after reaching maintenance SCIT dose (2000 U), the patients will receive an additional allergen injection as part of the study. Patients will be randomized into the 3 different groups with 10 participants per group. Patients will receive a single ultrasound-guided injection with Polvac™ Grass+Rye ILIT (0.1 ml, 400 U) or SCIT (0.5 ml, 2000 U). FNA of lymph node tissue will be conducted at baseline and at 2, 6 or 24 hours after allergen injection. Venous blood will be sampled at baseline, after 2, 6 or 24 hours, after 7 days, and after 28 days of allergen injection.
Inclusion / Exclusion criteria:	Included are patients between 18 and 55 years of age with grass-pollen allergy and undergoing pre-seasonal short-term-scheme Polvac™ Grass+Rye SCIT at the USZ Allergy Unit for treatment of allergic rhinoconjunctivitis. Exclusion criteria are significant allergy to house dust mite or pet animal allergens, uncontrolled asthma, upper airway disease, severe pulmonary disease, recent allergic reactions, immunosuppression, cardiovascular disease, malignant disease, conspicuous lymph node size or morphology, use of ACE-blockers and any other diseases or conditions rendering the treatment of anaphylactic reactions difficult.
Measurements and procedures:	A single injection of grass pollen allergen extract (Polvac™ Grass+Rye) will be administered by ILIT or SCIT. FNA of material from lymph node tissue will be aspirated 6 hours after allergen injection. Opposite inguinal lymph node tissue will be aspirated by FNA as a baseline comparison. Venous blood will be sampled at baseline, after 2, 6 or 24 hours, after 7 days, and after 28 days of allergen injection. For safety reasons, as to exclude respiratory cases, peak flow measurement will be performed before treatment and 40 minutes after treatment.
Study Product / Intervention:	A single dose of grass pollen allergen extract (Polvac™ Grass+Rye from Bencard AG, Switzerland) will be injected via ultrasound-guided ILIT or SCIT route. Different doses will be injected according to the 2 study arms: (i) ILIT 0.1 ml, 400 U or SCIT 0.5 ml, 2000 U.
Control Intervention:	No control intervention is planned.
Number of Participants with Rationale:	For this pilot, unblinded study with three treatment groups, no sample size calculation is made. We assume that 10 patients per treatment group will enable us to describe immunological features of the three treatments.
Study Duration:	01.09.2023 to 31.12.2024
Study Schedule:	Plan: October 2023 First-Participant-In; March 2024 Last-Participant-Out.
Investigators:	Emma Widmer, Department of Dermatology, University of Zurich, Raemistrasse 100, 8091 Zurich, Switzerland Lara Šošić, MD, Department of Dermatology, University of Zurich, Raemistrasse 100, 8091 Zurich, Switzerland Claudia Lang, MD, USZ Allergy Unit, The Circle 59, 8058 Zurich-Flughafen, Switzerland
Study Centre(s):	Department of Dermatology, University Hospital Zurich, Zurich
Statistical Considerations:	All data are analysed and reported with descriptive statistics.
GCP Statement:	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, ICH-GCP, as well as all national legal and regulatory requirements.

ABBREVIATIONS

AE	Adverse Event
ASR	Annual Safety Report
ARC	Allergic Rhinoconjunctivitis
BASEC	Business Administration System for Ethical Committees, (https://submissions.swissethics.ch/en/)
CA	Competent Authority (e.g. Swissmedic)
CRF	Case Report Form
ClinO	Ordinance on Clinical Trials in Human Research *(in German: KlinV, in French: OClin, in Italian: OSRUm)*
eCRF	Electronic Case Report Form
CTCAE	Common terminology criteria for adverse events
DSUR	Development safety update report
FNA	Fine needle aspiration
GCP	Good clinical practice
IB	Investigator's Brochure
Ho	Null hypothesis
HRA	Federal Act on Research involving Human Beings *(in German: HFG, in French: LRH, in Italian: LRUm)*
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IIT	Investigator-initiated Trial
ILIT	Intralymphatic immunotherapy
ITT	Intention to treat
PI	Principal Investigator
SCIT	Subcutaneous Immunotherapy
SDV	Source Data Verification
SmPc	Summary of Product Characteristics
SOP	Standard Operating Procedure
SPC	Summary of product characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
USZ	University Hospital Zurich

STUDY SCHEDULE

Table 1. Illustrating the study schedule with all interventions divided into the five visits. Patients will receive 6 SCIT injections as standard of care (not part of the study). 4-6 weeks later, the patients will be included in the clinical trials for assessment of immunological characteristics after a single ILIT or SCIT with subsequent fine needle aspiration (FNA), adverse event (AE) monitoring, and venous blood collection.

Visit / time point	**1** **Screening** < 6 weeks of last SCIT	**2** **AIT** Day 0	**3** **FNA** Day 0-1	**4** **Follow-up** Day 7	**5** **Follow up** Day 28
Required personnel	Nurse/MD	MD	MD	Nurse/MD	Nurse/MD
Informed consent	x				
Randomization	x				
Medical history	x				
Physical exam	x				
Incl./excl. criteria	x				
Blood samples		x	x	x	x
Pregnancy test		x			
AEs/Concomitant medication		x			x
Peakflow measurement		x*			
Injection: Study drug administration		x			
FNA (2x)			x**		
AE monitoring (1h observation at allergy unit after injection)		x			
Dispensing rescue medication		x			

* Peakflow measurement will be performed immediately before injection and 40 minutes after injection

** The FNA is performed 2, 6 or 24 hours after the Polvac™ ILIT or SCIT, and the injected (ILIT) or draining (SCIT) lymph node is used for FNA. For baseline reference, a contralateral lymph node is also aspirated.

1. STUDY ADMINISTRATIVE STRUCTURE

1.1 Sponsor

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1.2 Medical Investigators

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1.5 IT Management

Not applicable.

1.6 Data Safety Monitoring Committee

Not applicable.

1.7 Partners at the USZ Allergy Unit

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1.8 Further Partners

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2. ETHICAL AND REGULATORY ASPECTS

The clinical study will only begin once approval from CEC and Swissmedic has been received. Any additional requirements imposed by the authorities will be implemented.

2.1 Study registration

The trial will be registered on SNCTP via BASEC.

2.2 Categorisation of study

Interventional study with investigational medicinal product (IMP) for intralymphatic allergen immunotherapy. Clinical trial, Risk category B.

Allocation to Risk Category B because of off-label use of the allergen, i.e., intralymphatic administration and dosage change of investigational medicinal product (IMP).

2.3 Independent Ethics Committee (IEC)

Approval will be requested from the cantonal ethical committee (*Kantonale Ethikkomission Zürich*) via BASEC and by the CA *Swissmedic*.

The Sponsor will report any premature termination or interruption of the trial to the IEC and Swissmedic within 15 days. The regular end of the study is reported by the Sponsor to the IEC as well to Swissmedic within 90 days, the final study report will be submitted within one year after study end. The Sponsor will submit an annual safety report to the IEC.

Substantial amendments are only implemented after approval of the IEC and CA respectively.

Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the Sponsor and the IEC/CA. Such deviations shall be documented and reported to the Sponsor and the IEC/CA as soon as possible.

All non-substantial amendments are communicated to the CA as soon as possible if applicable and to the IEC within the ASR.

2.4 Competent Authorities (CA)

Approval will be requested from the CA *Swissmedic* according to study category B before the start of the clinical trial.

2.5 Ethical Conduct of the Study

The study will be carried out in accordance to the protocol and with principles enunciated in the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP) issued by ICH, and Swiss Law and Swiss regulatory authority's requirements. The IEC and regulatory authorities will receive Annual Safety (ASR) and interim Reports and be informed about study stop/end in agreement with local requirements.

2.6 Declaration of interest

The Department of Dermatology of the University Hospital of Zurich (Director Professor Dr. med. Thomas Kündig) will support the study with infrastructure and overhead.

Prof. Thomas Kündig has served as medical advisor for Allergy Therapeutics (Worthing, UK), which is providing the investigational drug (Polvac™ Grass+Rye) to be used in this study. The Sponsor of this study, Pål Johansen, has received research funding from Allergy Therapeutic for pre-clinical research with products or developments from Allergy Therapeutics. However, the efficacy of the study drug (Polvac™ Grass+Rye) itself in comparison to other study drugs plays no role in this study for which reason the relationship to Allergy Therapeutics should have no influence on the overall analysis of the study.

Other researchers participating in this study have no conflicts of interest. No bias in the study is expected from conflicts of interest.

2.7 Patient Information and Informed Consent

The medical investigators explains to each study participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may

entail. Each subject is informed that the participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical assistance and treatment. The subjects are informed that he/she can ask any question, and consult with family members, friends, their treating physicians, or other experts before deciding about their participation in the study. Enough time is given to the subjects before signing the informed consent form.

The subjects are informed that authorised individuals other than their treating physician may examine his/her medical records.

All subjects are given a subject information sheet and a consent form describing the study and providing sufficient information for the subjects to make an informed decision about their participation in the study.

The formal consent of a subject, using the approved consent form, is obtained before the subject is submitted to any investigation procedure.

The subject should read, understand, and voluntarily agree before signing and dating the informed consent form, and is given a copy of the signed document. The consent form is signed and dated by the subject and the Principle-Investigator (or his designee). The signed consent form it is retained as part of the investigation records.

The patients will be compensated for potential travel cost only.

2.8 Participant privacy and confidentiality

The investigators affirm and uphold the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. Especially, anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals.

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited.

For data verification purposes, authorised representatives of the Sponsor, a competent authority (e.g. Swissmedic), or the competent ethics committee (*Kantonale Ethikkomission Zürich*) may require direct access to parts of the medical records relevant to the study, including participants' medical history.

The identity of the participants in the study will be coded with a participant number. The handling of data, including data quality control, will comply with regulatory guidelines (e.g. International Conference on Harmonisation (ICH) and Good Clinical Practice (GCP). The project will comply with the General Data Protection Regulation (GDPR) and The Data Protection Act.

2.9 Early termination of the study

The Principle-Investigator or the Sponsor may terminate the study prematurely according to certain circumstances, for example:

- ethical concerns,
- insufficient participant recruitment,
- when the safety of the participants is doubtful or at risk, respectively,
- alterations in accepted clinical practice that make the continuation of a clinical trial unwise,
- early evidence of benefit or harm of the experimental intervention

The Sponsor will report any premature termination of the trial to the IEC.

2.10 Protocol amendments

Substantial amendments are only implemented after approval of the IEC and CA respectively.

Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the sponsor and the IEC/CA. Such deviations will be documented and reported to the sponsor and the IEC/CA as soon as possible.

All non-substantial amendments are communicated to the CA as soon as possible if applicable and to the IEC within the ASR.

3. BACKGROUND AND RATIONALE

3.1 Background and Rationale

Allergic rhinitis is an important public health concern; it affects more than 20% of Western populations (8). According to the definition proposed by the ARIA (Allergic Rhinitis and its Impact on Asthma) Initiative, allergic rhinitis is an IgE-mediated inflammatory reaction of the nasal epithelium with the corresponding symptoms, which is precipitated by exposure to an allergen (9). IgE mediated allergy can be treated by subcutaneous (SCIT) or sublingual (SLIT) allergen-specific immunotherapy (1-3). While SLIT comprises daily tablets or drops for three years, SCIT is performed by weekly-to-monthly injections over the same time period, resulting in 18 to approx. 50 injections, depending on the chosen scheme. However, AIT, and especially SCIT is not used by the majority of allergic individuals with allergic rhinitis, mainly due to the long duration of the therapy, each injection associated with a doctor visit and the risk of severe systemic allergic reactions associated with the allergen administration (4-6).

Intralymphatic immunotherapy (ILIT), the administration of the allergens directly to an inguinal lymph node, is an investigational method developed to shorten treatment duration and improve safety while maintaining efficacy.

Clinical trials with only three intralymphatic injections that are given with 1-2 month intervals showed the same effect as 3 years of subcutaneous or sublingual immunotherapy (7). Indeed, the treatment effect as measured by clinical symptoms, allergen provocation tests and symptom-medication scores have shown comparable efficacy and safety to that of three years of SCIT (10-12)

The effects were typically explained by the more efficient delivery of allergen to the lymphatic system with ILIT as compared to other routes (13, 14). Other routes (SLIT and SCIT) depend on the drainage and uptake of injected allergen into lymph node for antigen presentation, resulting in only a minor fraction of the administered allergen arriving in the lymph node. Moreover, because fewer and lower doses are used in ILIT than in SCIT, less AIT-associated side effects may be expected. Hence, ILIT could raise patient compliance and improve management of allergies in many patients afraid or reluctant to receive conventional AIT.

While efficacy of ILIT is becoming increasingly recognized, the mechanism by which ILIT acts is not known and has not been investigated. Since a different tissue is targeted than when using SCIT or SLIT, it may be assumed that other cells are directly involved or providing bystander effects. For instance, antigen-presenting cells in the subcutaneous (SCIT) or mucosal (SLIT) tissues are surely different from those residing in the lymph nodes. Since the presentation of proteins in vaccines and immunotherapy products differs in quality and effect size between different antigen-presenting cells (e.g. macrophages, dendritic cells, monocytes, B cells and neutrophils), antigen presentation after ILIT, SLIT and SCIT mostly likely differs. Moreover, inflammatory cells such as neutrophils have versatile bystander functions and contribute to adaptive immunity by various way. The migration of neutrophils from the periphery (as in SCIT) to lymphoid organs has been linked to upregulation of the chemokine receptor CCR7, but it is unknown to what extent such inflammatory cells get involved when allergen is injected directly to lymph nodes (ILIT). Therefore, the current study aims to analyse and characterise local and systemic immunological changes upon a single ILIT injection in grass-pollen allergic patients receiving immunotherapy. We will characterize the immunological potential induced by ILIT and compare this to the immunological events observed upon SCIT. By these means, we expect to reveal some of the mechanisms by which ILIT exerts its effects and thereby address unmet needs in AIT. The present small study with limited patient numbers has a focus on the major ILIT-induced changes in leukocyte numbers, activation, migration and function. The results should inform the strategy for bigger follow-up studies to optimize ILIT timing and dosing.

The following question will be addressed:

- Is immunity by SCIT and ILIT generated by the same mechanisms and only weaker in SCIT due to less allergen reaching the lymph nodes or does ILIT result in more efficient activation of specialized lymph node cells, different from what is seen after SCIT?
- Which innate immune cells are involved in ILIT and SCIT?
- Why does SCIT require higher doses and longer treatment times as compared to ILIT?
- How do innate and adaptive immune responses correlate?

3.2 Investigational Product (treatment) and Indication

Polvac™ Grass+Rye, a grass- allergen extract with microcrystalline tyrosine (MCT) adjuvant for treatment of allergic rhinitis will be used. The active ingredient is a standardized grass pollen allergen extract from 13 species of grass combined with the excipient MCT adjuvant as from Allergy Therapeutics Ltd (cf. Appendix 001 for further details). The allergen extract has been treated with glutaraldehyde as to reduce allergic side effects, i.e., to increase the safety. Bencard AG (Greifensee, Switzerland) is providing the product in Switzerland. Polvac™, is a marketed product that has been approved by the Swiss medical product agency *Swissmedic* (#46489 and #65479) and adheres to GMP. The product consists of needle-like crystalline structures of an average size of 20 micrometers (15). Polvac™ is approved for SCIT, which includes 6 pre-seasonal injections with 1-2 week intervals for 3-4 years, adding up to a total of 18 to 24 injections for the treatment to be completed (16-18). In the current study, for SCIT, Polvac™ will be used as described by the provider (Appendix 001). For ILIT, the samples will be processed as described by the provider, but administered at one fifth of the dose used for SCIT.

3.3 Preclinical Evidence

ILIT has been successfully performed in various mouse models using purified ovalbumin, bee venom allergen extract or purified phospholipase A1 (Api m 1), recombinant Fel d 1 cat allergen, and birch and grass pollen allergen extracts (19-25). One ILIT study in mice revealed that approximately 100-fold higher allergen doses were found in lymph nodes after ILIT as compared with a subcutaneous injection of the same allergen dose (20). Accordingly, similar therapeutic effects after ILIT with Api m 1 could be reached with only 1% of dose needed after SCIT with the same allergen (20). In another mouse ILIT study, only 0.1% of the SCIT dose was required to obtain comparable results (20, 26).

3.4 Clinical Evidence to Date

ILIT has been tested in clinical phase I and II studies in patients with sensitization to grass and tree allergens (7, 27-39). In addition, ILIT has been tested for treatment of allergic rhinitis in patients sensitised to house dust mite, cats and dogs (ref). Generally, three ILIT injections are performed with a time interval of 4-12 weeks between the injections (33, 40), as to allow for the development of antigen-specific immune responses, similar to the immunological mechanism of childhood vaccines. Shorter intervals showed no clinical improvement despite indications of immunological tolerance (31, 32, 40).

Three recent systematic reviews and meta-analysis of 10-17 clinical trials about ILIT for allergic rhinoconjunctivitis showed a benefit toward symptom alleviation and reduced medication use, as well as comparable safety and efficacy to both SCIT and SLIT (10-12). One clinical investigation demonstrated that ILIT with only 1/1000th of the SCIT dose produced comparable immunological or clinical effects (7), and the follow-up of the same study patients showed long-term allergen tolerance of ILIT in 29 patients that received grass pollen ILIT 19 years earlier (41). Most recently, a five year follow-up DBPC study in patients that received birch and grass pollen ILIT was published (42). The authors reported long-lasting effect of ILIT as measured by the registration of combined symptoms and medications score (cSMS), a nasal provocation test, and assessment of immunological parameters such as IgG4 and IgE. One study concluded that high-dose ILIT (up to 10'000 SQ-U, corresponding to the maintenance of SCIT) was safe in patients that recently underwent SCIT, suggesting high-dose ILIT in patients as add-on treatment for patients receiving SCIT could be an option to further improve symptom control (28).

To date, there has been one published clinical ILIT trial where Polvac™ ILIT was applied in 19 hay fever patients (43). One double-blind, placebo controlled clinical trial with 60 patients receiving Polvac™ Grass+Rye ILIT or placebo ILIT is ongoing in our clinic in Zurich (BASEC Nr. 2021-02301), with no severe adverse events occurring after a total of 180 injections, 90 thereof in the Polvac™ group. Local adverse events (AEs) were transient and included erythema and swelling at the site of injection (**Fig. 1**). Typically, more AEs were observed or perceived after second or third ILIT than after the first ILIT. , Since the study is still blinded, no segregation of AEs in placebo and active treatment is not yet possible. This trial, which goal it is to test the treatment efficacy of Polvac™ ILIT, is performed with the same PI (Pål Johansen) and with one of the study doctors (Lara Sosic) as the planned new project described in this study protocol and ethics application.

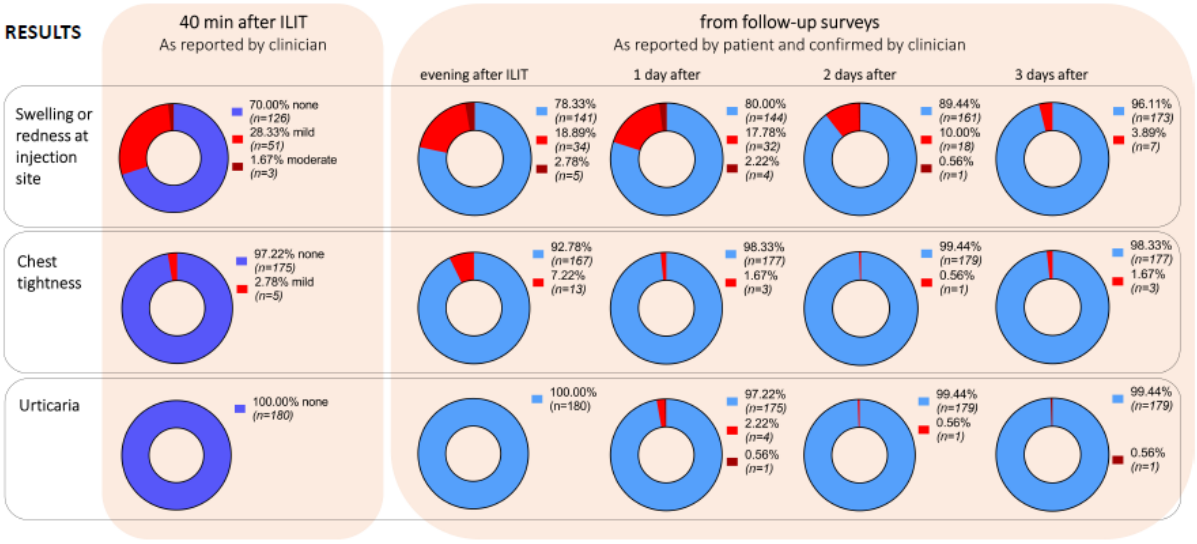

Figure 1. Registration of adverse events within an ongoing placebo-controlled study of Polvac ILIT in patients with allergic rhinitis due to sensitisation to grass pollen allergens (BASEC Nr. 2021-02301). Safety parameters were analysed by clinician 40 minutes post ILIT or by patients 1, 2 and 3 days post ILIT. The pie charts illustrate the percentage and numbers of events graded as none (blue), mild (red) and dark red (moderate). In the current project and application, a comparable injection will be performed in a comparable patient cohort. In the current study, however, only one injection will be made, while in the illustrated study, three injections were made (Lara Sosic, unpublished data).

3.5 Rationale for the dosage, route, regimen

We will include grass pollen allergy patients with pre-seasonal short-term and pre-seasonal injection scheme with Polvac™ SCIT at the USZ Allergy Unit (Head: Prof. Peter Schmid-Grendelmeier). After the patients have received the full cycle of SCIT (six injections) as a part of standard care, they will be randomized into one of the two study groups for one additional injection, which is to be given 4-6 weeks after the last SCIT cycle injection. Depending on the group allocation, patients will receive allergen by SCIT (or by ILIT. *Figure 2* illustrates schematically the study design and *Figure 3* shows the flow chart for the study participants.

Polvac™ Doses:

- SCIT (n=15): 0.5 ml Polvac™ (max maintenance dose, 2000 U).
- ILIT (n=15): 0.1 ml Polvac™ (400 U).

The SCIT dose is chosen as this is the recommended and standardized treatment dose for patients receiving SCIT with Polvac™ for treatment of grass pollen allergy. The ILIT dose is 1/5th of the maintenance SCIT dose. This has been safely administered in four patients treated off-label with Polvac™ at the USZ allergy in 2023 (Lara Sosic and Peter Schmid, personal communication). These four patients received three injections in a dose escalating manner and with four-week intervals. The doses were 1/15th, 1/10th and finally 1/5th of the maintenance SCIT dose. An ILIT dose of 1/15th of the maintenance SCIT dose was safely administered three times in 30 patients in the aforementioned ongoing study with Polvac™ ILIT (BASEC 2021-02301).

3.6 Explanation for choice of comparator (or placebo)

No comparator or placebo is used in the current project.

3.7 Risks / Benefits

Benefits

- The study should improve our understanding of the immune responses stimulation after SCIT and ILIT, and could lead to novel developments in AIT, and especially, ILIT.
- All study participants will get one additional AIT injection (SCIT or ILIT), which may contribute to and even further consolidate the success of their therapy.
- Results from previous studies suggest that an ILIT injection on top of a full SCIT cycle may further improve the therapeutic efficacy.

- The study participants receiving an ILIT injection would get to know a new route of AIT. In the future, they may use this as basis for discussion with their treating allergist regarding a possible change in the treatment regimen, as an alternative to SCIT. The benefits being reduced treatment times and lower allergen doses with potentially less AIT-associated side effects.

Risks

The SmPc for Polvac™ is the reference sheet for evaluation of AEs (see Appendix 002). The following known SCIT-associated are mentioned

- AE: itch or swelling and rubor at the injection site that resolves without treatment by next day (27%, 125 events in 462 injections of 154 patients)
- AE: allergic symptoms - urticaria, tightness of chest, drop in FEV1 >40% (2%, 11 of 462 injections) treated with an antihistamine or a short acting beta-2 agonist. Every clinic offering AIT is equipped to deal with this AE, and patients are required to stay in the clinic for 60 minutes for observation to deal with these incidents.
- Unexpected AE: some few patients reported fatigue within 6 hours
- Severe AE: systemic allergic symptoms that required hospitalization: drop in blood pressure, generalized urticaria or deterioration by 40% of peak flow or FEV1 (0.2%, 1 of 462 injections)
- Generation of autoimmune antibodies may be a potential long-term effect of AIT. This has not been reported in any of the AIT trials with Polvac™.
- One study reported one patient with hypothyroidism with auto-antibodies among 72 patients treated by ILIT (patients were not treated with Polvac™ but with a comparable alum-based product).
- In all AIT procedures (ILIT, SCIT or SLIT), there is a risk of anaphylactic reactions that typically happens within minutes of the injections. This is a SAE that will required hospitalization.
- The fine-needle aspiration (FNA) of the previously injected lymph node is a minimally invasive procedure and comparable to the ILIT procedure, hence, the potential AEs of ILIT injection and FNA are similar: infection and bleeding (hematoma). These problems are very rare, and antiseptic and ultrasound-guided procedures further minimizes the risk.

All SAE will be reported to the sponsor within 24 hours. SAEs will be followed up until one month after the end of the treatment period.

To minimize the risk of any allergic AEs, the patients will receive an emergency set (“Allergie Notfallset”) for self-medication in case of side effects after the patient has left the study site. The emergency set consists of two tablets of Xyzal® 5 mg and two tablets of Spiricort® 50 mg (Appendix 003). Patients will be advised on when and how to use the emergency set. Furthermore after ILIT treatment the patients have to stay one hour at the allergy unit for observation.

3.8 Justification of choice of study population

We plan to include 30 participants with allergic rhinitis due to sensitization to grass pollen allergens. We chose to include patients already undergoing AIT and not sensitized-only patients as to reduce risk of allergic side effects and to enable the injection of a higher allergen dose. As compared to a low dose, a high allergen dose is expected to trigger stronger immune responses thereby facilitating assessment of such responses, the primary objective of the study.

4. STUDY OBJECTIVES

4.1 Overall Objective

The overall purpose of this study is to widely characterize immunological changes induced by ILIT and to evaluate these changes in comparison to SCIT by descriptive statistics. In brief, the study will assess quantity and quality of ILIT- and SCIT-induced immunological changes in lymph nodes and in blood. The two following theories will be investigated addressing the question: Why does SCIT require higher doses and longer than ILIT?

- Quantitative theory: Immunity by SCIT and ILIT is generated by the same cell types and mechanisms, but only a fraction of the SCIT allergen arrives in the regional lymph nodes, hence

weaker effect of SCIT.

- Qualitative theory: ILIT results in a more efficient activation of specialized lymph node cells, different from the vaccination effect activated after SCIT.

The current study is a pilot study including small patient numbers as a basis for bigger follow up studies with a focus on the major ILIT induced immunological changes. The study seeks primarily to determine hallmarks in immunological response (analysis of cell populations and cell culture expression patterns) in lymph nodes and peripheral blood after ILIT versus SCIT.

4.2 Primary Objective

The primary objective is to compare inflammatory responses in blood and lymph nodes from patients receiving grass pollen allergen ILIT or SCIT. No single primary endpoint is defined, as numerous readouts will be performed as part of a wide screening of inflammatory responses.

4.3 Secondary Objectives

As a single secondary endpoint is not defined in this pilot study, primary and secondary endpoints melt together in the overall objective of analysing a wide panel of inflammatory responses to a single ILIT or SCIT.

4.4 Safety Objectives

Although the risk is low when AIT is performed appropriately, allergic reactions do occasionally occur. Part of the inflammatory characterization of ILIT and SCIT will therefore be to assess also local and systemic reaction associated with ILIT and SCIT treatment.

5. STUDY OUTCOMES

5.1 Primary Outcome

The FNA material of lymph node tissue at baseline and 2, 6 or 24 hours after allergen injection (via ILIT or SCIT) will be evaluated for changes in cellular composition and functions.

Immunological changes in peripheral blood at baseline, after 2, 6 or 24 hours, after 7 days and after 28 days of allergen ILIT or SCIT will be analysed by measuring cellular composition and functions in whole blood and in culture blood cells as well as inflammatory proteins and antibodies in serum.

5.2 Secondary Outcomes

To analyse the effect of dose on the measured immunological and inflammatory responses after a single ILIT injection (400 U).

To analyse differences between ILIT and SCIT-induced inflammatory and immunological responses.

5.3 Other Outcomes of Interest

None.

5.4 Safety Outcomes

To analyse local and systemic reactions after ILIT and SCIT (assessed by clinician during on site monitoring 60 min after the intervention and by study participant at home for the next two days).

6. STUDY DESIGN

6.1 General study design and justification of design

This is a prospective, comparative, clinical open label pilot study. From patients with grass pollen allergy who undergo a pre-seasonal SCIT scheme with Polvac™ Grass+RyeSCIT at the USZ Allergy Unit, 30 patients will be recruited for the study that is illustrated in Figure 2.

Four to six weeks after end of SCIT treatment (at maximal maintenance dose), the patients will be randomized to one out of two treatment groups and receive one additional allergen injection. Fifteen patients will be allocated to receive another subcutaneous injection with 0.5 ml (2000 U) Polvac™

Grass+Rye. The other 15 patients will be allocated to receive an intralymphatic injection of 0.1 ml Polvac™ Grass+Rye with 400 U allergen. Both SCIT and ILIT injections are performed by ultrasound-guidance as to assure injection into the lymph node (ILIT) or 1 cm next to the lymph node (SCIT).

The FNA of lymph node tissue will be conducted at 2, 6 or 24 hours after allergen injection. At the same time, FNA of a contralateral lymph nodes will be performed as baseline. Venous blood will be sampled at all visits: at baseline as well as after 2, 6 or 24 hours, after 7 days and after 28 days of the allergen ILIT or SCIT. The FNA is performed by ultrasound-guidance.

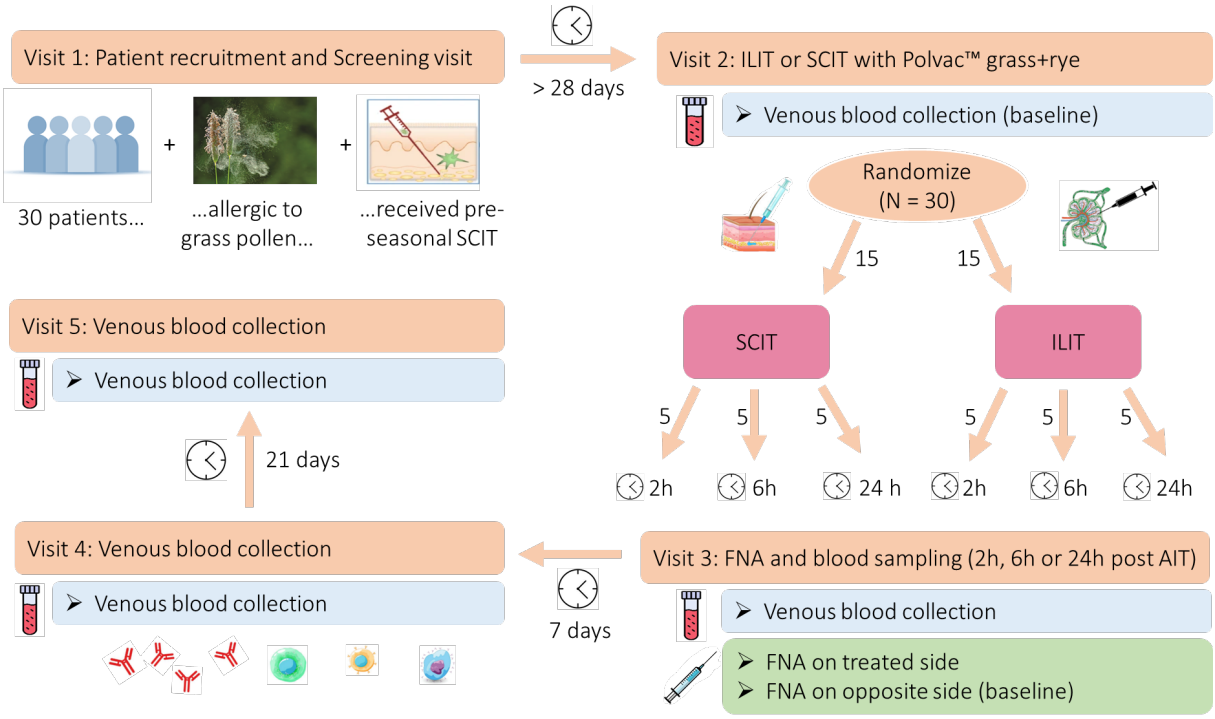

Figure 2. Study design illustrated schematically the open-label clinical pilot study for assessment of immunological and inflammatory responses to a single ILIT or SCIT in patients receiving grass pollen immunotherapy with Polvac™ Grass+Rye.

6.2 Methods of minimising bias

6.2.1 Randomisation

All patients who give consent for participation and who fulfil the inclusion criteria will be randomized to either SCIT or ILIT treatment group with a 1:1 allocation. The randomisation list will be computer-generated by a statistician using a permuted block design (groups of six). Before trial initiation, randomisation envelopes will be given to the study physician who will then be responsible of envelope storage and appropriate use. Allocation concealment will be ensured as the randomisation envelopes will not be opened before the patient has been recruited into the trial, which takes place after screening has been completed.

6.2.2 Blinding procedures

Not applicable. The study is not blinded.

6.2.3 Other methods of minimising bias

Patients will be randomized into two different groups. We will avoid age bias by choosing only patients from 18 to 55 years. Neutral recruiting will take place at USZ Allergy Unit.

6.3 Unblinding Procedures (Code break)

Not applicable. The study is not blinded.

7. STUDY POPULATION

7.1 Eligibility criteria

Participants fulfilling all of the following inclusion criteria are eligible for the study:

- Patients who have seasonal grass-pollen-induced rhinoconjunctivitis as confirmed by patient history and type-1-sensitization to grass-pollen in skin and/or serum
- Patients that undergo pre-seasonal short-term scheme with Polvac™ SCIT at the USZ Allergy Unit in autumn and winter 2023 for treatment of allergic rhinoconjunctivitis.
- Informed Consent as documented by signature.
- Patients are between 18 and 55 years of age when they sign the informed consent.

The presence of any one of the following exclusion criteria will disqualify the patient from participating the study:

Allergy related exclusion criteria:

- Known or suspected allergy to additives to the study product
- Known intolerance or allergy to phenol
- Planned depot steroid injection for treatment of allergic rhinoconjunctivitis
- Uncontrolled asthma or severe asthma with post bronchodilator FEV_1<70%, decided by the investigator
- Pulmonary disease with post bronchodilator FEV_1 < 70 % of predicted
- Pulmonary disease, perennial or seasonal, with daily use of more than 800 microgram inhaled budesonide/day (or equivalent)
- Treatment with omalizumab or other biologics for allergy, AD, urticaria or asthma.
- Allergic reaction within the last 4 days or anaphylaxis within last month before planned ILIT or SCIT injection.

Exclusion criteria based on serious comorbidities as judged by the recruiting physician:

- Autoimmune or collagen disease
- Disease or conditions rendering the treatment of anaphylactic reactions difficult (symptomatic coronary heart diseases, severe arterial hypertension, and treatment with beta-blockers)
- Concomitant infection with fever or other signs/symptoms of an acute or chronic infection at treatment visit
- Vaccination of any kind two weeks before or after the ILIT or SCIT injections.
- Known cardiovascular disease, i.e., not even NYHA class I.
- Use of ACE-blockers.
- Recent or on-going hepatic or renal disease
- Any malignant disease
- Conspicuous lymph node size or morphology
- Immuno- or chemotherapy during the last 15 years.
- Increased bleeding tendency
- Any other than study medication with an effect of interfering with the immune response
- Chronic obstructive or restrictive lung disease
- Skin diseases with barrier defect in the inguinal areas
- Alcohol or drug abuse
- Women who are pregnant and breast feeding

7.2 Recruitment and screening

We will recruit 30 patients directly at the USZ Allergy Unit. All 30 participants must have a type-1-sensitisation to grass pollen allergens and be undergoing pre-seasonal short-term SCIT scheme with Polvac™ at the USZ Allergy Unit in winter 2023/2024.

Patients will be invited to a screening visit 1 to 60 days before the intervention, where an extensive interview will be conducted and the informed consent will be signed by the patient and the medical investigator. The informed consent will be uploaded to the clinical information system (KISIM) and filed

into the trial master file (TMF). At the screening visit there will be a medical interview assessing all inclusion and exclusion criteria and a short clinical exam (auscultation of the lungs and the heart, blood pressure measurement).

7.3 Assignment to study groups

The 30 patients will be randomized to the two different equally-sized treatment groups (ILIT 400 U and SCIT 2000 U), with each 15 patients per group.

7.4 Criteria for withdrawal / discontinuation of participants

If a patient is seen to be ineligible for study intervention because of change in inclusion or exclusion criteria, the patient may be excluded from the trial at the discretion of the physician. The patient can at any time chose to withdraw the informed consent and thereby be released from the study. Replacement of a study participant is possible if the timeframe of 4-6 weeks between last dose of the short-term scheme and start of the additional allergen injection in the framework of the study can be complied. Replacement of a participant that has withdrawn consent is possible within the time frame of the study.

8. STUDY INTERVENTION

8.1 Identity of Investigational Products

Four weeks after patients have received the full cycle of SCIT with Polvac™ Grass+Rye as a part of standard care treatment, they will be randomized to receive one additional treatment.

All study participants will receive again the same allergen extract (Polvac™ Grass+Rye). Depending on the group allocation, they will receive the allergen by the subcutaneous (SCIT) or by intralymphatic (ILIT) injections.

The following Polvac™ doses are used:

- SCIT (n=15): 0.5 ml Polvac™ (2000 U).
- ILIT (n=15): 0.1 ml Polvac™ (400 U).

8.1.1 Experimental Intervention

The treatment medication (Polvac™ Grass+Rye) will be prepared aseptically as described (Appendix 001). The medication that is stored with the pharma team GCP lab in a temperature-logged fridge (2-8 °C) is placed at room temperature 2 hours prior to use. The medical investigator will order the drug from the pharma team to be provided on the day of treatment or on the day before treatment if treatment will take place early next morning (before 09:00 a.m.). **Table 2** summarizes the characteristics of the Polvac™ Grass+Rye preparation used in standard subcutaneous (SCIT) administration and the experimental intralymphatic (ILIT) administration.

Table 2. Characterisation of Polvac™ Grass+Rye preparation used in standard subcutaneous (SCIT) administration and the experimental intralymphatic (ILIT) administration.

	Polvac Grass+Rye SCIT	**Polvac Grass+Rye ILIT**
Route of injection	subcutaneous	intralymphatic
Initial U/ml	4000 U/ml	4000 U/ml
Dilution	none	none
Dose volume / injection	0.5 ml	0.1 ml
Phl p 5 / injection	900 ng	180 ng
U / injection	2000	400

To prepare Polvac™ for SCIT (2000 U), the 1 ml medication vial (4000 U/ml) is shaken well for 10 seconds. The head of the vial is disinfected and 0.5 ml of the medication is drawn into a 1 ml syringe with 23G hypodermic needle assuring no air in the syringe or needle. The medication is used no later than 4 hours after the preparation. Replica of the SCIT dose or of the ILIT high dose (see below) are made if more than one injection is to be performed. The remaining medication is discarded. The time

point for preparation is documented in the CRF.

To prepare Polvac™ for ILIT (400 U), the 1 ml medication vial (4000 U/ml) is shaken well for 10 seconds. The head of the vial is disinfected and 0.1 ml of the medication is drawn into a 1 ml syringe with 19G hypodermic needle. The medication is used no later than 4 hours after the preparation. Before injection, the needle is changed to a 23G hypodermic needle, assuring no air in the syringe or needle. Replica of the dose are made if more than one ILIT is to be performed, and the remaining medication is discarded. The time point for preparation is documented in the drug accountability log (Appendix 004).

Commercially available Polvac™ will be purchased from Bencard AG (Greifensee, Switzerland) and kept in a temperature-logged fridge (2-8 °C).

After preparation of the syringes, these will be labelled with the study name (ILIT-FNA-2023), patient number, and the expiry time point (e.g., “ILIT-FNA-2023, #21, Exp 21.12.23, 10:30h”).

8.1.2 Control Intervention (standard/routine/comparator treatment)

No control intervention is used in this study.

8.1.3 Packaging, Labelling and Supply (re-supply)

All medication is stored in a temperature-logged fridge with the pharma team GCP lab at the Department of Dermatology.

The medical investigator will request needed medication from the pharma team at the Department of Dermatology.

The treatment medication will be prepared aseptically under laminar flow between 30 minutes and 4 hours before use. After preparation, the medication is discarded or it is stored in a temperature-logged fridge with the pharma team at the Department of Dermatology.

The IMP Polvac™ has market authorization (MA) and has been released for the market. However, as the IMP Polvac™ will be administrated in different doses for the study, a simplified Investigator Medicinal Product Dossier (sIMPD) is provided (Appendix 003). The drug substance remains unchanged.

8.1.4 Storage Conditions

Polvac™ will be kept in a temperature-logged fridge (2-8 °C) in the Department of Dermatology. The medication is collected and brought to room temperature at least 30 minutes and not longer than 4 hours before administration.

The time points for removal of medication from fridge and the administration time are documented in a drug accountability log (one for all patients).

8.2 Administration of experimental and control interventions

8.2.1 Experimental Intervention

8.2.1.1 Allergen Injection

The immunotherapy is performed by an experienced physician at the USZ Circle Allergy Unit (Department of Dermatology). Both SCIT and ILIT injections are performed using 1 ml syringes with 23G needles and for both SCIT and ILIT, inguinal lymph nodes are identified using ultrasound guidance. For SCIT, a volume of 0.5 ml undiluted Polvac™ is injected subcutaneously ca. 1 cm from a lymph node (**Fig. 3**). For ILIT a volume of 0.1 ml undiluted Polvac™ is injected directly into a lymph node. The lymph nodes are tagged by marking the site of injection, ultrasound position and video recording of injection with the ultrasound machine.

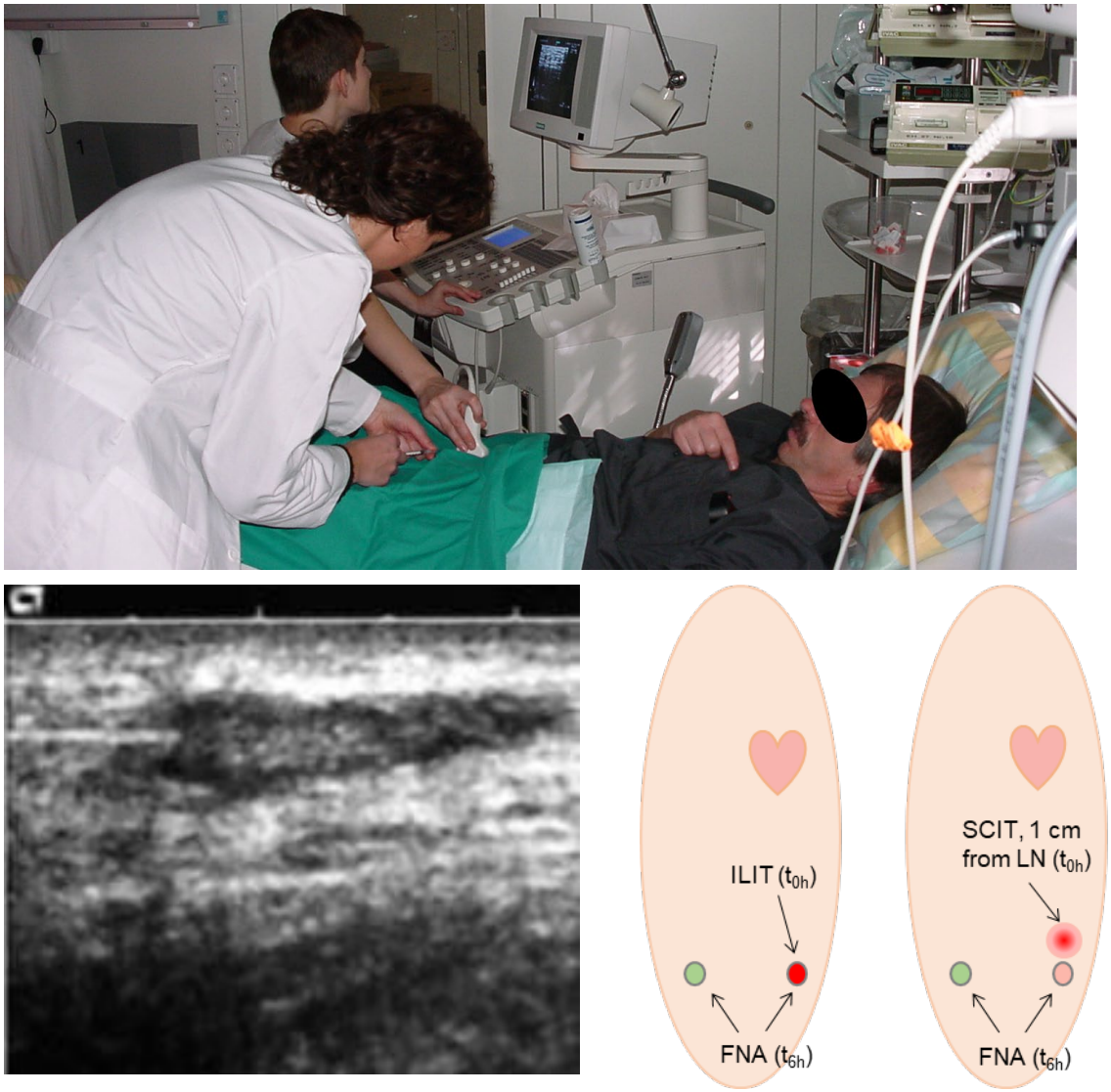

Figure 3: By ILIT administration, the study drug is injected into an inguinal lymph node under ultrasound guidance (top image), which allows targeting of the needle to the lymph node (lower left). In the current study, the study drug, Polvac™ Grass+Rye, is injected into an inguinal lymph node (ILIT) or it is injected subcutaneously ca. 1 cm from an inguinal lymph node (SCIT) is illustrated in the scheme (lower right). Two, six or 24 hours after injection, a fine-needle aspiration (FNA) of the treated (LIT) or involved (SCIT) LN is performed. As baseline control, the contralateral LN is also aspirated (green dot).

8.2.1.2 Fine needle aspiration (FNA)

The FNA is minimally invasive and an alternative to more invasive methods such as incisional or excisional biopsy for sampling of lymph node tissue (**Fig. 3**). Just recently, we used FNA in a similar way on sentinel lymph nodes in skin cancer patients (44).

An experienced investigator will perform the FNA. The skin above the lymph node, will be disinfected and a sterile needle (23-25g), attached to the 5 ml syringe, fixed in a FNA gun, will be inserted into the lymph node cortex under colour Doppler ultrasound guidance while avoiding important tissues and organs, such as large blood vessels and nerves. The syringe plunger is drawn to set the seal at a 2 ml scale in order to maintain negative pressure in the syringe. After rotating and aspirating the syringe 5 times, the needle is rotated to aspirate in a fan shape way at different trajectories in the lymph node. The needle is withdrawn when ca. 0.1 ml tissue is present in the syringe. The puncture site is covered with sterile gauze and pressure is applied with appropriate force for 10 min. Patients are observed for 30 min of observation and released when no adverse reaction.

The FNA sample will be rinsed with dimethyl sulfoxide (DMSO)-containing live cell freezing medium (10% DMSO and 90% fetal bovine serum (FBS)), put into a cryotube and frozen in -80°C.

8.2.1.3 Blood collection and preservation of cells and blood serum

First venous blood collection is performed at baseline before the immunotherapy. Second blood collection is performed 2, 6 or 24 hours post injection. Third and fourth blood collection are performed 7 and 28 days after immunotherapy. Each time, 25 ml venous blood is collected for preparation of PBMCs and 5 ml blood is collected for preparation of serum. Both samples are processed by routine in-house methods within 2 hours for preparation of serum and cells. The produced serum is aliquoted in 1 ml samples and frozen at – 20 °C for later analysis. Whole blood is centrifuged on Ficoll and the PBMCs isolated and washed in PBS before re-suspended in DMSO-containing live cell freezing medium. 1 ml aliquots of the cells are slow-frozen in cryotubes and finally stored at -80 °C before further analysis.

8.2.2 Control Intervention

No control intervention is used in this pilot study.

8.3 Dose modifications

We already have experiences with dosing Polvac™ from an ongoing study using Polvac™ at our department (BASEC Number: 2021-02301) and with off-label use of up-dosing Polvac™. The ILIT doses used in the trial described in the current protocol is based on the experience from the past trial (133 U) and from off-label treatment of four patients in February to April 2023 (133 U, 200 U and 400 U).

We inject the study dose after patients finished a full cycle of pre-seasonal short-term SCIT scheme with Polvac™ (the patients have therefore reached and tolerated the maximal maintenance dose of 2000 U). The maximal dose in the current study will not exceed the maintenance dose for SCIT (2000 U) and will be much lower for ILIT (400 U) and should therefore be well tolerated by the study participants.

8.4 Compliance with study intervention

Monitoring of subject compliance will not be done. We only include patients who have finished pre-seasonal SCIT with six injections at the USZ Allergy Unit. Patients who opt for SCIT are usually highly motivated because their burden of allergy-suffering is high. The effort for the patient associated with our study is relatively modest. The five personal visits include 1) screening visit (which can be combined with a SCIT injection already planned), 2) allergen injection, 3) FNA and blood samplings 2-24 hours post injection, 4) follow-up after 7 days, and 5) follow-up after 28 days. We expect that the patients will be motivated for the study, because they potentially will benefit from this one additional injection and might also benefit from future ILIT applications (such as off-label interventions).

8.5 Data Collection and Follow-up for withdrawn participants

In accordance with the Declaration of Helsinki, the investigator must explain to the patient that they have the right to withdraw from the study at any time, and that this will in no way prejudice their future treatment. We are required by law to report on adverse events and other side effects; this law takes precedence over the GDPR as it concerns public safety. Even if a patient withdraws from the project, we will have to keep safety data after treatment. However, all other clinical data that do not relate to safety of the applied treatment, will be deleted at a written request of a patient. A subject that discontinues will always be asked about the reason(s) for discontinuation and the presence of any adverse events. The timing and reason for any kind of withdrawal will be recorded in the patient medical file (KISIM) and deposited in the TMF. The sponsor should inform Swissmedic and IEC within 24 hours after decision of a treated patient to discontinue the study and also the consequences for the study persons as well as for the continued investigations will be declared.

Intervention (SCIT or ILIT) within the study is not necessary for the routine therapy for the patients, therefore, the study intervention don’t need to be repeated in case of a withdrawal.

8.6 Trial specific preventive measures

All the preventive measures described below will be recorded in the CRF.

Prior to the allergen injection, a pregnancy testing (if applicable) will be performed.

The patients will receive an emergency set (“Allergie Notfallset”) to take home in case of systemic side effects (Appendix 003). The emergency set consists of two tablets of Xyzal® 5 mg (Levocetirizine dihydrochloride) and two tablets of Spiricort® 50 mg (Prednisolone). Patients will be given an instruction about when and how to use the emergency set. Fenistil gel 1% will be given for local allergic reactions restricted to the site of injection.

After ILIT treatment the patients have to stay one hour at the allergy unit for observation.

During the study (from allergen injection to blood samplings after 28 d) patients cannot receive another allergen injection. To avoid allergen exposure from the environment, the study allergen is seasonal, and therefore not in the environment during the whole study period.

8.7 Concomitant Interventions (treatments)

Besides the allergen administration (via ILIT or SCIT), the FNA (2x) and blood collection (4x) a pregnancy test, 2x peak flow measurement and a physical examination will be performed.

8.8 Study Drug Accountability

Commercially available Polvac™ will be purchased from Bencard AG (Switzerland). The study drug will be kept in a temperature-logged fridge (2-8 °C) with the pharma team at the Department of Dermatology. The pharma team will keep a log for received and used study medication.

The medical investigator will order drug from the pharma team, which will log study drug provision with date and time point (DD.MM.YYYY, HH:MM h). The time point for preparation of study drug is documented in the log with the pharma team and in the CRF (ILIT 400U and for SCIT 2000 U; directly syringed by the medical investigator).

8.9 Return or Destruction of Study Drug

At the end of the study, rest doses of Polvac™ are kept in the research lab in Wagistrasse 18 for use in non-clinical research.

9. STUDY ASSESSMENTS

9.1 Study flow chart

The study flow chart is illustrated in **Figure 4**.

9.2 Assessments of outcomes

9.2.1 Assessment of primary outcome

FNA material of lymph node tissue at baseline and 6 hours after allergen injection (via ILIT or SCIT) will be evaluated for changes in lymph node cell populations and cell culture expression patterns.

Immunological changes (analysis of cell populations and cell cultures) in peripheral blood at baseline, after 2, 6 and 24 hours and at 7 and 28 days after allergen injection (via ILIT or SCIT).

9.2.1.1 Analysis of allergen-specific antibodies in serum by ImmunoCAP

The concentrations of IgE, IgG and IgG4 against grass-pollen allergens as well as tryptase are determined in serum using the ImmunoCAP system (ThermoFisher Scientific, Phadia, Waltham, US-MA) according to the manufacturer's instructions. This is a routine diagnostic method in our department.

9.2.1.2 Analysis of innate cell reactions in serum by antibody arrays

PBMCs cryotubes are thawed and immediately washed in pre-warmed culture medium supplemented with calf serum, glutamate and antimicrobial agents before restimulated with allergen in vitro. Firstly, the cells are cultured for up to 5 days in the presence of grass pollen allergens and the supernatants are analyzed for cytokines, e.g. IL-2, IL-4, IL-5, IFN-gamma, IL-10, and TGF-beta. The cytokines are measured by ELISA using a TECAN™ plate reader. Secondly, and based on the ELISA results, the cells are cultured with allergens and transferred to multiscreen HTS membrane plates for detection of cytokine-secreting cells by ELISPOT using a AID EliSpot/FluoroSpot Reader.

9.2.1.3 Analysis of T-cell activation by flow cytometry

PBMCs are also analyzed by flow cytometry after allergen-restimulation in vitro. Briefly, the cells are typically cultured 18-20h in the presence of grass pollen allergens, the last 4-6 hours of the incubation, the compound brefeldin A is added to prevent the golgii apparatus to release newly produced proteins. Then, the cell as stained for with fluorescent antibodies to detect lymphocytes, lymphocyte activation surface proteins. The cell are then fixed, permeabilised and stained to detect intracellular transcription factors such as Foxp3, STAT-1 and STAT-3 and STAT-6 as well as intracellular cytokines such as IL-4, IL-10 and IFN-gamma. The exact cytokines may depend on the result from the above-mentioned ELISA

studies. The cells are acquired on a BD LSR-Fortessa Cell analyzer and the data analyzed using FlowJo LCC software.

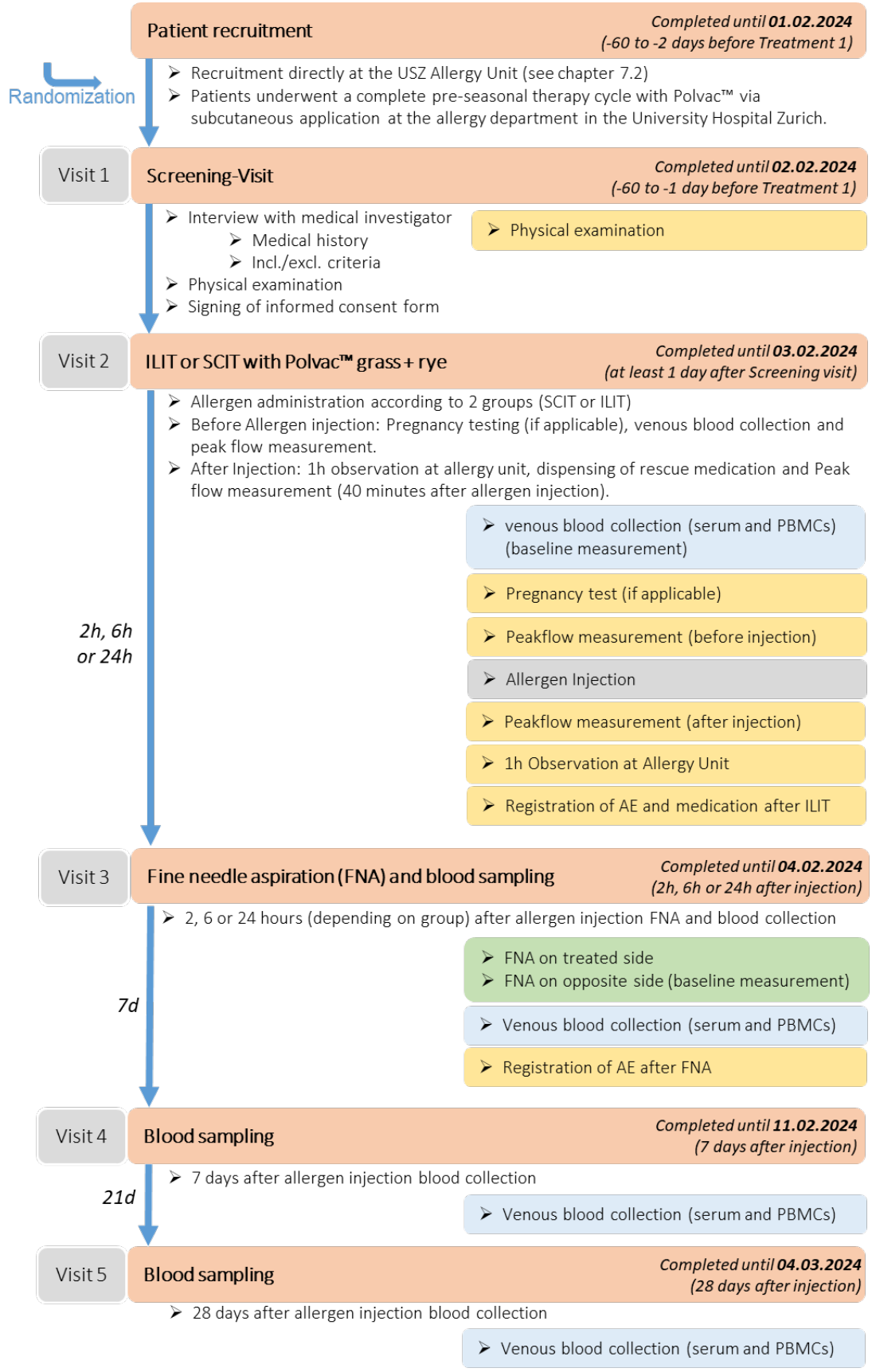

Figure 4. Patient flow chart illustrating study visits with timing, interventions, and assessments for patients treated by ILIT or SCIT for grass pollen-mediated allergic rhinoconjunctivitis.

9.2.1.4 Multiparameter cellular analysis by mass cytometry (CyTOF)

PBMCs as well as lymph node cells are also analyzed by mass spectrum cytometry (45, 46) Again, cryotubes are thawed and cell immediately washed with complete medium. The cells are incubated with a viability dye washed extensively, and stained for a wide panel of extracellular antigens that will enable us to detect the phenotype of all possible leucocytes. After fixation and permeabilisation, the cells are patient-wise barcoded using the commercial Barcoding Kit (Fluidigm) and pooled before staining for intracellular antigens. Cell DNA is then stained using Cell-ID™ Intercalator-Ir (Fluidigm) overnight at 4°C, frozen at -80°C using a cryopreservation box, thawed, supplemented with Four Element Calibration Beads (Fluidigm) and acquired on a Helios™ mass cytometer. Files are concatenated, normalized, and debarcoded using the CyTOF software v6.7.1014. The data are analyzed by descriptive statistics as well as multivariant test methods with dimension reduction, e.g. PCA, t-SNA and UMAP (47).

9.2.2 Assessment of secondary outcomes

Differences in immunological response after injection of ILIT 400 U and SCIT 2000 U via two routes to demonstrate a possible route dependency.

9.2.3 Assessment of other outcomes of interest

None.

9.2.4 Assessment of safety outcomes

9.2.4.1 Adverse events

For reporting of adverse event information see 10.1.2.

To minimise the risk for AE the following precautions are carried out:

- The patients will receive an emergency set (“Allergie Notfallset”) to take home in case of allergic side effects. The emergency set consists of two tablets of Xyzal® 5 mg and two tablets of Spiricort® 50 mg. Patients will be advised on when and how to use the emergency set.
- The patients will receive Fenistil gel 1% to use in case of local allergic side effects
- After the allergen injection the patients have to stay one hour at the allergy unit for observation of systemic allergic reactions as well as allergic skin reaction oat the site of injection.
- The patients will be advised to call the medical investigators upon any delayed AEs or otherwise medical emergency if systemic allergic reactions (e.g. asthma).

9.2.4.2 Laboratory parameters

Before the allergen injection a pregnancy testing (if applicable) is performed. Pregnant participants are excluded from the clinical study.

9.2.4.3 Vital signs

A physical examination is done in a semi-structured way; several items are checked on a list in the CRF, and details can be entered if required.

The following vital signs will be assessed (e.g. heartbeat, lung auscultation and blood pressure).

9.2.5 Assessments in participants who prematurely stop the study

Most allergic events are resolved within a week, and we do not expect to have to follow patients for longer. If an AE is registered in a patients CRF this will be addressed by attending staff at the next visit. Patients will be able to contact staff by e-mail during the trial. If a patient should experience allergic symptoms severe enough to warrant hospitalization this will be recorded in the CRF.

Our injection in the framework of the study is not necessary for the routine therapy for the patients, therefore they don’t need to be repeated in case of a withdrawal.

9.3 Procedures at each visit

9.3.1 Visit 1: Screening visit (20 minutes)

Completed until 02.02.2024 (-29 to 1 days before Visit 2)

- Interview with medical investigator
    - Medical history
    - Check of Inclusion/Exclusion criteria

    - Physical examination
- Reading and signing of informed consent by participant and medical investigator

9.3.2 Visit 2 (120 minutes)

Completed until 03.02.2024 (at least 1 day after screening visit)

- Pregnancy testing (if applicable)
- Peak Flow before and 40 min after injection
- Venous blood collection before injection (5 ml serum and 25 ml EDTA)
- Injection of Polvac™ Grass+Rye (SCIT 2000U or ILIT 400 U)
- Immediately after injection
    - 1 h observation at the allergy unit of the University Hospital Zurich
    - Dispense of rescue medications
- 2, 6 or 24 hours after allergen injection the following parameters are obtained:
    - FNA of ILIT-treated inguinal lymph node or lymph node adjacent to SCIT injection
    - FNA of non-involved inguinal lymph node on the opposite side, which had not been treated (baseline measurement)
    - Venous blood collection (5 ml serum and 25 ml EDTA)

9.3.3 Visit 3 (30 minutes)

Completed until 04.02.2024 (2, 6 or 24 hours after SCIT/ILIT))

- Venous blood collection before injection (5 ml serum and 25 ml EDTA)
- FNA of ILIT-treated inguinal lymph node or lymph node adjacent to SCIT injection
- FNA of non-involved inguinal lymph node on the opposite side, which had not been treated (baseline measurement)

9.3.4 Visit 4 (10 minutes)

Completed until 24.03.2024 (7 days after injection)

- Venous blood collection (5 ml serum and 25 ml EDTA).

9.3.5 Visit 5 (20 minutes)

Completed until 31.03.2024 (28 days after injection)

- Venous blood collection (5 ml serum and 25 ml EDTA).
- Study termination

10. SAFETY

10.1 Drug studies

During the entire duration of the study, all adverse events (AE) and all serious adverse events (SAEs) are collected, fully investigated, and documented in source documents and case report forms (CRF).

10.1.1 Definition and assessment of (serious) AEs and other safety related events

An **AE** is any untoward medical occurrence in a patient, or a clinical investigation participant administered a pharmaceutical product and which does not necessarily have a causal relationship with the study procedure. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. [ICH E6 1.2]

A **SAE** is classified as any untoward medical occurrence that:

- results in death,
- is life-threatening,
- requires in-patient hospitalization or prolongation of existing hospitalisation,
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect.

In addition, important medical events that may not be immediately life-threatening or result in death, or require hospitalisation, but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed above should also usually be considered serious. [ICH E2A]

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation, or development of drug dependency or drug abuse.

SAEs should be followed until resolution or stabilisation. Participants with ongoing SAEs at study termination (including safety visit) will be further followed up until recovery or until stabilisation of the disease after termination.

10.1.1.1 Assessment of Causality

Investigators and Sponsor make a causality assessment of the event to the study drug, based on the criteria listed in the ICH E2A guidelines:

Relationship	Description
Unrelated	This category is applicable to those AEs which, after careful medical consideration at the time of evaluation, are judged to be clearly and incontrovertibly due to extraneous causes (disease, environment, etc) and do not meet the criteria for study medication relationship listed under remote, possible, or probable.
Unlikely	In general, this category is applicable to those AEs which, after careful medical consideration at the time they are evaluated, are judged to be remotely related to the test study medication. An AE may be considered remote if, or when, for example: (i) it does not follow a reasonable temporal sequence from administration; (ii) it could readily have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient; (iii) it does not follow a known response pattern to the suspected study medication.
Plausible	(Must have the first 2 criteria): This category is applicable to those AEs which, after careful medical consideration at the time they are evaluated, the connection with the test study medication administration appears unlikely but cannot be ruled out with certainty. An AE may be considered plausible if, or when: (i) it follows a reasonable temporal sequence from administration; (ii) it could readily have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient; (iii) it follows a known response pattern to the suspected study medication.
Probable	(Must have the first 3 criteria): This category is applicable to those AEs, which, after careful medical consideration at the time they are evaluated, the connection with the test study medication administration appears to, with a high degree of certainty, be related to the test study medication. An AE may be considered probable if: (i) it follows a reasonable temporal sequence from administration; (ii) it could not be reasonably explained by the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient; (iii) it disappears or decreases on cessation or reduction in dose. There are important exceptions when an AE does not disappear upon discontinuation of the study medication, yet study medication-relatedness exists, e.g. bone marrow depression, fixed study medication eruptions, and tardive dyskinesias; (iv) it follows a known response pattern to the suspected study medication; (v) it reappears upon rechallenge.
Not assessable	When not possible to assign the event to any of the criteria mentioned above.

10.1.1.2 Unexpected Adverse Drug Reaction

An "unexpected" adverse drug reaction is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for drugs that are not yet approved and Product Information for approved drugs, respectively). [ICH E2A]

10.1.1.3 Suspected Unexpected Serious Adverse Reactions (SUSARs)

The Principle-Investigator evaluates any SAE that has been reported regarding seriousness, causality, and expectedness. If the event is related to the investigational product and is both serious and unexpected, it is classified as a SUSAR.

10.1.1.4 Assessment of Severity

For all AEs, serious as well as non-serious, the investigator must make an assessment of severity:

- Mild: awareness of sign or symptom, but easily tolerated and cause no interference with daily activities.
- Moderate: discomfort enough to cause interference with daily activities.
- Severe: inability to perform normal daily activities.

10.1.2 Reporting of SAE and other safety related events

10.1.2.1 Reporting of SAEs

The study doctor or the principal investigator will report all SAEs immediately and within a maximum of 24 hours to the Sponsor of the study (via notification by phone/email) The Sponsor will re-evaluate the SAE and return the form to the site.
The study doctor or the PI will report SAEs resulting in death to the Ethics Committee via BASEC within 7 days. The sponsor-representative will inform all SAEs to *Swissmedic* within 7 days of the event.

10.1.2.2 Reporting of SUSARs

A SUSAR will be reported by the principal investigator to the Ethics Committee via BASEC and to Swissmedic by the Sponsor within 7 days, if the event is fatal, or within 15 days (all other events).

10.1.2.3 Reporting of immediate safety and protective measures

All suspected new risks and relevant new aspects of known adverse reactions that require immediate safety-related measures, are reported by the study doctor to the Sponsor within 24 hours.

The principal investigator will notify the Ethics Committee and the sponsor will notify the *Swissmedic* within 7 days if immediate safety and protective measures had to be taken during the trial conduct. The circumstances necessitating the safety-protective will also be reported.

10.1.2.4 Reporting and handling of pregnancies

Before the allergen injection a pregnancy testing (if applicable) is performed. Pregnant participants are excluded from the clinical study. All interventions except one blood sampling (28 days) are done on the day of pregnancy testing.

Any pregnancy within 28 days after allergen injection will be reported to the Sponsor within 24 hours. The course and outcome of the pregnancy should be followed up carefully, and any abnormal outcome regarding the mother or the child should be documented and reported.

10.1.2.5 Periodic reporting of safety

The ASR / Development safety update report (DSUR) is written in compliance to ICH Harmonised Guideline E2F for commercial and non-commercial sponsor.

An ASR / DSUR is submitted once a year to the IEC via Investigator and to Swissmedic via Sponsor.

The start date for the ASR/DSUR is the date the sponsor's first authorisation to conduct a clinical trial.

The ASR / DSUR is submitted to the IEC and to Swissmedic throughout the duration of the clinical trial, and the last ASR / DSUR submission will cover the Last Patient Last Visit.

The sponsor will submit an annual report of SUSAR and SAE to *Swissmedic* as well as to the Pharmacovigilance department of the medicine supplier Allergy Therapeutics.

10.1.3 Follow up of AEs and SAEs

Most allergic events are resolved within a week, and we do not expect to have to follow patients for longer. If an adverse event is registered in a patients CRF this will be addressed by attending staff at the next visit. Patients will be able to contact staff by e-mail during the trial. If a patient should experience allergic symptoms severe enough to warrant hospitalization this will be recorded in the CRF.

10.2 Assessment, notification and reporting on the use of radiation sources

Not applicable.

11. STATISTICAL METHODS

11.1 Hypothesis

This study is a pilot study which goal is to describe the effect of ILIT on the immune cell composition and immune cell function in lymph nodes and in blood. We will compare this with the effect SCIT on the same, but not to confirm a hypothesis of a certain difference.

We will use a wide screening with several methods to describe the immune responses.

11.2 Determination of Sample Size

Not applicable for a pilot study.

11.3 Statistical criteria of termination of trial

There is no statistical criteria for stopping the study.

11.4 Planned Analyses

The investigators and the appointed statistician will be responsible for the statistical analysis of the collected data. The statistical software used will be Graph Pad Prism®, SPSS®, or open-source software “R”.

We will apply descriptive statistics to describe frequencies, proportions and numbers for the various analysis to be performed. The data will be described with an average (mean or median) and with variances (confidential intervals or standard deviations or standard errors) depending on the type of data.

Data will typically be illustrated with scatter plots or with box plots with 90 and 10% whiskers. For multiparametric analysis, the results from different tests will transformed/normalized (e.g. z-score normalization) to generate heat maps, and for the hierarchical clustering of treatments and phenotypes, we will apply The R Project with Euclidean metrics.

For comparative testing of the different treatment groups (SCIT versus ILIT), ANOVA is applied for continuous parametric data that fulfil the criteria for ANOVA. Kruskal-Wallis analysis are applied for non-parametric ranked data.

For changes over time within patients, dependent (paired) t-test may be used. No sex/gender difference analysis is planned.

11.5 Handling of missing data and drop-outs

There will be no imputation of missing data. We will not use false data. There will not be unused data. We will strive to publish all data.

12. QUALITY ASSURANCE AND CONTROL

The Sponsor is responsible for implementing and maintaining quality assurance and quality control systems with written SOPs and Working Instructions. The Sponsor is responsible for proper training of all involved study personnel.

12.1 Data handling and record keeping / archiving

12.1.1 Case Report Forms

Study data is recorded in Case Report Forms on paper (pCRF). Data entry will be done by the investigators who are responsible for the correctness of the data. If a person is enrolled to participate in the study, the personal record will be encoded, and the person given a randomization number. For each enrolled study participant a pCRF is maintained. All patient information and test results are recorded in the CRF. Only the participant number and the year of birth will be entered in the pCRF. The encryption process used for the pCRF are in accordance with Swiss guidelines for encryption. Hence, only the investigators and authorized persons have access to data.

The pCRFs are kept current to reflect subject status at each phase during the course of study. The investigators at the site are responsible for pCRF entries and have to sign their entries with initials and date of the entry according the specifications of the pCRF.

The informed consent will be obtained in wet ink during the screening visit and then uploaded to the clinical information system (KISIM) and filed into the TMF for recordkeeping.

Data transfer in excel tables for statistical evaluation is checked by double data entry. A printout of the excel sheet is added to the study documents and every data change in the electronic data should be assigned a new printout.

The randomization list will be stored electronically as a series of files labelled with the participant number that contains information on the randomization, with password-protected, logged access and an audit trail that will be stored electronically for 15 years. As this is an open-label trial, any physician can access the randomization list, and request details of the allocation of an individual patient.

12.1.2 Specification of source documents

All data will be registered in a series of pCRFs including patient questionnaires and laboratory analysis that will be considered as source data. The source documents include the electronic medical records on site, as well as the study-specific documents and data collected during the visits, which are recorded directly via questionnaires or by the study-specific tests. The relevant source documents from the electronic medical records are printed out, signed by the investigator and collected in a separate folder. This folder is stored in a lockable cabinet to which only the study team has access.

Data from the pCRF are filed in to the TMF for recordkeeping and additionally in the clinical information system (KISIM).

12.1.3 Record keeping / archiving

All study documents (essential documents and site documents) are archived in the Department of Dermatology of the University Hospital Zurich for a minimum of 10 years after study termination or premature termination of the clinical trial.

12.2 Data management

The project site will retain all essential documents according to national legal and regulatory requirements, including source data and CRF. Source data are all information in original records and certified copies of original records of clinical findings, observations, or other activities necessary for the reconstruction and evaluation of this project. All essential documents are stored until 10 years after the termination of the project, during which time the project leader guarantees access and availability of the data at any time.

12.3 Monitoring

The trial monitoring in Zurich is done by the Clinical Trials Center, USZ.

12.4 Audits and Inspections

The investigators and sponsor allow direct access to source documents for monitoring, audit and inspection by GCP units, health authorities, ethics committees, data authorities and other medical autothrities. All involved parties must keep the participant data strictly confidential.

12.5 Confidentiality, Data Protection

Health-related personal data captured during this project from participants are strictly confidential and disclosure to third parties is prohibited. Coding will safeguard participants' confidentiality.

The electronic case report forms (CRFs) will be entered into an excel-based database. Confidentiality will be ensured by entering only coded data into the analysis set. The coded data will be password-protected and accessible to the sponsor, the medical investigators and the project researchers. The statistician will obtain copies of the Excel database after the data entry has been completed. Coded research data are not considered part of the essential documents and will be stored on a central data server at the USZ with restricted access: \\fs-group\der_forschungen\DER_FL_Immunotherapie

The code for data generated at USZ is kept with the sponsor.

12.6 Storage of biological material and related health data

Data from this trial will be stored for 10 years in electronic form after completion of the trial. The collected blood and FNA samples are to be used and analysed exclusively in the current study. No material or data transfer to other or future projects are foreseen. The collected blood samples and FNA in this study

is not identified by participant name but by a unique participant number and is appropriately stored in a restricted area only accessible to the authorized personnel.

13. PUBLICATION AND DISSEMINATION POLICY

A clinical study report will be prepared covering clinical aspects and summarizing all findings of the clinical study. The content has to be treated as strictly confidential. The information gathered in this study is considered confidential and is the property of the sponsor. Within 12 months of completion of the trial, trial results will be entered into the international clinical trials registry (SNCTP and clinicaltrials.gov).

We aim to publish the results of this study in a peer reviewed journal, irrespective of the outcome.

There is a publication delay of up to 3 months while partners or interested parties may evaluate the commercial potential of results from this clinical trial. Authorship will follow Vancouver rules. Partners in the project will be authors on the publication; contributors will be mentioned under acknowledgements (there is a group of researchers that is credited without being authors. that is where they may be).

We confirm if gender effects are observed, they will be published in the final study report. If none are observed, this will be published as well.

14. FUNDING AND SUPPORT

14.1 Funding

The Department of Dermatology of the University Hospital of Zurich (Professor Thomas Kündig) will support the study with CHF ca. CHF 30’000 if required through University funds. The sponsor will guarantee for ca. 50’000 through overhead from various project-unrelated contracted research with biotech.

The Truus und Gerrit van Riemsdijk Stifung, Vaduz will support the study with 20’000 CHF.

We submitted a project-grant application to EMDO Stiftung (CHF 50’000), and we are planning an application to Theodor und Ida Herzog Egli Stiftung for the same amount (submission deadline 30.4.2023).

14.2 Other Support

14.2.1 Cooperation with other institutes

All clinical and experimental infrastructures required to conduct the study are available in Department of Dermatology, USZ/UZH and at the associated lab at SIAF, Davos.

We will collaborate with Prof. Dr. med. Peter Schmid-Grendelmeier and his team the Allergy Unit USZ CIRCLE for screening, recruitment, treatment and monitoring of patients included in the study. Such a collaboration is already existing as part of already running randomized double-blind placebo controlled trial on the efficacy of grass pollen ILIT. Hence, all personnel and infrastructure required in the current project, is already established and available.

We will also collaborate closely with the UZH-Institute SIAF, Davos, for the analysis of blood and lymph node cells by mass cytometry (CyTOF). Since four years, SIAF as being building of expertise on CyTOF through Dr. Reihane Ziadlou and Prof Dr. med. Charlotte Brüggen.

Finally, we are and will consult Prof. Hans Jürgen Hoffmann from the Department of Respiratory Diseases and Allergy at the University of Aarhus, Denmark. Prof. Hoffmann is worldwide perhaps recognized as the most experienced researcher on ILIT. We have discussed the trial design for the current study with Prof. Hoffmann.

15. INSURANCE

Insurance will be provided by the Sponsor /

Patients will be covered by an insurance (*Pauschalvertrag*) for clinical trials, issued by Zurich *Versicherungs-Gesellschaft* AG. The policy number is 14.970.888.

16. REFERENCES

1. Varney VA, Gaga M, Frew AJ, Aber VR, Kay AB, Durham SR. Usefulness of immunotherapy in patients with severe summer hay fever uncontrolled by antiallergic drugs. BMJ. 1991;302(6771):265-9.

2. Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. J Allergy Clin Immunol. 1998;102(4 Pt 1):558-62.

3. Lockey RF. "ARIA": global guidelines and new forms of allergen immunotherapy. J Allergy Clin Immunol. 2001;108(4):497-9.

4. Lockey RF, Benedict LM, Turkeltaub PC, Bukantz SC. Fatalities from immunotherapy (IT) and skin testing (ST). J Allergy Clin Immunol. 1987;79(4):660-77.

5. Lockey RF, Turkeltaub PC, Olive ES, Hubbard JM, Baird-Warren IA, Bukantz SC. The Hymenoptera venom study. III: Safety of venom immunotherapy. J Allergy Clin Immunol. 1990;86(5):775-80.

6. Stewart GE, Lockey RF. Systemic reactions from allergen immunotherapy. J Allergy Clin Immunol. 1992;90(4 Pt 1):567-78.

7. Senti G, Prinz Vavricka BM, Erdmann I, Diaz MI, Markus R, McCormack SJ, et al. Intralymphatic allergen administration renders specific immunotherapy faster and safer: a randomized controlled trial. Proc Natl Acad Sci U S A. 2008;105(46):17908-12.

8. Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. Eur Respir J. 2004;24(5):758-64.

9. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). Allergy. 2008;63 Suppl 86:8-160.

10. Aini NR, Mohd Noor N, Md Daud MK, Wise SK, Abdullah B. Efficacy and safety of intralymphatic immunotherapy in allergic rhinitis: A systematic review and meta-analysis. Clin Transl Allergy. 2021;11(6):e12055.

11. Werner MT, Bosso JV. Intralymphatic immunotherapy for allergic rhinitis: A systematic review and meta-analysis. Allergy Asthma Proc. 2021;42(4):283-92.

12. Hoang MP, Seresirikachorn K, Chitsuthipakorn W, Snidvongs K. Intralymphatic immunotherapy for allergic rhinoconjunctivitis: a systematic review and meta-analysis. Rhinology. 2021;59(3):236-44.

13. Senti G, Johansen P, Kündig TM. Intralymphatic immunotherapy. Curr Opin Allergy Clin Immunol. 2009;9(6):537-43.

14. Senti G, Johansen P, Kündig TM. Intralymphatic immunotherapy: from the rationale to human applications. Curr Top Microbiol Immunol. 2011;352:71-84.

15. Baldrick P, Richardson D, Wheeler AW. Review of L-tyrosine confirming its safe human use as an adjuvant. J Appl Toxicol. 2002;22(5):333-44.

16. Heath MD, Mohsen MO, de Kam PJ, Carreno Velazquez TL, Hewings SJ, Kramer MF, et al. Shaping Modern Vaccines: Adjuvant Systems Using MicroCrystalline Tyrosine (MCT. Front Immunol. 2020;11:594911.

17. Padró C, Gutiérrez D, Moreno F, Parra A, Rial MJ, Lleonart R, et al. Effectiveness and safety of a microcrystalline tyrosine-adjuvanted Dermatophagoides pteronyssinus allergoid immunotherapy in adult patients with allergic asthma and rhinitis: A real-life prospective observational study. Immun Inflamm Dis. 2022;10(5):e585.

18. Roger A, Malet A, Moreno V, Parra A, Gutiérrez D, Lleonart R, et al. Real-life effect of a microcrystalline tyrosine adjuvanted mite immunotherapy in patients with allergic rhinitis. Immunotherapy. 2020;12(1):53-62.

19. Johansen P, Kündig TM. Intralymphatic immunotherapy and vaccination in mice. J Vis Exp. 2014(84):e51031.

20. Martínez-Gómez JM, Johansen P, Erdmann I, Senti G, Crameri R, Kündig TM. Intralymphatic injections as a new administration route for allergen-specific immunotherapy. Int Arch Allergy Immunol. 2009;150(1):59-65.

21. Johansen P, Senti G, Martinez Gomez JM, Storni T, von Beust BR, Wüthrich B, et al. Toll-like receptor ligands as adjuvants in allergen-specific immunotherapy. Clin Exp Allergy. 2005;35(12):1591-8.

22. Johansen P, von Moos S, Mohanan D, Kündig TM, Senti G. New routes for allergen immunotherapy. Hum Vaccin Immunother. 2012;8(10):1525-33.

23. Johansen P, Senti G, Martínez Gómez JM, Wüthrich B, Bot A, Kündig TM. Heat denaturation, a simple method to improve the immunotherapeutic potential of allergens. Eur J Immunol. 2005;35(12):3591-8.

24. Maloy KJ, Erdmann I, Basch V, Sierro S, Kramps TA, Zinkernagel RM, et al. Intralymphatic immunization enhances DNA vaccination. Proc Natl Acad Sci U S A. 2001;98(6):3299-303.

25. Martínez-Gómez JM, Johansen P, Rose H, Steiner M, Senti G, Rhyner C, et al. Targeting the MHC class II pathway of antigen presentation enhances immunogenicity and safety of allergen immunotherapy. Allergy. 2009;64(1):172-8.

26. Smith KA, Tam VL, Wong RM, Pagarigan RR, Meisenburg BL, Joea DK, et al. Enhancing DNA vaccination by sequential injection of lymph nodes with plasmid vectors and peptides. Vaccine. 2009;27(19):2603-15.

27. Konradsen JR, Grundström J, Hellkvist L, Tran TAT, Andersson N, Gafvelin G, et al. Intralymphatic immunotherapy in pollen-allergic young adults with rhinoconjunctivitis and mild asthma: A randomized trial. J Allergy Clin Immunol. 2020;145(3):1005-7.e7.

28. Hellkvist L, Hjalmarsson E, Weinfeld D, Dahl Å, Karlsson A, Westman M, et al. High-dose pollen intralymphatic immunotherapy: Two RDBPC trials question the benefit of dose increase. Allergy. 2022;77(3):883-96.

29. Hellkvist L, Hjalmarsson E, Kumlien Georén S, Karlsson A, Lundkvist K, Winqvist O, et al. Intralymphatic immunotherapy with 2 concomitant allergens, birch and grass: A randomized, double-blind, placebo-controlled trial. J Allergy Clin Immunol. 2018;142(4):1338-41.e9.

30. Hylander T, Latif L, Petersson-Westin U, Cardell LO. Intralymphatic allergen-specific immunotherapy: an effective and safe alternative treatment route for pollen-induced allergic rhinitis. J Allergy Clin Immunol. 2013;131(2):412-20.

31. Hylander T, Larsson O, Petersson-Westin U, Eriksson M, Kumlien Georén S, Winqvist O, et al. Intralymphatic immunotherapy of pollen-induced rhinoconjunctivitis: a double-blind placebo-controlled trial. Respir Res. 2016;17:10.

32. Witten M, Malling HJ, Blom L, Poulsen BC, Poulsen LK. Is intralymphatic immunotherapy ready for clinical use in patients with grass pollen allergy? J Allergy Clin Immunol. 2013;132(5):1248-52.e5.

33. Weinfeld D, Westin U, Hellkvist L, Mellqvist UH, Jacobsson I, Cardell LO. A preseason booster prolongs the increase of allergen specific IgG4 levels, after basic allergen intralymphatic immunotherapy, against grass pollen seasonal allergy. Allergy Asthma Clin Immunol. 2020;16:31.

34. Thompson CP, Silvers S, Shapiro MA. Intralymphatic immunotherapy for mountain cedar pollinosis: A randomized, double-blind, placebo-controlled trial. Ann Allergy Asthma Immunol. 2020;125(3):311-8.e2.

35. Skaarup SH, Schmid JM, Skjold T, Graumann O, Hoffmann HJ. Intralymphatic immunotherapy improves grass pollen allergic rhinoconjunctivitis: A 3-year randomized placebo-controlled trial. J Allergy Clin Immunol. 2021;147(3):1011-9.

36. Skaarup SH, Graumann O, Schmid J, Bjerrum AS, Skjold T, Hoffmann HJ. The number of successful injections associates with improved clinical effect in intralymphatic immunotherapy. Allergy. 2021;76(6):1859-61.

37. Ahlbeck L, Ahlberg E, Nyström U, Björkander J, Jenmalm MC. Intralymphatic allergen immunotherapy against pollen allergy: A 3-year open follow-up study of 10 patients. Ann Allergy Asthma Immunol. 2018;121(5):626-7.

38. Ahlbeck L, Ahlberg E, Stuivers L, Björkander J, Nyström U, Retsas P, et al. Intralymphatic immunotherapy with birch and grass pollen extracts. A randomized double-blind placebo-controlled clinical trial. Clin Exp Allergy. 2023.

39. Ahlbeck L, Ahlberg E, Björkander J, Aldén C, Papapavlou G, Palmberg L, et al. Intralymphatic immunotherapy with one or two allergens renders similar clinical response in patients with allergic rhinitis due to birch and grass pollen. Clin Exp Allergy. 2022;52(6):747-59.

40. Kündig TM, Johansen P, Bachmann MF, Cardell LO, Senti G. Intralymphatic immunotherapy: time interval between injections is essential. J Allergy Clin Immunol. 2014;133(3):930-1.

41. Adlany YK, Šošić L, Senti G, Lang CCV, Wüthrich B, Kündig TM, Johansen P. Quality of life in allergic rhinitis patients treated with intralymphatic immunotherapy (ILIT): A 19-year follow-up. Journal

of Allergy and Clinical Immunology: Global. 2023;2(1):43-50.

42. Hjalmarsson E, Hellkvist L, Karlsson A, Winquist O, Kumlien Georén S, Westin U, et al. A five-year open follow up of a randomized, double-blind placebo-controlled trial of intralymphatic immunotherapy for birch and grass reveals remaining beneficial effects. J Investig Allergol Clin Immunol. 2022:0.

43. Park HJ, Kim SH, Shin YS, Park CH, Cho ES, Choi SJ, et al. Intralymphatic immunotherapy with tyrosine-adsorbed allergens: a double-blind, placebo-controlled trial. Respir Res. 2021;22(1):170.

44. Tastanova A, Ramelyte E, Balázs Z, Menzel U, Beisel C, Krauthammer M, et al. Collection and preprocessing of fine needle aspirate patient samples for single cell profiling and data analysis. STAR Protoc. 2021;2(2):100581.

45. Verdier J, Fayet OM, Hemery E, Truffault F, Pinzón N, Demeret S, et al. Single-cell mass cytometry on peripheral cells in Myasthenia Gravis identifies dysregulation of innate immune cells. Front Immunol. 2023;14:1083218.

46. Leonard C, Montamat G, Davril C, Domingues O, Hunewald O, Revets D, et al. Comprehensive mapping of immune tolerance yields a regulatory TNF receptor 2 signature in a murine model of successful Fel d 1-specific immunotherapy using high-dose CpG adjuvant. Allergy. 2021;76(7):2153-65.

47. Rønn Olsen L, Bligaard Pedersen C, Leipold M, Maecker H. Cytometrist's primer to mass cytometry data analysis. 2021; verfügbar über: https://biosurf.org/cytof_cytometrist.html.

17. APPENDICES

001. Product description Polvac™

002. SmPC Polvac™

003. Product description of rescue medication

1. Product description “Allergie-Notfallset”
2. Product description “Fenistil Gel 1%”

004. Drug Accountability Log

005. CRF and questionnaire for participants

006. Patient questionnaires in German (days 0-3 post ILIT)