

CME Review Article

Nasal allergen challenge studies of allergic rhinitis: a guide for the practicing clinician



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Learning Objectives:

At the conclusion of this activity, participants should be able to:

- Describe nasal allergen challenge (NAC) methodology
- Discuss how the NAC study designs, objective measures and biological sampling techniques inform studies of the pathophysiology of allergic rhinitis and clinical efficacy of novel therapies for allergic rhinitis

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Allergic Rhinitis

Allergic rhinitis (AR) is an IgE-mediated inflammatory nasal disorder that involves hyperactive nasal mucosa, obstruction of the nasal passages, and symptoms of rhinorrhea, sneezing, nasal pruritus, and congestion.¹ AR is also commonly associated with conjunctivitis, itchy palate, and aggravation of comorbid asthma.¹ Traditionally, AR has been classified as perennial, with symptoms occurring year round, or seasonal, with symptoms occurring at particular times of the year.² Perennial symptoms are most commonly associated with dust mite, cockroach, and molds, whereas seasonal symptoms may be induced by pollens.² However, differentiating seasonal and perennial symptoms often proves to be difficult because many patients have multiple sensitizations.

The Allergic Rhinitis and Its Impact on Asthma (ARIA) classification provides a definition of AR that considers both severity and duration of symptoms.¹ This classifies rhinitis symptoms as intermittent or persistent and mild or moderate-severe.¹ Although AR is often dismissed as a minor discomfort, it currently affects 10% to 25% of the population worldwide and has been found to have significant socioeconomic effects.¹

Many experimental models have been developed and are continuously being improved for the development of novel medications for the treatment of AR. This review focuses particularly on experimental models of direct nasal allergen challenge (NAC) in humans and their utility for research and evaluation of new therapeutic strategies. It also aims to provide a guide for clinicians to use while reviewing articles that use these models.

Experimental Models of AR in Humans

Experimental models of AR have been developed to resemble the symptoms, signs, and inflammatory processes found with natural allergen exposure, such as park, environmental exposure unit (EEU), and NAC studies. Phase 3 efficacy outpatient studies are typically randomized, double-blind, parallel-group, placebo-controlled trials designed to comply with Food and Drug Administration (FDA) regulations because the goal is often to bring a new drug to market.^{3,4} This design uses the participant's own natural environment, in the outpatient setting, as a vehicle for allergen delivery across multiple sites and provides the advantage of enrolling large numbers of participants to observe season-long response to medication.^{3,4} Limitations of standard phase 3 efficacy studies include the variable natural pollen production during the study period, weather conditions, inconsistent pollen exposure within the study population, potential concurrent exposure to multiple allergens, and the reliability of participant dosing and reporting.⁴ Some studies perform participant matching through the inclusion of an observation season, but variability in pollen levels from year to year may affect results, and studies with or without an observation season typically require a large number of participants to achieve statistical power.³ Despite limitations, this model is considered the standard for drug validation and final approval.^{1,4}

Park studies use an outdoor setting where participants stay at a park for the study day to be exposed to environmental allergens during the pollen season, usually in a randomized, double-blind, and placebo-controlled manner.⁵ Studies in park settings control certain variables that standard phase 3 studies do not, such as ensuring similar pollen exposure levels among participants, accurate timing of symptom reporting, precise timing of study drug administration, and verification of adherence.⁴ However, park studies have their caveats. Because they are necessarily conducted in season, participants enter park studies with preexisting symptoms.³ Pollen counting during park studies, whether performed on site or estimated from counts made at a central location, can be challenging because of the variety of species of pollen present in the outdoor air, pollen fragmentation, and uncontrollable wind

currents.⁴ Several park studies have provided useful clinical data in examining antiallergic medications, and the model has been used to determine important pharmacodynamic parameters, such as onset of action.⁴

EEUs are specialized facilities, sometimes referred to as chambers, where a particular allergen is delivered into the ambient air and several participants are exposed concurrently.³ EEU studies allow for the control of several factors that limit park studies, such as allergen specificity, pollen levels, air quality, humidity, and temperature.⁴ Clinical findings of EEU studies have been found to be consistent through repetition of the same protocol in the same unit on separate occasions by the Vienna Challenge Chamber and the Kingston EEU.⁶ EEUs have been used to validate efficacy and establish pharmacologic parameters for many important therapeutics for AR, including immunotherapies, antihistamines, and nasal steroids.⁴

Occasionally, it is important to perform more complex physiologic sampling. The performance of NAC studies does not require the specialized equipment and space necessary to establish an EEU, further allowing for these techniques to be integrated into multicenter studies. Described in further detail below, the NAC involves the introduction of allergen to the nose and several hours to days of follow-up. NAC of allergic individuals has been found to produce nasal symptoms and increase levels of histamine and tryptase in the nose.⁷ Nasal congestion can also be objectively measured using acoustic rhinometry or peak nasal inspiratory flow.⁸ Thus, NAC has the advantage of focusing on a smaller number of participants at a time, enabling the collection of more detailed data and biological samples.

Utility of NAC in Evaluating Novel Therapies

NAC has played an important role in evaluating new therapies for AR efficacy, onset of action, and prophylactic therapy use. NAC also facilitates the identification and validation of mechanisms of action through the collection of biological samples. The effects on mediator release of the widely used third-generation antihistamine fexofenadine were evaluated using NAC.⁹ NAC studies have also revealed the efficacy of topical nasal corticosteroids¹⁰ and the effects of subcutaneous vs sublingual immunotherapy on IgE, cytokines, and eosinophilia production.¹¹

Typical NAC Protocols

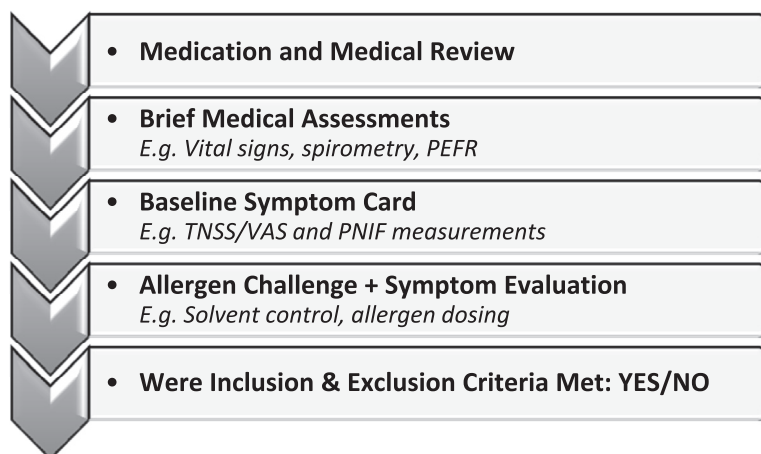
Screening Visit

The screening visit is necessary to evaluate the suitability of a potential participant before enrollment in the study (Fig 1). Skin testing and a history of AR on exposure to a particular allergen is required for inclusion.¹²

Common exclusion criteria include any chronic medical condition that may represent a health hazard, pregnancy and lactation, active AR, and structural nasal abnormalities that may interfere with sample collection. Participants are usually asked to restrict intake of certain medications that may interfere with nasal inflammation, such as corticosteroids, anti-histamines, and decongestants, or to adhere to pre-specified washout periods before the study visits. Spirometry to measure the forced expiratory volume in 1 second can be included as a safety measure.¹³

At screening, a direct allergen challenge is often performed to confirm reactivity. Some centers use a single allergen dose,¹⁴ whereas others perform a titration challenge to determine the dose required to produce adequate symptoms.¹⁵ Allergen extracts that contain no additives are used in some centers for NAC, with an initial nasal wash sometimes performed to confirm the absence of nonspecific hyperreactivity.¹⁴ Another option is to use the standardized allergen extracts offered in different preparations, such

Screening Visit



Nasal Allergen Challenge (NAC) Visit

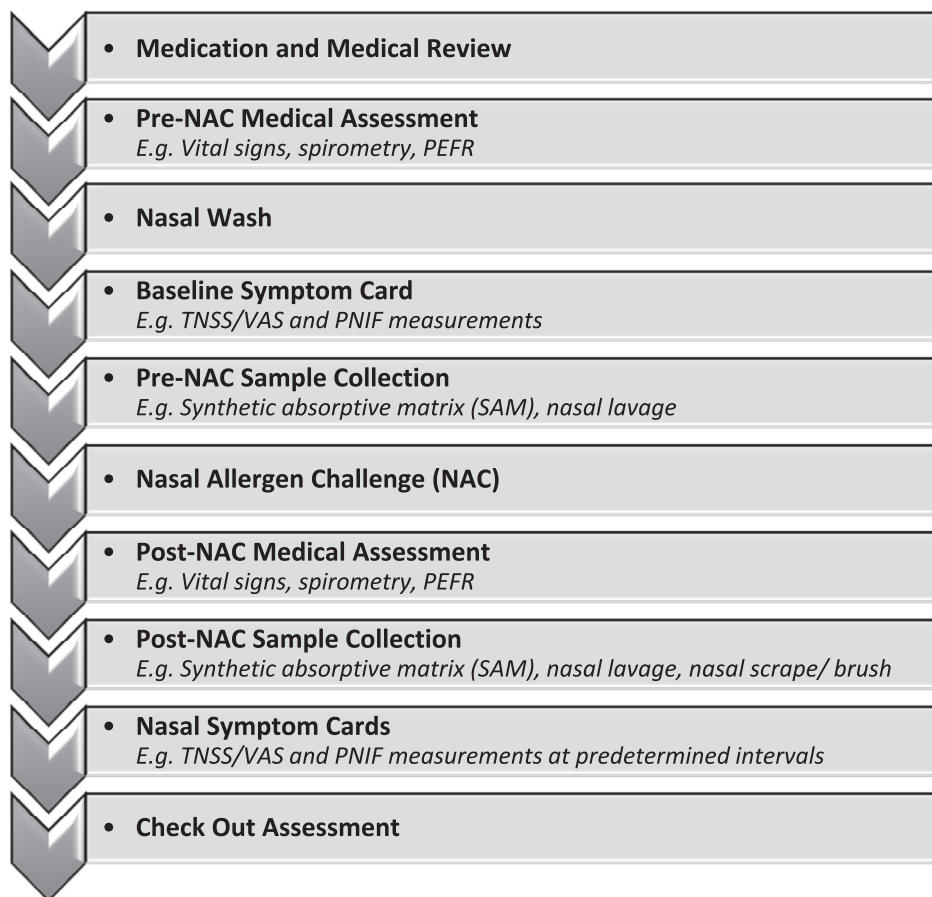


Figure 1. The nasal allergen challenge (NAC) model is composed of 2 main visits: the screening visit and the NAC visit. The screening visit is necessary for reviewing the qualifying criteria as a safety measure and to ensure the symptoms experienced are directly attributed to allergic rhinitis and not any other comorbidity. If the protocol involves titration of the allergen, this might be included as part of the screening visit. The NAC visit includes a medical assessment and a saline wash of the nasal cavity before the allergen challenge. Symptoms are recorded and samples collected at baseline and at specific intervals after the allergen challenge.

as lyophilized powder or in aqueous solution with added phenol and/or glycerin. These additives preserve the allergen and maintain potency, but it is important to prepare fresh allergen dilutions each time to avoid diluting the preservatives in the stock solution.^{16,17} The presence of these compounds, however, may cause

nonspecific reactions in some participants.¹⁸ Thus, the diluent control must be administered as the first dose during the screening visit to eliminate participants who have a significant nonspecific reaction to the diluent itself. After this confirmation, the lowest allergen concentration is introduced, and participants measure

their nasal symptoms using both subjective and objective tools, which will be discussed in more detail below. The starting or lowest allergen concentration can be determined by the size of the wheal measured during skin testing, either 1/100 of the concentration that resulted in a positive skin test result or the lowest concentration of a standardized dilution curve.¹⁹ Increasing concentrations of allergen are administered until the participants meet a prespecified qualifying symptom level, at which point the participant is eligible for the NAC at this concentration. Alternatively, if a nontitrated challenge procedure is to be followed, one can proceed directly to the one-time allergen delivery determined by the protocol.¹⁴

Allergen Delivery

The allergen can be administered using a variety of methods. Allergens in powder form can be delivered using a nebulizer.²⁰ Aqueous allergens can be delivered by spraying, pipetting, dripping, or using paper disks placed in the nose with forceps.^{14,15,21} The area of nasal mucosa exposed to the allergen is limited when using paper disks, pipetting and dripping methods, and paper disks may cause local irritation.²² Nasal spray devices are becoming the preferred method because they are capable of reaching the turbinate region with high-deposition efficiencies without causing local irritation.²³

Baseline NAC Visit

A nasal wash should be performed before the start of the allergen challenge to cleanse the nasal cavity. Baseline subjective and objective measurements of AR are recorded, and the allergen is administered. The dose and schedule of allergen administration vary widely within different NAC protocols. Protocols can include the highest allergen concentration administered during the titrated challenge, a cumulative concentration of all doses from the titrated challenge, a standard dose that produces symptoms in most participants, or a dose calculated from skin testing. Both the single and cumulative allergen concentrations have been found to be reproducible if NAC visits are separated by at least 7 days for the mucosa to return to normal state.^{14,15}

During the NAC visit, measurements are recorded at set intervals to monitor symptom development. If used in a clinical trial, the NAC can be repeated after treatment to assess efficacy, determine onset, and/or determine duration of action. Long-acting medications or late-phase reactions can be studied by monitoring the symptoms for 12 to 24 hours after the NAC, giving a more complete picture of the pharmacodynamics. Biological samples can also be collected at preset time points.

Duration of Allergen Exposure

NAC protocols are limited to acute allergen exposure compared with park and EEU models that allow continuous exposure for a prolonged period.³ To address this issue, protocols have been developed that involve allergen challenge once daily for 1 week.²⁴ This method was useful in studying the dose-response relationship of corticosteroid treatment.²⁴

One issue that remains somewhat controversial is the effect of repeated allergen exposure in this model on biological and pharmacologic outcomes. Connell²⁵ first described the effect in 1969, calling it the “priming effect,” suggesting that repeated environmental exposure to ragweed lowers the nasal threshold of reactivity of the nasal mucosa. Priming is thought to be the result of an influx of inflammatory cells during the late phase of allergic rhinitis.²⁶ The effects of priming can be nonspecific.^{25,27} For example, if a cat allergic participant is exposed to a cat before study entry (ie, already primed), the level of allergen required to activate their symptoms during a noncat allergen study would be lower

than that required for a nonprimed person. Although studies that followed found similar effects, some reported the lack of the priming phenomenon, which may be attributed to the method of allergen introduction, evaluation of the response, or seasonal effects.^{28–30} The consequences of the priming effect in the NAC model are yet to be determined. Current studies are focused on comparing various NAC procedures to determine the optimal methods for reproducible responses.

Subjective and Objective Measurements of AR Symptoms

Symptoms experienced by the participants can be quantified by subjective and objective measures at regular time points. Participants can be asked to assign their subjective symptoms scores according to various rating scales and composite scores can be generated. The Total Nasal Symptom Score (TNSS) is measured on a 3-point scale (range, 0–3) and is the sum of nasal congestion, sneezing, nasal itching, and rhinorrhea. The FDA recommends TNSS as the standard method for evaluating AR in clinical trials because of its reliable and repeatable outcome.³¹ An alternate method, the visual analogue scale (VAS), provides participants with a 10-cm or 0- to 100-point scale that ranges from no symptom to worst symptom ever for each of the nasal symptoms. The VAS is recommended by ARIA and is considered complementary to other objective and subjective measurements.^{32,33}

Peak nasal inspiratory flow (PNIF) is an objective method for assessing nasal patency. It makes use of a specially designed face mask that measures the peak air flow when the participant inhales maximally through the nose with lips tightly closed. The PNIF has the advantage of being noninvasive and simple and can be used by participants independently after training by qualified site personnel. The outcome is a direct representation of nasal patency, being affected mainly by congestion.^{15,34} The PNIF correlates strongly with other objective measurements yet is easier to use when repeated measurements are needed and thus provides a better assessment of symptoms over time.^{33,35}

Some studies use peak expiratory flow rate, measured by forcefully expiring through the mouth into a peak flow meter, a quick method for assessing the effect of the allergen on participants with mild to moderate asthma.^{36,37}

Rhinomanometry is another objective measure of nasal airway resistance that quantifies the effect of the allergen and medication by measuring airflow and transnasal pressure. Several variations of the technique are used, depending on whether the sensor is placed near the anterior or posterior/choanal area of the nasal cavity.³⁸ The nasal airflow measurement is considered active or passive, depending on whether the participant is asked to breathe freely or follow a set airflow protocol.^{38,39} Nasal fractures, bleeding mucosa, and frequent sneezing may affect the efficiency of the device.

Furthermore, acoustic rhinometry is an objective, safe, and noninvasive method that makes use of the resonance of sound waves in the nasal cavity. Sound waves are emitted and reflected off the mucosa to be captured by a microphone. Use of the time elapsed provides an estimate of the nasal cavity's minimal cross-sectional area (MCA). The MCA usually relates to the level of the inferior turbinate, and any change in MCA corresponds to a change in size of the turbinate as a result of inflammation.^{40,41} Acoustic rhinometry correlates with magnetic resonance imaging and is thus reliable although more technically demanding than PNIF.^{8,42} MCA or PNIF measurements before and after treatment can be incorporated into an index used to compare nasal congestion. The Nasal Congestion Index is calculated using any of the 2 measurements, measurement after treatment–measurement at baseline// measurement at baseline, and provides another objective method of comparing medications' efficacy in reversing nasal congestion.⁴³ Thus, subjective and objective measures of NAC-induced symptoms

are often used to determine the effectiveness of various treatments compared with placebo or active treatment.

Biological Samples: Pros, Cons, and Optimal Uses

One of the clear advantages of the NAC procedure over standard phase 3 trials, park studies, or EEU studies is the ability to collect biological samples at various time points after allergen challenge. Some of the most common samples collected include blown secretions, secretions absorbed on a matrix, nasal lavage, and nasal scrapings or brushings.

Blown Secretions

Blown secretion samples are spontaneously ejected from the nose by the participant and consist of mucus, fluids, and cells.⁴⁴ The weights of blown secretions are considered to be representative of the severity of rhinorrhea after NAC.⁴⁵ Brooks et al⁴⁶ found that antihistamines and systemic corticoids reduced the weight of blown secretions concurrent with decreases in sneezing. The secretion fluid has been used to evaluate cytokines, such as interleukin (IL) 5, IL-16, and soluble ST2 (the decoy receptor of IL-33). These cytokines have been found to be increased in allergic participants in season and after the NAC, helping to reveal the comparability of NAC to real life.⁴⁵ Drawbacks of blown secretions include the fact that the area of the nose from which the cells and secretions originate is unknown and that they can be difficult to obtain from healthy, nonallergic persons.⁴⁴ To overcome this, some researchers have used techniques in which saline is sprayed into the nose before blowing.⁴⁴ However, the spray-blow technique is then subject to a variety of concerns regarding the unknown sample dilution factor and potential dilution of mediator levels below their limit of detection.

Nasal Lavage

Also known as a nasal wash, this method is a dilution technique.⁴⁴ Nasal lavage involves rinsing the nasal cavity with saline solution and recollecting as much of the fluid as possible.⁴⁷ To accomplish this, typically 1 of 2 techniques is used. The traditional nasal lavage method involves introducing saline fluid to the nose and asking the volunteer to tilt their head back, closing their nasopharynx with the soft palate.⁴⁴ The volunteer is asked to retain the fluid for approximately 10 seconds and then blow their nose into the desired container.⁴⁴ Alternatively, the nasal pool technique may be used, in which the participant is asked to sit in a forward-flexed neck position and a nasal pool device, such as an olive, is placed in the nose to act as a stopper against fluid leakage.⁴⁷ The nasal cavity is flushed with saline using a syringe.⁴⁷ Nasal lavage yields a fluid phase, which can be used to quantify mediators and cytokines, and a cellular phase, which can be used for inflammatory cell analysis, such as monitoring eosinophil influx.^{7,48,49} Disadvantages of the lavage technique include the previously mentioned dilution of nasal secretions and with it extremely low mediator levels and varying dilution factors.⁴⁴ Common methods of correcting the unknown dilution factor include standardizing to total protein, albumin, and secretory IgA, of which total protein is the most stable.⁵⁰ Despite technical issues, nasal lavage sampling is a useful technique that takes advantage of the detailed opportunities afforded by the NAC procedure.

Modified Matrix Method

To overcome some of the challenges, including dilution of the nasal secretions, a modified matrix method has been developed.⁴⁹ This method is referred to as an absorption technique because it relies on the properties of various media to take up the secretions from the nose, while changing the composition as little as

possible.⁴⁴ Various sampling media have been proposed, including filter papers, synthetic absorptive matrix, and polyurethane foam. Unfortunately, there is no consensus across studies as to which method is superior. Riechelmann et al⁴⁴ found that filter papers provide good absorption when rhinorrhea is present but may be dry (no sample) in healthy controls, suggesting polyurethane foam to be more reliable. Although the filter paper method has limitations, Sim et al⁵¹ used it to reveal significant up-regulation of IL-1 β , IL-5, IL-6, and granulocyte-macrophage colony-stimulating factor concurrent with the elevation of symptom scores after NAC. Erin et al¹⁰ also used filter paper to reveal a reduction in postchallenge cytokine levels after corticosteroid treatment. Synthetic absorptive matrix has been used successfully in studies of new experimental therapies.⁵² Some obstacles of the modified matrix method include small sample volume and the differing placement of absorptive matrix media in the nose across studies, ranging from the nasal septum to the inferior turbinate.^{14,44,51} Despite varying materials and protocols, absorptive matrix sampling is becoming the criterion standard for nasal cytokine measurement during NAC because of its minimally invasive nature while also providing the opportunity for repeated collection of undiluted samples.

Nasal Scraping and Brushing

These methods allow for the collection of samples from the nasal mucosa, including epithelial cells, mast cells, goblet cells, surface inflammatory cells, and secretions.⁵³ The standard site of collection for both techniques is the medial surface of the inferior turbinate.⁵³ Scrapings use a curette, usually plastic, and brushings use a plastic coated steel wire with nylon bristles, such as a cyto-brush.⁵³ Both techniques have been used for cytologic testing, messenger RNA analyses, and epigenetics.^{54–57} Currently, there is no agreement on which method is superior. Lin et al⁵⁸ found that scraping samples differentiate those with symptoms of AR from controls better than the cytobrush based on leukocyte and eosinophil quantification. Baccarelli et al⁵⁵ used nasal brushing to find associations between nasal cell DNA methylation, markers of lung inflammation, and wheezing in children with asthma.⁵⁵ Nasal scrapings and brushings are more invasive than absorption techniques but less invasive than biopsies, and they have the undeniable advantage of providing cell or tissue samples that can be used for a wide variety of analyses.

Interpretation of Research Study Results

The efficacy of a medication is easily assessed by comparing the symptoms recorded after an NAC before and after treatment. Reductions in TNSS can be used to compare one medication to another or complete a dose-finding assessment for pilot efficacy data. Efficacy can also be monitored for 12 to 24 hours to establish the duration of action of the treatment and its effectiveness in controlling symptoms and airflow measurements.⁵⁶

Comparing the concentrations of proinflammatory cytokines in biological samples, before and after allergen challenge is important in understanding the mode of action in participants with or without medication.⁴⁴ Early- and late-phase markers, such as tryptase and IL-5 and IL-13, respectively, can aid in our understanding of how various interventions affect the nasal mucosa.⁴⁵ Further study of the cell count, particularly eosinophils from nasal lavage samples, offers an insight into the inflammatory cells responsible for the symptoms and the phase they are most active in.⁴⁸

Modification of gene expression after immunotherapy or other treatments can be studied using samples collected by nasal brushings or scraping. RNA isolation from nasal cells and subsequent reverse transcription is used to generate complementary DNA for quantitative polymerase chain reaction during such a study.⁵⁹ Primers are then designed to target genes that code for

proinflammatory cytokines and chemokines, for which the expression is thought to be decreased by immunotherapy. Gene expression results are then correlated with the measured cytokine levels on the protein level to confirm that a decrease in gene expression directly resulted in reduced cytokine concentrations.⁵⁹

Conclusion

The NAC method is effective for modeling AR in phase 2 and 3 clinical trials. Participants experience similar symptoms that are reported in natural exposure models but with the advantage of tailoring the allergen dose to each participant, ensuring consistent individual symptoms and allergen exposure throughout the study. Subjective and objective measurements are easily recorded to provide comparisons before and after allergen challenge, with and without treatment. This enables the evaluation of a treatment's efficacy, duration, and mechanism of action.

NAC studies do not require dedicated infrastructure and are easier to set up for use in multicenter trials, allowing for the inclusion of a wider range of participants allergic to different allergens in different geographical regions. NAC protocols are being improved regularly and remain a favored method of study of AR medications, particularly at the proof-of-concept stage or initial phase 2 evaluations of a novel compound.

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