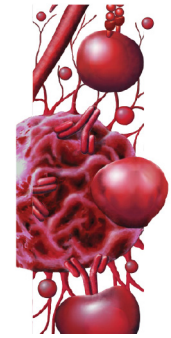
Perspective

Immunotherapy



From genomes to diaries: a 3-year prospective, real-life study of ragweed-specific sublingual immunotherapy

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During the last decades, the prevalence of allergy has dramatically increased. Allergen-specific immunotherapy is the only currently available medical intervention that has the potential to affect the natural course of the disease, but there are still many questions and unmet needs hindering its widespread use to fulfill its treatment potential and maximize its benefits for the society. To provide a comprehensive phenome-wide overview in sublingual immunotherapy, using ragweed allergy as a target, we planned and carried out a longitudinal, prospective, observational, open-label study (DesensIT). In this paper we present challenges of using deep and comprehensive phenotypes embracing biological, clinical and patient-reported outcomes in allergen-specific immunotherapy and show how we designed the DesensIT project to optimize data collection, processing and evaluation.

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During the last decades the prevalence of allergy has dramatically increased. While at the beginning of the 20th century allergy was considered as a rare disease, today the most common form of allergic disease, allergic rhinoconjunctivitis (ARC), has a prevalence of about 25% in Europe and according to a prediction of the European Academy of Allergy and Clinical Immunology, within the next few decades more than half of the European population will have some type of allergy [1–3]. Patients with ARC can have a profound impairment in their quality of life and work or school performance. The impact of allergic disease, however, is detrimental not only for individual sufferers, but also for the whole society. ARC results several million lost workdays and missed school days

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each year in Europe and also significantly reduced performance of people at work with untreated allergy resulting in a significant burden on health economics and macroeconomics. In this way, society now confronts increasing burden on a scale that will soon become unaffordable requiring an immediate action [1,4,5].

Allergen-specific immunotherapy (AIT) is the only currently available medical intervention that has the potential to affect the natural course of the disease [1,3,6,7]. Symptomatic treatment is available for ARC, but a lot of patients report insufficient symptom control [8]. In addition, pharmacotherapy has no effect on the progression of the disease and even after years of a continuous, effective treatment, symptoms relapse very shortly after ceasing daily use of medication [9]. The benefits of AIT, however, continue several years after discontinuation of the treatment and it has been shown that AIT is able to prevent the progression of allergic diseases to more serious forms and reduce the risk of new sensitizations [10–13].

The original administration form of AIT was by subcutaneous injection. Later, sublingual immunotherapy (SLIT) was introduced which represents an alternative mode of treatment that may afford a safe, convenient and effective treatment modality for the management of allergic respiratory disease. Due to its safety, SLIT enables patients to self-administer their daily dose at home, become more or less out from the supervision of the doctor, highlighting the importance of build-up a continuous collaborative nature of doctor–patient relationship [1,3].

Several double-blind, placebo-controlled studies justified the effectiveness of SLIT in different types of allergy [3,14,15]. Although during the last two decades, hundreds of additional studies have been carried out about different aspects of AIT, it is clear that there are still many questions and unmet needs hindering its widespread use to fulfill its treatment potential and to maximize its benefits for the society.

In order to provide comprehensive, cross-domain knowledge about SLIT in respiratory allergy with special emphasis on genetics and self-reported patient diaries, we planned and carried out a longitudinal, prospective, observational, open-label study, which we named DesensIT. DesensIT aimed to depict real-world patient experiences and to reveal the possible relationships between variables which were individually detected at multiple levels from inherited factors, immunological parameters up to subjective patient reported outcomes and global evaluation of efficacy attributed to AIT. Indeed, incorporating the patient perspective is even more important for better assessment of a specific treatment and healthcare quality. Monitoring patient reported outcomes is also expected to be presented for regulatory bodies as it can raise evidence to a higher level. Especially, in similar diseases as ARC it is essential to capture well-structured data, due to the lack of standardized objective end points and the challenging interpretation of the clinical improvement mainly relying on subjective narrative of the patients.

In the followings we present some of the problems about AIT today and show how we designed the DesensIT project to optimize the data collection, processing and evaluation.

Problems & unmet needs in allergen specific SLIT

Lack of objective diagnostic/predictive biomarkers for SLIT

In the clinical context of AIT the first question accumulates around the eligibility of the patients, namely the accurate selection of candidates who more likely expect benefit from the therapy. Presently there are no objective biomarkers which can predict whether a patient will respond beneficially to the therapy, will develop unpleasant adverse reactions or after several years of therapy his/her symptoms will not improve [3,16,17]. Regarding the cost of the therapy and the possible side effects during the administrations this can have a serious harmful effect to the patients and also to the physician–patient relationship. An additional difficulty is that due to the preventive nature of immunotherapy in seasonal allergies, the accurate assessment of the possible effects should be evaluated prior the season when the natural exposition does not occur and there are no symptoms.

Lack of reliable end point biomarkers

The evaluation of clinical improvement based traditionally on changes in subjective clinical and immunological parameters during SLIT. The clinical data (i.e., data collected by the health experts) are generated at different levels and on different scale. For evaluation of allergy-related health condition as well as of AIT effectiveness several scoring systems have been developed, although they are built up quite heterogeneous and make any direct comparisons of observations hard. Each score is intended to give a depiction of actual condition in a defined exposed period taking into consideration of symptom severity, concomitant medication and their impact on everyday life [18].

Immunological readouts include circulating allergen-specific immunoglobulin or intensity of allergen-evoked reactions during skin test, or in more sophisticated *in vitro* test systems like basophil activation test or ELISPOT assay for monitoring cytokine producing specific immune cells [11,19–25]. Presently, however, there are not any

documented data available from comprehensive studies about correlation of those changes at an individual patient level.

According to the recommendation of the World Allergy Organization, to assess the primary outcome combined symptom and concomitant medication score should be considered. Data should be collected during natural exposition (e.g., during pollen season), and scores are calculated with appropriate method for balanced combination of symptoms and medication [3]. Different algorithms have been developed for calculating adjusted symptom and medication scores, but none is universally accepted and to date only one has been standardized and formally validated [26,27].

A fundamental barrier of individual evaluation of patients is that the currently used scores have been developed for trials and it does not necessarily applicable for practicing clinicians who might follow only a few dozen patients during a treatment cycle. The therapeutic response of AIT in seasonal allergies is usually calculated at the end of treatment against a reference point, representing the indicators averaged over the same periods of several years. Evaluation period and data collection time is also critical, as a comprehensive meta-analysis showed that pollen exposure substantially influences the magnitude of treatment effect [28]. Indeed, the variable exposure strongly influences the assessment of subjective response to different treatments and it cannot be controlled adequately by starting with baseline observations of untreated condition.

High dropout rate

One of the main reasons why SLIT cannot achieve its maximal beneficial potential in the society is the high rate of dropout. In different studies the dropout rates vary widely and can be as high as 93% over a 3-year period as was found in a large Dutch study and 87% in two other studies [29–31]. The major issues influencing patient adherence to SLIT are thought to be the feeling of inefficacy, cost and side effects. The personality of the doctor and the psychological attitude of the patient can also affect the dropout. The high dropout rate is detrimental for AIT because an insufficient duration prevents the occurrence of the immunological changes that underlie the clinical efficacy since in most cases the persistence of the clinical effects requires at least a 3-year long treatment [32,33]. Therefore, improved adherence to SLIT is an unmet need that should be targeted.

Insufficient knowledge about the mechanism

Although characterizing the mechanism of action of immunotherapy has been the focus of intense investigation during the past several decades, and our knowledge about it increased significantly, there are still unknown processes and unanswered questions [1,3,21,34]. One important sign of it is the lack of biomarkers which would be capable to indicate the response of the patients to the therapy. Better understanding of the immunologic mechanisms underlying AIT would help the development of not only biomarkers but also novel targeted vaccines with improved efficacy and safety.

Several studies have indicated that AIT is more cost effective than pharmacotherapy, however, the experts agree that the data are still insufficient and additional pharmacoeconomic studies are needed [1,3,35,36].

Need for sequential, supportive IT solutions

Typically the first SLIT drops are administered in the office of doctors, but afterward they are applied by the patients at home for a couple of years. The visits are typically irregular. For controlling side effects, maintaining the motivation of the patients and the possible application of short-term predictive immunological biomarkers, regular visits would be necessary. In some cases it would be even crucial that the patients remain in close, nearly daily contact with the doctor. The continuous contact between patients and physicians can be achieved by an interactive, web-based patient diary connected to the physician, which can support real-time tracking the treatment-related events and allows communication at different levels.

Insufficient public awareness

Although AIT is the only available method that offers the possibility of reducing long-term allergic disease burden and even can cure the disease or at least stop in its progression, it is still underused. For example, there are more than 100 million people who suffer from respiratory allergy in Europe and between 30 and 60 million in the USA, only a small fraction of them initiate AIT annually [1,3]. The main reasons include the above mentioned problems but also the lack of awareness of AIT in the general population and the insufficient experience of physicians taking care of allergic subjects [37].

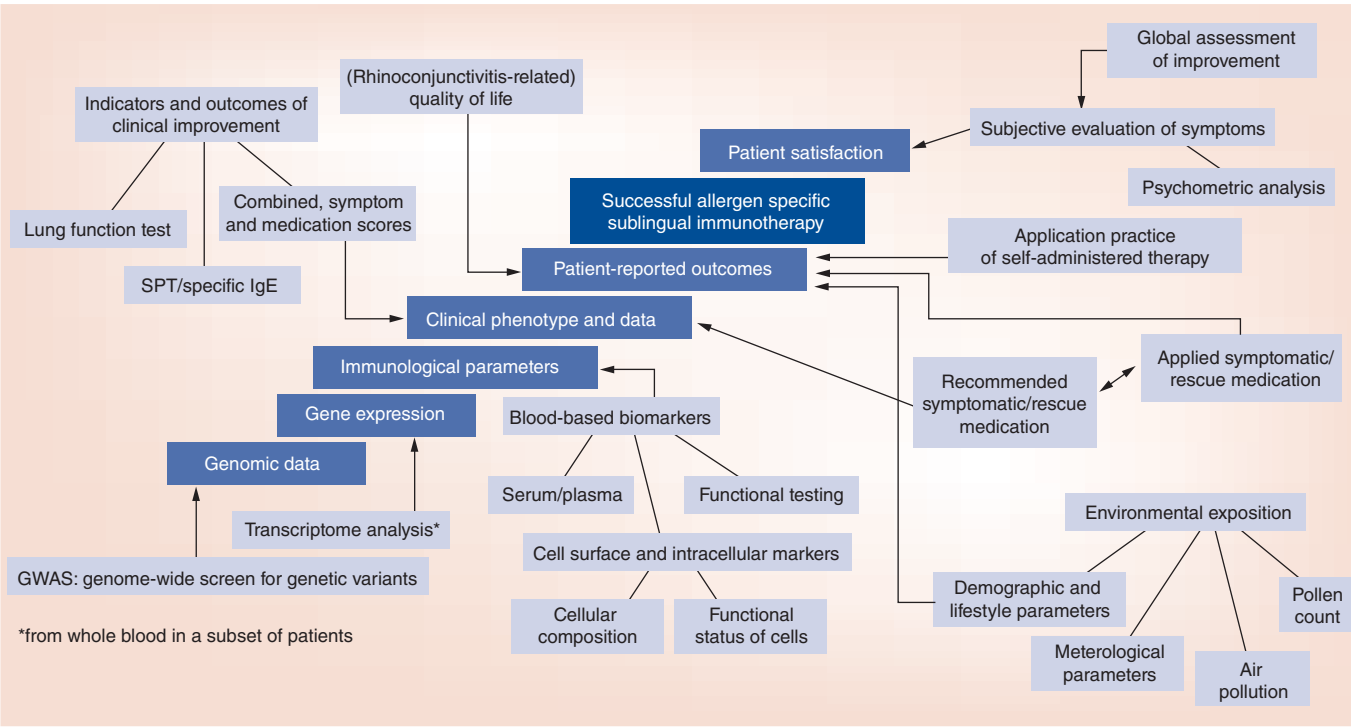

Figure 1. Concept map of data sources and data types in the DesensIT project.

Considerations about the study design in the DesensIT project

The main aim of the DesensIT project was to collect and evaluate data in SLIT to ARC to provide additional information, to address the above mentioned questions and to develop multivariate, system-based predictive models for multiple treatment responses with focus on personalized medicine ('personalized SLIT'), particularly on the use of genetic data and self-reported patient data. Specifically, the set of predictor variables includes novel factors, such as genome-wide variation data, gene expression data and psychological factors. The response set contains widely used outcome scores, immunological data, comprehensive clinical information, medication data and also novel patient-reported end points, including full repertoire of adverse effects and detailed information about attrition. Figure 1 shows a concept map of sources and data types of relevant information in the DesensIT project.

As a model we selected ragweed which is the dominant cause of seasonal allergy in South and Central Europe with an estimated prevalence of more than 20% in some areas. The ragweed allergy causes significant morbidity, associates with significant disease progression and adversely affects the economy [38,39].

As several earlier double bind placebo-controlled clinical trials provided definitive proofs about the effectiveness of SLIT [1,3,10–13], thus, in this study our aim was not to present additional proofs for the effectiveness of the therapy, but clarify factors influencing effectiveness.

Our study design was also influenced by ethical aspects. According to the general consensus, to achieve maximal and long-term effects SLIT must be carried out at least for 3 years. As SLIT is typically recommended for patients whose allergic symptoms cannot be adequately controlled by pharmacotherapy and environmental controls, or have a risk of developing more serious disease, like asthma, the employment of placebo would raise some ethical issues due to the availability of a disease modifying therapy with a proven effectiveness that should be administered for a relative long time. Considering this, we thought it ethically questionable to give patients placebo for 3 years. In addition, envisaging a placebo arm may be discouraging for the volunteers, and it can cause participation bias losing patients with the more serious disease. Additionally, the size of placebo-effect related to SLIT is reported to be very low (1.3% or less), especially if compared with other administration routes, namely in case of subcutaneous injection where the placebo effect can be as high as 25% in pollen allergic patients [40].

Based on these considerations we designed a longitudinal, prospective, observational open-labeled study without using placebo-treated or untreated controls. As a therapeutic agent we selected Staloral (ragweed 300 IR/ml),

which has been on the market for more than two decades and its efficiency has been strongly underscored [41]. Moreover, the dosing regimen and the practice of application have also been well established. Therefore in that case we could focus on revealing as much subjective clinical variables as possible until the completion of the full course of immunotherapy. Based on these repeatedly recorded data, rank-lists can be obtained from multidimensional comparisons enabling variables from different component (in time and level) of therapy response, like quality of life, global assessment, combined scores encompassing symptoms and use of medication, visual analog scale (VAS) measurements, clinical data and immunological measurements.

For the better adaptation to the real-life practice, DesensIT was planned to play a role as an external observer. Instead of designation of documentation to a limited, very specific content, including data related solely to clinical symptoms and medication, DesensIT was planned to perform a comprehensive data collection, preferring patient-reported outcomes, recording variables, those not only far beyond the usual practice, but also the majority of information should come from the patient, without involvement or interpretation by healthcare professionals. In addition to the clinical data, several immunological and genomic measurements were planned in different time points of the study. These inputs would be ready to be used for reconstitution of clinical scores, which are usually recommended for clinical evaluation and make it possible to generate new ones.

Particularly remarkable is the effect of varying exposition respective to the timing of visits.

To compensate the apparent lack of placebo group, multiple reference points were planned, and the participants described not only their actual impressions about the last couple of days, but also those related to the last season.

In order to better approximate the real-life situation, the open-label DesensIT study broke with the strict restrictions approach of clinical studies, and although it was implemented according to the evidence-based practice, it focused rather on the individual dynamics of allergic condition along the whole course of treatment in a personalized way.

Aims & work packages in the DesensIT project

The main aims of the DesensIT project were the followings:

- Monitor wide range of biomarkers to track how patients respond to the therapy, including gene expression data, immunological markers, clinical information and daily, self-reported patient data;
- Comprehensive collection of adverse effects of the therapy;
- Exploration of multivariate biomarkers which can help physicians to identify patients who more likely can expect benefit from the SLIT;
- Collection of information about the mechanism of SLIT;
- Evaluation of the effect of the psychological characteristics of the patients on therapy adherence and compliance, response to the therapy, symptoms and quality of life;
- Exploration of factors influencing dropout rate except for cost of the therapy;
- Develop tools which can help physician in interactively following the patient and making decisions on initiation of the therapy, abrogation of the therapy or suggesting steps for controlling adverse effects;
- Increasing public awareness of SLIT.

The strategy & workflow of the DesensIT project

The activities of the DesensIT project cover the complete lifecycle of a multicentric study: protocols for logistics of biological samples, recruitment of patients, maintenance of a biobank, clinical and omic measurements, clinical informatics for routine medical data, statistical data analysis and medical decision support. These activities were supported by the work packages (WP) presented in Table 1 and detailed below.

Work packages in details

WP1. Selection of patients for the study

Participants were selected from patients of five Hungarian allergic outpatient centers with documented ragweed allergy and/or with clinical history for at least 2 years with peak symptoms in August-September and agreed in participating in the DesensIT study. Those patients were preferred who had moderate-severe seasonal ARC according to Allergic Rhinitis and its Impact on Asthma criteria [27,42] and whose respiratory symptoms remain troublesome despite avoidance or adequate pharmacologic therapy and interfering with usual daily activities or with sleep during the ragweed pollen season. Only adult and adolescent patients with age 14–65 years were included due

Table 1. Work packages in the DesensIT project.

Work package numbers	Activities
WP1	Selection of patients
WP2	Collection of immunological and clinical data
WP3	Collection of genetic and genomic data
WP4	Application of ragweed specific sublingual immunotherapy
WP5	Development of a psychological questionnaire for study participants
WP6	Creating logistics for collection, processing and storage of biological samples; development of a biobank
WP7	Collection of environmental data that can influence the therapy response
WP8	Development of an interactive web-based, electronic patient SLIT-diary
WP9	Development of a comprehensive web-based interface for the collection of data for physicians
WP10	Data quality control and data preprocessing
WP11	Statistical data analysis
WP12	Development of decision support systems for personalized SLIT
WP13	Project management, dissemination, training and ethical issues

SLIT: Sublingual immunotherapy.

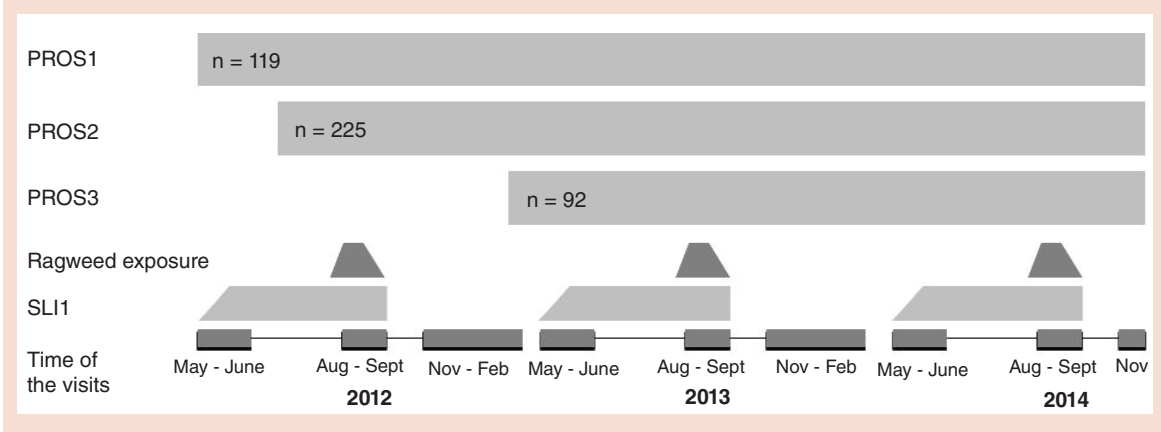

Figure 2. Schematic representation of experimental group design in DesensIT. PROS1, 2 and 3 represent the three groups of patients who entered the project in a time-shifted manner. PROS1 patients entered the project and started the therapy in May–June, 2012. PROS2 patients entered the project in the late summer to early fall of 2012 were examined during the ragweed season and started the therapy in 2013. PROS3 group entered the project and also started the therapy in May–June 2013.
N: Number of patients involved in the project in each patient group.

to the their ability to fill the questionnaires and psychology test and give informed consent and comply with study procedures unattended. To avoid dropout due to therapy cost, the therapeutic agent was offered to the participants for free of charge.

Mainly from logistic reasons, three groups of subjects were involved in a time-shifted manner (see Figure 2): PROS1 group was launched in the recommended therapy initiation period, 3 months before the season started; PROS2 participants recruited in symptomatic time period in the season; and PROS3 group follows PROS1 after 1 year. The demographic and clinical characteristics of the study groups at the treatment initiation are presented in Table 2.

Exclusion criteria were applied considering the follow-up nature of the study and general recommendation of the manufacturer (see in the Supplementary Information).

The study protocol was approved by institutional and national ethics committees (10130/2012/EKU), and written consent was obtained from the patients included.

Table 2. Demographic and clinical characteristics of the study groups at the treatment initiation.

Demographic and clinical characteristics	PROS1	PROS2	PROS3	All
Total (n)	119	225	92	436
Male (n, %)	52 (43.7)	100 (44.4)	42 (45.7)	194 (44.5)
Female (n, %)	67 (56.3)	125 (55.6)	50 (54.3)	242 (55.5)
Age (mean [SD] year)	40.1 (12.3)	39.4 (12.4)	37.7 (11.9)	39.2 (12.3)
Time since diagnosis				
Missing (n)	7	11	11	29
Time since diagnosis (mean [SD] year)	14.6 (9.0)	12.2 (8.3)	14.9 (8.8)	13.4 (8.6)
Sensibilization				
Total (n)	114	222	90	426
Ambrosia (n, %)	114 (100)	222 (100)	90 (100)	426 (100)
Artemisia (n, %)	60 (52.6)	116 (52.3)	46 (51.1)	222 (52.1)
Grass (n, %)	54 (47.4)	68 (30.6)	33 (36.7)	155 (36.4)
Betula (n, %)	25 (21.9)	43 (19.4)	31 (34.4)	99 (23.2)
Ambrosia + Artemisia (n, %)	60 (52.6)	115 (51.8)	46 (51.1)	221 (51.9)
Total (n)	112	214	62	388
RTSS last year (mean, SD)	2.0 (0.5)	1.9 (0.7)	1.9 (0.8)	1.9 (0.6)
Comorbidities				
Total (n)	114	222	90	426
Asthma (n, %)	25 (21.9)	51 (23.0)	18 (20.0)	94 (22.1)
Sinusitis (n, %)	6 (5.3)	29 (13.1)	22 (24.4)	57 (13.4)
Atopic dermatitis (n, %)	2 (1.8)	23 (10.4)	7 (7.8)	32 (7.5)
Food allergy (n, %)	6 (5.3)	20 (9.0)	8 (8.9)	34 8.0)

Rhinitis total symptom score (RTSS) ranges between 0 and 3: the total of the individual symptom scores graded by the participants on the scale from no symptoms: 0 to severe symptoms: 3, and it were divided by the number of 7 symptoms.

WP2. Collection of clinical & immunological data

Collection of clinical data

The following data were recorded at SLIT initiation: demographic data, mainly allergy-centric clinical history and characteristics, any previous data on sensitization profile (see below), symptoms and regularly or casually used symptomatic medications. Both patients and involved clinicians received instructions about uniformed SLIT protocol, education materials and procedures to be followed to manage any unusual or unexpected events. Attention was drawn toward importance of regularly administration of drops, commonly occurring local and gastrointestinal side-effects and on the preventive nature of preseasonal application of SLIT. The study design and schedule of visits is summarized in Figure 2.

We attempted to standardize the scheme of the meetings between doctors and patients and perform the visits according to a scheme. Regular visits occurred three-times a year: preseasonal (May–June), seasonal (August–October) and postseasonal (November–February) visits. During the visits the patients first completed questionnaires alone, then lung function tests, physical examination and discussion of the allergy related status were carried out in order to enrich the data with contextual information and to minimize the invalid and incomplete patient-reported data by targeted questions of the doctors. The practically focused schema-based interviews applying prefilled forms could effectively increase the proportion of useful data and the face-to-face time was thereby optimized comparing to linear approach of data collection of usual processes.

Eligible patients were registered, and medical history and status, especially focused on allergy-related factors were recorded. If a screening visit overlapped with the symptomatic period, skin prick test (SPT) as a diagnostic procedure was postponed until next visit arranged during pollen-free winter months to reassure that ragweed is a relevant sensitizer.

During follow-up visits the following data were recorded:

- Any deviation from planned schedule of SLIT treatment;

- Drug compliance, any adverse events recorded associated with the application;
- Patient reported symptoms;
- Progression of rhinoconjunctivitis-specific health-related quality of life;
- Concomitant symptomatic medications;
- Results of physical exams. Lung function testing was performed with recording forced expiratory volume 1, forced vital capacity, forced expiratory flow 25–75 and peak expiratory flow. Peak nasal inspiratory flow was determined to obtain objective variable for nasal obstruction.

Blood samples were also collected on these visits. After the second and the third year of treatment, patients were asked for global evaluation of immunotherapy considering improvements of symptoms and changed demand for rescue medication.

Independently from the data recorded on the visits, from the preseasonal visit through the end of the therapy, patients were also followed with daily resolution using an electronic diary (see below).

Skin prick test

A diagnosis of ragweed allergy was made in subjects with positive results of specific serum IgE and/or SPTs in presence of a positive clinical history for allergic respiratory symptoms with a peak in August–September months. Sensitization against the same array of antigens was determined in two different time points: before therapy initiation and after the season following the 2 or 3 years of immunotherapy.

Obviously, timing of the first SPTs was different on the time-shifted initiated groups: in May–June 2012 and 2013 prior to initialization and in winter after recruitment visit in case of PROS1, PROS3 and PROS2 patients, respectively. Second test was performed in the same visit period for all groups, namely in November 2014. Those relatively common allergens were included whose sensibilization and evoked symptoms can interfere with the assessment of change in clinical variables attributed to ragweed specific immunotherapy (e.g., botanical relative to *Artemisia vulgaris*) and those, whose evoked symptoms are distinguishable easily due to the different seasonal occurrence (*Betula pendula, Graminaceae*).

Brief subscription of the SPT can be found in the Supplementary Information.

Immunological measurements

Two main methods were used for the measurement of the immunological parameters, namely ELISA and flow cytometry. Several cluster of differentiation (CD) markers, cytokines and transcription factors were selected for measurement by flow cytometry to follow the changes of different immunological cells during the study (see more details in the Supplementary Information). Allergen specific IgA, IgE, IgG1 and IgG4 and total IgE levels were measured with ELISA.

WP3. Collection of genomic data

Two types of genetic data were measured in the patients. All the patients were screened for 1 million genetic variants in their genomic DNA. Furthermore, total RNA was isolated from blood of the patients and whole genome gene expression measurements were carried out in two time points, before and after the SLIT. Both measurements were carried out by different Affymetrix microarrays.

WP4. Application of ragweed specific SLIT

A standardized ragweed extract (Staloral ragweed 300 IR/ml) was used for immunotherapy. During the build-up phase, the participants were administered daily increasing doses as follows: 1, 2, 4, 6, 8 to 10 drops of 10 IR/ml solution. After reaching the maintenance dose (4 drops of 300 IR/ml solution), the participants administered the allergen every day during the maintenance phase until end of October. The patients were told to hold the drops of allergen under their tongue for 2 min before swallowing or spitting out.

WP5. Development of a psychological questionnaire

To address the questions how the psychological characteristics of the patients influence their therapy adherence and compliance, response to the therapy, symptoms and quality of life a psychological questionnaire was developed. The questionnaire consists of 146 questions selected from different psychological tests; the majority of them

Table 3. The groups of questions and the measured psychological characteristics in the psychological questionnaire.

Groups of questions	What does it measure?	Subscales
Barratt Impulsiveness Scale	Impulsivity	–
Selected questions from Rahe Brief Stress and Coping Inventory	Health motivation and inclination for coping	Coping Health motivation
Somatosensory Amplification Scale	Tendency to experience a somatic sensation as intense, noxious and disturbing	–
Trust	Trust in the medical profession	–
Hospital Anxiety and Depression Scale	Dimensional representation of mood	Anxiety Depression
Shortened version of Temperament and Character Inventory	Biological independent personality traits	Reward dependence Self-directedness Harm avoidance Cooperativeness Self-transcendence Novelty seeking Persistence

were validated earlier for Hungarian language [43,44]. The groups of questions and the measured psychological characteristics are presented in Table 3.

Upon entering the study, all patients completed the psychological questionnaire.

WP6. Creating logistics for collection, processing & storage of biological samples; development of a biobank

The logistics of the study is visualized and described in Figure 3.

All biological materials were planned to be stored for long-term. The developed biobank and the connected clinical and laboratory data are suitable also for future researches.

WP7. Collection of environmental data that can influence the therapy response

The ragweed pollen concentration has a direct, causal, quantitative effect on allergy symptoms, thus its monitoring and usage in the data analysis is essential. Consistently to the daily patient-reported data, we accessed the hourly pollen concentration data for Ragweed (*Ambrosia artemisiifolia*) collected and reported by the National Public Health and Medical Officer Service. Because the ratio of monoallergy for ragweed is generally low and less than 10% in our population, we also accessed concentration data for additional pollens frequently present in polyallergic patients. Specifically, we used data for *Chenopodioideae, Cannabaceae, Asterales, Rumex, Poaceae, Plantago, Urticaceae* and *Artemisia*.

Effects of pollen concentrations on allergic symptoms are also influenced by meteorological factors and air pollution, thus we also collected hourly data about meteorological entities, such as air temperature, humidity, wind speed and also about air pollution, such as CO, NO, NO_2, SO_2, O_3 and PM10 (inhalable particulate matter with a diameter between 2.5 and 10 μm) [45–47].

WP8. Patient-reported data by web-based SLIT-diary

To support self-reported data from home environment, we developed a web-based patient diary, which assists free, continuous tracking of all allergy and SLIT treatment-related activities (SLIT-diary). Through the SLIT-diary, from the preseasonal visit through the end of the therapy at the end of the ragweed season, patients were asked to confirm once a day the completion of intake of the drops and record adverse events, medication, allergy-related well-being on a VAS and numerical evaluation of symptoms. In order to avoid enrichment of invalid and missing data due to inconvenience, the software was designed to provide an ergonomic and personalized platform. It is able to recall a part of previously recorded data (e.g., name of medicine, profile of symptoms, place of stay, etc.) and offer an adaptively developed list for most typical adverse events noticed by other patients.

The SLIT-diary is also prepared to support the appearance and use of automated detectors of allergic symptoms, such as sneezing/nose blowing/coughing detectors by wearable electronics or red eye detection by mobile phones.

The SLIT-diary also supports advanced real-time monitoring, assisted interaction and personalized, automated reporting between patients and physicians. To support monitoring notifications it can be asked for various statistical aggregates or data patterns both by the patients and the physicians. These notifications can automatically trigger

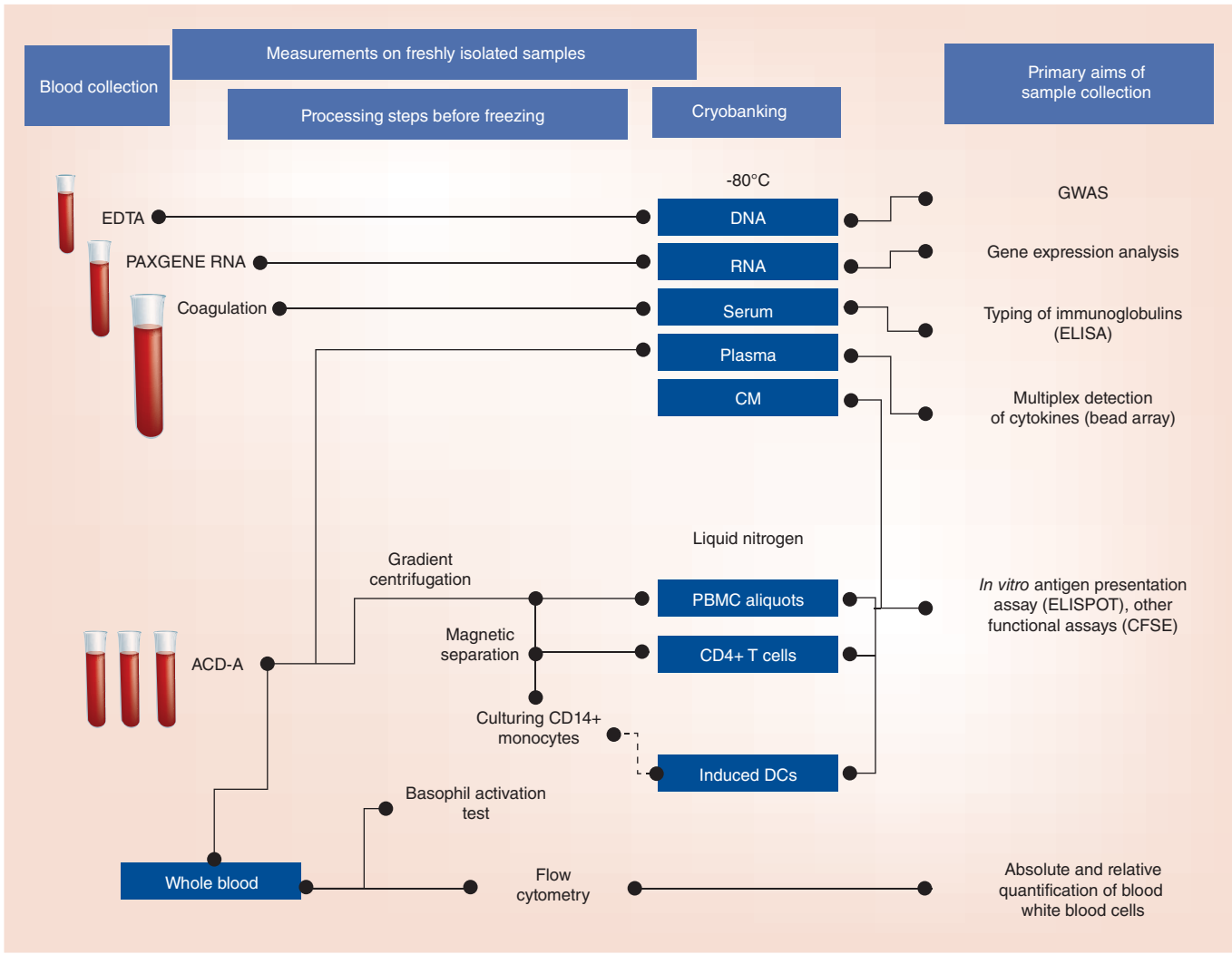

Figure 3. Sample collection and processing pipeline in the DESENSIT study. Venous blood samples were drawn into different types of tubes on the specified visits, shipped temperature controlled and processed within a couple of hours after collection. Preparation of samples for flow cytometry was started immediately after arrival of blood samples to the laboratory. The serum and plasma from each patient was divided in 8–12 equal aliquots which were stored at -80°C until measurements. PBMC aliquots isolated by density gradient centrifugation were stored at -196°C liquid nitrogen. For genomic measurements DNA was isolated from blood. For RNA isolation the blood was collected in PAXgene Blood RNA System, according to the instruction of the manufacturer.
ACD: Acid citrate dextrose (anticoagulant); CM: Conditioned media; CFSE: Carboxyfluorescein diacetate succinimidyl ester; DC: Dendritic cell; PBMC: Peripheral blood mononuclear cell.

communications with the physician at different levels, such as internal messages, emails or visit request. Furthermore, in a transparent and controllable way for the patient to respect privacy, wide range of statistical aggregates as SLIT-patient profiles are computed and reported to support the visits to the physician (see WP9 about the integrated system for the physicians).

WP9. Development of a web-based system for the collection of data for physicians

The comprehensive SLIT-related clinical descriptors (SLIT-phenome) and the breadth of the patient-reported data posed a dual challenge for the physicians participating in the DesensIT project. Especially, the patient-reported, home-collected, continuous flow of information presents a novel challenge in many areas of personalized medicine. To support this dual goal, we developed a web-based system, which integrates the collection and entry of clinical descriptors according to the protocol described in WP2 and the SLIT-diary described in WP8.

Although the patient-reported data are independent from many clinical data recorded during the visits at physicians and serve as important side information to complement it, the self-reported patient data can also be used to derive automatically objective values for certain clinically relevant attributes and quantities. Thus, the integrated system automatically computes wide range of statistical aggregates from the data collected by the SLIT-diary. On one hand these aggregates compose a patient profile about efficiency of the treatment, about potential adverse effects and also about compliance of the patient. On the other hand, these aggregates are offered for the physicians as default values, which can be modified based on the consultation with the patient at regular visits. The self-reported data, the derived aggregates and the corresponding approved or modified clinical attributes are managed by a unified IT framework and stored in a unified database.

WP10. Data quality control & data preprocessing

The multicentric aspect of the study, the complexity of the SLIT-related clinical descriptors and the patient-reported data called for a dedicated quality control over the nonmolecular data. Especially the patient-reported data and the dependent, partly overlapping/redundant clinical attributes required careful management and synchronization. To cope with these challenges, we developed a quality control protocol, which was implemented and supported by the integrated data collection system, thus all the changes in the data could be traced back formally.

Patient-reported data also brought up the issue of the coding of adverse effects and special actions (preventive/supportive medications), for which we developed special taxonomies.

WP11. Statistical data analysis

The DesensIT project collected wide range of phenotypic and genetic information:

- Demographic data;
- Clinical/allergic status before SLIT treatment;
- Psychological factors;
- Patient-reported data about
  - compliance,
  - quality of life,
  - medications,
  - adverse effects
- Genome-wide variation data;
- Genome-wide gene expression (GEX) data;

Following a phenome-wide approach, heterogeneous, comprehensive sets of outcomes were considered:

- (Single occasion after 2 years) genome-wide gene expression data in SLIT treatment (used as differential data compared with the baseline level before treatment);
- (Yearly) clinical/allergic status in SLIT treatment before and after ragweed season
  - Standard ARC questionnaires/scores: Rhinitis Total Symptom Score/VAS/Allergy Control Score/ Rhinoconjunctivitis Quality of Life Questionnaire,
  - Immunological status,
- (Daily) multiple ARC scores;
- (Daily) patient-reported data
  - (SLIT) compliance,
  - (Other than SLIT) medications,
  - Quality of life,
  - Adverse effects.

Potential interaction, effect modifier and confounder variables are as follows:

- (Hourly) pollen concentration for ragweed and other pollens;
- (Hourly) environmental variables;
- (Hourly) air pollution variables.

The exploration of markers relevant for these outcomes, covering effectiveness of the treatment and adverse effects or risk of dropout is challenged by multiple factors:

- Multitask setting, because the relevant response variables have a rich interdependency structure;
- High-dimensionality of the predictor variables, such as GWAS or GEX data;
- Small sample size, because of logistic and budgetary reasons the study could involve only about 400 participants;
- Heterogeneous domains with different sample sizes and systematically missing data.

To cope with these challenges we adopted a Bayesian systems-based methodology. It is based on probabilistic graphical models, which provide a formal, structural representation for the set of multivariate dependencies and independencies. A popular class of probabilistic graphical models, Bayesian networks, uses directed acyclic graphs to represent multivariate dependencies and conditional independencies; Bayesian networks also offer a rich language for representing causal relations [48–50]. The Bayesian inference over structural properties of Bayesian networks was put forward decades ago, which was successfully applied to characterize a complete domain using a posteriori probabilities of pairwise relations [51], and it was also useful to characterize the relevance of explanatory variables for a given target [52,53].

WP12. Development of decision support systems for personalized SLIT

The systems-based probabilistic models formalized as Bayesian networks from data analysis encompass both predictive genetic factors and wide range of outcomes, such as various ARC symptoms, high-level ARC scores or adverse effects. Additionally, the importance/relevance of various outcomes can be quantified patient-by-patient using patient-specific utilities/losses in decision networks [54]. Figure 4 shows a conceptual model demonstrating the interplay of inferring factors of interpatient variability in AIT outcomes. Briefly, the ordered domains of patient outcomes represent a causal chain from biological variables through symptoms, functional status and general health perceptions to overall quality of life. Explanation of the relationship between quality of life and relating, explicitly measurable parameters is often challenging, especially if even the severity of symptoms that occur in seasonal allergies are difficult to evaluate themselves without the contribution of the patient, due to their temporal variability and self-reported nature. Indeed, besides the severity of symptoms and applied medication, the expected impact by the observed patient should also take into account during the evaluation of adequate control of symptoms. Hence health perceptions about the satisfaction with allergy-related health and applied therapy are essential as well as how symptoms and functional abilities are valued.

The personalized decision support models are available both for the physicians in the data management system and for the patients in the SLIT-diary. Physicians can use this functionality to decide about the cost-effectivity of SLIT treatment for an actual patient. Patients can use these models to check the expected changes (improvements) of their actual status in various scenarios of compliance.

WP13. Project management, dissemination, training & ethical issues

Detailed protocols and educational materials were prepared for participant physicians and patient informed forms for patients. Also, all the physicians participated in face to face trainings. Ethical applications were presented to institutional and national review committees. During the study all the project participants were in close contact through web-based tools. Additionally, consortium members and scientific coordinators had face to face scientific management meetings twice a year and contacted with regular teleconferences according to demands.

Conclusion

In the present paper we describe the DesensIT project which was planned and carried out in order to collect and evaluate data related to allergen specific SLIT.

During this project we have collected heterogeneous clinical, psychological, genomic, immunologic and environmental data for more than 3 years and from more than 400 participants, developed data collection systems and established a biobank which can be used for further measurements and evaluations.

In future publications we plan to present the different results of our evaluations including the potential biomarkers identified in the study; what immunological and gene expression changes occurred during the therapy; how different factors, like genetic variations, environmental factors and the psychological characteristics of the patients influenced the respond to the therapy; how different factors influenced the adverse effects of the therapy; what factors influenced

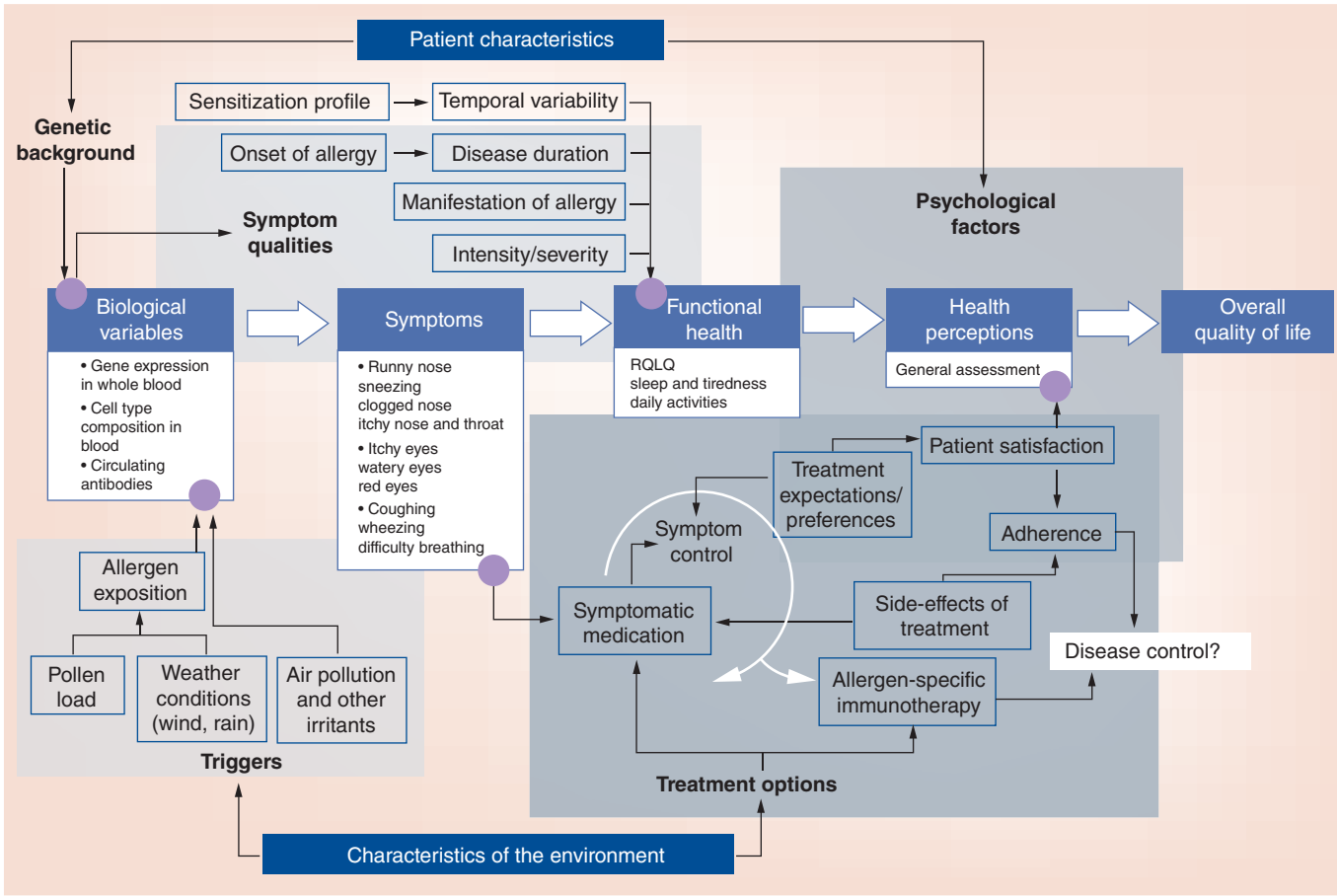

Figure 4. Interplay of inferring factors of interpatient variability in allergen-specific immunotherapy outcomes. The conceptual model demonstrates the multilevel interaction of treatment effects attributed to allergen-specific immunotherapy in context of allergic rhinoconjunctivitis. Gathering of individual time-series data in the observational DESENSIT study enables to explore the internal relationship of variables and to estimate the outcome as a specific, disease-modifying effect size of the treatment. Here we propose the leading causal relationships between biological and clinical variables which may determine the success of allergen-specific immunotherapy. The main goal would be individualized prediction of treatment effects and link them to measures that describe their impact on health-related quality of life.

the patient adherence to SLIT; what were our experiences regarding patient-reported data; what consequences can be drawn from the results, etc. We think that our results will contribute to the better utilization of the potential of AIT in allergic diseases and increase the awareness and acceptance of this therapy in the society.

Future perspective

AIT soon can become a forerunner of future medicine/medical approaches in many respects, such as real-life monitoring of efficiency and side-effects and using personalized drugs and treatments.

The DesensIT project already demonstrated many aspects of such future studies, especially collecting comprehensive phenotype information, in a phenome-wide approach including clinical, biological and patient-reported data. Within the next 10 years, based on such 'deep', in other words, detailed phenotype and environmental data and high throughput genomic, proteomic, metabolomic and immunologic approaches, different types of novel targets will be identified which can help physicians in selection of patients who more likely will respond beneficially to the therapy. Studies will also identify more objective biomarkers which can monitor clinical changes during the therapy and/or predict whether the therapy should be continued, terminated or alternative therapy should be applied.

In the next 10 years our knowledge about the cellular and molecular mechanism of immunotherapy will improve which gives rise to the possibility of development of novel targeted therapy with improved efficacy and safety. The novel biomarkers and the different therapeutic possibilities will also contribute to more personalized treatments.

The different levels of data collected by the high throughput studies and the progression of IT and bioinformatic methods will contribute to the development of user friendly decision support systems which will help physicians in selection from the treatment possibilities for the different patients.

In 10 years the portable/wearable medical devices will be significantly more advanced and their usage more widespread. They will continuously be able to monitor the health status of the patients, such as the number of sneezing, coughing, itching and blinking or to measure the redness/inflammation of the eyes or the degree of nasal congestion. Together with automatically collected health-related environmental data through sensors and internet connections (weather, air pressure, pollen counts, etc.), they will be able to provide personalized, real-time suggestions and continuous, cheaper and more effective healthcare.

Executive summary

Allergen-specific immunotherapy

- Allergen-specific immunotherapy (AIT) is the only currently available medical intervention that has the potential to affect the natural course of the disease.
- There are still many questions and unmet needs hindering its widespread use to fulfill its treatment potential and to maximize its benefits for the society.

Problems & unmet needs in allergen-specific sublingual immunotherapy

- Lack of objective diagnostic/predictive biomarkers for sublingual immunotherapy (SLIT).
- Lack of reliable end point biomarkers.
- The dropout rate is high.
- Knowledge about the mechanism of AIT is insufficient.
- Continuous contact between patients and physicians should be achieved.
- The public awareness of AIT is insufficient.

Aim of the DesensIT project

- The main aim of the DesensIT project was to collect and evaluate data in SLIT to allergic rhinoconjunctivitis to address the above mentioned problems and to develop multivariate, system-based predictive models for multiple treatment responses with focus on personalized medicine, particularly on the use of genetic, immunologic and self-reported patient data.

Work packages in the DesensIT project

- To target the above mentioned aims, 13 work packages were planned which involved selection of the patients, application of ragweed specific SLIT, collection of biological samples, clinical, environmental and laboratory data including results of genetic and immunologic measurements, development of different IT tools like electronic SLIT-diary, web-based interface for data collection for physicians, statistical analysis, development of decision support systems for personalized SLIT, project management, dissemination, training and ethical issues.

Ethical conduct of the research

The study protocol was approved by institutional and national ethics committees (10130/2012/EKU), and written consent was obtained from the patients included.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: **www.futuremedicine.com/doi/suppl/full/10.2217/imt-2017-0093**

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